

TEXTBOOK

Dirk Haller *Editor*

The Gut Microbiome in Health and Disease

A Textbook

Second Edition

 Springer

Editor

Dirk Haller

The Gut Microbiome in Health and Disease

A Textbook

Second Edition 2026



Editor

Dirk Haller

Chair of Nutrition and Immunology, School of Life Sciences, Technical University of Munich, Freising, Germany

ISBN 978-3-032-26409-1 e-ISBN 978-3-032-26410-7

<https://doi.org/10.1007/978-3-032-26410-7>

© The Editor(s) (if applicable) and The Author(s), under exclusive license to Springer Nature Switzerland AG 2018, 2026

This work is subject to copyright. All rights are solely and exclusively licensed by the Publisher, whether the whole or part of the material is concerned, specifically the rights of translation, reprinting, reuse of illustrations, recitation, broadcasting, reproduction on microfilms or in any other physical way, and transmission or information storage and retrieval, electronic adaptation, computer software, or by similar or dissimilar methodology now known or hereafter developed. Unless otherwise expressly agreed by the Publisher in writing, the Publisher does not authorize use or reproduction of all or any part of this work in any manner for the purpose of training, fine-tuning, evaluating, or developing artificial intelligence models, technologies, or systems. In accordance with Article 4(3) of the EU Directive 2019/790 on copyright and related rights in the Digital Single Market, the Publisher expressly reserves all rights with respect to this work under the text and data mining exception of the directive.

The use of general descriptive names, registered names, trademarks, service marks, etc. in this publication does not imply, even in the absence of a specific statement, that such names are exempt from the

relevant protective laws and regulations and therefore free for general use.

The publisher, the authors and the editors are safe to assume that the advice and information in this book are believed to be true and accurate at the date of publication. Neither the publisher nor the authors or the editors give a warranty, expressed or implied, with respect to the material contained herein or for any errors or omissions that may have been made. The publisher remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

This Springer imprint is published by the registered company Springer Nature Switzerland AG

The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

Preface

In 2013, the German Research Foundation (DFG) began funding the Priority Program (SPP) 1656 entitled “Intestinal Microbiota” and thereby consolidated a concerted action of the German Society of Hygiene and Medical Microbiology (DGHM) to support microbiome research in Germany. An interdisciplinary network of scientists, including microbiologists, gastroenterologists, immunologists, nutrition scientists, and physicians, worked together over the past 12 years to achieve novel insights into the role of the gut microbiome in health and diseases. Recent advances in this field stimulated the second generation of coordinated research yielding into microbiome-centered Collaborative Research Centers (CRCs) funded by the German Research Foundation (DFG), including CRC 1182 “*Origin and function of metaorganisms*” in Kiel, CRC 1371 “*Microbiome Signatures – functional relevance in the digestive tract*” at the Technical University of Munich, and SPP 2474 “*Illuminating gene function in the human gut microbiome.*” In addition, the German Excellence Strategy actively supports microbiome research through dedicated funding of Clusters of Excellence (“Controlling microbes to fight Infections” in Tübingen and “Balance of the microverse” in Jena) in understanding microbial communities and their impact on health and the environment. Beyond the numerous scientific accomplishments, the consortia made an effort to use their complementary expertise in educating the next generation of young researchers. Many colleagues contributed to the Second Edition of this book, providing a comprehensive reflection of cutting-edge knowledge on the gut microbiome and its functions in human health and diseases, covering basic principles of the gut microbial ecosystem (the impact of diet on composition and metabolic activities as well as its evolution over time and life stages), its reciprocal interaction with the mucosal immune system, and the clinical implementation related to diagnosis and therapy. Considering the needs for a continuous education process of students and health

professionals, the Second Edition of this book is supported by the CRC 1371 and provides a structured and modern overview about the methodologies applied as well as the scientific and clinical aspects of microbiome-host interactions, highlighting perspectives on historic developments and controversies in the field.

Dirk Haller
Freising, Germany

Contents

1 Intestinal Microbiome in Health and Disease: Introduction

Dirk Haller

2 Composition and Function of the Human Gut Microbiome

Thomas C. A. Hitch and Thomas Clavel

3 Microbiome and Early Life

Niels van Best and Mathias W. Hornef

4 Sequencing-Based Microbiome Profiling

Amélia Camarinha-Silva, Melanie Schirmer and W. Florian Fricke

5 Evolutionary Perspectives on the Human Gut Microbiome

Shauni Doms, Mathilde Poyet, Mathieu Groussin and John F. Baines

6 Microbiome and Diet

Francesca De Filippis and Danilo Ercolini

7 Microbiome and Gut Immunity: The Epithelium

Claudia Günther and Dirk Haller

8 The Interplay of the Microbiome with the Innate Immune System

Till Strowig and Andreas Diefenbach

9 Microbiome and Gut Immunity: T Cells

Martina Palatella, Sabrina Wagner, Dirk H. Busch and Jochen Huehn

10 Microbiome and Gut Immunity: B Cells

Oliver Pabst

11 Microbiome and Diseases: Inflammatory Bowel Diseases

Kai Hildner and Dirk Haller

12 Microbiome and Diseases: Allergy

Yacine Amar, Valentina Faihs, Miriam Hils, Martin Köberle and Tilo Biedermann

13 Microbiome and Diseases: Graft-Versus-Host Disease

D. Weber, H. Poeck and E. Holler

14 Microbiome and Diseases: Infection

Romana R. Gerner, Éva de Hoog-Almasi and Bärbel Stecher

15 Microbiome and Diseases: Colorectal Cancer

Sebastian Zeissig, Florian Jokisch, Kenneth Peuker and Klaus-Peter Janssen

16 Microbiome and Diseases: Metabolic Disorders

Thomas Clavel and Josef Ecker

17 Microbiome and Diseases: Steatotic Liver Disease

Ina Bergheim and Kai Markus Schneider

18 Microbiome and Diseases: Gut-Brain Axis and Neurological Disorders

Juliana de Castilhos and Christoph K. Stein-Thoeringer

19 Clinical Diagnostics for Microbiome-Based Therapies

Andreas Hiergeist and André Gessner

20 Fecal Microbiota Transplantation

Lea-Maxie Haag, Franziska Nowak and Britta Siegmund

21 Gnotobiology

Marijana Basic and André Bleich

22 Functional Metabolites from the Human Gut Microbiota

Karin Kleigrewe, Lisa Göbner and Tobias A. M. Gulder

23 Microbiome-Based Therapies: Phage Intervention

Joshua Lemuel Hadi, Adrian Thaqi, Julia Ammann,
Mohammadali Khan Mirzaei and Li Deng

1. Intestinal Microbiome in Health and Disease: Introduction

Dirk Haller¹ 

(¹) Chair of Nutrition and Immunology, School of Life Sciences,
Technical University of Munich, Munich, Germany

 **Dirk Haller**

Email: dirk.haller@tum.de

Abstract

Over the past 20 years, research has shown that the intestinal microbiome, the community of microorganisms living in our gut, plays a crucial role in human health. New sequencing technologies in the mid-2000s, revealed that healthy people differ widely in their gut microbial composition and that distinct changes of these microbial patterns are linked to diseases. We understand that the interplay between the intestinal microbiome and the body is complex, bidirectional and modulated by factors such as diet, drugs, and host immune and metabolic functions. However, when the functional balance between the microbiome and its host is disturbed, infectious and non-communicable diseases can develop.

Keywords Microbiome – Intestine – Infectious – Chronic non-communicable diseases

At the end of the nineteenth century, Robert Koch and Louis Pasteur developed the concept that transmissible human diseases are caused by

microbial infections and, thereby, revolutionized the view of physicians on how to prevent and treat epidemics. More than 100 years later, the next conceptual revolution implies that naturally occurring communities of “commensal” microbes in and on human body sites affect health and the development of numerous diseases. Over the past decade, large science consortia in Europe (MetaHIT; Metagenomics of the Human Intestinal Tract) and the USA (Human Microbiome Project) have started to acquire data on the genomic potentials, phylogenetic relationships, and functional properties of microbial communities, collectively called microbiome, in healthy and diseased human populations. Next generation sequencing (NGS) and the development of novel bioinformatic approaches combined with the implementation of clinical and experimental studies leveraged an enormous boost of scientific activities leading to almost 200,000 publications indexed under the search term “microbiome” in the database of the US National Library of Medicine (PubMed) (Fig. [1.1](#)). A wide variety of disorders, including infectious as well as immune- and metabolically driven diseases, are associated with microbiome changes in the most densely colonized body site—the digestive tract. The gut provides an explicitly large interface to the environment and is critically involved in immune and metabolic homeostasis, providing the conceptual basis that these spatially adapted communities of microorganisms affect human health. Immune, metabolic, and xenobiotic receptors sense and process microbial signals, and thereby contribute to a mutualistic relationship between the microbiome and the host. It is a plausible hypothesis that members of the intestinal microbiome, considered as the *forgotten organ*, co-evolved with the mammalian host, leading to a symbiotic interdependence of this metaorganism. However, at present approximately 70% of the gut bacteria are not yet cultured and 40% of the genetic information reflects unknown functionality, exemplifying the need to better understand the functional relationships of microbial communities with their host at the mechanistic level. An essential question arising from many human studies is whether microbiome alterations are the cause or simply the consequence of pathologies.

Given the substantial inter-individual variations in the microbiome of human populations and the pleiotropy of confounding factors, the correlative NGS-based analyses in cross-sectional studies require validation in well-controlled intervention studies using a careful selection of participants. The implementation of prospective (longitudinal) and treatment naïve early onset or birth cohorts may help to identify disease-relevant microbiome signatures in a progressive fashion and at very early stages. In addition to a better stratification of human phenotypes in healthy and diseased populations, the implementation of standardized protocols for sampling and analysis is needed to improve the reproducibility and comparability of microbiome signatures at a meaningful taxonomic resolution. Bioinformatic algorithms need to cope with the inherent complexity and the implementation of machine-learning algorithms is a growing need. In addition to the sequencing-based knowledge gain, the development of large-scale bacterial strain isolation strategies is required to access the large repertoire of yet uncultured taxa. The implementation of these isolates in simplified biosynthetic consortia will further help narrow the functional knowledge gap associated with the sparse understanding of microbiome-host circuits. First approvals of microbiome-related therapies targeting *Clostridioides difficile* infections indicate the successful translation of microbiome research into clinical application. The future aim of microbiome research must be to further develop a profound mechanistic understanding of the complex and functionally interrelated relationship of complex microbial communities with their human host, ultimately leading to novel prognostic tools and therapeutic applications for targeted clinical implementation.

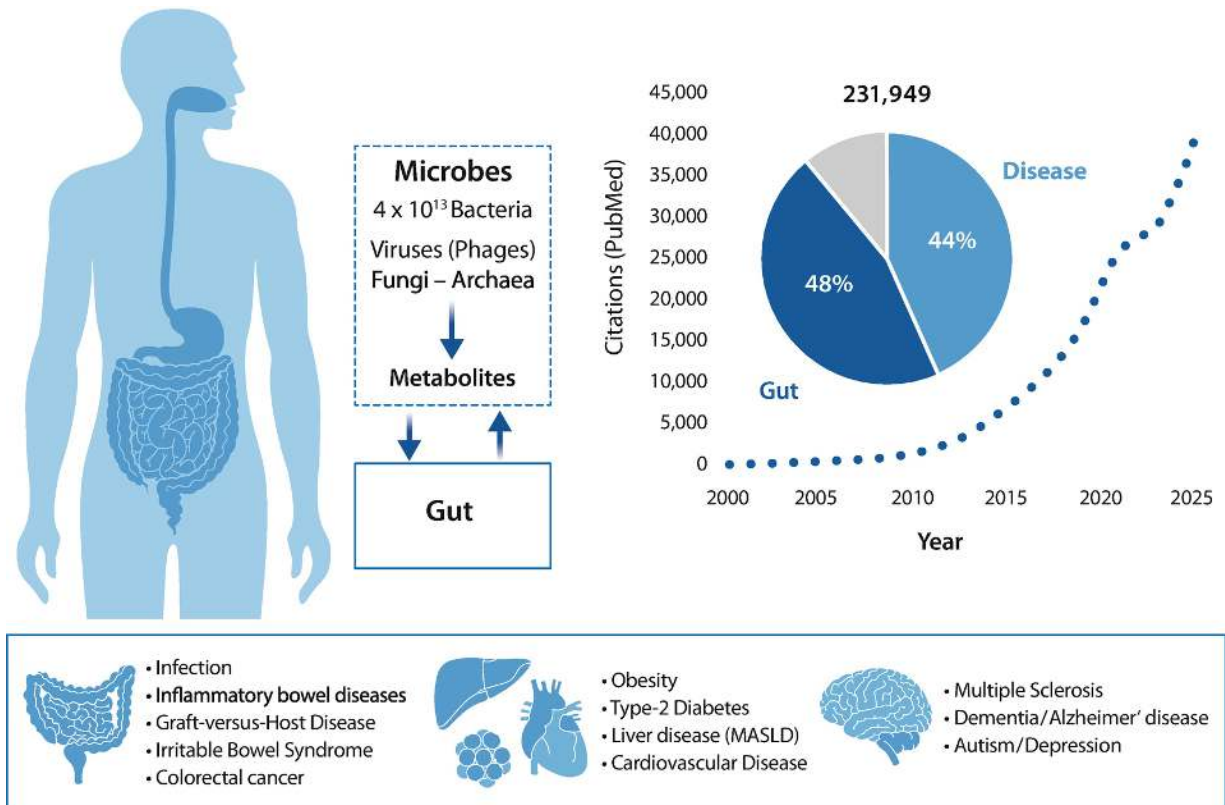


Fig. 1.1 Microbiome–host interactions in the gut. Citations were obtained by searching the database of the US National Library of Medicine (www.ncbi.nlm.nih.gov/pmc). The term “microbiome” retrieved more than 230,00 publications at the end of 2025. The pie chart illustrates the relative contribution (in %) of different aspects in microbiome research related to diseases and the importance of the gut microbial ecosystem. A selection of diseases is highlighted to illustrate the clinical relevance of the gut microbiome. The annual contribution of publications related to disease categories is displayed between 2000 and 2025. Abbreviation: MASLD Metabolic dysfunction-associated steatotic liver disease

2. Composition and Function of the Human Gut Microbiome

Thomas C. A. Hitch^{1,2}✉ and Thomas Clavel¹✉

- (¹) Functional Microbiome Research Group, Institute of Medical Microbiology, RWTH University Hospital, Aachen, Germany
(²) Department of Anaesthesia and Intensive Care, The Chinese University of Hong Kong, Hong Kong, Hong Kong

✉ **Thomas C. A. Hitch**

Email: thitch@cuhk.edu.hk

✉ **Thomas Clavel (Corresponding author)**

Email: tclavel@ukaachen.de

Abstract

The human gastrointestinal tract harbors a plethora of microorganisms, most of which belong to the domain Bacteria. Owing to manifold effects on host physiology and host health, there is a growing interest in better understanding the role and function of gut microbial communities. Microbiota composition changes along the gastrointestinal tract in response to changes in the physicochemical conditions and substrate availability. Moreover, large interindividual differences are observed. One major function of the gut microbiota is the conversion of indigestible dietary carbohydrates and host-derived glycans to short-chain fatty acids, which provide energy to the host and have many regulatory functions. Microbiome analysis has led to the notion of a “core microbiome,” which encodes functions shared by human

individuals. Gut microbial community members interact with each other and with the host, constituting a functional microbial ecosystem. However, there are still major gaps in our understanding of the molecular mechanisms underlying such interactions.

Keywords Taxonomy – Community – Functionality – Digestion

This new edition is based on the previous manuscript, written entirely by Prof. Dr. Michael Blaut (DIfE, Potsdam, Germany), who gave his permission to the current authors to re-use the text and to make any changes and corrections they deemed appropriate to update the content.

2.1 Introduction

Microbial life (bacteria, archaea, fungi, and viruses) has conquered essentially every habitat on earth and may therefore be considered ubiquitous. They occupy environments that differ profoundly in their physicochemical conditions and the substrates available for growth. Microbial habitats range from marine and sweet water environments, deep-sea hydrothermal vents, soil, and air to plants and animals. The microbes thriving in a given habitat are optimally adapted to the conditions prevailing therein. Some microbial communities withstand even harsh conditions such as radiation, high temperature, high salinity, and low or high pH. The ability of microorganisms to colonize essentially all habitats on earth reflects four billion years of evolution. Depending on the environment, microbes may be phototrophic, chemotrophic, lithotrophic, autotrophic, heterotrophic, and combinations thereof, indicating a high metabolic variability. Besides playing essential roles in the global cycles of carbon, nitrogen, and sulfur, microorganisms also occur in and on animals and humans. In humans, they occupy various body sites, including the skin, nose, throat, as well as the urogenital and gastrointestinal tracts. Due to the enclosed nature of the intestinal tract, it uniquely offers an anaerobic environment with a constant temperature and is rich in nutrients,

allowing both facultative and strict anaerobes to survive and flourish. These communities consist of members from each domain of life, with thousands of different bacteria (Leviatan et al., [2022](#)) and viruses (Camarillo-Guerrero et al., [2021](#)) being identified, along with hundreds of fungi (Yan et al., [2024](#)) and Archaea (Chibani et al., [2022](#)). The selection of these communities has occurred over millennia, with evidence existing that the intestinal microbial communities coevolved with their respective host (Suzuki et al., [2022](#)).

2.2 Distribution of Microbial Communities in the Human Gastrointestinal Tract

Environmental conditions in the human gastrointestinal tract are not uniform but differ considerably between the stomach and colon. It is therefore not surprising that the microbial communities resident in the various sections of the digestive tract differ in several aspects, including cell density, composition, and metabolic activity.

2.2.1 Stomach

Between meals, the pH in the stomach of healthy adults is typically 1–2, but increases following food ingestion. Transit time through the stomach, determined in eight healthy subjects with a magnet tracking system, ranged between 5 and 133 min, with a median of 56 min (Worsoe et al., [2011](#)). Transit time is influenced by food consistency (shorter for fluids than for solid and unchewed food), osmolarity (longer for monosaccharides compared to polysaccharides), nutrient composition (longer for fats and carbohydrates), and energy density (longer for high-energy diets). The low pH of gastric juice largely prevents the growth of ingested microbes, explaining the low density of $<10^3$ microbial cells per ml of gastric content. However, a culture-independent survey of microbial 16S rRNA gene sequences in 23 gastric mucosa biopsy samples revealed a diverse community consisting of members from the phyla *Bacillota* (formerly Firmicutes), *Bacteroidota* (formerly Bacteroidetes), *Pseudomonadota* (formerly

Proteobacteria), *Actinomycetota* (formerly Actinobacteria), and *Fusobacteriota* (formerly Fusobacteria) (Fig. [2.1](#)), along with minor components of other phyla (Bik et al., [2006](#)). A high proportion of the detected sequences were assigned to oral bacteria, such as *Prevotellaceae*, *Streptococcus*, and *Porphyromonas* spp. It may be surmised that the main habitat of many of these species is the oral cavity, from where they get into to stomach by swallowing. Nineteen of the 23 subjects harbored *Helicobacter pylori*, although prevalence depends on studies and their scale (Bik et al., [2006](#)). This organism resides in the mucus layer of the stomach and is known to secure its survival in the gastric environment by the production of urease, which catalyzes the release of ammonia (and carbon dioxide) from urea, resulting in an increase of the pH in the immediate environment of the cell. Bacteria isolated from gastric contents include *Lactobacillus* and *Streptococcus* spp., which are capable of surviving at relatively low pH.

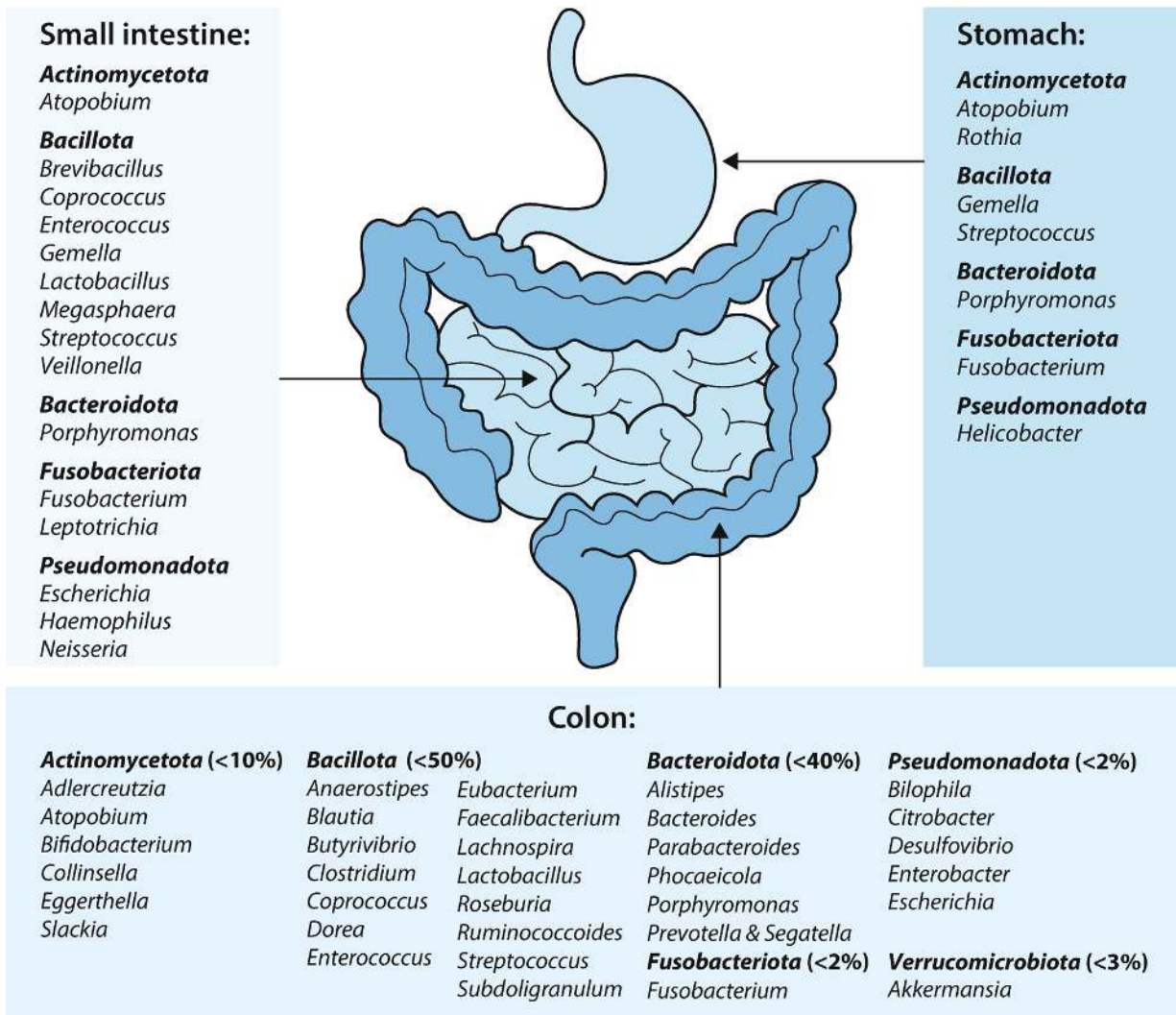


Fig. 2.1 Major bacterial genera in the various sections of the gastrointestinal tract

2.2.2 Small Intestine

The small intestine represents the longest part of the digestive tract, with changing conditions and increasing bacterial cell densities along its course. The relatively short residence time of intestinal contents, namely, 209–391 min with a median of 255 min (Worsoe et al., [2011](#)), limits the growth of microorganisms to high density, in particular in the duodenum. Bacterial cell counts increase from the duodenum to the terminal ileum from approximately 10^4 to 10^8 per mL of intestinal content (Booijink et al., [2010](#); Finegold et al., [1983](#)), and also the number of taxa detectable with culture-independent methods increases (Hayashi et al., [2005](#)). Catheter-based collection of duodenal, jejunum,

and ilial content from patients was reported to contain *Streptococcaceae*, *Veillonellaceae*, and *Actinomycetaceae*. *Prevotellaceae* is another dominant member in proximal samples, but it reduces toward the distal small intestine while *Pasteurellaceae* increases in distal samples (Fig. 2.1). These communities were also observed to be distinct to stool samples from the same individuals (Shalon et al., 2023; An et al., 2024). A large fraction of microorganisms in the small intestine are aerotolerant anaerobes, facultative anaerobes, or microaerophiles, but their proportion decreases from the duodenum to the terminal ileum as oxygen becomes more limited and the redox potential decreases. It is important to note that the number of studies investigating the small intestinal microbiota is considerably smaller than the number dealing with the colonic or fecal microbiota. This is largely due to the invasive nature of sampling, however new technology, such as smart pills, may change this, allowing for direct sampling (Shalon et al., 2023).

2.2.3 Colon and Feces

Owing to a relatively long mean colonic transit time of 28–35 h (Metcalf et al., 1987; Asnicar et al., 2021; Procházková et al., 2023), colonic microorganisms have more time to proliferate. The absorption of water and ions during passage of colonic contents also contributes to an increase in bacterial density from cecum (10^8 mL^{-1}) to distal colon (10^{11} mL^{-1}) (Sender et al., 2016b). Greater water absorption occurs in people with longer transit times, leading to a direct association of transit time and the Bristol stool scale, a measure of stool consistency (Asnicar et al., 2021). The colon is the most densely microbially populated body site. The total number of microbial cells inhabiting the human body has previously been estimated to exceed the number of host cells by a factor of 10 (Savage, 1977). A more recent publication is in conflict with this estimate. Based on thorough considerations, the total number of microbial cells harbored by a reference male person (20–30 years of age with a weight of 70 kg and a height of 170 cm) was estimated to be in the range of 4×10^{13} with more than 99% of these

cells residing in the colon, while the number of human body cells is approximately 3×10^{13} , with red blood cells contributing 84% to this number (Sender et al., [2016a](#)). Hence, the number of microbial cells in the human body is 1.3-fold higher than the number of body cells. However, if only nucleated cells are considered (0.3×10^{13}), this ratio increases to a factor of 10.

Although there is no dispute about the fact that the number of microbial species residing within the colon is quite high when compared with other microbial habitats, estimates of the number of microbial species present within each person's colonic contents or feces have varied substantially, especially since the advent of next generation sequencing methods due to the detection of spurious taxa (Clavel et al., [2016](#); Reitmeier et al., [2021](#)). The most recent evidence provided an estimated total diversity of 3594 bacterial species within the human gut (Leviatan et al., [2022](#)). From this, 4624 fecal samples were screened and identified to each contain 214 ± 64 species, ranging from only 20 up to 402 species per individual. While the communities within the gut vary greatly between people, especially at the strain level, most of the species within a gut microbiota belong to only a few bacterial phyla: *Bacillota*, *Bacteroidota*, *Actinomycetota*, *Pseudomonadota*, *Verrucomicrobiota*, and *Fusobacteriota* (Almeida et al., [2019](#))

Other members of the human gut microbiota include methanogenic Archaea and eukaryotic yeasts, whose abundance, based on the proportion of these organisms' genes in the intestinal metagenome, is in the range of 0.8% and 0.1%, respectively (Qin et al., [2010](#)). The Archaea are represented by only 18 species (Chibani et al., [2022](#)). The most common archaeon within the human gut is *Methanobrevibacter smithii*, which converts H_2 and CO_2 or formate to methane. Methane has been shown to reduce inflammation by preventing the generation of reactive oxygen species (ROS) (Boros et al., [2012](#)) and immune signaling in colitis models (Zhang et al., [2016](#)). The amount of methane excreted by humans in breath is variable, suggesting that, while methanogenic populations are prevalent across individuals

(Hoegenauer et al., [2022](#)), they account for dominant populations only in every other person (Florin et al., [2000](#)). Some archaeal strains have also been shown to degrade trimethylamine N-oxide (TMAO) (Ramezani et al., [2018](#)), a microbial metabolite associated with cardiovascular disease (Fromentin et al., [2022](#)).

Among eukaryotic intestinal microorganisms, fungi are the most prominent members of the intestinal microbiota (Huffnagle & Noverr, [2013](#)). Intestinal fungi, referred to as gut mycobiome, have been much less studied than intestinal prokaryotes. Analysis of fecal samples from 98 healthy individuals led to the identification of 66 fungal genera and an estimated number of 184 species (Hoffmann et al., [2013](#)). *Saccharomyces*, *Candida*, and *Cladosporium* were the most prevalent genera, being found in 89%, 57%, and 42% of the samples, respectively. This is supported by the recent isolation 12,453 strains of fungi, representing 206 species, from fecal samples (Yan et al., [2024](#)). While their isolation confirms their existence within this ecosystem, it does not clarify if these are residents of the gut or merely transient. Even though fungi are considered important members of microbial communities in the human gut and have been implicated in immune response development and chronic inflammation (Sokol et al., [2017](#)), knowledge about their role in health and disease is still very limited in comparison to the bacterial members of the community.

While viruses are not considered to be organisms, they can readily be detected in fecal samples, termed the virome (Li et al., [2022](#); Minot et al., [2011](#)). Current research has identified 82,141 viral species across 8130 fecal samples from infants, adding to existing repositories built using genomic and metagenomic data from the intestine of adult subjects (Gregory et al., [2020](#)). Bacteriophages, the viruses that infect bacteria, form a dominant part of viral communities and are the subject of intense research due to both, their contribution to the ecological dynamics within gut microbial communities and their potential use in the clinic (Camarillo-Guerrero et al., [2021](#); Duan et al., [2022](#)). Whilst further research is needed to understand the functional impact of specific viruses on the microbiota, the virome can alter the microbial

community within the gut with causal consequences for the host (Mao et al., [2024](#)).

While species richness in the human gut is high, the majority of microbes have been identified based on the reconstruction of partial genomes from metagenomic data and lack a cultured representative. For example, it is currently estimated that approximately half of the taxonomic and functional diversity of human gut bacteria remains to be described (Hitch et al., [2021](#); Almeida et al. [2019](#); Thomas & Segata, [2019](#)). This is a major obstacle for understanding the molecular mechanisms underlying microbe-microbe and microbe-host interactions, and highlights the importance of intensifying cultivation work, including public access and detailed description of novel taxa (Hitch et al., [2025](#)).

2.3 Factors Influencing Variability in the Microbiota

The microbiota of each individual is shaped by the internal and external pressures placed upon the ecosystem. The factors that influence gut microbial communities include those that are immutable to a person, such as the length of the GI tract or diversity of the bile acid pool, or those that are changeable, such as diet.

2.3.1 Host-Related Effects on the Microbiota

Colonization of the human intestinal tract starts at birth (Kennedy et al., [2023](#)), during which the first host-driven effect on the microbiota is made. A study of 46 infants born via caesarean section, and 74 who were vaginally delivered, identified distinct differences. Vaginally delivered infants showed a higher relative abundance of *Bifidobacterium*, while the gut microbiota of caesarean section infants contained more *Klebsiella* and *Staphylococcus* (Reyman et al., [2019](#)). While the effects of birth by caesarian section on fecal microbial communities tend to vanish after a few months of life, health consequences exist in children, such as an increased risk of developing

allergies, even though long-term outcomes are less clear (Sandall et al., [2018](#)). The naïve state of the infant gut makes it highly susceptible to change. Studying 903 infants over the first 46 months of life, factors such as the presence of a furry pet in the household or siblings were identified to alter the infants' microbiota (Stewart et al., [2018](#)). For more information on the human gut microbiome during early life, see Chap. [3](#).

Past early life, the gut microbiota reaches a stable state, until the age of ~60, where significant changes have been observed. A comparison of 161 elderly (>65 years old) and 9 younger (<46 years old) people identified a microbiota dominated by *Bacteroides* spp. in the elderly, distinct from that of the younger people. Refinement of the age categories past 'elderly' has provided greater insight into the diversity of ageing microbiota. Comparison of 69 people, including 24 centenarians, who live past 100, identified a decrease in microbial taxa, including *Coproccus*, *Roseburia*, and *Faecalibacterium* with increasing age, but an increase in other taxa, such as *Oscillospira*, *Eggerthella*, and *Akkermansia* (Biagi et al., [2016](#)). Recent work has confirmed a link between the gut microbiome and host longevity, including viral and fungal communities (Johansen et al., [2023](#); Pang et al., [2023](#); Pu et al., [2024](#)). However, more work is needed to reach a consensus about the specific microbiome signatures associated with host lifespan and the factors that influence them.

One factor that alters the microbiota throughout life is diet. The resources available to a community directly impact the growth of strains. An example of this is *Roseburia intestinalis*, which encodes a range of enzymes specific to the utilization of dietary β -mannans (La Rosa et al., [2019](#)). Due to this, dietary supplementation with β -mannans leads to a bloom in *R. intestinalis*. By understanding the enzymes encoded by specific microbes, it is possible to develop molecules that target the growth of individual species within the gut. Targeted treatment of milled Norway Spruce produced an acetylated galactoglucomannan which matched the enzymes encoded on polysaccharide utilization loci (PULs) within *Roseburia* and

Faecalibacterium spp. (Michalak et al., [2020](#)). Further details on the impact of diet on the microbiota can be found in Chap. [6](#).

The diet of an individual is, in part, driven by the availability of foods in a region, such as seaweed in Japan (Tasse et al., [2010](#)). The geographical region has been shown to be a key factor associated with microbiota profiles (Yatsunenko et al., [2012](#)). So far, the majority of gut microbiota research has been conducted on high-income populations in America, Europe, and China. The current exclusion of many populations from being studied prevents us from gaining a comprehensive understanding of gut microbial communities. A common example of this is the Hadza tribe, a population of hunter-gatherers in Tanzania, whose microbiome contains enriched diversity compared to European cohorts (Schnorr et al., [2014](#)). The gut microbiota of the Hadza contains 124 species that are vanishing within industrialized populations (Carter et al., [2023](#)). A study on bacterial strains from diverse populations identified industrialization as a driver of horizontal gene transfer between microbes (Groussin et al., [2021](#)). Therefore, without the collection of rural strains prior to industrialization, this unique diversity may be lost. To combat this, projects such as the Global Microbiome Conservancy (<https://microbiomeconservancy.org>) and the Microbiota Vault (www.microbiotavault.org) have been established to collect and store microbial diversity for the future.

2.3.2 Core and Accessory Microbiome

The intestinal tract selects for microbes that have the capacity to grow within the confines of the gut. Since bacteria in the gut share the same environment, it can be surmised that they have certain gene functions in common in addition to the so-called housekeeping functions required by all living cells. Therefore, it is not surprising that some microbes consistently occur within the gut of humans. These taxa have been referred to as the “core microbiome” due to their ubiquitous nature across large cohorts (Tap et al., [2009](#); Qin et al., [2010](#)). They commonly encode metabolic activities that are found in, nearly every subject. Conversely, the “accessory microbiome” is a term used to

discuss taxa that are less common but may be important for certain functions or in specific conditions, such as those converting xenobiotics (Haiser et al., [2014](#)) or species like *Fusobacterium nucleatum* that play a role in the development of colorectal cancer (Zepeda-Rivera et al., [2024](#)) or *Oxalobacter formigenes* that provides protection against calcium stone disease (Kaufman et al., [2008](#)). The accessory microbiome encodes for less general functionality, including conversion of polyphenols, utilization of porphyrin from marine red algae (see Sect. [2.4.2](#)), and many other functions that can be strain-dependent (Atkinson et al., [2004](#); Hehemann et al., [2010](#); Wolin & Miller, [1983](#)). The idea of a core microbiome at a functional level is a useful concept as it reflects distinct environmental influences in a given habitat.

The term “core” has also been applied at differing levels of specificity. While a genus, such as *Bacteroides*, may be deemed core, this does not apply to all species within the genus (Maier et al., [2018](#)). The complexity of such terms expands when considering strain-level variability. While *Limosilactobacillus reuteri* is a common member of mammalian gut microbiota, host-specific adaptations have evolved to provide strains a selective advantage to survive within their respective host (Oh et al., [2010](#)). Due to this, the origin of a strain must be considered when selecting representatives of an ecosystem.

2.4 Functional Adaptation of Microbes to the Intestinal Environment

The term metagenome refers to the collective genome of all members of the microbiota in a given habitat. The proteins encoded in the human intestinal metagenome roughly reflect the functions required by intestinal microbes to cope with the gut environment. A large proportion of the genes identified in the microbiome are related to fundamental functions required by every cell to grow and divide. Genes and proteins related to energy generation, synthesis of cellular components, and reproduction are found in every bacterial cell and are usually well conserved among bacteria. Examples include ribosomal

proteins, RNA polymerase, and ATP synthase. Metabolic pathways, including glycolysis and the tricarboxylic acid cycle are widely but not universally distributed among bacteria in general and intestinal bacteria in particular. For example, *Bifidobacterium* spp. degrade hexoses using the unique fructose-6-phosphate phosphoketolase pathway rather than by glycolysis. Moreover, in many strict anaerobes, the tricarboxylic acid cycle is incomplete, and the remaining enzymes of the cycle preferentially fulfil anabolic functions. Physiology and the metabolic capacity of intestinal microorganisms are adapted to the conditions prevailing in the digestive tract, but they also evolve over time under the influence of host-driven and external factors.

2.4.1 Physicochemical Conditions in the Digestive Tract and Electron Transport

The intestine, especially the colon, is characterized by low oxygen partial pressure and highly reduced conditions with a redox potential (E_h) of approximately -215 mV, a value measured in the large intestine of pigs (Hornich & Chrastova, [1981](#)). Therefore, >99% of human fecal bacteria and also methanogenic Archaea are strict anaerobes. They cannot grow in the presence of oxygen because critical enzymes become inactivated under oxic conditions (Chavez-Arroyo et al., [2025](#)). Even though facultative aerobic or aerotolerant bacteria make up less than 1% of microbial cells in the human distal gut, they play an important role. In particular, facultative anaerobes such as the *Enterobacteriaceae* are capable of utilizing oxygen as a terminal electron acceptor. For example, *Escherichia coli* is capable of expressing two ubiquinol-dependent oxidases, one of which has a low affinity for oxygen and a high turnover rate (cytochrome *o*-type oxidase), while the other one (cytochrome *d*-type oxidase) has a high affinity for oxygen and a low turnover rate. If no oxygen is available, *E. coli* can gain energy anaerobically either by anaerobic electron transport using nitrate, trimethylamine-*N*-oxide, dimethyl sulfoxide, or fumarate as terminal electron acceptors. If none of these is available, *E. coli* gains energy by mixed acid fermentation. The metabolism of this facultative anaerobe is

regulated in such a way that the most efficient mode of energy generation is turned on while less efficient, alternative modes are turned off. Bacteria that tolerate oxygen but cannot gain energy by respiration are called aerotolerant. Lactic acid bacteria, for example, exclusively gain energy by substrate-level phosphorylation involving the conversion of carbohydrates such as lactose to lactic acid and some minor fermentation products. Despite the scarcity of oxygen in the human intestinal tract, small amounts of oxygen become available by swallowing air during meals and by diffusion of oxygen from the blood circulation to the mucosal surface. In addition to facultative aerobes, which tolerate high oxygen partial pressures, some gut bacteria previously considered strict anaerobes are able to utilize oxygen as long as nanomolar concentrations are not exceeded (Baughn & Malmay, [2004](#)). *Bacteroides fragilis* and other *Bacteroides* spp. harbor a high-affinity cytochrome *bd*-type oxidase, allowing ATP generation by aerobic respiration. The term anaerobes has been coined for such bacteria (Baughn & Malmay, [2004](#)). *Faecalibacterium prausnitzii* is an oxygen-sensitive organism that nevertheless grows near the mucosal surface where the oxygen partial pressure is relatively high. This is possible because *F. prausnitzii* transfers electrons to extracellular flavins and thiols, present in the gut, to reduce oxygen (Khan et al., [2012](#)). However, even though this mechanism enables the organism to reoxidize reduced electron carriers, ATP generation is less efficient than aerobic respiration.

2.4.2 Alternative Electron Acceptors

Anaerobes capable of transferring electrons derived from oxidation reactions onto external electron acceptors do not have to use valuable intermediates such as pyruvate as electron acceptors. For example, under anoxic conditions, *E. coli* and other *Enterobacteriaceae* are capable of using nitrate or trimethylamine-*N*-oxide as an electron acceptor. Nitrate may be formed in the inflamed gut in which NO levels are increased because of an upregulated nitric oxide synthase (iNOS). NO reacts with reactive oxygen species to peroxynitrate (ONOO⁻),

which isomerizes to nitrate. Accordingly, nitrate produced under inflammatory conditions not only stimulates the growth of *Salmonella* but also that of *E. coli* (Lopez et al., [2012](#); Winter et al., [2013](#)). Various sulfur compounds, including sulfate, thiosulfate, and tetrathionate, may also serve as electron acceptors. Sulfate reaching the colon may be of dietary origin, but the majority is derived from sulfated mucins by the action of bacterial sulfatases (Christl et al., [1992](#)). Sulfate-reducing bacteria in the human gut include species of the genera *Desulfovibrio*, *Desulfobacter*, and *Desulfobulbus* (Nava et al., [2012](#)). Since sulfate (SO_4^{2-}) is a poor electron acceptor, it first needs to be activated to adenosine-5'-phosphosulfate (APS) ($\text{SO}_4^{2-} + \text{ATP} \rightarrow \text{APS} + \text{PP}_i$). APS is subsequently reduced to sulfite (SO_3^{2-}) in a two-electron transfer reaction and thereafter to sulfide (S^{2-}) in a six-electron transfer reaction. Some bacteria, including *Bilophila wadsworthia*, are capable of gaining sulfite from sulfonates such as taurine (Carbonero et al., [2012](#)). Therefore, bile acids conjugated with taurine stimulate the growth of this organism. Trimethylamine-*N*-oxide, another electron acceptor used by *Enterobacteriaceae*, is formed by the oxidation of trimethylamine as catalyzed by host monooxygenases (Bennett et al., [2013](#)). Trimethylamine, in turn, originates from the bacterial degradation of choline or carnitine in the human intestine and may be associated in the long run (under adverse dietary conditions) with the development of cardiovascular complications (Fromentin et al., [2022](#)).

2.4.3 Utilization of Hydrogen and Formate

Metabolic cross-feeding processes between different microbes, including phylogenetically distant species, are essential for ecosystem stability in the human gut. In addition to the production of short-chain fatty acids (SCFA) (see Sect. [2.5.2](#)), the gut microbiota produces intermediary molecules such as formic acid and the gases H_2 and CO_2 , which are partly excreted and partly further utilized within the ecosystem. H_2 is produced by various bacterial populations in the colon. For example, *Enterobacteriaceae* such as *E. coli* produce H_2 and CO_2 from formate, catalyzed by formate-hydrogen lyase, while strict

anaerobes such as *Clostridiales* species may release H₂ in the course of pyruvate oxidation as catalyzed by pyruvate:ferredoxin oxidoreductase. The reduced ferredoxin produced in this reaction is used by hydrogenase for the reduction of two protons to produce H₂. The latter and formate play a role in methanogenesis and acetogenesis, which are essential metabolic functions in the human gut. Archaea, including the prevalent species *Methanobrevibacter smithii* produce CH₄ from H₂ and CO₂ ($\text{CO}_2 + 4\text{H}_2 \rightarrow \text{CH}_4 + 2\text{H}_2\text{O}$) or formate ($4\text{HCOOH} \rightarrow 3\text{CO}_2 + \text{CH}_4 + 2\text{H}_2\text{O}$). Homoacetogenic bacteria such as *Blautia* spp. can also take advantage of H₂ and CO₂ and/or formate produced by other species for their own metabolism (Bernalier et al., [1996](#); Liu et al., [2008](#)). Using the Wood-Ljungdahl pathway, these bacteria catalyze the formation of acetate ($2\text{CO}_2 + 4\text{H}_2 \rightarrow \text{CH}_3\text{-COOH} + 2\text{H}_2\text{O}$ or $4\text{HCOOH} \rightarrow \text{CH}_3\text{-COOH} + 2\text{CO}_2 + 2\text{H}_2\text{O}$) (Ragsdale, [2006](#)). H₂ is an important product of anaerobic fermentation as it enables anaerobes to reoxidize electron carriers without the need to use intermediates such as pyruvate as electron acceptors, enabling a higher ATP gain per hexose metabolized compared to other fermentations.

2.4.4 Gut Microbes Evolve with Their Host

The physiochemical features of the gut described above vary depending on the host species, which influence the structure and functions of the microbial community and how single members evolve. The microbiome has been linked to the evolutionary history of their host based on the dietary habits of the host species (Ley et al., [2008](#)). A well-studied example of host-specific adaptation is *Limosilactobacillus reuteri* for which strains group phylogenetically depending on the host they were cultured from (Oh et al., [2010](#)). To understand if these differences translate to preferential colonization of hosts, a mixture of host-specific strains from mice, chicken, pigs, and humans was used to colonize these hosts. Both mice and chickens were preferentially colonized by their host-specific strains, confirming the theory that strain-specific adaptation has occurred to facilitate enhanced colonization of the environment they reside (Duar et al., [2017](#)). Similar findings were

obtained using complex communities, demonstrating that mouse-derived species outcompete those from the human gut when administered to gnotobiotic mice (Seedorf). Experimental evolution studies, e.g., in *Drosophila melanogaster*, have demonstrated that within-host evolution, including the influence of diet, contributes to the emergence of strains with a beneficial effect on the host (Martino et al., [2018](#)). Strain level variation also occurs within a host species, such as humans, for which it has been linked to migration patterns out of Africa (Suzuki et al., [2022](#)). This has led to common gut microbes such as *Segatella copri* and *B. longum* diversifying with their hosts, as observed by their geographical distribution. Comparison of *B. longum* strains for potential use as probiotics identified that those isolated from Bangladesh encoded two gene clusters involved in utilization of plant-derived polysaccharides compared to a USA isolated strain (Barratt et al., [2022](#)). In addition to coevolution (see Chap. [5](#)), the diversity of the microbiota can change due to factors such as a shift in lifestyle, such as industrialization and the occurrence of ‘western diet’ (de Goffau et al., [2022](#)). These changes have been proposed as the reason for reduced *S. copri* prevalence and strain-level diversity within the global north (Tett et al., [2019](#)). The microbiota of non-westernized populations is more likely to include strains from multiple clades of *S. copri*, each clade encoding different patterns of CAZymes. These genomic differences facilitate the use of different plant-derived polysaccharides, suggesting the increased plant fiber in non-westernized populations may have directed the evolution of the *S. copri* clades (De Filippis et al., [2019](#)).

2.4.5 Horizontal Gene Transfer Facilitates Metabolic Adaptation of Microbes

A major environmental factor the gut microbiota must adapt to is the host’s diet, which may change suddenly due to the availability or preference of the host. One way in which the microbiome can adapt to sustained changes in diet is the sharing of functions via horizontal gene transfer (Groussin et al., [2021](#)). Several lines of evidence indicate that PULs can be horizontally transferred among members of the intestinal

microbiota (Tasse et al., [2010](#)) and between bacteria resident in the digestive tract and bacteria ingested with food (Hehemann et al., [2010](#)). *Zobellia galactanivorans*, a marine member of the *Bacteroidota*, is capable of degrading the sulfated polysaccharide porphyrin, which is present in marine red algae, and utilizing it as a growth substrate. There is evidence that the genes encoding the porphyranases, agarases, and accessory proteins required for porphyrin degradation have been transferred to the gut bacterium *Bacteroides plebeius*. Interestingly, this species was isolated exclusively from Japanese individuals (Kitahara et al., [2005](#)) who consumed porphyrin-containing seaweed and thereby probably also ingested *B. plebeius* present on it. Intestinal microbiome analyses revealed that the genes encoding porphyranase and agarase are frequently found in Japanese subjects but not in North American individuals.

2.5 Main Metabolic Activities of the Intestinal Microbiota

Besides conferring colonization resistance on the host and protecting against pathogens, the gut microbiota primes the immune system and provides enzymes that expand the metabolic capacity of the host. A major function of the intestinal microbiota is the conversion of dietary and endogenous substrates that escape digestion, including carbohydrates, proteins, secondary plant metabolites, and xenobiotics. The conversion of these substrates supports the growth of intestinal microbes by providing energy and metabolites for anabolic reactions or benefits them indirectly by enhancing their competition with other species. The gut microbiota's metabolic capacity has been proposed to rival that of the liver, encompassing a wide range of reactions that reflect the low redox potential and the scarcity of oxygen available in most parts of the intestinal tract.

2.5.1 Substrates of the Intestinal Microbiota

Even though intestinal bacteria differ in how they generate energy, they share the same environment, i.e., the physicochemical conditions (pH, redox potential, temperature) at a given intestinal site, and they are dependent on the substrates coming from the diet (Table 2.1) or endogenous substrates provided by the host (Table 2.2). However, some bacterial population groups cross-feed other community members by converting these primary substrates into products that can be utilized further by bacteria that depend on these substrates; examples include lactate, formate, and hydrogen.

Table 2.1 Substrates of dietary origin utilized by the intestinal microbiota

Dietary origin		
Category	Class	Composition or representative compounds
Polysaccharides	Resistant starch:	
	Amylose	$\alpha(1 \rightarrow 4)$ Glucan
	Amylopectin	$\alpha(1 \rightarrow 4)$, $\alpha(1 \rightarrow 6)$ Glucan (branched)
	Cellulose	$\beta(1 \rightarrow 4)$ Glycan
	Pectins	$\alpha(1 \rightarrow 4)$, $\alpha(1 \rightarrow 6)$ Galacturonan (methylesters) $\alpha(1 \rightarrow 4)$ Galactan and mixed-linked arabinans
	Pentosans	$\beta(1 \rightarrow 4)$ Xylan with some arabinose and uronic side chains
	Hexosans	$\beta(1 \rightarrow 4)$ Glucomannan, $\beta(1 \rightarrow 3)$, $\beta(1 \rightarrow 4)$ Glycan (single or mixed)
	Xyloglycans	$\beta(1 \rightarrow 4)$ Glucan with $\beta(1 \rightarrow 6)$ -linked xylose side chains
	Galactomannans (Guar gum)	$\beta(1 \rightarrow 4)$ Mannans with $\alpha(1 \rightarrow 6)$ -linked galactose side chains
	Chitin	$\beta(1 \rightarrow 4)$ <i>N</i> -Acetylglucosamine
	Laminarin	$\beta(1 \rightarrow 3)$ Glucans
	Inulin	$\beta(1 \rightarrow 2)$ Fructan
Oligosaccharides	Stachyose	$\alpha(1 \rightarrow 6)$ Galactosyl raffinose
	Raffinose	$\alpha(1 \rightarrow 6)$ Galactosyl sucrose
	Lactose	$\beta(1 \rightarrow 4)$ Galactosyl glucose

Dietary origin		
Category	Class	Composition or representative compounds
	Lactulose	$\beta(1 \rightarrow 4)$ Galactosyl fructose
Sugar alcohols	Sorbitol	
	Xylitol	
Secondary plant metabolites	Flavonoids	Quercetin, Luteolin, Cyanidin, Daidzein
	Tannins	Polymers of ellagic acid, gallic acid, pyrogalllic acid
	Glucosinolates	Glucoraphanin, Sinigrin, Sinalbin, Glucobrassicin
	Lignin	Cross-linked macromolecule formed from paracoumaryl, coniferyl, and sinapyl alcohol
Proteins Fats	Sarcoplasmatic and myofibrillar proteins Triglycerides Cholesterol Phospholipids	

Table 2.2 Substrates of the intestinal microbiota provided by the host

Host		
Category	Class	Composition or representative compounds
Glycoproteins	Mucus	Protein backbone with characteristic carbohydrates (fucose, sialic acid, <i>N</i> -acetyl-galactosamine, <i>N</i> -acetyl-galactosamine) preferentially linked to serine and threonine residues
	Hyaluronate	Polymer of glucuronic acid $\beta(1 \rightarrow 3)$ <i>N</i> -acetyl-galactosamine
	Chondroitin sulfate	Polymer of glucuronic acid $\beta(1 \rightarrow 3)$ <i>N</i> -acetyl-galactosamine, the latter being sulfated in C4 and/or C6
	Mucosal surface glycoproteins	Fucosylated proteins
Proteins	Digestive enzymes	Trypsin
		Chymotrypsin
		Leucine aminopeptidase
		Elastase
		Lipase
		Nucleic acid hydrolase

Host		
Category	Class	Composition or representative compounds
	Desquamated epithelial cells Immune molecules	Secretory IgA
Bile acids	Primary bile salts	Taurocholate, glycocholate Taurochenodeoxycholate, glycochenodeoxycholate
	Secondary bile salts	Deoxycholate and its taurine or glycine conjugates Lithocholate and its taurine or glycine conjugates

There is evidence that the intestinal microbiota coevolved with the respective animal host, suggesting that the bacteria resident in the digestive tract are optimally adapted to the specific environment and the nutritional habits of the host species. In humans, one of the main functions of the gut microbiota is the breakdown of dietary components that escape digestion by host enzymes. Nondigestible polysaccharides include resistant starch, plant cell wall components such as cellulose (β -[1 $\rightarrow$ 4] D-glucose), β -[1 $\rightarrow$ 3, 1 $\rightarrow$ 4] glucans, and pectins (α -[1 $\rightarrow$ 4]-linked *D-galacturonic acid* esterified by methanol to varying degree) as well as inulin (β -[2 $\rightarrow$ 1] fructose with a chain-terminating *glucosyl* moiety), which serves as a storage polysaccharide in various plants (Table 2.1). Some of these polymeric carbohydrates occur in conjunction with lignin and are referred to as dietary fibers, which represent the main substrate source for intestinal bacteria. However, the extent to which dietary fiber becomes utilized by intestinal bacteria depends on the physicochemical properties of the polymeric components, in particular on water solubility, water-binding capacity, and viscosity. These properties in turn depend on their chemical structure: type of carbohydrate units present, the way in which they are linked, and the degree of branching and polymerization. Dietary fiber may be categorized into structural polysaccharides originating from plant cell walls such as cellulose, pectin, xylan,

mannan, and β -glucan and into storage carbohydrates such as inulin and starch.

Starch is the main carbohydrate source in a typical human diet. Humans are equipped with α -amylase, which is produced in salivary glands and the pancreas and catalyzes the breakdown of starch to maltotriose and maltose. However, certain forms of starch, referred to as resistant starch, escape digestion because the glycosidic bonds cannot be accessed by human enzymes. Raw potatoes, green bananas, legumes, and unprocessed grains are typical sources of resistant starch. Moreover, heating and cooling of starch-containing foods such as potatoes and noodles may lead to the formation of retrograded starch, which represents one form of resistant starch. The intestinal microbiome has the capacity to depolymerize resistant starch and to utilize the cleavage products as sources of energy and carbon. Other indigestible dietary carbohydrates originate from whole-grain products, legumes, vegetables, fruits, and nuts. For the sake of completeness, it has to be mentioned that in addition to such complex carbohydrates, some mono- or oligosaccharides to a greater or lesser extent escape digestion in the small intestine and therefore become substrates of the intestinal microbiota. These include sugar alcohols such as sorbitol and xylitol, disaccharides such as lactulose (4-*O*- β -D-galactopyranosyl-D-fructofuranose), as well as oligosaccharides such as stachyose (α -D-galactopyranosyl-[1 $\rightarrow$ 6]- α -D-galactopyranosyl-[1 $\rightarrow$ 6]- α -D-glucopyranosyl-[1 $\rightarrow$ 2]- β -D-fructofuranoside), fructooligosaccharides, galactooligosaccharides, and xylooligosaccharides. In human and animal studies, the latter three have been demonstrated to stimulate the growth of bacterial population groups considered to be beneficial and to have health-promoting properties. They are referred to as prebiotics. However, the original concept of prebiotics has recently been revisited to include a wider diversity of compounds and to determine criteria that clearly define them, including a link between the chemical structure and effects on microbial communities (Deehan et al., [2020](#); Gibson et al., [2017](#)).

2.5.2 Breakdown of Complex Carbohydrates

Humans and other mammals lack the enzymes required for the breakdown of the large variety of complex dietary carbohydrates. However, the human gut microbiome provides a wide range of depolymerizing enzymes enabling the host to take advantage of dietary fiber by utilizing the bacterial degradation products. Metagenomic studies revealed that the human colonic microbiome, in comparison to all sequenced microbial genomes, is enriched in genes involved in the breakdown of dietary polysaccharides, whereas genes encoding other functions, such as energy production and lipid metabolism, are underrepresented. Genes representing more than 80 different glycoside hydrolase families, also referred to as carbohydrate-active enzymes (CAZymes), were identified in the distal human gut microbiome (Gill et al., [2006](#)). High-throughput functional screens enabled the isolation of 310 clones exhibiting β -glucanase, hemicellulase, galactanase, amylase, or pectinase activities, with 26 clones being particularly efficient in the degradation of raw plant polysaccharides (Tasse et al., [2010](#)). Seventy-three CAZymes from 35 different enzyme families were discovered, 32 of which were highly homologous to prevalent genes found in the gut microbiome of 20 human individuals. The results obtained in this study are consistent with the occurrence of horizontal gene transfer among intestinal bacteria (Tasse et al., [2010](#)).

The first step in the utilization of nondigestible polymeric carbohydrates by intestinal bacteria requires their breakdown, which results in the formation of oligomeric and monomeric carbohydrates (Fig. [2.2](#)). This process involves various enzyme families such as glycoside hydrolases, polysaccharide lyases, glycosyltransferases, and carbohydrate esterases (Cantarel et al., [2009](#); Flint et al., [2012](#)). These CAZymes may also be categorized according to the type of substrates they act on, namely, plant cell wall components such as cellulose, pectins, xylans, β -glucans, and mannans, or storage carbohydrates such as inulin and fructooligosaccharides.

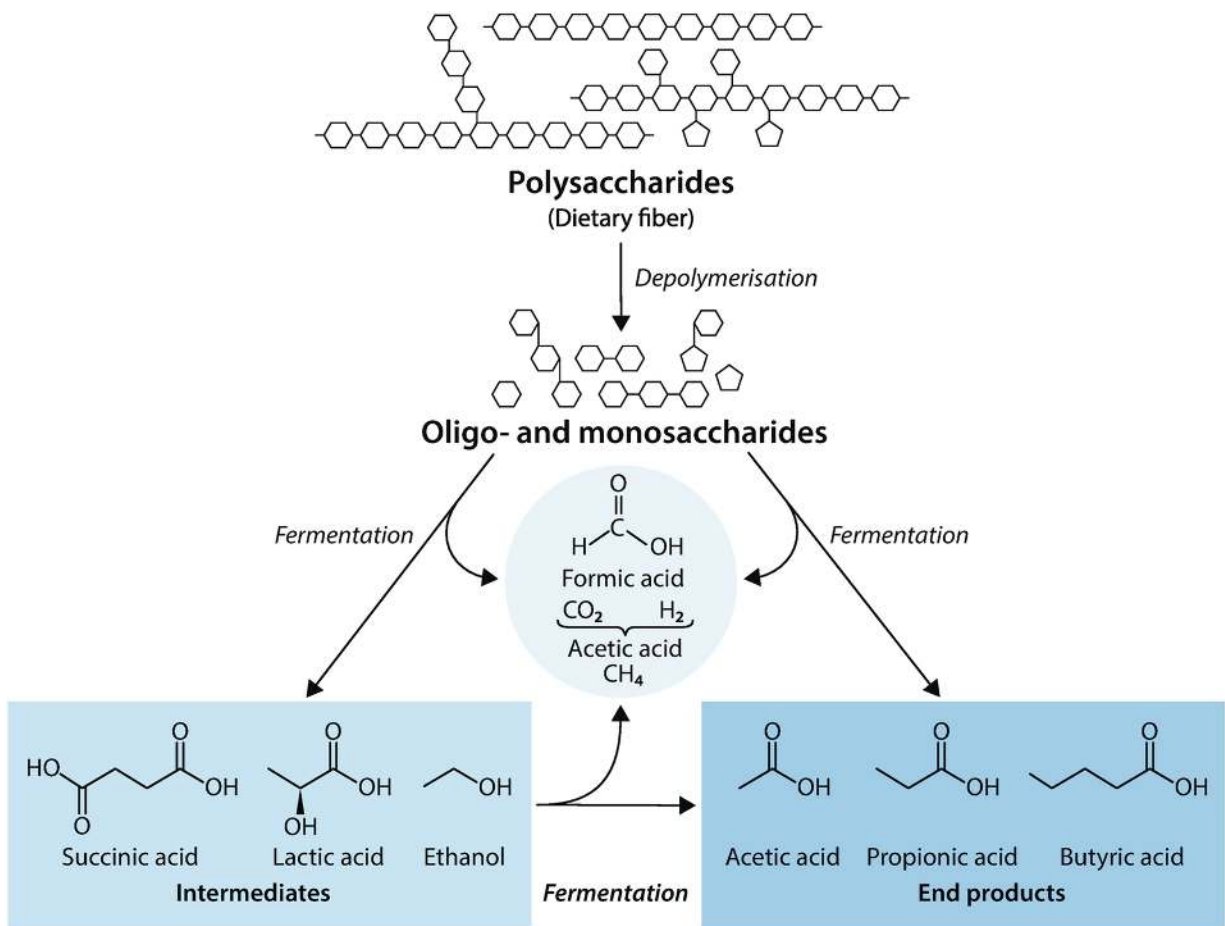


Fig. 2.2 Major steps in the breakdown of complex carbohydrates by the colonic microbiota

CAZymes can also act on host-glycans, such as mucins. It is also important to note that in addition to the enzymes catalyzing the depolymerization of glycans, auxiliary proteins are required for substrate binding, transport, and regulation. They act hand in hand and efficiently provide bacterial substrates for energy generation. During early life, the microbiota is adept at utilizing human-milk oligosaccharides (HMOs) (Schalich et al., [2024](#)). Due to this, supplementing infant formula with HMOs during early life leads to a *Bifidobacterium*-rich community (Dinleyici et al., [2023](#)). However, strain level specificity is critical for HMO utilization. One study identified variable growth of *Bifidobacterium pseudocatenulatum* on different HMOs (Sanchez-Gallardo et al., [2024](#)). These were linked to specific glycosyl hydrolases that were not encoded by all strains, giving those strains encoding them a selective advantage.

Early on, it was recognized that *Bacteroidaceae* species play an important role in the degradation of nondigestible carbohydrates in the human colon (Salyers et al., [1977a](#)). *Bacteroides thetaiotaomicron* has been used as a model organism to study carbohydrate utilization in detail. Transcriptome analysis of *B. thetaiotaomicron* recovered from mono-associated mice fed either a polysaccharide-rich or a simple sugar diet revealed that the organism not only induces glycoside hydrolases in a diet-dependent manner but also expresses outer membrane proteins engaged in polysaccharide binding (Sonnenburg et al., [2005](#)). *B. thetaiotaomicron* preferably utilizes simple carbohydrates or host glycans when dietary polysaccharides are not available, indicating a high degree of metabolic flexibility. Two sets of proteins are involved in starch utilization by *B. thetaiotaomicron*. One set, which is referred to as the starch utilization system (Sus), encompasses seven proteins contributing to starch degradation (SusABCDEFG) and a regulatory protein (SusR). The *mal* (maltose) regulon with the regulatory protein MalR also plays a role in starch utilization besides controlling the expression of *malA*, an α -glucosidase gene (Cho et al., [2001](#)). Deletion of *malR* attenuates *sus* gene expression, indicating that SusR, in conjunction with MalR, controls their expression. SusR, activated by maltose, maltotriose, or longer glucose oligomers, binds to the promoter region located upstream of *susB* and thereby activates the transcription of *susBCDEFG*. As *susA* is located upstream of *susB*, it has its own promoter (Reeves et al., [1997](#)) (Fig. [2.3](#)). SusDEF are lipoproteins anchored in the outer membrane, where they form a complex capable of binding starch (Shipman et al., [2000](#)). SusG, which is also anchored in the outer membrane, is an α -amylase cleaving starch molecules bound to SusDEF (Shipman et al., [1999](#)). The products formed by SusG are sufficiently small to reach the periplasm through the TonB-dependent transporter SusC located in the outer membrane. TonB is a complex in the cytoplasmic membrane of Gram-negative bacteria; it promotes the transport of various nutrients, including complexed iron and cobalamin, across the outer membrane (Schauer et al., [2008](#)). Interestingly, SusD is required for the binding and the

subsequent uptake of starch through the TonB-dependent SusC complex. SusA and SusB, which are located in the periplasm, exhibit α -amylase and α -glucosidase activity, respectively, resulting in the release of mainly maltotriose and maltose, which are produced from the starch chunks released by SusG and transported into the periplasm. In turn, maltose and maltotriose are subsequently transported into the cytoplasm for further degradation and fermentation.

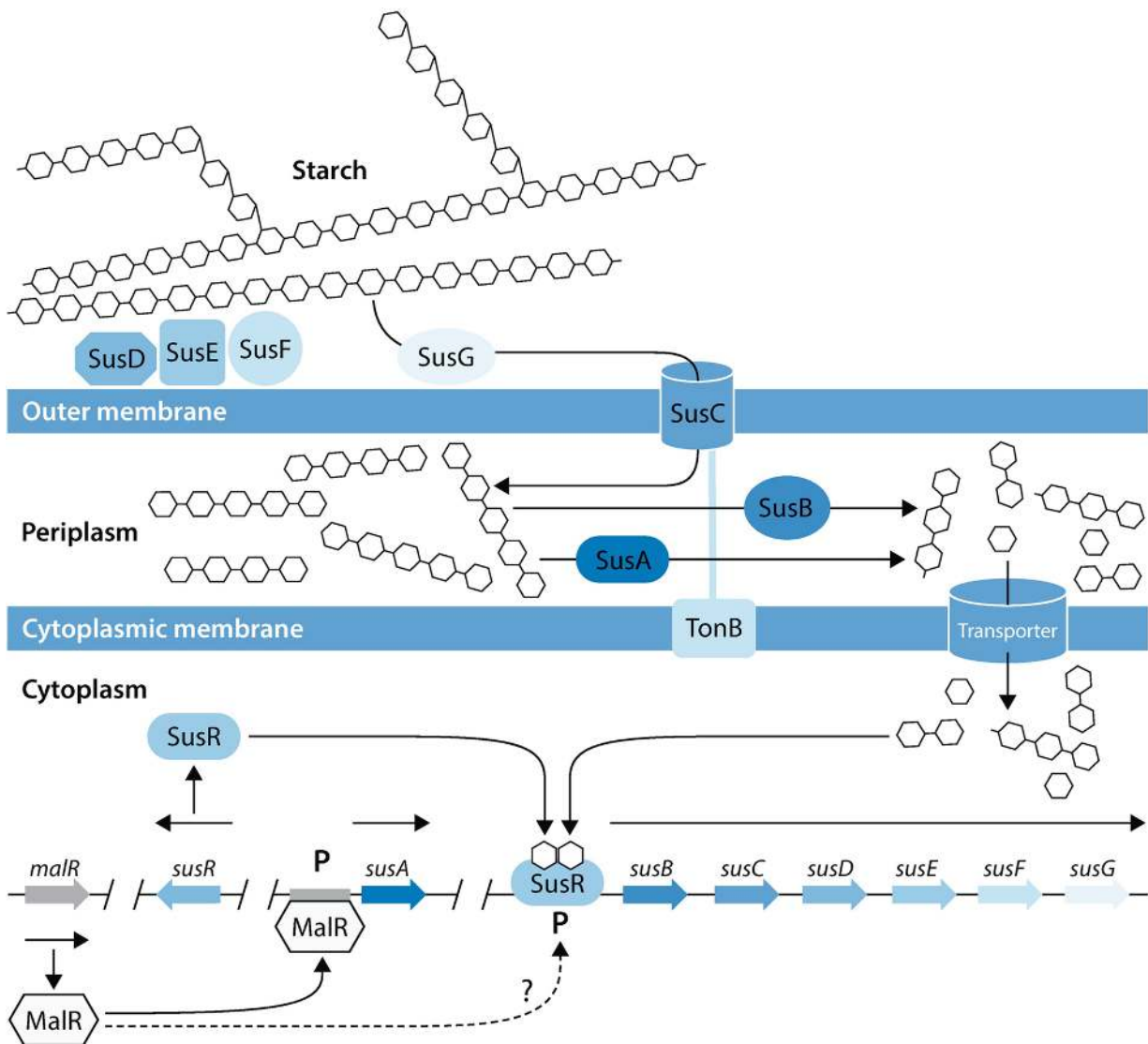


Fig. 2.3 Starch utilization by *Bacteroides thetaiotaomicron* as catalyzed by the starch utilization system (Sus). Mal refers to the maltose regulon

Inspection of the genome of *B. thetaiotaomicron* revealed the presence of approximately 90 *sus*-like gene loci, referred to as polysaccharide utilization loci (PULs) accounting for 18% of this organism's genome (Martens et al., [2008](#); Sonnenburg et al., [2005](#)). Approximately two thirds of these *Sus*-like PULs probably serve the degradation of plant-derived dietary polysaccharides, while one third plays a role in the degradation of host-derived glycans such as present in mucins and other glycoproteins. The most studied bacteria that uses host glycan is *Akkermanisa muciniphila*, which was isolated in the early 2000s from human feces by using mucin as a sole carbon source (Derrien et al., [2004](#)). Mucin utilization is facilitated by the encoding of a wide range of glycoside hydrolases, including sialidases and fucosidases (Kim et al., [2022](#)). *A. muciniphila* has become of increased interest due to its association with beneficial effects on host metabolism. This led to its potential as a probiotic being evaluated. Ingestion of either live or pasteurized *A. muciniphila* was shown, both in human (Depommier et al., [2019](#)) and mouse trials (Everard et al., [2013](#)), to have minimal effect on the microbiome while improving host metabolic parameters, including insulin sensitivity and plasma cholesterol levels. Due to these results, one strain (Muc) of *A. muciniphila* has been approved, in pasteurized form, as a food supplement and categorized as a novel food by the European Food Safety Authority (EFSA) (Turck et al., [2021](#)).

Studies into the breakdown of complex carbohydrates by intestinal bacteria have largely concentrated on *Bacteroidota* even though it is clear that members of the *Bacillota*, in particular *Ruminococcaceae*/*Oscillospiraceae* and *Lachnospiraceae*, also play an important role in polysaccharide degradation (Flint et al., [2012](#)). For example, in human subjects the consumption of a diet rich in resistant starch favors intestinal bacteria related to *Ruminococcus bromii* (Abell et al., [2008](#)). *R. bromii* not only outperformed *B. thetaiotaomicron* in the degradation of resistant starch but also promoted its utilization by other starch-degrading bacteria, including *Agathobacter rectalis* (synonym *Eubacterium rectale*), *Bifidobacterium adolescentis*, and *B.*

thetaiotaomicron (Ze et al., [2012](#)). Therefore, bacteria related to *R. bromii* have been proposed to play a critical role in the initial steps of resistant starch degradation. However, amylases have also been identified in *Roseburia inulinivorans* and other *Roseburia* species (Flint et al., [2012](#)). While *Bacteroides* species employ several proteins anchored in the outer membrane to capture and cleave starch, amylolytic Gram-positive gut bacteria such as *R. inulinivorans* take advantage of amylases that are bound to the bacterial cell wall. In conjunction with a variable number of carbohydrate-binding modules on the cell surface, they effectively bind and cleave starch (Ramsay et al., [2006](#)). Interestingly, starch also induces the formation of flagella in *R. inulinivorans*, which possibly helps the organism to reach the substrates (Scott et al., [2011](#)). Nine to 13 putative glycohydrolase genes were identified in the genomes of *Roseburia* spp. and *A. rectale*, but their exact roles are not yet clear (Ze et al., [2012](#)). A considerable number of intestinal *Bacillota* play a role in the degradation of complex carbohydrates of plant origin. For example, human strains of *Ruminococcus albus*, *Roseburia intestinalis*, and *Faecalibacterium prausnitzii* utilize galactomannan, xylan, and pectin, respectively (Chassard et al., [2007](#); Lopez-Siles et al., [2012](#); Salyers et al., [1977b](#)). A β -fructofuranosidase in *R. inulinivorans* catalyzes the depolymerization of fructans of different chain lengths. This activity is linked to an ATP-dependent sugar carrier, and expression of the corresponding genes was increased in the presence of inulin. Various mucin degraders including *Ruminococcus torques* have also been identified among the *Bacillota* (Hoskins, [1993](#)). It may be concluded that *Bacillota* play an important role in the degradation of both plant and host glycans, but in comparison with glycan-degrading *Bacteroidota*, knowledge on mechanistic details is relatively limited.

2.5.3 Utilization of Proteins by Intestinal Bacteria

Based on measurements of nitrogen digestibility in ileostomized patients, it has been estimated that 5–10% of the ingested dietary protein reaches the colon (Darragh & Hodgkinson, [2000](#)). Endogenous

proteins, in particular digestive host enzymes, are an additional source of protein for bacterial fermentation in the colon. The proteolytic activity of host proteases decreases from proximal to distal colon, indicating that they become inactivated due to bacterial proteolysis (Gibson et al., [1989](#)). Depending on diet, the total daily amount of protein entering the colon has been estimated to vary between 6 and 18 g (Cummings & Macfarlane, [1991](#); Yao et al., [2016](#)). Most colonic bacteria prefer carbohydrates over proteins for energy generation, but many species are also capable of utilizing proteins, peptides, and amino acids, alternatively or simultaneously. Protein can be used for energy generation and for biosynthetic purposes as they deliver both carbon and nitrogen for microbial growth. Protein degradation in the colon occurs in several steps and involves different bacteria. The first step in protein utilization is proteolysis, which results in the release of peptides and amino acids (Fig. [2.4](#)). Strains of *B. fragilis* and *Phocaeicola vulgatus* use cell-bound proteases for the cleavage of proteins, while many species secrete extracellular proteases. Strains of *Lactobacillus*, *Streptococcus*, *Staphylococcus*, *Clostridium*, and *Bifidobacterium* have been identified to secrete proteases that degrade dietary proteins, including gluten (Caminero et al., [2014](#)). In addition to targeted dietary proteins, microbial proteases can target host cells and molecules, altering human health. Proteolytic activity has been associated with inflammatory bowel diseases (Galipeau et al., [2021](#); Mills et al., [2022](#)). In particular, *Enterococcus* and *Staphylococcus* spp. produce proteases that activate PAR2, promoting intestinal permeability and inflammation (Rondeau et al., [2024](#)). Host molecules secreted into the gut lumen for defense can also be targeted by microbial proteases, such as those from *Tomasiella immunophila*, which degrade SIgA (Lu et al., [2024](#)).

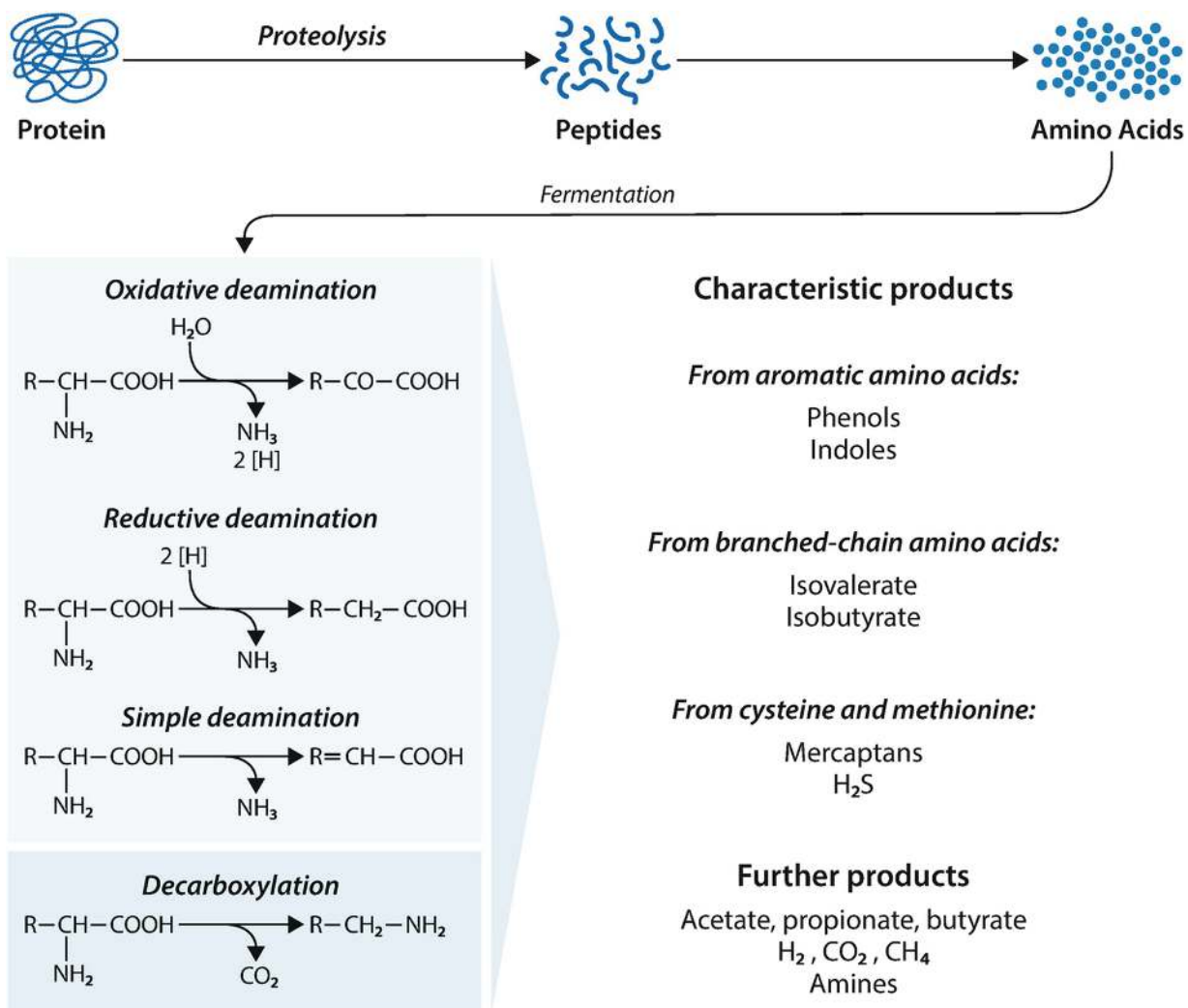


Fig. 2.4 Bacterial proteolysis in the large intestine

The pathways used by colonic bacteria for amino acid fermentation in the colon may differ among bacterial species. However, the initial steps of bacterial amino acid breakdown are restricted to only a few: oxidative deamination, reductive deamination, simple deamination, and decarboxylation resulting in α -oxo-acids, carboxylic acids, enoates, and (poly)amines, respectively (Fig. 2.4). Intestinal bacteria such as *Clostridium sticklandii* utilize pairs of different amino acids: one of the amino acids is subjected to oxidative deamination, while the other one undergoes reductive deamination, a process referred to as Stickland reaction. As a consequence of the release of ammonia, the luminal pH increases from proximal to distal colon, indicating protein becoming

more important as an energy source as carbohydrates become increasingly exhausted (Macfarlane et al., [1992](#)). Amino acid fermentation results in the formation of SCFA, formate, H_2 , and CO_2 , with the latter three serving as potential substrates for methanogenesis and homoacetogenesis, i.e., the same products also formed from carbohydrates. Products characteristic of amino acid fermentation include branched-chain fatty acids such as isovalerate and isobutyrate, which are formed from branched-chain amino acids; phenols and indoles, which stem from the fermentation of the aromatic amino acids tyrosine, phenylalanine, and tryptophan; and amines. Colonic fermentation of the sulfur-containing amino acids cysteine and methionine gives rise to hydrogen sulfide (H_2S) and mercaptans. A high protein intake is accompanied by increased bacterial sulfide generation in the human colon (Magee et al., [2000](#)).

2.5.4 Formation of Short-Chain Fatty Acids by Bacterial Fermentation in the Colon

One of the major host-relevant and abundant metabolites produced by the microbiota are SCFAs. These are both intermediates in metabolic pathways, such as acetate which may be used by other bacteria or the host, while others are end products, namely propionate and butyrate. Acetate, propionate, and butyrate are formed at an approximate molar ratio of 60:23:17. Total SCFA concentrations in the colon are in the range of 90–120 mM (Cummings et al., [1987](#)). However, both the molar ratios and the concentrations of colonic SCFA are highly variable and depend on the type and amount of dietary fiber ingested, although they can also be produced from protein and fatty acid metabolism. The majority (95%) of the SCFA formed by the colonic microbiota becomes absorbed (Topping & Clifton, [2001](#)). Following their absorption, acetate and butyrate may become oxidized in body tissues providing energy to the host. SCFA, preferentially butyrate, provide up to 70% of the energy required by colonic epithelial cells (Roediger, [1980](#)). Propionate may serve as a gluconeogenic substrate in the liver and acetate as a substrate for lipogenesis (Cummings, [1995](#)). However, SCFA also play

various regulatory roles. For example, by inhibiting histone deacetylase, butyrate influences gene expression, which in colon cancer cells results in cell cycle arrest and activates apoptosis (Lazarova et al., [2013](#)). Thus, SCFA play an important role in maintaining homeostasis of the colonic mucosa. SCFA have also been recognized as ligands of the G-protein-coupled receptors FFAR2 (free fatty acid receptor 2) and FFAR3, earlier referred to as GPR43 and GPR41 (Brown et al., [2003](#)), which are expressed in ileal and colonic enteroendocrine L cells, adipocytes, and immune cells. FFAR2 activation triggers the release of leptin from adipocytes (Xiong et al., [2004](#)) and the excretion of peptide YY (Tazoe et al., [2008](#)) and glucagon-like peptide from enteroendocrine cells (Tolhurst et al., [2012](#)). Since these molecules reduce appetite (Wren & Bloom, [2007](#)), they have been proposed to play a role in the control of appetite regulation. However, recent animal studies cast some doubts on such a role of intestinal FFAR (Lin et al., [2012](#); Tang et al., [2015](#)).

The majority of intestinal bacteria produce acetate, but the quantities they produce vary dramatically. Homofermentative and heterofermentative lactic acid bacteria, such as *Lactobacillaceae* spp., produce no or only small amounts of acetate. Another major group of lactate-producing intestinal bacteria, the bifidobacteria, produces considerable amounts of acetate in addition to lactate ($2 \text{ glucose} \rightarrow 2 \text{ lactate} + 3 \text{ acetate}$). Major propionate producers include various *Bacteroides* species, *Veillonella parvula*, *Dialister succinatiphilus*, *Phascolarctobacterium succinatutens*, *Selenomonas ruminantium*, and *Megasphaera elsdenii*, many of which also produce succinate as a by-product or an intermediate that can be taken up again to be converted to propionate. Three different propionate formation pathways have been identified: the methylmalonyl-CoA pathway, the acrylate pathway, and the propanediol pathway. Primers targeting genes characteristic of either pathway were used to test the presence of the corresponding genes in representative human gut species (Reichardt et al., [2014](#)). The majority of bacterial species were found to use the methylmalonyl-CoA pathway.

Butyrate-producing strains include *Anaerobutyricum hallii*, *Anaerostipes caccae*, *R. intestinalis*, *A. rectale*, and *F. prausnitzii* (Duncan et al., [2002](#), [2004](#)). Four pathways for butyrate production have been identified, but genome analysis identified the most common being the acetyl-coenzyme A pathway (Vital et al., [2014](#)). The utilization of acetate to produce butyrate, but inability to utilize as a sole energy source, has been observed for *Roseburia* and *Faecalibacterium* species (Duncan et al., [2002](#)). *Intestinimonas* spp. can also produce butyrate and acetate from the amino acid lysine (Bui et al., [2015](#)). Genome analysis identified the lysine pathway to occur sporadically within all major phyla of human gut bacteria, particularly in *Fusobacterium* and *Porphyromonas* spp. (Vital et al., [2014](#)).

2.5.5 Triglyceride Degradation and Cholesterol Conversion

Whilst carbohydrates and proteins are the main substrates for microbial communities in the human intestine, lipids, including not only dietary fat but also host-derived lipids such as bile acids (see Sect. [2.5.6](#)), are also converted by gut bacteria with consequences on their bioavailability and bioactivity. Obesity is increasingly becoming a burden on high-income countries, caused in part by the high fat content in the diet, and the gut microbiome plays a role in the development of this disease and comorbidities (see Chap. [16](#)). The prevailing knowledge about fat absorption is that it occurs within the small intestine, specifically in the proximal region (Borgstrom et al., [1957](#); Clark et al., [1973](#)). However, fat absorption differs greatly depending on the amount of fat and the microbiota of the host (Kübeck et al., [2016](#)). Fat excretion was shown to increase with increasing dietary intake, suggesting that the exposure of bacteria to fat in the colon can reach non-negligible levels. Whilst germfree mice are resistant to high-fat diet-induced weight gain when the diet contains lard and sucrose (Bäckhed et al., [2007](#)), those fed a palm oil-based high-fat diet develop obesity (Fleissner et al., [2010](#); Kübeck et al., [2016](#)). This clearly shows that the type of fat plays an important role in microbe-host interactions.

The production of lipases by the gut microbiota is one route by which it modulates host response to diet. Triglyceride lipases (EC:3.1.1.3) catalyze the release of free fatty acids and glycerol from triglyceride molecules. Oral administration of a transgenic lipase-producing bacterium was shown to significantly increase fat absorption in pigs and improve steatorrhea (Drouault et al., [2002](#)). Carp, *Cyprinus carpio*, fed with LipG1, a microbial lipase isolated from the gut of carp, significantly increased lipolytic activity and weight gain when fed palm oil, a cheap and industrially advantageous feed (Ran et al., [2015](#)). The benefit to the microbes producing these lipases is currently unclear as no evidence has been provided that they take up either the fatty acids or glycerol subunit.

In addition to triglycerides, cholesterol is an important component of dietary fats. The gut microbiota is capable of converting dietary sterols, including modification of cholesterol into coprostanol, which is excreted in feces (Eyssen et al., [1973](#); Gérard et al., [2007](#)). In a study of 633 patients, the conversion rate of cholesterol to coprostanol varied from <5% up to >80% in some individuals (Benno et al., [2005](#)). The cholesterol dehydrogenase, *ismA*, is responsible for the conversion of cholesterol and is encoded by *Eubacterium coprostanoligenes* and its close relatives, many of which remain uncultured (Kenny et al., [2020](#)). The presence of these *ismA* encoding species within a patient's microbiota was associated with decreased fecal cholesterol and increased coprostanol.

2.5.6 Conversion of Bile Acids

Even though bile acids are not a major source of energy for the gut microbiota, they may suppress the growth of bacteria sensitive to these detergents. They play a role in fat digestion and are ligands for the farnesoid X receptor (FXR), the liver X receptor (LXR), and the G-protein-coupled receptor TGR5; and they undergo conversion by the gut microbiota (Jones et al., [2008](#)). They are synthesized in the liver from cholesterol, conjugated with glycine or taurine, and subsequently stored in the gall bladder. When required, they are excreted into the gut

to solubilize dietary fat and support the formation of micelles. Many intestinal bacteria can deconjugate the primary bile acids to the corresponding unconjugated forms. Many intestinal bacteria, including *Clostridium perfringens*, *Lactiplantibacillus plantarum*, *Lactobacillus johnsonii*, *Bifidobacterium bifidum*, *B. longum*, and *B. adolescentis*, harbor bile salt hydrolase genes (Ridlon et al., [2006](#); Wylensek et al. [2020](#)). Metagenomic analysis revealed that bile salt hydrolases are enriched in the human gut microbiome and that they are present in all major bacterial divisions as well as in the archaeal methanogens *M. smithii* and *M. stadtmanae*, suggesting that this activity is relevant to survival in the mammalian gastrointestinal tract (Jones et al., [2008](#)). The unconjugated bile acids may be further converted by bile acid dehydroxylases and hydroxysteroid dehydrogenases. Intestinal bacteria harboring enzymes involved in bile acid conversion include *E. lenta*, *C. perfringens*, *B. producta*, *B. fragilis*, *B. thetaiotaomicron*, *E. coli*, *Clostridium sardiniense*, *Paeniclostridium sordellii*, *Clostridium innocuum*, *Clostridium scindens*, *Clostridium hylemonae*, *Paraclostridium bifermentans*, *Hathewayia limosa*, *Clostridium leptum*, and *Clostridium paraputrificum* (Ridlon et al., [2006](#)). Intestinal bacteria such as *Ruminococcus gnavus* may favor the growth of *Bacteroides* spp. owing to their ability to detoxify the secondary bile acid deoxycholic acid by converting it to the corresponding 3- β -hydroxy bile acid epimer isodeoxycholic acid (Devlin & Fischbach, [2015](#)). Dehydroxylation of bile acids, which occurs in a position-specific and stereoselective way, was studied in *Clostridium scindens* in detail. The eight genes required for bile acid dehydroxylation are organized in the *bai* (bile acid-inducible) operon (*baiBCDEAFGHI*) encoding the 27 kDa 3 α -hydroxysteroid dehydrogenase (BaiA), the 58 kDa bile acid CoA ligase (BaiB), the 70 kDa 3-dehydro-4-chenodeoxycholic acid/cholic acid steroid oxidoreductase (BaiCD), the 72 kDa 3-dehydro-4-ursodeoxycholic acid/7-epi cholic acid steroid oxidoreductase (BaiH), the 19.5 kDa 7 α -dehydratase (BaiE), a hypothetical 22 kDa 7 β -dehydratase (BaiI), the 47.5 kDa bile acid CoA hydrolase and a hypothetical bile acid CoA transferase (BaiF), and the 50 kDa transmembrane protein (BaiG),

which catalyzes H⁺-dependent bile acid transport (Ridlon et al., [2006](#)). It is important to note that none of the bacterial enzymes acting on the bile acids cleaves the steroid ring structure.

Potential benefits for intestinal bacteria may arise from the utilization of the glycine or taurine moieties of bile acids as a carbon or nitrogen source. Utilization of taurine by *B. longum* as a nitrogen source is in accordance with the finding that the bile salt hydrolase gene is co-transcribed with the glutamine synthetase adenylyltransferase gene (*glnE*), which is part of the nitrogen regulation cascade (Tanaka et al., [2000](#)). There is evidence that taurine stimulates growth of the colitogenic *B. wadsworthia* by providing the electron acceptor for sulfite reduction.

2.5.7 Microbial Interaction with Xenobiotics

In addition to carbohydrates, proteins, and fat, a diet may contain non-nutritive metabolites, both natural, such as polyphenols found in grains, fruits, and vegetables, and artificial, such as medicinal drugs.

Polyphenols such as lignans and flavonoids have been reported to exert beneficial health effects. Therefore, their uptake, bioavailability, and biological activities in humans have been studied (Clavel et al., [2006b](#); Culp et al., [2024](#); Hollman & Katan, [1999](#)). The chemical structure of polyphenols and the composition of the intestinal microbiota affect the fate of these compounds in the digestive tract. Polyphenols are usually glycosylated, and, depending on the extent of absorption, they pass into the colon where they undergo conversion by intestinal bacteria. For example, intestinal bacteria convert the lignan secoisolariciresinol diglucoside (SDG) to enterolactone in several steps (Axelson et al., [1982](#)). Various *Bacteroides* and *Clostridium* spp. are capable of deglycosylating SDG, but *Thomasclavelia saccharogumia* turned out to be the most effective species of the strains tested (Clavel et al., [2006a](#), [2007](#)). “*Butyribacterium methylotrophicum*,” *B. producta*, *Eubacterium callanderi*, and *E. limosum* are capable of catalyzing the second step, namely, the *O*-demethylation of matairesinol, whereas *E. lenta* dehydroxylates the *O*-demethylated matairesinol to enterodiol

(third step) (Clavel et al. [2006](#)). The last step in this pathway, the conversion of enterodiol to enterolactone, is catalyzed by *Lactonifactor longoviformis* (Clavel et al., [2007](#)). Many polyphenols other than lignans can be metabolized by gut bacteria or influence microbiome composition and function, and we are only beginning to understand how complex these interactions are (Clavel [2013](#)).

The gut microbiota also interacts with xenobiotics taken medicinally, with many inhibiting bacterial growth (Maier et al., [2018](#)). An example is metformin, a common therapeutic drug for type 2 diabetes that significantly alters the microbiota. This effect is mediated by the enhanced growth of some species, including *A. muciniphila* and *Bifidobacterium adolescentis*, but no effect on *E. coli* (Wu et al., [2017](#)). An example of a drug metabolized by the gut microbiome is digoxin, a common medicine for heart conditions. It was observed that 16.4% of digoxin was secreted into the metabolized dihydrodigoxin form (Greenwood et al., [1975](#)). Cultivation work identified *E. lenta* as a singular species able to degrade digoxin (Saha et al., [1983](#)). The cardiac glycoside reductase (cgr) operon was later identified to encode two genes that use digoxin as an alternative electron acceptor (Haiser et al., [2013](#)). This degradation likely occurs due to digoxin originating as a plant toxin, which the gut microbiota has evolved to protect the host against (Koppel et al., [2018](#)). In addition to the modification of therapeutic drugs, a study of representative gut bacteria identified many bioaccumulate the drugs, storing them internally without modification (Klünemann et al., [2021](#)). This can alter the dose received by the host, limiting the therapeutic benefit.

2.6 Conclusions

The microbial communities inhabiting the human intestinal tract play a major role in the breakdown of dietary components that cannot be utilized by the host and in the conversion of host metabolites. By expanding the metabolic capacity of the host and interacting with the host immune system and metabolic processes, the gut microbiota

profoundly affects host physiology. Even though metagenomics, transcriptomics, and proteomics have increased our knowledge about important functions of the intestinal microbiota, there still is a gap in our understanding of the exact molecular mechanisms underlying microbe-microbe or host-microbe interactions. Therefore, identifying the exact role of members of the gut microbiota and their competitive or cooperative links is of major importance. Considerable differences in microbiota composition among human individuals and populations impede the elucidation of the mechanisms underlying the role of intestinal bacteria in various diseases. Moreover, it often is not possible to find out whether disease-related changes in the gut microbiota are cause or consequence of the disease. We should strive to identify microorganisms that play critical roles in physiological and pathophysiological processes, with the ultimate goal to identify the bacterial molecules involved and their targets in the host.

Controversy Analysis of human fecal samples revealed differences in the relative abundance of key taxa. Three robust patterns or clusters, referred to as enterotypes, were identified (Arumugam et al., [2011](#)). The three enterotypes are characterized by differences in the relative abundance of *Bacteroidaceae*, *Prevotellaceae*, or *Ruminococcaceae*. Recent work has proposed the existence of a nested *Bacteroidaceae* enterotype, characterized by low population density and referred to as Bact2, which is associated with adverse health conditions (Vieira-Silva et al., 2020). While the enterotypes did not correlate with gender, age, or body weight, long-term dietary habits were reported to influence the enterotype (Wu et al., [2011](#)). Several studies confirmed that the fecal microbiota of human subjects can be categorized into two of the three proposed enterotypes, namely, *Bacteroidaceae* and *Prevotellaceae*, while the *Ruminococcaceae* enterotype was usually not found. In a Korean study, 72% of the fecal samples collected from Korean monozygotic twin pairs belonged to the same enterotype and 2 years later, the affiliation with either enterotype was still the same for 80% of the individuals (Lim et al.,

[2014](#)). However, this also means that 20% of the subjects changed from one enterotype to the other during this time. Interestingly, a more recent study in Taiwanese adults, which was based on the analysis of 181 fecal samples, identified *Escherichia* rather than *Ruminococcaceae* as a representative genus of a third enterotype (Liang et al., [2017](#)). These inconsistencies and others have led the enterotype concept to be challenged (Knights et al., [2014](#)). The issues with the concept can be summed up as: Dominant taxonomic groups including *Ruminococcaceae* and *Bacteroidaceae* are highly variable among individuals belonging to the same enterotype. Available datasets propose continuous abundance in gradients rather than discrete clusters despite the fact that the absence of *Prevotellaceae* as observed in Italian children (De Filippo et al., [2010](#)) inevitably results in discrete clustering. Most importantly, the affiliation of human subjects with a given enterotype may vary over time arguing against the notion that enterotypes are discrete states (Knights et al., [2014](#)). Indeed, a large cohort study revealed that the microbiota profile of most study subjects corresponded to one of the enterotypes, while others had intermediate profiles, impeding a clear assignment to an enterotype (Huse et al., [2012](#)). Based on these findings, it may be concluded that the enterotype concept, however appealing it may appear, does not really promote a better understanding of the gut microbiome.

Metagenomic analysis allows the reconstruction of partial genomes from environmental samples (Pasolli et al., [2019](#)). While initially such analysis was limited to simple ecosystems, with only the dominant taxa being reconstructed (Tyson et al., [2004](#)) it is now commonly applied to diverse ecosystems, supposedly allowing a range of microbes to be accurately reconstructed (Nayfach et al., [2021](#)). Sample specific reconstructions of metagenome-assembled genomes (MAGs) has been proposed to capture strain-level differences (Zachariasen et al., [2024](#)). This has led to strain level analysis being conducted using MAGs to represent strains from specific samples (Nayfach et al., [2019](#)). However, the accuracy of such

methods has been thrown into doubt by comparison of MAGs to isolate genomes and showing that 10–30% of both the core and accessory genes of a strain are missing within MAGs, preventing them from accurately representing the strain's functionality (Meziti et al., [2021](#)). While many genes may be missing, MAGs can provide initial insight into the taxonomic diversity within a sample, including as-yet uncultured taxa (Parks et al., [2022](#)). One study used functional insights from a MAG to construct growth conditions specific to that taxon, allowing for its isolation and further study (Pope et al., [2011](#)). While MAGs provide taxonomic insights, skepticism about their use to study strain-level diversity remains, but may be overcome with the application of long-read sequencing which enhances the ability to capture the entire functional repertoire of a strain (Yaffe & Relman, [2020](#); Treichel et al., [2026](#)).

History

The ubiquitous existence of microorganisms only became evident with the invention of the light microscope by Antonie van Leeuwenhoek (1632–1723) and the studies of Louis Pasteur (1822–1895) and Robert Koch (1843–1910), which revealed that bacteria catalyze reactions and may cause infections. Theodor Escherich (1857–1911) was one of the first researchers, who became interested in the role of intestinal bacteria in the digestive tract, in particular of infants. He isolated a fecal bacterium that later on was named after him, namely, *Escherichia coli*. For a long period of time, bacteria have primarily been perceived as culprits even though most bacteria known to date are non-pathogenic. This might explain that the intestinal fermentation was considered a detrimental process. The British surgeon William Arbuthnot-Lane (1856–1943) removed the colon from some of his patients because he assumed that the colonic fermentation led to an “autointoxication.” For a long time, the investigation of the intestinal microbiota was impeded, because adequate methods for handling strict anaerobes were not yet available. So the exploration of the ecosystem only started after

pioneers such as Robert E. Hungate (1906–2004) and Sydney M. Finegold (1921–2018) developed methods for the isolation and handling of strict anaerobes (Hungate, [1969](#); Sugihara et al., [1974](#)). These early researchers and others laid the foundation for the field. They isolated and described a considerable number of bacterial species and tried for the first time to link the gut microbiota to health and disease (Finegold et al., [1975](#)). The development of cultivation-independent methods (Amann et al., [1995](#)) facilitated and accelerated the characterization of various microbial habitats, including that of human fecal samples (Suau et al., [1999](#)). The shift from sequencing marker genes (16S rRNA gene, ITS) to shotgun sequencing all environmental DNA has enabled comprehensive studies of the microbial gene sequences in an ecosystem. In conjunction with transcriptomics and metabolomics, it is possible to characterize the metabolic potential of intestinal microbial communities (Almeida et al. [2021](#); Dumas et al., [2006](#); Gill et al., [2006](#); Jiang et al., [2016](#)). However, the prediction of gene functions depends on the correct annotation, which in turn is largely based on work of scientists who previously isolated bacteria and characterized their enzymes and genes. Isolation of new community members and identification of new gene functions can be tedious and usually receive little appreciation by the scientific community. Due to the large workload required, few genes are studied extensively, meaning that currently it is estimated that ~50% of genes lack an assigned function (Thomas & Segata, [2019](#)). Advances in bioinformatic approaches have also allowed for the reconstruction of partial genomes that represent species from metagenomic data (Tyson et al., [2004](#)). This has facilitated the identification of previously unknown taxonomic groups, although the lack of cultured representatives prevents further mechanistic study (Parks et al., [2022](#); Leviatan et al., [2022](#)). Therefore, to study both the microbes and their functionality, it is still necessary to isolate as many bacteria as possible, describe and validly name novel taxa, and study their genes and enzymes.

Highlights

- The digestive tract of human and animals is colonized by microbial communities encompassing bacteria, archaea, fungi, and viruses, referred to as gastrointestinal microbiota.
- The gastrointestinal microbiota has coevolved with the host, and its members are well adapted to the different sections of the digestive tract, which differ in the physicochemical conditions and the availability of substrates. The composition of the intestinal microbiota at strain level is highly variable among humans.
- The majority of intestinal microorganisms are strictly anaerobic bacteria, which gain energy by fermenting dietary fiber and endogenous substrates mainly to short-chain fatty acids, carbon dioxide, molecular hydrogen, and methane.
- The collective genome (metagenome) of all members of the intestinal microbiota encodes the functions of all community members. While a large proportion of functions are shared among the microbiomes of human individuals, some activities are only observed in certain human populations.
- Bacterial groups in the intestinal tract interact with each other and with the host. The majority of interactions are of mutualistic or commensal character. However, the molecular mechanisms underlying such interactions have yet to be fully understood.

References

Abell, G. C., Cooke, C. M., Bennett, C. N., Conlon, M. A., & McOrist, A. L. (2008). Phylotypes related to *Ruminococcus bromii* are abundant in the large bowel of humans and increase in response to a diet high in resistant starch. *FEMS Microbiology Ecology*, 66, 505–515.

[\[PubMed\]](#)

Almeida, A., Mitchell, A. L., Boland, M., Vazquez-Baeza, Y., Finn, R. D., Knight, R., & Pollard, K. S. (2019). A new genomic blueprint of the human gut microbiota. *Nature*, 568(7753), 499–504.

<https://doi.org/10.1038/s41586-019-0965-1>

[\[PubMed\]](#)

Almeida, A., Nayfach, S., Boland, M., Strozzi, F., Beracochea, M., Shi, Z. J., Pollard, K. S., Sakharova, E., Parks, D. H., Hugenholtz, P., Segata, N., Kyrpides, N. C., Finn, R. D. (2021). A unified catalog of 204,938 reference genomes from the human gut microbiome. *Nature Biotechnol*, 39, 105–114. <https://doi.org/10.1038/s41587-020-0603-3>

Amann, R. I., Ludwig, W., & Schleifer, K.-H. (1995). Phylogenetic identification and *in situ* detection of individual microbial cells without cultivation. *Microbiological Reviews*, 59, 143–169. [\[PubMed\]](#)

An, R., Wilms, E., Gerritsen, J., Kim, H. K., Pérez, C. S., Besseling-van der Vaart, I., Jonkers, D. M. A. E., Rijkers, G. T., de Vos, W. M., Masclee, A. A. M., Zoetendal, E. G., Troost, F. J., & Smidt, H. (2024). Spatio-temporal dynamics of the human small intestinal microbiome and its response to a synbiotic. *Gut Microbes*, 16(1), 2350173. <https://doi.org/10.1080/19490976.2024.2350173>

Arumugam, M., Raes, J., Pelletier, E., Le Paslier, D., Yamada, T., Mende, D. R., Fernandes, G. R., Tap, J., Bruls, T., Batto, J. M., Bertalan, M., Borruel, N., Casellas, F., Fernandez, L., Gautier, L., Hansen, T., Hattori, M., Hayashi, T., Kleerebezem, M., Kurokawa, K., Leclerc, M., Levenez, F., Manichanh, C., Nielsen, H. B., Nielsen, T., Pons, N., Poulain, J., Qin, J., Sicheritz-Ponten, T., Tims, S., Torrents, D., Ugarte, E., Zoetendal, E. G., Wang, J., Guarner, F., Pedersen, O., de Vos, W. M., Brunak, S., Doré, J., MetaHIT Consortium, Antolín, M., Artiguenave, F., Blottiere, H. M., Almeida, M., Brechot, C., Cara, C., Chervaux, C., Cultrone, A., Delorme, C., Denariáz, G., Dervyn, R., Foerstner, K. U., Friss, C., van de Guchte, M., Guedon, E., Haimet, F., Huber, W., van Hylckama-Vlieg, J., Jamet, A., Juste, C., Kaci, G., Knol, J., Lakhdari, O., Layec, S., Le Roux, K., Maguin, E., Mérieux, A., Melo Minardi, R., M'rini, C., Muller, J., Oozeer, R., Parkhill, J., Renault, P., Rescigno, M., Sanchez, N., Sunagawa, S., Torrejon, A., Turner, K., Vandemeulebrouck, G., Varela, E., Winogradsky, Y., Zeller, G., Weissenbach, J., Ehrlich, S. D., Bork, P. (2011). Enterotypes of the human gut microbiome. *Nature*, 473, 174–180.

Asnicar, F., Leeming, E. R., Dimidi, E., Mazidi, M., Franks, P. W., Al Khatib, H., Valdes, A. M., Davies, R., Bakker, E., Francis, L., Chan, A., Gibson, R., Hadjigeorgiou, G., Wolf, J., Spector, T. D., Segata, N., & Berry, S. E. (2021). Blue poo: impact of gut transit time on the gut microbiome using a novel marker. *Gut*, 70(9), 1665–1674. <https://doi.org/10.1136/gutjnl-2020-323877>. PMID: 33722860; PMCID: PMC8349893.

Atkinson, C., Berman, S., Humbert, O., & Lampe, J. W. (2004). In vitro incubation of human feces with daidzein and antibiotics suggests interindividual differences in the bacteria responsible for equol production. *The Journal of Nutrition*, 134, 596–599. [\[PubMed\]](#)

Axelsson, M., Sjövall, J., Gustafsson, B. E., & Setchell, K. D. R. (1982). Origin of lignans in mammals and identification of a precursor from plants. *Nature*, 298, 659–660. <https://doi.org/10.1038/298659a0> [\[PubMed\]](#)

Bäckhed, F., Manchester, J. K., Semenkovich, C. F., & Gordon, J. I. (2007). Mechanisms underlying the resistance to diet-induced obesity in germ-free mice. *Proceedings of the National Academy of Sciences of the United States of America*, 104(3), 979–984. <https://doi.org/10.1073/pnas.0605374104>.

Barratt, M. J., Nuzhat, S., Ahsan, K., Frese, S. A., Arzamasov, A. A., Sarker, S. A., Islam, M. M., Palit, P., Islam, M. R., Hibberd, M. C., Nakshatri, S., Cowardin, C. A., Guruge, J. L., Byrne, A. E., Venkatesh, S.,

Sundaresan, V., Henrick, B., Duar, R. M., Mitchell, R. D., Casaburi, G., Prambs, J., Flannery, R., Mahfuz, M., Rodionov, D. A., Osterman, A. L., Kyle, D., Ahmed, T., Gordon, J. I. (2022) Bifidobacterium infantis treatment promotes weight gain in Bangladeshi infants with severe acute malnutrition. *Science Translational Medicine*, 14(640), eabk1107. <https://doi.org/10.1126/scitranslmed.abk1107>. Epub 2022 Apr 13. PMID: 35417188; PMCID: PMC9516695.

Baughn, A. D., & Malamy, M. H. (2004). The strict anaerobe *Bacteroides fragilis* grows in and benefits from nanomolar concentrations of oxygen. *Nature*, 427, 441–444. [\[https://doi.org/10.1038/nature02285\]](https://doi.org/10.1038/nature02285) [\[PubMed\]](#)

Bennett, B. J., de Aguiar Vallim, T. Q., Wang, Z., Shih, D. M., Meng, Y., Gregory, J., Allayee, H., Lee, R., Graham, M., Crooke, R., et al. (2013). Trimethylamine-N-oxide, a metabolite associated with atherosclerosis, exhibits complex genetic and dietary regulation. *Cell Metabolism*, 17, 49–60. [\[PubMed\]](#)

Benno, P., Midtvedt, K., Alam, M., Collinder, E., Norin, E., & Midtvedt, T. (2005). Examination of intestinal conversion of cholesterol to coprostanol in 633 healthy subjects reveals an age- and sex-dependent pattern. *Microbial Ecology in Health and Disease*, 17(4), 200–204. <https://doi.org/10.1080/08910600500519854>

Bernalier, A., Willems, A., Leclerc, M., Rochet, V., & Collins, M. D. (1996). *Ruminococcus hydrogenotrophicus* sp. nov., a new H₂/CO₂-utilizing acetogenic bacterium isolated from human feces. *Archives of Microbiology*, 166, 176–183. [\[PubMed\]](#)

Biagi, E., Franceschi, C., Rampelli, S., Severgnini, M., Ostan, R., Turroni, S., et al. (2016). Gut microbiota and extreme longevity. *Current Biology*, 26(11), 1480–1485. <https://doi.org/10.1016/j.cub.2016.04.016> [\[PubMed\]](#)

Bik, E. M., Eckburg, P. B., Gill, S. R., Nelson, K. E., Purdom, E. A., Francois, F., Perez-Perez, G., Blaser, M. J., & Relman, D. A. (2006). Molecular analysis of the bacterial microbiota in the human stomach. *Proceedings of the National Academy of Sciences of the United States of America*, 103, 732–737. [\[PubMed\]](#)

Booijink, C. C., El-Aidy, S., Rajilic-Stojanovic, M., Heilig, H. G., Troost, F. J., Smidt, H., Kleerebezem, M., De Vos, W. M., & Zoetendal, E. G. (2010). High temporal and inter-individual variation detected in the human ileal microbiota. *Environmental Microbiology*, 12, 3213–3227. [\[PubMed\]](#)

Borgstrom, B., Dahlqvist, A., Lundh, G., Sjovall, J. (1957). Studies of intestinal digestion and absorption in the human. *Journal of Clinical Investigation*, 36(10), 1521–1536. <https://doi.org/10.1172/JCI103549>. PMID: 13475490; PMCID: PMC1072752

Boros, M., Ghyczy, M., Érces, D., Varga, G., Tokés, T., Kupai, K., Torday, C., & Kaszaki, J. (2012). The anti-inflammatory effects of methane*. *Critical Care Medicine*, 40, 1269. [\[PubMed\]](#)

Braune, A., Engst, W., & Blaut, M. (2016). Identification and functional expression of genes

encoding flavonoid O- and C-glycosidases in intestinal bacteria. *Environmental Microbiology*, 18, 2117–2129.

[[PubMed](#)]

Brown, A. J., Goldsworthy, S. M., Barnes, A. A., Eilert, M. M., Tcheang, L., Daniels, D., Muir, A. I., Wigglesworth, M. J., Kinghorn, I., Fraser, N. J., et al. (2003). The orphan G protein-coupled receptors GPR41 and GPR43 are activated by propionate and other short chain carboxylic acids. *The Journal of Biological Chemistry*, 278, 11312–11319.

[[PubMed](#)]

Bui, T., Ritari, J., Boeren, S. et al. (2015). Production of butyrate from lactate by the human gut bacterium *Anaerostipes caccae*. *Nature Communications*, 6(1), 10062. <https://doi.org/10.1038/ncomms10062>

Camarillo-Guerrero, L. F., Almeida, A., Rangel-Pineros, G., Finn, R. D., & Lawley, T. D. (2021). Massive expansion of human gut bacteriophage diversity. *Cell*, 184(4), 1098–1109. <https://doi.org/10.1016/j.cell.2021.01.029>

[[PubMed](#)]

Caminero, A., Herrán, A. R., Nistal, E., Pérez-Andrés, J., Vaquero, L., Vivas, S., et al. (2014). Diversity of the cultivable human gut microbiome involved in gluten metabolism: Isolation of microorganisms with potential interest for coeliac disease. *FEMS Microbiology Ecology*, 88(2), 309–319. <https://doi.org/10.1111/1574-6941.12295>

[[PubMed](#)]

Cantarel, B. L., Coutinho, P. M., Rancurel, C., Bernard, T., Lombard, V., & Henrissat, B. (2009). The Carbohydrate-Active EnZymes database (CAZy): An expert resource for glycogenomics. *Nucleic Acids Research*, 37, D233–D238.

[[PubMed](#)]

Carbonero, F., Benefiel, A. C., Alizadeh-Ghamsari, A. H., & Gaskins, H. R. (2012). Microbial pathways in colonic sulfur metabolism and links with health and disease. *Frontiers in Physiology*, 3, 448.

[[PubMed](#)]

Carter, M. M., Olm, M. R., Merrill, B. D., Dahan, D., Tripathi, S., Spencer, S. P., Yu, F. B., Jain, S., Neff, N., Jha, A. R., Sonnenburg, E. D., & Sonnenburg, J. L. (2023). Ultra-deep sequencing of Hadza hunter-gatherers recovers vanishing gut microbes. *Cell*, 186(14), 3111–3124.e13. <https://doi.org/10.1016/j.cell.2023.05.046>. Epub 2023 Jun 21. PMID: 37348505; PMCID: PMC10330870

Culp, E. J., Nelson, N.T., Verdegaal, A. A., Goodman, A. L., (2024). Microbial transformation of dietary xenobiotics shapes gut microbiome composition. *Cell*, 187(22), 6327–6345.e20. <https://doi.org/10.1016/j.cell.2024.08.038>. Epub 2024 Sep 24. PMID: 39321800; PMCID: PMC11531382.

Chassard, C., Goumy, V., Leclerc, M., Del'homme, C., & Bernalier-Donadille, A. (2007). Characterization of the xylan-degrading microbial community from human faeces. *FEMS Microbiology Ecology*, 61, 121–131.

[[PubMed](#)]

Chavez-Arroyo, A., Radlinski, L. C., Bäumler AJ., (2025) Principles of gut microbiota assembly.

Trends Microbiol, 33(7), 718–726. <https://doi.org/10.1016/j.tim.2025.02.014>. Epub 2025 Mar 14. PMID: 40089422.

Chibani, C. M., Mahnert, A., Borrel, G., Almeida, A., Werner, A., Brugère, J. F., Gribaldo, S., Finn, R. D., Schmitz, R. A., & Moissl-Eichinger, C. (2022) A catalogue of 1,167 genomes from the human gut archaeome. *Nature Microbiology*, 7(1), 48–61. <https://doi.org/10.1038/s41564-021-01020-9> Epub 2021 Dec 30. Erratum in: *Nat Microbiol*. 2022 Feb;7(2):339. 10.1038/s41564-022-01061-8. PMID: 34969981; PMCID: PMC8727293

Cho, K. H., Cho, D., Wang, G. R., & Salyers, A. A. (2001). New regulatory gene that contributes to control of *Bacteroides thetaiotaomicron* starch utilization genes. *Journal of Bacteriology*, 183, 7198–7205.

[[PubMed](#)]

Christl, S. U., Gibson, G. R., & Cummings, J. H. (1992). Role of dietary sulphate in the regulation of methanogenesis in the human large intestine. *Gut*, 33, 1234–1238.

[[PubMed](#)]

Clark, S. B., Lawergren, B., & Martin, J. V. (1973). Regional intestinal absorptive capacities for triolein: an alternative to markers. *American Journal of Physiology*, 225(3), 574–585. <https://doi.org/10.1152/ajplegacy.1973.225.3.574>. PMID: 4726491

Clavel, T., Henderson, G., Engst, W., Doré, J., & Blaut, M. (2006). Phylogeny of human intestinal bacteria that activate the dietary lignan secoisolariciresinol diglucoside. *FEMS Microbiol Ecol*, 55(3), 471–478. <https://doi.org/10.1111/j.1574-6941.2005.00057.x>. PMID: 16466386.

Clavel, T., Borrmann, D., Braune, A., Dore, J., & Blaut, M. (2006a). Occurrence and activity of human intestinal bacteria involved in the conversion of dietary lignans. *Anaerobe*, 12, 140–147.

[[PubMed](#)]

Clavel, T., Dore, J., & Blaut, M. (2006b). Bioavailability of lignans in human subjects. *Nutrition Research Reviews*, 19, 187–196.

[[PubMed](#)]

Clavel, T., Lippman, R., Gavini, F., Dore, J., & Blaut, M. (2007). *Clostridium saccharogumia* sp. nov. and *Lactonifactor longoviformis* gen. nov., sp. nov., two novel human faecal bacteria involved in the conversion of the dietary phytoestrogen secoisolariciresinol diglucoside. *Systematic and Applied Microbiology*, 30, 16–26.

[[PubMed](#)]

Clavel, M. (2013). Phenolics in human nutrition: importance of the intestinal microbiome for isoflavone and lignan bioavailability, In *Handbook of Natural Products*; Ramawat, Merillon (Eds); Elsevier, ISBN 978-3-642-221-43-9/44-6; https://doi.org/10.1007/978-3-642-22144-6_94

Clavel, T., Lagkouvardos, I., & Hiergeist, A. (2016). Microbiome sequencing: challenges and opportunities for molecular medicine. *Expert Review of Molecular Diagnostics*, 16(7), 795–805. <https://doi.org/10.1080/14737159.2016.1184574>. Epub 2016 May 14. PMID: 27125906

Cummings, J. H. (1995). Short chain fatty acids. In G. R. Gibson & G. T. Macfarlane (Eds.), *Human*

colonic bacteria: Role in nutrition, physiology and pathology (pp. 101–130). CRC Press.

Cummings, J. H., & Macfarlane, G. T. (1991). The control and consequences of bacterial fermentation in the human colon. *The Journal of Applied Bacteriology*, 70, 443–459.

[[PubMed](#)]

Cummings, J. H., Pomare, E. W., Branch, W. J., Naylor, C. P., & Macfarlane, G. T. (1987). Short chain fatty acids in human large intestine, portal, hepatic and venous blood. *Gut*, 28, 1221–1227.

[[PubMed](#)]

Darragh, A. J., & Hodgkinson, S. M. (2000). Quantifying the digestibility of dietary protein. *The Journal of Nutrition*, 130, 1850S–1856S.

[[PubMed](#)]

de Goffau, M. C., Jallow, A. T., Sanyang, C., Prentice, A. M., Meagher, N., Price, D. J., Revill, P. A., Parkhill, J., Pereira, D. I. A., & Wagner, J. (2022). Gut microbiomes from Gambian infants reveal the development of a non-industrialized Prevotella-based trophic network. *Nature Microbiology*, 7(1), 132–144. <https://doi.org/10.1038/s41564-021-01023-6>. Epub 2021 Dec 31. PMID: 34972822; PMCID: PMC8727306.

De Filippo, C., Cavalieri, D., Di Paola, M., Ramazzotti, M., Poullet, J. B., Massart, S., Collini, S., Pieraccini, G., & Lionetti, P. (2010). Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. *Proceedings of the National Academy of Sciences of the United States of America*, 107, 14691–14696.

[[PubMed](#)]

Deehan, E. C., Yang, C., Perez-Muñoz, M. E., Nguyen, N. K., Cheng, C. C., Triador, L., Zhang, Z., Bakal, J. A., & Walter, J. (2020) Precision Microbiome Modulation with Discrete Dietary Fiber Structures Directs Short-Chain Fatty Acid Production. *Cell Host Microbe*, 27(3), 389–404.e6. <https://doi.org/10.1016/j.chom.2020.01.006>. Epub 2020 Jan 30. PMID: 32004499.

Depommier, C., et al. (2019). Supplementation with *Akkermansia muciniphila* in overweight and obese human volunteers: a proof-of-concept exploratory study. *Nature Medicine*, 25, 1096–1103.

[[PubMed](#)]

Derrien, M., Vaughan, E. E., Akermans, A. D., & de Vos, W. M. (2004). *Akkermansia muciniphila* gen. nov., sp. nov., a human intestinal mucin-degrading bacterium. *International Journal of Systematic and Evolutionary Microbiology*, 54(5), 1469–1476. <https://doi.org/10.1099/ijs.0.02873-0>

[[PubMed](#)]

Devlin, A. S., & Fischbach, M. A. (2015). A biosynthetic pathway for a prominent class of microbiota-derived bile acids. *Nature Chemical Biology*, 11, 685–690.

[[PubMed](#)]

Dinleyici, M., Barbieur, J., Dinleyici, E. C., & Vandenplas, Y. (2023). Functional effects of human milk oligosaccharides (HMOs). *Gut Microbes*, 15(1). <https://doi.org/10.1080/19490976.2023.2186115>

Drouault, S., Juste, C., Marteau, P., Renault, P., & Corthier, G. (2002). Oral treatment with *Lactococcus lactis* expressing *Staphylococcus hyicus* lipase enhances lipid digestion in pigs with

induced pancreatic insufficiency. *Applied and Environmental Microbiology*, 68(6):3166–3168. <https://doi.org/10.1128/AEM.68.6.3166-3168.2002>. PMID: 12039786; PMCID: PMC123933.

Duar, R. M., Lin, X. B., Zheng, J., Martino, M. E., Grenier, T., Pérez-Muñoz, M. E., et al. (2017). Lifestyles in transition: Evolution and natural history of the genus *Lactobacillus*. *FEMS Microbiology Reviews*, 41(Supp_1), S27–S48. <https://doi.org/10.1093/femsre/fux030> [PubMed]

Duan, Y., Young, R., & Schnabl, B. (2022). Bacteriophages and their potential for treatment of gastrointestinal diseases. *Nature Reviews Gastroenterology & Hepatology*, 19(2), 135–144. <https://doi.org/10.1038/s41575-021-00536-z>. Epub 2021 Nov 15. PMID: 34782783; PMCID: PMC8966578

Dumas, M. E., Barton, R. H., Toyé, A., Cloarec, O., Blancher, C., Rothwell, A., Fearnside, J., Tatoud, R., Blanc, V., Lindon, J. C., et al. (2006). Metabolic profiling reveals a contribution of gut microbiota to fatty liver phenotype in insulin-resistant mice. *Proceedings of the National Academy of Sciences of the United States of America*, 103, 12511–12516. [PubMed]

Duncan, S. H., Barcenilla, A., Stewart, C. S., Pryde, S. E., & Flint, H. J. (2002). Acetate utilization and butyryl coenzyme A (CoA): Acetate-CoA transferase in butyrate-producing bacteria from the human large intestine. *Applied and Environmental Microbiology*, 68, 5186–5190. [PubMed]

Duncan, S. H., Louis, P., & Flint, H. J. (2004). Lactate-utilizing bacteria, isolated from human feces, that produce butyrate as a major fermentation product. *Applied and Environmental Microbiology*, 70, 5810–5817. [PubMed]

Everard, A., et al. (2013). Cross-talk between *Akkermansia muciniphila* and intestinal epithelium controls diet-induced obesity. *Proceedings of the National Academy of Sciences*, 110, 9066–9071.

Eyssen, H. J., Parmentier, G. G., Compennolle, F. C., De Pauw, G., Piessens-Denef M. (1973). Biohydrogenation of sterols by *Eubacterium* ATCC 21,408--Nova species. *European Journal of Biochemistry*, 36(2), 411–421. <https://doi.org/10.1111/j.1432-1033.1973.tb02926.x>. PMID: 4730962

De Filippis, F., Pasolli, E., Tett, A., Tarallo, S., Naccarati, A., De Angelis, M., Neviani, E., Cocolin, L., Gobbetti, M., Segata, N., Ercolini, D. (2019). Distinct Genetic and Functional Traits of Human Intestinal *Prevotella copri* Strains Are Associated with Different Habitual Diets. *Cell Host Microbe*, 25(3):444–453.e3. <https://doi.org/10.1016/j.chom.2019.01.004>. Epub 2019 Feb 21. PMID: 30799264.

Finegold, S. M., Flora, D. J., Attebery, H. R., & Sutter, V. L. (1975). Fecal bacteriology of colonic polyp patients and control patients. *Cancer Research*, 35, 3407–3417. [PubMed]

Finegold, S. M., Sutter, V. L., & Mathisen, G. E. (1983). Normal indigenous intestinal flora. In D. J. Hentges (Ed.), *Human intestinal microflora in health and disease* (pp. 3–31). Academic Press.

Fleissner, C. K., Huebel, N., Abd El-Bary, M. M., Loh, G., Klaus, S., & Blaut, M. (2010). Absence of intestinal microbiota does not protect mice from diet-induced obesity. *British Journal of Nutrition*, 104(6), 919–929. <https://doi.org/10.1017/S0007114510001303>. Epub 2010 May 5. PMID: 20441670.

Flint, H. J., Scott, K. P., Duncan, S. H., Louis, P., & Forano, E. (2012). Microbial degradation of complex carbohydrates in the gut. *Gut Microbes*, 3, 289–306.
[PubMed]

Florin, T. H., Zhu, G., Kirk, K. M., & Martin, N. G. (2000). Shared and unique environmental factors determine the ecology of methanogens in humans and rats. *The American Journal of Gastroenterology*, 95, 2872–2879.
[PubMed]

Fromentin, S., et al. (2022). Microbiome and metabolome features of the cardiometabolic disease spectrum. *Nature Medicine*, 28, 303–314.
[PubMed]

Galipeau HJ, Caminero A, Turpin W, Bermudez-Brito M, Santiago A, Libertucci J, Constante M, Raygoza Garay JA, Rueda G, Armstrong S, Clarizio A, Smith MI, Surette MG, Bercik P; CCC Genetics, Environmental, Microbial Project Research Consortium; Croitoru K, Verdu EF. (2021). Novel fecal biomarkers that precede clinical diagnosis of ulcerative colitis. *Gastroenterology*, 160(5), 1532–1545. <https://doi.org/10.1053/j.gastro.2020.12.004>. Epub 2020 Dec 10. PMID: 33310084

Gérard, P., Lepercq, P., Leclerc, M., Gavini, F., Raibaud, P., & Juste, C. (2007). Bacteroides sp. strain D8, the first cholesterol-reducing bacterium isolated from human feces. *Applied and Environmental Microbiology*, 73(18), 5742–5749. <https://doi.org/10.1128/AEM.02806-06>. Epub 2007 Jul 6. PMID: 17616613; PMCID: PMC2074900.

Gibson, S. A., McFarlan, C., Hay, S., & MacFarlane, G. T. (1989). Significance of microflora in proteolysis in the colon. *Applied and Environmental Microbiology*, 55, 679–683.
[PubMed]

Gill, S. R., Pop, M., Deboy, R. T., Eckburg, P. B., Turnbaugh, P. J., Samuel, B. S., Gordon, J. I., Relman, D. A., Fraser-Liggett, C. M., & Nelson, K. E. (2006). Metagenomic analysis of the human distal gut microbiome. *Science*, 312, 1355–1359.
[PubMed]

Gibson, G. R., Hutkins, R., Sanders, M. E., Prescott, S. L., Reimer, R. A., Salminen, S. J., Scott, K., Stanton, C., Swanson, K. S., Cani, P. D., Verbeke, K., & Reid, G. (2017). Expert consensus document: The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. *Nature Reviews Gastroenterology & Hepatology*, 14(8), 491–502. <https://doi.org/10.1038/nrgastro.2017.75>. Epub 2017 Jun 14. PMID: 28611480.

Greenwood, H., Snedden, W., Hayward, R. P., & Landon, J. (1975). The measurement of urinary digoxin and dihydrodigoxin by radioimmunoassay and by mass spectroscopy. *Clinica Chimica Acta*, 62(2), 213–224. [https://doi.org/10.1016/0009-8981\(75\)90230-2](https://doi.org/10.1016/0009-8981(75)90230-2). PMID: 1170963.

Gregory, A. C., et al. (2020). The Gut Virome Database reveals age-dependent patterns of virome diversity in the human gut. *Cell Host & Microbe*, 28(5), 724–740. <https://doi.org/10.1016/j.chom.2020.08.003>

Groussin, M., Poyet, M., Sistiaga, A., Kearney, S. M., Moniz, K., Noel, M., Hooker, J., Gibbons, S. M., Segurel, L., Froment, A., Mohamed, R. S., Fezeu, A., Juimo, V. A., Lafosse, S., Tabe, F. E., Girard, C., Iqaluk, D., Nguyen, L. T. T., Shapiro, B. J., Lehtimäki, J., Ruokolainen, L., Kettunen, P. P., Vatanen, T., Sigwazi, S., Mabulla, A., Domínguez-Rodrigo, M., Nartey, Y. A., Agyei-Nkansah, A., Duah, A., Awuku, Y. A., Valles, K. A., Asibey, S. O., Afihene, M. Y., Roberts, L. R., Plymoth, A., Onyekwere, C. A., Summons, R. E., Xavier, R. J., & Alm, E. J. (2021). Elevated rates of horizontal gene transfer in the industrialized human microbiome. *Cell*, 184(8), 2053–2067.e18. <https://doi.org/10.1016/j.cell.2021.02.052>. Epub 2021 Mar 31. PMID: 33794144.

Haiser, H. J., et al. (2013). Predicting and manipulating cardiac drug inactivation by the human gut bacterium *eggerthella lenta*. *Science*, 341(6143), 295–298. <https://doi.org/10.1126/science.1235872> [PubMed]

Haiser, H. J., Seim, K. L., Balskus, E. P., & Turnbaugh, P. J. (2014). Mechanistic insight into digoxin inactivation by *eggerthella lenta* augments our understanding of its pharmacokinetics. *Gut Microbes*, 5(2), 233–238. <https://doi.org/10.4161/gmic.27915>. Epub 2014 Jan 23. PMID: 24637603; PMCID: PMC4063850.

Hayashi, H., Takahashi, R., Nishi, T., Sakamoto, M., & Benno, Y. (2005). Molecular analysis of jejunal, ileal, caecal and recto-sigmoidal human colonic microbiota using 16S rRNA gene libraries and terminal restriction fragment length polymorphism. *Journal of Medical Microbiology*, 54, 1093–1101. [PubMed]

Hehemann, J. H., Correc, G., Barbeyron, T., Helbert, W., Czjzek, M., & Michel, G. (2010). Transfer of carbohydrate-active enzymes from marine bacteria to Japanese gut microbiota. *Nature*, 464, 908–912. <https://doi.org/10.1038/nature08937> [PubMed]

Hitch, T. C. A., Afrizal, A., Riedel, T., Kioukis, A., Haller, D., Lagkouvardos, I., Overmann, J., & Clavel, T. (2021). Recent advances in culture-based gut microbiome research. *International Journal of Medical Microbiology*, 311(3), 151485. <https://doi.org/10.1016/j.ijmm.2021.151485>. Epub 2021 Feb 25. PMID: 33689954.

Hitch, T. C. A., Masson, J. M., Pauvert, C., Bosch, J., Nüchtern, S., Treichel, N. S., Baloh, M., Razavi, S., Afrizal, A., Kousetzi, N., Aguirre, A. M., Wylensek, D., Coates, A. C., Jennings, S. A. V., Panyot, A., Viehof, A., Schmitz, M. A., Stuhmann, M., Deis, E. C., Bisdorf, K., Chiotelli, M. D., Lissin, A., Schober, I., Witte, J., Cramer, T., Riedel, T., Wende, M., Winter, K. A., Amend, L., Riva, A., Trinh, S., Mitchell, L., Hartman, J., Berry, D., Seitz, J., Bossert, L. C., Grognot, M., Allers, T., Strowig, T., Pester, M., Abt, B., Reimer, L. C., Overmann, J., & Clavel, T. (2025). HiBC: a publicly available collection of bacterial strains isolated from the human gut. *Nature Communications*, 16(1), 4203. <https://doi.org/10.1038/s41467-025-59229-9>. PMID: 40328737; PMCID: PMC12056005

Hoegenauer, C., Hammer, H. F., Mahnert, A., Moissl-Eichinger, C. (2022). Methanogenic archaea in

the human gastrointestinal tract. *Nature Reviews Gastroenterology & Hepatology*, 19(12), 805–813. <https://doi.org/10.1038/s41575-022-00673-z>. Epub 2022 Sep 1. PMID: 36050385

Hoffmann, C., Dollive, S., Grunberg, S., Chen, J., Li, H., Wu, G. D., Lewis, J. D., & Bushman, F. D. (2013). Archaea and fungi of the human gut microbiome: Correlations with diet and bacterial residents. *PLoS One*, 8, e66019.
[PubMed]

Hollman, P. C., & Katan, M. B. (1999). Dietary flavonoids: Intake, health effects and bioavailability. *Food and Chemical Toxicology*, 37, 937–942.
[PubMed]

Hornich, M., & Chrastova, V. (1981). The redox potential of the large intestine in swine in relation to swine dysentery. *Veterinary Medicine (Praha)*, 26, 593–598.

Hoskins, L. C. (1993). Mucin degradation in the human gastrointestinal tract and its significance to enteric microbial ecology. *European Journal of Gastroenterology & Hepatology*, 5, 205–213.

Huffnagle, G. B., & Noverr, M. C. (2013). The emerging world of the fungal microbiome. *Trends in Microbiology*, 21, 334–341.
[PubMed]

Hungate, R. E. (1969). A roll tube method for cultivation of strict anaerobes. In J. R. Norris & D. W. Ribbons (Eds.), *Methods in microbiology* (p. 117). Academic Press.

Huse, S. M., Ye, Y., Zhou, Y., & Fodor, A. A. (2012). A core human microbiome as viewed through 16S rRNA sequence clusters. *PLoS One*, 7, e34242.
[PubMed]

Jiang, Y., Xiong, X., Danska, J., & Parkinson, J. (2016). Metatranscriptomic analysis of diverse microbial communities reveals core metabolic pathways and microbiome-specific functionality. *Microbiome*, 4, 2.
<https://doi.org/10.1186/s40168-015-0146-x> [PubMed]

Johansen, J., Atarashi, K., Arai, Y., Hirose, N., Sørensen, S. J., Vatanen, T., Knip, M., Honda, K., Xavier, R. J., Rasmussen, S., & Plichta, D. R. (2023). Centenarians have a diverse gut virome with the potential to modulate metabolism and promote healthy lifespan. *Nature Microbiology*, 8(6), 1064–1078. <https://doi.org/10.1038/s41564-023-01370-6>. Epub 2023 May 15. PMID: 37188814. Epub 2023 Apr 6. PMID: 37117794. PMID: 30322585. Epub 2016 Feb 3. PMID: 26843508; PMCID: PMC5532459

Jones, B. V., Begley, M., Hill, C., Gahan, C. G., & Marchesi, J. R. (2008). Functional and comparative metagenomic analysis of bile salt hydrolase activity in the human gut microbiome. *Proceedings of the National Academy of Sciences of the United States of America*, 105, 13580–13585.
[PubMed]

Kaufman, D. W., Kelly, J. P., Curhan, G. C., Anderson, T. E., Dretler, S. P., Preminger, G. M., & Cave, D. R. (2008). Oxalobacter formigenes may reduce the risk of calcium oxalate kidney stones. *Journal of*

the American Society of Nephrology, 19(6), 1197–203. <https://doi.org/10.1681/ASN.2007101058>. Epub 2008 Mar 5. PMID: 18322162; PMCID: PMC2396938

Kenny, D. J., Plichta, D. R., Shungin, D., Koppel, N., Hall, A. B., Fu, B., Vasan, R. S., Shaw, S. Y., Vlamakis, H., Balskus, E. P., & Xavier, R. J. (2020). Cholesterol Metabolism by Uncultured Human Gut Bacteria Influences Host Cholesterol Level. *Cell Host Microbe*, 28(2), 245–257.e6. <https://doi.org/10.1016/j.chom.2020.05.013>. Epub 2020 Jun 15. PMID: 32544460; PMCID: PMC7435688

Kennedy, K. M., de Goffau, M. C., Perez-Munoz, M. E., Arrieta, M. C., Backhed, F., Bork, P., Braun, T., Bushman, F. D., Dore, J., de Vos, W. M., Earl, A. M., Eisen, J. A., Elovitz, M. A., Ganai-Vonarburg, S. C., Ganzle, M. G., Garrett, W. S., Hall, L. J., Hornef, M. W., Huttenhower, C., Konnikova, L., Lebeer, S., Macpherson, A. J., Massey, R. C., McHardy, A. C., Koren, O., Lawley, T. D., Ley, R. E., O'Mahony, L., O'Toole, P. W., Pamer, E. G., Parkhill, J., Raes, J., Rattei, T., Salonen, A., Segal, E., Segata, N., Shanahan, F., Sloboda, D. M., Smith, G. C., Sokol, H., Spector, T. D., Surette, M. G., Tannock, G. W., Walker, A. W., Yassour, M., & Walter, J. (2023). Questioning the fetal microbiome illustrates pitfalls of low-biomass microbial studies. *Nature*, 613(7945), 639–649. <https://doi.org/10.1038/s41586-022-05546-8>. Epub 2023 Jan 25. PMID: 36697862; PMCID: PMC11333990.

Khan, M. T., Duncan, S. H., Stams, A. J., van Dijk, J. M., Flint, H. J., & Harmsen, H. J. (2012). The gut anaerobe *Faecalibacterium prausnitzii* uses an extracellular electron shuttle to grow at oxic-anoxic interphases. *The ISME Journal*, 6, 1578–1585. [\[PubMed\]](#)

Kim, J. S., Kang, S. W., Lee, J. H., Park, S. H., & Lee, J. S. (2022). The evolution and competitive strategies of *Akkermansia muciniphila* in gut. *Gut Microbes*, 14(1). <https://doi.org/10.1080/19490976.2021.2025017>

Kitahara, M., Sakamoto, M., Ike, M., Sakata, S., & Benno, Y. (2005). *Bacteroides plebeius* sp. nov. and *Bacteroides coprocola* sp. nov., isolated from human faeces. *International Journal of Systematic and Evolutionary Microbiology*, 55, 2143–2147. [\[PubMed\]](#)

Klünemann, M., Andrejev, S., Blasche, S., Mateus, A., Phapale, P., Devendran, S., Vappiani, J., Simon, B., Scott, T. A., Kafkia, E., Konstantinidis, D., Zirngibl, K., Mastorilli, E., Banzhaf, M., Mackmull, M. T., Hövelmann, F., Nesme, L., Brochado, A. R., Maier, L., Bock, T., Periwal, V., Kumar, M., Kim, Y., Tramontano, M., Schultz, C., Beck, M., Hennig, J., Zimmermann, M., Sévin, D. C., Cabreiro, F., Savitski, M. M., Bork, P., Typas, A., & Patil, K. R. (2021). Bioaccumulation of therapeutic drugs by human gut bacteria. *Nature*, 597(7877), 533–538. <https://doi.org/10.1038/s41586-021-03891-8>. Epub 2021 Sep 8. PMID: 34497420; PMCID: PMC7614428.

Knights, D., Ward, T. L., McKinlay, C. E., Miller, H., Gonzalez, A., McDonald, D., & Knight, R. (2014). Rethinking “enterotypes”. *Cell Host & Microbe*, 16, 433–437.

Koppel, N., Bisanz, J. E., Pandelia, M.-E., Turnbaugh, P. J., & Balskus, E. P. (2018). Discovery and characterization of a prevalent human gut bacterial enzyme sufficient for the inactivation of a family of plant toxins, *eLife*, 7, e33953.

Kübeck, R., Bonet-Ripoll, C., Hoffmann, C., Walker, A., Maria Müller, V., Luise Schüppel, V., Lagkouvardos, I., Scholz, B., Karl-Heinz, E., Daniel, H., Schmitt-Kopplin, P., Haller, D., Clavel, T. &

Klingenspor, M. (2016). Dietary fat and gut microbiota interactions determine diet-induced obesity in mice, *Molecular Metabolism*, 5(12), 1162–1174

La Rosa, S. L., Leth, M. L., Michalak, L., Hansen, M. E., Pudlo, N. A., Glowacki, R., Pereira, G., Workman, C. T., Arntzen, M. Ø, Pope, P. B., Martens, E. C., Hachem, M. A., & Westereng, B. (2019) The human gut Firmicute *Roseburia intestinalis* is a primary degrader of dietary β -mannans. *Nature Communications*, 10(1), 905. <https://doi.org/10.1038/s41467-019-08812-y>. PMID: 30796211; PMCID: PMC6385246

Lazarova, D. L., Chiaro, C., Wong, T., Drago, E., Rainey, A., O'Malley, S., & Bordonaro, M. (2013). CBP activity mediates effects of the histone deacetylase inhibitor butyrate on WNT activity and apoptosis in colon cancer cells. *Journal of Cancer*, 4, 481–490.
[PubMed]

Leviatan, S., Shoer, S., Rothschild, D. et al. (2022). An expanded reference map of the human gut microbiome reveals hundreds of previously unknown species. *Nature Communications*, 13, 386. <https://doi.org/10.1038/s41467-022-31502-1>

Ley, R. E., Hamady, M., Lozupone, C., Turnbaugh, P. J., Ramey, R. R., Bircher, J. S., Schlegel, M. L., Tucker, T. A., Schrenzel, M. D., Knight, R., & Gordon, J. I. (2008). Evolution of mammals and their gut microbes. *Science*, 320, 1647–1651.
[PubMed]

Li, R., Wang, Y., Hu, H., Tan, Y., & Ma, Y. (2022). Metagenomic analysis reveals unexplored diversity of archaeal virome in the human gut. *Nature Communications*, 13(1):7978. <https://doi.org/10.1038/s41467-022-35735-y>. PMID: 36581612; PMCID: PMC9800368

Liang, C., Tseng, H. C., Chen, H. M., Wang, W. C., Chiu, C. M., Chang, J. Y., Lu, K. Y., Weng, S. L., Chang, T. H., Chang, C. H., et al. (2017). Diversity and enterotype in gut bacterial community of adults in Taiwan. *BMC Genomics*, 18, 932.
<https://doi.org/10.1186/s12864-016-3261-6>[PubMed]

Lim, M. Y., Rho, M., Song, Y. M., Lee, K., Sung, J., & Ko, G. (2014). Stability of gut enterotypes in Korean monozygotic twins and their association with biomarkers and diet. *Scientific Reports*, 4, 7348.
[PubMed]

Lin, H. V., Frassetto, A., Kowalik, E. J., Jr., Nawrocki, A. R., Lu, M. M., Kosinski, J. R., Hubert, J. A., Szeto, D., Yao, X., Forrest, G., & Marsh, D. J. (2012). Butyrate and propionate protect against diet-induced obesity and regulate gut hormones via free fatty acid receptor 3-independent mechanisms. *PLoS One*, 7, e35240.
[PubMed]

Liu, C., Finegold, S. M., Song, Y., & Lawson, P. A. (2008). Reclassification of *Clostridium coccoides*, *Ruminococcus hansenii*, *Ruminococcus hydrogenotrophicus*, *Ruminococcus luti*, *Ruminococcus productus* and *Ruminococcus schinkii* as *Blautia coccoides* gen. nov., comb. nov., *Blautia hansenii* comb. nov., *Blautia hydrogenotrophica* comb. nov., *Blautia luti* comb. nov., *Blautia producta* comb. nov., *Blautia schinkii* comb. nov. and description of *Blautia wexlerae* sp. nov., isolated from human faeces. *International Journal of Systematic and Evolutionary Microbiology*, 58, 1896–1902.

[[PubMed](#)]

Lopez, C. A., Winter, S. E., Rivera-Chavez, F., Xavier, M. N., Poon, V., Nuccio, S. P., Tsolis, R. M., & Baumber, A. J. (2012). Phage-mediated acquisition of a type III secreted effector protein boosts growth of salmonella by nitrate respiration. *MBio*, 3, e00143–e00112.

[[PubMed](#)]

Lopez-Siles, M., Khan, T. M., Duncan, S. H., Harmsen, H. J., Garcia-Gil, L. J., & Flint, H. J. (2012). Cultured representatives of two major phylogroups of human colonic *Faecalibacterium prausnitzii* can utilize pectin, uronic acids, and host-derived substrates for growth. *Applied and Environmental Microbiology*, 78, 420–428.

[[PubMed](#)]

Lu, Q., et al. (2024). A host-adapted auxotrophic gut symbiont induces mucosal immunodeficiency. *Science*, 385(6716), eadk2536. <https://doi.org/10.1126/science.adk2536>

Macfarlane, G. T., Gibson, G. R., & Cummings, J. H. (1992). Comparison of fermentation reactions in different regions of the human colon. *The Journal of Applied Bacteriology*, 72, 57–64.

[[PubMed](#)]

Magee, E. A., Richardson, C. J., Hughes, R., & Cummings, J. H. (2000). Contribution of dietary protein to sulfide production in the large intestine: An in vitro and a controlled feeding study in humans. *The American Journal of Clinical Nutrition*, 72, 1488–1494.

[[PubMed](#)]

Maier, L., et al. (2018). Extensive impact of non-antibiotic drugs on human gut bacteria. *Nature*, 555(7698), 623–628. <https://doi.org/10.1038/nature25979>

[[PubMed](#)]

Mao, X., Larsen, S. B., Zachariassen, L. S. F., Brunse, A., Adamberg, S., Mejia, J. L. C., Larsen, F., Adamberg, K., Nielsen, D. S., Hansen, A. K., Hansen, C. H. F., Rasmussen, T. S. (2024). Transfer of modified gut viromes improves symptoms associated with metabolic syndrome in obese male mice. *Nature Communications*, 15(1), 4704. <https://doi.org/10.1038/s41467-024-49152-w>. PMID: 38830845; PMCID: PMC11148109

Martens, E. C., Chiang, H. C., & Gordon, J. I. (2008). Mucosal glycan foraging enhances fitness and transmission of a saccharolytic human gut bacterial symbiont. *Cell Host & Microbe*, 4, 447–457.

Martino, M. E., Joncour, P., Leenay, R., Gervais, H., Shah, M., Hughes, S., Gillet, B., Beisel, C., & Leulier, F. (2018). Bacterial Adaptation to the Host's Diet Is a Key Evolutionary Force Shaping Drosophila-Lactobacillus Symbiosis. *Cell Host Microbe*, 24(1), 109–119.e6. <https://doi.org/10.1016/j.chom.2018.06.001>. PMID: 30008290; PMCID: PMC6054917.

Meziti, A., Rodriguez-R, L. M., Hatt, J. K., Peña-Gonzalez, A., Levy, K., & Konstantinidis, K. T. (2021). The Reliability of Metagenome-Assembled Genomes (MAGs) in Representing Natural Populations: Insights from Comparing MAGs against Isolate Genomes Derived from the Same Fecal Sample. *Applied and Environmental Microbiology*, 87(6), e02593–20. <https://doi.org/10.1128/AEM.02593-20>. PMID: 33452027; PMCID: PMC8105024.

Metcalf, A. M., Phillips, S. F., Zinsmeister, A. R., MacCarty, R. L., Beart, R. W., & Wolff, B. G. (1987). Simplified assessment of segmental colonic transit. *Gastroenterology*, 92, 40–47. [\[PubMed\]](#)

Michalak, L., Gaby, J. C., Lagos, L., La Rosa, S. L., Hvidsten, T. R., Tétard-Jones, C., Willats, W. G. T., Terrapon, N., Lombard, V., Henrissat, B., Dröge, J., Arntzen, M. Ø., Hagen, L. H., Øverland, M., Pope, P. B., Westereng, B. (2020). Microbiota-directed fibre activates both targeted and secondary metabolic shifts in the distal gut. *Nature Communications*, 11(1):5773. <https://doi.org/10.1038/s41467-020-19585-0>. PMID: 33188211; PMCID: PMC7666174.

Mills, R. H., Dulai, P.S., Vázquez-Baeza, Y., Saucedo, C., Danie, I. N., Gerner R. R., Batachari, L. E., Malfavon, M. Zhu, Q., Weldon, K., Humphrey, G., Carrillo-Terrazas, M., Goldasich, L. D., Bryant, M., Raffatellu, M., Quinn, R. A., Gewirtz, A. T., Chassaing, B., Chu, H., Sandborn, W. J., Dorrestein, P. C., Knight, R., & Gonzalez D. J. (2022). Multi-omics analyses of the ulcerative colitis gut microbiome link *Bacteroides vulgatus* proteases with disease severity. *Nature Microbiology*, 7(2), 262–276. <https://doi.org/10.1038/s41564-021-01050-3>. Epub 2022 Jan 27. PMID: 35087228; PMCID: PMC8852248.

Minot, S., Sinha, R., Chen, J., Li, H., Keilbaugh, S. A., Wu, G. D., Lewis, J. D., & Bushman, F. D. (2011). The human gut virome: Inter-individual variation and dynamic response to diet. *Genome Research*, 21, 1616–1625. [\[PubMed\]](#)

Nava, G. M., Carbonero, F., Croix, J. A., Greenberg, E., & Gaskins, H. R. (2012). Abundance and diversity of mucosa-associated hydrogenotrophic microbes in the healthy human colon. *The ISME Journal*, 6, 57–70. [\[PubMed\]](#)

Nayfach, S., Shi, Z. J., Seshadri, R., Pollard, K. S., & Kyrpides, N. C. (2019). New insights from uncultivated genomes of the global human gut microbiome. *Nature*, 568(7753), 505–510. <https://doi.org/10.1038/s41586-019-1058-x>. Epub 2019 Mar 13. PMID: 30867587; PMCID: PMC6784871

Nayfach, S., Roux, S., Seshadri, R. et al. (2021). A genomic catalog of Earth's microbiomes. *Nature Biotechnology*, 39, 499–509. <https://doi.org/10.1038/s41587-020-0718-6>

Oh, P., Benson, A., Peterson, D. et al. (2010). Diversification of the gut symbiont *Lactobacillus reuteri* as a result of host-driven evolution. *ISME J* 4, 377–387 <https://doi.org/10.1038/ismej.2009.123>

Pang, S., Chen, X., Lu, Z., Meng, L., Huang, Y., Yu, X., Huang, L., Ye, P., Chen, X., Liang, J., Peng, T., Luo, W., & Wang, S. (2023). Longevity of centenarians is reflected by the gut microbiome with youth-associated signatures. *Nature Aging*, 3(4), 436–449. <https://doi.org/10.1038/s43587-023-00389-y>. Epub 2023 Apr 6. PMID: 37117794. PMID: 30322585. Epub 2016 Feb 3. PMID: 26843508; PMCID: PMC5532459

Parks, D. H., Chuvochina, M., Rinke, C., Mussig, A. J., Chaumeil, P.-A., & Hugenholtz, P. (2022). GTDB: an ongoing census of bacterial and archaeal diversity through a phylogenetically consistent, rank normalized and complete genome-based taxonomy. *Nucleic Acids Research*, 50(D1), D785–D794, <https://doi.org/10.1093/nar/gkab776>

Pasolli, E., et al. (2019). Extensive unexplored human microbiome diversity revealed by over 150,000 genomes from metagenomes spanning age, geography, and lifestyle. *Cell*, 176(3), 649–662. <https://doi.org/10.1016/j.cell.2019.01.001> [PubMed]

Pope, P. B., Smith, W., Denman, S. E., Tringe, S. G., Barry, K., Hugenholtz, P., McSweeney, C. S., McHardy, A. C., & Morrison, M. (2011). Isolation of Succinivibrionaceae implicated in low methane emissions from Tammar wallabies. *Science*, 333(6042), 646–648. <https://doi.org/10.1126/science.1205760>. Epub 2011 Jun 30. PMID: 21719642

Procházková, P., Falony, G., Dragsted, L. O., Licht, T. R., Raes, J., & Roager, H. M. (2023). Advancing human gut microbiota research by considering gut transit time. *Gut*, 72(1), 180–191. <https://doi.org/10.1136/gutjnl-2022-328166>. PMID: 33722860; PMCID: PMC8349893

Pu, L., Pang, S., Mu, W., Chen, X., Zou, Y., Wang, Y., Ding, Y., Yan, Q., Huang, Y., Chen, X., Peng, T., Luo, W., & Wang, S. (2024). The gut mycobiome signatures in long-lived populations. *iScience*, 27(8), 110412. <https://doi.org/10.1016/j.isci.2024.110412>. PMID: 39081291; PMCID: PMC11284699.

Qin, J., Li, R., Raes, J., Arumugam, M., Burgdorf, K. S., Manichanh, C., Nielsen, T., Pons, N., Levenez, F., Yamada, T., et al. (2010). A human gut microbial gene catalogue established by metagenomic sequencing. *Nature*, 464, 59–65. <https://doi.org/10.1038/nature08821> [PubMed]

Ragsdale, S. W. (2006). Metals and their scaffolds to promote difficult enzymatic reactions. *Chemical Reviews*, 106, 3317–3337. [PubMed]

Ramezani, A., et al. (2018). Gut colonization with methanogenic archaea lowers plasma trimethylamine N-oxide concentrations in apolipoprotein e^{-/-} Mice. *Scientific Reports*, 8, 14752. [PubMed]

Ramsay, A. G., Scott, K. P., Martin, J. C., Rincon, M. T., & Flint, H. J. (2006). Cell-associated alpha-amylases of butyrate-producing Firmicute bacteria from the human colon. *Microbiology*, 152, 3281–3290. [PubMed]

Ran, C., He, S., Yang, Y., Huang, L., Zhou, Z. (2015). A Novel Lipase as Aquafeed Additive for Warm-Water Aquaculture. *PLoS One*, 10(7), e0132049. <https://doi.org/10.1371/journal.pone.0132049>. PMID: 26147311; PMCID: PMC4492967.

Reeves, A. R., Wang, G. R., & Salyers, A. A. (1997). Characterization of four outer membrane proteins that play a role in utilization of starch by *Bacteroides thetaiotaomicron*. *Journal of Bacteriology*, 179, 643–649. [PubMed]

Reichardt, N., Duncan, S. H., Young, P., Belenguer, A., McWilliam Leitch, C., Scott, K. P., Flint, H. J., & Louis, P. (2014). Phylogenetic distribution of three pathways for propionate production within the human gut microbiota. *The ISME Journal*, 8, 1323–1335. [PubMed]

Reitmeier, S., Hitch, T. C. A., Treichel, N., Fikas, N., Hausmann, B., Ramer-Tait, A. E., Neuhaus, K., Berry, D., Haller, D., Lagkouvardos, I., & Clavel, T. (2021). Handling of spurious sequences affects the outcome of high-throughput 16S rRNA gene amplicon profiling. *ISME Communications*, 1(1), 31. <https://doi.org/10.1038/s43705-021-00033-z>. PMID: 37938227; PMCID: PMC9723555

Reyman M, van Houten MA, van Baarle D, Bosch AATM, Man WH, Chu MLJN, Arp K, Watson RL, Sanders EAM, Fuentes S, Bogaert D. (2019). Impact of delivery mode-associated gut microbiota dynamics on health in the first year of life. *Nature Communications* 10(1), 4997. <https://doi.org/10.1038/s41467-019-13014-7>. Erratum in: *Nat Commun*. 2019 Nov 25;10(1):5352. doi: 10.1038/s41467-019-13373-1. PMID: 31676793; PMCID: PMC6825150.

Ridlon, J. M., Kang, D. J., & Hylemon, P. B. (2006). Bile salt biotransformations by human intestinal bacteria. *Journal of Lipid Research*, 47, 241–259.
[PubMed]

Roediger, W. E. (1980). Role of anaerobic bacteria in the metabolic welfare of the colonic mucosa in man. *Gut*, 21, 793–798.
[PubMed]

Rondeau, L. E., Da Luz, B. B., Santiago, A., Bermudez-Brito, M., Hann, A., De Palma, G., Jury, J., Wang, X., Verdu, E. F., Galipeau, H. J., Rolland, C., Deraison, C., Ruf, W., Bercik, P., Vergnolle, N., Caminero, A. (2024). Proteolytic bacteria expansion during colitis amplifies inflammation through cleavage of the external domain of PAR2. *Gut Microbes*, 16(1), 2387857. <https://doi.org/10.1080/19490976.2024.2387857>. Epub 2024 Aug 22. PMID: 39171684; PMCID: PMC11346554.

Saha, J. R., Butler, V. P. Jr., Neu, H. C., & Lindenbaum, J. (1983). Digoxin-inactivating bacteria: identification in human gut flora. *Science*, 220(4594), 325–327. <https://doi.org/10.1126/science.6836275>. PMID: 6836275.

Salyers, A. A., Palmer, J. K., & Wilkins, T. D. (1977a). Laminarinase (beta-glucanase) activity in *Bacteroides* from the human colon. *Applied and Environmental Microbiology*, 33, 1118–1124.
[PubMed]

Salyers, A. A., West, S. E., Vercellotti, J. R., & Wilkins, T. D. (1977b). Fermentation of mucins and plant polysaccharides by anaerobic bacteria from the human colon. *Applied and Environmental Microbiology*, 34, 529–533.
[PubMed]

Sanchez-Gallardo, R., Bottacini, F., O'Neill, I. J., Esteban-Torres, M., Moore, R., McAuliffe, F. M., Cotter, P. D., & van Sinderen, D. (2024). Selective human milk oligosaccharide utilization by members of the *Bifidobacterium pseudocatenulatum* taxon. *Applied and Environmental Microbiology*, 90, e00648-24. <https://doi.org/10.1128/aem.00648-24>

Sandall, J., Tribe, R. M., Avery, L., Mola, G., Visser, G. H., Homer, C. S., Gibbons, D., Kelly, N. M., Kennedy, H. P., Kidanto, H., Taylor, P., & Temmerman, M. (2018). Short-term and long-term effects of caesarean section on the health of women and children. *Lancet*, 13, 392(10155), 1349–1357. [https://doi.org/10.1016/S0140-6736\(18\)31930-5](https://doi.org/10.1016/S0140-6736(18)31930-5). PMID: 30322585. Epub 2016 Feb 3. PMID: 26843508; PMCID: PMC5532459

Savage, D. C. (1977). Microbial ecology of the gastrointestinal tract. *Annual Review of Microbiology*, 31, 107–133.

[[PubMed](#)]

Schauer, K., Rodionov, D. A., & de Reuse, H. (2008). New substrates for TonB-dependent transport: Do we only see the ‘tip of the iceberg’? *Trends in Biochemical Sciences*, 33, 330–338.

[[PubMed](#)]

Schnorr, S. L., Candela, M., Rampelli, S., Centanni, M., Consolandi, C., Basaglia, G., Turrone, S., Biagi, E., Peano, C., Severgnini, M., Fiori, J., Gotti, R., De Bellis, G., Luiselli, D., Brigidi, P., Mabulla, A., Marlowe, F., Henry, A. G., Crittenden, A. N. (2014). Gut microbiome of the Hadza hunter-gatherers. *Nature Communications*, 5(1), 3654 <https://doi.org/10.1038/ncomms4654>. PMID: 24736369; PMCID: PMC3996546.

Schalich, K. M., Buendia, M. A., Kaur, H., Choksi, Y. A., Washington, M. K., Codreanu, G. S., Sherrod, S. D., McLean, J. A., Peek, Jr. R. M., Acra, S. A., Townsend, S. D., & Yan, F. (2024). A human milk oligosaccharide prevents intestinal inflammation in adulthood via modulating gut microbial metabolism. *mBio* 15, e00298–24

Scott, K. P., Martin, J. C., Chassard, C., Clerget, M., Potrykus, J., Campbell, G., Mayer, C. D., Young, P., Rucklidge, G., Ramsay, A. G., & Flint, H. J. (2011). Substrate-driven gene expression in *Roseburia inulinivorans*: Importance of inducible enzymes in the utilization of inulin and starch. *Proceedings of the National Academy of Sciences of the United States of America*, 108(Suppl 1), 4672–4679.

[[PubMed](#)]

Sender, R., Fuchs, S., & Milo, R. (2016a). Are we really vastly outnumbered? Revisiting the ratio of bacterial to host cells in humans. *Cell*, 164, 337–340.

[[PubMed](#)]

Sender, R., Fuchs, S., & Milo, R. (2016b). Revised estimates for the number of human and bacteria cells in the body. *PLoS Biology*, 14, e1002533.

[[PubMed](#)]

Shalon, D., Culver, R. N., Grembi, J. A., Folz, J., Treit, P. V., Shi, H., Rosenberger, F. A., Dethlefsen, L., Meng, X., Yaffe, E., Aranda-Díaz, A., Geyer, P. E., Mueller-Reif, J. B., Spencer, S., Patterson, A. D., Triadafilopoulos, G., Holmes, S. P., Mann, M., Fiehn, O., Relman, D. A., & Huang, K. C. (2023). Profiling the human intestinal environment under physiological conditions. *Nature*, 617(7961), 581–591. <https://doi.org/10.1038/s41586-023-05989-7>. PMID: 37165188; PMCID: PMC10191855.

Shipman, J. A., Cho, K. H., Siegel, H. A., & Salyers, A. A. (1999). Physiological characterization of SusG, an outer membrane protein essential for starch utilization by *Bacteroides thetaiotaomicron*. *Journal of Bacteriology*, 181, 7206–7211.

[[PubMed](#)]

Shipman, J. A., Berleman, J. E., & Salyers, A. A. (2000). Characterization of four outer membrane proteins involved in binding starch to the cell surface of *Bacteroides thetaiotaomicron*. *Journal of Bacteriology*, 182, 5365–5372.

[[PubMed](#)]

Sokol, H., Leducq, V., Aschard, H., Pham, H. P., Jegou, S., Landman, C., Cohen, D., Liguori, G., Bourrier, A., Nion-Larmurier, I., Cosnes, J., Seksik, P., Langella, P., Skurnik, D., Richard, M. L., & Beaugerie, L. (2017). Fungal microbiota dysbiosis in IBD. *Gut*, 66(6), 1039–1048. <https://doi.org/10.1136/gutjnl-2015-310746>. Epub 2016 Feb 3. PMID: 26843508; PMCID: PMC5532459

Sonnenburg, J. L., Xu, J., Leip, D. D., Chen, C. H., Westover, B. P., Weatherford, J., Buhler, J. D., & Gordon, J. I. (2005). Glycan foraging in vivo by an intestine-adapted bacterial symbiont. *Science*, 307, 1955–1959. [\[PubMed\]](#)

Sonnenburg, E. D., Zheng, H., Joglekar, P., Higginbottom, S. K., Firbank, S. J., Bolam, D. N., & Sonnenburg, J. L. (2010). Specificity of polysaccharide use in intestinal bacteroides species determines diet-induced microbiota alterations. *Cell*, 141, 1241–1252. [\[PubMed\]](#)

Stewart, C. J., et al. (2018). Temporal development of the gut microbiome in early childhood from the TEDDY study. *Nature*, 562(7728), 583–588. <https://doi.org/10.1038/s41586-018-0617-x> [\[PubMed\]](#)

Suau, A., Bonnet, R., Sutren, M., Godon, J. J., Gibson, G. R., Collins, M. D., & Doré, J. (1999). Direct analysis of genes encoding 16S rRNA from complex communities reveals many novel molecular species within the human gut. *Applied and Environmental Microbiology*, 65, 4799–4807. [\[PubMed\]](#)

Suzuki, T. A., Fitzstevens, J. L., Schmidt, V. T., Enav, H., Huus, K. E., Mbong Ngwese, M., Grieshammer, A., Pfeleiderer, A., Adegbite, B. R., Zinsou, J. F., Esen, M., Velavan, T. P., Adegnika, A. A., Song, L. H., Spector, T. D., Muehlbauer, A. L., Marchi, N., Kang, H., Maier, L., Blekhman, R., Séguirel, L., Ko, G., Youngblut, N. D., Kremsner, P., & Ley, R. E. (2022). Codiversification of gut microbiota with humans. *Science*, 377(6612), 1328–1332. <https://doi.org/10.1126/science.abm7759>. Epub 2022 Sep 15. PMID: 36108023; PMCID: PMC10777373.

Sugihara, P. T., Sutter, V. L., Attebery, H. R., Bricknell, K. S., & Finegold, S. M. (1974). Isolation of *Acidaminococcus fermentans* and *Megasphaera elsdenii* from normal human feces. *Applied Microbiology*, 27, 274–275. [\[PubMed\]](#)

Tanaka, H., Hashiba, H., Kok, J., & Mierau, I. (2000). Bile salt hydrolase of *Bifidobacterium longum*-biochemical and genetic characterization. *Applied and Environmental Microbiology*, 66, 2502–2512. [\[PubMed\]](#)

Tang, C., Ahmed, K., Gille, A., Lu, S., Grone, H. J., Tunaru, S., & Offermanns, S. (2015). Loss of FFA2 and FFA3 increases insulin secretion and improves glucose tolerance in type 2 diabetes. *Nature Medicine*, 21, 173–177. [\[PubMed\]](#)

Tap, J., et al. (2009). Towards the human intestinal microbiota phylogenetic core. *Environmental Microbiology*, 11(10), 2574–2584. <https://doi.org/10.1111/j.1462-2920.2009.01982.x> [\[PubMed\]](#)

Tasse, L., Bercovici, J., Pizzut-Serin, S., Robe, P., Tap, J., Klopp, C., Cantarel, B. L., Coutinho, P. M., Henrissat, B., Leclerc, M., et al. (2010). Functional metagenomics to mine the human gut microbiome for dietary fiber catabolic enzymes. *Genome Research*, 20, 1605–1612.

[\[PubMed\]](#)

Tazoe, H., Otomo, Y., Kaji, I., Tanaka, R., Karaki, S. I., & Kuwahara, A. (2008). Roles of short-chain fatty acids receptors, GPR41 and GPR43 on colonic functions. *Journal of Physiology and Pharmacology*, 59(Suppl 2), 251–262.

[\[PubMed\]](#)

Tett, A., et al. (2019). The *Prevotella copri* complex comprises four distinct clades that are common in non-Westernized populations. *Cell Host & Microbe*, 26(5), 666–679. <https://doi.org/10.1016/j.chom.2019.08.018>

Thomas, A.M., & Segata, N. (2019). Multiple levels of the unknown in microbiome research. *BMC Biology*, 17, 48. <https://doi.org/10.1186/s12915-019-0667-z>

Tolhurst, G., Heffron, H., Lam, Y. S., Parker, H. E., Habib, A. M., Diakogiannaki, E., Cameron, J., Grosse, J., Reimann, F., & Gribble, F. M. (2012). Short-chain fatty acids stimulate glucagon-like peptide-1 secretion via the G-protein-coupled receptor FFAR2. *Diabetes*, 61, 364–371.

[\[PubMed\]](#)

Topping, D. L., & Clifton, P. M. (2001). Short-chain fatty acids and human colonic function: Roles of resistant starch and nonstarch polysaccharides. *Physiological Reviews*, 81, 1031–1064.

[\[PubMed\]](#)

Treichel, N. S., Pauvert, C., Séneca, J., Pjevac, P., Berry, D., Penders, J., Hitch, T. C. A., Clavel, T. (2026). Benchmarking of shotgun sequencing depth reveals the potential and limitations of shallow metagenomics and strain-level analysis. *Nature Microbiol*, 11(5), 1233–1244. <https://doi.org/10.1038/s41564-026-02334-2>. Epub 2026 Apr 21. PMID: 42014453; PMCID: PMC13171431.

Turck, D., et al. (2021). Safety of pasteurised *Akkermansia muciniphila* as a novel food pursuant to Regulation (EU) 2015/2283. *EFSA Journal*, 19, 6780.

Tyson, G. W., Chapman, J., Hugenholtz, P., Allen, E. E., Ram, R. J., Richardson, P. M., Solovyev, V. V., Rubin, E. M., Rokhsar, D. S., & Banfield, J. F. (2004). Community structure and metabolism through reconstruction of microbial genomes from the environment. *Nature*, 428(6978), 37–43. <https://doi.org/10.1038/nature02340>

Vital, M., Howe, A. C., & Tiedje, J. M. (2014). Revealing the bacterial butyrate synthesis pathways by analyzing (meta)genomic data. *mBio*, 5(2). <https://doi.org/10.1128/mBio.00889-14>

Winter, S. E., Winter, M. G., Xavier, M. N., Thiennimitr, P., Poon, V., Keestra, A. M., Laughlin, R. C., Gomez, G., Wu, J., Lawhon, S. D., et al. (2013). Host-derived nitrate boosts growth of *E. coli* in the inflamed gut. *Science*, 339, 708–711.

[\[PubMed\]](#)

Wolin, M. J., & Miller, T. L. (1983). Carbohydrate fermentation. In D. J. Hentges (Ed.), *Human intestinal microflora in health and disease* (p. 19). Academic Press.

Worsoe, J., Fynne, L., Gregersen, T., Schlageter, V., Christensen, L. A., Dahlerup, J. F., Rijkhoff, N. J., Laurberg, S., & Krogh, K. (2011). Gastric transit and small intestinal transit time and motility assessed by a magnet tracking system. *BMC Gastroenterology*, 11, 145.

[[PubMed](#)]

Wren, A. M., & Bloom, S. R. (2007). Gut hormones and appetite control. *Gastroenterology*, 132, 2116–2130.

[[PubMed](#)]

Wu, G. D., Chen, J., Hoffmann, C., Bittinger, K., Chen, Y. Y., Keilbaugh, S. A., Bewtra, M., Knights, D., Walters, W. A., Knight, R., et al. (2011). Linking long-term dietary patterns with gut microbial enterotypes. *Science*, 334, 105–108.

[[PubMed](#)]

Wu, H., et al. (2017). Metformin alters the gut microbiome of individuals with treatment-naïve type 2 diabetes, contributing to the therapeutic effects of the drug. *Nature Medicine*, 23(7), 850–858. <https://doi.org/10.1038/nm.4345>

[[PubMed](#)]

Wylensek, D., Hitch, T. C. A., Riedel, T., Afrizal, A., Kumar, N., Wortmann, E., Liu, T., Devendran, S., Lesker, T. R., Hernández, S. B., Heine, V., Buhl, E. M., M D'Agostino, P., Cumbo, F., Fischöder, T., Wyschkon, M., Looft, T., Parreira, V. R., Abt, B., Doden, H. L., Ly, L., Alves, J. M. P., Reichlin, M., Flisikowski, K., Suarez, L. N., Neumann, A. P., Suen, G., de Wouters, T., Rohn, S., Lagkouvardos, I., Allen-Vercoe, E., Spröer, C., Bunk, B., Taverne-Thiele, A. J., Giesbers, M., Wells, J. M., Neuhaus, K., Schnieke, A., Cava, F., Segata, N., Elling, L., Strowig, T., Ridlon, J. M., Gulder, T. A. M., Overmann, J., & Clavel, T. (2020). A collection of bacterial isolates from the pig intestine reveals functional and taxonomic diversity. *Nature Communications*, 11(1), 6389. <https://doi.org/10.1038/s41467-020-19929-w>. PMID: 33319778; PMCID: PMC7738495

Xiong, Y., Miyamoto, N., Shibata, K., Valasek, M. A., Motoike, T., Kedzierski, R. M., & Yanagisawa, M. (2004). Short-chain fatty acids stimulate leptin production in adipocytes through the G protein-coupled receptor GPR41. *Proceedings of the National Academy of Sciences of the United States of America*, 101, 1045–1050.

[[PubMed](#)]

Yatsunencko, T., Rey, F. E., Manary, M. J., Trehan, I., Dominguez-Bello, M. G., Contreras, M., Magris, M., Hidalgo, G., Baldassano, R. N., Anokhin, A. P., Heath, A. C., Warner, B., Reeder, J., Kuczynski, J., Caporaso, J. G., Lozupone, C. A., Lauber, C., Clemente, J. C., Knights, D., Knight, R., & Gordon, J. I. (2012). Human gut microbiome viewed across age and geography. *Nature*, 486(7402), 222–227. <https://doi.org/10.1038/nature11053>

Yan, Q., Li, S., Yan, Q., Huo, X., Wang, C., Wang, X., Sun, Y., Zhao, W., Yu, Z., Zhang, Y., Guo, R., Lv, Q., He, X., Yao, C., Li, Z., Chen, F., Ji, Q., Zhang, A., Jin, H., Wang, G., Feng, X., Feng, L., Wu, F., Ning, J., Deng, S., An, Y., Guo, D. A., Martin, F. M., & Ma, X. (2024). A genomic compendium of cultivated human gut fungi characterizes the gut mycobiome and its relevance to common diseases. *Cell*, 187(12), 2969–2989.e24. <https://doi.org/10.1016/j.cell.2024.04.043>. Epub 2024 May 21. PMID: 38776919.

Yao, C. K., Muir, J. G., & Gibson, P. R. (2016). Review article: Insights into colonic protein

fermentation, its modulation and potential health implications. *Alimentary Pharmacology & Therapeutics*, 43, 181–196.

Yaffe, E., & Relman, D. A. (2020). Tracking microbial evolution in the human gut using Hi-C reveals extensive horizontal gene transfer, persistence and adaptation. *Nature Microbiol*, 5(2), 343–353. <https://doi.org/10.1038/s41564-019-0625-0>. Epub 2019 Dec 23. PMID: 31873203; PMCID: PMC6992475.

Zachariasen, T., Russel, J., Petersen, C. et al. (2024). MAGinator enables accurate profiling of de novo MAGs with strain-level phylogenies. *Nature Communications*, 15, 5734. <https://doi.org/10.1038/s41467-024-49958-8>

Ze, X., Duncan, S. H., Louis, P., & Flint, H. J. (2012). *Ruminococcus bromii* is a keystone species for the degradation of resistant starch in the human colon. *The ISME Journal*, 6, 1535–1543. [\[PubMed\]](#)

Zepeda-Rivera, M., Minot, S. S., Bouzek, H., Wu, H., Blanco-Míguez, A., Manghi, P., Jones, D. S., LaCourse, K. D., Wu, Y., McMahon, E. F., Park, S. N., Lim, Y. K., Kempchinsky, A. G., Willis, A. D., Cotton, S. L., Yost, S. C., Sicinska, E., Kook, J. K., Dewhirst, F.E., Segata, N., Bullman, S., & Johnston, C. D. (2024). A distinct *Fusobacterium nucleatum* clade dominates the colorectal cancer niche. *Nature*, 628(8007):424–432. <https://doi.org/10.1038/s41586-024-07182-w>. Epub 2024 Mar 20. PMID: 38509359; PMCID: PMC11006615

Zhang, X., et al. (2016). Methane limit LPS-induced NF- κ B/MAPKs signal in macrophages and suppress immune response in mice by enhancing PI3K/AKT/GSK-3 β -mediated IL-10 expression. *Scientific Reports*, 6, 29359. [\[PubMed\]](#)

3. Microbiome and Early Life

Niels van Best^{1, 2} and Mathias W. Hornef¹ 

- (1) Institute of Medical Microbiology, RWTH Aachen University, Aachen, Germany
- (2) Institute of Nutrition and Translational Research in Metabolism (NUTRIM), Department of Medical Microbiology, Infectious Diseases and Infection Prevention, Maastricht University Medical Centre, Maastricht, The Netherlands

 **Mathias W. Hornef**
Email: mhornef@ukaachen.de

Abstract

Under physiological conditions, the fetus is protected from exposure to viable microorganisms. With rupture of membranes and passage through the birth canal, the neonate becomes exposed to bacteria that colonize maternal internal and external body surfaces and the environment. These bacteria start to establish the enteric microbiota, initially characterized by low bacterial diversity and high interindividual variation. This makes the neonatal and early infant microbiota particularly vulnerable to exogenous interference. On the other hand, the low colonization resistance allows the interventional modification of the early microbiota by oral administration of beneficial bacteria (probiotics). With time, additional bacterial species colonize the intestine and increase the diversity of the microbiota composition. The composition is shaped by dietary, environmental, and genetic factors, ultimately leading to a mature and highly diverse enteric microbiota that remains relatively stable throughout life. In this

chapter, we will discuss the establishment of the enteric microbiota after birth and the current understanding of its influence on immune maturation and disease susceptibility. We will also address interventional strategies that, particularly during early life, might be able to improve long-term health.

Keywords Birth – Neonate – Cesarean section – Bifidobacteria – Immune maturation – Antibiotics – Breast-feeding

3.1 Establishment of the Enteric Microbiota After Birth

During pregnancy, the fetal body is enclosed by the amniotic membranes that physically separate it from the uterus cavity and preserve a sterile environment (see text box “[controversy](#)”). Although the fetal body is protected from direct contact with viable microorganisms, microbial metabolites and microbial stimulatory molecules derived from the maternal gut microbiota, as well as host cytokines, reach the fetal tissue across the fetal-maternal interface of the placenta. This means that changes of the maternal microbiota or inflammatory processes in the maternal body may drive immune maturation and prime immune cells of the developing fetus. For example, transient colonization of germ-free pregnant dams with an auxotroph *E. coli* bacterium increased the number of mucosal type 3 innate lymphoid cells and F4/80⁺CD11c⁺ mononuclear cells (Gomez de Agüero et al., [2016](#)). Also, acetate supplementation of germ-free dams enhanced the thymic output of CD4 T lymphocytes in the offspring (Hu et al., [2019](#)). Finally, interleukin (IL)-6 induced during a transient enteric infection of the dam during pregnancy was shown to reach fetal intestinal epithelial stem cells and enhance the postnatal resistance of the offspring to enteric infection (Hu et al., [2019](#)).

The first exposure to bacteria starts with rupture of membranes that in most cases happens few hours or even less prior to birth. It releases the amniotic fluid that surrounds the fetus and facilitates

access of bacteria that ascend from the vaginal mucosa into the uterine cavity. Although this process is well known, the number and kind of bacteria that reach the fetus at this early time point have not been specifically studied. With increasing uterine contraction and opening of the cervix, the fetal body enters the birth canal. The majority of neonates pass the birth canal with the occiput ahead (so-called dorsoanterior occiput presentation), which means that the nasal and oral openings move along the densely colonized posterior mucosal surfaces of the birth canal and pass in close proximity to the maternal anus. This transient but intense physical contact may facilitate efficient transfer of bacteria from maternal mucosal surfaces to the newborn.

The very early microbiota composition is similar at different body sites and segregates only thereafter, most likely due to site-specific selective determinants (Chu et al., [2017](#)). An early report described that the enteric microbiota of vaginally delivered neonates resembles the maternal vaginal microbiota (Dominguez-Bello et al., [2010](#)). Later, metagenomic analyses allowing the detection of shared strains between mother and infant identified the mother's fecal rather than vaginal microbiota as the main source of the emerging infant microbiota (Mitchell et al., [2020](#)). This is consistent with the stronger effect of the transfer of fecal material (FMT) as compared to vaginal seeding to reconstitute the baby's gut microbiota after caesarean section (Dominguez-Bello et al., [2016](#); Dubois et al., [2024](#); Mueller et al., [2023](#)).

Soon after delivery, the neonate starts suckling at the mother's breasts, which intensifies the contact with bacteria colonizing the maternal skin, another important source of bacteria. Regular breast-feeding maintains the transfer and ingestion of resident bacteria. Clearly, breast milk is not sterile but contains significant numbers of bacteria (Martin et al., [2003](#); Toscano et al., [2017](#)). The existence of an entero-mammary pathway, i.e., the direct transfer of viable bacteria from the mother's intestine to the breast gland, that would reinforce the transfer of gut bacteria to the neonate has been proposed (Rodriguez, [2014](#)). However, compelling evidence or a mechanistic description of such a transfer of viable bacteria through the systemic

circulation has not yet been provided. Alternatively, ascending colonization by bacteria from the maternal skin and infant oral cavity through the opening of the milk ducts that drain the breast milk to the skin surface could explain the presence of bacteria in breast milk samples. Consistently, bacteria found in breast milk, such as *Streptococci* and *Staphylococci*, represent known constituents of the skin and oral microbiota. Breastfeeding undoubtedly exerts a major influence on the establishment of the enteric microbiota, but this may not primarily be due to its role as a source for new bacterial taxa, but rather its important prebiotic influence. Breast milk constituents exert a major influence on bacterial growth within the intestinal lumen. For example, and as further detailed below, human milk oligosaccharides (HMOs) selectively foster the growth of bifidobacteria, prominent members of the enteric microbiota of breast-fed neonates (Heppner et al., [2024](#); Kotloff et al., [2013](#); Lawson et al., [2020](#); Penders et al., [2006](#)).

Beside the maternal fecal, vaginal and skin microbiota, bacteria from the maternal oral cavity are also transmitted to the neonatal host (Bogaert et al., [2023](#)). With this, the mother clearly represents the most important source of bacteria early after birth, with more than 60% of the microbiota composition attributed to this source (Bogaert et al., [2023](#)). With time, the strain sharing rate decreases and reaches less than 50% at 1 year (Selma-Royo et al., [2024](#)). Paternal bacterial strains also account for a significant number of the infant's microbiota, although with a somewhat delayed kinetic (Dubois et al., [2024](#)).

Later, bacteria from other sources, such as siblings, pets, and livestock or the direct environment, may also become part of the developing microbiota (Azad et al., [2013](#); Hill et al., [2017](#); van Best et al., [2015](#)). At this early period during the development of the microbiota, the bacterial diversity is still relatively low, and thus newly incoming bacteria do not have to compete for nutrients and space. Thus, in the neonate, exposure to most types of bacteria may result in successful colonization. The term used to describe this situation is low "colonization resistance" (Brugiroux et al., [2016](#); Olsan et al., [2017](#); Woelfel et al., [2024](#)). We will see later that this characteristic of a

microbiota differs dramatically between neonates and adults. On the one hand, low colonization resistance allows rapid establishment of an increasingly complex microbiota that fulfills the metabolic and immunological requirements of the developing individual. On the other hand, it also renders the neonate particularly susceptible to infection.

Thus, the early neonatal enteric microbiota is of low diversity and characterized by low colonization resistance. In addition, it is highly individual. Microbiota analysis of 14 neonates in a study revealed major differences between all individuals but two—a pair of twin babies (Palmer et al., [2007](#)). These results again stress the importance of the mother as a source of bacteria for the initial microbiota, but also indicate that the early process of colonization mostly depends on exogenous influences (Hill et al., [2017](#)). Bacteria that first colonize the neonate's intestine have also been named pioneer bacteria and are thought to play a particularly important role (Karlsson et al., [2011](#)). Indeed, the succession of colonization by individual pioneer species may critically influence the final microbiota composition (Feng et al., [2020](#); Martinez et al., [2018](#)). Another aspect of the microbiota besides its composition is its density, i.e., the number of bacteria at a given anatomical site. The ability of many bacteria to multiply rapidly is well known. Fast-proliferating bacteria can divide every 20 min under nutrient-rich conditions, theoretically resulting in more than 1×10^9 bacteria from just one bacterium within 12 h. The high lactose and fat concentration in milk promote the growth of many bacteria. It might therefore not be surprising that although the bacterial diversity of the enteric microbiota requires years to develop (see below), the density reaches its threshold values already within few days (Palmer et al., [2007](#); Torow et al., [2023b](#)).

Over the time following birth, additional bacterial species become members of the infant enteric microbiota. This process is supported by changes in the environmental conditions within the intestinal lumen. For example, oxygen is present in the postnatal intestine, limiting early colonization to oxygen-resistant, so-called facultatively anaerobic bacteria. Their presence and metabolism soon reduce the oxygen

concentration, allowing subsequent colonization by strictly anaerobic bacteria such as bifidobacteria, *Clostridium* spp., and other Bacillota (formerly Firmicutes) (Reinhardt et al., [2009](#); van Best et al., [2015](#)). Also, changes in the diet, such as the cessation of breastfeeding and the introduction of solid food, offer new nutritional niches. This leads to a shift toward Bacteroidota (formerly Bacteroidetes) and Bacillota (formerly Firmicutes) (Backhed et al., [2015](#); Roswall et al., [2021](#)). The succession of bacteria that colonize and expand as part of the enteric microbiota thus follows a certain order of distinct phases and developmental trajectory, although the speed in individual infants may vary and most regulatory mechanisms and circuits are still unknown (Roswall et al., [2021](#); Stewart et al., [2018](#)). Notably, the succession of colonization by individual bacteria might impact the final microbial composition due to the priority effect, illustrating the need for an undisturbed establishment of the enteric microbiota (Feng et al., [2020](#); Martinez et al., [2018](#); Sprockett et al., [2018](#)). This step-by-step process increases the diversity of the enteric microbiota reaching values similar to adult individuals approximately at the age of 2–3 years (Roswall et al., [2021](#); Yatsunenko et al., [2012](#)). At the same time, the individual variation decreases generating something like a typical “mature” adult composition of the enteric microbiota (Yatsunenko et al., [2012](#)). The underlying mechanisms are not entirely clear, but probably this development results from the overall more similar metabolic and environmental conditions in the adult intestine (Figs. [3.1](#) and [3.2](#)).

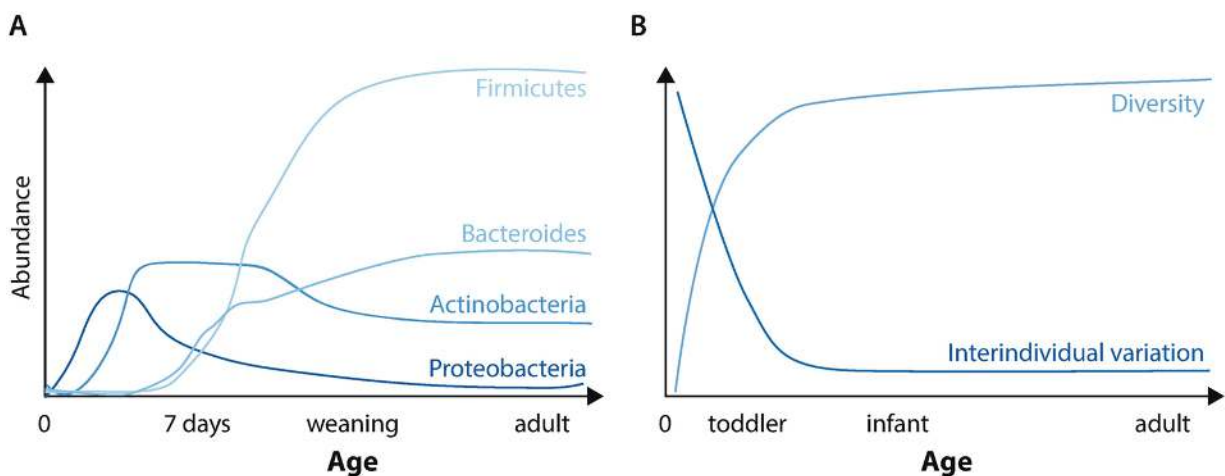


Fig. 3.1 Establishment of the enteric microbiota. **(a)** Bacterial composition. The dominant bacterial species within the microbiota change over time depending on, for example, the susceptibility of certain obligate anaerobic bacteria to luminal oxygen or the dietary substrates before or after weaning (i.e., breast milk versus solid food). **(b)** Overall microbial diversity. Shortly after birth, the neonate's microbiome exhibits a high individuality (i.e., all babies are different) but low diversity (i.e., the microbiome consists of few bacterial species). This changes rapidly with age, and approximately at 2–3 years of age in humans, a mature, highly diverse microbiome is established. Modified after **(a)** (Reinhardt et al., [2009](#)), **(b)** (Yatsunenکو et al., [2012](#))

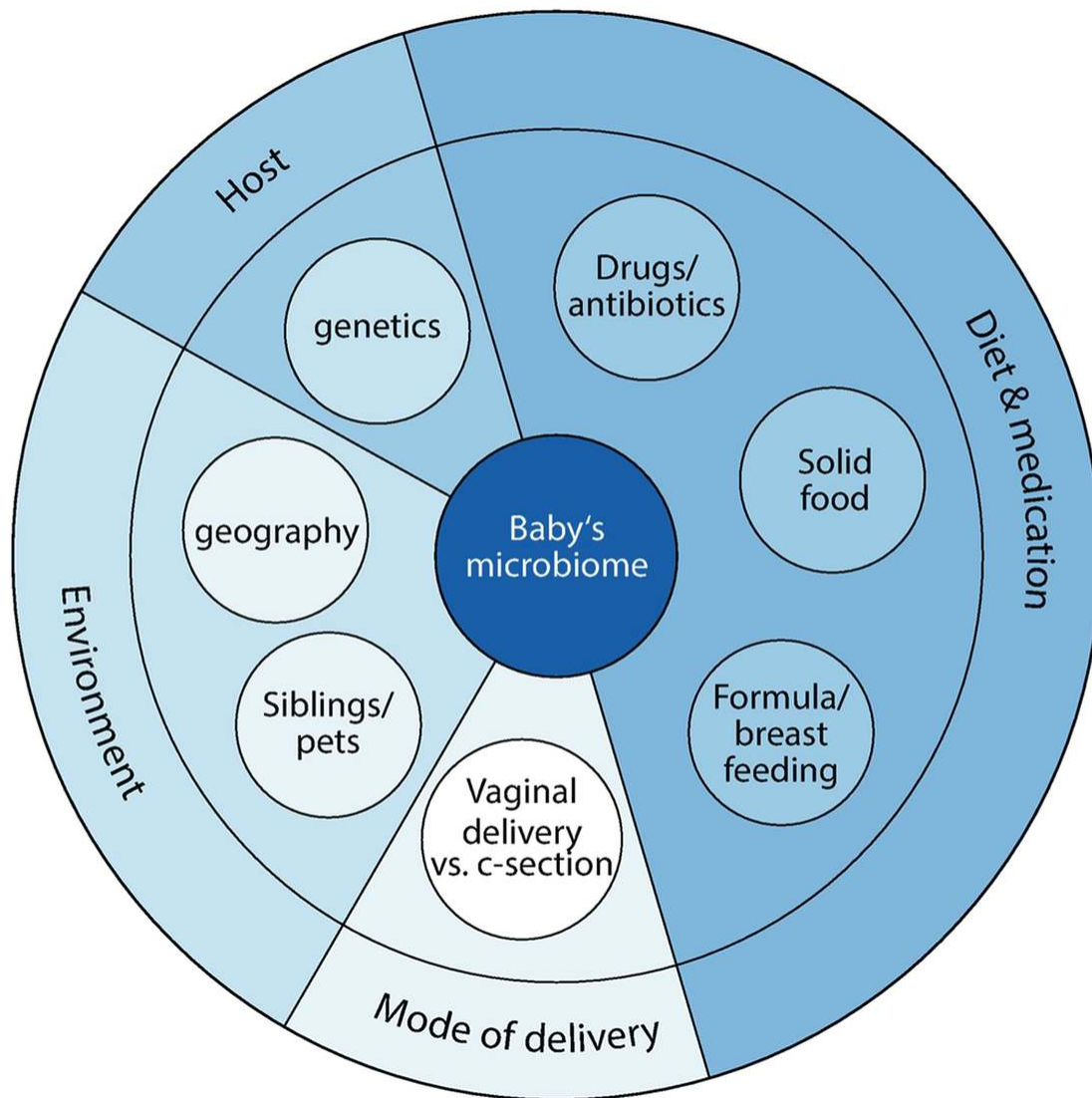


Fig. 3.2 Factors influencing the neonate's enteric microbiota. The mode of delivery (vaginal delivery versus caesarean section), diet (breast milk versus formula), medication (antibiotics), environmental exposure (siblings, pets, geography), and host genetics impact on the neonate's enteric microbiota

3.2 Factors Influencing the Infant Microbiota

A number of factors influence the establishment and maturation of the enteric microbiota after birth. Most of them alter the timing or quality of important exogenous determinants. While determinants present throughout human evolution, such as environmental exposure and low personal and food hygiene, might appear beneficial with respect to their effect on the establishment of a diverse and competitive enteric microbiota, we must be aware that they are associated with a significant risk for infection with pathogenic microorganisms (Table [3.1](#)).

Table 3.1 Factors identified to influence the composition of the enteric microbiota and their impact on the abundance of specific commensal bacteria within the infant's enteric microbiome

Factor	Observed change	References
Geography	Differences in the microbiota composition have been noted, but the overall postnatal development is similar	Fallani et al. (2010), Vatanen et al. (2016), and Yatsunenکو et al. (2012)
Mode of delivery	Neonates born by vaginal delivery are exposed to the maternal fecal and vaginal microbiota whereas cesarean section-born neonates have an initial exposure to maternal skin and environmental bacteria. Differences in the microbiota composition are detected until approximately 1 year	Backhed et al. (2015), Dogra et al. (2015), Dominguez-Bello et al. (2010), Hesla et al. (2014), Hill et al. (2017), Jakobsson et al. (2014), Mitchell et al. (2020), Penders et al. (2013), Roswall et al. (2021), Shao et al. (2019), and Stewart et al. (2018)
Preterm birth	Enhanced abundance of <i>Staphylococcus</i> spp. and members of the <i>Enterobacteriaceae</i> , lower colonization by bifidobacteria	Arbolea et al. (2015), Hill et al. (2017), Roze et al. (2017), and Toubon et al. (2025)
Breast or formula feeding, solid food	Breast milk enhances the abundance of bifidobacteria and lactobacilli. Formula feeding increases microbial diversity, lowers the abundance of bifidobacteria, and increases taxa of the Bacteroidota (formerly Bacteroidetes). The introduction of solid food and plant oligosaccharides promotes expansion of the phyla Bacteroidota and Bacillota (formerly Firmicutes)	Backhed et al. (2015), Fallani et al. (2010), Kashtanova et al. (2016), Koenig et al. (2011), Penders et al. (2013), and Stewart et al. (2018)

Factor	Observed change	References
Early-life antibiotic treatment	Reduced diversity, loss of certain species, increased abundance of bacteria with intrinsic antibiotic resistance like <i>Enterococcus</i> spp.	Arboleya et al. (2015), Ozkul et al. (2020), Penders et al. (2006), Ruiz et al. (2017), Russell et al. (2012), Rutten et al. (2015), Stefka et al. (2014), and Thiemann et al. (2016)
Pets and siblings	Increased diversity by exposure to pets and siblings	Azad et al. (2013) and Stewart et al. (2018)
Genetics	Individual loci, such as the ABO locus, influence the microbiota composition	Goodrich et al. (2014) and Zhernakova et al. (2024)

As described above, the newborn under physiological conditions first encounters bacteria from the maternal rectal and vaginal mucosa during passage through the birth canal. As expected, this exposure markedly changes when the neonate is born by cesarean section (Dominguez-Bello et al., [2010](#); Mitchell et al., [2020](#); Shao et al., [2019](#); Stewart et al., [2018](#)). Cesarean section represents a surgical intervention to rescue the child's and mother's life in the event of acute bleeding, infection, impaired fetal blood circulation, or anatomical obstruction of the birth canal. It requires anesthesia of the mother and antibiotic prophylaxis to prevent infection at the site of skin incision. Several studies have shown that the early enteric microbiota of neonates delivered by cesarean section differs significantly during the first months after birth (Backhed et al., [2015](#); Dogra et al., [2015](#); Hesla et al., [2014](#); Jakobsson et al., [2014](#); Mitchell et al., [2020](#); Penders et al., [2013](#); Roswall et al., [2021](#); Shao et al., [2019](#); Stewart et al., [2018](#)). It exhibits a reduced abundance of *Bifidobacterium* spp. and *Bacteroides* spp., a reduced diversity of members of the Bacteroidota phylum and enhanced numbers of *Enterococcus* spp.; *Streptococcus* spp.; *Staphylococcus* spp.; and members of the *Enterobacteriaceae* (Shao et al., [2019](#)). Not surprisingly, the early microbiota after cesarean section resembles the maternal skin microbiota, the site of the first contact with a colonized body surface, with enhanced numbers of *Staphylococcus* spp., *Corynebacterium* spp., and *Cutibacterium* (formerly *Propionibacterium*) spp. (Dominguez-Bello et al., [2010](#)). At

approximately 1 year after birth, the enteric microbiota of caesarean section-born and vaginally delivered babies becomes largely indistinguishable (Shao et al., [2019](#); Stewart et al., [2018](#)). The observed differences might not only result from the alteration in the early exposure to bacteria. Cesarean section is also associated with an altered perinatal cytokine milieu (Liao et al., [2017](#); Lotz et al., [2006](#); Ulas et al., [2017](#)), more medical interventions, the hospital environment, reduced early mother–child contact, delayed start of breastfeeding, and pre-incision antibiotic prophylaxis (Chalmers et al., [2009](#)). Two randomized cohort studies compared the effect of antibiotics given during caesarean section before incision versus after cord clamping and found no difference in the offspring's microbiota composition (Dierikx et al., [2022](#); Kamal et al., [2019](#)). Another study compared the enteric microbiota of vaginally delivered neonates without antibiotic administration with vaginally delivery neonates that received intrapartum antibiotic prophylaxis (IAP) to prevent group B streptococcus (GBS) infection and caesarean section-born neonates with antibiotic prophylaxis prior to skin incision. This study found that the microbiota after caesarean section differed from that of unexposed vaginally delivered neonates, but not from vaginally delivered neonates that had received IAP (Stearns et al., [2017](#)). Although antibiotics clearly play a role, it is unclear whether they act more on the mother's or the neonate's microbiota. Interestingly, cesarean section was shown to increase the risk of a number of non-communicable diseases such as asthma (Huang et al., [2015](#)), allergic disease (Magnus et al., [2011](#)), obesity (Kuhle et al., [2015](#)), diabetes (Cardwell et al., [2008](#)), and coeliac disease (Decker et al., [2010](#)). The enhanced disease susceptibility might result from an altered microbiota or other independent cesarean section-associated factors. Notably, despite the recommendation of the WHO to perform cesarean delivery for no more than 15% of deliveries (Karlstrom et al., [2013](#); WHO, [2015](#)), the rate in most industrialized countries rises continuously and now reaches 30%.

Based on their findings from the analysis of vaginally delivered versus caesarean section-born neonates, the group of Maria

Dominguez-Bello proposed a procedure named “vaginal seeding” to compensate for the lack of contact with bacteria of the maternal vaginal mucosa in neonates born by cesarean section (Dominguez-Bello et al., [2016](#)). Exposure of caesarean section-born neonates to vaginal fluid partially restored the microbiota maturation (Dominguez-Bello et al., [2016](#); Zhou et al., [2023](#)). Other studies indicated that caesarean section-born infants display delayed *Bacteroides* spp. colonization owing to the lack of maternal fecal microbial transfer (Korpela et al., [2020](#)). Only ~12% of the early gut bacteria of cesarean section-born neonates originated from the mother, compared to ~74% in vaginally delivered infants (Shao et al., [2019](#); Zhang, Li, et al., [2021a](#)). Oral supplementation with maternal fecal microbiota early after birth significantly restored key taxa like *Bacteroides* spp. in cesarean-section born neonates, underscoring the critical role of maternal gut-derived microbes in establishing a healthy infant microbiome (Dubois et al., [2024](#); Korpela et al., [2020](#)).

The best-studied and most powerful factor influencing the enteric microbiota composition in both infants and adults is diet. In neonates, this particularly addresses the issue of breastfeeding versus formula feeding. Whereas past centuries employed wet nurses to replace mothers who were unable or unwilling to breast-feed their babies, companies today offer formula diets that mimic the composition of the human breast milk. Nevertheless, breastfeeding is associated with an enhanced abundance of bifidobacteria and lactobacilli (Kashtanova et al., [2016](#)). In contrast, formula feeding results in higher microbial diversity, lower abundance of bifidobacteria, and higher numbers of bacteria of the phylum Bacteroidota (formerly Bacteroidetes) (Bezirtzoglou et al., [2011](#); Fallani et al., [2010](#)). Also, the succession of microbial patterns observed over time was disrupted in those fed infant formula (Gregory et al., [2016](#)).

Although major efforts have been made to make formulas as similar to human breast milk as possible, significant differences remain. For example, human breast milk and, in particular, colostrum, the breast milk produced during the first days after birth, contains enormous

concentrations of secretory (S) immunoglobulin A (SIgA). This SIgA is at least in part directed against commensal bacteria and contributes to the establishment of a beneficial microbiota composition and may also protect from infection (Cullender et al., [2013](#); Johnson-Hence et al., [2023](#); Lindner et al., [2015](#); Palm et al., [2014](#)). This is facilitated by the recruitment of intestinal mucosal plasma cells to the mammary gland that secrete SIgA but also IgG and IgM specific for gut bacteria (Caballero-Flores et al., [2019](#); Lindner et al., [2015](#)). Breast milk also contains human milk oligosaccharides (HMO), unconjugated complex carbohydrates with nutritional effects on bacteria (Yu et al., [2013](#)). HMOs increase colonization with *Bifidobacterium* spp. and impede colonization by pathogenic bacteria (Asakuma et al., [2011](#)). This potent “bifidogenic” effect is explained by the fact that bifidobacteria encode an HMO-degrading enzyme cluster and cross-feed metabolites to other bifidobacterial species (Heppner et al., [2024](#); Lawson et al., [2020](#)). The effect is so strong that oral administration of HMOs facilitated even colonization of adult individuals with a fully competitive microbiota with *B. infantis* (Button et al., [2022](#)). Efforts are made to identify specific carbohydrate molecules that mimic HMOs and confer a beneficial effect (Matsuki et al., [2016](#)). Recent work suggests that the composition of HMOs in breast milk can vary considerably among mothers, influencing which bifidobacterial strains thrive in the infant gut (Barnett et al., [2023](#)). Some HMO types (e.g., lacto-N-hexaose) are linked to higher *Bifidobacterium* abundance, whereas others (e.g., 6'-sialyllactose) may differentially affect microbial colonization patterns (Barnett et al., [2023](#)).

Following the initial period of sole breastfeeding (recommended by the WHO to last for 4–6 months after birth) or formula feeding, solid food is introduced. This introduces a major shift in the enteric microbiota composition. The introduction of plant oligosaccharides promotes expansion of the phyla Bacteroidota (formerly Bacteroidetes) and Bacillota (formerly Firmicutes) (Koenig et al., [2011](#)). More recent data indicate that diversification of the diet during the weaning period supports a more stable and mature gut microbiome in infancy

(Homann et al., [2021](#)). In particular, solid food increases microbial richness and favors bacteria that can metabolize complex polysaccharides, thereby driving the transition toward an adult-like gut community (Homann et al., [2021](#)).

Another potent and well-established factor is the administration of antibiotics either perinatally to the mother or after birth to the neonate (Arboleya et al., [2015](#); Penders et al., [2006](#); Rutten et al., [2015](#); Thiemann et al., [2016](#)). As described above, administration of a single dose of a prophylactic antibiotic (in most cases a second-generation cephalosporin) is recommended for all mothers undergoing cesarean section to prevent infection at the site of the surgical skin incision. Also, many countries recommend intrapartum antibiotic prophylaxis (IAP) with penicillin to all mothers with a positive vaginal swab for group B streptococci (GBS, also named *Streptococcus agalactiae*). GBS colonizes the urogenital tract of approximately one-third of women and represents the most frequent causative agent of neonatal sepsis and meningitis (Ahmadzia & Heine, [2014](#)). Transmission occurs during birth by direct contact with colonized body surfaces. Third, some pregnant females develop fever with signs of ascending bacterial infection of the amniotic sac and placental tissue (chorioamnionitis) prior to birth (Goldenberg et al., [2000](#)). This condition is associated with preterm birth and neonatal sepsis and requires antibiotic treatment. In all three scenarios, both the mother and the neonate are exposed to the antibiotic. Additionally, the neonate after birth may exhibit clinical or laboratory signs of infection, warranting antibiotic therapy. Notably, neonates and, in particular, preterm-born neonates are highly vulnerable to infection, and thus a delay in the initiation of an adequate antibiotic treatment may risk major complications or even death of the newborn. The alteration of the neonate's microbiota following antibiotic therapy depends on the substance used (i.e., its antibacterial spectrum), as well as the dose and regimen. Generally, antibiotic treatment increases the abundance of bacterial species with intrinsic antibiotic resistance, such as *Enterococcus* spp., *Staphylococcus* spp., and members of the *Enterobacteriaceae*. Importantly, exposure to

antibiotics after birth has been associated with an enhanced susceptibility to food allergy and asthma in preclinical models (Russell et al., [2012](#); Stefka et al., [2014](#)). The underlying mechanisms have not been resolved. Recent studies have identified differences between neonatal antibiotic regimens and IAP. Neonatal antibiotics (e.g., for suspected infections) can suppress beneficial microorganisms such as bifidobacteria, while promoting overgrowth by opportunistic bacteria (e.g., *Enterobacteriaceae*), with partial recovery over subsequent weeks (Pivrcova et al., [2022](#)). Intrapartum antibiotics, on the other hand, reduce the mother's bacterial load at delivery, limiting direct microbial transfer to the infant. Both scenarios can shift the early microbiota trajectory and may affect long-term immune or metabolic health.

Additional factors exert a major influence on the microbiota composition. Preterm birth enhances the abundance of *Staphylococcus* spp. and *Enterobacteriaceae* and lowers the colonization by *Bifidobacterium* spp. (Arbolea et al., [2015](#); Hill et al., [2017](#); Roze et al., [2017](#)). However, preterm birth is simultaneously associated with birth by cesarean section, prolonged hospitalization, risk of infection, and antibiotic administration, and it remains unclear whether any of these factors or the premature intestinal tissue per se is responsible for the observed change in the microbiota composition. Although with a minor effect size, genetic factors are also associated with the microbiota composition, as determined by comparing monozygotic with dizygotic twin pairs or using genome-wide association studies (GWAS) (Goodrich et al., [2014](#); Zhernakova et al., [2024](#)). For example, the ABO blood group locus was associated with the abundance of *Faecalibacterium prausnitzii* (Zhernakova et al., [2024](#)).

3.3 Postnatal Establishment of Host–Microbial Immune Homeostasis

Bacterial colonization of previously sterile mucosal surfaces after birth challenges the regulatory mechanisms of the innate immune system to prevent inappropriate immune receptor stimulation by microbial

pattern molecules. Numerous studies have identified mechanisms that contribute to establishing and maintaining immune homeostasis and restricting the secretion of proinflammatory mediators (Hornef & Torow, [2020](#); Torow et al., [2023a](#)). For example, the presence of immunomodulatory mediators such as the epidermal growth factor (EGF), intestinal alkaline phosphatase (IAP), and the secretory leukocyte protease inhibitor (SLPI) in amniotic fluid and breast milk dampens postnatal mucosal immune stimulation. Also, enhanced expression of regulatory molecules of innate immune signaling prevents an exaggerated innate immune response during the postnatal time period (Good et al., [2012](#); Vereecke et al., [2010](#)).

Immunomodulatory cytokines such as interleukin (IL)-10 also contribute to maintaining immune homeostasis during early microbial colonization (Glocker et al., [2009](#)). In addition, inhibitory mechanisms activated upon first microbial encounter, such as the postnatal induction of innate immune tolerance, have been described. Postnatal exposure to bacterial endotoxin induces a state of innate immune non-responsiveness of the intestinal epithelium (Chassin et al., [2010](#); Lotz et al., [2006](#)). Interestingly, postnatal acquisition of innate immune tolerance is also observed at systemic body sites (Ulas et al., [2017](#)).

The neonate's mucosal adaptive immune system differs significantly from that of adult individuals (Hornef & Torow, [2020](#); Torow et al., [2023a](#)). The development of lymphoid tissues and stromal support structures and the homing of thymus- and bone marrow-derived cells continue during the first days and weeks of life (Lindner et al., [2015](#); South et al., [1967](#); Thome et al., [2016](#); Torow et al., [2015](#)). Modulatory mechanisms such as T regulatory cells and maternal SIgA delay early postnatal cell maturation and acquisition of effector function in response to microbial colonization under physiological conditions (Torow et al., [2015](#)). The emergence of M cells and activated dendritic cells determines the onset of adaptive immune priming in the neonatal Peyer's patch in mice (Torow et al., [2023b](#)). Yet, the neonatal immune system is able to react and mount a protective systemic immune response to pathogen challenge (Forsthuber et al., [1996](#); Sarzotti et al.,

[1996](#)). It may therefore be more appropriate to speak about a “distinct neonatal” as opposed to “immature” immune system of the newborn host. Not unexpectedly, both systems, the establishment of the enteric microbiota and the maturation of the adaptive immune system, closely interfere and influence each other. The critical role of this interaction is illustrated by the finding that a single dose of an antibiotic in early life can have a lasting effect on immune cell numbers, humoral immunity, and the susceptibility to mucosal inflammation (Ozkul et al., [2020](#); Ruiz et al., [2017](#)). However, the whole complexity of this interaction is still incompletely understood.

An example for the sequence of immune maturation events and the expansion of immune cell populations after birth and the influence of dietary and microbial signals is the role of HMOs and bifidobacteria on the developing immune system (Henrick et al., [2021](#)). HMOs in breast milk promote the abundance of bifidobacteria in the neonatal intestine due to their HMO utilization ability. In turn, bifidobacteria produce metabolites such as indole-3-lactic acid, which in turn act on naïve CD4⁺ T lymphocytes and promote the polarization of T regulatory and T helper (Th)1 cells and suppress pathogenic Th2 and Th17 immune responses via upregulation of the immunomodulatory molecule galectin-1. Accordingly, reduced HMO concentrations in breast milk have been associated with reduced numbers of tolerogenic RORγt⁺ T regulatory cells in the neonate (Li et al., [2023](#)).

B cell maturation and antibody production represent another maturation event, as they are stimulated by early microbial exposure. A study analyzed the influence of the early enteric microbiota on B cell effector function, namely, the production and secretion of IgE (Cahenzli et al., [2013](#)). The authors found that mucosal B cells in the absence of an enteric microbiota (in germ-free mice) switch to IgE resulting in elevated serum IgE levels. Interestingly, the presence of a diverse enteric microbiota during a postnatal time window was required to inhibit IgE induction and prevent oral-induced systemic anaphylaxis. Consistent with this, another study reported that B cells arising during an early time window after birth form a network of self-sustaining IgA

memory compartments with unique specificities and substantially contribute to the homeostatic IgM and IgA repertoire in adult mice (Vergani et al., [2022](#)).

The third example addresses yet another aspect of immune maturation, namely, the postnatal homing of immune cells to peripheral tissue sites. During a defined postnatal time window, the enteric microbiota influences the number and function of embryonic macrophages in the colonic mucosal tissue, which are in turn critical to facilitate residency of invariant natural killer cells (iNKT) (Gensollen et al., [2021](#)). These iNKTs promote the antimicrobial response to enteropathogens. However, in the absence of enteric bacteria, enhanced numbers of iNKTs were found in colonic and lung tissue associated with enhanced susceptibility to colitis and allergic asthma. Early postnatal microbial colonization was required to regulate chemokine expression and restrict mucosal iNKT cell homing associated with lower disease susceptibility. Notably, bacterial colonization at later time points was unable to compensate the phenotype (Olszak et al., [2012](#)).

In summary, the enteric microbiota exerts important influences on the development and maturation of the mucosal innate and adaptive immune system. The described examples highlight the particular and non-redundant role of the postnatal period and the early microbiota establishment to reach long-term immune homeostasis and health. This is supported by the finding that the protective effect of a farm environment on childhood asthma depends on the microbiota maturation during the first year of life (Depner et al., [2020](#)). The existence of a critical time period has been described as “neonatal window of opportunity.” It might explain the studies that identify the strong effect of microbiota-influencing factors such as postnatal antibiotic use, mode of delivery, or livestock exposure on a farm for the incidence of allergic and metabolic diseases (Cox et al., [2014](#); Depner et al., [2013](#); Huang et al., [2015](#)). Notably, we here only discuss the effect of viable bacteria as part of the enteric microbiota. Microbial constituents and metabolites might reach the embryonic and fetal organisms via the placental circulation and influence its development (Gomez de Agüero

et al., [2016](#)). Further, the effects of the microbiota are not restricted to the mucosal immune system, but also include aspects of the systemic immune system (Hergott et al., [2016](#)).

3.4 Dysbiosis and Disease

Alterations in the microbiota composition and diversity have been observed in various disease models, and comparative analyses have revealed significant differences between healthy individuals and patients suffering from inflammatory, metabolic, and immune-mediated diseases. Notably, many factors can explain these microbiota differences, and a direct causative contribution has been demonstrated for only few conditions.

One of the conditions for which the enteric microbiota is thought to contribute to the disease etiology is necrotizing enterocolitis of the newborn (NEC) (Hackam & Sodhi, [2022](#)). This condition is almost exclusively found in preterm-born neonates (i.e., neonates that are born below the 37th week of gestation) and characterized by a severe inflammation and necrosis of colonic tissue. Despite surgical intervention, it is associated with high mortality and morbidity. Interestingly, its incidence and severity increase with low gestational age of the preterm neonate, and it usually starts with a delay of 1–2 weeks after birth, i.e., after bacterial colonization of the intestine. An inappropriate immune stimulation of the still “immature” intestinal mucosa by members of the microbiota is thought to drive the tissue inflammation. Consistently, a number of immunoregulatory mechanisms that are thought to protect the mature neonate mucosa from inappropriate microbiota-induced immune stimulation were shown to be absent or functionally impaired in the preterm intestine. Also, a significantly altered microbiota was noted in preterm neonates, and some changes were associated with the development of NEC (Roze et al., [2017](#)). For example, a high abundance of *Enterobacteriaceae* in the human preterm intestine was demonstrated, and the outer cell membrane constituent of Gram-negative bacteria, lipopolysaccharide

(LPS), and its receptor, Toll-like receptor (TLR)4, were identified to drive mucosal inflammation in animal models of NEC (Egan et al., [2016](#)). On the other side, maternal SIgA was shown to protect from NEC (Gopalakrishna et al., [2019](#)).

Infections represent the leading cause of death in the age group of neonates and young infants below the age of 1 year worldwide (Collaborators, [2016](#)). Among them, gastrointestinal and pulmonary infections account for the majority of cases (Liu et al., [2012](#)). The still maturing innate and adaptive immune system and the absence of immune memory from previous exposures might contribute to this enhanced susceptibility. Additionally, the establishment of a mature and diverse enteric microbiota was shown to be required for immune maturation and resistance to infection (Lubin et al., [2023](#)). The previously described low colonization resistance of the early microbiota that results from low bacterial diversity might also play a direct role. The low colonization resistance might also contribute to the remarkably different spectra of pathogenic microorganisms that affect neonates. The most common causative agents of neonatal sepsis, group B streptococci (GBS, also called *S. agalactiae*), *Listeria monocytogenes*, and *E. coli* K1 (capsule type 1) are rarely observed in adults. The major causes of infant gastroenteritis are rotavirus, enterohemorrhagic, and enteropathogenic *E. coli*, but these pathogens rarely cause disease in adults (Kotloff et al., [2013](#)).

The incidence of autoimmune diseases and allergies in industrialized countries has steadily increased during the last 30 years. Given the strong influence of our modern Western lifestyle on the microbiota composition, a possible causative role of the microbiota has been suggested. A recent study observed a significantly higher abundance of *Bacteroides* spp. in the microbiota of Finnish and Estonian as compared to Russian children (Vatanen et al., [2016](#)). The low immunostimulatory potential of *Bacteroides* lipopolysaccharide (LPS) was suggested to preclude immune education and explain the enhanced incidence of autoimmune diseases in Western countries.

Furthermore, additional evidence indicates that early-life microbial exposures may modulate disease susceptibility. For instance, another study suggested that the presence of siblings and household pets lowers the incidence of food allergies by a microbiota-dependent effect (Koplin et al., [2012](#)). Conversely, the administration of antibiotics during early life through its effect on the enteric microbiota was proposed to contribute to the increase in metabolic and immune-mediated diseases (Cox et al., [2014](#); Rawat et al., [2023](#); Zhang, Borbet, et al., [2021b](#)). Indeed, a higher incidence of early signs of allergy was observed in both animal studies as well as human patient cohorts after repeated antibiotic treatment prior to or shortly after birth (Lapin et al., [2015](#); Russell et al., [2012](#); Stefka et al., [2014](#)). Changes in the microbiota composition induced by antibiotic exposure are thought to contribute to enhanced susceptibility to allergic diseases (Russell et al., [2012](#); Stefka et al., [2014](#)). The mechanisms involved, however, have not been identified, and other studies did not confirm this effect.

3.5 Probiotics and Prebiotics

The oral administration of specific bacterial species might occupy open niches and thus enhance colonization resistance, preventing infection with pathogenic microorganisms. Inflammatory diseases induced by an altered microbiota (then called dysbiosis) might also be targeted by correcting the microbiota composition through oral administration of beneficial bacteria. These beneficial bacteria are called probiotic bacteria, and the WHO defines probiotic bacteria as “viable microorganisms that exert a health-promoting effect on the host organism.” It is important to keep in mind that probiotics, by and large, represent commensal bacteria or yeast. The most commonly used probiotic agents are *Saccharomyces cerevisiae* (the brewer’s yeast), *Lactobacillus* and *Bifidobacterium* species, as well as *E. coli*.

In addition to the administration of probiotic bacteria, the nutritional supplementation with substances that foster the growth of beneficial bacteria (so-called prebiotics) has become a therapeutic

strategy. As described, breast milk and its constituents strongly promote the growth of beneficial neonatal bacteria such as bifidobacteria and can be considered a natural prebiotic. The concept of combining probiotics with prebiotics (synbiotics) has gained traction, particularly in neonatal care. Synbiotics seek to optimize both the colonization potential of the introduced microbes and the gut environment that fosters their growth. In addition, so-called postbiotics—non-viable microbial products or metabolic byproducts that confer health benefits—have begun to appear in infant nutrition research. While definitive neonatal outcomes for postbiotics remain under investigation, early studies in experimental models suggest they could modulate immune function and gut barrier integrity without the viability concerns posed by live organisms (Lemoine et al., [2023](#); Zhang et al., [2024](#)).

The prophylactic value of all three strategies has been tested for possible dysbiosis-related diseases of the neonate discussed in the previous chapter. In several studies, premature neonates received Gram-positive bacteria in order to prevent the overgrowth of Gram-negative bacteria and the stimulation of the immature intestinal mucosa, leading to NEC. A recent meta-analysis concluded that postnatal oral administration of probiotic bacteria to preterm human neonates provides a significant benefit, lowering the incidence and severity of NEC (Sharif et al., [2020](#); Ullah et al., [2024](#); Wu et al., [2024](#)). However, not all probiotic bacteria confer a beneficial effect, and further studies are needed to better define the optimal prophylactic strategy. Besides probiotics, the administration of breast milk as a physiological form of prebiotic (see above) has been shown to protect from NEC (Miller et al., [2018](#); Quigley et al., [2024](#)).

Low colonization resistance in neonates and young children was also addressed by oral administration of viable beneficial bacteria. Indeed, a recent prospective controlled study in rural India revealed a strong protective effect of daily oral *Lactobacillus plantarum* plus fructooligosaccharide administration on the incidence of bacterial sepsis and lower respiratory tract infection (Panigrahi et al., [2017](#)). The

underlying mechanisms that allow outcompetition of enteropathogenic bacteria have been analyzed in preclinical studies (Eberl et al., [2021](#); Kim et al., [2017](#); Thiemann et al., [2016](#)). Additionally, the incidence of allergies in children born by cesarean section was reduced following oral administration of probiotic bacteria (Hesser et al., [2024](#); Kuitunen et al., [2009](#)). It is interesting that neonates and young infants appear to exhibit a greater responsiveness to the administration of probiotic bacteria as compared to adult individuals. In fact, the vast majority of studies on adult patients with various diseases failed to show a significant benefit of oral treatment with probiotic bacteria. Thus, the lower colonization resistance in neonates might make neonates more vulnerable to disturbance of the microbiota composition, but at the same time render them more susceptible to the beneficial effects by oral administration of probiotic bacteria.

Over the past few years, additional evidence has underscored the importance of selecting specific probiotic regimens for preterm neonates. For instance, observational data demonstrated that supplementation with *Bifidobacterium*- and *Lactobacillus*-containing probiotic mixtures can shift the preterm infant gut microbiota toward a healthier, *Bifidobacterium*-dominated profile, along with reduced pathogen overgrowth (Alcon-Giner et al., [2020](#); van Best et al., [2020](#)). These findings align with large-scale meta-analyses suggesting that multi-strain probiotics effectively lower NEC incidence and severity in very low birth weight infants (Morgan et al., [2020](#)). In a more recent randomized controlled trial (RCT) of extremely preterm infants, it was shown that a multi-species probiotic mixture accelerated microbiome maturation and decreased intestinal inflammation, highlighting the potential for improved clinical outcomes in this vulnerable population (Samara et al., [2022](#)).

Next-generation microbial therapies are also on the horizon, including emerging strains (e.g., *Akkermansia muciniphila*, certain *Bacteroides* spp.) and genetically engineered bacteria designed to enhance gut barrier function or combat specific pathogens. While their clinical use in neonates remains experimental, preliminary data from

adult trials suggest these next-generation approaches may offer more targeted benefits than current probiotics.

The enhanced susceptibility of the neonate and infant host should also raise concerns on possible safety aspects and secondary long-term effects of the administration of probiotic bacteria. Invasive infections caused by orally administered probiotic bacteria have been rarely reported, and orally administered bacteria disappeared from the infant microbiota shortly after cessation of oral administration (Bazanella et al., [2017](#)). Nevertheless, comparative studies are needed to define the beneficial effect of each individual probiotic regimen. Robust quality control data are required before we treat this vulnerable patient population. Finally, there is growing interest in tailoring probiotic and prebiotic interventions to each infant's baseline microbiome and clinical risk factors. Early findings indicate that not all neonates effectively engraft the same probiotic strains, suggesting that rapid stool analysis could guide individualized approaches. Although still in its infancy, this precision medicine approach may optimize benefits and reduce unnecessary interventions in neonatal care.

3.6 Future Perspectives

Although much has been learned during the last years and we now have an approximate idea of the development of the early enteric microbiota and influencing factors, much remains to be investigated. In particular, we only marginally understand how and to what extent the host directs and shapes the establishment of the enteric microbiota. Since essential physiological processes critically depend on signals from the enteric microbiota, it is likely that the host ensures the presence of the right bacteria providing the right signals at the right time. A better understanding of these mechanisms might help us to identify the overall beneficial aspects of the microbiota. Importantly, this is not restricted to bacteria, but may also involve phages, fungi, and archaea.

A large number of studies performed during the last few years demonstrated differences in microbiota composition between healthy

individuals and patients and discussed a possible causative role in disease etiology or progression. However, few investigations were able to demonstrate a causal role and/or insight into the mechanism(s) involved. In many cases, the microbiota alterations might result from disease-associated behavioral changes or treatment-related factors.

One example illustrates the problems, but also the surprises that might emerge from this type of analysis. First a large study identified differences in the microbiota between healthy and type 2 diabetic patients suggesting a role of the microbiota in the etiology of the disease (Qin et al., [2012](#)). Then, the commonly administered antidiabetic drug, metformin, was identified to have caused much of the observed microbiota alteration (Forslund et al., [2015](#)). Finally, the metformin-induced microbiota changes were shown to at least in part account for the therapeutic benefit of the drug (Wu et al., [2017](#)). Thus, we have to overcome the descriptive analysis of pure differences and move forward to identify and culture the responsible microbial species, analyze their metabolic profiles, and reveal underlying molecular mechanisms (Beresford-Jones et al., [2022](#); Lagkouvardos et al., [2016](#)).

Microbiota alterations might confer improved health or disease in different ways. The increased abundance of one commensal species might occupy the niche of a pathogenic one and thus prevent colonization (Brugiroux et al., [2016](#); Osbelt et al., [2024](#)). Beneficial bacteria might contribute to nutrient metabolism and provide beneficial metabolic substrates or produce immunostimulatory molecules that promote immune homeostasis (Round et al., [2011](#)). Even the bacterial production of human mediator homologues was described, which might act at very low concentrations and significantly influence physiological function (Cohen et al., [2017](#)).

Important in the context of the discussion of the neonatal, developing enteric microbiota is the fact that it displays a much higher individual variation, is dynamic in its composition, and is vulnerable to exogenous factors such as antibiotics (Cox et al., [2014](#); Ozkul et al., [2020](#); Ruiz et al., [2017](#)). We therefore have to exert caution when administering antibiotics—if not strictly medically indicated—and

other microbiota-modifying agents to neonates and young children. Few studies have started to address possible side effects both during treatment and in the long term. At the same time, this situation opens the possibility of (possibly lasting) interventional modification to prevent metabolic diseases and infections (Panigrahi et al., [2017](#)). Thus, although many questions still need to be addressed, the microbiota represents a promising future target for prophylactic and interventional strategies, and neonates and young infants represent the most promising age group.

Infants born during the COVID-19 pandemic have exhibited gut microbiomes noticeably distinct from those of pre-pandemic cohorts (Korpela et al., [2024](#)). Compared to previous years, infants born during the lockdown often had lower community and hospital exposure, which may have limited their contact with common environmental and maternal microbes. Meanwhile, extended social distancing likely diminished the exchange of microbes via older siblings, relatives, and daycare settings. However, these measures may also have curtailed beneficial microbial exposures from the environment, possibly delaying the development of a more complex infant gut microbiota. Overall, while rigorous hygiene and limited social contacts helped protect neonates from infectious risks, they simultaneously altered the routes of early-life microbial colonization. Long-term health consequences of these pandemic-induced alterations remain to be fully clarified, but preliminary data suggest some shift in immune-related outcomes (Korpela et al., [2024](#)). Understanding how short-term social measures affect the microbiota underlines the delicate balance between infection control and beneficial microbial exposures.

The One Health paradigm emphasizes that human, animal, and environmental health are deeply interconnected. Infants acquire microbes from diverse sources, including siblings, pets, and the broader natural environment, and these exposures can substantially shape their early gut microbiome (Olm et al., [2022](#)). For example, living near green spaces and interacting with animals have been associated with a more diverse microbiota in infancy (Nielsen et al., [2020](#)). This phenomenon

also ties in with the so-called “farm effect,” in which children raised on farms show reduced rates of allergies and asthma due in part to early, repeated contact with farm-related microorganisms (Depner et al., [2020](#); von Mutius, [2016](#)). Alongside these macro-level factors, smaller-scale changes—such as maternal diet, hygiene practices, and antibiotic usage—can amplify or diminish beneficial microbial exposures. Consequently, supporting infant microbiome health entails looking beyond the immediate home or hospital setting and considering the collective microbial ecosystem shared by humans, animals, and the environment. Understanding these interactions helps explain why pandemic social-distancing measures or the modernization of childcare practices might trigger ripple effects on childhood immune health. In turn, encouraging moderate exposures to safe environmental and animal microbes, while remaining vigilant about pathogenic risks, can foster balanced microbiome development. Ultimately, a One Health approach reminds us that infant gut colonization is not solely a matter of maternal-neonatal biology, but also of public health, urban planning, and sustainable relationships with our ecosystems.

Controversy It is generally believed that the fetus in utero develops in the absence of viable microorganisms. Some years ago, several groups challenged this view and proposed the existence of a placental microbiome, i.e., the presence of viable commensal bacteria in the placenta and fetus with colonization of the fetal gastrointestinal tract. For example, one group found low numbers of culturable bacteria in human cord blood samples after elective cesarean section (Jimenez et al., [2005](#)) and meconium samples of healthy neonates born by either vaginal delivery or cesarean section (Jimenez et al., [2008](#)). Others used PCR-based methods and detected bacterial DNA in placental and fetal tissue (Aagaard et al., [2014](#); Bassols et al., [2016](#); Mishra et al., [2021](#); Rackaityte et al., [2020](#); Rautava et al., [2012](#)). Also, the culture of bacteria from fetal tissue and the visualization of bacteria-like structures by electron microscopy (Rackaityte et al., [2020](#)) as well as the detection of T-lymphocytes reactive to certain bacterial species

(Mishra et al., [2021](#)) were reported. The maternal oral cavity was proposed to represent the reservoir of bacteria colonizing the placenta and fetus (Pax et al., [2024](#)). Based on these results, the presence of a low-richness and low-diversity fetal microbiota was suggested (Collado et al., [2016](#)). However, do the reported findings really support the existence of a “fetal microbiota” and of “microbial transfer at the feto-maternal interface”? PCR-based methods detect DNA and are, by definition, unable to differentiate between viable bacteria and dead bacteria or remnants of bacterial DNA. Modern high-throughput PCR methods are extremely sensitive and able to detect minute amounts of DNA. Low DNA concentrations were shown to be present in commercial enzyme preparations, column material, and even buffer solutions. In addition, mucosal body surfaces in the oral cavity, large intestine, and urogenital tract are densely colonized, and we know that transient bacteremia occurs following minor manipulation of mucosal tissues, for example, after brushing the teeth or during labor. Moreover, bacteria can proliferate very rapidly, and thus, meconium released hours after birth is not expected to be sterile. Finally, low numbers of microorganisms are ubiquitously found in our environment, and tight precautions have to be put in place to avoid contamination of the samples during the analytical process (Lauder et al., [2016](#)). Thus, the detection of bacterial DNA and the culture of low bacterial numbers in clinical specimens obtained after birth does not prove the existence of a bona fide microbiota. Indeed, a number of studies failed to detect a specific signal of a fetal or placental microbiota when re-analyzing existing data and accounting for environmental bacterial DNA and batch effects (de Goffau et al., [2021](#); Kennedy et al., [2023](#); Kennedy et al., [2021](#); Leiby et al., [2018](#); Theis et al., [2020](#)). The most compelling argument, however, comes from animal studies and human medicine: The sterile recovery of mature mouse embryos can be used to generate germ-free animals. Similarly, the delivery of human neonates suspected to suffer from severe combined immunodeficiency (SCID) by c-section and transfer into a sterile environment maintained culture-negative conditions for

several days (Barnes et al., [1969](#)). Thus, the current findings do not provide sufficient evidence to support the existence of a fetal microbiota (Hornef & Penders, [2017](#); Kennedy et al., [2023](#); Perez-Munoz et al., [2017](#); Walter & Hornef, [2021](#)).

History

In the mid-nineteenth century, microbiologists like Robert Koch and Louis Pasteur identified the first pathogenic bacteria and started to gather knowledge on the etiology of infectious disease and the epidemiology and transmission of microorganisms. This led to the development of methods and behavioral habits to avoid contact with pathogens and lower the incidence of infections. Technical inventions made during the last 100 years, such as water toilets and sewage treatment, the production and distribution of drinking water, personal hygiene, and improved housing conditions (e.g., separate rooms for livestock and humans), and food storage (e.g., the introduction of refrigerators that reduce microbial growth and allow prolonged food storage) contributed to this. In parallel, the vast majority of the population started to live in an urban setting with reduced contact with livestock and a microbe-rich farming environment. Both factors significantly lowered the incidence of life-threatening infections in industrialized countries, accompanied by an increase in life expectancy and a reduction in morbidity and mortality. Public and personal hygiene as a strategy to avoid contact with pathogenic microorganisms culminated in habits such as sterilizing the pacifier or milk bottle from babies and infants prior to use and the overuse of disinfectants. Also, antibiotics were used widely also during early childhood often with little medical evidence for their benefit (e.g., to treat infections that are typically caused by viruses). Today, we learn that these measures alter our microbiota and deprive us from exposure to nonpathogenic microorganisms and microbial constituents that contribute to maturation of our immune system and host-microbial homeostasis (Deckers et al., [2021](#); Depner et al., [2020](#); Pacheco Da Silva et al., [2024](#)). We start to understand that this

deprivation from microbial exposure, in particular during early childhood, might be associated with secondary effects and contribute to the increasing incidence of noncommunicable inflammatory and immune-mediated diseases such as allergic disease, inflammatory bowel disease, and diabetes (Riedler et al., [2001](#); Vatanen et al., [2016](#)). This concept was initially proposed in the so-called hygiene hypothesis that particularly focused on the influence of infections during the postnatal and infant period for immune-mediated disease susceptibility in later life (Strachan, [1989](#)). It was later extended to also include changes in our exposure to commensal bacteria (Rook, [2010](#)). Today, we aim at identifying the functional mechanisms that contribute to enhanced disease susceptibility (Vatanen et al., [2016](#); Zhang, Li, et al., [2021a](#)). We also aim at reestablishing microbial exposure after birth and during early childhood to prevent or treat immune-mediated diseases, of course, without increasing the risk of exposure to pathogenic microorganisms (Bogaert et al., [2023](#); Carpen et al., [2022](#); Dubois et al., [2024](#); Song et al., [2021](#)).

Highlights

- First exposure to viable bacteria starts with rupture of membranes during birth, resulting in contact with maternal fecal, urogenital, and skin bacteria.
- The early postnatal enteric microbiota is characterized by low diversity and high interindividual variation.
- Microbial diversity increases by incorporation of new bacterial taxa, reaching an adult-like microbiota in humans at approximately 2–3 years of age.
- Important exogenous factors influencing the microbiota composition are the mode of delivery, diet (breast-feeding versus formula feeding), and antibiotic use.
- The neonatal/infant microbiota is significantly more susceptible than the adult microbiota to exogenous alteration, e.g., by antibiotic treatment or administration of probiotic bacteria.

References

- Aagaard, K., Ma, J., Antony, K. M., Ganu, R., Petrosino, J., & Versalovic, J. (2014). The placenta harbors a unique microbiome. *Science Translational Medicine*, 6(237), 237ra265. <https://doi.org/10.1126/scitranslmed.3008599>
- Ahmadzia, H. K., & Heine, R. P. (2014). Diagnosis and management of group B streptococcus in pregnancy. *Obstetrics and Gynecology Clinics of North America*, 41(4), 629–647. <https://doi.org/10.1016/j.ogc.2014.08.009> [PubMed]
- Alcon-Giner, C., Dalby, M. J., Caim, S., Ketskemety, J., Shaw, A., Sim, K., Lawson, M. A. E., Kiu, R., Leclaire, C., Chalklen, L., Kujawska, M., Mitra, S., Fardus-Reid, F., Belteki, G., McColl, K., Swann, J. R., Kroll, J. S., Clarke, P., & Hall, L. J. (2020). Microbiota supplementation with bifidobacterium and lactobacillus modifies the preterm infant gut microbiota and metabolome: An observational study. *Cell Reports Medicine*, 1(5), 100077. <https://doi.org/10.1016/j.xcrm.2020.100077> [PubMed]
- Arbolea, S., Sanchez, B., Milani, C., Duranti, S., Solis, G., Fernandez, N., de los Reyes-Gavilan, C. G., Ventura, M., Margolles, A., & Gueimonde, M. (2015). Intestinal microbiota development in preterm neonates and effect of perinatal antibiotics. *The Journal of Pediatrics*, 166(3), 538–544. <https://doi.org/10.1016/j.jpeds.2014.09.041> [PubMed]
- Asakuma, S., Hatakeyama, E., Urashima, T., Yoshida, E., Katayama, T., Yamamoto, K., Kumagai, H., Ashida, H., Hirose, J., & Kitaoka, M. (2011). Physiology of consumption of human milk oligosaccharides by infant gut-associated bifidobacteria. *The Journal of Biological Chemistry*, 286(40), 34583–34592. <https://doi.org/10.1074/jbc.M111.248138> [PubMed]
- Azad, M. B., Konya, T., Maughan, H., Guttman, D. S., Field, C. J., Sears, M. R., Becker, A. B., Scott, J. A., & Kozyrskyj, A. L. (2013). Infant gut microbiota and the hygiene hypothesis of allergic disease: Impact of household pets and siblings on microbiota composition and diversity. *Allergy, Asthma and Clinical Immunology*, 9(1), 15. <https://doi.org/10.1186/1710-1492-9-15>
- Backhed, F., Roswall, J., Peng, Y., Feng, Q., Jia, H., Kovatcheva-Datchary, P., Li, Y., Xia, Y., Xie, H., Zhong, H., Khan, M. T., Zhang, J., Li, J., Xiao, L., Al-Aama, J., Zhang, D., Lee, Y. S., Kotowska, D., Colding, C., et al. (2015). Dynamics and stabilization of the human gut microbiome during the first year of life. *Cell Host & Microbe*, 17(6), 852. <https://doi.org/10.1016/j.chom.2015.05.012>
- Barnes, R. D., Fairweather, D. V., Holliday, J., Keane, C., Piesowicz, A., Soothill, J. F., & Tuffrey, M. (1969). A germfree infant. *Lancet*, 1(7587), 168–171. [https://doi.org/10.1016/s0140-6736\(69\)91187-8](https://doi.org/10.1016/s0140-6736(69)91187-8) [PubMed]
- Barnett, D. J. M., Endika, M. F., Klostermann, C. E., Gu, F., Thijs, C., Nauta, A., Schols, H. A., Smidt, H., Arts, I. C. W., & Penders, J. (2023). Human milk oligosaccharides, antimicrobial drugs, and the gut

microbiota of term neonates: Observations from the KOALA birth cohort study. *Gut Microbes*, 15(1), 2164152. <https://doi.org/10.1080/19490976.2022.2164152>
[PubMed]

Bassols, J., Serino, M., Carreras-Badosa, G., Burcelin, R., Blasco-Baque, V., Lopez-Bermejo, A., & Fernandez-Real, J. M. (2016). Gestational diabetes is associated with changes in placental microbiota and microbiome. *Pediatric Research*, 80(6), 777–784. <https://doi.org/10.1038/pr.2016.155>
[PubMed]

Bazanella, M., Maier, T. V., Clavel, T., Lagkouvardos, I., Lucio, M., Maldonado-Gomez, M. X., Autran, C., Walter, J., Bode, L., Schmitt-Kopplin, P., & Haller, D. (2017). Randomized controlled trial on the impact of early-life intervention with bifidobacteria on the healthy infant fecal microbiota and metabolome. *The American Journal of Clinical Nutrition*, 106(5), 1274–1286. <https://doi.org/10.3945/ajcn.117.157529>
[PubMed]

Beresford-Jones, B. S., Forster, S. C., Stares, M. D., Notley, G., Viciani, E., Browne, H. P., Boehmler, D. J., Soderholm, A. T., Kumar, N., Vervier, K., Cross, J. R., Almeida, A., Lawley, T. D., & Pedicord, V. A. (2022). The Mouse Gastrointestinal Bacteria Catalogue enables translation between the mouse and human gut microbiotas via functional mapping. *Cell Host & Microbe*, 30(1), 124–138e128. <https://doi.org/10.1016/j.chom.2021.12.003>

Bezirtzoglou, E., Tsiotsias, A., & Welling, G. W. (2011). Microbiota profile in feces of breast- and formula-fed newborns by using fluorescence in situ hybridization (FISH). *Anaerobe*, 17(6), 478–482. <https://doi.org/10.1016/j.anaerobe.2011.03.009>
[PubMed]

Bogaert, D., van Beveren, G. J., de Koff, E. M., Lusarreta Parga, P., Balcazar Lopez, C. E., Koppensteiner, L., Clerc, M., Hasrat, R., Arp, K., Chu, M., de Groot, P. C. M., Sanders, E. A. M., van Houten, M. A., & de Steenhuijsen Pijters, W. A. A. (2023). Mother-to-infant microbiota transmission and infant microbiota development across multiple body sites. *Cell Host & Microbe*, 31(3), 447–460e446. <https://doi.org/10.1016/j.chom.2023.01.018>

Brugiroux, S., Beutler, M., Pfann, C., Garzetti, D., Ruscheweyh, H. J., Ring, D., Diehl, M., Herp, S., Lotscher, Y., Hussain, S., Bunk, B., Pukall, R., Huson, D. H., Munch, P. C., McHardy, A. C., McCoy, K. D., Macpherson, A. J., Loy, A., Clavel, T., et al. (2016). Genome-guided design of a defined mouse microbiota that confers colonization resistance against *Salmonella enterica* serovar Typhimurium. *Nature Microbiology*, 2, 16215. <https://doi.org/10.1038/nmicrobiol.2016.215>
[PubMed]

Button, J. E., Autran, C. A., Reens, A. L., Cosetta, C. M., Smriga, S., Ericson, M., Pierce, J. V., Cook, D. N., Lee, M. L., Sun, A. K., Alousi, A. M., Koh, A. Y., Rechtman, D. J., Jenq, R. R., & McKenzie, G. J. (2022). Dosing a synbiotic of human milk oligosaccharides and *B. infantis* leads to reversible engraftment in healthy adult microbiomes without antibiotics. *Cell Host & Microbe*, 30(5), 712–725e717. <https://doi.org/10.1016/j.chom.2022.04.001>

Caballero-Flores, G., Sakamoto, K., Zeng, M. Y., Wang, Y., Hakim, J., Matus-Acuna, V., Inohara, N., & Nunez, G. (2019). Maternal immunization confers protection to the offspring against an attaching

and effacing pathogen through delivery of IgG in breast milk. *Cell Host & Microbe*, 25(2), 313–323e314. <https://doi.org/10.1016/j.chom.2018.12.015>

Cahenzli, J., Koller, Y., Wyss, M., Geuking, M. B., & McCoy, K. D. (2013). Intestinal microbial diversity during early-life colonization shapes long-term IgE levels. *Cell Host & Microbe*, 14(5), 559–570. <https://doi.org/10.1016/j.chom.2013.10.004>

Cardwell, C. R., Stene, L. C., Joner, G., Cinek, O., Svensson, J., Goldacre, M. J., Parslow, R. C., Pozzilli, P., Brigis, G., Stoyanov, D., Urbonaite, B., Sipetic, S., Schober, E., Ionescu-Tirgoviste, C., Devoti, G., de Beaufort, C. E., Buschard, K., & Patterson, C. C. (2008). Caesarean section is associated with an increased risk of childhood-onset type 1 diabetes mellitus: A meta-analysis of observational studies. *Diabetologia*, 51(5), 726–735. <https://doi.org/10.1007/s00125-008-0941-z> [PubMed]

Carpen, N., Brodin, P., de Vos, W. M., Salonen, A., Kolho, K. L., Andersson, S., & Helve, O. (2022). Transplantation of maternal intestinal flora to the newborn after elective cesarean section (SECFLOR): Study protocol for a double blinded randomized controlled trial. *BMC Pediatrics*, 22(1), 565. <https://doi.org/10.1186/s12887-022-03609-3> [PubMed]

Chalmers, B., Kaczorowski, J., Levitt, C., Dzakpasu, S., O'Brien, B., Lee, L., Boscoe, M., Young, D., Maternity Experiences Study Group of the Canadian Perinatal Surveillance System, & Public Health Agency of Canada. (2009). Use of routine interventions in vaginal labor and birth: Findings from the Maternity Experiences Survey. *Birth*, 36(1), 13–25. <https://doi.org/10.1111/j.1523-536X.2008.00291.x> [PubMed]

Chassin, C., Kocur, M., Pott, J., Duerr, C. U., Gutle, D., Lotz, M., & Hornef, M. W. (2010). miR-146a mediates protective innate immune tolerance in the neonate intestine. *Cell Host & Microbe*, 8(4), 358–368. <https://doi.org/10.1016/j.chom.2010.09.005>

Chu, D. M., Ma, J., Prince, A. L., Antony, K. M., Seferovic, M. D., & Aagaard, K. M. (2017). Maturation of the infant microbiome community structure and function across multiple body sites and in relation to mode of delivery. *Nature Medicine*, 23(3), 314–326. <https://doi.org/10.1038/nm.4272> [PubMed]

Cohen, L. J., Esterhazy, D., Kim, S. H., Lemetre, C., Aguilar, R. R., Gordon, E. A., Pickard, A. J., Cross, J. R., Emiliano, A. B., Han, S. M., Chu, J., Vila-Farres, X., Kaplitt, J., Rogoz, A., Calle, P. Y., Hunter, C., Bitok, J. K., & Brady, S. F. (2017). Commensal bacteria make GPCR ligands that mimic human signalling molecules. *Nature*, 549(7670), 48–53. <https://doi.org/10.1038/nature23874> [PubMed]

Collaborators GBD 2015 HIV. (2016). Estimates of global, regional, and national incidence, prevalence, and mortality of HIV, 1980–2015: The Global Burden of Disease Study 2015. *The Lancet. HIV*, 3(8), e361–e387. [https://doi.org/10.1016/S2352-3018\(16\)30087-X](https://doi.org/10.1016/S2352-3018(16)30087-X)

Collado, M. C., Rautava, S., Aakko, J., Isolauri, E., & Salminen, S. (2016). Human gut colonisation may be initiated in utero by distinct microbial communities in the placenta and amniotic fluid. *Scientific Reports*, 6, 23129. <https://doi.org/10.1038/srep23129>

[[PubMed](#)]

Cox, L. M., Yamanishi, S., Sohn, J., Alekseyenko, A. V., Leung, J. M., Cho, I., Kim, S. G., Li, H., Gao, Z., Mahana, D., Zarate Rodriguez, J. G., Rogers, A. B., Robine, N., Loke, P., & Blaser, M. J. (2014). Altering the intestinal microbiota during a critical developmental window has lasting metabolic consequences. *Cell*, 158(4), 705–721. <https://doi.org/10.1016/j.cell.2014.05.052>

[[PubMed](#)]

Cullender, T. C., Chassaing, B., Janzon, A., Kumar, K., Muller, C. E., Werner, J. J., Angenent, L. T., Bell, M. E., Hay, A. G., Peterson, D. A., Walter, J., Vijay-Kumar, M., Gewirtz, A. T., & Ley, R. E. (2013). Innate and adaptive immunity interact to quench microbiome flagellar motility in the gut. *Cell Host & Microbe*, 14(5), 571–581. <https://doi.org/10.1016/j.chom.2013.10.009>

de Goffau, M. C., Charnock-Jones, D. S., Smith, G. C. S., & Parkhill, J. (2021). Batch effects account for the main findings of an in utero human intestinal bacterial colonization study. *Microbiome*, 9(1), 6. <https://doi.org/10.1186/s40168-020-00949-z>

[[PubMed](#)]

Decker, E., Engelmann, G., Findeisen, A., Gerner, P., Laass, M., Ney, D., Posovszky, C., Hoy, L., & Hornef, M. W. (2010). Cesarean delivery is associated with celiac disease but not inflammatory bowel disease in children. *Pediatrics*, 125(6), e1433–e1440. <https://doi.org/10.1542/peds.2009-2260>

[[PubMed](#)]

Deckers, J., Marsland, B. J., & von Mutius, E. (2021). Protection against allergies: Microbes, immunity, and the farming effect. *European Journal of Immunology*, 51(10), 2387–2398. <https://doi.org/10.1002/eji.202048938>

[[PubMed](#)]

Depner, M., Ege, M. J., Genuneit, J., Pekkanen, J., Roponen, M., Hirvonen, M. R., Dalphin, J. C., Kaulek, V., Krauss-Etschmann, S., Riedler, J., Braun-Fahrlander, C., Roduit, C., Lauener, R., Pfefferle, P. I., Weber, J., von Mutius, E., & PASTURE Study Group. (2013). Atopic sensitization in the first year of life. *The Journal of Allergy and Clinical Immunology*, 131(3), 781–788. <https://doi.org/10.1016/j.jaci.2012.11.048>

[[PubMed](#)]

Depner, M., Taft, D. H., Kirjavainen, P. V., Kalanetra, K. M., Karvonen, A. M., Peschel, S., Schmausser-Hechfellner, E., Roduit, C., Frei, R., Lauener, R., Divaret-Chauveau, A., Dalphin, J. C., Riedler, J., Roponen, M., Kabesch, M., Renz, H., Pekkanen, J., Farquharson, F. M., Louis, P., et al. (2020). Maturation of the gut microbiome during the first year of life contributes to the protective farm effect on childhood asthma. *Nature Medicine*, 26(11), 1766–1775. <https://doi.org/10.1038/s41591-020-1095-x>

[[PubMed](#)]

Dierikx, T., Berkhout, D., Eck, A., Tims, S., van Limbergen, J., Visser, D., de Boer, M., de Boer, N., Touw, D., Benninga, M., Schierbeek, N., Visser, L., Knol, J., Roeselers, G., de Vries, J., & de Meij, T. (2022). Influence of timing of maternal antibiotic administration during caesarean section on infant microbial colonisation: A randomised controlled trial. *Gut*, 71(9), 1803–1811. <https://doi.org/10.1136/gutjnl-2021-324767>

[PubMed]

Dogra, S., Sakwinska, O., Soh, S. E., Ngom-Bru, C., Bruck, W. M., Berger, B., Brussow, H., Lee, Y. S., Yap, F., Chong, Y. S., Godfrey, K. M., Holbrook, J. D., & GUSTO Study Group. (2015). Dynamics of infant gut microbiota are influenced by delivery mode and gestational duration and are associated with subsequent adiposity. *mBio*, 6(1), e02419–e02414. <https://doi.org/10.1128/mBio.02419-14>
[PubMed]

Dominguez-Bello, M. G., Costello, E. K., Contreras, M., Magris, M., Hidalgo, G., Fierer, N., & Knight, R. (2010). Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. *Proceedings of the National Academy of Sciences of the United States of America*, 107(26), 11971–11975. <https://doi.org/10.1073/pnas.1002601107>
[PubMed]

Dominguez-Bello, M. G., De Jesus-Laboy, K. M., Shen, N., Cox, L. M., Amir, A., Gonzalez, A., Bokulich, N. A., Song, S. J., Hoashi, M., Rivera-Vinas, J. I., Mendez, K., Knight, R., & Clemente, J. C. (2016). Partial restoration of the microbiota of cesarean-born infants via vaginal microbial transfer. *Nature Medicine*, 22(3), 250–253. <https://doi.org/10.1038/nm.4039>
[PubMed]

Dubois, L., Valles-Colomer, M., Ponsero, A., Helve, O., Andersson, S., Kolho, K. L., Asnicar, F., Korpela, K., Salonen, A., Segata, N., & de Vos, W. M. (2024). Paternal and induced gut microbiota seeding complement mother-to-infant transmission. *Cell Host & Microbe*, 32(6), 1011–1024e1014. <https://doi.org/10.1016/j.chom.2024.05.004>

Eberl, C., Weiss, A. S., Jochum, L. M., Durai Raj, A. C., Ring, D., Hussain, S., Herp, S., Meng, C., Kleigrewe, K., Gigl, M., Basic, M., & Stecher, B. (2021). E. coli enhance colonization resistance against Salmonella Typhimurium by competing for galactitol, a context-dependent limiting carbon source. *Cell Host & Microbe*, 29(11), 1680–1692e1687. <https://doi.org/10.1016/j.chom.2021.09.004>

Egan, C. E., Sodhi, C. P., Good, M., Lin, J., Jia, H., Yamaguchi, Y., Lu, P., Ma, C., Branca, M. F., Weyandt, S., Fulton, W. B., Nino, D. F., Prindle, T., Jr., Ozolek, J. A., & Hackam, D. J. (2016). Toll-like receptor 4-mediated lymphocyte influx induces neonatal necrotizing enterocolitis. *The Journal of Clinical Investigation*, 126(2), 495–508. <https://doi.org/10.1172/JCI83356>
[PubMed]

Fallani, M., Young, D., Scott, J., Norin, E., Amarri, S., Adam, R., Aguilera, M., Khanna, S., Gil, A., Edwards, C. A., Dore, J., & Other Members of the INFABIO Team. (2010). Intestinal microbiota of 6-week-old infants across Europe: geographic influence beyond delivery mode, breast-feeding, and antibiotics. *Journal of Pediatric Gastroenterology and Nutrition*, 51(1), 77–84. <https://doi.org/10.1097/MPG.0b013e3181d1b11e>
[PubMed]

Feng, L., Raman, A. S., Hibberd, M. C., Cheng, J., Griffin, N. W., Peng, Y., Leyn, S. A., Rodionov, D. A., Osterman, A. L., & Gordon, J. I. (2020). Identifying determinants of bacterial fitness in a model of human gut microbial succession. *Proceedings of the National Academy of Sciences of the United States of America*, 117(5), 2622–2633. <https://doi.org/10.1073/pnas.1918951117>
[PubMed]

Forslund, K., Hildebrand, F., Nielsen, T., Falony, G., Le Chatelier, E., Sunagawa, S., Prifti, E., Vieira-Silva, S., Gudmundsdottir, V., Pedersen, H. K., Arumugam, M., Kristiansen, K., Voigt, A. Y., Vestergaard, H., Hercog, R., Costea, P. I., Kultima, J. R., Li, J., Jorgensen, T., et al. (2015). Disentangling type 2 diabetes and metformin treatment signatures in the human gut microbiota. *Nature*, 528(7581), 262–266. <https://doi.org/10.1038/nature15766>

[PubMed]

Forsthuber, T., Yip, H. C., & Lehmann, P. V. (1996). Induction of TH1 and TH2 immunity in neonatal mice. *Science*, 271(5256), 1728–1730. <https://doi.org/10.1126/science.271.5256.1728>

[PubMed]

Gensollen, T., Lin, X., Zhang, T., Pyzik, M., See, P., Glickman, J. N., Ginhoux, F., Waldor, M., Salmi, M., Rantakari, P., & Blumberg, R. S. (2021). Embryonic macrophages function during early life to determine invariant natural killer T cell levels at barrier surfaces. *Nature Immunology*, 22(6), 699–710. <https://doi.org/10.1038/s41590-021-00934-0>

[PubMed]

Glocker, E. O., Kotlarz, D., Boztug, K., Gertz, E. M., Schaffer, A. A., Noyan, F., Perro, M., Diestelhorst, J., Allroth, A., Murugan, D., Hatscher, N., Pfeifer, D., Sykora, K. W., Sauer, M., Kreipe, H., Lacher, M., Nustede, R., Woellner, C., Baumann, U., et al. (2009). Inflammatory bowel disease and mutations affecting the interleukin-10 receptor. *The New England Journal of Medicine*, 361(21), 2033–2045.

<https://doi.org/10.1056/NEJMoa0907206>

[PubMed]

Goldenberg, R. L., Hauth, J. C., & Andrews, W. W. (2000). Intrauterine infection and preterm delivery. *The New England Journal of Medicine*, 342(20), 1500–1507. <https://doi.org/10.1056/NEJM200005183422007>

[PubMed]

Gomez de Agüero, M., Ganai-Vonarburg, S. C., Fuhrer, T., Rupp, S., Uchimura, Y., Li, H., Steinert, A., Heikenwalder, M., Hapfelmeier, S., Sauer, U., McCoy, K. D., & Macpherson, A. J. (2016). The maternal microbiota drives early postnatal innate immune development. *Science*, 351(6279), 1296–1302.

<https://doi.org/10.1126/science.aad2571>

[PubMed]

Good, M., Siggers, R. H., Sodhi, C. P., Afrazi, A., Alkhudari, F., Egan, C. E., Neal, M. D., Yazji, I., Jia, H., Lin, J., Branca, M. F., Ma, C., Prindle, T., Grant, Z., Shah, S., Slagle, D., 2nd, Paredes, J., Ozolek, J., Gittes, G. K., & Hackam, D. J. (2012). Amniotic fluid inhibits Toll-like receptor 4 signaling in the fetal and neonatal intestinal epithelium. *Proceedings of the National Academy of Sciences of the United States of America*, 109(28), 11330–11335. <https://doi.org/10.1073/pnas.1200856109>

[PubMed]

Goodrich, J. K., Waters, J. L., Poole, A. C., Sutter, J. L., Koren, O., Blekhman, R., Beaumont, M., Van Treuren, W., Knight, R., Bell, J. T., Spector, T. D., Clark, A. G., & Ley, R. E. (2014). Human genetics shape the gut microbiome. *Cell*, 159(4), 789–799. <https://doi.org/10.1016/j.cell.2014.09.053>

[PubMed]

Gopalakrishna, K. P., Macadangdang, B. R., Rogers, M. B., Tometich, J. T., Firek, B. A., Baker, R., Ji, J.,

Burr, A. H. P., Ma, C., Good, M., Morowitz, M. J., & Hand, T. W. (2019). Maternal IgA protects against the development of necrotizing enterocolitis in preterm infants. *Nature Medicine*, 25(7), 1110–1115. <https://doi.org/10.1038/s41591-019-0480-9>
[PubMed]

Gregory, K. E., Samuel, B. S., Houghteling, P., Shan, G., Ausubel, F. M., Sadreyev, R. I., & Walker, W. A. (2016). Influence of maternal breast milk ingestion on acquisition of the intestinal microbiome in preterm infants. *Microbiome*, 4(1), 68. <https://doi.org/10.1186/s40168-016-0214-x>
[PubMed]

Hackam, D. J., & Sodhi, C. P. (2022). Bench to bedside - new insights into the pathogenesis of necrotizing enterocolitis. *Nature Reviews. Gastroenterology & Hepatology*, 19(7), 468–479. <https://doi.org/10.1038/s41575-022-00594-x>

Henrick, B. M., Rodriguez, L., Lakshmikanth, T., Pou, C., Henckel, E., Arzoomand, A., Olin, A., Wang, J., Mikes, J., Tan, Z., Chen, Y., Ehrlich, A. M., Bernhardsson, A. K., Mugabo, C. H., Ambrosiani, Y., Gustafsson, A., Chew, S., Brown, H. K., Prambs, J., et al. (2021). Bifidobacteria-mediated immune system imprinting early in life. *Cell*, 184(15), 3884–3898 e3811. <https://doi.org/10.1016/j.cell.2021.05.030>
[PubMed]

Heppner, N., Reitmeier, S., Heddes, M., Merino, M. V., Schwartz, L., Dietrich, A., List, M., Gigl, M., Meng, C., van der Veen, D. R., Schirmer, M., Kleigrew, K., Omer, H., Kiessling, S., & Haller, D. (2024). Diurnal rhythmicity of infant fecal microbiota and metabolites: A randomized controlled interventional trial with infant formula. *Cell Host & Microbe*, 32(4), 573–587 e575. <https://doi.org/10.1016/j.chom.2024.02.015>

Hergott, C. B., Roche, A. M., Tamashiro, E., Clarke, T. B., Bailey, A. G., Laughlin, A., Bushman, F. D., & Weiser, J. N. (2016). Peptidoglycan from the gut microbiota governs the lifespan of circulating phagocytes at homeostasis. *Blood*, 127(20), 2460–2471. <https://doi.org/10.1182/blood-2015-10-675173>
[PubMed]

Hesla, H. M., Stenius, F., Jaderlund, L., Nelson, R., Engstrand, L., Alm, J., & Dicksved, J. (2014). Impact of lifestyle on the gut microbiota of healthy infants and their mothers-the ALADDIN birth cohort. *FEMS Microbiology Ecology*, 90(3), 791–801. <https://doi.org/10.1111/1574-6941.12434>
[PubMed]

Hesser, L. A., Puente, A. A., Arnold, J., Ionescu, E., Mirmira, A., Talasani, N., Lopez, J., Maccio-Maretto, L., Mimee, M., & Nagler, C. R. (2024). A synbiotic of *Anaerostipes caccae* and lactulose prevents and treats food allergy in mice. *Cell Host & Microbe*, 32(7), 1163–1176 e1166. <https://doi.org/10.1016/j.chom.2024.05.019>

Hill, C. J., Lynch, D. B., Murphy, K., Ulaszewska, M., Jeffery, I. B., O'Shea, C. A., Watkins, C., Dempsey, E., Mattivi, F., Tuohy, K., Ross, R. P., Ryan, C. A., O'Toole, P. W., & Stanton, C. (2017). Evolution of gut microbiota composition from birth to 24 weeks in the INFANTMET Cohort. *Microbiome*, 5(1), 4. <https://doi.org/10.1186/s40168-016-0213-y>
[PubMed]

Homann, C. M., Rossel, C. A. J., Dizzell, S., Bervoets, L., Simioni, J., Li, J., Gunn, E., Surette, M. G., de Souza, R. J., Mommers, M., Hutton, E. K., Morrison, K. M., Penders, J., van Best, N., & Stearns, J. C. (2021). Infants' first solid foods: Impact on gut microbiota development in two intercontinental cohorts. *Nutrients*, 13(8), 2639. <https://doi.org/10.3390/nu13082639>
[PubMed]

Hornef, M., & Penders, J. (2017). Does a prenatal bacterial microbiota exist? *Mucosal Immunology*, 10(3), 598–601. <https://doi.org/10.1038/mi.2016.141>
[PubMed]

Hornef, M. W., & Torow, N. (2020). 'Layered immunity' and the 'neonatal window of opportunity' - timed succession of non-redundant phases to establish mucosal host-microbial homeostasis after birth. *Immunology*, 159(1), 15–25. <https://doi.org/10.1111/imm.13149>
[PubMed]

Hu, M., Eviston, D., Hsu, P., Marino, E., Chidgey, A., Santner-Nanan, B., Wong, K., Richards, J. L., Yap, Y. A., Collier, F., Quinton, A., Joung, S., Peek, M., Benzie, R., Macia, L., Wilson, D., Ponsonby, A. L., Tang, M. L. K., O'Hely, M., et al. (2019). Decreased maternal serum acetate and impaired fetal thymic and regulatory T cell development in preeclampsia. *Nature Communications*, 10(1), 3031. <https://doi.org/10.1038/s41467-019-10703-1>
[PubMed]

Huang, L., Chen, Q., Zhao, Y., Wang, W., Fang, F., & Bao, Y. (2015). Is elective cesarean section associated with a higher risk of asthma? A meta-analysis. *Journal of Asthma: Official Journal of the Association for the Care of Asthma*, 52(1), 16–25. <https://doi.org/10.3109/02770903.2014.952435>
[PubMed]

Jakobsson, H. E., Abrahamsson, T. R., Jenmalm, M. C., Harris, K., Quince, C., Jernberg, C., Bjorksten, B., Engstrand, L., & Andersson, A. F. (2014). Decreased gut microbiota diversity, delayed Bacteroidetes colonisation and reduced Th1 responses in infants delivered by caesarean section. *Gut*, 63(4), 559–566. <https://doi.org/10.1136/gutjnl-2012-303249>
[PubMed]

Jimenez, E., Fernandez, L., Marin, M. L., Martin, R., Odriozola, J. M., Nueno-Palop, C., Narbad, A., Olivares, M., Xaus, J., & Rodriguez, J. M. (2005). Isolation of commensal bacteria from umbilical cord blood of healthy neonates born by cesarean section. *Current Microbiology*, 51(4), 270–274. <https://doi.org/10.1007/s00284-005-0020-3>
[PubMed]

Jimenez, E., Marin, M. L., Martin, R., Odriozola, J. M., Olivares, M., Xaus, J., Fernandez, L., & Rodriguez, J. M. (2008). Is meconium from healthy newborns actually sterile? *Research in Microbiology*, 159(3), 187–193. <https://doi.org/10.1016/j.resmic.2007.12.007>
[PubMed]

Johnson-Hence, C. B., Gopalakrishna, K. P., Bodkin, D., Coffey, K. E., Burr, A. H. P., Rahman, S., Rai, A. T., Abbott, D. A., Sosa, Y. A., Tometich, J. T., Das, J., & Hand, T. W. (2023). Stability and heterogeneity in the antimicrobiota reactivity of human milk-derived immunoglobulin A. *The Journal of Experimental Medicine*, 220(8), e20220839. <https://doi.org/10.1084/jem.20220839>

[PubMed]

Kamal, S. S., Hyldig, N., Krych, L., Greisen, G., Krogfelt, K. A., Zachariassen, G., & Nielsen, D. S. (2019). Impact of early exposure to cefuroxime on the composition of the gut microbiota in infants following cesarean delivery. *The Journal of Pediatrics*, 210, 99–105e102. <https://doi.org/10.1016/j.jpeds.2019.03.001>

[PubMed]

Karlsson, C. L., Molin, G., Cilio, C. M., & Ahrne, S. (2011). The pioneer gut microbiota in human neonates vaginally born at term-a pilot study. *Pediatric Research*, 70(3), 282–286. <https://doi.org/10.1203/PDR.0b013e318225f765>

[PubMed]

Karlstrom, A., Lindgren, H., & Hildingsson, I. (2013). Maternal and infant outcome after caesarean section without recorded medical indication: Findings from a Swedish case-control study. *BJOG*, 120(4), 479–486; discussion 486. <https://doi.org/10.1111/1471-0528.12129>

[PubMed]

Kashtanova, D. A., Popenko, A. S., Tkacheva, O. N., Tyakht, A. B., Alexeev, D. G., & Boytsov, S. A. (2016). Association between the gut microbiota and diet: Fetal life, early childhood, and further life. *Nutrition*, 32(6), 620–627. <https://doi.org/10.1016/j.nut.2015.12.037>

[PubMed]

Kennedy, K. M., Gerlach, M. J., Adam, T., Heimesaat, M. M., Rossi, L., Surette, M. G., Sloboda, D. M., & Braun, T. (2021). Fetal meconium does not have a detectable microbiota before birth. *Nature Microbiology*, 6(7), 865–873. <https://doi.org/10.1038/s41564-021-00904-0>

[PubMed]

Kennedy, K. M., de Goffau, M. C., Perez-Munoz, M. E., Arrieta, M. C., Backhed, F., Bork, P., Braun, T., Bushman, F. D., Dore, J., de Vos, W. M., Earl, A. M., Eisen, J. A., Elovitz, M. A., Ganai-Vonarburg, S. C., Ganzle, M. G., Garrett, W. S., Hall, L. J., Hornef, M. W., Huttenhower, C., et al. (2023). Questioning the fetal microbiome illustrates pitfalls of low-biomass microbial studies. *Nature*, 613(7945), 639–649. <https://doi.org/10.1038/s41586-022-05546-8>

[PubMed]

Kim, Y. G., Sakamoto, K., Seo, S. U., Pickard, J. M., Gilliland, M. G., 3rd, Pudlo, N. A., Hoostal, M., Li, X., Wang, T. D., Feehley, T., Stefka, A. T., Schmidt, T. M., Martens, E. C., Fukuda, S., Inohara, N., Nagler, C. R., & Nunez, G. (2017). Neonatal acquisition of Clostridia species protects against colonization by bacterial pathogens. *Science*, 356(6335), 315–319. <https://doi.org/10.1126/science.aag2029>

[PubMed]

Koenig, J. E., Spor, A., Scalfone, N., Fricker, A. D., Stombaugh, J., Knight, R., Angenent, L. T., & Ley, R. E. (2011). Succession of microbial consortia in the developing infant gut microbiome. *Proceedings of the National Academy of Sciences of the United States of America*, 108(Suppl 1), 4578–4585.

<https://doi.org/10.1073/pnas.1000081107>

[PubMed]

Koplin, J. J., Dharmage, S. C., Ponsonby, A. L., Tang, M. L., Lowe, A. J., Gurrin, L. C., Osborne, N. J., Martin, P. E., Robinson, M. N., Wake, M., Hill, D. J., Allen, K. J., & HealthNuts, I. (2012).

Environmental and demographic risk factors for egg allergy in a population-based study of infants. *Allergy*, 67(11), 1415–1422. <https://doi.org/10.1111/all.12015> [PubMed]

Korpela, K., Helve, O., Kolho, K. L., Saisto, T., Skogberg, K., Dikareva, E., Stefanovic, V., Salonen, A., Andersson, S., & de Vos, W. M. (2020). Maternal fecal microbiota transplantation in cesarean-born infants rapidly restores Normal gut microbial development: A proof-of-concept study. *Cell*, 183(2), 324–334e325. <https://doi.org/10.1016/j.cell.2020.08.047> [PubMed]

Korpela, K., Hurley, S., Ford, S. A., Franklin, R., Byrne, S., Lunjani, N., Forde, B., Neogi, U., Venter, C., Walter, J., Hourihane, J., O'Mahony, L., & CORAL Study Group. (2024). Association between gut microbiota development and allergy in infants born during pandemic-related social distancing restrictions. *Allergy*, 79(7), 1938–1951. <https://doi.org/10.1111/all.16069> [PubMed]

Kotloff, K. L., Nataro, J. P., Blackwelder, W. C., Nasrin, D., Farag, T. H., Panchalingam, S., Wu, Y., Sow, S. O., Sur, D., Breiman, R. F., Faruque, A. S., Zaidi, A. K., Saha, D., Alonso, P. L., Tamboura, B., Sanogo, D., Onwuchekwa, U., Manna, B., Ramamurthy, T., et al. (2013). Burden and aetiology of diarrhoeal disease in infants and young children in developing countries (the Global Enteric Multicenter Study, GEMS): A prospective, case-control study. *Lancet*, 382(9888), 209–222. [https://doi.org/10.1016/S0140-6736\(13\)60844-2](https://doi.org/10.1016/S0140-6736(13)60844-2) [PubMed]

Kuhle, S., Tong, O. S., & Woolcott, C. G. (2015). Association between caesarean section and childhood obesity: A systematic review and meta-analysis. *Obesity Reviews*, 16(4), 295–303. <https://doi.org/10.1111/obr.12267> [PubMed]

Kuitunen, M., Kukkonen, K., Juntunen-Backman, K., Korpela, R., Poussa, T., Tuure, T., Haahtela, T., & Savilahti, E. (2009). Probiotics prevent IgE-associated allergy until age 5 years in cesarean-delivered children but not in the total cohort. *The Journal of Allergy and Clinical Immunology*, 123(2), 335–341. <https://doi.org/10.1016/j.jaci.2008.11.019> [PubMed]

Lagkouvardos, I., Pukall, R., Abt, B., Foesel, B. U., Meier-Kolthoff, J. P., Kumar, N., Bresciani, A., Martinez, I., Just, S., Ziegler, C., Brugiroux, S., Garzetti, D., Wenning, M., Bui, T. P., Wang, J., Hugenholtz, F., Plugge, C. M., Peterson, D. A., Hornef, M. W., et al. (2016). The Mouse Intestinal Bacterial Collection (miBC) provides host-specific insight into cultured diversity and functional potential of the gut microbiota. *Nature Microbiology*, 1(10), 16131. <https://doi.org/10.1038/nmicrobiol.2016.131> [PubMed]

Lapin, B., Piorkowski, J., Ownby, D., Freels, S., Chavez, N., Hernandez, E., Wagner-Cassanova, C., Pelzel, D., Vergara, C., & Persky, V. (2015). Relationship between prenatal antibiotic use and asthma in at-risk children. *Annals of Allergy, Asthma & Immunology*, 114(3), 203–207. <https://doi.org/10.1016/j.anai.2014.11.014>

Lauder, A. P., Roche, A. M., Sherrill-Mix, S., Bailey, A., Laughlin, A. L., Bittinger, K., Leite, R., Elovitz, M.

A., Parry, S., & Bushman, F. D. (2016). Comparison of placenta samples with contamination controls does not provide evidence for a distinct placenta microbiota. *Microbiome*, 4(1), 29. <https://doi.org/10.1186/s40168-016-0172-3> [PubMed]

Lawson, M. A. E., O'Neill, I. J., Kujawska, M., Gowrinadh Javvadi, S., Wijeyesekera, A., Flegg, Z., Chalklen, L., & Hall, L. J. (2020). Breast milk-derived human milk oligosaccharides promote Bifidobacterium interactions within a single ecosystem. *The ISME Journal*, 14(2), 635–648. <https://doi.org/10.1038/s41396-019-0553-2> [PubMed]

Leiby, J. S., McCormick, K., Sherrill-Mix, S., Clarke, E. L., Kessler, L. R., Taylor, L. J., Hofstaedter, C. E., Roche, A. M., Mattei, L. M., Bittinger, K., Elovitz, M. A., Leite, R., Parry, S., & Bushman, F. D. (2018). Lack of detection of a human placenta microbiome in samples from preterm and term deliveries. *Microbiome*, 6(1), 196. <https://doi.org/10.1186/s40168-018-0575-4> [PubMed]

Lemoine, A., Tounian, P., Adel-Patient, K., & Thomas, M. (2023). Pre-, pro-, syn-, and postbiotics in infant formulas: What are the immune benefits for infants? *Nutrients*, 15(5), 1231. <https://doi.org/10.3390/nu15051231> [PubMed]

Li, X., Ning, X., Rui, B., Wang, Y., Lei, Z., Yu, D., Liu, F., Deng, Y., Yuan, J., Li, W., Yan, J., & Li, M. (2023). Alterations of milk oligosaccharides in mothers with gestational diabetes mellitus impede colonization of beneficial bacteria and development of RORgammat(+) Treg cell-mediated immune tolerance in neonates. *Gut Microbes*, 15(2), 2256749. <https://doi.org/10.1080/19490976.2023.2256749> [PubMed]

Liao, S. L., Tsai, M. H., Yao, T. C., Hua, M. C., Yeh, K. W., Chiu, C. Y., Su, K. W., Huang, S. Y., Kao, C. C., Lai, S. H., & Huang, J. L. (2017). Caesarean section is associated with reduced perinatal cytokine response, increased risk of bacterial colonization in the airway, and infantile wheezing. *Scientific Reports*, 7(1), 9053. <https://doi.org/10.1038/s41598-017-07894-2> [PubMed]

Lindner, C., Thomsen, I., Wahl, B., Ugur, M., Sethi, M. K., Friedrichsen, M., Smoczek, A., Ott, S., Baumann, U., Suerbaum, S., Schreiber, S., Bleich, A., Gaboriau-Routhiau, V., Cerf-Bensussan, N., Hazanov, H., Mehr, R., Boysen, P., Rosenstiel, P., & Pabst, O. (2015). Diversification of memory B cells drives the continuous adaptation of secretory antibodies to gut microbiota. *Nature Immunology*, 16(8), 880–888. <https://doi.org/10.1038/ni.3213> [PubMed]

Liu, L., Johnson, H. L., Cousens, S., Perin, J., Scott, S., Lawn, J. E., Rudan, I., Campbell, H., Cibulskis, R., Li, M., Mathers, C., Black, R. E., & Child Health Epidemiology Reference Group of WHO and Unicef. (2012). Global, regional, and national causes of child mortality: An updated systematic analysis for 2010 with time trends since 2000. *Lancet*, 379(9832), 2151–2161. [https://doi.org/10.1016/S0140-6736\(12\)60560-1](https://doi.org/10.1016/S0140-6736(12)60560-1) [PubMed]

Lotz, M., Gutle, D., Walther, S., Menard, S., Bogdan, C., & Hornef, M. W. (2006). Postnatal acquisition of endotoxin tolerance in intestinal epithelial cells. *The Journal of Experimental Medicine*, 203(4), 973–984. <https://doi.org/10.1084/jem.20050625>
[PubMed]

Lubin, J. B., Green, J., Maddux, S., Denu, L., Duranova, T., Lanza, M., Wynosky-Dolfi, M., Flores, J. N., Grimes, L. P., Brodsky, I. E., Planet, P. J., & Silverman, M. A. (2023). Arresting microbiome development limits immune system maturation and resistance to infection in mice. *Cell Host & Microbe*, 31(4), 554–570 e557. <https://doi.org/10.1016/j.chom.2023.03.006>

Magnus, M. C., Haberg, S. E., Stigum, H., Nafstad, P., London, S. J., Vangen, S., & Nystad, W. (2011). Delivery by Cesarean section and early childhood respiratory symptoms and disorders: The Norwegian mother and child cohort study. *American Journal of Epidemiology*, 174(11), 1275–1285. <https://doi.org/10.1093/aje/kwr242>
[PubMed]

Martin, R., Langa, S., Reviriego, C., Jiminez, E., Marin, M. L., Xaus, J., Fernandez, L., & Rodriguez, J. M. (2003). Human milk is a source of lactic acid bacteria for the infant gut. *The Journal of Pediatrics*, 143(6), 754–758. <https://doi.org/10.1016/j.jpeds.2003.09.028>
[PubMed]

Martinez, I., Maldonado-Gomez, M. X., Gomes-Neto, J. C., Kittana, H., Ding, H., Schmaltz, R., Joglekar, P., Cardona, R. J., Marsteller, N. L., Kembel, S. W., Benson, A. K., Peterson, D. A., Ramer-Tait, A. E., & Walter, J. (2018). Experimental evaluation of the importance of colonization history in early-life gut microbiota assembly. *eLife*, 7, e36521. <https://doi.org/10.7554/eLife.36521>
[PubMed]

Matsuki, T., Tajima, S., Hara, T., Yahagi, K., Ogawa, E., & Kodama, H. (2016). Infant formula with galacto-oligosaccharides (OM55N) stimulates the growth of indigenous bifidobacteria in healthy term infants. *Beneficial Microbes*, 7(4), 453–461. <https://doi.org/10.3920/BM2015.0168>
[PubMed]

Miller, J., Tonkin, E., Damarell, R. A., McPhee, A. J., Suganuma, M., Suganuma, H., Middleton, P. F., Makrides, M., & Collins, C. T. (2018). A systematic review and meta-analysis of human milk feeding and morbidity in very low birth weight infants. *Nutrients*, 10(6), 707. <https://doi.org/10.3390/nu10060707>
[PubMed]

Mishra, A., Lai, G. C., Yao, L. J., Aung, T. T., Shental, N., Rotter-Maskowitz, A., Shepherdson, E., Singh, G. S. N., Pai, R., Shanti, A., Wong, R. M. M., Lee, A., Khyriem, C., Dutertre, C. A., Chakarov, S., Srinivasan, K. G., Shadan, N. B., Zhang, X. M., Khalilnezhad, S., et al. (2021). Microbial exposure during early human development primes fetal immune cells. *Cell*, 184(13), 3394–3409 e3320. <https://doi.org/10.1016/j.cell.2021.04.039>
[PubMed]

Mitchell, C. M., Mazzoni, C., Hogstrom, L., Bryant, A., Bergerat, A., Cher, A., Pochan, S., Herman, P., Carrigan, M., Sharp, K., Huttenhower, C., Lander, E. S., Vlamakis, H., Xavier, R. J., & Yassour, M. (2020). Delivery mode affects stability of early infant gut microbiota. *Cell Reports Medicine*, 1(9), 100156. <https://doi.org/10.1016/j.xcrm.2020.100156>

[[PubMed](#)]

Morgan, R. L., Preidis, G. A., Kashyap, P. C., Weizman, A. V., Sadeghirad, B., McMaster Probiotic, P., & Synbiotic Work, G. (2020). Probiotics reduce mortality and morbidity in preterm, low-birth-weight infants: A systematic review and network meta-analysis of randomized trials.

Gastroenterology, 159(2), 467–480. <https://doi.org/10.1053/j.gastro.2020.05.096>

[[PubMed](#)]

Mueller, N. T., Differding, M. K., Sun, H., Wang, J., Levy, S., Deopujari, V., Appel, L. J., Blaser, M. J., Kundu, T., Shah, A. A., Dominguez Bello, M. G., & Hourigan, S. K. (2023). Maternal bacterial engraftment in multiple body sites of cesarean section born neonates after vaginal seeding-a randomized controlled Trial. *mBio*, 14(3), e0049123. <https://doi.org/10.1128/mbio.00491-23>

[[PubMed](#)]

Nielsen, C. C., Gascon, M., Osornio-Vargas, A. R., Shier, C., Guttman, D. S., Becker, A. B., Azad, M. B., Sears, M. R., Lefebvre, D. L., Moraes, T. J., Turvey, S. E., Subbarao, P., Takaro, T. K., Brook, J. R., Scott, J. A., Mandhane, P. J., Tun, H. M., & Kozyskyj, A. L. (2020). Natural environments in the urban context and gut microbiota in infants. *Environment International*, 142, 105881. <https://doi.org/10.1016/j.envint.2020.105881>

[[PubMed](#)]

[[PubMed](#)]

Olm, M. R., Dahan, D., Carter, M. M., Merrill, B. D., Yu, F. B., Jain, S., Meng, X., Tripathi, S., Wastyk, H., Neff, N., Holmes, S., Sonnenburg, E. D., Jha, A. R., & Sonnenburg, J. L. (2022). Robust variation in infant gut microbiome assembly across a spectrum of lifestyles. *Science*, 376(6598), 1220–1223.

<https://doi.org/10.1126/science.abj2972>

[[PubMed](#)]

Olsan, E. E., Byndloss, M. X., Faber, F., Rivera-Chavez, F., Tsolis, R. M., & Baumler, A. J. (2017). Colonization resistance: The deconvolution of a complex trait. *The Journal of Biological Chemistry*, 292(21), 8577–8581. <https://doi.org/10.1074/jbc.R116.752295>

[[PubMed](#)]

Olszak, T., An, D., Zeissig, S., Vera, M. P., Richter, J., Franke, A., Glickman, J. N., Siebert, R., Baron, R. M., Kasper, D. L., & Blumberg, R. S. (2012). Microbial exposure during early life has persistent effects on natural killer T cell function. *Science*, 336(6080), 489–493. <https://doi.org/10.1126/science.1219328>

[[PubMed](#)]

[[PubMed](#)]

Osbelt, L., Almasi, E. D. H., Wende, M., Kienesberger, S., Voltz, A., Lesker, T. R., Muthukumarasamy, U., Knischewski, N., Nordmann, E., Bielecka, A. A., Giralt-Zuniga, M., Kaganovitch, E., Kuhne, C., Baier, C., Pietsch, M., Musken, M., Greweling-Pils, M. C., Breinbauer, R., Flieger, A., et al. (2024). *Klebsiella oxytoca* inhibits *Salmonella* infection through multiple microbiota-context-dependent mechanisms. *Nature Microbiology*, 9(7), 1792–1811. <https://doi.org/10.1038/s41564-024-01710-0>

[[PubMed](#)]

[[PubMed](#)]

Ozkul, C., Ruiz, V. E., Battaglia, T., Xu, J., Roubaud-Baudron, C., Cadwell, K., Perez-Perez, G. I., & Blaser, M. J. (2020). A single early-in-life antibiotic course increases susceptibility to DSS-induced colitis. *Genome Medicine*, 12(1), 65. <https://doi.org/10.1186/s13073-020-00764-z>

[PubMed]

Pacheco Da Silva, E., Weinmann, T., Gerlich, J., Weinmayr, G., Genuneit, J., Nowak, D., von Mutius, E., Vogelberg, C., Radon, K., & Forster, F. (2024). Exposure profiles for the long-term use of disinfectants and cleaning products and asthma. *Allergy*, 80(4), 996–1005. <https://doi.org/10.1111/all.16456>

[PubMed]

Palm, N. W., de Zoete, M. R., Cullen, T. W., Barry, N. A., Stefanowski, J., Hao, L., Degnan, P. H., Hu, J., Peter, I., Zhang, W., Ruggiero, E., Cho, J. H., Goodman, A. L., & Flavell, R. A. (2014). Immunoglobulin A coating identifies colitogenic bacteria in inflammatory bowel disease. *Cell*, 158(5), 1000–1010. <https://doi.org/10.1016/j.cell.2014.08.006>

[PubMed]

Palmer, C., Bik, E. M., DiGiulio, D. B., Relman, D. A., & Brown, P. O. (2007). Development of the human infant intestinal microbiota. *PLoS Biology*, 5(7), e177. <https://doi.org/10.1371/journal.pbio.0050177>

[PubMed]

Panigrahi, P., Parida, S., Nanda, N. C., Satpathy, R., Pradhan, L., Chandel, D. S., Baccaglini, L., Mohapatra, A., Mohapatra, S. S., Misra, P. R., Chaudhry, R., Chen, H. H., Johnson, J. A., Morris, J. G., Paneth, N., & Gewolb, I. H. (2017). A randomized synbiotic trial to prevent sepsis among infants in rural India. *Nature*, 548(7668), 407–412. <https://doi.org/10.1038/nature23480>

[PubMed]

Pax, K., Buduneli, N., Alan, M., Meric, P., Gurlek, O., Dabdoub, S. M., & Kumar, P. S. (2024). Placental TLR recognition of salivary and subgingival microbiota is associated with pregnancy complications. *Microbiome*, 12(1), 64. <https://doi.org/10.1186/s40168-024-01761-9>

[PubMed]

Penders, J., Thijs, C., Vink, C., Stelma, F. F., Snijders, B., Kummeling, I., van den Brandt, P. A., & Stobberingh, E. E. (2006). Factors influencing the composition of the intestinal microbiota in early infancy. *Pediatrics*, 118(2), 511–521. <https://doi.org/10.1542/peds.2005-2824>

[PubMed]

Penders, J., Gerhold, K., Stobberingh, E. E., Thijs, C., Zimmermann, K., Lau, S., & Hamelmann, E. (2013). Establishment of the intestinal microbiota and its role for atopic dermatitis in early childhood. *The Journal of Allergy and Clinical Immunology*, 132(3), 601–607e608. <https://doi.org/10.1016/j.jaci.2013.05.043>

[PubMed]

Perez-Munoz, M. E., Arrieta, M. C., Ramer-Tait, A. E., & Walter, J. (2017). A critical assessment of the “sterile womb” and “in utero colonization” hypotheses: Implications for research on the pioneer infant microbiome. *Microbiome*, 5(1), 48. <https://doi.org/10.1186/s40168-017-0268-4>

[PubMed]

Pivrcova, E., Kotaskova, I., & Thon, V. (2022). Neonatal diet and gut microbiome development after C-section during the first three months after birth: A systematic review. *Frontiers in Nutrition*, 9, 941549. <https://doi.org/10.3389/fnut.2022.941549>

[[PubMed](#)]

Qin, J., Li, Y., Cai, Z., Li, S., Zhu, J., Zhang, F., Liang, S., Zhang, W., Guan, Y., Shen, D., Peng, Y., Zhang, D., Jie, Z., Wu, W., Qin, Y., Xue, W., Li, J., Han, L., Lu, D., et al. (2012). A metagenome-wide association study of gut microbiota in type 2 diabetes. *Nature*, 490(7418), 55–60. <https://doi.org/10.1038/nature11450>

[[PubMed](#)]

Quigley, M., Embleton, N. D., Meader, N., & McGuire, W. (2024). Donor human milk for preventing necrotising enterocolitis in very preterm or very low-birthweight infants. *Cochrane Database of Systematic Reviews*, 9(9), CD002971. <https://doi.org/10.1002/14651858.CD002971.pub6>

[[PubMed](#)]

Rackaityte, E., Halkias, J., Fukui, E. M., Mendoza, V. F., Hayzelden, C., Crawford, E. D., Fujimura, K. E., Burt, T. D., & Lynch, S. V. (2020). Viable bacterial colonization is highly limited in the human intestine in utero. *Nature Medicine*, 26(4), 599–607. <https://doi.org/10.1038/s41591-020-0761-3>

[[PubMed](#)]

Rautava, S., Kainonen, E., Salminen, S., & Isolauri, E. (2012). Maternal probiotic supplementation during pregnancy and breast-feeding reduces the risk of eczema in the infant. *The Journal of Allergy and Clinical Immunology*, 130(6), 1355–1360. <https://doi.org/10.1016/j.jaci.2012.09.003>

[[PubMed](#)]

Rawat, S. S., Narain, N. P., Marathe, S. M., Sonawale, S. B., & Veligandla, K. C. (2023). Early-life antibiotics and childhood obesity: Yeast probiotics as a strategy to modulate gut microbiota. *Cureus*, 15(3), e36795. <https://doi.org/10.7759/cureus.36795>

[[PubMed](#)]

Reinhardt, C., Reigstad, C. S., & Backhed, F. (2009). Intestinal microbiota during infancy and its implications for obesity. *Journal of Pediatric Gastroenterology and Nutrition*, 48(3), 249–256.

<https://doi.org/10.1097/mpg.0b013e318183187c>

[[PubMed](#)]

Riedler, J., Braun-Fahrlander, C., Eder, W., Schreuer, M., Waser, M., Maisch, S., Carr, D., Schierl, R., Nowak, D., von Mutius, E., & ALEX Study Team. (2001). Exposure to farming in early life and development of asthma and allergy: A cross-sectional survey. *The Lancet*, 358(9288), 1129–1133.

[https://doi.org/10.1016/S0140-6736\(01\)06252-3](https://doi.org/10.1016/S0140-6736(01)06252-3)

Rodriguez, J. M. (2014). The origin of human milk bacteria: Is there a bacterial entero-mammary pathway during late pregnancy and lactation? *Advances in Nutrition*, 5(6), 779–784. <https://doi.org/10.3945/an.114.007229>

[[PubMed](#)]

Rook, G. A. (2010). 99th Dahlem conference on infection, inflammation and chronic inflammatory disorders: Darwinian medicine and the ‘hygiene’ or ‘old friends’ hypothesis. *Clinical and Experimental Immunology*, 160(1), 70–79. <https://doi.org/10.1111/j.1365-2249.2010.04133.x>

[[PubMed](#)]

Roswall, J., Olsson, L. M., Kovatcheva-Datchary, P., Nilsson, S., Tremaroli, V., Simon, M. C., Kiilerich,

P., Akrami, R., Kramer, M., Uhlen, M., Gummesson, A., Kristiansen, K., Dahlgren, J., & Backhed, F. (2021). Developmental trajectory of the healthy human gut microbiota during the first 5 years of life. *Cell Host & Microbe*, 29(5), 765–776. e763. <https://doi.org/10.1016/j.chom.2021.02.021>

Round, J. L., Lee, S. M., Li, J., Tran, G., Jabri, B., Chatila, T. A., & Mazmanian, S. K. (2011). The Toll-like receptor 2 pathway establishes colonization by a commensal of the human microbiota. *Science*, 332(6032), 974–977. <https://doi.org/10.1126/science.1206095>
[PubMed]

Roze, J. C., Ancel, P. Y., Lepage, P., Martin-Marchand, L., Al Nabhani, Z., Delannoy, J., Picaud, J. C., Lapillonne, A., Aires, J., Durox, M., Darmaun, D., Neu, J., Butel, M. J., & Nutrition EPIPAGE 2 study group and EPIFLORE Study Group. (2017). Nutritional strategies and gut microbiota composition as risk factors for necrotizing enterocolitis in very-preterm infants. *The American Journal of Clinical Nutrition*, 106(3), 821–830. <https://doi.org/10.3945/ajcn.117.152967>
[PubMed]

Ruiz, V. E., Battaglia, T., Kurtz, Z. D., Bijmens, L., Ou, A., Engstrand, I., Zheng, X., Iizumi, T., Mullins, B. J., Muller, C. L., Cadwell, K., Bonneau, R., Perez-Perez, G. I., & Blaser, M. J. (2017). A single early-in-life macrolide course has lasting effects on murine microbial network topology and immunity. *Nature Communications*, 8(1), 518. <https://doi.org/10.1038/s41467-017-00531-6>
[PubMed]

Russell, S. L., Gold, M. J., Hartmann, M., Willing, B. P., Thorson, L., Wlodarska, M., Gill, N., Blanchet, M. R., Mohn, W. W., McNagny, K. M., & Finlay, B. B. (2012). Early life antibiotic-driven changes in microbiota enhance susceptibility to allergic asthma. *EMBO Reports*, 13(5), 440–447. <https://doi.org/10.1038/embor.2012.32>
[PubMed]

Rutten, N. B., Rijkers, G. T., Meijssen, C. B., Crijns, C. E., Oudshoorn, J. H., van der Ent, C. K., & Vlieger, A. M. (2015). Intestinal microbiota composition after antibiotic treatment in early life: The INCA study. *BMC Pediatrics*, 15, 204. <https://doi.org/10.1186/s12887-015-0519-0>
[PubMed]

Samara, J., Moossavi, S., Alshaikh, B., Ortega, V. A., Pettersen, V. K., Ferdous, T., Hoops, S. L., Soraisham, A., Vayalumkal, J., Dersch-Mills, D., Gerber, J. S., Mukhopadhyay, S., Puopolo, K., Tompkins, T. A., Knights, D., Walter, J., Amin, H., & Arrieta, M. C. (2022). Supplementation with a probiotic mixture accelerates gut microbiome maturation and reduces intestinal inflammation in extremely preterm infants. *Cell Host & Microbe*, 30(5), 696–711.e695. <https://doi.org/10.1016/j.chom.2022.04.005>

Sarzotti, M., Robbins, D. S., & Hoffman, P. M. (1996). Induction of protective CTL responses in newborn mice by a murine retrovirus. *Science*, 271(5256), 1726–1728. <https://doi.org/10.1126/science.271.5256.1726>
[PubMed]

Selma-Royo, M., Dubois, L., Manara, S., Armanini, F., Cabrera-Rubio, R., Valles-Colomer, M., Gonzalez, S., Parra-Llorca, A., Escuriet, R., Bode, L., Martinez-Costa, C., Segata, N., & Collado, M. C. (2024). Birthmode and environment-dependent microbiota transmission dynamics are

complemented by breastfeeding during the first year. *Cell Host & Microbe*, 32(6), 996–1010 e1014. <https://doi.org/10.1016/j.chom.2024.05.005>

Shao, Y., Forster, S. C., Tsaliki, E., Vervier, K., Strang, A., Simpson, N., Kumar, N., Stares, M. D., Rodger, A., Brocklehurst, P., Field, N., & Lawley, T. D. (2019). Stunted microbiota and opportunistic pathogen colonization in caesarean-section birth. *Nature*, 574(7776), 117–121. <https://doi.org/10.1038/s41586-019-1560-1>
[PubMed]

Sharif, S., Meader, N., Oddie, S. J., Rojas-Reyes, M. X., & McGuire, W. (2020). Probiotics to prevent necrotising enterocolitis in very preterm or very low birth weight infants. *Cochrane Database of Systematic Reviews*, 10(10), CD005496. <https://doi.org/10.1002/14651858.CD005496.pub5>
[PubMed]

Song, S. J., Wang, J., Martino, C., Jiang, L., Thompson, W. K., Shenhav, L., McDonald, D., Marotz, C., Harris, P. R., Hernandez, C. D., Henderson, N., Ackley, E., Nardella, D., Gillihan, C., Montacuti, V., Schweizer, W., Jay, M., Combellick, J., Sun, H., et al. (2021). Naturalization of the microbiota developmental trajectory of Cesarean-born neonates after vaginal seeding. *The Medicus*, 2(8), 951–964e955. <https://doi.org/10.1016/j.medj.2021.05.003>

South, M. A., Warwick, W. J., Wolheim, F. A., & Good, R. A. (1967). The IgA system. 3. IgA levels in the serum and saliva of pediatric patients--evidence for a local immunological system. *The Journal of Pediatrics*, 71(5), 645–653. [https://doi.org/10.1016/s0022-3476\(67\)80199-9](https://doi.org/10.1016/s0022-3476(67)80199-9)
[PubMed]

Sprockett, D., Fukami, T., & Relman, D. A. (2018). Role of priority effects in the early-life assembly of the gut microbiota. *Nature Reviews. Gastroenterology & Hepatology*, 15(4), 197–205. <https://doi.org/10.1038/nrgastro.2017.173>

Stearns, J. C., Simioni, J., Gunn, E., McDonald, H., Holloway, A. C., Thabane, L., Mousseau, A., Schertzer, J. D., Ratcliffe, E. M., Rossi, L., Surette, M. G., Morrison, K. M., & Hutton, E. K. (2017). Intrapartum antibiotics for GBS prophylaxis alter colonization patterns in the early infant gut microbiome of low risk infants. *Scientific Reports*, 7(1), 16527. <https://doi.org/10.1038/s41598-017-16606-9>
[PubMed]

Stefka, A. T., Feehley, T., Tripathi, P., Qiu, J., McCoy, K., Mazmanian, S. K., Tjota, M. Y., Seo, G. Y., Cao, S., Theriault, B. R., Antonopoulos, D. A., Zhou, L., Chang, E. B., Fu, Y. X., & Nagler, C. R. (2014). Commensal bacteria protect against food allergen sensitization. *Proceedings of the National Academy of Sciences of the United States of America*, 111(36), 13145–13150. <https://doi.org/10.1073/pnas.1412008111>
[PubMed]

Stewart, C. J., Ajami, N. J., O'Brien, J. L., Hutchinson, D. S., Smith, D. P., Wong, M. C., Ross, M. C., Lloyd, R. E., Doddapaneni, H., Metcalf, G. A., Muzny, D., Gibbs, R. A., Vatanen, T., Huttenhower, C., Xavier, R. J., Rewers, M., Hagopian, W., Toppari, J., Ziegler, A. G., et al. (2018). Temporal development of the gut microbiome in early childhood from the TEDDY study. *Nature*, 562(7728), 583–588. <https://doi.org/10.1038/s41586-018-0617-x>
[PubMed]

Strachan, D. P. (1989). Hay fever, hygiene, and household size. *BMJ*, 299(6710), 1259–1260.

<https://doi.org/10.1136/bmj.299.6710.1259>

[PubMed]

Theis, K. R., Romero, R., Greenberg, J. M., Winters, A. D., Garcia-Flores, V., Motomura, K., Ahmad, M. M., Galaz, J., Arenas-Hernandez, M., & Gomez-Lopez, N. (2020). No consistent evidence for microbiota in murine placental and fetal tissues. *mSphere*, 5(1), e00933–e00919. <https://doi.org/10.1128/mSphere.00933-19>

[PubMed]

Thiemann, S., Smit, N., & Strowig, T. (2016). Antibiotics and the intestinal microbiome: Individual responses, resilience of the ecosystem, and the susceptibility to infections. *Current Topics in Microbiology and Immunology*, 398, 123–146. https://doi.org/10.1007/82_2016_504

[PubMed]

Thome, J. J., Bickham, K. L., Ohmura, Y., Kubota, M., Matsuoka, N., Gordon, C., Granot, T., Griesemer, A., Lerner, H., Kato, T., & Farber, D. L. (2016). Early-life compartmentalization of human T cell differentiation and regulatory function in mucosal and lymphoid tissues. *Nature Medicine*, 22(1), 72–77. <https://doi.org/10.1038/nm.4008>

[PubMed]

Torow, N., Yu, K., Hassani, K., Freitag, J., Schulz, O., Basic, M., Brennecke, A., Sparwasser, T., Wagner, N., Bleich, A., Lochner, M., Weiss, S., Forster, R., Pabst, O., & Hornef, M. W. (2015). Active suppression of intestinal CD4(+)TCRalpha(+) T-lymphocyte maturation during the postnatal period. *Nature Communications*, 6, 7725. <https://doi.org/10.1038/ncomms8725>

[PubMed]

Torow, N., Hand, T. W., & Hornef, M. W. (2023a). Programmed and environmental determinants driving neonatal mucosal immune development. *Immunity*, 56(3), 485–499. <https://doi.org/10.1016/j.immuni.2023.02.013>

[PubMed]

Torow, N., Li, R., Hitch, T. C. A., Mingels, C., Al Bounny, S., van Best, N., Stange, E. L., Simons, B., Maie, T., Ruttger, L., Gubbi, N., Abbott, D. A., Benabid, A., Gadermayr, M., Runge, S., Treichel, N., Merhof, D., Rosshart, S. P., Jehmlich, N., et al. (2023b). M cell maturation and cDC activation determine the onset of adaptive immune priming in the neonatal Peyer's patch. *Immunity*, 56(6), 1220–1238e1227. <https://doi.org/10.1016/j.immuni.2023.04.002>

[PubMed]

Toscano, M., De Grandi, R., Peroni, D. G., Grossi, E., Facchin, V., Comberiati, P., & Drago, L. (2017). Impact of delivery mode on the colostrum microbiota composition. *BMC Microbiology*, 17(1), 205. <https://doi.org/10.1186/s12866-017-1109-0>

[PubMed]

Toubon, G., Patin, C., Delannoy, J., Roze, J. C., Barbut, F., Ancel, P. Y., Charles, M. A., Butel, M. J., Lepage, P., & Aires, J. (2025). Very preterm gut microbiota development from the first week of life to 3.5 years of age: A prospective longitudinal multicenter study. *Microbiology Spectrum*, 13(4), e0163624. <https://doi.org/10.1128/spectrum.01636-24>

[PubMed]

Ulas, T., Pirr, S., Fehlhaber, B., Bickes, M. S., Loof, T. G., Vogl, T., Mellinger, L., Heinemann, A. S., Burgmann, J., Schoning, J., Schreek, S., Pfeifer, S., Reuner, F., Vollger, L., Stanulla, M., von Kockritz-Blickwede, M., Glander, S., Barczyk-Kahlert, K., von Kaisenberg, C. S., et al. (2017). S100-alarmin-induced innate immune programming protects newborn infants from sepsis. *Nature Immunology*, 18(6), 622–632. <https://doi.org/10.1038/ni.3745>

[PubMed]

Ullah, S., Khan, I., Lnu, A., Ullah Khan, J., Syed, A., Shafiq, F., Khan, M., & Khan, F. R. (2024). Probiotics vs. Placebo: Preventing necrotizing enterocolitis in a premature infant. *Cureus*, 16(9), e68848.

<https://doi.org/10.7759/cureus.68848>

[PubMed]

van Best, N., Hornef, M. W., Savelkoul, P. H., & Penders, J. (2015). On the origin of species: Factors shaping the establishment of infant's gut microbiota. *Birth Defects Research. Part C, Embryo Today*, 105(4), 240–251. <https://doi.org/10.1002/bdrc.21113>

[PubMed]

van Best, N., Trepels-Kottek, S., Savelkoul, P., Orlikowsky, T., Hornef, M. W., & Penders, J. (2020). Influence of probiotic supplementation on the developing microbiota in human preterm neonates. *Gut Microbes*, 12(1), 1–16. <https://doi.org/10.1080/19490976.2020.1826747>

[PubMed]

Vatanen, T., Kostic, A. D., d'Hennezel, E., Siljander, H., Franzosa, E. A., Yassour, M., Kolde, R., Vlamakis, H., Arthur, T. D., Hamalainen, A. M., Peet, A., Tillmann, V., Uibo, R., Mokurov, S., Dorshakova, N., Ilonen, J., Virtanen, S. M., Szabo, S. J., Porter, J. A., et al. (2016). Variation in microbiome LPS immunogenicity contributes to autoimmunity in humans. *Cell*, 165(6), 1551.

<https://doi.org/10.1016/j.cell.2016.05.056>

[PubMed]

Vereecke, L., Sze, M., Mc Guire, C., Rogiers, B., Chu, Y., Schmidt-Supprian, M., Pasparakis, M., Beyaert, R., & van Loo, G. (2010). Enterocyte-specific A20 deficiency sensitizes to tumor necrosis factor-induced toxicity and experimental colitis. *The Journal of Experimental Medicine*, 207(7), 1513–1523. <https://doi.org/10.1084/jem.20092474>

[PubMed]

Vergani, S., Muleta, K. G., Da Silva, C., Doyle, A., Kristiansen, T. A., Sodini, S., Krausse, N., Montano, G., Kotarsky, K., Nakawesi, J., Akerstrand, H., Vanhee, S., Gupta, S. L., Bryder, D., Agace, W. W., Lahl, K., & Yuan, J. (2022). A self-sustaining layer of early-life-origin B cells drives steady-state IgA responses in the adult gut. *Immunity*, 55(10), 1829–1842e1826. <https://doi.org/10.1016/j.immuni.2022.08.018>

[PubMed]

von Mutius, E. (2016). The microbial environment and its influence on asthma prevention in early life. *The Journal of Allergy and Clinical Immunology*, 137(3), 680–689. <https://doi.org/10.1016/j.jaci.2015.12.1301>

Walter, J., & Hornef, M. W. (2021). A philosophical perspective on the prenatal in utero microbiome

debate. *Microbiome*, 9(1), 5. <https://doi.org/10.1186/s40168-020-00979-7>
[PubMed]

WHO (2015). WHO statement on caesarean section rates. *Geneva: World Health Organization*.
<https://www.who.int/publications/i/item/WHO-RHR-15.02>

Woelfel, S., Silva, M. S., & Stecher, B. (2024). Intestinal colonization resistance in the context of environmental, host, and microbial determinants. *Cell Host & Microbe*, 32(6), 820–836. <https://doi.org/10.1016/j.chom.2024.05.002>

Wu, H., Esteve, E., Tremaroli, V., Khan, M. T., Caesar, R., Manneras-Holm, L., Stahlman, M., Olsson, L. M., Serino, M., Planas-Felix, M., Xifra, G., Mercader, J. M., Torrents, D., Burcelin, R., Ricart, W., Perkins, R., Fernandez-Real, J. M., & Backhed, F. (2017). Metformin alters the gut microbiome of individuals with treatment-naive type 2 diabetes, contributing to the therapeutic effects of the drug. *Nature Medicine*, 23(7), 850–858. <https://doi.org/10.1038/nm.4345>
[PubMed]

Wu, W. H., Chiang, M. C., Fu, R. H., Lai, M. Y., Wu, I. H., Lien, R., & Lee, C. C. (2024). Impact of clinical use of probiotics on preterm-related outcomes in infants with extremely low birth weight. *Nutrients*, 16(17), 2995. <https://doi.org/10.3390/nu16172995>
[PubMed]

Yatsunenkov, T., Rey, F. E., Manary, M. J., Trehan, I., Dominguez-Bello, M. G., Contreras, M., Magris, M., Hidalgo, G., Baldassano, R. N., Anokhin, A. P., Heath, A. C., Warner, B., Reeder, J., Kuczynski, J., Caporaso, J. G., Lozupone, C. A., Lauber, C., Clemente, J. C., Knights, D., et al. (2012). Human gut microbiome viewed across age and geography. *Nature*, 486(7402), 222–227. <https://doi.org/10.1038/nature11053>
[PubMed]

Yu, Z. T., Chen, C., Kling, D. E., Liu, B., McCoy, J. M., Merighi, M., Heidtman, M., & Newburg, D. S. (2013). The principal fucosylated oligosaccharides of human milk exhibit prebiotic properties on cultured infant microbiota. *Glycobiology*, 23(2), 169–177. <https://doi.org/10.1093/glycob/cws138>
[PubMed]

Zhang, C., Li, L., Jin, B., Xu, X., Zuo, X., Li, Y., & Li, Z. (2021a). The effects of delivery mode on the gut microbiota and health: State of art. *Frontiers in Microbiology*, 12, 724449. <https://doi.org/10.3389/fmicb.2021.724449>
[PubMed]

Zhang, X., Borbet, T. C., Fallegger, A., Wipperfman, M. F., Blaser, M. J., & Muller, A. (2021b). An antibiotic-impacted microbiota compromises the development of colonic regulatory T cells and predisposes to dysregulated immune responses. *mBio*, 12(1), e03335–e03320. <https://doi.org/10.1128/mBio.03335-20>
[PubMed]

Zhang, W., Zhang, Y., Zhao, Y., Li, L., Zhang, Z., Hettinga, K., Yang, H., & Deng, J. (2024). A comprehensive review on dietary polysaccharides as prebiotics, synbiotics, and postbiotics in

infant formula and their influences on gut microbiota. *Nutrients*, 16(23), 4122. <https://doi.org/10.3390/nu16234122>
[PubMed]

Zhernakova, D. V., Wang, D., Liu, L., Andreu-Sanchez, S., Zhang, Y., Ruiz-Moreno, A. J., Peng, H., Plomp, N., Del Castillo-Izquierdo, A., Gacesa, R., Lopera-Maya, E. A., Temba, G. S., Kullaya, V. I., van Leeuwen, S. S., Lifelines Cohort, S., Xavier, R. J., de Mast, Q., Joosten, L. A. B., Riksen, N. P., et al. (2024). Host genetic regulation of human gut microbial structural variation. *Nature*, 625(7996), 813–821. <https://doi.org/10.1038/s41586-023-06893-w>
[PubMed]

Zhou, L., Qiu, W., Wang, J., Zhao, A., Zhou, C., Sun, T., Xiong, Z., Cao, P., Shen, W., Chen, J., Lai, X., Zhao, L. H., Wu, Y., Li, M., Qiu, F., Yu, Y., Xu, Z. Z., Zhou, H., Jia, W., et al. (2023). Effects of vaginal microbiota transfer on the neurodevelopment and microbiome of cesarean-born infants: A blinded randomized controlled trial. *Cell Host & Microbe*, 31(7), 1232–1247e1235. <https://doi.org/10.1016/j.chom.2023.05.022>

4. Sequencing-Based Microbiome Profiling

Amélia Camarinha-Silva¹✉, Melanie Schirmer²✉ and W. Florian Fricke¹✉

(¹) University of Hohenheim, Stuttgart, Germany

(²) Technical University Munich, Munich, Germany

✉ **Amélia Camarinha-Silva**

Email: amelia.silva@uni-hohenheim.de

✉ **Melanie Schirmer**

Email: melanie.schirmer@tum.de

✉ **W. Florian Fricke (Corresponding author)**

Email: w.florian.fricke@uni-hohenheim.de

Abstract

Traditional microbiological research largely depends on the cultivation and characterization of microorganisms under laboratory conditions. However, with the establishment of new sequencing-based methods over the last two decades that expanded the accessible fraction of the microbiota to non-cultivable members, microbiome research has gained significant momentum and popularity. Today, next-generation sequencing allows even smaller research groups to carry out massively parallel sequencing at affordable costs. Selective amplification and sequencing of universal phylogenetic marker genes, such as those of the small subunit ribosomal RNA, still represent a cornerstone of the

taxonomic composition analysis that is typically used to describe and compare microbiome samples. At the same time, shotgun sequencing of metagenomes (DNA) that represent all members of a microbial community is becoming increasingly popular as a more expensive but also more comprehensive alternative to amplicon sequencing. In addition, sequencing RNA at the community level, so-called metatranscriptomics, provides a direct functional readout by profiling gene expression. All of these approaches generate large amounts of sequence data, which require bioinformatic support for processing, analysis, and visualization. The following chapter provides an overview of the typical steps involved in microbiome projects, starting from sample collection and storage, over 16S rRNA and other marker gene amplification, amplicon, and metagenome as well as metatranscriptomic sequencing to bioinformatic sequence analysis.

Keywords Microbiome analysis – Shotgun metagenomics – Amplicon sequencing – Taxonomic profiling – Bioinformatics pipelines

4.1 Introduction

Historically, the detection and characterization of the human microbiome, as well as microbes in other biological specimens, relied heavily on microscopy and cultivation-based techniques. These approaches, while foundational, were inherently limited to microorganisms that could be successfully cultivated under standard, typically aerobic, laboratory conditions. This paradigm began to shift in the 1970s with the utilization of the universal small subunit ribosomal RNA gene for phylogenetic comparisons, which culminated in drawing the first comprehensive *tree of life* (Pace, [1997](#)). In particular, the 16S rRNA gene continues to be used as a molecular marker for differentiating bacterial microbes based on sequence similarity, enabling the identification of both cultured and uncultured microorganisms and uncovering previously unknown members of

complex microbial communities, such as the human microbiome (Knight et al., [2018](#)).

With the advent of next-generation sequencing (NGS) technologies in the early 2000s and subsequent advances in sequencing methodologies, microbiome research has experienced a profound transformation. Technical and economical innovations have enabled widespread access to affordable, high-throughput, large-scale analyses of human microbiome samples, often encompassing thousands of specimens and individual microbial taxa per sample. Different sequencing technologies provide complementary benefits, with short-read sequencing platforms that generate millions of high-accuracy reads facilitating the deep profiling of microbial communities, whereas long-read sequencing platforms allow for the assembly of larger genomic fragments, providing detailed structural and functional insights into individual microbial genomes and microbiome members (Pinto & Bhatt, [2024](#)).

Among sequencing-based microbiome analysis approaches, 16S rRNA gene amplicon sequencing remains one of the most widely utilized methods. As a targeted approach that selectively amplifies and sequences specific phylogenetic marker regions, it offers a cost-efficient, widely accessible means of profiling bacterial microbiota members. Alternative targets for amplicon sequencing have been explored for fungi, eukaryotic species, or other microbiome fractions. However, amplicon sequencing is inherently limited in scope, addressing the question of “who is there?” while only allowing taxonomy-based estimates about other, functional microbiome features at best (Douglas et al., [2020](#)). Additional limitations include the restricted taxonomic resolution—for 16S rRNA gene amplicon sequencing typically to the genus level—and the lack of information about other genetic microbiome features, such as operons, genes, regulatory regions, mobile genetic elements etc.

Whole-genome shotgun (WGS) metagenomic sequencing has emerged as a complementary approach that overcomes many of these limitations. By sequencing the complete mixture of genomic DNA

fragments in a sample, i.e., the metagenome, without prior target amplification, WGS metagenomics enables the characterization of the entire microbiome at high taxonomic and functional resolution, capturing viruses, bacteria, fungi, and other community members, including many previously unexplored members of the human microbiota (Pasolli et al., [2019](#)). Additionally, this approach provides insights into the functional potential of microbiomes through gene detection and analysis and the assembly of global, human, and planetary gene catalogues (Coelho et al., [2022](#)). Metagenomic reads can be analyzed directly or assembled into metagenome-assembled genomes (MAGs), facilitating both reference-based and reference-free analyses (Pinto & Bhatt, [2024](#)).

Building upon these methods, metatranscriptomics extends the scope of microbiome research to include the transcriptional activity of microbial communities (Ojala et al., [2023](#)). By sequencing and quantifying RNA transcripts, metatranscriptomics provides a dynamic view of microbiome activity, complementing the genomic insights of metagenomics. Especially when combined (Lloyd-Price et al., [2019](#)), these approaches provide researchers with powerful means to address not only questions about “who is there?” (amplicon sequencing) but also “who CAN do what?” (metagenomics) and “who IS doing what?” (metatranscriptomics).

In this chapter, we focus on sequencing-based methods for microbiome analysis, including 16S rRNA gene amplicon sequencing, metagenomics, and metatranscriptomics, in the context of other complementary “omics” technologies, such as culturomics, metaproteomics, and metabolomics. We discuss laboratory workflows for nucleic acid preparation and sequencing library construction, and we provide an overview of the bioinformatics pipelines commonly employed in sequence data analysis.

4.2 Extraction of Nucleic Acids from Microbiomes for Sequencing

Preserving the integrity of microbiome samples is critical for accurate downstream analyses, as nucleic acids, proteins, and metabolites degrade rapidly at room temperature. Sample preservation methods must stabilize nucleic acids (for metagenomics and metatranscriptomics), proteins (for metaproteomics), and metabolites (for metabolomics) while preventing degradation or metabolic changes. Microbial activity during collection can lead to shifts in gene abundance, gene expression, protein modification, and metabolite turnover, potentially skewing results (Mordant & Kleiner, [2021](#)). The gold standard for microbiome sample preservation involves immediate snap freezing in liquid nitrogen, followed by storage at ultra-low temperatures (-70°C to -80°C), which minimizes alterations in microbial composition and prevents metabolite degradation and protein denaturation (Li et al., [2023](#)). Snap-freezing in liquid nitrogen is particularly effective for RNA stabilization, although its practicality is often constrained by the availability of specialized equipment and the expense of cold storage and transport. In remote or non-clinical settings where such conditions are infeasible, chemical preservation agents such as RNeasy[®], OMNIgene[®], or DNA/RNA Shield[®] are frequently used to stabilize DNA and RNA during collection and transport (Nearing et al., [2021](#)). Standardization of protocols tailored to specific study objectives is essential for improving reproducibility and comparability across studies. Furthermore, clinically relevant applications, such as fecal microbiota transplantation (FMT), underscore the importance of preserving viable microbes. Stool banks typically employ cryopreservation in glycerol at -80°C , but FMT effects may also involve non-viable microbial components, such as bacterial metabolites or extracellular vesicles (Ott et al., [2017](#)).

Efficient nucleic acid extraction from microbiome samples (Fig. [4.1](#)) poses unique challenges due to the structural diversity of microbial cells. Many microorganisms, particularly Gram-positive bacteria and spores, possess robust cell walls that resist lysis, leading to biases in downstream analyses (Pollock et al., [2018](#)). Chemical lysis agents, including detergents (e.g., SDS, Triton X-100), chaotropic agents (e.g.,

guanidine thiocyanate), and chelators (e.g., EDTA), disrupt cell membranes and stabilize nucleic acids by inhibiting nuclease activity. Enzymatic treatments with lysozyme, proteinase K, or specialized enzymes targeting fungal or archaeal cell walls enhance lysis efficiency, but their efficacy varies across microbial taxa. Mechanical disruption, often via bead beating, is indispensable for breaking open thick-walled organisms or spores but requires optimization to prevent nucleic acid shearing and sample overheating (de Boer et al., [2010](#)). Simultaneous extraction of DNA, RNA, and proteins from microbiome samples is advantageous for integrative multi-omics studies, particularly when sample material is limited (Hummon et al., [2007](#)). The TRIzol[®] protocol, based on phenol-chloroform extraction, allows the recovery of RNA, DNA, and proteins in separate phases, maximizing the utility of limited sample material, facilitating comprehensive analyses of metagenomics, metatranscriptomics, and metaproteomics from the same sample (Chomczynski & Sacchi, [2006](#)). Despite its broad utility, the protocol's reliance on toxic reagents and labor-intensive workflows limits its scalability for high-throughput studies.

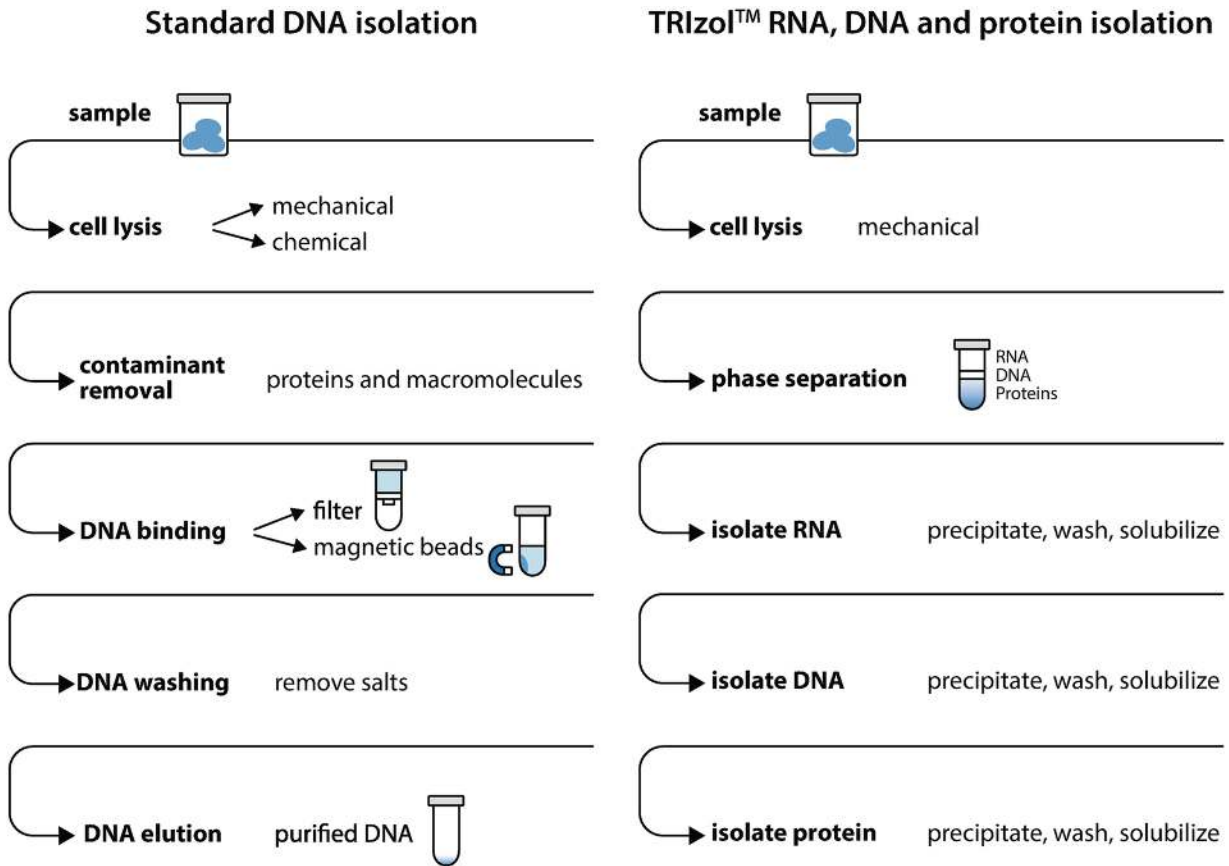


Fig. 4.1 Protocol overview for the processing of microbiome samples, using standard commercial kits for the isolation of metagenomic DNA or the TRIzol™-based protocol for the simultaneous isolation of RNA, DNA, and protein

Commercial extraction kits have simplified the isolation of metagenomic DNA and RNA, providing user-friendly protocols that handle diverse sample types, including feces, soil, and tissues. These kits often incorporate a combination of chemical, enzymatic, and mechanical lysis steps, followed by DNA and/or RNA binding to silica membranes or magnetic beads under high-salt conditions, and ethanol washes to remove contaminants, ensuring high-purity DNA/RNA suitable for downstream applications. Automated extraction systems are improving both the efficiency and reliability of microbiome sample processing. Optimizing microbiome sample collection, storage, and nucleic acid extraction is essential for generating high-quality data that accurately reflects microbial communities. Inefficient lysis or incomplete contaminant removal can lead to inconsistencies in

microbiome composition data, complicating cross-study comparisons (Brooks et al., [2015](#)). Furthermore, RNA degradation should be minimized by reducing freeze-thaw cycles and processing samples as quickly as possible once thawed. As microbiome research continues to evolve, the development and adoption of standardized, high-efficiency protocols will be critical for advancing our understanding of microbial ecosystems and their roles in health and disease. In practice, the success of all nucleic acid extraction protocols is also largely dependent on the removal of host DNA and RNA from the sample. The presence of nucleic acids from the host can affect the success of downstream applications such as metagenomics, metaproteomics, and metatranscriptomics, either directly by inhibiting amplification reactions or indirectly by reducing the fraction of microbial data in the resulting data output, effectively increasing the cost of sequencing (Ojala et al., [2023](#)).

4.3 Targeted Amplicon Sequencing

Targeted amplicon sequencing leverages conserved genetic markers to profile microbial communities by amplifying regions that carry sufficient phylogenetic information for taxonomic differentiation (Fig. [4.2](#)). These markers are selected based on two critical features: (i) conserved (~universally present) regions with minimal sequence variation between taxa, which serve as binding sites for the same set of (~universal) primers during PCR amplification; and (ii) hypervariable regions, which display sequence variability even among closely related organisms, providing the phylogenetic signal necessary for taxonomic assignment. These structural requirements have been extensively studied for the bacterial 16S rRNA gene, for which degenerate primers with broad binding affinities across bacterial taxa have been developed (Claesson et al., [2010](#)). However, in eukaryotes, the greater sequence variation, even among closely related taxa, has restricted the application of such universal approaches, leading to marker selections that often target smaller taxonomic groups, such as fungi (Lücking et

al., [2021](#)) or protozoa (Stensvold, [2024](#)). Today, 16S rRNA gene amplicon sequencing remains the most affordable, simple, and widely employed method for human taxonomic microbiome profiling, although metagenomic approaches continue to gain traction for broader microbiome analysis (Knight et al., [2018](#)).

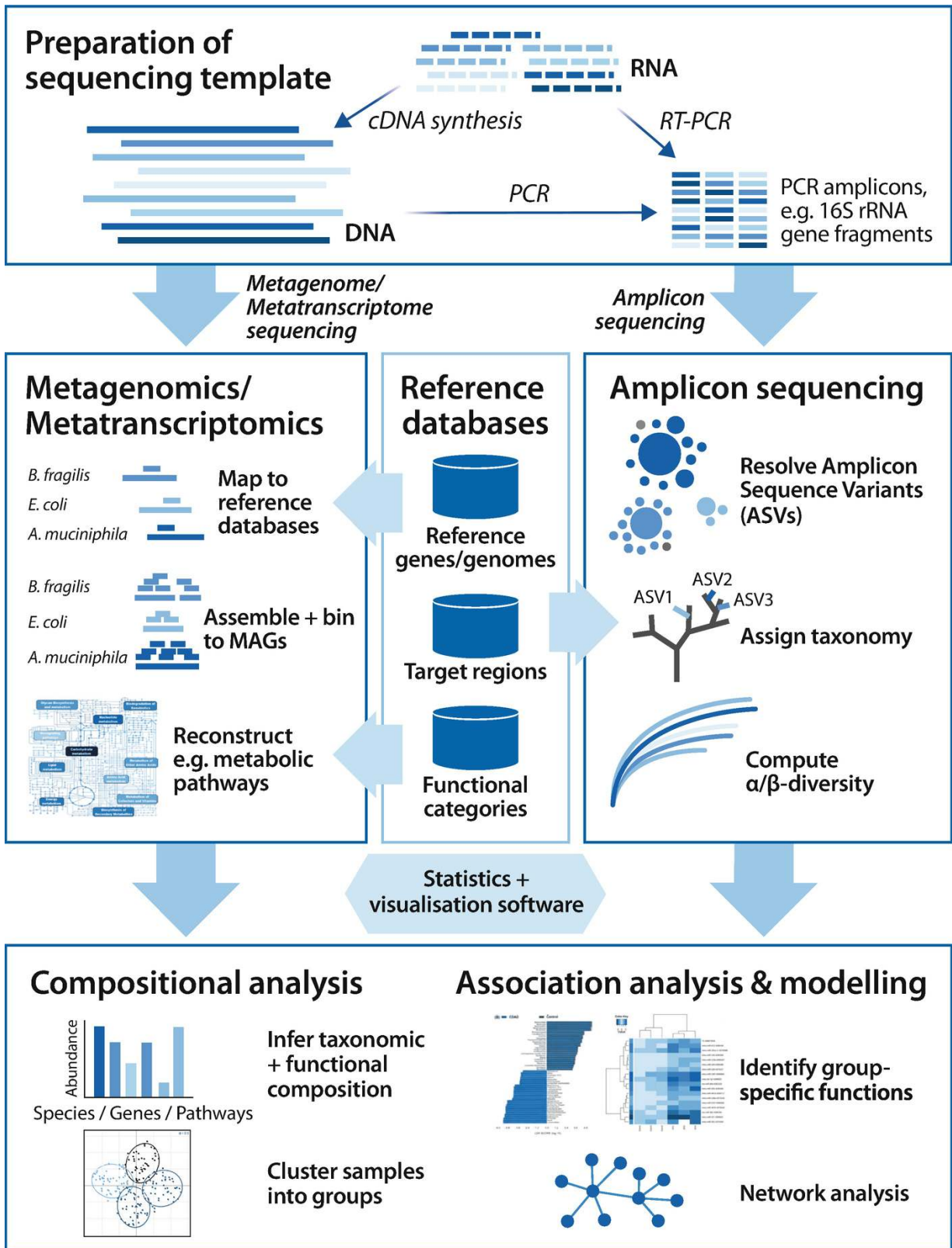


Fig. 4.2 Workflow overview for amplicon, metagenome, or metatranscriptome sequencing-based microbiome projects

Advances in sequencing technologies, particularly the divergent development of short-read and long-read platforms, have significantly shaped the capabilities and limitations of targeted amplicon sequencing. Short-read platforms produce reads spanning a few hundred base pairs, which are sufficient to cover individual hypervariable regions of the 16S rRNA gene. However, length restrictions often require the selection of specific hypervariable regions of the 16S rRNA gene for amplification, and these regions can vary in their phylogenetic informativeness. As a result, different targeted regions may yield distinct community compositions, leading to variations in detected taxa or relative abundance profiles (Walker et al., [2015](#)). This region-dependent variability introduces batch effects and poses challenges for cross-study comparisons, even when analyzing similar sample types (Claesson et al., [2010](#)). Long-read sequencing platforms, on the other hand, can sequence the entire 16S rRNA gene (>1500 bp), the larger 23S rRNA gene (>2900 bp), or even both, offering far superior phylogenetic resolution (Srinivas et al., [2025](#)). However, as these platforms produce fewer reads at higher relative costs per base pair, the resulting lower read count that is typically available per sample reduces the sensitivity to capture a broader diversity of a microbiome sample, including low-abundant microbiota members.

For eukaryotic microbiomes, the 18S rRNA gene has been used to assess diversity (Meyer et al., [2010](#)). However, its widespread use is limited by the longer lengths of target regions compared to the sequencing capabilities, at least of short-read sequencing platforms (Amaral-Zettler et al., [2009](#)), and by the greater variability in rRNA gene copy numbers across eukaryotic species compared to bacteria (Prokopowich et al., [2003](#)). In fungi, the internal transcribed spacer (ITS) regions—ITS1 and ITS2—located within the ribosomal RNA operon are the most commonly used markers for taxonomic profiling (Lücking et al., [2021](#)). These regions exhibit high interspecific variability, providing robust classification and serving as the primary

fungal barcode as proposed by the Fungal Barcoding Consortium (Schoch et al., [2012](#)).

The cost-effectiveness of targeted amplicon sequencing is primarily attributed to its capacity for multiplexing—simultaneously analyzing multiple samples in a single sequencing reaction. This “metabarcoding” is achieved by incorporating sample-specific sequence barcodes into one or both PCR primers, enabling the tagging of amplicons during amplification. Barcode systems, which utilize hundreds of unique sequence tags (8–12 nucleotides in length), allow for the pooling and concurrent sequencing of hundreds of samples in a single run (Fadrosh et al., [2014](#)).

4.4 Generating Phylogenetic Profiles of Specific Microbiome Members

Target amplicon sequencing-based microbiome analysis hinges on the identification and quantification of sequence similarities among amplicons to infer phylogenetic relationships. Amplicons with sequence similarities exceeding a defined threshold are grouped and assigned to the same taxon. For example, 16S rRNA gene fragment amplicons with at least 97% sequence similarity are typically assigned to the same bacterial species. These similarity thresholds are empirically established through in silico comparisons of genome database entries. However, the relationship between sequence similarity and taxonomy is more complicated for most other amplicons besides the 16S rRNA gene, including ITS (Lücking et al., [2021](#)). Moreover, multi-copy target amplicons, like the 16S rRNA gene, may exhibit sequence variability among copies (Větrovský & Baldrian, [2013](#)), further complicating phylogenetic inferences.

For bacterial microbiome profiling, amplicon sequence reads have long been taxonomically assigned based on the concept of “Operational Taxonomic Units” (OTUs). OTUs represent biological taxa defined by statistical clustering of sequences that meet a minimum similarity threshold (Schmidt et al., [2014](#)). OTU generation typically follows a

two-step process ribosomal RNA gene database project:known as “open-reference OTU picking” (Rideout et al., [2014](#)): sequences are first compared against a reference database (“closed-reference OTU picking”), and unmatched sequences are subsequently clustered de novo. When an OTU matches a reference sequence, its taxonomic lineage is adopted.

In recent years, the concept of “Amplicon Sequence Variants” (ASVs) has gained prominence as a refinement over OTUs. ASVs employ error-correction algorithms, such as DADA2 (Callahan et al., [2016](#)), to model and correct platform-specific sequencing error rates and distinguish true biological sequence variations from sequencing errors. The error profiles enable precise discrimination between biological variation and sequencing artefacts, which can inflate OTU-based microbiome profiles (Knight et al., [2018](#)). As a result, ASV-based analysis enhances microbial community resolution, often achieving species-level distinction—in contrast to OTU clustering, which may combine multiple taxa into a single OTU but is also less likely to split different rRNA genes from the same genome into separate clusters (Schloss, [2021](#)). As ASVs theoretically represent exact biological sequences, ASV-based profiles more accurately reflect the true microbial community and can be directly compared across datasets, unlike OTUs, which depend on study-specific clustering parameters. ASV methods are particularly adept at identifying rare taxa, even in low-biomass samples, offering improved sensitivity over OTU-based methods (Fasolo et al., [2024](#)).

Both OTU and ASV approaches allow the identification of previously unknown taxa without representatives in reference databases. For instance, analyzing fecal samples from two patient cohorts may reveal a previously unidentified bacterial group (e.g., an ASV or OTU with <97% similarity to database entries). Although the species may remain unnamed, its higher-level classification, such as belonging to a specific bacterial family, could provide valuable insights and identify biomarkers for the cohort.

4.5 Metagenomic and Metatranscriptomic Shotgun Sequencing

Metagenomics refers to the analysis of DNA mixtures from microbial communities, i.e., instead of focusing on individual microbial genomes or selected target regions from multiple genomes, the entire “meta” genome of all microorganisms combined is studied (Eisen, [2007](#)). Because of the required sequencing depth to provide sufficient resolution for hundreds of genomes simultaneously, metagenomics usually relies on short-read next-generation sequencing platforms that provide the best cost-per-base pair ratio. In order to generate an even distribution of all microbial genomes in the metagenomic sequence dataset, DNA isolates need to be sheared into fragments of equal length. The term “shotgun sequencing” refers to the generation of sequencing libraries by randomly fragmenting DNA templates, irrespective of differences between microbial genomes, such as specific sequence motifs, G + C contents, secondary structures, etc. As such, the generation of metagenomic sequencing libraries is not fundamentally different from those generated for single microbial or eukaryotic genomes, i.e., template DNA is mechanically or enzymatically sheared and resulting fragments are ligated to adaptors used for sequencing, amplified, and sequenced (Fleischmann et al., [1995](#)).

Metagenomic shotgun sequencing has the potential to characterize the entire genetic potential of a microbiome sample. As genomic DNA isolates from the sample are directly submitted to sequencing, there is no restriction imposed by PCR amplification of specific target loci that is required for amplicon sequence analysis. As a result, at least theoretically, the complete genome sequences, including all protein-coding and non-coding sequences from all organisms in the sample, can be obtained, including viral, bacterial, archaeal, fungal, and other eukaryotic microorganisms. However, the lack of a targeted amplification step also means less control over the sequencing template. Human DNA, which is typically not the target of microbiome projects, can represent a considerable problem for metagenomic

shotgun sequencing of microbiome samples. The “contamination” of microbiome samples with human DNA is less of a problem for microbiota analyses of fecal samples with relatively few human cells or DNA, which is one reason why fecal microbiota analysis has become so popular. However, blood in stool samples can pose a significant problem and substantially increase the amount of human DNA. Also, as human cells contain roughly 1000x more DNA than bacterial cells, human DNA contamination is more problematic for other types of microbiome samples, such as epidermal or mucosal samples, vaginal or skin swabs, biopsies or tissue samples, among other reasons, resulting in fewer available metagenomic sequence data from these sample types. Removing human DNA from metagenomes using selective hybridization before sequencing or increasing the sequencing effort for problematic samples has been proposed to address this problem; bioinformatically, the removal of human reads from sequence data poses less of a problem (Ferretti et al., [2017](#)).

Metatranscriptomics refers to the analysis of transcriptional activities of microbial communities by whole-transcriptome shotgun sequencing or “RNA-Seq.” After isolating RNA from microbiome samples, reverse transcription into cDNA is performed, typically using random hexamer primers. cDNA fragments can be sequenced using the same methods and platforms as metagenomic DNA. For metatranscriptomics, which aims at sequencing the mRNA fraction of the microbiome, an additional rRNA depletion step is required as rRNA accounts for approximately 80% of the extracted total RNA (Westermann & Vogel, [2021](#)). Generally, this metatranscriptomic approach has its own specific challenges, as it can be difficult to extract sufficient amounts of pure RNA devoid of DNA, effectively deplete rRNA from diverse bacterial community members and to evenly reverse-transcribe this RNA to high-quality cDNA that can be sequenced (Pinto & Bhatt, [2024](#)).

4.6 Bioinformatic Analyses to Infer Taxonomic and Functional Profiles of the Entire Microbiome

Two major approaches dominate metagenomic shotgun sequence analyses: read-based and assembly-based methods. Read-based methods directly map individual sequencing reads to reference databases, while assembly-based methods first reconstruct longer contiguous sequences (contigs) or complete metagenome-assembled genomes (MAGs) from overlapping reads for subsequent analysis. For read-based metagenomic sequence analyses, both reference-dependent and independent methods exist that can vary with respect to the computational demands, sensitivity for the detection of individual taxa, and accuracy of the resulting taxonomic profiles. Similarly, metatranscriptomic shotgun sequencing reads can be aligned to functional reference databases, such as UniRef (Suzek et al., [2015](#)), to infer functional abundance profiles. At the gene family level, this typically results in millions of gene families when profiling complex microbial communities, such as the gut microbiome. These gene families can subsequently be summarized into higher functional categories, such as MetaCyc metabolic pathways or Gene Ontology (GO) categories (Franzosa et al., [2018](#)). Several widely used bioinformatic platforms support 16S rRNA gene amplicon and metagenomic/metatranscriptomic shotgun sequence analysis, including open-source and proprietary software that is available for download and local installation or web-based execution on online servers (Table [4.1](#)).

Table 4.1 Bioinformatic support platforms

<i>16S rRNA gene amplicon sequence analysis</i>		
Mothur	Open-source software package for all steps involved in amplicon sequencing-based microbiota analysis	Schloss et al. (2009)
QIIME 2	Open-source software package for all steps involved in amplicon sequencing-based microbiota analysis	Bolyen et al. (2019)

DADA2	Open-source software package for sequence error correct and the generation of amplicon sequence variants (ASVs)	Callahan et al. (2016)
Silva	Reference database of small and large subunit rRNA sequences for all three domains of life	Quast et al. (2013)
Greengenes2	Reference database of small and large subunit rRNA sequences for all three domains of life	McDonald et al. (2024)
<i>Metagenomic and metatranscriptomic sequence analysis</i>		
MetaPhlAn4	Open-source software for read and reference-based taxonomic profiling of metagenomic sequence data	Blanco-Míguez et al. (2023)
Kraken2/Bracken	Open-source software for read-based, reference-independent taxonomic profiling of metagenomic sequence data	Lu et al. (2022)
mOTUs2	Open-source software for read-based, reference-independent profiling of metagenomic sequence data	Milanese et al. (2019)
HUMANn3	Open-source software for metabolic pathway prediction from metagenomic or metatranscriptomic sequence data	Franzosa et al. (2018)
MetaCyc	Reference database of metabolic pathways with a web portal for query, visualization, and analysis tools	Caspi et al. (2020)

First, reads are trimmed using the fastq quality scores, and any reads below a certain length threshold are filtered. In addition, host reads (e.g. human reads) are removed by mapping against the corresponding host reference genome. In read-based analyses of metagenomic data, quality-controlled sequencing reads are compared to reference datasets that may include universal marker genes, clade-specific marker genes, or whole genomes. Universal marker gene-based methods, such as mOTUs2 (Milanese et al., [2019](#)), provide similar broad taxonomic coverage as 16S rRNA gene analyses, but typically involve small gene sets with almost universal prevalence in microbial genomes and are particularly useful for identifying both known and novel organisms. Clade- or species-specific marker genes, as available for example through the bioBakery tool set (Beghini et al., [2021](#)), are derived from comparative genome analyses and offer high specificity and sensitivity for identifying known taxa but may miss uncharacterized organisms. Mapping reads to whole-genome

references can allow for the detection of genetic variants or gene content, which are not easily accessible through gene-based methods, but are computationally demanding. Available tools, such as Kraken2 (Lu & Salzberg, [2020](#)), therefore characterize reads and reference genomes based on the prevalence of short sequence “words” (k-mers), allowing for the rapid classification of reads, even across large metagenome and reference genome datasets. Read-based functional metagenome profiling, e.g., with HUMAnN3 (Beghini et al., [2021](#)), is performed by mapping reads to gene databases to estimate the relative abundance of gene categories and associated metabolic pathways in a sample. HUMAnN can take advantage of the taxonomic profiles by first mapping reads against a custom database of pangenomes (the entire set of genes from all strains within a clade, e.g., a species) from all organisms present in the sample. This sample-specific pangenome database is much smaller than comprehensive functional databases, thus substantially reducing the search space. Furthermore, from the match, the profiled gene families can be attributed to a particular species. The remainder of reads that do not map during the pangenome search can subsequently be searched against comprehensive functional databases to profile gene families that are contributed by organisms that were not captured in the taxonomic profiles. Similarly, for the analysis of metatranscriptomic data, reads can be directly mapped against functional databases. If paired metagenomic data is available from the same sample, HUMAnN3 uses the metagenomic taxonomic profiles for a pangenome search prior to the database search to infer functional profiles.

In contrast, assembly-based analyses reconstruct contigs by aligning individual overlapping metagenomics reads and then grouping or “binning” contigs into MAGs using individual genome-specific features such as k-mer frequencies, co-abundance and genome sequencing depths (Tyson et al., [2004](#)). MAGs can be generated with short and long-read sequencing technologies, which improve assembly accuracy by resolving repetitive regions, and have been used to identify

and characterize unknown members of the human microbiota (Pasolli et al., [2019](#)).

Metagenomic sequencing offers near-complete phylogenetic resolution, enabling the differentiation of subspecific taxa or strains. Although the term “strain” has historically been variably defined based on microbial culturing, comparative genomics, and metagenomics (Van Rossum et al., [2020](#)), setting appropriate sequence similarity thresholds enables strain tracking within a host over time or across different individuals and environments following transmission (Podlesny et al., [2022](#)). Strain-level analysis is valuable for understanding microbiome dynamics, such as the processes of microbial establishment, stability, and variability within and between individuals or body sites.

4.7 Other Omics Technologies: Insights into Gene Expression and Metabolic Activities

Cultivation remains a keystone of microbiological research, enabling detailed experimental studies and providing foundational insights into microbial physiology and interactions. However, traditional methods for isolating bacteria are labor-intensive, difficult to scale, and lack integration between phenotype and genotype (Huang et al., [2023](#)). Culturomics has emerged as a high-throughput strategy to address this challenge, combining advanced methods such as automated liquid handling, mass spectrometry, and genomic sequencing to isolate and identify previously uncharacterized microbial species (Lagier et al., [2012](#)). This approach has expanded microbial culture collections and revealed that over 73% of the metagenomic species in human gut microbiota could be cultivated under optimized conditions (Browne et al., [2016](#)). Despite these advances, significant gaps remain. For instance, more than 70% of species in the Unified Human Gastrointestinal Genome (UHGG) collection lack a cultured representative (Almeida et al., [2021](#)). Integrating automation and machine learning has further improved workflows, accelerating the discovery of novel species and

optimizing culture conditions to mimic natural environments more effectively (Huang et al., [2023](#)).

Metabolomics has transformed our understanding of microbial metabolic activity by quantifying small molecules such as short-chain fatty acids (SCFAs) and bile acids, which are critical in regulating host physiology and linking microbial functions to health and disease (Patejko et al., [2017](#)). Recent advancements in nuclear magnetic resonance (NMR) and mass spectrometry (MS), particularly liquid chromatography-mass spectrometry (LC-MS/MS), have improved sensitivity and resolution, enabling researchers to map complex metabolic networks and microbial contributions to diseases (Pathmasiri et al., [2024](#)). Targeted and untargeted metabolomic approaches now allow for the comprehensive profiling of thousands of metabolites in complex biological systems. The field has also benefited from integrating computational tools and machine learning, enabling the simultaneous analysis of thousands of metabolites in biological samples such as plasma, feces, and urine (Pathmasiri et al., [2024](#)). Moreover, the establishment of metabolite databases, like the Human Metabolome Database (Wishart et al., [2022](#)), has standardized analyses, improving reproducibility and cross-study comparisons. Integrating metabolomics with other omics can expose metabolic pathways linking microbial activity and host responses, and give insights into host-microbiome interactions.

Metaproteomics provides insights into the functional state of microbiomes by characterizing the proteins expressed by microbial communities. Using advanced liquid chromatography and mass spectrometry techniques, metaproteomics identifies active enzymes and proteins that reflect microbial metabolic activity (Wilmes et al., [2015](#)). Innovations such as data-independent acquisition (DIA) and tandem mass tag (TMT) labeling have improved protein quantification accuracy, enabling detailed functional analyses of microbiomes (Zhao et al., [2023](#)). This approach allows researchers to link microbial proteins to nutrient metabolism, immune modulation, and host-microbiome interactions. The integration of genomics, culturomics, metabolomics,

and metaproteomics offers a multidimensional view of microbiomes, bridging compositional data with functional information. These insights can provide the basis for identifying novel therapeutic targets, advancing precision medicine, and understanding microbiome-host interactions.

Box 4.1: The Terminology of Microbiome Research

- OTU (operational taxonomic unit): Group of sequences that are separated from others by using clustering approaches under a specific sequence identity threshold.
- ASV (amplicon sequence variant): Single sequence determined from amplicon sequence data by error correction.
- MAG (metagenome-assembled genome): Group of contigs representing an individual genome in a microbiome.
- Microbiome: the entire habitat, including the microorganisms, their genes, genomes, proteomes, and metabolomes.
- Microbiota: the group of microorganisms (bacteria, archaea, or lower eukaryotes) inhabiting a specific niche. The microbiota is defined based on taxonomic profiles derived from amplicon, metagenomic or metatranscriptomic sequencing.
- Metagenome: the collection of genes and genomes of the microbiota members and their encoded functions.
- Metatranscriptomics: the study of the expressed RNAs with information on the regulation and expression profiles of the microbial communities.
- Metaproteomics: protein identification and characterization of a specific sample at a given point in time, reflecting host and microbial protein content of the sample.
- Metabolomics: quantitative and qualitative determination of the metabolites of a microorganism, a tissue, a single cell, or a complex sample involving contributions by the host and microorganisms (“meta-metabolomics”).
- Strain: subspecies taxonomic lineage with a variable definition used to differentiate and track individual microbiota members

across space and time.

Box 4.2: Microbiome Research: Hype and Hubris of an Emerging Field

Human microbiome research continues to rapidly expand, with hundreds of new publications being released on the subject per week. This rapid growth and the enthusiasm that accompanies it come at the cost of several important weaknesses, limitations, and inaccuracies:

- **Terminology:** A number of poorly or inconsistently defined expressions are widely found in the literature on microbiome research. Following recommendations made by Marchesi and Ravel ([2015](#)), Box 4.1 provides an overview of the essential microbiome vocabulary.
- **Controls:** Due to the sensitivity of sequencing-based microbiota analyses, particularly 16S rRNA gene amplicon sequencing, and the omnipresence of bacteria in the environment, laboratory, and even consumables, there is a huge risk of reporting false-positive microbiome results, particularly when studying low-biomass samples. Enthusiastic early microbiome studies may have been misinterpreting contamination, e.g., in the case of a now widely debunked placenta microbiome (Kennedy et al., [2023](#)). As a consequence, extreme care should be applied when processing microbiome samples, and negative and positive controls should be included, processed, sequenced, and analyzed side by side with samples to assess potential contamination and the efficacy of amplicon, metagenomic, or metatranscriptomic sequencing-based microbiome studies.
- **Quantitative microbiome analyses:** The vast majority of microbiome studies today continue to characterize samples based on compositional data, including amplicon and metagenome sequencing-based taxonomic and functional studies, although increasing evidence points to quantitative microbiome parameters, such as fecal microbiome load, as a major confounder of disease

associations (Nishijima et al., [2025](#)). A variety of methods have been successfully used to measure absolute microbiota abundance, including flow cytometry-based cell counting, quantitative PCR, or internal standard provision (e.g., spike-in DNA), which should be more broadly applied to all microbiome projects.

- **Transparency and reproducibility:** As datasets grow larger and bioinformatic and biostatistic protocols become more complex, full and open access to microbiome sequence data, source data, and background sample information (so-called metadata), as well as to scripts and programs used for data processing, should be mandatory. Data release should follow the Findable, Accessible, Interoperable, and Reusable (FAIR) principles. Full transparency is a critical prerequisite to ensure reproducibility and maintain integrity of the field.

Box 4.3: Summary and Highlights

- We are only starting to realize the importance of the microbiome for human health. However, the future and long-term success of microbiome research will depend to a large extent on standard operating procedures (SOPs), i.e., community-validated, standardized, and efficient protocols, for sample collection, storage and preservation, isolation, sequencing, and bioinformatic analyses.
- Successful nucleic acid extraction protocols involve steps to eliminate inhibitory substances, unwanted DNA or RNA from microbiome samples, as well as nucleic acids from the host to improve the outcome of downstream analysis.
- Targeted amplicon sequencing can be used to generate sequence-based phylogenetic and taxonomic profiles of specific microbiome members and components, while metagenomic shotgun sequencing provides insights into both phylogenetic and functional profiles of the entire microbiota.

- Bioinformatic sequence analysis for microbiome analysis relies on the identification and quantification of sequence similarities to infer phylogenetic relationships and shared functionalities. With new AI-based tools, structural predictions (e.g., AlphaFold) have introduced a paradigm shift, where now functional comparisons of genes are feasible based on structural similarity.
- Microbiome research is an active, growing field in the process of developing the best set of experimental and bioinformatic tools, protocols, and parameters to study and determine the ecological, physiological, immunological, and clinical relevance of the microbiome.

References

Almeida, A., et al. (2021). A unified catalog of 204,938 reference genomes from the human gut microbiome. *Nature Biotechnology*, 39(1), 105–114.

[\[PubMed\]](#)

Amaral-Zettler, L. A., et al. (2009). A method for studying protistan diversity using massively parallel sequencing of V9 hypervariable regions of small-subunit ribosomal RNA genes. *PLoS One*, 4(7), e6372.

[\[PubMed\]](#)

Beghini, F., et al. (2021). Integrating taxonomic, functional, and strain-level profiling of diverse microbial communities with bioBakery 3. *eLife*, 10, e65088. Available at: <https://doi.org/10.7554/eLife.65088>

[\[PubMed\]](#)

Blanco-Míguez, A., et al. (2023). Extending and improving metagenomic taxonomic profiling with uncharacterized species using MetaPhlAn 4. *Nature Biotechnology*, 41(11), 1633–1644.

[\[PubMed\]](#)

Bolyen, E., et al. (2019). Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nature Biotechnology*, 37(8), 852–857.

[\[PubMed\]](#)

Brooks, J. P., et al. (2015). The truth about metagenomics: Quantifying and counteracting bias in 16S rRNA studies. *BMC Microbiology*, 15, 66.

[\[PubMed\]](#)

Browne, H. P., et al. (2016). Culturing of “unculturable” human microbiota reveals novel taxa and extensive sporulation. *Nature*, 533(7604), 543–546.

[\[https://doi.org/10.1038/nature17645\]](https://doi.org/10.1038/nature17645)[\[PubMed\]](#)

Callahan, B. J., et al. (2016). DADA2: High-resolution sample inference from Illumina amplicon data. *Nature Methods*, 13(7), 581–583.

[\[PubMed\]](#)

Caspi, R., et al. (2020). The MetaCyc database of metabolic pathways and enzymes - a 2019 update. *Nucleic Acids Research*, 48(D1), D445–D453.

[\[PubMed\]](#)

Chomczynski, P., & Sacchi, N. (2006). The single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction: Twenty-something years on. *Nature Protocols*, 1(2), 581–585.

[\[PubMed\]](#)

Claesson, M. J., et al. (2010). Comparison of two next-generation sequencing technologies for resolving highly complex microbiota composition using tandem variable 16S rRNA gene regions. *Nucleic Acids Research*, 38(22), e200.

[\[PubMed\]](#)

Coelho, L. P., et al. (2022). Towards the biogeography of prokaryotic genes. *Nature*, 601(7892), 252–256.

[\[https://doi.org/10.1038/s41586-021-04233-4\]](https://doi.org/10.1038/s41586-021-04233-4)[\[PubMed\]](#)

de Boer, R., et al. (2010). Improved detection of microbial DNA after bead-beating before DNA isolation. *Journal of Microbiological Methods*, 80(2), 209–211.

[\[PubMed\]](#)

Douglas, G. M., et al. (2020). PICRUSt2 for prediction of metagenome functions. *Nature Biotechnology*, 38(6), 685–688.

[\[PubMed\]](#)

Eisen, J. A. (2007). Environmental shotgun sequencing: Its potential and challenges for studying the hidden world of microbes. *PLoS Biology*, 5(3), e82.

[\[PubMed\]](#)

Fadrosh, D. W., et al. (2014). An improved dual-indexing approach for multiplexed 16S rRNA gene sequencing on the Illumina MiSeq platform. *Microbiome*, 2(1), 6.

[\[https://doi.org/10.1186/2049-2618-2-6\]](https://doi.org/10.1186/2049-2618-2-6)[\[PubMed\]](#)

Fasolo, A., et al. (2024). ASV vs OTUs clustering: Effects on alpha, beta, and gamma diversities in microbiome metabarcoding studies. *PLoS One*, 19(10), e0309065.

[\[PubMed\]](#)

Ferretti, P., et al. (2017). Experimental metagenomics and ribosomal profiling of the human skin microbiome. *Experimental Dermatology*, 26(3), 211–219.

[\[PubMed\]](#)

Fleischmann, R. D., et al. (1995). Whole-genome random sequencing and assembly of *Haemophilus*

influenzae Rd. *Science*, 269(5223), 496–512.

[\[PubMed\]](#)

Franzosa, E. A., et al. (2018). Species-level functional profiling of metagenomes and metatranscriptomes. *Nature Methods*, 15(11), 962–968.

[\[PubMed\]](#)

Huang, Y., et al. (2023). High-throughput microbial culturomics using automation and machine learning. *Nature Biotechnology*, 41(10), 1424–1433.

[\[PubMed\]](#)

Hummon, A. B., et al. (2007). Isolation and solubilization of proteins after TRIzol extraction of RNA and DNA from patient material following prolonged storage. *BioTechniques*, 42(4), 467–70, 472.

[\[PubMed\]](#)

Kennedy, K. M., et al. (2023). Questioning the fetal microbiome illustrates pitfalls of low-biomass microbial studies. *Nature*, 613(7945), 639–649.

[\[https://doi.org/10.1038/s41586-022-05546-8\]](https://doi.org/10.1038/s41586-022-05546-8) [\[PubMed\]](#)

Knight, R., et al. (2018). Best practices for analysing microbiomes. *Nature Reviews. Microbiology*, 16(7), 410–422.

[\[PubMed\]](#)

Lagier, J.-C., et al. (2012). Microbial culturomics: Paradigm shift in the human gut microbiome study. *Clinical Microbiology and Infection: The Official Publication of the European Society of Clinical Microbiology and Infectious Diseases*, 18(12), 1185–1193.

[\[PubMed\]](#)

Li, X.-M., et al. (2023). Effects of stool sample preservation methods on gut microbiota biodiversity: New original data and systematic review with meta-analysis. *Microbiology Spectrum*, 11(3), e0429722.

[\[PubMed\]](#)

Lloyd-Price, J., et al. (2019). Multi-omics of the gut microbial ecosystem in inflammatory bowel diseases. *Nature*, 569(7758), 655–662.

[\[https://doi.org/10.1038/s41586-019-1237-9\]](https://doi.org/10.1038/s41586-019-1237-9) [\[PubMed\]](#)

Lu, J., & Salzberg, S. L. (2020). Ultrafast and accurate 16S rRNA microbial community analysis using Kraken 2. *Microbiome*, 8(1), 124.

[\[https://doi.org/10.1186/s40168-020-00900-2\]](https://doi.org/10.1186/s40168-020-00900-2) [\[PubMed\]](#)

Lu, J., et al. (2022). Metagenome analysis using the Kraken software suite. *Nature Protocols*, 17(12), 2815–2839.

[\[PubMed\]](#)

Lücking, R., et al. (2021). Fungal taxonomy and sequence-based nomenclature. *Nature Microbiology*, 6(5), 540–548.

[\[PubMed\]](#)

Marchesi, J. R., & Ravel, J. (2015). The vocabulary of microbiome research: A proposal. *Microbiome*, 3, 31.

[\[https://doi.org/10.1186/s40168-015-0094-5\]](https://doi.org/10.1186/s40168-015-0094-5) [\[PubMed\]](#)

McDonald, D., et al. (2024). Greengenes2 unifies microbial data in a single reference tree. *Nature Biotechnology*, 42(5), 715–718.

[\[PubMed\]](#)

Meyer, A., et al. (2010). Fast evolving 18S rRNA sequences from Solenogastres (Mollusca) resist standard PCR amplification and give new insights into mollusk substitution rate heterogeneity. *BMC Evolutionary Biology*, 10, 70.

[\[PubMed\]](#)

Milanese, A., et al. (2019). Microbial abundance, activity and population genomic profiling with mOTUs2. *Nature Communications*, 10(1), 1014.

[\[PubMed\]](#)

Mordant, A., & Kleiner, M. (2021). Evaluation of sample preservation and storage methods for metaproteomics analysis of intestinal microbiomes. *Microbiology Spectrum*, 9(3), e0187721.

[\[PubMed\]](#)

Nearing, J. T., Comeau, A. M., & Langille, M. G. I. (2021). Identifying biases and their potential solutions in human microbiome studies. *Microbiome*, 9(1), 113.

[\[https://doi.org/10.1186/s40168-021-01059-0\]](https://doi.org/10.1186/s40168-021-01059-0) [\[PubMed\]](#)

Nishijima, S., et al. (2025). Fecal microbial load is a major determinant of gut microbiome variation and a confounder for disease associations. *Cell*, 188(1), 222–236.e15.

[\[PubMed\]](#)

Ojala, T., et al. (2023). Current concepts, advances, and challenges in deciphering the human microbiota with metatranscriptomics. *Trends in Genetics: TIG*, 39(9), 686–702.

[\[PubMed\]](#)

Ott, S. J., et al. (2017). Efficacy of sterile fecal filtrate transfer for treating patients with clostridium difficile infection. *Gastroenterology*, 152(4), 799–811.e7.

[\[PubMed\]](#)

Pace, N. R. (1997). A molecular view of microbial diversity and the biosphere. *Science*, 276(5313), 734–740.

[\[PubMed\]](#)

Pasolli, E., et al. (2019). Extensive unexplored human microbiome diversity revealed by over 150,000 genomes from metagenomes spanning age, geography, and lifestyle. *Cell*, 176(3), 649–662.e20.

[\[PubMed\]](#)

Patejko, M., Jacyna, J., & Markuszewski, M. J. (2017). Sample preparation procedures utilized in microbial metabolomics: An overview. *Journal of Chromatography. B, Analytical Technologies in the Biomedical and Life Sciences*, 1043, 150–157.

[\[PubMed\]](#)

Pathmasiri, W., et al. (2024). Untargeted metabolomics reveal signatures of a healthy lifestyle. *Scientific Reports*, 14(1), 13630.

[\[PubMed\]](#)

Pinto, Y., & Bhatt, A. S. (2024). Sequencing-based analysis of microbiomes. *Nature Reviews. Genetics*, 25(12), 829–845.

[\[PubMed\]](#)

Podlesny, D., et al. (2022). Metagenomic strain detection with SameStr: Identification of a persisting core gut microbiota transferable by fecal transplantation. *Microbiome*, 10(1), 53.

[\[https://doi.org/10.1186/s40168-022-01251-w\]](https://doi.org/10.1186/s40168-022-01251-w) [\[PubMed\]](#)

Pollock, J., et al. (2018). The madness of microbiome: Attempting to find consensus “Best Practice” for 16S microbiome studies. *Applied and Environmental Microbiology*, 84(7), e02627–e02617.

Available at: <https://doi.org/10.1128/AEM.02627-17>

[\[PubMed\]](#)

Prokopowich, C. D., Gregory, T. R., & Crease, T. J. (2003). The correlation between rDNA copy number and genome size in eukaryotes. *Genome*, 46(1), 48–50.

[\[PubMed\]](#)

Quast, C., et al. (2013). The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools. *Nucleic Acids Research*, 41(Database issue), D590–D596.

[\[PubMed\]](#)

Rideout, J. R., et al. (2014). Subsampled open-reference clustering creates consistent, comprehensive OTU definitions and scales to billions of sequences. *PeerJ*, 2, e545. <https://doi.org/10.7717/peerj.545>. eCollection 2014. PMID: 25177538, PMCID: PMC4145071

Schloss, P. D. (2021). Amplicon sequence variants artificially split bacterial genomes into separate clusters. *mSphere*, 6(4), e0019121.

[\[PubMed\]](#)

Schloss, P. D., et al. (2009). Introducing mothur: Open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Applied and Environmental Microbiology*, 75(23), 7537–7541.

[\[PubMed\]](#)

Schmidt, T. S. B., Matias Rodrigues, J. F., & von Mering, C. (2014). Ecological consistency of SSU rRNA-based operational taxonomic units at a global scale. *PLoS Computational Biology*, 10(4), e1003594.

[\[PubMed\]](#)

Schoch, C. L., et al. (2012). Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. *Proceedings of the National Academy of Sciences of the United States of America*, 109(16), 6241–6246.

[\[PubMed\]](#)

Srinivas, M., et al. (2025). Evaluating the efficiency of 16S-ITS-23S operon sequencing for species level resolution in microbial communities. *Scientific Reports*, 15(1), 2822.

[\[PubMed\]](#)

Stensvold, C. R. (2024). Metabarcoding in gut protozoology. *Trends in Parasitology*, 40(12), 1173–1182.

[\[PubMed\]](#)

Suzek, B. E., et al. (2015). UniRef clusters: a comprehensive and scalable alternative for improving sequence similarity searches. *Bioinformatics*, 31(6), 926–932.

[\[PubMed\]](#)

Tyson, G. W., et al. (2004). Community structure and metabolism through reconstruction of microbial genomes from the environment. *Nature*, 428(6978), 37–43.

[\[https://doi.org/10.1038/nature02340\]](https://doi.org/10.1038/nature02340) [\[PubMed\]](#)

Van Rossum, T., et al. (2020). Diversity within species: Interpreting strains in microbiomes. *Nature Reviews. Microbiology*, 18(9), 491–506.

[\[PubMed\]](#)

Větrovský, T., & Baldrian, P. (2013). The variability of the 16S rRNA gene in bacterial genomes and its consequences for bacterial community analyses. *PLoS ONE*, 8(2), e57923.

[\[PubMed\]](#)

Walker, A. W., et al. (2015). 16S rRNA gene-based profiling of the human infant gut microbiota is strongly influenced by sample processing and PCR primer choice. *Microbiome*, 3, 26.

[\[https://doi.org/10.1186/s40168-015-0087-4\]](https://doi.org/10.1186/s40168-015-0087-4) [\[PubMed\]](#)

Westermann, A. J., & Vogel, J. (2021). Cross-species RNA-seq for deciphering host-microbe interactions. *Nature Reviews. Genetics*, 22(6), 361–378.

[\[PubMed\]](#)

Wilmes, P., Heintz-Buschart, A., & Bond, P. L. (2015). A decade of metaproteomics: Where we stand and what the future holds. *Proteomics*, 15(20), 3409–3417.

[\[PubMed\]](#)

Wishart, D. S., et al. (2022). HMDB 5.0: the Human Metabolome Database for 2022. *Nucleic Acids Research*, 50(D1), D622–D631.

[\[PubMed\]](#)

Zhao, J., et al. (2023). Data-independent acquisition boosts quantitative metaproteomics for deep characterization of gut microbiota. *NPJ Biofilms and Microbiomes*, 9(1), 4.

[\[PubMed\]](#)

5. Evolutionary Perspectives on the Human Gut Microbiome

Shauni Doms^{1,2}✉, Mathilde Poyet¹✉, Mathieu Groussin³✉ and John F. Baines^{1,2}✉

(1) Section of Evolutionary Medicine, Institute of Experimental Medicine, Kiel University, Kiel, Germany

(2) Max-Planck-Institute for Evolutionary Biology, Plön, Germany

(3) Institute of Clinical Molecular Biology, Kiel University, Kiel, Germany

✉ **Shauni Doms**

Email: doms@evolbio.mpg.de

✉ **Mathilde Poyet**

Email: m.poyet@iem.uni-kiel.de

✉ **Mathieu Groussin**

Email: m.groussin@ikmb.uni-kiel.de

✉ **John F. Baines (Corresponding author)**

Email: baines@evolbio.mpg.de

Abstract

The renewed interest in human gut microbiome research, spawned by modern developments in metagenomics, resulted in many fascinating new results, but confusion and seeming contradictions are still common in this nascent field. As for other subdisciplines of biology, evolutionary biology serves as a unifying principle for studying host–

microbe interactions. However, the range of perspectives offered by evolution is often not considered or fully appreciated in human gut microbiome research. In this chapter, we provide a broad overview of evolutionary perspectives on the human gut microbiome, which range from the origin of holobionts to strain-level microbial evolution within a host's lifetime.

Keywords Human gut microbiome – Evolutionary biology – Holobiont – Hologenome – Phyllosymbiosis – Host genetics – Metagenomics – Industrialization – Within-host evolution – Evolutionary medicine

The realization of the human gut microbiota as an important determinant for health and disease, together with the relative ease of generating metagenomic sequencing-based profiles of microbial communities, resulted in tremendous renewed interest in microbiome research from many different areas within biology. This attention resulted in many fascinating new results but also led to some degree of “microbiomania” (Eisen, [2014](#)) and confusion among researchers coming from diverse backgrounds, in part due to misunderstandings and the inherent complexity of describing and interpreting metagenomic data.

One frequent source of confusion is also one of the most fundamental questions that concerns human gut microbiome researchers, namely, “What are the origins and consequences of variability in the gut microbiome?” Part of the challenge in answering this basic question is that a second, equally important question is often not carefully considered: “At what level are you referring to?” Indeed, the gut microbiota is a complex ecological community, whose differences can range from subtle to dramatic, independently in terms of both (1) composition and/or structure and (2) their potential impact on the host. Thus, many considerations must be taken when designing and interpreting the results of comparative studies. Excellent review material is available on a wide range of topics, including diet, development, genetics, disease, ecology and evolution (Ley et al., [2006](#);

Spor et al., [2011](#); Singh et al., [2017](#); Hall et al., [2017](#); Goodrich et al., [2017](#)). Still, the full scope of evolutionary considerations for gut microbiome research, which range from the origins of “holobionts” (see definitions in Box [5.1](#)) to strain-level microbial evolution within a host’s lifetime, is often not sufficiently appreciated. Thus, in this chapter, we provide a broad overview of evolutionary perspectives on the gut microbiome and, following the lead of Dobzhansky’s oft-quoted essay (Dobzhansky, [1973](#)) and other renditions (Varki, [2006](#)), here we boldly assert that “nothing in gut microbiome research makes sense, except in the light of evolution.”

5.1 On the Origin of Holobionts and Their Hologenomes

An appropriate place to begin our considerations is at the origin of eukaryotes and multicellular organisms, as these entities arose in a world already dominated by microbes and were likely shaped by interactions with them throughout their evolution (Margulis, [1993](#); Zilber-Rosenberg & Rosenberg, [2008](#); McFall-Ngai et al., [2013](#)) (Fig. [5.1](#)). The existence of these ancient interactions is evidenced by, e.g., the existence of mitochondria and chloroplasts and the conservation of microbial pattern recognition receptor pathways across animals (Franzenburg et al., [2012](#)). Contemporary observations, on the other hand, find every plant and animal examined to be associated with diverse microbes, ranging from strict to loose associations. A largely agreed-upon term to describe these entities, comprised of macrobes and their associated microbes, is the “holobiont” (Margulis & Fester, [1991](#); Gordon et al., [2013](#)). In turn, the collective genomic content of the host genome, the organelles, and the associated microbiome is referred to as the “hologenome” (Zilber-Rosenberg & Rosenberg, [2008](#)).

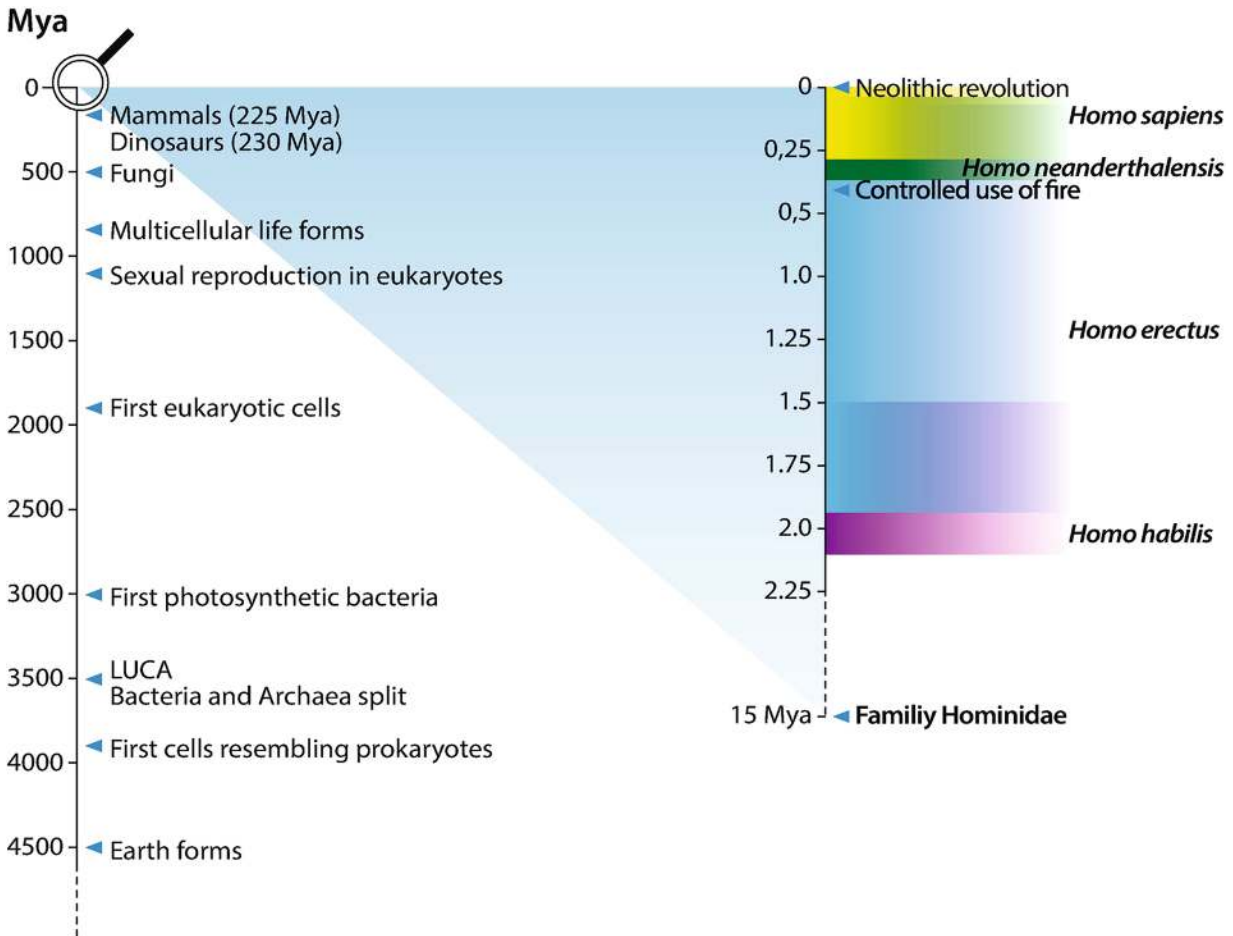


Fig. 5.1 Timeline of the history of life on earth, highlighting key events in the evolution of the human holobiont. *Homo habilis* lived between 2.1 and 1.5 Mya. Fossil evidence dates *Homo erectus* from about 1.9 Mya to about 150,000 BC. Neanderthals, *Homo neanderthalensis*, lived from about 350,000 to 40,000 BC. Our species, *Homo sapiens*, first appeared about 300,000 BC. We conservatively dated the first controlled use of fire at 400 Kya based on unequivocal archaeological evidence (Karkanas et al., [2007](#)). Less conclusive archaeological evidence suggests that fire use by *Homo erectus* may have occurred as early as one million years ago (Berna et al., [2012](#)). The Neolithic Revolution began about 10,000 BC

The utility, definition, and implications of the hologenome concept have been a matter of discussion (Moran & Sloan, [2015](#); Theis et al., [2016](#)) (Box [5.2](#)). However, after careful consideration and definition of the term, viewing evolutionary processes through the lens of holobionts and their hologenomic content offers a number of valuable principles that aid in conceptual understanding of host–microbiome interactions and, importantly, help guide research (Bordenstein & Theis, [2015](#)). Among these principles is that holobionts and

hologenomes represent a critical unit of biological organization, which includes the nuclear genome, organelles, and microbiome. Beneficial, neutral, and deleterious changes can occur at each of the above three sublevels and contribute to variation in holobiont traits, which is, in turn, the raw material upon which selection can act. Accordingly, variation in the microbiome can also contribute to adaptation and speciation (Bordenstein & Theis, [2015](#)).

5.2 The Bacterial 16S rRNA Gene and Shotgun Metagenomics as Tools for Studying Holobionts

Most of the data collected for characterizing the microbial component of holobionts to date is based on either bacterial 16S rRNA genes or shotgun metagenomic profiles. The 16S rRNA gene is a commonly used marker gene due to its evolutionary properties (Woese & Fox, [1977](#)), as it contains both conserved, slowly evolving regions and fast-evolving, hypervariable regions. While the former enables the design of “universal” primers (i.e., those that can amplify widely divergent bacterial taxa), the latter provides more fine-scale resolution to identify individual taxa. Shotgun metagenomics consists of sequencing the entire pool of DNA sequences from a given biospecimen, providing both taxonomic and functional information on the microbiome. Numerous tools and pipelines to process these data, from quality control to feature profiling and diversity metric calculation, were developed, such as Qiime2 for 16S (Bolyen et al., [2019](#)) and Metaphlan4 for metagenomics (Blanco-Míguez et al., [2023](#)). Reference databases, such as Greengenes for 16S rRNA gene data (McDonald et al., [2024](#)) or GTDB-Tk for metagenomic data (Parks et al., [2020](#)), can be used by these tools to calculate abundance profiles of microbial taxonomic ranks, ranging from phylum to species.

5.3 Concepts and Terms for Comparative Evolutionary Studies of Holobionts

Comparative studies discussed throughout the chapter, in large part, rely on overall bacterial community similarity/dissimilarity between individual samples (holobionts), as determined by shared presence and/or abundance of bacterial taxa determined by the 16S rRNA gene or metagenomic data. Differences in bacterial community composition between samples are typically quantified by various measures of beta diversity (Box [5.1](#)). Thus, we will mostly be discussing the “evolution” of the microbiome in terms of inferred bacterial community-level changes over time, based on comparing hosts at varying degrees of relatedness, ranging from among mammals to differences between individual humans.

A second important concept of microbiome evolution discussed in this chapter concerns the co-divergence, or sometimes co-speciation, between hosts and their microbial symbionts. Co-divergence can be observed when the topologies of the host phylogeny and the symbiont phylogeny are congruent (Groussin et al., [2020](#)) (Box [5.1](#)). Co-speciation events are signatures of a prolonged history of host–microbe associations, which may reflect a more intimate interplay between components of the holobionts (Fig. [5.2a](#)). Finally, the term phylosymbiosis describes a related phenomenon when the overall pattern of similarity among host-associated microbial communities parallels the phylogeny of their hosts (Fig. [5.2b](#)).

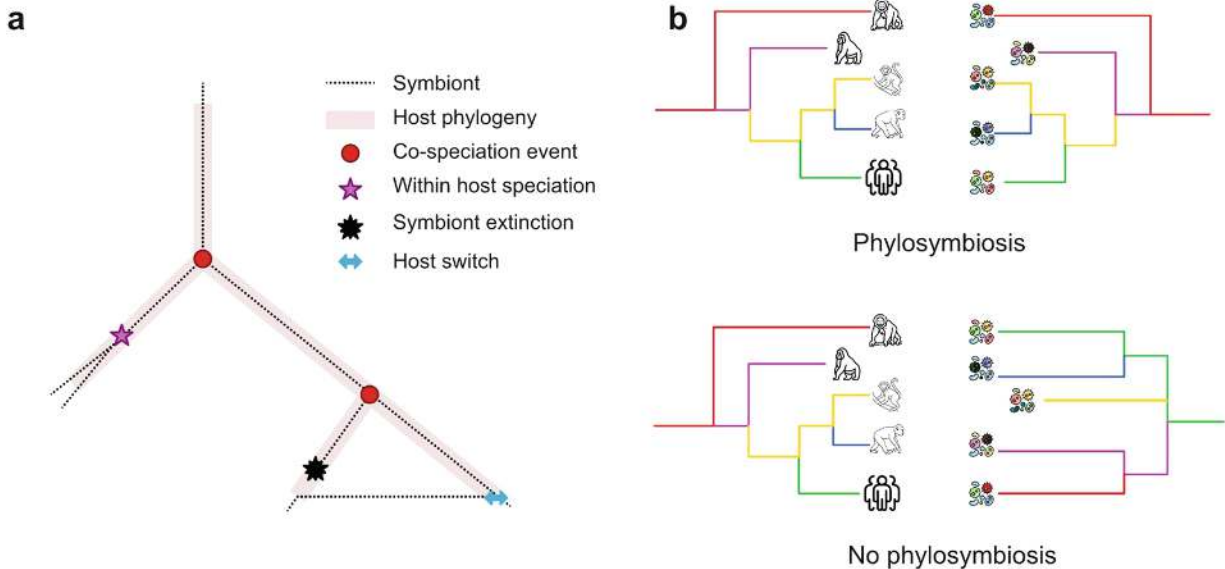


Fig. 5.2 Schematic overview of the evolutionary patterns of host-microbe evolution. **(a)** The four host-microbe evolutionary pathways. Four evolutionary mechanisms can be used to explain host-symbiont co-occurrence patterns. Individual symbionts and their hosts may co-speciate, resulting in co-phylogenetic patterns (red circle); symbionts may speciate within a single host (pink star), switch hosts (blue arrow), or go extinct (black double star). **(b)** Phylosymbiosis (top) versus stochastic community assembly (bottom). The host phylogeny is displayed on the left, and the phylogeny is based on the host-associated microbes on the right. The color of the branches indicates the host and associated community. In phylosymbiosis, the host phylogeny is congruent with the microbiome's phylogeny (top). However, if the phylogenies do not parallel each other, the assembly of the host's microbiome is likely to be a result of stochasticity (bottom)

5.4 The Gut Microbiome Across Mammalian Evolution

Phylogenetic comparison is one of the most common means of inference used to test evolutionary hypotheses, whereby similarities and differences between species are interpreted in terms of their evolutionary history. To understand the evolution of the gut microbiome during mammalian evolution, researchers have applied this method and analyzed the relative roles of evolutionary history and changes in diet across host species, which revealed many important insights (Ley et al., [2008a](#)). Overall, most studies found significant contributions of both host phylogeny and diet on the composition of the gut microbiome, with stronger effects for host phylogeny or diet being dependent on mammalian clades, comparative methods, and breadth of

host traits being sampled (Amato et al., [2019](#); Brown et al., [2023](#); Nielsen et al., [2023](#)). It was also found that host phylogeny and diet do not determine the composition of bacterial lineages at the same taxonomic rank, which may partly explain disparities between studies in terms of the relative impact of host phylogeny (Box [5.1](#)) and the environment/diet. For instance, major dietary shifts in Mammals are associated with the selection of ancient bacterial lineages, whereas associations with host phylogeny are more apparent among recent bacterial lineages (Groussin et al., [2017](#)). Analyses further confirmed that bacterial lineages from high-rank taxonomic groups that are involved in the digestion of a plant-based diet in herbivores were independently recruited from the environment (Ley et al., [2008a](#); Groussin et al., [2017](#)). In addition, multiple reports confirmed that bacterial lineages with lower taxonomic ranks, such as genera or species, display signatures of co-speciation (Box [5.1](#), Fig. [5.2a](#)) with their mammalian hosts (Groussin et al., [2017](#); Youngblut et al., [2019](#)). Intriguingly, co-speciating bacteria present in the human gut are associated with intestinal disorders such as inflammatory bowel disease (Groussin et al., [2017](#)).

5.5 Evolutionary Patterns Among Hominids

As highlighted above, it is important to consider the phylogenetic scale at which evolutionary changes among components of the hologenome take place. In this section, we will discuss the intriguing patterns that have emerged from focusing on microbiome comparisons among humans and our closest living relatives. In the first study examining fecal samples from free-living representatives of the great apes, i.e., humans (*Homo sapiens*), three chimpanzee subspecies (*Pan troglodytes troglodytes*, *P. t. schweinfurthii*, and *P. t. ellioti*), bonobos (*Pan paniscus*), and eastern and western lowland gorillas (*Gorilla beringei* and *G. gorilla*, respectively), Ochman et al. observed that the relationship of fecal microbial communities among these five species parallels the phylogeny of the host species (Ochman et al., [2010](#)). Together with

similar observations in numerous other unrelated animal hosts, these results helped inspire the term “phylosymbiosis” (Box [5.1](#), Fig [5.2b](#)), which refers to the phenomenon whereby the ecological relatedness of host-associated microbial communities parallels the phylogeny of their hosts (Brooks et al., [2016](#)). The existence of phylosymbiosis implies that factors such as vertical transmission and the accumulation of host genetic differences over time significantly impact gut bacterial community composition. A follow-up study of great apes by Moeller et al. provides an additional level of resolution by performing taxonomic profiling using a faster-evolving molecular barcode, the variable region of DNA gyrase subunit B (gyrB) (Moeller et al., [2016](#)). This reveals numerous individual taxa belonging to the families *Bacteroidaceae* and *Bifidobacteriaceae* to have phylogenies congruent with those of their host, which is consistent with co-speciation. Lineages belonging to the *Lachnospiraceae*, on the other hand, display more evidence of host switching. Interestingly, this may be related to *Lachnospiraceae* containing spore-forming members that can survive outside the gut, which is not the case for *Bacteroidaceae* and *Bifidobacteriaceae*. These observations demonstrate how evolutionary analyses help interpret contrasting patterns among individual members of the microbiome.

In another study, Moeller et al. provide an additional critical observation, namely, that in terms of community composition, the human gut microbiome has experienced accelerated evolution relative to that of other wild hominids (Moeller et al., [2014](#)). Specifically, the authors found that changes in bacterial abundances steadily accumulated among African apes, but humans, in particular, have experienced an accelerated loss of ancestral microbes over the course of hominization. Recent studies on the microbiome of non-human great apes and geographically diverse human populations use shotgun metagenomic sequencing to identify metagenome-assembled genomes (MAGs) (Sanders et al., [2023](#); Rühlemann et al., [2024](#)). Both studies confirm that phylosymbiosis in hominids is strongly supported by community structure and that humans have experienced an accelerated loss of bacterial clades. In addition, Sanders et al. found that co-

diversifying clades experienced elevated rates of extinction in humans, suggesting that host species-specific symbionts are particularly susceptible to elimination in humans. Most of these bacterial clades were lost from both industrialized and non-industrialized human populations, which suggests that the extinction occurred in humans' more distant evolutionary past. Rühlemann et al. found that microbial characteristics such as decreased genome size and gene counts, susceptibility to bile acids, and utilization of multiple simple carbohydrates were found to be depleted among bacteria with co-phylogenetic patterns, suggesting that the specialization of microbes to colonize the hominid gut and use complex carbohydrates derived from the host is reflected in these depletions.

Interesting possible explanations for the accelerated evolution of the human gut microbiome are recent culturally defined practices, e.g., meat consumption, the consumption of processed starches, and a hygienic living environment. For example, the “expensive tissue hypothesis” argues that a dietary increase in meat by *Homo* species throughout the Pleistocene period (2.5 Mya to 12 Kya) fostered the development of the large brain size by providing essential nutrition necessary for this evolutionary development, at the expense of larger guts, due to energy trade-offs (Aiello & Wheeler, [1995](#); Ley et al., [2008b](#)). A more recent comparative study across mammals calls the necessity of such an energetic trade-off in the context of brain evolution into question (Navarrete et al., [2011](#)). However, compared to wild apes, the human gut microbiome does consist of a higher relative abundance of the genus *Bacteroides*, a taxon associated with a diet rich in animal products (Wu et al., [2011](#); Moeller et al., [2014](#)), which is consistent with a change toward animal-based diets. A further cultural factor to consider is the habitual use of fire for food preparation beginning approximately 350,000 years ago (Shimelmitz et al., [2014](#)) (Fig. [5.1](#)), which may have reduced the selective pressure to retain certain microbes, such as those responsible for digesting more resistant components of plant-based nutrition (Gillings et al., [2015](#)). The use of fire could also presumably disrupt previous means of

transmission/acquisition of ancestral microbes. Thus, the evolution of the gut microbiome in hominids is not merely a reflection of genetic changes but also a response to dietary shifts, e.g., meat consumption, consumption of processed starches, and other recent culturally defined practices.

5.6 Variation in the Gut Microbiome Among Human Populations

Following dietary changes in our more distant human ancestors, the Neolithic Revolution (about 10 K BC) marks the transition from nomadic populations with foraging activities as main modes of subsistence to settled communities engaged in agricultural practices and animal domestication (Richards, [2002](#)). Evidence of adaptation to this transition has been left in the human genome, such as the amylase (AMY1) copy number. This enzyme hydrolyzes starch and correlates positively with human salivary protein levels. Individuals living in communities consuming a diet rich in starches, namely, agricultural societies, have higher copy numbers of AMY1 than individuals from communities that traditionally eat diets low in starch, like rain forest and circum-arctic hunter-gatherer groups (Perry et al., [2007](#); Ley et al., [2008b](#)). The selective pressure for an increase in amylase activity is also hypothesized to be in response to conflict with members of the gut microbiota, whereby greater energy gains can be achieved by faster uptake of starch monomers in the upper GI tract compared to the lower GI tract, where fermentation of starch by microbes occurs (Walter & Ley, [2011](#)).

Studies of the gut microbiome across human populations worldwide revealed drastic microbial shifts that occurred during ancient and recent human evolution. A decrease in taxonomic diversity in the gut microbiome of industrialized societies was observed early on when comparing the gut microbiome of Amerindian people in Venezuela, individuals living in rural communities in Malawi, and people residing in metropolitan areas of the USA (Yatsunenko et al.,

[2012](#)). This was further confirmed in follow-up studies that included populations from other geographies (Morton et al., [2015](#); Gomez et al., [2016](#)). Evolutionary investigations that included the gut microbiome of Great Apes also confirmed a loss of bacterial diversity along the human lineage, which greatly increased in populations living in industrialized societies (Fig [5.3a](#)) (Gomez et al., [2019](#); Rühlemann et al., [2024](#)). In addition, large shifts in microbial composition are observed among populations living with different lifestyles, from foraging to industrialized modes of subsistence (Smits et al., [2017](#)) (Fig [5.3b](#)). Several gut microbial markers of non-industrialized and industrialized societies have been identified, including *Prevotella* and *Spirochaetes*, which are enriched among non-industrialized groups, and *Bacteroides* and *Verrucomicrobia*, whose abundance is increased among industrialized populations (Fig [5.3c](#)) (Smits et al., [2017](#); Pasolli et al., [2019](#)). Metagenomic and metabolomic investigations also revealed a myriad of functional attributes that reflect host lifestyle and industrialization (Schnorr et al., [2014](#)). For instance, genes involved in carbohydrate metabolism, tryptophan metabolism, fimbrial functions, and degradation of complex pectins are differentially abundant between industrialized and non-industrialized groups (Pasolli et al., [2019](#); Tett et al., [2019](#)). Recent investigation of ancient gut microbiomes, reconstructed from 1000- to 2000-year-old palaeofeces, revealed multiple taxonomic and functional similarities with the microbiota of modern non-industrialized populations (Wibowo et al., [2021](#)). It suggests that differences in the gut microbiome between populations worldwide are driven by societal practices of industrialized lifestyles, such as regular consumption of low-fiber ultra-processed foods and increased exposure to antibiotics (Sonnenburg & Sonnenburg, [2019](#)). While industrialized lifestyles are very likely to play a role in driving changes in the gut microbiome during recent human evolution, the contribution of confounding factors such as host history, host genetics, and geography remains to be properly quantified. Interestingly, a recent study discovered several bacteria that show evidence of co-diversification with human lineages (Suzuki et al., [2022](#)).

This suggests that ancient human evolution, through migrations and population bottlenecks, imprinted the evolution of gut bacteria, with signals still present in the microbiome of modern worldwide societies.

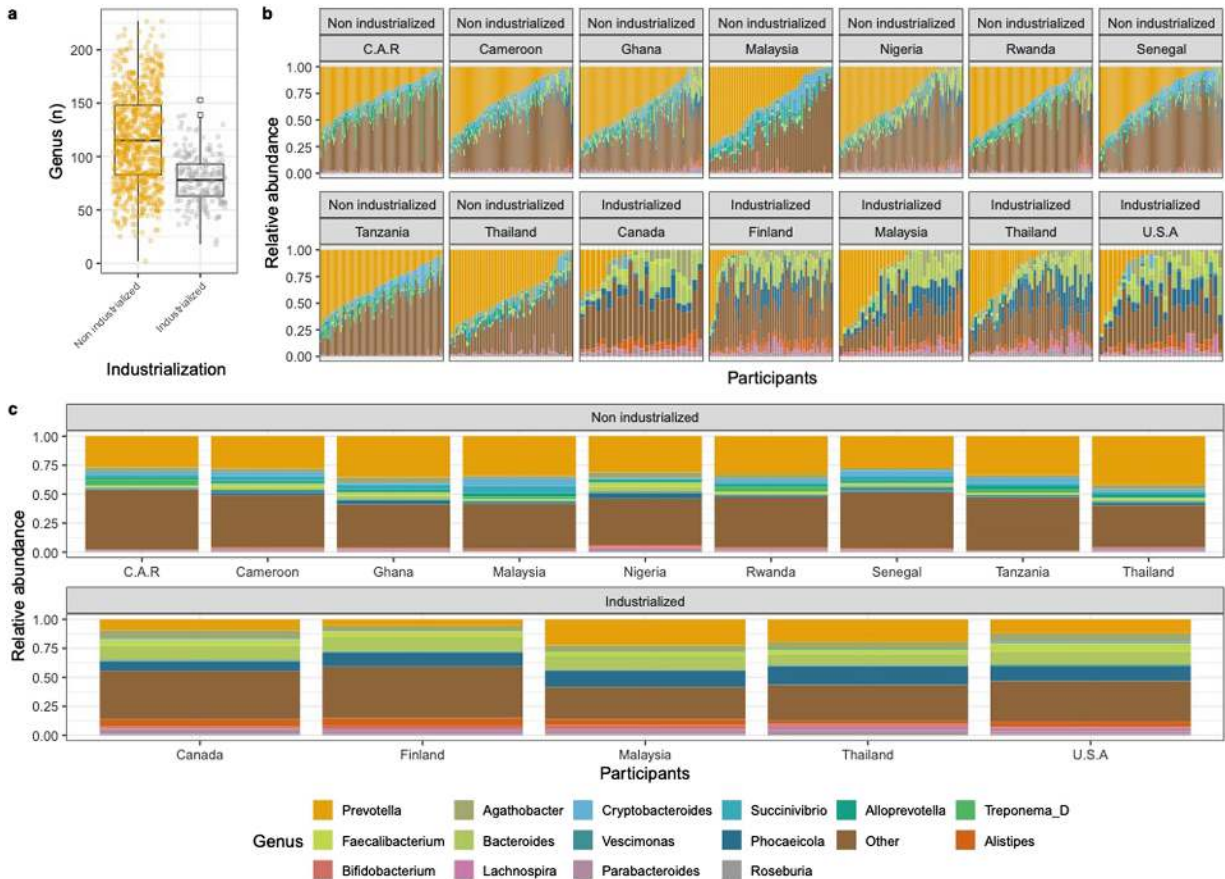


Fig. 5.3 Bacterial diversity and composition within the gut microbiome of worldwide industrialized and non-industrialized populations. **(a)** Number of unique genera in industrialized and non-industrialized individuals. Abundance data were normalized in counts per millions. **(b)** Genus-level composition of individuals from various populations across 12 countries. The 15 most abundant genera are shown. The samples are ordered in descending relative abundance of the most abundant genera. Both industrialized and non-industrialized populations were recruited in Thailand and Malaysia. **(c)** Average relative abundance of the 15 most abundant genera across populations. (Data are from Poyet et al. (2025))

5.7 Interindividual Variation

In the previous sections, we discussed species-specific aspects of the microbiome (Sects. 5.4 and 5.5) as well as its variability among human populations (Sect. 5.6). In this section, we will examine the origins of microbiome variation between individuals from the same population.

From an evolutionary perspective, this is of fundamental importance, as it is the differences between individuals that are ultimately converted to variation between populations and species.

Interindividual variation in the human gut microbiome arises from multiple sources, which can be roughly divided into genetic, environmental, lifestyle, and health-related factors. While the latter categories all influence the microbiome, we will focus here on host genetic influences, as it is the underlying host genetic architecture that has the greatest capacity to shape the long-term evolution of host-associated microbial traits. More explicitly, host genetics is most appropriately understood in the context of Darwin's conditions for evolution to occur by natural selection, extended to microbial symbionts (Hurst, [2017](#)): (i) there is variation in microbiome composition between individuals that produces phenotypic differences, (ii) this variation affects the chance of host survival and/or its reproductive success (i.e., fitness differences exist), and (iii) there is a heritable component of microbiome composition. Whether or not there is a heritable element to microbiomes is an important topic of research and discussion (Morris & Bohannan, [2024](#)).

Numerous studies have established that gut microbiome traits display significant heritabilities, reinforcing the idea that host genetics play a role in shaping microbial composition. Twin studies demonstrated that 8–12% of gut microbial taxa are heritable (Goodrich et al., [2016](#), n = 1126 twin pairs; Kurilshikov et al., [2021](#), n = 744 twin pairs), with heritability estimates ranging up to 42% for individual single taxa. Goodrich et al. further show that these heritable taxa are temporally stable over long periods, suggesting a smaller effect of environmental factors on their relative abundances. Complementing these studies, genome-wide association studies (GWAS) also enable SNP-based heritability estimates and, in addition, have identified specific genetic loci linked to specific microbial taxa. Well-replicated associations include the lactase (LCT) gene, which influences *Bifidobacterium* abundance (Blekhman et al., [2015](#); Goodrich et al., [2016](#); Kurilshikov et al., [2021](#); Lopera-Maya et al., [2022](#); Qin et al.,

[2022](#)), and the ABO blood group locus, which affects *Bacteroides* and *Faecalibacterium* abundances (Rühlemann et al., [2021](#); Kurilshikov et al., [2021](#)). These important studies demonstrate that host genetic variation contributes to the diversity of gut microbiomes between individuals. Thus, this heritable component provides a substrate for selection, meaning that certain bacterial traits could persist or shift over time, with the potential to influence host adaptation and health.

At this point, further clarification of the term “role of host genetics” is, however, warranted. As pointed out by Goodrich et al., it must be emphasized that estimates of genetic effects in human studies are based on the subset of human genes that actually contain functionally related variation (Goodrich et al., [2017](#)). Because of the important functions that gut microbes provide to the host and the challenge to contain and regulate them, many, if not most, genes involved in host-microbe interactions may experience predominantly negative or “purifying” selection (Box [5.1](#)) against mutations disrupting their function. As a consequence, these genes would not be expected to display appreciable variation within a host species. Therefore, estimates of the contribution of host genetic variation to microbiome diversity must be interpreted cautiously. Rather than indicating a fixed proportion of microbiome composition dictated by genetics, these estimates reflect the proportion of measurable variation explained within the constraints of current study designs. Furthermore, feasibility and technical aspects also limit the identification of host-microbial traits. Although it was previously hypothesized that bacteria inhabiting the intestinal mucosa may be more influenced by host genetics due to more intimate contact with the host (Spor et al., [2011](#)), the majority of studies are based on feces due to limited access to biopsy material. Indeed, subsequent studies found evidence of stronger genetic effects in the intestinal mucosa of mice (Linnenbrink et al., [2013](#); Doms et al., [2022](#)), and candidate genes observed to influence mucosal-attached communities (Rausch et al., [2011](#); Tong et al., [2014](#)) fail to be replicated in studies based on fecal material (Davenport, [2016](#)). Thus, it is likely that a portion of functionally related genetic variation influencing the

gut microbiome may be currently overlooked due to differences in habitats within the intestine.

5.8 Within-Host Evolution

In this final section, we transition to the microevolutionary processes that operate within an individual's lifetime. The vast majority of microbiome studies are conducted from an ecological perspective, focusing on community composition, diversity, and stability across individuals and environments. However, it is increasingly clear that the microbial populations comprising the gut microbiome undergo significant within-host (or “intrahost”) genetic evolution, shaped by mutation, horizontal gene transfer, and selection (Barreto & Gordo, [2023](#)).

Much of our knowledge on within-host evolution is derived from experimental mouse models, which provide controlled environments that enable microbial genetic changes to be tracked over time. Gnotobiotic mice colonized with a single bacterial strain or defined community are particularly useful for this purpose. For example, an experiment that monocolonized germ-free mice with *Escherichia coli* demonstrated rapid diversification within individual mice, with multiple strains being maintained due to trade-offs between resistance to bile salts and nutritional competence (Paepe et al., [2011](#)). Mouse models of inflammatory bowel disease (IBD) also reveal important related consequences of bacterial evolution. *E. coli* strains in inflamed mouse guts were shown to evolve higher motility and stress resistance, which are traits that enhance survival in an inflammatory environment but potentially contribute to the pathology of the host (Elhenawy et al., [2019](#); Barreto et al., [2020](#)).

While much of our understanding of within-host evolution has come from experimental mouse models, longitudinal studies in humans also now provide clear evidence that gut bacteria evolve within individual hosts. For example, in healthy human volunteers, it was shown that *Bacteroides fragilis*, a very common gut commensal,

acquires de novo mutations over time, with selection occurring on traits such as polysaccharide utilization and capsule synthesis (Zhao et al., [2019](#)). An important principle used both here and in the aforementioned mouse models is using signatures of parallel evolution to infer adaptation. Parallel evolution can be defined as the occurrence of identical or functionally similar mutations that arise independently in separate lineages exposed to similar environments—and hence selective pressures (Box [5.1](#)).

Using a strain-tracking approach applied to shotgun metagenomic sequence data, Kumbhari et al. ([2024](#)) identified hundreds of taxonomically diverse strain-level lineages that are enriched in IBD patients. Their study also revealed parallel genetic changes in pathways related to motility, oxidative stress, and others. These results thus substantiate the relevance of mouse models in understanding the nature of bacterial genetic adaptation to an inflamed environment. Importantly, however, the molecular phylogenetic approach used by Kumbhari et al. ([2024](#)) also enabled the estimation of divergence times between health- and IBD-associated lineages, which were found to be on the order of several million years (Kumbhari et al., [2024](#)). This suggests that past selective pressures—such as episodes of infectious gastroenteritis—may have played a role in shaping bacterial lineages that are now pre-adapted to inflammatory conditions like IBD.

5.9 Conclusion and Outlook

In this chapter, we have outlined the range of perspectives from which evolutionary considerations help understand the composition, function, and changes in the human gut microbiome. This spans from the origins of holobionts and host–microbe co-diversification to strain-level microbial evolution occurring within a host’s lifetime. Evolutionary biology will likely continue to play a key role in the future of microbiome research.

One important question to address, for example, is why host genetic variation for gut microbiome traits exists. A key example is the

association of the human *Lactase* (*LCT*) gene and *Bifidobacterium* abundance (Blekhman et al., [2015](#)), where variation exists as a by-product of adaptive evolution in the human genome (lactase persistence mutations evolved multiple times independently in response to the advent of dairy farming) (Goodrich et al., [2017](#)). A similar possibility pointed out by Blekhman et al. is the so-called bystander effect (Blekhman et al., [2015](#); Benson, [2015](#)), where alleles providing resistance against pathogens also happen to influence the commensal microbiome. It is also conceivable that such effects may not always represent mere “bystanders” but may also ensue “collateral damage” when the changes are undesirable. Given the prevalence of pathogen resistance-associated variation in the human genome (Fumagalli et al., [2009](#); Andrés et al., [2009](#)), systematically evaluating the overlap between pathogen- and gut microbiome-associated loci may represent a promising strategy to identify variation relevant to personalized medicine.

To date, most microbiome GWAS (mGWAS) studies have focused on bacterial abundances as phenotypes. However, a recent shift toward shotgun metagenomic sequencing allows researchers to assess not only taxonomic composition but also the functional potential of the microbiome and structural variations in microbial genes. This approach addresses the functional redundancy between microbial genomes, offering a more precise understanding of how microbial communities contribute to host traits. Additionally, it enables the emerging field of “quantitative hologenomics”, which integrates host and microbial genetic variation to dissect the genetic basis of complex phenotypic traits. Unlike traditional quantitative genetics, which focuses on a single species’ genetic diversity, quantitative hologenomics provides a framework to study holobiont-level interactions, offering novel insights into host–microbiome co-evolution and potential applications in precision medicine (Bordenstein et al., [2024](#)).

Beyond interindividual variation, it is now clear that microbial evolution is an ongoing process within individual hosts. It is thus important that evolutionary considerations be extended to the finest

scale, i.e., to the evolution of our own personal microbiomes. This insight offers particular promise for personalized medicine, whereby not only “dysbiosis” but also *evolutionary* processes that occur in the context of diseases involving the microbiome may ultimately be targeted for therapeutic purposes (Andreani et al., [2024](#)).

Box 5.1: Definitions

- *Beta diversity*: In ecology, this term refers to the ratio between regional and local species diversity. In microbiome research, the term typically refers to differences in community composition between samples.
- *Co-adaptation*: the process via which two or more species, genes, or phenotypic characteristics adapt together.
- *Co-divergence*: the parallel divergence of ecologically associated lineages within two distinct phylogenies.
- *Co-evolution*: when the process of natural selection reciprocally influences the development of two or more species.
- *Co-speciation*: a form of coevolution in which the speciation of one species dictates the speciation of another species.
- *Holobiont*: a host organism plus its associated microorganisms.
- *Hologenome*: the genomic content of the host genome, the organelles, and the associated microbiome.
- *Parallel evolution*: the occurrence of identical or functionally similar mutations that arise independently in separate lineages exposed to similar environments. This enables the inference of adaptation, following the logic that if evolution were driven purely by random mutation and genetic drift, then unique mutations would be expected in different individuals, due to the large number of possible genetic changes. If the same (or similar) genetic changes occur across multiple hosts, it suggests that they are not random but rather favored by natural selection.
- *Phylogeny*: inferred evolutionary relationships among various biological species or other entities.

- *Phylosymbiosis*: a phenomenon whereby the ecological relatedness of host-associated microbial communities parallels the phylogeny of their hosts.
- *Purifying selection*: also termed negative selection. This refers to the phenomenon whereby deleterious genetic variation, i.e., that which reduces an organism's fitness, is removed from populations through natural selection.
- *Symbiosis*: relationship between species where at least one of the species receives a net benefit.

Box 5.2: Controversy A large part of the controversy surrounding the hologenome concept relates to the term co-evolution. An almost ubiquitous statement appearing in the introductions of host-microbiome studies is that hosts and their associated microbes represent the outcome of millions of years of co-evolution. However, as Moran and Sloan point out, the definition of co-evolution is more restrictive, requiring that respective partners undergo reciprocal evolutionary change due to selective forces imposed on each other, and many other alternative phenomena can produce patterns of host-microbial association appearing consistent with co-evolution (Moran & Sloan, [2015](#)). Although co-evolution within mammalian holobionts has most certainly occurred, its likelihood is dependent on the mode of transmission of microbes, and the extent and nature of co-evolution are still largely to be determined.

A second contention pertains to whether the holobiont is the primary unit upon which natural selection acts or, rather, whether the original conception of the hologenome *requires* that it be the primary unit of selection (Moran & Sloan, [2015](#); Theis et al., [2016](#)). A problem with assuming that the holobiont is the primary unit of selection is that akin to problems with group selection, it requires selection at a higher level (i.e., the holobiont) to outweigh conflicts between its individual members. Given the diversity of the microbiota in terms of its composition and modes of transmission/acquisition, clear

opportunities for conflicts of interest exist, such as an individual microbe increasing its own transmission at the expense of the host. However, as noted by Theis et al., the hologenome concept does not require it to be the primary unit of selection but rather embraces multilevel selection, including those traits for which the holobiont could be a primary unit of selection (Theis et al., [2016](#)).

Box 5.3: Highlights

- Evolutionary considerations for gut microbiome research range from the origins of holobionts to strain-level microbial evolution within a host's lifetime.
- The concept of holobionts and their hologenomes provides a number of valuable principles that aid in the conceptual understanding of host–microbiome interactions and, importantly, help guide research.
- Humans have experienced accelerated evolution of their gut microbiome, most likely in response to dietary changes.
- Future research needs to account for bacterial strain-level evolution within the gut microbiome of individuals over their lifetime.

References

Aiello, L. C., & Wheeler, P. (1995). The expensive-tissue hypothesis: The brain and the digestive system in human and primate evolution. *Current Anthropology*, 36, 199–221.

[<https://doi.org/10.1086/204350>]

Amato, K. R., Sanders, J. G., Song, S. J., et al. (2019). Evolutionary trends in host physiology outweigh dietary niche in structuring primate gut microbiomes. *The ISME Journal*, 13, 576–587.

[<https://doi.org/10.1038/s41396-018-0175-0>]

[PubMed]

Andreani, N. A., Unterweger, D., Schreiber, S., & Baines, J. F. (2024). Evolutionary medicine for chronic inflammatory diseases of the gut: More than a clinical fantasy? *Gastroenterology*. <https://doi.org/10.1053/j.gastro.2024.10.016>

Andrés, A. M., Hubisz, M. J., Indap, A., et al. (2009). Targets of balancing selection in the human

genome. *Molecular Biology and Evolution*, 26, 2755–2764. <https://doi.org/10.1093/molbev/msp190>
[PubMed]

Barreto, H. C., & Gordo, I. (2023). Intrahost evolution of the gut microbiota. *Nature Reviews Microbiology*, 21, 590–603. <https://doi.org/10.1038/s41579-023-00890-6>
[PubMed]

Barreto, H. C., Sousa, A., & Gordo, I. (2020). The landscape of adaptive evolution of a gut commensal bacteria in aging mice. *Current Biology*, 30, 1102–1109.e5. <https://doi.org/10.1016/j.cub.2020.01.037>
[PubMed]

Benson, A. K. (2015). Host genetic architecture and the landscape of microbiome composition: Humans weigh in. *Genome Biology*, 16, 203. <https://doi.org/10.1186/s13059-015-0775-1>
[PubMed]

Berna, F., Goldberg, P., Horwitz, L. K., et al. (2012). Microstratigraphic evidence of in situ fire in the Acheulean strata of Wonderwerk Cave, Northern Cape province, South Africa. *Proceedings of the National Academy of Sciences*, 109, E1215–E1220. <https://doi.org/10.1073/pnas.1117620109>

Blanco-Míguez, A., Beghini, F., Cumbo, F., et al. (2023). Extending and improving metagenomic taxonomic profiling with uncharacterized species using MetaPhlAn 4. *Nature Biotechnology*, 41, 1633–1644. <https://doi.org/10.1038/s41587-023-01688-w>
[PubMed]

Blekhman, R., Goodrich, J. K., Huang, K., et al. (2015). Host genetic variation impacts microbiome composition across human body sites. *Genome Biology*, 16, 191. <https://doi.org/10.1186/s13059-015-0759-1>
[PubMed]

Bolyen, E., Rideout, J. R., Dillon, M. R., et al. (2019). Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nature Biotechnology*, 37, 852–857. <https://doi.org/10.1038/s41587-019-0209-9>
[PubMed]

Bordenstein, S. R., & Theis, K. R. (2015). Host biology in light of the microbiome: Ten principles of holobionts and hologenomes. *PLoS Biology*, 13. <https://doi.org/10.1371/journal.pbio.1002226>

Bordenstein, S. R., The Holobiont Biology Network, Gilbert, M. T. P., et al. (2024). The disciplinary matrix of holobiont biology. *Science*, 386, 731–732. <https://doi.org/10.1126/science.ado2152>
[PubMed]

Brooks, A. W., Kohl, K. D., Brucker, R. M., et al. (2016). Phyllosymbiosis: Relationships and functional effects of microbial communities across host evolutionary history. *PLoS Biology*, 14, e2000225. <https://doi.org/10.1371/journal.pbio.2000225>
[PubMed]

Brown, B. R. P., Goheen, J. R., Newsome, S. D., et al. (2023). Host phylogeny and functional traits

differentiate gut microbiomes in a diverse natural community of small mammals. *Molecular Ecology*, 32, 2320–2334. <https://doi.org/10.1111/mec.16874> [PubMed]

Davenport, E. R. (2016). Elucidating the role of the host genome in shaping microbiome composition. *Gut Microbes*, 7, 178–184. <https://doi.org/10.1080/19490976.2016.1155022> [PubMed]

Dobzhansky, T. (1973). Nothing in biology makes sense except in the light of evolution. *The American Biology Teacher*, 35, 125–129. <https://doi.org/10.2307/4444260>

Doms, S., Fokt, H., Rühlemann, M. C., et al. (2022). Key features of the genetic architecture and evolution of host-microbe interactions revealed by high-resolution genetic mapping of the mucosa-associated gut microbiome in hybrid mice. *eLife*, 11, e75419. <https://doi.org/10.7554/eLife.75419> [PubMed]

Eisen, J. A. (2014). The Tree of Life: Microbiomania and “overselling the microbiome.” In *Tree Life*. <https://phylogenomics.blogspot.com/p/blog-page.html>. Accessed 12 Feb 2025.

Elhenawy, W., Tsai, C. N., & Coombes, B. K. (2019). Host-specific adaptive diversification of Crohn’s disease-associated adherent-invasive *Escherichia coli*. *Cell Host & Microbe*, 25, 301–312.e5. <https://doi.org/10.1016/j.chom.2018.12.010>

Franzenburg, S., Fraune, S., Künzel, S., et al. (2012). MyD88-deficient Hydra reveal an ancient function of TLR signaling in sensing bacterial colonizers. *Proceedings of the National Academy of Sciences of the United States of America*, 109, 19374–19379. <https://doi.org/10.1073/pnas.1213110109> [PubMed]

Fumagalli, M., Cagliani, R., Pozzoli, U., et al. (2009). Widespread balancing selection and pathogen-driven selection at blood group antigen genes. *Genome Research*, 19, 199–212. <https://doi.org/10.1101/gr.082768.108> [PubMed]

Gillings, M. R., Paulsen, I. T., & Tetu, S. G. (2015). Ecology and evolution of the human microbiota: Fire, farming and antibiotics. *Genes*, 6, 841–857. <https://doi.org/10.3390/genes6030841> [PubMed]

Gomez, A., Petrzelkova, K. J., Burns, M. B., et al. (2016). Gut microbiome of coexisting BaAka pygmies and bantu reflects gradients of traditional subsistence patterns. *Cell Reports*, 14, 2142–2153. <https://doi.org/10.1016/j.celrep.2016.02.013> [PubMed]

Gomez, A., Sharma, A. K., Mallott, E. K., et al. (2019). Plasticity in the human gut microbiome defies evolutionary constraints. *mSphere*, 4. <https://doi.org/10.1128/msphere.00271-19>

Goodrich, J. K., Davenport, E. R., Beaumont, M., et al. (2016). Genetic determinants of the gut microbiome in UK twins. *Cell Host & Microbe*, 19, 731–743. <https://doi.org/10.1016/j.chom.2016>

[04.017](#)

Goodrich, J. K., Davenport, E. R., Clark, A. G., & Ley, R. E. (2017). The relationship between the human genome and microbiome comes into view. *Annual Review of Genetics*, 51, 413–433.

<https://doi.org/10.1146/annurev-genet-110711-155532>

[PubMed]

Gordon, J., Knowlton, N., Relman, D. A., et al. (2013). Superorganisms and holobionts. *Microbe*, 8, 152–153. <https://doi.org/10.1128/microbe.8.152.1>

Groussin, M., Mazel, F., Sanders, J. G., et al. (2017). Unraveling the processes shaping mammalian gut microbiomes over evolutionary time. *Nature Communications*, 8, 14319. <https://doi.org/10.1038/ncomms14319>

<https://doi.org/10.1038/ncomms14319>

[PubMed]

Groussin, M., Mazel, F., & Alm, E. J. (2020). Co-evolution and co-speciation of host-gut bacteria systems. *Cell Host & Microbe*, 28, 12–22. <https://doi.org/10.1016/j.chom.2020.06.013>

Hall, A. B., Tolonen, A. C., & Xavier, R. J. (2017). Human genetic variation and the gut microbiome in disease. *Nature Reviews. Genetics*, 18, 690–699. <https://doi.org/10.1038/nrg.2017.63>

[PubMed]

Hurst, G. D. D. (2017). Extended genomes: Symbiosis and evolution. *Interface Focus*, 7, 20170001.

<https://doi.org/10.1098/rsfs.2017.0001>

[PubMed]

Karkanas, P., Shahack-Gross, R., Ayalon, A., et al. (2007). Evidence for habitual use of fire at the end of the Lower Paleolithic: Site-formation processes at Qesem Cave, Israel. *Journal of Human Evolution*, 53, 197–212. <https://doi.org/10.1016/j.jhevol.2007.04.002>

<https://doi.org/10.1016/j.jhevol.2007.04.002>

[PubMed]

Kumbhari, A., Cheng, T. N. H., Ananthakrishnan, A. N., et al. (2024). Discovery of disease-adapted bacterial lineages in inflammatory bowel diseases. *Cell Host & Microbe*, 32, 1147–1162.e12.

<https://doi.org/10.1016/j.chom.2024.05.022>

Kurilshikov, A., Medina-Gomez, C., Bacigalupe, R., et al. (2021). Large-scale association analyses identify host factors influencing human gut microbiome composition. *Nature Genetics*, 53, 156–165. <https://doi.org/10.1038/s41588-020-00763-1>

<https://doi.org/10.1038/s41588-020-00763-1>

[PubMed]

Ley, R. E., Peterson, D. A., & Gordon, J. I. (2006). Ecological and evolutionary forces shaping microbial diversity in the human intestine. *Cell*, 124, 837–848. <https://doi.org/10.1016/j.cell.2006.02.017>

<https://doi.org/10.1016/j.cell.2006.02.017>

[PubMed]

Ley, R. E., Hamady, M., Lozupone, C., et al. (2008a). Evolution of mammals and their gut microbes. *Science*, 320, 1647–1651. <https://doi.org/10.1126/science.1155725>

<https://doi.org/10.1126/science.1155725>

[PubMed]

Ley, R. E., Lozupone, C. A., Hamady, M., et al. (2008b). Worlds within worlds: Evolution of the vertebrate gut microbiota. *Nature Reviews Microbiology*, 6, 776–788. <https://doi.org/10.1038/nrmicro1978>
[PubMed]

Linnenbrink, M., Wang, J., Hardouin, E. A., et al. (2013). The role of biogeography in shaping diversity of the intestinal microbiota in house mice. *Molecular Ecology*, 22, 1904–1916. <https://doi.org/10.1111/mec.12206>
[PubMed]

Lopera-Maya, E. A., Kurilshikov, A., Van Der Graaf, A., et al. (2022). Effect of host genetics on the gut microbiome in 7,738 participants of the Dutch Microbiome Project. *Nature Genetics*, 54, 143–151. <https://doi.org/10.1038/s41588-021-00992-y>
[PubMed]

Margulis, L. (1993). *Symbiosis in cell evolution: Microbial communities in the Archean and Proterozoic eons* (2nd ed.). Freeman.

Margulis, L., & Fester, R. (1991). *Symbiosis as a source of evolutionary innovation: Speciation and morphogenesis*. MIT Press.

McDonald, D., Jiang, Y., Balaban, M., et al. (2024). Greengenes2 unifies microbial data in a single reference tree. *Nature Biotechnology*, 42, 715–718. <https://doi.org/10.1038/s41587-023-01845-1>
[PubMed]

McFall-Ngai, M., Hadfield, M. G., Bosch, T. C. G., et al. (2013). Animals in a bacterial world, a new imperative for the life sciences. *Proceedings of the National Academy of Sciences*, 110, 3229–3236. <https://doi.org/10.1073/pnas.1218525110>

Moeller, A. H., Li, Y., Mpoudi Ngole, E., et al. (2014). Rapid changes in the gut microbiome during human evolution. *Proceedings of the National Academy of Sciences of the United States of America*, 111, 16431–16435. <https://doi.org/10.1073/pnas.1419136111>
[PubMed]

Moeller, A. H., Caro-Quintero, A., Mjungu, D., et al. (2016). Cospeciation of gut microbiota with hominids. *Science*, 353, 380–382. <https://doi.org/10.1126/science.aaf3951>
[PubMed]

Moran, N. A., & Sloan, D. B. (2015). The hologenome concept: Helpful or Hollow? *PLoS Biology*, 13, e1002311. <https://doi.org/10.1371/journal.pbio.1002311>

Morris, A. H., & Bohannan, B. J. M. (2024). Estimates of microbiome heritability across hosts. *Nature Microbiology*, 9, 3110–3119. <https://doi.org/10.1038/s41564-024-01865-w>
[PubMed]

Morton, E. R., Lynch, J., Froment, A., et al. (2015). Variation in rural African gut microbiota is strongly correlated with colonization by *Entamoeba* and subsistence. *PLoS Genetics*, 11, e1005658. <https://doi.org/10.1371/journal.pgen.1005658>

[[PubMed](#)]

Navarrete, A., van Schaik, C. P., & Isler, K. (2011). Energetics and the evolution of human brain size. *Nature*, 480, 91–93. <https://doi.org/10.1038/nature10629>

[[PubMed](#)]

Nielsen, D. P., Harrison, J. G., Byer, N. W., et al. (2023). The gut microbiome reflects ancestry despite dietary shifts across a hybrid zone. *Ecology Letters*, 26, 63–75. <https://doi.org/10.1111/ele.14135>

[[PubMed](#)]

Ochman, H., Worobey, M., Kuo, C.-H., et al. (2010). Evolutionary relationships of wild hominids recapitulated by gut microbial communities. *PLoS Biology*, 8, e1000546. <https://doi.org/10.1371/journal.pbio.1000546>

[[PubMed](#)]

Paepe, M. D., Gaboriau-Routhiau, V., Rainteau, D., et al. (2011). Trade-off between bile resistance and nutritional competence drives *Escherichia coli* diversification in the mouse gut. *PLoS Genetics*, 7, e1002107. <https://doi.org/10.1371/journal.pgen.1002107>

[[PubMed](#)]

Parks, D. H., Chuvochina, M., Chaumeil, P.-A., et al. (2020). A complete domain-to-species taxonomy for bacteria and archaea. *Nature Biotechnology*, 38, 1079–1086. <https://doi.org/10.1038/s41587-020-0501-8>

[[PubMed](#)]

Pasolli, E., Asnicar, F., Manara, S., et al. (2019). Extensive unexplored human microbiome diversity revealed by over 150,000 genomes from metagenomes spanning age, geography, and lifestyle. *Cell*, 176, 649–662.e20. <https://doi.org/10.1016/j.cell.2019.01.001>

[[PubMed](#)]

Perry, G. H., Dominy, N. J., Claw, K. G., et al. (2007). Diet and the evolution of human amylase gene copy number variation. *Nature Genetics*, 39, 1256–1260. <https://doi.org/10.1038/ng2123>

[[PubMed](#)]

Poyet, M., Rühlemann, M., Schaan, A. P., et al. (2025). Industrialization drives convergent microbial and physiological shifts in the human metaorganism. *BioRxiv*. 2025.10.20.683358.

Qin, Y., Havulinna, A. S., Liu, Y., et al. (2022). Combined effects of host genetics and diet on human gut microbiota and incident disease in a single population cohort. *Nature Genetics*, 54, 134–142. <https://doi.org/10.1038/s41588-021-00991-z>

[[PubMed](#)]

Rausch, P., Rehman, A., Kunzel, S., et al. (2011). Colonic mucosa-associated microbiota is influenced by an interaction of Crohn disease and FUT2 (Secretor) genotype. *Proceedings of the National Academy of Sciences*, 108, 19030–19035. <https://doi.org/10.1073/pnas.1106408108>

Richards, M. P. (2002). A brief review of the archaeological evidence for Palaeolithic and Neolithic subsistence. *European Journal of Clinical Nutrition*, 56, 16 p following 1262. <https://doi.org/10.1038/sj.ejcn.1601646>.

Rühlemann, M. C., Hermes, B. M., Bang, C., et al. (2021). Genome-wide association study in 8,956 German individuals identifies influence of ABO histo-blood groups on gut microbiome. *Nature Genetics*, 1–9. <https://doi.org/10.1038/s41588-020-00747-1>

Rühlemann, M. C., Bang, C., Gogarten, J. F., et al. (2024). Functional host-specific adaptation of the intestinal microbiome in hominids. *Nature Communications*, 15, 326. <https://doi.org/10.1038/s41467-023-44636-7>
[PubMed]

Sanders, J. G., Sprockett, D. D., Li, Y., et al. (2023). Widespread extinctions of co-diversified primate gut bacterial symbionts from humans. *Nature Microbiology*, 8, 1039–1050. <https://doi.org/10.1038/s41564-023-01388-w>
[PubMed]

Schnorr, S. L., Candela, M., Rampelli, S., et al. (2014). Gut microbiome of the Hadza hunter-gatherers. *Nature Communications*, 5, 3654. <https://doi.org/10.1038/ncomms4654>
[PubMed]

Shimelmitz, R., Kuhn, S. L., Jelinek, A. J., et al. (2014). “Fire at will”: The emergence of habitual fire use 350,000 years ago. *Journal of Human Evolution*, 77, 196–203. <https://doi.org/10.1016/j.jhevol.2014.07.005>
[PubMed]

Singh, R. K., Chang, H.-W., Yan, D., et al. (2017). Influence of diet on the gut microbiome and implications for human health. *Journal of Translational Medicine*, 15, 73. <https://doi.org/10.1186/s12967-017-1175-y>
[PubMed]

Smits, S. A., Leach, J., Sonnenburg, E. D., et al. (2017). Seasonal cycling in the gut microbiome of the Hadza hunter-gatherers of Tanzania. *Science*, 357, 802–806. <https://doi.org/10.1126/science.aan4834>
[PubMed]

Sonnenburg, J. L., & Sonnenburg, E. D. (2019). Vulnerability of the industrialized microbiota. *Science*, 366, eaaw9255. <https://doi.org/10.1126/science.aaw9255>
[PubMed]

Spor, A., Koren, O., & Ley, R. (2011). Unravelling the effects of the environment and host genotype on the gut microbiome. *Nature Reviews. Microbiology*, 9, 279–290. <https://doi.org/10.1038/nrmicro2540>
[PubMed]

Suzuki, T. A., Fitzstevens, J. L., Schmidt, V. T., et al. (2022). Codiversification of gut microbiota with humans. *Science*, 377, 1328–1332. <https://doi.org/10.1126/science.abm7759>
[PubMed]

Tett, A., Huang, K. D., Asnicar, F., et al. (2019). The *Prevotella copri* complex comprises four distinct clades underrepresented in westernized populations. *Cell Host & Microbe*, 26, 666–679.e7. <https://doi.org/10.1016/j.chom.2019.08.018>

Theis, K. R., Dheilly, N. M., Klassen, J. L., et al. (2016). Getting the hologenome concept right: An eco-evolutionary framework for hosts and their microbiomes. *mSystems*, 1, e00028-16. <https://doi.org/10.1128/mSystems.00028-16>

Tong, M., McHardy, I., Ruegger, P., et al. (2014). Reprogramming of gut microbiome energy metabolism by the *FUT2* Crohn's disease risk polymorphism. *The ISME Journal*, 8, 2193–2206. <https://doi.org/10.1038/ismej.2014.64>
[PubMed]

Varki, A. (2006). Nothing in glycobiology makes sense, except in the light of evolution. *Cell*, 126, 841–845. <https://doi.org/10.1016/j.cell.2006.08.022>
[PubMed]

Walter, J., & Ley, R. (2011). The human gut microbiome: Ecology and recent evolutionary changes. *Annual Review of Microbiology*, 65, 411–429. <https://doi.org/10.1146/annurev-micro-090110-102830>
[PubMed]

Wibowo, M. C., Yang, Z., Borry, M., et al. (2021). Reconstruction of ancient microbial genomes from the human gut. *Nature*, 594, 234–239. <https://doi.org/10.1038/s41586-021-03532-0>
[PubMed]

Woese, C. R., & Fox, G. E. (1977). Phylogenetic structure of the prokaryotic domain: The primary kingdoms. *Proceedings of the National Academy of Sciences*, 74, 5088–5090. <https://doi.org/10.1073/pnas.74.11.5088>

Wu, G. D., Chen, J., Hoffmann, C., et al. (2011). Linking long-term dietary patterns with gut microbial enterotypes. *Science*, 334, 105–108. <https://doi.org/10.1126/science.1208344>
[PubMed]

Yatsunenko, T., Rey, F. E., Manary, M. J., et al. (2012). Human gut microbiome viewed across age and geography. *Nature*, 486, 222–227. <https://doi.org/10.1038/nature11053>
[PubMed]

Youngblut, N. D., Reischer, G. H., Walters, W., et al. (2019). Host diet and evolutionary history explain different aspects of gut microbiome diversity among vertebrate clades. *Nature Communications*, 10, 2200. <https://doi.org/10.1038/s41467-019-10191-3>
[PubMed]

Zhao, S., Lieberman, T. D., Poyet, M., et al. (2019). Adaptive evolution within gut microbiomes of healthy people. *Cell Host & Microbe*, 25, 656–667.e8. <https://doi.org/10.1016/j.chom.2019.03.007>

Zilber-Rosenberg, I., & Rosenberg, E. (2008). Role of microorganisms in the evolution of animals and plants: The hologenome theory of evolution. *FEMS Microbiology Reviews*, 32, 723–735. <https://doi.org/10.1111/j.1574-6976.2008.00123.x>
[PubMed]

6. Microbiome and Diet

Francesca De Filippis^{1,2}  and Danilo Ercolini^{1,2} 

(1) Division of Microbiology, Department of Agricultural Sciences,
University of Naples Federico II, Portici (NA), Italy

(2) Task Force on Microbiome Studies, University of Naples Federico
II, Naples, Italy

 **Francesca De Filippis**

Email: francesca.defilippis@unina.it

 **Danilo Ercolini (Corresponding author)**

Email: ercolini@unina.it

Abstract

The importance of gut microbiome in influencing human health has been widely assessed. The gut microbiome may vary according to several extrinsic factors, among which diet can be considered one of the most important. Substrates provided through diet are metabolized by the gut microbiome, with the possible production of beneficial or harmful metabolites. In the past decades, dietary habits in the Western world have strongly changed, with an increase in the consumption of foods of animal origin and a decrease in the intake of fiber and complex polysaccharides. These changes in the diet impacted our microbial symbionts, possibly playing a role in the development of several diseases. The understanding of these relationships will allow, in a next future, a targeted modulation of the gut microbiome through ad hoc dietary interventions for therapeutic or preventive purposes. In this chapter, recent findings about the existing interconnections between

gut microbiome, diet, and human health are discussed, highlighting possible future perspectives.

Keywords Non-Westernized populations – Dietary nutrients – Polyphenols – Fibre – Short-chain fatty acids – Strain-level diversity

6.1 The Human Gut Microbiome

Gut microbiota of healthy adults is commonly dominated by two bacterial *phyla*, *Bacillota* (previously *Firmicutes*) and *Bacteroidota* (previously *Bacteroidetes*), with interindividual variability in their proportions. *Proteobacteria*, *Actinobacteria*, and *Verrucomicrobia* are present at lower levels (Lozupone et al., [2012](#)). In spite of the great inter- and intraindividual variability, in the past years, a classification of subjects based on the most abundant genera in their gut microbiome was proposed. Some years ago, it was observed that all the subjects may be classified in three discrete clusters, named “enterotypes,” based on the prevalence of *Prevotella*, *Bacteroides*, or *Ruminococcus* in their gut microbiome (Arumungam et al., [2011](#)). However, the “enterotype” concept was lately criticized, since a rigorous categorization may lead to an oversimplified vision of the gut microbiome (Knights et al., [2014](#); Jeffery et al., [2012](#)). On the contrary, although this classification may be attractive for understanding microbial variation in health and disease, the existence of a smooth gradient of the dominant *taxa* is more plausible, where the abundance of dominant genera varies continuously in the human population going from one enterotype to another. The important role of gut microbiota in influencing human well-being is widely recognized. It plays a primary function in host health by shaping the development of the immune system, metabolizing dietary nutrients and drugs, and synthesizing vitamins, bioactive molecules, and other beneficial or detrimental metabolites.

In the past decades, gut dysbiosis has been linked to the development of several kinds of diseases, including obesity (Turnbaugh et al., [2006](#); Le Chatelier et al., [2013](#)), diabetes (Qin et al., [2012](#)),

inflammatory bowel disease (Marchesi et al., [2016](#)), and cardiovascular diseases (Koeth et al., [2013](#)).

Moreover, the gut microbiome may influence human behavior by the bidirectional communication path between the gastrointestinal (GI) and the central nervous system, namely, the gut–brain axis. This happens by a microbiome-mediated production of molecules that have neuroactive effects, such as serotonin and γ -aminobutyric acid (GABA). Nutrients and microbial metabolites interact with the enteroendocrine cells (EECs) located along the GI tract and containing most of the nutrient receptors. Interactions with EEC receptors are crucial in mechanisms such as the regulation of appetite and insulin secretion (Furness et al., [2013](#)). Indeed, recent studies suggest that bacterial proteins may influence the appetite-controlling pathways, acting locally in the gut with a short-term effect on satiation (Breton et al., [2016](#)). Moreover, plasmatic levels of specific bacterial proteins may activate host anorexigenic circuitries with a long-term regulation of the feeding pattern (Breton et al., [2016](#)).

6.2 Diet-Induced Signatures in Gut Microbiota Composition and Functions in Rural Populations Around the World

Diet can be considered as the primary factor influencing gut microbiota composition and functionality. The first studies highlighting the key role played by diet found an association of *Bacteroides* with a high-fat/low-fiber diet, while a high abundance of *Prevotella* was linked to a diet rich in fiber and low in foods of animal origin (David et al., [2014](#); Wu et al., [2011](#)). Indeed, a short-term dietary switch from high-fat/low-fiber to high-fiber/low-fat diet causes reproducible changes in the gut microbiome that are, however, not long-lasting. On the contrary, long-term, habitual diet is the primary factor shaping our gut microbiome (David et al., [2014](#); Wu et al., [2011](#)). Over recent decades, modern dietary patterns in Western countries have undergone major compositional changes, with an increase in the consumption of red

meat, high-fat foods, and refined sugars. This “Westernization” of the diet, together with changes in the lifestyle, has surely partially contributed to the higher incidence of inflammatory disorders, such as obesity, diabetes, cardiovascular diseases, and allergies. The study of agrarian populations living in South America or Africa helped to understand the complex relationships between habitual diet and the gut microbiome (Table 6.1). Comparison of the gut microbiomes of agrarian and Western populations may help in understanding how dietary changes have affected the gut symbionts. All these studies consistently found that the composition of the gut microbiota dramatically differs between urbanized, Western people eating a high-fat and protein diet and rural populations still consuming a subsistent, agrarian diet, with low intake of products of animal origin and high consumption of fruit, vegetables, fibrous tubers, and roots, suggesting that some taxa disappeared in industrialized populations, with yet unknown consequences for human health (Segata, 2015; Sonnenburg & Sonnenburg, 2019).

Table 6.1 Principal gut microbiome signatures reported in traditional populations across the world

Traditional population	Location	Dietary habits	Gut microbiota signatures ^a	Reference
Burkinabe	Africa	Agricultural products	↑ <i>Bacteroidetes</i> , <i>Prevotella</i> , <i>Xylanibacter</i> , <i>Butyrivibrio</i> , <i>Treponema</i> ↓ <i>Firmicutes</i> , <i>Bacteroides</i> , <i>Enterobacteriaceae</i>	De Filippo et al. (2010)
Malawian	Africa	Agricultural products	↑ <i>Prevotella</i>	Yatsunenکو et al. (2012)
Guahibo Amerindians	South America	Agricultural products	↑ <i>Prevotella</i>	Yatsunenکو et al. (2012)
Hazda	Africa	Hunting and gathering	↑ <i>Prevotella</i> , <i>Succinivibrio</i> , <i>Treponema</i> , <i>Eubacterium</i> ↓ <i>Bacteroides</i> , <i>Blautia</i> , <i>Dorea</i>	Schnorr et al. (2014)

Traditional population	Location	Dietary habits	Gut microbiota signatures ^a	Reference
BaAka	Africa	Hunting and gathering	↑ Bacteroidetes, <i>Prevotella</i> , <i>Treponema</i>	Gomez et al. (2016b)
Bantu	Africa	Agricultural products	↑ Firmicutes	Gomez et al. (2016b)
Tunapuco	South America	Agricultural products	↑ <i>Treponema</i> , <i>Prevotella</i>	Obregon-Tito et al. (2015)
Matses	South America	Hunting and gathering	↑ <i>Eubacterium</i> , <i>Lachnospira</i> , <i>Catenibacterium</i> , <i>Treponema</i> , <i>Clostridium</i>	Obregon-Tito et al. (2015)
Rural South Africans	Africa	Agricultural products	↑ <i>Prevotella</i> , <i>Oscillospira</i> , <i>Succinivibrio</i> , <i>Treponema</i>	Ou et al. (2013)
Arctic Inuit	Canada	Animal fats and proteins rich diet	↓ <i>Prevotella</i>	Girard et al. (2017)
Yanomami Amerindian	South America	Hunting and gathering	↑ <i>Prevotella</i> , <i>Spirochaeta</i> , <i>Desulfovibrio</i> , <i>Helicobacter</i>	Clemente et al. (2015)
Asaro and Sausi	Papua New Guineas	Agricultural products	↑ <i>Prevotella</i> , <i>Streptococcus</i> , <i>Bacteroides</i> , <i>Odoribacter</i> ↓ <i>Parabacteroides</i> , <i>Alistipes</i> , <i>Bilophila</i>	Martínez et al. (2015)
Adadle woreda district population	Ethiopia	Agropastoralists	↑ <i>Streptococcaceae</i> , <i>Bifidobacteriaceae</i> , <i>Erysipelatoclostridiaceae</i> ↓ <i>Bacteroidaceae</i> , <i>Prevotellaceae</i>	Yersin et al. (2023)
Betsimisaraka and Tsimihety	Madagascar	Agricultural products	↑ <i>Succinatimonas</i> , <i>Eggerthella</i> , <i>Treponema succinifaciens</i> , <i>Collinsella</i> ↓ <i>Bacteroides uniformis</i> , <i>Alistipes putredinis</i> , <i>Parabacteroides diastonis</i>	Pasolli et al. (2019)

^aThe increase or decrease refers to a comparison with Western subjects

Most of these studies highlighted the loss of microbial diversity in Westernized populations (Carter et al., [2023](#); Clemente et al., [2015](#);

Martínez et al., [2015](#); Obregon-Tito et al., [2015](#); Schnorr et al., [2014](#); De Filippo et al., [2010](#)) and overlapping signatures in gut microbiota composition of the traditional population studied (Table [6.1](#)).

Prevotella, *Xylanibacter*, *Treponema*, *Succinivibrio*, *Lachnospira*, and other fiber-degrading bacteria were reported as enriched in traditional populations consuming an agrarian diet (Fig. [6.1](#)). *Treponema* is a genus belonging to the *Spirochaetes*. It was only reported to be present in the gut microbiota of nonhuman primates (Gomez et al., [2016a](#); Ley et al., [2008](#); Ochman et al., [2010](#)) and of human traditional populations (Schnorr et al., [2014](#); De Filippo et al., [2010](#); Obregon-Tito et al., [2015](#); Gomez et al., [2016b](#); Ou et al., [2013](#)) suggesting that these symbionts were lost in urban, Westernized people. The genomes of two distinct strains of *Treponema* from gut metagenomes of South Americans were reconstructed (Obregon-Tito et al., [2015](#)). These strains turned out to be clearly different from known pathogenic *Treponema* strains with their genomic potential suggesting a selective adaptation to the gut environment including the capability to use complex polysaccharides, abundant in the diet of non-Westernized subjects.

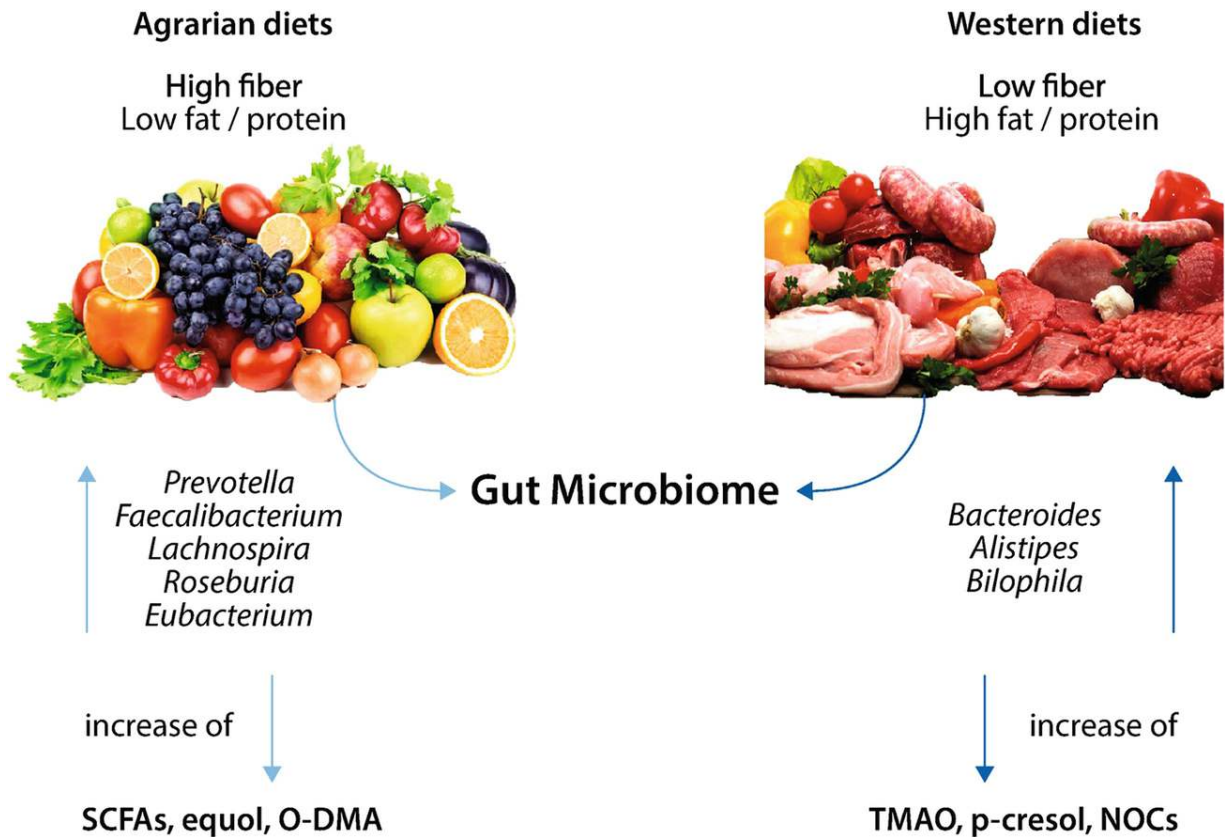


Fig. 6.1 Effect of diet on gut microbiota and metabolome

Gomez et al. ([2016b](#)) characterized the gut microbiome of two traditional African populations (BaAka and Bantu) with different diets and lifestyles: BaAka are hunter-gatherers living in the rainforest and consuming a diet rich in fibrous tubers, such as manioc roots and wild yams, while Bantu have switched to subsistence agriculture in the past century. Interestingly, when comparing their gut microbiomes with those of American control subjects, they found that Bantus showed a gut microbiota composition intermediate between BaAka and Americans. Indeed, a decrease in the abundance of fiber-degrading bacteria was observed from BaAka to Americans, with intermediate levels in Bantu, reflecting the different subsistence patterns and highlighting the effect of a recent transition to a more modern lifestyle in Bantu (Gomez et al., [2016b](#)).

On the contrary, the gut microbiota of Western subjects is often enriched in bile-tolerant microorganisms, such as *Bacteroides*, *Alistipes*,

and *Bilophila*, associated with the consumption of a diet rich in fat and protein and poor in indigestible carbohydrates (Fig. [6.1](#)). Consistently, migration from a non-Western country to the USA is associated with immediate loss of gut microbiome diversity and function, with a displacing of native strains and functions with those typical of US citizen. These effects increase with duration of U.S. residence, and are linked with an increase in obesity across generations (Vangay et al., [2018](#)).

Such a difference in microbiota composition between rural and Western populations results in different functionality. Indeed, metagenomes of Hadza hunter-gatherer and urbanized Italians showed different potential activity (Rampelli et al., [2015](#)). The Hadza microbiome contains a more diverse pool of genes that encode for enzymes able to break down a broader set of polysaccharides compared to Italians. On the contrary, metabolic pathways related to the degradation of several xenobiotics of human origin (e.g., naphthalene, xylene, or benzoate), ubiquitous in industrialized environments, were enriched in the Italian gut microbiome (Rampelli et al., [2015](#)). Moreover, Western diet, richer in fat and proteins, led to the selection of a gut microbiome with a higher capacity to degrade amino acids and convert primary bile acids in secondary bile acids, as well as simple sugars (Yatsunenko et al., [2012](#)).

6.3 Diet-Mediated Production of Beneficial or Detrimental Metabolites by the Gut Microbiome

Different dietary habits and gut microbiomes promote the production of a distinctive fecal metabolome in traditional and Western populations. Undigested dietary components reach the large intestine, where they are fermented by the microbial community to produce a wide pattern of metabolites, reflecting both the chemical diversity of the available substrates and the different metabolic potential of the gut microbiota. Most studies focused on fecal levels of short-chain fatty

acids (SCFAs). The three most abundant SCFAs, acetic, propionic, and butyric acids, are produced by bacterial fermentation of indigestible polysaccharides and are normally present in a molar ratio of about 3:1:1 (Louis et al., [2014](#)). Nondigestible carbohydrates include the structural polysaccharides of plant cell walls (non-starch polysaccharides, such as celluloses, xylans, pectins, and glucans), resistant starch, and soluble oligosaccharides (e.g., fructo-oligosaccharides) (Flint et al., [2012](#)). SCFAs are an important energy source for human colonocytes and have often been associated with several health-promoting effects, such as anti-inflammatory and anticarcinogenic (Louis et al., [2014](#); O'Keefe, [2016](#)). Indeed, the abundance of fecal SCFAs was significantly higher in the agrarian and rural populations studied around the world (De Filippo et al., [2010](#); Ou et al., [2013](#); Schnorr et al., [2014](#)), and this is undoubtedly correlated with the substantial enrichment of fiber in their diet, which selects for a fiber-degrading and SCFA-producing gut microbiota. Besides SCFAs, gut microbiota can produce other metabolites, with a potentially beneficial effect on the host's health (Fig. [6.1](#)).

Plant cell walls englobe a variety of complex micronutrients, collectively named phytochemicals, which are unabsorbed in the upper gastrointestinal tract. In the colon, microbial fermentation releases these compounds, and some bacteria can metabolize and convert them to a wide range of bioactive molecules (Cardona et al., [2013](#)). As an example, the soy isoflavone, daidzein, can be converted to equol by some gut bacteria, such as species belonging to *Faecalibacterium*, *Eubacterium*, *Bifidobacterium*, and *Clostridium*. According to some scientific evidence, equol may possess anticarcinogenic properties (Cardona et al., [2013](#)). Another polyphenol class, ellagitannins, is metabolized by some microbial species into urolithin A, B, or C, which have antioxidant and anti-inflammatory properties, while resveratrol can be metabolized into glucuronides and sulphates, which may have an anti-inflammatory activity and seem to be linked with a reduction of cholesterol (Krishnamurthy et al., [2023](#)).

However, due to the wide variety of chemical molecules commonly included in the phytochemical class, it can be expected that different gut microbiota members may be differently involved in their degradation, with the consequent production of a wide range of bioactive metabolites.

Gut microbiota can also produce detrimental metabolites (Fig. [6.1](#)). High-protein intake results in an increase in degradation of proteins in the colon and consequently higher levels of fecal amino acid-derived products, such as branched-chain fatty acids and phenylacetic acid (Ou et al., [2013](#); Russell et al., [2011](#)). High levels of branched-chain amino acids in the blood have been associated with insulin resistance and the development of type 2 diabetes (Pedersen et al., [2016](#)). In addition, some bacteria, such as *Bacteroides* spp., can ferment aromatic amino acids to produce phenylacetic acid, phenols, indoles, and *p*-cresol, associated with a pro-inflammatory and carcinogenic effect (Louis et al., [2014](#)). Sulfate-reducing bacteria (e.g., *Desulfovibrio* spp.) produce sulfides through the catabolism of sulfur amino acids and taurine. Sulfides are pro-inflammatory and toxic for colonocytes (O'Keefe, [2016](#)). Moreover, amine derived from microbial fermentation of proteins in the colon can be nitrosated to produce *N*-nitroso compounds (NOCs), which exert a carcinogenic and mutagenic effect and are correlated to the incidence of colorectal cancer (Loh et al., [2011](#)). Indeed, increased fecal NOCs and phenylacetic acid and decreased SCFA levels can be found after administration of a high-protein/low-carbohydrate diet (Russell et al., [2011](#)). In addition, a high-fat diet leads to an increase in bile secretion and consequent higher quantity of bile acids in the colon. Bile salt hydrolases can cleave glycine and taurine residues from the primary bile acids, converting them into several secondary bile acids, mainly deoxycholic and lithocholic acids. Ou et al. ([2013](#)) observed that microbial genes encoding for secondary bile acid production and fecal secondary bile acid concentration were more abundant in African Americans compared to rural native Africans, also showing lower colorectal cancer risk. Accordingly, when African Americans and rural Africans switched their diets for 2 weeks, a

reduction in fecal secondary bile acids and in colonic mucosal inflammation markers was observed in African Americans, associated with the decrease in the abundance of *Bilophila wadsworthia*. On the contrary, the high-fat intervention in rural Africans was linked to an increase in *Fusobacterium nucleatum*, previously found in human colon cancer tissues (O'Keefe et al., [2015](#)).

Another important microbial metabolite associated with a detrimental effect for host health is trimethylamine-*N*-oxide (TMAO; Fig. [6.1](#)). Intestinal microbiota catabolism of choline, phosphatidylcholine, and L-carnitine produces trimethylamine (TMA), which is further oxidized in the liver, resulting in TMAO. The latter is considered a risk factor in the development of cardiovascular diseases (CVDs) and atherosclerosis (Tang et al., [2013](#); Wang et al., [2011](#)).

6.4 Gut Microbiome and Dietary Habits in the Western World

Western diets are not necessarily detrimental to the gut microbiome. In Westernized countries, healthier dietary patterns such as vegetarian and vegan diets are becoming increasingly popular. According to an American poll in 2016, approximately 3.3% of American adults are vegetarians or vegans. This percentage increases to 6% when considering only young adults (18–34 years), while only 2% of people 65 years or older are vegetarians. Accordingly, sales of alternative meat products reached \$553 million in 2012, an 8% increase in 2 years (Melina et al., [2016](#)). A well-planned vegetarian diet contains vegetables, fruits, whole grains, legumes, nuts, and seeds, besides some products of animal origin, such as eggs, milk, and derivatives thereof. Both vegetarian and vegan diets are devoid of flesh foods (such as meat, poultry, wild game, seafood, and their products). In addition, vegans do not consume any food of animal origin. The adoption of a vegetarian diet may cause a reduced intake of certain nutrients; however, deficiencies can be readily avoided by appropriate dietary planning and, if necessary, the consumption of supplements (Melina et al., [2016](#)).

Although the spread of these dietary patterns is growing, only few studies addressed the question if these diets select for distinctive traits in the gut microbiome. Gut microbiome and metabolome were studied in a cohort of 153 vegetarian, vegan, and omnivore Italians (De Filippis et al., [2016](#)). Vegans and vegetarians showed a higher abundance of plant-degrading bacteria, such as *Lachnospira* and *Prevotella*. Moreover, these genera were positively correlated to the fecal levels of the three main SCFAs and negatively correlated with the urinary concentration of TMAO, with the former being higher and the latter being lower in vegetarians/vegans compared to omnivores (De Filippis et al., [2016](#)). Increased plasma levels of metabolites likely derived from microbial catabolism of plant polyphenolic compounds as well as increased abundance of equol were found in a small cohort of vegans (Wu et al., [2016](#)). However, differences in equol concentration were not linked to the consumption of foods rich in equol precursors (such as soy-based products). Indeed, only about 30–40% of Western adults are likely able to convert isoflavones to equol, regardless of the dietary intake, compared to about the 70% of Asians (Magee, [2011](#)). Thus, these data emphasize that both the consumption of the right substrates and the presence of specific microbial metabolic capacity jointly determine the potential development of a health-promoting metabolome.

More recently, in a large meta-analysis of >21,000 omnivore, vegetarian, and vegan individuals from different countries, microbiome signatures associated with the dietary patterns were identified (Fackelmann et al., [2025](#)), confirming previous findings. Omnivore gut microbiome was characterized by pro-inflammatory taxa, such as *Ruminococcus torques*, *Bilophila wadsworthia*, and *Alistipes putredinis*, negatively correlated with cardiometabolic health parameters. On the contrary, subjects consuming plant-based diets showed higher abundance of known fiber-degrading and butyrate-producing taxa (*Lachnospiraceae*, *Butyricicoccus*, *Roseburia hominis*). In addition, they suggested that the consumption of dairy products is the main driver differentiating the vegan and vegetarian gut microbiome. Indeed, several dairy-associated taxa (e.g., *Streptococcus thermophilus*,

Lactobacillus acidophilus, *Lactobacillus delbrueckii*, *Lactococcus lactis*, *Lacticaseibacillus paracasei*, and *Lacticaseibacillus rhamnosus*) were enriched in vegetarians' microbiome (Fackelmann et al., [2025](#)).

Beyond the strict vegetarian or vegan regimes, the consumption of a healthy and diverse dietary pattern, such as that based on the Mediterranean-style, can help to develop a health-associated microbiome and metabolome, without completely banning meat and other products of animal origin. The Mediterranean diet has been recognized by UNESCO as intangible cultural heritage (<http://www.unesco.org/culture/ich/RL/00884>). The Mediterranean dietary pattern is characterized by high intake of fruit, vegetables, legumes, nuts, and whole grains, moderate consumption of fish, and low intake of saturated fat, meat, and dairy products (Trichopoulou et al., [1995](#)). It has been demonstrated to be beneficial for the treatment of obesity, type 2 diabetes, and inflammatory and cardiovascular diseases (Santoro et al., [2014](#); Estruch et al., [2013](#); Salas-Salvadó et al., [2011](#)). Studying the gut microbiota and metabolome in an Italian cohort of adults with different dairy habits, De Filippis et al. ([2016](#)) also evaluated the adherence level to the Mediterranean diet, calculating the Mediterranean dietary score (Agnoli et al., [2011](#)). They observed a progressive increase in the concentration of fecal SCFA going from low-adherence to high-adherence subjects (Fig. [6.2a](#)). Intriguingly, even when considering the omnivore group alone, the higher the adherence to the Mediterranean diet, the higher the concentration of fecal SCFAs (Fig. [6.2b](#)).

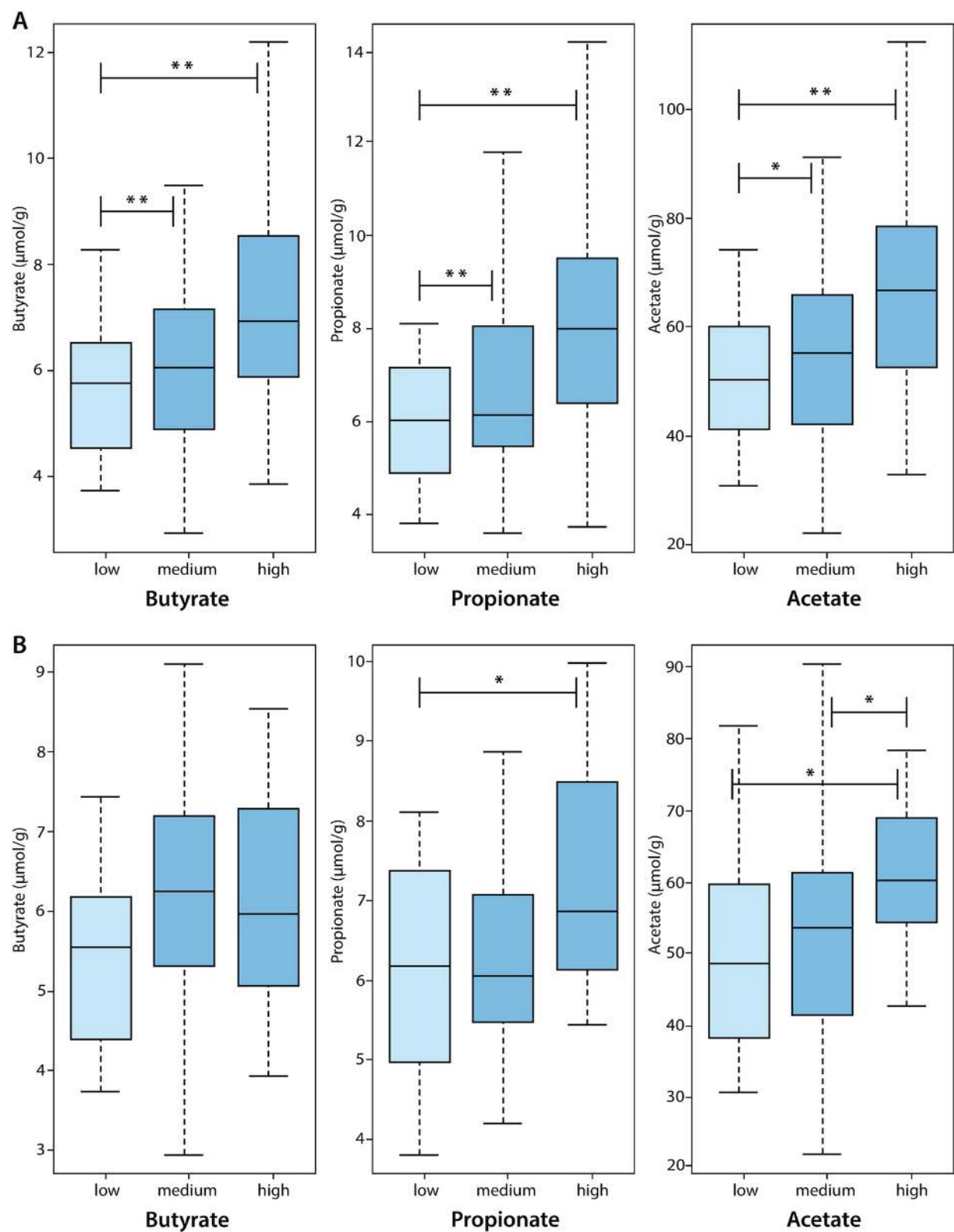


Fig. 6.2 Box plots showing the fecal concentrations of butyrate, propionate, and acetate in Italian omnivores, vegetarians, and vegans analyzed in De Filippis et al. (2016). Panel (a) shows the data for the subjects grouped according to adherence level to the Mediterranean diet, while panel (b)

shows the data for the omnivore subjects. (* $p < 0.05$ and ** $p < 0.01$). (Reproduced from De Filippis et al. (2016) with permission from BMJ Publishing Group Ltd.)

The link among Mediterranean diet, gut microbiome, and healthy metabolome was subsequently confirmed by a larger cohort study on the US population, which found that the Mediterranean diet adherence was positively associated with the abundance of fiber-metabolizing and SCFA-producing taxa, including *Faecalibacterium prausnitzii*, *Eubacterium eligens*, and *Bacteroides cellulosilyticus*. On the contrary, there was a negative correlation with species such as *Ruminococcus torques* and *Clostridium leptum* (Wang et al., 2021). Exploring the gut microbiome functional potential, the same authors found an increased abundance of genes related to pectin and hemicellulose degradation in subjects with higher adherence, while those with lower adherence showed an enrichment of the secondary bile acid biosynthesis potential (Wang et al., 2021), identified as hepatotoxic and potentially carcinogenic (Wolf et al., 2022).

Although the bacterial fraction of the gut microbiome is the most studied, there is evidence that diet may also affect the eukaryotic part. A recent survey of >56,000 gut metagenomes highlighted that subjects with a diet richer in plant-based foods have higher abundance of the protozoal *Blastocystis* and its abundance was also correlated with a healthiest cardiometabolic profile (Piperni et al., 2024).

Therefore, a healthy dietary pattern, such as the Mediterranean-style diet, can provide appropriate substrates and shape the gut microbiome to promote the production of health-related metabolites even in an overall omnivore-type diet.

6.5 Modulation of Gut Microbiome Through Diet: Possible Implications for Human Health

Several studies tried to address the question of whether we can modulate the microbiome through diet. Indeed, the presence/abundance of specific gut microbiota members was shown to

be adjustable through a specific dietary intervention. However, the consumption of Westernized diets may induce changes in the gut microbiota composition that are not reversible. Indeed, experiments on humanized mice showed that the consumption of a diet low in microbiota-accessible carbohydrates (low-MACs) over several generations results in a progressive loss of microbial diversity in the offspring, which is not recoverable after the reintroduction of dietary MACs (Sonnenburg et al., [2016](#)).

Meslier et al. ([2020](#)) carried out a 2-month intervention with a personalized, isocaloric Mediterranean diet, substituting nutrients and foods of Western diets (e.g., refined sugars, animal proteins, saturated fats) with those typical of the Mediterranean diet (e.g., fiber from whole grains, fruit and vegetables, vegetable proteins from legumes, mono- and poly-unsaturated fats from fish, olive oil, and nuts), thus increasing the subjects' adherence to the Mediterranean diet, without changing the total caloric intake. The intervention led to an increase in the fiber-degrading *Faecalibacterium prausnitzii* and in genes related to carbohydrate degradation, as well as a decrease in the potentially proinflammatory *Ruminococcus gnavus*. In addition, the treated group showed higher levels of urolithins and a decrease in insulin resistance and bile acid levels.

In addition, a 1-year, multi-countries (UK, France, Netherlands, Italy, Poland) intervention with a Mediterranean diet in an elderly population found positive changes in the gut microbiota composition, that were correlated with an improvement of several markers of frailty and of the cognitive function, while negatively associated with inflammatory markers including C-reactive protein and interleukin-17 (Ghosh et al., [2020](#)). The changes in the gut microbiota composition were reflected in a lower abundance of genes related to the production of pro-inflammatory metabolites, such as p-cresol and secondary bile acids.

However, response to dietary intervention is often person-specific and has been shown to be strongly linked to the gut microbiota composition. Indeed, Kovatcheva-Datchary and co-workers ([2015](#))

demonstrated that a dietary intervention with barley kernel fiber may induce changes in the gut microbiome that are associated with improved glucose metabolism. Nevertheless, the supplementation did not have the same effect on all the subjects, and the authors suggested that responders have peculiar traits in their gut microbiota, such as higher *Prevotella* abundance, and that this microbe may be responsible for increased glycogen storage in the liver. On the contrary, subjects showing lower abundance of *P. copri* did not show a metabolic response to the fiber supplementation. Accordingly, people eating identical meals showed different metabolic responses, consistently associated with gut microbiota features. Integration of blood parameters, dietary habits, and anthropometric and gut microbiota features in a complex algorithm enables an accurate prediction of postprandial glycemic response, linked to type 2 diabetes development (Zeevi et al., [2015](#)). Moreover, such an algorithm may be successfully used in setting up personalized dietary interventions to lower postprandial blood glucose.

Since the application of generalized medical practices did not always ensure consistent results among subjects, the possibility of a personalized nutrition strategy has recently emerged. The knowledge acquired in the past decades about gut microbiome composition and functionality, and the presence of diet-responsive members, will be used in the near future in personalized therapeutic approaches based on a targeted modulation of intestinal microbial communities.

6.6 Evidences of Strain-Level Diversity in the Gut Microbiome and Associations with Diet

Most of the available studies provide a picture of the gut microbiome composition at the species or genus level. However, with the increasing affordability of high-throughput sequencing technologies and the development of novel bioinformatic tools, the number of available microbial genomes, either coming from isolates or reconstructed from metagenomes (e.g., Metagenome-Assembled genomes, MAGs), is rising steadily. This allowed big pangenome comparison studies and the

identification of several different strains from the same species, with huge inter- and intra-individual diversity (Anderson & Bisanz, [2023](#)). This diversity may have important implications in the relationships with human health. One of the most explicative examples is *Prevotella copri*. *P. copri* has been considered for several years a beneficial microbe, since its abundance is usually higher in subjects consuming diets rich in fiber. However, contrasting results are reported in the literature regarding its associations with health. Some evidence suggests positive implications of *P. copri* in glucose homeostasis (Yang et al., [2024](#); Kovatcheva-Datchary et al., [2015](#); De Vadder et al., [2016](#)). On the contrary, other studies associated it with a pro-inflammatory activity (Scher et al., [2013](#); Iljazovic et al., [2021](#)), while its ability to produce branched-chain amino acids (BCAAs) aggravated glucose intolerance and insulin resistance (Pedersen et al., [2016](#)). These apparently contrasting results may be explained by the presence of different strains with distinct functions. Indeed, analyzing the *P. copri* pangenome, it was highlighted that the genetic signatures of *P. copri* strains in fiber-rich diets were enriched in genes related to complex carbohydrate degradation, while strains harboring genes involved in BCAA biosynthesis were rather associated with an omnivore diet (De Filippis et al., [2019](#)), suggesting that diet may be effective in modulating intra-species diversity. Interestingly, a wider genome comparison of *P. copri* highlighted the presence of 13 different phylogenetic clades (namely *Segatella copri* complex; Blanco-Míguez et al., [2023](#)).

Pangenome analyses were also applied to *Faecalibacterium* genomes from isolates and MAGs (De Filippis et al., [2020](#)), identifying the presence of 22 different *Faecalibacterium*-like species-level genome bins (SGBs). Among them, 12 SGBs were globally spread in the human gut. Indeed, different species may co-occur in the same subject, with lower diversity in Western populations, possibly due to changes in diet and lifestyle, which led to a decrease in *Faecalibacterium* diversity. *Faecalibacterium* SGBs also showed different genomic potential in the utilization of complex polysaccharides, suggesting that higher SGB diversity may be related to increased ability to use plant-based foods. In

addition, the study also demonstrated that some SGBs can be further divided in different strains, distributed according to the subject's geographical origin.

Therefore, strain-level diversity should be taken into account for the evaluation of diseases associated with dysbiosis and in the interpretation of diet-driven changes in the gut microbiome, as well as for the development of novel, personalized nutritional therapies.

Controversy Recent studies highlighted the possibility of boosting changes in the gut microbiome through specific and appropriate dietary interventions. Although this opportunity is tantalizing, there are several issues to consider. All intervention studies must face the presence of many confounding factors, e.g., different physical activity or lifestyle, and consumption of alcohol, drugs, or medications in the cohort studied, all of which may obscure the effect of the treatment. Thus, controlling for external factors in microbiome studies is extremely important, disentangling the effect of the treatment from the effects of confounders. Moreover, high intersubject variability in the gut microbiome composition exists. Interindividual differences in the gut microbiome at baseline may lead to subject-specific responses to the same treatment. Indeed, results reported in the literature are often contrasting, and even in the same study, different subjects in the cohort may show distinct outcomes in response to the same dietary intervention. Integration of blood parameters, dietary habits, and anthropometric and gut microbiota features in complex algorithms can help to accurately predict metabolic response to different meals and to formulate subject-specific dietary regimes. Personalized nutrition strategies based on individual gut microbiome features are recently emerging and in a next future will allow developing new therapeutic or disease-preventive approaches based on a targeted modulation of gut microbiome through diet.

History

Gut microbiota plays an important role in regulating human health/disease status, affecting the development of the immune system, metabolizing dietary nutrients and drugs, and synthesizing vitamins, bioactive molecules, or other beneficial or detrimental metabolites. Several factors may influence gut microbiome, leading to gut dysbiosis, which was linked to the development of several diseases. Diet may be considered the primary factor influencing gut microbiota composition and functionality. In the recent past, dietary habits in the Western world have strongly changed, with increased consumption of animal-origin fat/proteins and reduced intake of complex polysaccharides. Comparison of the gut microbiome of urbanized, Western subjects with traditional, rural populations living in Africa and South America highlighted several differences, suggesting that our microbiome coevolved with humans, shaped by diet and lifestyle. Westernized subjects show lower microbial diversity and lose specific fiber-degrading bacteria in their gut microbiome. Differences in gut microbiota composition reflect its functionality. Indeed, gut microbiome produces several health-promoting (e.g., short-chain fatty acids, equol) or detrimental (e.g., sulfides, N-nitroso compounds, trimethylamine) metabolites, depending on the substrates that are made available through the diet. Specific dietary interventions may induce changes in the gut microbiome. However, recent studies highlighted subject-specific responses to the same intervention, possibly due to specific features of the gut microbiome.

Highlights

- Gut microbiome may influence host health through the production of beneficial or detrimental metabolites, depending on the type of substrates provided by diet.
- What is food for us is also food for our microbial symbionts; thus, the gut microbiome is strongly influenced by the habitual diet.

- Changes in dietary habits and lifestyles in Western populations may have affected their gut microbiome.
- Short dietary intervention may induce changes in the gut microbiome.
- Recent research provides the basis for the development of new therapeutic strategies based on a targeted modulation of the gut microbiome through diet.

References

Agnoli, C., Krogh, V., Grioni, S., et al. (2011). A priori-defined dietary patterns are associated with reduced risk of stroke in a large Italian cohort. *The Journal of Nutrition*, 141, 1552–1558.

[[PubMed](#)]

Anderson, B. D., & Bisanz, J. E. (2023). Challenges and opportunities of strain diversity in gut microbiome research. *Frontiers in Microbiology*, 14, 1117122.

[[PubMed](#)]

Arumungam, M., Raes, J., Pelletier, E., et al. (2011). Enterotypes of the human gut microbiome. *Nature*, 473(7346), 174–180.

[<https://doi.org/10.1038/nature09944>]

Blanco-Míguez, A., Gálvez, E. J. C., Pasolli, E., et al. (2023). Extension of the *Segatella copri* complex to 13 species with distinct large extrachromosomal elements and associations with host conditions. *Cell Host & Microbe*, 31(11), 1804–1819.

Breton, J., Tennoune, N., Lucas, N., et al. (2016). Gut commensal *E. coli* proteins activate host satiety pathways following nutrient-induced bacterial growth. *Cell Metabolism*, 23(2), 324–334.

[[PubMed](#)]

Cardona, F., Andrés-Lacueva, C., Tulipani, S., et al. (2013). Benefits of polyphenols on gut microbiota and implications in human health. *The Journal of Nutritional Biochemistry*, 24, 1415–1422.

[[PubMed](#)]

Carter, M. M., Olm, M. R., Merrill, B. D., et al. (2023). Ultra-deep sequencing of Hadza hunter-gatherers recovers vanishing gut microbes. *Cell*, 186(14), 3111–3124.e13.

[[PubMed](#)]

Clemente, J. C., Pehrsson, E. C., Blaser, M. J., et al. (2015). The microbiome of uncontacted Amerindians. *Science Advances*, 1(3), e1500183. <https://doi.org/10.1126/sciadv.1500183>

[[PubMed](#)]

David, L. A., Maurice, C. F., Carmody, R. N., et al. (2014). Diet rapidly and reproducibly alters the human gut microbiome. *Nature*, 505(7484), 559–563.
[\[https://doi.org/10.1038/nature12820\]](https://doi.org/10.1038/nature12820)[\[PubMed\]](#)

De Filippis, F., Pellegrini, N., Vannini, L., et al. (2016). High-level adherence to a Mediterranean diet beneficially impacts the gut microbiota and associated metabolome. *Gut*, 65(11), 1812–1821.
[\[PubMed\]](#)

De Filippis, F., Pasolli, E., Tett, A., et al. (2019). Distinct genetic and functional traits of human intestinal *Prevotella copri* strains are associated with different habitual diets. *Cell Host & Microbe*, 25(3), 444–453.

De Filippis, F., Pasolli, E., & Ercolini, D. (2020). Newly explored *Faecalibacterium* diversity is connected to age, lifestyle, geography, and disease. *Current Biology*, 30(24), 4932–4943.e4.
[\[PubMed\]](#)

De Filippo, C., Cavalieri, D., Di Paola, M., et al. (2010). Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. *Proceedings of the National Academy of Sciences of the United States of America*, 107(33), 14691–14696.
[\[PubMed\]](#)

De Vadder, F., Kovatcheva-Datchary, P., Zitoun, C., et al. (2016). Microbiota-Produced Succinate Improves Glucose Homeostasis via Intestinal Gluconeogenesis. *Cell Metabolism*, 24(1), 151–157.
[\[PubMed\]](#)

Estruch, R., Ros, E., Salas-Salvadó, J., et al. (2013). Primary prevention of cardiovascular disease with a Mediterranean diet. *The New England Journal of Medicine*, 368(14), 1279–1290.
[\[PubMed\]](#)

Fackelmann, G., Manghi, P., Carlino, N., et al. (2025). Gut microbiome signatures of vegan, vegetarian and omnivore diets and associated health outcomes across 21,561 individuals. *Nature Microbiology*, 10, 41–52.
[\[PubMed\]](#)

Flint, H. J., Scott, K. P., Duncan, S. H., et al. (2012). Microbial degradation of complex carbohydrates in the gut. *Gut Microbes*, 3(4), 289–306.
[\[PubMed\]](#)

Furness, J. B., Rivera, L. R., Cho, H.-J., et al. (2013). The gut as a sensory organ. *Nature Reviews Gastroenterology & Hepatology*, 10, 729–740.

Ghosh, T. S., Rampelli, S., Jeffery, I. B., et al. (2020). Mediterranean diet intervention alters the gut microbiome in older people reducing frailty and improving health status: The NU-AGE 1-year dietary intervention across five European countries. *Gut*, 69(7), 1218–1228.
[\[PubMed\]](#)

Girard, C., Tromas, N., Amyot, M., et al. (2017). Gut microbiome of the Canadian arctic Inuit. *mSphere*, 2(1), e00297–16.
[\[PubMed\]](#)

Gomez, A., Petrzalkova, K. J., Burns, M. B., et al. (2016a). Gut microbiome of coexisting BaAka pygmies and Bantu reflects gradients of traditional subsistence patterns. *Cell Reports*, 14(9), 2142–2153.

[\[PubMed\]](#)

Gomez, A., Rothman, J. M., Petrzalkova, K., et al. (2016b). Temporal variation selects for diet-microbe co-metabolic traits in the gut of Gorilla spp. *The ISME Journal*, 10, 514–526.

[\[PubMed\]](#)

Iljazovic, A., Roy, U., Gálvez, E. J., et al. (2021). Perturbation of the gut microbiome by *Prevotella* spp. enhances host susceptibility to mucosal inflammation. *Mucosal Immunology*, 14(1), 113–124.

[\[PubMed\]](#)

Jeffery, I. B., Claesson, M. J., O'Toole, P. W., & Shanahan, F. (2012). Categorization of the gut microbiota: Enterotypes or gradients? *Nature Reviews Microbiology*, 10(9), 591–592.

[\[PubMed\]](#)

Knights, D., Ward, T. L., McKinlay, C. E., et al. (2014). Rethinking “enterotypes”. *Cell Host & Microbe*, 16(4), 433–437.

Koeth, R. A., Wang, Z., Levison, B. S., et al. (2013). Intestinal microbiota metabolism of L-carnitine, a nutrient in red meat, promotes atherosclerosis. *Nature Medicine*, 19(5), 576–585.

[\[PubMed\]](#)

Kovatcheva-Datchary, P., Nilsson, A., Akrami, R., et al. (2015). Dietary fiber-induced improvement in glucose metabolism is associated with increased abundance of *Prevotella*. *Cell Metabolism*, 22(6), 971–982.

[\[PubMed\]](#)

Krishnamurthy, H. K., Pereira, M., Bosco, J., et al. (2023). Gut commensals and their metabolites in health and disease. *Frontiers in Microbiology*, 14, 1244293.

[\[PubMed\]](#)

Le Chatelier, E., Nielsen, T., Qin, J., et al. (2013). Richness of human gut microbiome correlates with metabolic markers. *Nature*, 500(7464), 541–546.

[\[https://doi.org/10.1038/nature12506\]](https://doi.org/10.1038/nature12506) [\[PubMed\]](#)

Ley, R. E., Hamady, M., Lozupone, C., et al. (2008). Evolution of mammals and their gut microbes. *Science*, 320, 1647–1651.

[\[PubMed\]](#)

Loh, Y. H., Jakszyn, P., Luben, R. N., et al. (2011). N-nitroso compounds and cancer incidence: The European Prospective Investigation into Cancer and Nutrition (EPIC)-Norfolk Study. *The American Journal of Clinical Nutrition*, 93(5), 1053–1061.

[\[PubMed\]](#)

Louis, P., Hold, G. L., & Flint, H. J. (2014). The gut microbiota, bacterial metabolites and colorectal cancer. *Nature Reviews Microbiology*, 12(10), 661–672.

[\[PubMed\]](#)

Lozupone, C. A., Stombaugh, J. I., Gordon, J. I., et al. (2012). Diversity, stability and resilience of the human gut microbiota. *Nature*, 489(7415), 220–230.

[<https://doi.org/10.1038/nature11550>][[PubMed](#)]

Magee, P. J. (2011). Is equol production beneficial to health? *The Proceedings of the Nutrition Society*, 70(1), 10–18.

[[PubMed](#)]

Marchesi, J. R., Adams, D. H., Fava, F., et al. (2016). The gut microbiota and host health: A new clinical frontier. *Gut*, 65, 330–339.

[[PubMed](#)]

Martínez, I., Stegen, J. C., Maldonado-Gómez, M. X., et al. (2015). The gut microbiota of rural Papua New Guineans: Composition, diversity patterns, and ecological processes. *Cell Reports*, 11(4), 527–538.

[[PubMed](#)]

Melina, V., Craig, W., & Levin, S. (2016). Position of the academy of nutrition and dietetics: Vegetarian diets. *Journal of the Academy of Nutrition and Dietetics*, 116(12), 1970–1980.

[[PubMed](#)]

Meslier, V., Laiola, M., Roager, H. M., et al. (2020). Mediterranean diet intervention in overweight and obese subjects lowers plasma cholesterol and causes changes in the gut microbiome and metabolome independently of energy intake. *Gut*, 69(7), 1258–1268.

[[PubMed](#)]

O’Keefe, S. J. (2016). Diet, microorganisms and their metabolites, and colon cancer. *Nature Reviews Gastroenterology & Hepatology*, 13(12), 691–706.

O’Keefe, S. J., Li, J. V., Lahti, L., et al. (2015). Fat, fibre and cancer risk in African Americans and rural Africans. *Nature Communications*, 6, 6342. <https://doi.org/10.1038/ncomms7342>

[[PubMed](#)]

Obregon-Tito, A. J., Tito, R. Y., Metcalf, J., et al. (2015). Subsistence strategies in traditional societies distinguish gut microbiomes. *Nature Communications*, 6, 6505. <https://doi.org/10.1038/ncomms7505>

[[PubMed](#)]

[[PubMed](#)]

Ochman, H., Worobey, M., Kuo, C. H., et al. (2010). Evolutionary relationships of wild hominids recapitulated by gut microbial communities. *PLoS Biology*, 8(11), e10000546. <https://doi.org/10.1371/journal.pbio.1000546>

[[PubMed](#)]

Ou, J., Carbonero, F., Zoetendal, E. G., et al. (2013). Diet, microbiota, and microbial metabolites in colon cancer risk in rural Africans and African Americans. *The American Journal of Clinical Nutrition*, 98(1), 111–120.

[[PubMed](#)]

Pasolli, E., Asnicar, F., Manara, S., et al. (2019). Extensive unexplored human microbiome diversity revealed by over 150,000 genomes from metagenomes spanning age, geography, and lifestyle. *Cell*,

176(3), 649–662.e20.

[[PubMed](#)]

Pedersen, H. K., Gudmundsdottir, V., Nielsen, H. B., et al. (2016). Human gut microbes impact host serum metabolome and insulin sensitivity. *Nature*, 535(7612), 376–381.

[<https://doi.org/10.1038/nature18646>][[PubMed](#)]

Piperni, E., Nguyen, L. H., Manghi, P., et al. (2024). Intestinal *Blastocystis* is linked to healthier diets and more favorable cardiometabolic outcomes in 56,989 individuals from 32 countries. *Cell*, 187(17), P4554–P4570.

Qin, J., Li, Y., Cai, Z., et al. (2012). A metagenome-wide association study of gut microbiota in type 2 diabetes. *Nature*, 490(7418), 55–60.

[<https://doi.org/10.1038/nature11450>][[PubMed](#)]

Rampelli, S., Schnorr, S. L., Consolandi, C., et al. (2015). Metagenome sequencing of the Hadza hunter-gatherer gut microbiota. *Current Biology*, 25(13), 1682–1693.

[[PubMed](#)]

Russell, W. R., Gratz, S. W., Duncan, S. H., et al. (2011). High-protein, reduced-carbohydrate weight-loss diets promote metabolite profiles likely to be detrimental to colonic health. *The American Journal of Clinical Nutrition*, 93(5), 1062–1072.

[[PubMed](#)]

Salas-Salvadó, J., Bulló, M., Babio, N., et al. (2011). Reduction in the incidence of type 2 diabetes with the Mediterranean diet results of the PREDIMED-Reus nutrition intervention randomized trial. *Diabetes Care*, 34(1), 14–19.

[[PubMed](#)]

Santoro, A., Pini, E., Scurti, M., et al. (2014). Combating inflammaging through a Mediterranean whole diet approach: The NU-AGE project's conceptual framework and design. *Mechanisms of Ageing and Development*, 136-137, 3–13.

[[PubMed](#)]

Scher, J. U., Sczesnak, A., Longman, R. S., et al. (2013). Expansion of intestinal *Prevotella copri* correlates with enhanced susceptibility to arthritis. *eLife*, 2, e01202.

[[PubMed](#)]

Schnorr, S. L., Candela, M., Rampelli, S., et al. (2014). Gut microbiome of the Hadza hunter-gatherers. *Nature Communications*, 5, 3654. <https://doi.org/10.1038/ncomms4654>

[[PubMed](#)]

Segata, N. (2015). Gut microbiome: Westernization and the disappearance of intestinal diversity. *Current Biology*, 25(14), R611–R613.

[[PubMed](#)]

Sonnenburg, E. D., & Sonnenburg, J. L. (2019). The ancestral and industrialized gut microbiota and implications for human health. *Nature Reviews Microbiology*, 17, 383–390.

[[PubMed](#)]

Sonnenburg, E. D., Smits, S. A., Tikhonov, M., et al. (2016). Diet-induced extinctions in the gut microbiota compound over generations. *Nature*, 529(7585), 212–215.

[\[https://doi.org/10.1038/nature16504\]](https://doi.org/10.1038/nature16504) [\[PubMed\]](#)

Tang, W. H. W., Wang, Z., Levison, B. S., et al. (2013). Intestinal microbial metabolism of phosphatidylcholine and cardiovascular risk. *The New England Journal of Medicine*, 368, 1575–1584.

[\[PubMed\]](#)

Trichopoulou, A., Kouris-Blazos, A., Wahlqvist, M. L., et al. (1995). Diet and overall survival in elderly people. *BMJ*, 311, 1457–1460.

[\[PubMed\]](#)

Turnbaugh, P. J., Ley, R. E., Mahowald, M. A., et al. (2006). An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature*, 444(7122), 1027–1031.

[\[https://doi.org/10.1038/nature05414\]](https://doi.org/10.1038/nature05414) [\[PubMed\]](#)

Vangay, P., Johnson, A. J., Ward, T. L., et al. (2018). US immigration westernizes the human gut microbiome. *Cell*, 175(4), 962–972.e10.

[\[PubMed\]](#)

Wang, Z., Klipfell, E., Bennett, B. J., et al. (2011). Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease. *Nature*, 472(7341), 57–63.

[\[https://doi.org/10.1038/nature09922\]](https://doi.org/10.1038/nature09922) [\[PubMed\]](#)

Wang, D. D., Nguyen, L. H., Li, Y., et al. (2021). The gut microbiome modulates the protective association between a Mediterranean diet and cardiometabolic disease risk. *Nature Medicine*, 27(2), 333–343.

[\[PubMed\]](#)

Wolf, P. G., Byrd, D. A., Cares, K., et al. (2022). Bile acids, gut microbes, and the neighborhood food environment - a potential driver of colorectal cancer health disparities. *mSystems*, 7(1), e0117421.

[\[PubMed\]](#)

Wu, G. D., Chen, J., Hoffmann, C., et al. (2011). Linking long term dietary patterns with gut microbial enterotypes. *Science*, 334(6052), 105–108.

[\[PubMed\]](#)

Wu, G. D., Compher, C., Chen, E. Z., et al. (2016). Comparative metabolomics in vegans and omnivores reveal constraints on diet-dependent gut microbiota metabolite production. *Gut*, 65, 63–72.

[\[PubMed\]](#)

Yang, C., Lan, R., Zhao, L., et al. (2024). *Prevotella copri* alleviates hyperglycemia and regulates gut microbiota and metabolic profiles in mice. *mSystems*, 9(7), e00532–24.

[\[PubMed\]](#)

Yatsunenko, T., Rey, F. E., Manary, M. J., et al. (2012). Human gut microbiome viewed across age and geography. *Nature*, 486(7402), 222–227.

[\[https://doi.org/10.1038/nature11053\]](https://doi.org/10.1038/nature11053)[\[PubMed\]](#)

Yersin, S., Garneau, J. R., Schneeberger, P. H. H., et al. (2023). Gut microbiomes of agropastoral children from the Adadle region of Ethiopia reflect their unique dietary habits. *Scientific Reports*, 13, 21342.

[\[PubMed\]](#)

Zeevi, D., Korem, T., Zmora, N., et al. (2015). Personalized nutrition by prediction of glycemic responses. *Cell*, 163(5), 1079–1094.

[\[PubMed\]](#)

7. Microbiome and Gut Immunity: The Epithelium

Claudia Günther¹✉ and Dirk Haller²✉

- (1) Centre for Personalised Medicine and Research (CESAR),
Universitätsklinikum Erlangen – Medizinische Klinik I, Erlangen,
Germany
- (2) Chair of Nutrition and Immunology, School of Life Sciences,
Technical University of Munich, Munich, Germany

✉ **Claudia Günther**

Email: c.guenther@uk-erlangen.de

✉ **Dirk Haller (Corresponding author)**

Email: dirk.haller@tum.de

Abstract

The intestinal epithelium plays a crucial role in maintaining a homeostatic relationship between the host and the microbiota while serving as the first line of defense against pathogenic microbes and their associated agents. To fulfill this dual role, intestinal epithelial cells (IECs) have evolved diverse mechanisms for responding to both commensal and non-commensal microbes. These cells not only form a physical barrier that prevents the translocation of potentially harmful microorganisms from the gut into surrounding tissues but also secrete antimicrobial peptides and mucus to regulate microbial composition and localization. Various host receptors integrate microbial factors and immune signals to sustain epithelial cell homeostasis. Mitochondrial

function is an emerging mechanism in the regulation of intestinal epithelial cell differentiation, and mitochondrial deficiency contributes to impaired barrier integrity and injury. Beyond their barrier role, IECs translate luminal signals from the intestinal surface to the underlying mucosal immune system, facilitating immune responses and maintaining gut homeostasis. Disruptions in any of these functions can have profound effects on intestinal homeostasis and are implicated as critical factors in the development of intestinal inflammation. In this chapter, we will explore the multifaceted role of the intestinal epithelium in host-microbe interactions and its impact on intestinal health and disease.

Keywords Inflammatory Bowel Diseases – Intestinal epithelial cells – Mitochondrial function – Tissue injury and regeneration

7.1 Introduction

The primary function of the gastrointestinal (GI) tract is to facilitate the digestion and absorption of nutrients from the intestinal lumen. Besides these food-derived nutrients, exogenous microorganisms such as bacteria, fungi, and viruses can also enter the gut. The abundance of innate and adaptive immune cells that reside together with trillions of beneficial commensal microorganisms in the GI tract requires an effective barrier in order to define host-microbial interactions and to preserve tissue homeostasis. This critical barrier is formed by the intestinal epithelium, a dynamic layer that serves as both a physical and biochemical barrier at the interface between the host and its microbial-rich environment (Fig. [7.1](#)). By protecting the underlying tissues while simultaneously interacting with the microbial community, the intestinal epithelium plays a central role in balancing immune tolerance and defense mechanisms, ensuring the stability of the gut ecosystem. The intestinal epithelium is the most regenerative tissue in the human body, illustrated by the fact that self-renewal of this single-cell-layered interface (30–40 m²) is completed every 3–5 days, most likely as a

protective mechanism against injuries and infections. Under homeostatic conditions of self-renewal, but also in response to injury and regeneration, crypt-based stem cells maintain the flow of specialized intestinal epithelial cells (IEC) that exert diverse functions (van der Flier & Clevers, [2009](#)), including mature secretory (e.g., Paneth cells, goblet cells, enteroendocrine cells) and absorptive cells (columnar-shaped enterocytes). The life cycle of intestinal epithelial cells is determined by the time span in which these cells migrate from their place of origin to the villus tip, where these cells are exposed to the luminal environment. Cells that reach this status are expelled from the epithelial layer through a complex cytoskeletal remodeling process. The organization of the epithelium is adapted to the specific functional requirements of the different regions of the intestinal tract. To allow efficient nutrient absorption, the small intestine is folded to form a large number of tubular invaginations, denoted as crypts, and fingerlike villus structures (Marshman et al., [2002](#); Ziv & Bendayan, [2000](#)). This enables the host to dramatically enlarge the surface area for nutrient absorption. In the lower small intestine (ileum), the epithelial surface is interrupted by the Peyer's patches (PP), aggregated lymphoid follicles embedded within the submucosa (Mowat, [2003](#)). These patches are rich in immune cells, including B cells, T cells, and dendritic cells, forming a critical part of the mucosal immune system (Jung et al., [2010](#)). The epithelial layer covering Peyer's patches includes specialized microfold (M) cells, which facilitate the transport of luminal antigens to underlying antigen-presenting cells (APCs) (Corr et al., [2008](#); Mabbott et al., [2013](#); Miller et al., [2007](#)). M cells are described as IECs that allow luminal contents to pass through and encounter antigen-presenting cells (APCs) below. Absorption of water and compacting of stool for rapid excretion take place in the large intestine (Kvietys & Granger, [2010](#)). Since villi would hamper this process, the colonic epithelium is arranged into multiple extended crypts associated with a flat luminal surface (Barker, [2014](#)). The colon also contains gut-associated lymphoid tissue (isolated lymphoid follicles) resembling Peyer's patches of the small intestine (Gibbons & Spencer, [2011](#)).

Beyond its architectural complexity, the intestinal epithelium is composed of a highly heterogeneous population of cells with diverse functions (Barker, [2014](#); Gunther et al., [2013](#); Sancho et al., [2003](#); van der Flier & Clevers, [2009](#)). The life cycle of an intestinal epithelial cell starts at the crypt base, where small populations of adult stem cells regularly divide to produce highly proliferative progenitors known as transit-amplifying (TA) cells. TA cells spend approximately 2 days in the crypt before they undergo cell cycle arrest and differentiate into specialized intestinal epithelial cells (Barker, [2014](#); Crosnier et al., [2006](#)). Differentiation of these precursor cells occurs along the crypt-villus axis. Intestinal epithelial cells can be subdivided into two major groups: cells of the absorptive lineage and cells imprinted for the secretory lineage (Barker, [2014](#); van der Flier & Clevers, [2009](#)). Senescent epithelial cells reach the tip of the villus (or surface epithelium in the colon) where they are shed into the intestinal lumen, which allows a continual epithelial turnover (Bullen et al., [2006](#); Marchiando et al., [2011](#)). The balance of cell shedding at the villus tip and renewal of stem cells in the crypts is indispensable to maintaining small intestinal morphology and functionality. The current model of cell shedding predicts that overcrowding due to proliferation and migration induces live cell extrusion at the villus tip to maintain homeostatic cell numbers in the epithelium (Eisenhoffer et al., [2012](#)).

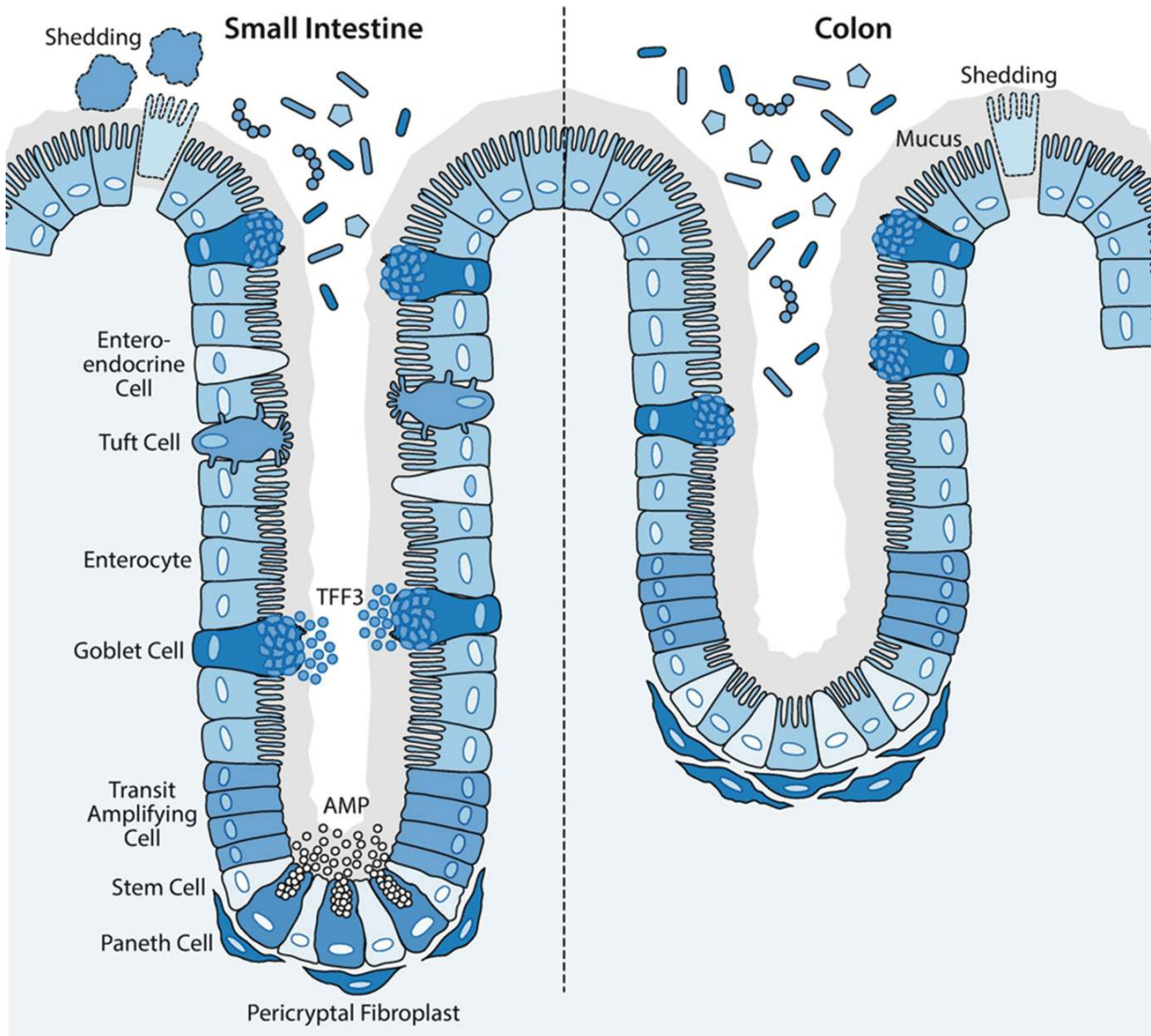


Fig. 7.1 Structure of the intestinal epithelium. In the small intestine (left panel), stem cells are located between Paneth cells at the crypt base. Stem cells continuously generate a pool of proliferating transit-amplifying cells (TA cells). These TA cells give rise to differentiated IECs with specialized functions. Enterocytes, goblet cells, enteroendocrine cells, and tuft cells replace cells that are lost by shedding at the villus tip. Paneth cells are located in the crypt next to stem cells. This epithelial monolayer is covered by a thick mucus layer, which protects the intestinal epithelium. Both Paneth cells and pericryptal fibroblasts supply essential factors (including WNT, the Notch ligand delta-like 4 (DLL4), epidermal growth factor (EGF), and Noggin) to regulate the survival and function of the intestinal stem cells. In the colon, crypts lack Paneth cells (right panel)

7.2 Intestinal Epithelial Cells: Regulators of Barrier Function and Immune Homeostasis

As mentioned above, intestinal epithelial stem cells can give rise to cells of either the secretory or absorptive lineage. Besides these main cell types, researchers have described some lesser-known cell types, such as tuft cells, cup cells, and Peyer's patch-associated M cells (Gerbe et al., [2012](#); Madara, [1982](#); Man et al., [2004](#); Ramirez & Gebert, [2003](#)). These specialized epithelial cells provide a tight barrier and achieve frontline defense by an incessant production of an array of secreted factors that limit direct contact between the epithelium and infectious agents. A breach in barrier integrity is associated with inflammatory pathologies and requires immediate action to restore barrier function and to induce mucosal healing. Injury-associated stem cells, tissue-related factors, and the microbial milieu are imperative in orchestrating tissue regeneration and barrier restoration. Loss of Lgr5⁺ stem cells in response to severe (often transmural) injury requires additional regenerative capacities in the intestine, involving quiescent slow-cycling cells located at the +4 position in the crypt, de-differentiated epithelial cells, and reserve stem cells. Mitochondrial functions are critically involved in epithelial stem cell homeostasis and differentiation, and mitochondrial signaling and metabolism contribute to the defence against infections. Thus, the epithelium is at the frontline of the intricate intersection of microbiome, immune system, and metabolism, and mitochondria are emerging as intriguing interceptors of milieu signals in the intestinal tissue. Commensal enteric microbes have evolved strategies to enforce barrier and immune functions, but at the same time pathogens developed strategies to evade defense mechanisms, suggesting a co-evolution of microbes and host in the gut (Fig. [7.2](#)).

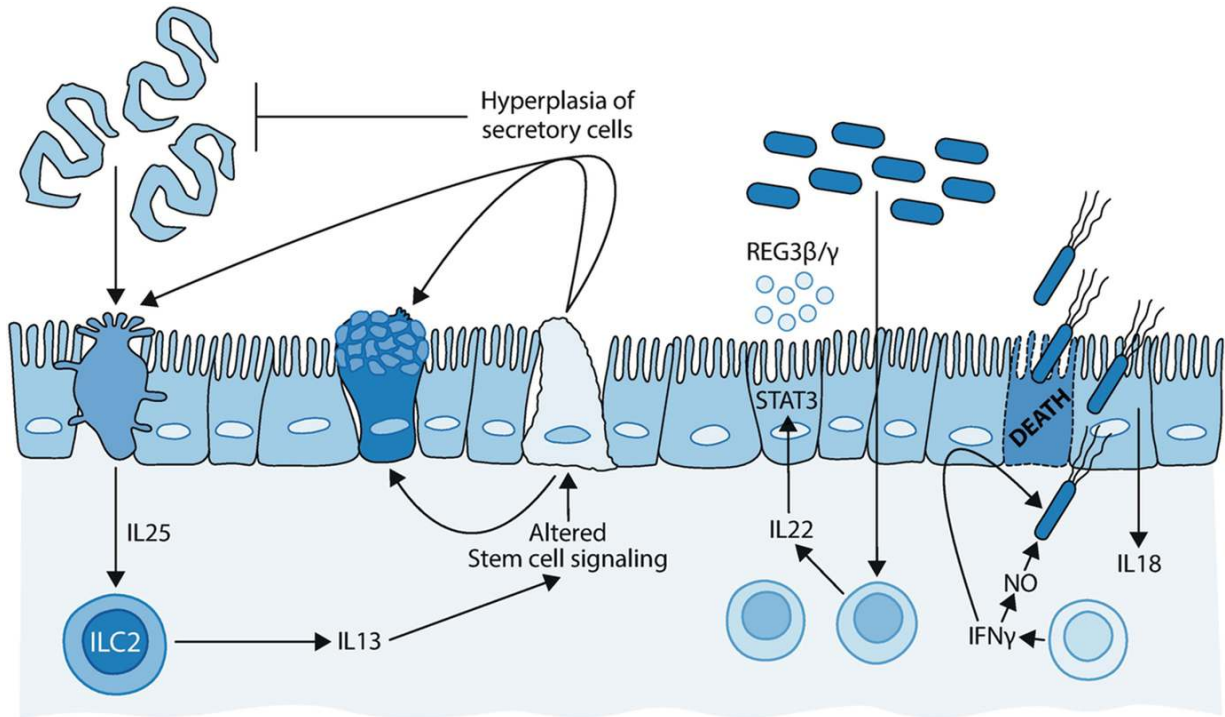


Fig. 7.2 Intestinal epithelial barrier function is essential for maintaining intestinal homeostasis. The intestinal epithelium can sense and respond to luminal contents by various direct and indirect mechanisms. IECs can directly respond to pathogens by the production of antimicrobial peptides or mucus. Elimination of infected cells by various cell death mechanisms can be initiated by IEC intrinsic signaling pathways or by factors produced by the underlying immune cell compartment. IECs are capable of producing and releasing immunogenic molecules that can shape the mucosal immune system. Tuft cells, ILC2s, and pericryptal fibroblasts can control differentiation of IEC progenitors into cells of the secretory lineage upon infection to efficiently clear pathogens. NO nitric oxide (antimicrobial activity)

7.2.1 Stem Cells

The intestinal epithelium is composed of functionally distinct epithelial cells located in defined anatomical zones of the crypt-villus axis. Stem cells reside at the crypt bottom and are responsible for the self-renewal of the epithelial cell monolayer (Barker, [2014](#)). The most prominent stem cell populations are crypt base columnar cells (CBCs) and +4 cells, named after their location at the fourth position from the crypt base; however, additional populations of active and quiescent stem cells appear to be able to give rise to each other. Lgr5 responds to Wnt signaling-associated R-Spondins and comprises the most accepted marker for actively proliferating intestinal stem cells (ISCs).

Olfactomedin 4 (Olfm4) is an alternative marker for highly proliferative

intestinal stem cells, co-expressed with, but not restricted to, Lgr5+ stem cells. In the small intestine, Lgr5+ CBC cells are located between Paneth cells with an estimated number of 5–16 per crypt, giving rise to ~250 new cells daily. Slowly dividing cell populations within the Lgr5+ ISC compartment exist and contribute to the rapidly dividing ISC pool, helping to promote regeneration. After rapid cell division, progenitor cells leave the transit amplifying zone to undergo lineage commitment and thereby acquire specialized functions during terminal differentiation. A highly complex network of transcription factors regulates IEC differentiation, including hairy/enhancer of split 1 (Hes1) and Atonal homologue 1 (Atoh1, also called Math1), and gives rise to terminally differentiated absorptive and secretory cell types, respectively. Major cell types include enterocytes, enteroendocrine cells, goblet cells, Paneth cells, and Tuft cells. Diverse sub-cryptal smooth muscle cells provide bone morphogenic protein inhibitors and other niche factors that are essential to maintain the niche (McCarthy et al., [2023](#)). Recent data showed that, unlike other secretory cell types, goblet and Paneth cells are not independently specified, but that niche factors, especially BMP signaling, distinguish goblet from Paneth phenotypes (Bhattacharya et al., [2025](#)). Interestingly, enteric glial cells (EGCs) constitute a key part of the intestinal stem cell niche. GFAP⁺ EGCs provide key WNT stem cell niche signals to promote regeneration (Baghdadi et al., [2022](#)).

7.2.2 Enterocytes

The majority (around 80%) of cells bordering the intestinal lumen are absorptive enterocytes (alternatively termed columnar cells) (de Santa Barbara et al., [2003](#); Snoeck et al., [2005](#)). Enterocytes provide the physical barrier of the intestinal epithelium by forming close contacts via tight junction molecules. Since efficient absorption and transport of nutrients from the luminal side across the epithelium is their main function, enterocytes have further enlarged their luminal surface by carrying apically located microvilli (microscopic cellular membrane protrusions). These microvilli can be nicely visualized by electron

microscopy, but not light microscopy; that is why they are mostly referred to as brush border (due to their nappy appearance by light microscopy) (Ziv & Bendayan, [2000](#)). However, enterocytes, apart from their participation in digestive processes, exert more than a passive barrier function and are often directly involved in immune processes. Enterocytes express class I and II MHC molecules, which allow them to deliver and present foreign antigens to the underlying gut-associated lymphoid tissue to respond appropriately to luminal antigens (immunity versus tolerance) (Campbell et al., [1999](#); Hershberg et al., [1997](#); Kambayashi & Laufer, [2014](#); Shao et al., [2005](#)). In order to act as antigen-presenting cells (APCs), enterocytes must be able to internalize and process antigens and contact lymphocytes within the epithelium (mainly intraepithelial lymphocytes) and T cells in the underlying lamina propria via basolateral projection (Dahan et al., [2007](#); Vitale et al., [2016](#)). Recently, it has been shown that MHC class II antigen presentation by intestinal epithelial cells fine-tunes bacteria-reactive CD4 T-cell responses (Heuberger et al., [2024](#)). However, the exact role of MHC II expression (expression induced under inflammatory conditions) by IECs is not fully (if at all) understood, and it remains debated whether and how IECs can affect mucosal immunity. During homeostasis, enterocytes do not express costimulatory molecules, resulting in the induction of anergy and thus tolerance. Under pathologic conditions, such as inflammation or infections, enterocytes function as professional APCs and stimulate immune responses. In addition, enterocytes are capable of producing a variety of proteins with antimicrobial activity (such as C-type lectin regenerating islet-derived protein III, Reg3 γ) and antimicrobial peptides (e.g., β -defensins that are expressed by colonic enterocytes) and thus contribute to the antimicrobial defense (Gallo & Hooper, [2012](#); Wittkopf et al., [2015](#)).

7.2.3 Enteroendocrine Cells

The gastrointestinal (GI) tract produces over 30 different hormones, earning it the designation of “the largest endocrine organ” in the body (Gunawardene et al., [2011](#)). Enteroendocrine cells, also known as

neuroendocrine cells, are key players in this system. These specialized cells are characterized by the presence of secretory vesicles, which are either large dense-core vesicles (LDCVs) or the smaller synaptic-like microvesicles (SLMVs) similar to those found in neurons. This structural similarity underscores their role as a critical link between the central and enteric neuroendocrine systems, enabling the coordination of various gut functions through hormone secretion (Buffa et al., [1978](#); Furness et al., [2013](#); Sternini & Rozengurt, [2025](#)). These hormones coordinate diverse functions of the gut, such as secretion (Serotonin (5-HT) stimulates mucus and electrolyte release), motility (somatostatin modulates peristalsis, slowing gut movement), and growth and repair (Glucagon-like peptide-2 (GLP-2) promotes enterocyte proliferation and intestinal repair) (Gunawardene et al., [2011](#)). Enteroendocrine cells are also capable of sensing microbiota-derived metabolites and structures like indoles (Gribble & Reimann, [2016](#)) or lipopolysaccharides (LPS) (Nguyen et al., [2014](#)). Highlighting the crosstalk between the immune and endocrine system, the GLP-1 receptor is expressed on multiple immune cell populations, including intestinal intraepithelial lymphocytes (Hadjiyanni et al., [2010](#); Yusta et al., [2015](#)). Hence, an immunoendocrine axis has been suggested with EECs orchestrating an intestinal metabolic-inflammatory response (Moran et al., [2008](#); Worthington et al., [2018](#)). Moreover, EECs have been proposed to relay nutritional information to the intestinal stem cell niche to regulate the proliferative capacity of the crypt (McCauley et al., [2023](#)). This is particularly relevant, as crypt-resident stem and progenitor cells are not directly exposed to the luminal environment, but are metabolically shielded by differentiated cells (Kaiko et al., [2016](#)) and thus, cannot directly adapt to nutritional challenges. Despite their critical role, EECs constitute only about 1% of the IEC population, making them a relatively minor yet essential component of the intestinal lining (Barker et al., [2008](#); Gunawardene et al., [2011](#)). These cells are dispersed throughout the mucosa, with a higher concentration in the upper small intestine. EECs are a highly heterogeneous population, comprising up to 15 subtypes defined by their unique

morphology and the specific peptide hormones or marker genes they express (Furness et al., [2013](#); Fothergill & Furness, [2018](#); Gehart et al., [2019](#)). This diversity allows EECs to finely tune gastrointestinal functions in response to a wide range of stimuli, maintaining homeostasis and coordinating the gut's interactions with the nervous and immune systems. Through their multifunctional roles, enteroendocrine cells play a pivotal part in integrating gut activity with systemic physiological processes, emphasizing their importance beyond the gastrointestinal tract.

7.2.4 Goblet Cells

A key element of the intestinal strategy for maintaining a homeostatic host-microbial relationship is to limit the contact between the dense luminal microbial community and the epithelial cell surface (Hansson, [2020](#); Belkaid & Hand, [2014](#)). To this end, specialized secretory cells called goblet cells secrete mucus that overlies the epithelium and forms a first line of defense in the form of a physical and chemical barrier (Nyström et al., [2021](#)). The proportion of goblet cells among all other intestinal epithelial cells increases from the proximal small intestine (around 4%) to the distal colon (around 16%) (van der Flier & Clevers, [2009](#)). Equally, the composition and thickness of the protective mucus layer vary along the longitudinal axis of the gastrointestinal tract, reflecting the physiological requirements and microbial load (Ermund et al., [2013](#); Atuma et al., 2001). While acting as a physical barrier, the mucus layer also forms a habitat for bacteria, particularly in the colon, where the mucus layer is divided into a mostly sterile stratified inner layer and a loose outer layer providing nutrients and attachment sites (Gustafsson & Johansson, [2022](#); Martens et al., [2018](#)). Mucins form the major building blocks of mucus and are composed of a polymeric protein backbone structure, linked to numerous hygroscopic and hydrophilic oligosaccharide side chains that assemble into a viscous gel-like matrix. The enormous structural variation in *O*-glycans on mucins forms a glycode and interspecies communicator between microbes and the host (Johansson et al., [2008](#); Gabius & Roth, [2017](#)). An

additional functional role of mucus was recently introduced, showing that proximally *O*-glycosylated mucus encapsulates fecal material and the microbiota to modulate microbiota structure and function (Bergstrom et al., [2020](#)). Mice with a targeted inactivation of the most abundant secreted gastrointestinal mucin (Muc2) lack this bacteria-free zone and develop spontaneous inflammation and adenomas (Van der Sluis et al., [2006](#); Velcich et al., [2002](#)). Furthermore, defective mucin *O*-glycosylation leads to an aberrant mucus layer, increased bacterial penetration, and colitis-associated cancer in mice (Bergstrom et al., [2016](#)). Impaired mucus characteristics have been associated with inflammatory bowel diseases (IBD), CAC, and CRC (Coleman & Haller, [2021](#)). The above demonstrates the importance of the mucus layer in maintaining intestinal homeostasis and a symbiotic relationship with the microbiota. Recent evidence further highlights the importance of goblet cell differentiation for intestinal homeostasis. Niu et al. (2025) demonstrated that colonic epithelial-derived FGF1 acts as a local regulatory signal, driving intestinal stem cell commitment toward the goblet cell lineage. This goblet cell enrichment was shown to enhance mucus barrier formation and significantly suppress inflammation in models of IBD (Lin et al., [2025](#)).

7.2.5 Paneth Cells

A second immune mechanism that limits bacteria-epithelial cell contact is the secretion of antimicrobial peptides (AMPs) by gut epithelial cells. Paneth cells are the main source of endogenous AMPs like α -defensins, lysozyme, or phospholipase A (Ayabe et al., [2004](#); Salzman, [2010](#)). Morphologically, they contain an extensive endoplasmic reticulum and Golgi network structures that already point toward an intensive secretory activity (Bevins & Salzman, [2011](#); Porter et al., [2002](#)). Within Paneth cells, AMPs are stored in large apical secretory granules, from which they can be released by exocytosis into the gut lumen and thereby contribute to host defense against a broad spectrum of bacteria, fungi, and some viruses (Elphick & Mahida, [2005](#); Wehkamp & Stange, [2006](#)). Some of them are constitutively released into the mucus

layer, whereas the secretion of others is induced in response to specific external stimuli (Bevins & Salzman, [2011](#)). Some AMPs, such as the α -defensins (a Paneth cell-specific AMP), are secreted as precursors and have to be processed (cleaved by matrix metalloproteinase-7) to acquire full antimicrobial activity (Wilson et al., [2009](#)). A recent study showed a novel interaction between the aryl hydrocarbon receptor (AhR) and alpha-defensin 1. Activation of AhR enhances alpha-defensin 1 expression by targeting its promoter, which helps restore microbial balance and reduce colitis symptoms, thereby highlighting its role in regulating gut immunity (Palrasu et al., [2025](#)). In addition to these AMPs, Paneth cell granules contain IgA (immunoglobulin A) and cytokines (e.g., TNF- α) (Ouellette, [2010](#)). TNF plays important roles in limiting infection, but is also linked to sepsis. The mechanisms underlying these paradoxical roles are unclear. TNF can limit the antimicrobial activity of Paneth cells, leading to bacterial translocation from the gut and subsequent sepsis and organ failure, a process that is mitigated in mice lacking the TNF receptor specifically in PCs. Mechanistically, TNF activates the interferon pathway and disrupts the unfolded protein response (UPR) in PCs, reducing antimicrobial peptide production and contributing to the detrimental effects of sepsis (Wallaeys et al., [2024](#)). Paneth cells are not evenly distributed in the gut epithelium. They are restricted to the small intestine, where they are located at the crypt base together with intestinal stem cells (Barker et al., [2008](#)). In contrast to all other differentiated epithelial cell types, Paneth cells escape from the migration toward the villus tip and instead settle down at the crypt base, where they can survive for more than 3 weeks (Bjerknes & Cheng, [1981](#), [2005](#); Ireland et al., [2005](#)). The relocation of Paneth cells at the crypt base has an important function, since Paneth cells constitute the niche for stem cells in intestinal crypts (Sato et al., [2011](#)). Paneth cells express EGF (epidermal growth factor), TGF α (transforming growth factor alpha), Wnt3, and the Notch ligand DLL4 (delta-like canonical notch ligand 4), all essential signals for stem cell maintenance. Dysfunction and death of Paneth cells have been associated with intestinal inflammation both in mice and men (Adolph

et al., [2013](#); Bevins & Salzman, [2011](#); Cadwell et al., [2008](#); Gunther et al., [2011](#); Kaser & Blumberg, [2008](#); Kobayashi et al., [2005](#); Wehkamp et al., [2007](#)).

7.2.6 Tuft Cells

Tuft cells, also known as brush cells due to their microvillus-covered apical surface, have long been a mysterious component of the intestinal epithelium. Although they constitute only 0.4–1% of intestinal epithelial cells, their specialized roles in immune sensing and defense against parasitic infections have positioned them as key players in gut immunity (Gerbe & Jay, [2016](#); Gerbe et al., [2012](#), [2016](#)). It is currently believed that tuft cells, Paneth cells, and goblet cells have a unique precursor, which would classify them as members of the secretory lineage (Gerbe et al., [2012](#)). This differentiation pathway highlights the essential function of the secretory lineage in maintaining gut homeostasis, with tuft cells contributing unique immune functions. Indeed, Tuft cells are the principal source of interleukin-25 (IL-25) in the gut, a cytokine critical for initiating type 2 immune responses, particularly in response to intestinal protozoa and helminth infections (Gronke & Diefenbach, [2016](#); Kokrashvili et al., [2009](#); von Moltke et al., [2016](#)). IL-25 secretion by tuft cells triggers a cascade that promotes the activation and proliferation of type 2 innate lymphoid cells (ILC2s), leading to the production of IL-13. This cytokine, in turn, drives goblet cell hyperplasia and mucus production, enhancing the expulsion of parasites from the intestine (Gronke & Diefenbach, [2016](#)). In addition to their immunological role, tuft cells produce and secrete endogenous opioids, indicating their involvement in neuroimmune crosstalk within the gut (Kokrashvili et al., [2009](#)). This neurochemical signaling potentially modulates pain perception and motility during parasitic infections, further emphasizing their multifaceted role in gut physiology. Despite their rarity, tuft cells are central to orchestrating type 2 immune responses and maintaining gut homeostasis. Their ability to integrate immune, epithelial, and neuronal signals

underscores their importance in the dynamic environment of the intestinal mucosa.

7.3 Mitochondria Control Epithelial Cell Homeostasis

Mitochondria are double-membraned, dynamic organelles that are essential in providing a cellular energy supply and metabolites for major biosynthetic pathways. In addition to their contribution to cellular metabolism, mitochondria function as a central intersection of stress and survival signaling (Rath et al., [2018](#)). Unfolded protein responses (UPR) are cornerstones in the homeostatic regulation of organelle function. Mitochondrial UPR is essential in maintaining cellular metabolism and function (Grootjans et al., [2016](#)). Using novel mouse models in which loss of Hsp60 causes UPRmt activation and subsequent mitochondrial dysfunction specifically in intestinal stem cells (ISC) or IECs, the crucial role of mitochondrial metabolism for intestinal stemness, Paneth cell functionality, and the proliferative capacity of IECs was demonstrated. Deficiency in mitochondrial OXPHOS upon deletion of Hsp60 causes loss of Lgr5+ ISCs and emergence of non-granular, dysfunctional Paneth cells, accompanied by tissue aberrations reminiscent of mucosal wound healing (Berger et al., [2016](#)). In line with the high energy demand of the inflamed mucosa, loss of metabolic circuits between microbiome and host, including butyrate, carnitine, purine, and tryptophan metabolism, are involved in the development of chronic inflammation, supporting the idea that mitochondrial exhaustion contributes to functional perturbation in IBD (Rath & Haller, [2022](#)). Among the multifaceted mechanisms of metabolic signal integration, and despite the complex nature of chemical ligands present in the microbial milieu, the aryl hydrocarbon receptor (AhR) plays a pivotal role in regulating tissue homeostasis and intestinal inflammation (Stockinger et al., [2021](#)). The epithelium is at the frontline of the intricate intersection of microbiome, immune

system, and metabolism, and mitochondria are emerging as intriguing interceptors of milieu signals in the intestinal tissue.

7.4 Interaction Between the Epithelium and Immune Cells

Despite its barrier function, the intestinal epithelium has an important function in translating luminal signals from the barrier surface to the underlying mucosal immune system (Artis, [2008](#); Gunther et al., [2016](#); Okumura & Takeda, [2017](#); Peterson & Artis, [2014](#); Ramanan & Cadwell, [2016](#)). IECs are capable of releasing immunogenic molecules that can shape the mucosal immune system and antimicrobial effectors that strongly influence the luminal microbiota both in the steady state and during inflammation or infection (Fig. [7.2](#)). Moreover, IECs not only produce and release various factors affecting immune cells they also respond to factors produced by the subjacent lymphocytes. This communication between the intestinal epithelium and resident immune cells might be particularly crucial to exert an appropriate immune or epithelial response during microbial challenge.

During intestinal infection, lamina propria cells and IECs express a wide range of inflammatory cytokines, providing constant signals about the activation status of both compartments, which are crucial for defense against pathogens (Wittkopf et al., [2014](#)). Pathogen colonization resistance can be regulated by either direct inhibition by the microbiome or indirectly by microbiome-mediated immune responses (Buffie & Pamer, [2013](#); Kamada et al., [2013](#)). For example, commensal microbes help restrict invading pathogens such as *Salmonella typhimurium* by priming the immune system, enhancing IFN- γ production by innate and adaptive immune cells (Buffie & Pamer, [2013](#); Thiemann et al., [2017](#)). IFN- γ can induce defense mechanisms in the intestinal epithelium, such as secretion of antimicrobial peptides (Farin et al., [2014](#)) and generation of mucus-filled vacuoles by goblet cells and mucus release into the gut lumen (Raetz et al., [2013](#); Songhet et al., [2011](#)). Similarly, IL-22, released by lymphocytes (including

mainly CD4⁺ T cells but also $\gamma\delta$ T cells and subsets of innate lymphoid cells (ILCs)) in the early phase of infections, plays an important role in the communication between IECs and lamina propria cells. One of the main functions of IL-22 is to support and maintain the gastrointestinal epithelial barrier as well as to facilitate barrier defense mechanisms against bacterial pathogens such as *Clostridium difficile* (Hasegawa et al., [2014](#)), *Citrobacter rodentium* (Munoz et al., [2015](#); Zheng et al., [2008](#)), and *Toxoplasma gondii* (Munoz et al., [2015](#)). Defense mechanisms supported by IL-22 include epithelial cell survival, proliferation of IECs, and release of proteins with antimicrobial activity (e.g., REG3 γ) (Pickert et al., [2009](#); Wittkopf et al., [2015](#)). IL-22 acts directly on IECs, activating STAT3, which promotes proliferation, wound healing, and secretion of REG3 γ to control infections like *C. rodentium* (Hall, [1997](#); Wittkopf et al., [2015](#)). IECs can also directly sense intestinal pathogens and respond through various mechanisms, such as programmed cell death or cytokine release. To facilitate this, IECs express inflammasome components, which form cytosolic multi-protein complexes that initiate innate immune responses against invasive pathogens by activating inflammatory pathways (Blazejewski et al., [2017](#); Sellin et al., [2015](#); Strowig et al., [2012](#)). This includes the activation of cell death and rapid expulsion of infected IECs as well as caspase-1-dependent processing and release of pro-inflammatory interleukin-1 β and interleukin-18 (Rauch et al., [2017](#)). IL-18, in turn, synergizes with IL-23 to promote IL-22 expression by ILCs. As mentioned above, elevated levels of IL-22 act on the intestinal epithelium to enhance antimicrobial defense. While these examples illustrate direct functions of IECs induced by pathogens or immune cell-derived cytokines, factors released by immune cells in response to infections can also shift epithelial cell differentiation toward the secretory lineage to improve innate immune responses (Gerbe et al., [2016](#); Grencis & Worthington, [2016](#)). Interleukin-33 (IL-33) is an IL-1-like cytokine with host-protective functions. It can be expressed by both hematopoietic and non-hematopoietic cells, such as IECs and pericryptal fibroblasts, and it is particularly expressed at barrier tissues

(Mahapatro et al., [2016](#); Oboki et al., [2010](#)). In the gut, IL-33 is involved in helminth expulsion and defense against pathogenic bacteria such as *Salmonella typhimurium* (Alves-Filho et al., [2010](#); Rostan et al., [2015](#)). IL-33 reprograms intestinal epithelial progenitor cell differentiation toward a specific expansion of cells of the secretory lineage, such as goblet and Paneth cells. This is achieved by the modulation of cell-intrinsic Notch signaling in the stem cell compartment (Mahapatro et al., [2016](#)). Whether IL-33 directly acts on IECs or indirectly via the release of IL-13 by innate lymphoid cells (ILC2s) is still not clear. IL-33 also promotes the expansion of tuft cells. Interestingly, after helminth infection, tuft cell-derived IL-25 activates ILC2 to secrete IL-13, which acts either directly or indirectly via IL-33 on epithelial crypt progenitors to promote differentiation of tuft and goblet cells, leading to increased frequencies of both (von Moltke et al., [2016](#)). Expression of Toll-like receptors along the GI epithelium may also contribute to innate sensing mechanisms of infectious and commensal microbes (Haller et al., [2002](#); Rakoff-Nahoum et al., [2006](#); Price et al., [2018](#)). This complex interplay between immune cells and IECs illustrates how the intestine maintains homeostasis while responding to infections, balancing host defense and tissue repair.

7.5 Epithelial Dysfunctions in the Pathogenesis of IBD

IBD are lifelong conditions that impose a substantial individual and socio-economic burden. Alongside other chronic immune and metabolic disorders, their incidence and prevalence are rising globally, following patterns associated with industrialization. Crohn's disease and ulcerative colitis are the two main clinical phenotypes of IBD, characterized by recurrent inflammation and increased risk of colon cancer. Host genetic susceptibility and environmental cues are cornerstones in the idiopathic etiology of these complex diseases, and the intestinal microbiome emerged as a common link. Loss of barrier functions and failure to mount immune tolerance likely contribute to

bacterial translocation, chronic exposure to luminal antigens, and chronic inflammation of the intestinal mucosa. Deficiencies in IEC homeostasis and function contribute to all of these detrimental steps.

7.5.1 Paneth Cells in Crohn's Disease

It has been shown that several genes that increase susceptibility to Crohn's disease directly implicate Paneth cells and their antimicrobial peptides (Fig. 7.3) (Wehkamp & Stange, 2010). One of the first genes that was described in that context was NOD2 (*nucleotide-binding oligomerization domain-containing 2*), a gene that is commonly mutated in individuals with Crohn's disease (around one-third of adult CD patients carry at least one heterozygous *NOD2* mutation; detailed information can be found in (Hugot et al., 2001; Ogura et al., 2001). Individuals who carry either homozygous or heterozygous germ-line variations of NOD2 have a 40-fold increased risk of developing ileal Crohn's disease (Cuthbert et al., 2002). In general, NOD2 is a protein that is expressed in macrophages, dendritic cells, as well as IECs, including Paneth cells (Ogura et al., 2003). NOD2 is an intracellular receptor for muramyl dipeptide, which can be found in bacterial peptidoglycans (Girardin et al., 2003a, b; Inohara et al., 2003). Activation of this receptor, therefore, provokes immune responses to intracellular bacteria. Animal studies uncovered that NOD2 signaling is linked to expression of antimicrobial peptides (defensins) and is important for host immunity to *Yersinia pseudotuberculosis*, *Listeria monocytogenes*, and *Citrobacter rodentium* (Kobayashi et al., 2005; Sorbara & Philpott, 2011). Moreover, the challenge of NOD2-deficient animals with *Helicobacter hepaticus*, a pathogen that is associated with IBD, results in the development of pathological lesions, called granulomas, that are characteristically associated with Crohn's disease (Biswas et al., 2010). Notably, NOD2 is well known for its potential to interact with the autophagy protein ATG16L1 (Travassos et al., 2010). NOD1 and NOD2 recruit ATG16L1 to the plasma membrane and can target cyto-invasive bacteria at the point of entry. Autophagy, in general, is a pathway in which cellular material, including organelles, is

sequestered in double-membrane-coated vesicles and transported to lysosomes for degradation and recycling. Thus, it is not surprising that the packing and secretion of granules within Paneth cells is strongly influenced by autophagy. Patients carrying homozygous ATG16L1 risk alleles and mice with hypomorphic expression of ATG16L1 are both associated with Paneth cell and goblet cell abnormalities (Adolph et al., [2013](#); Cadwell et al., [2008](#), [2010](#); Lassen et al., [2014](#)). Additionally, deletion of the unfolded protein response (UPR) transcription factor X-box binding protein-1 (*Xbp1*) in intestinal epithelial cells results in endoplasmic reticulum (ER) stress, Paneth cell impairment, and spontaneous enteritis (Kaser et al., [2008](#)). Importantly, variants of XBP1 have also been associated with an increased risk of Crohn's disease (Kaser et al., [2008](#)). XBP1 is a transcription factor involved in maintaining ER function and required for the expansion of the endoplasmic reticulum, which is essential for the functionality of highly secretory cells such as Paneth cells. It is believed that XBP1 deficiency induces ER stress in Paneth cells, leading to an epithelium that is overly reactive to bacterial products (such as flagellin) and inflammatory mediators (such as TNF- α), which is associated with Paneth cell dysfunction.

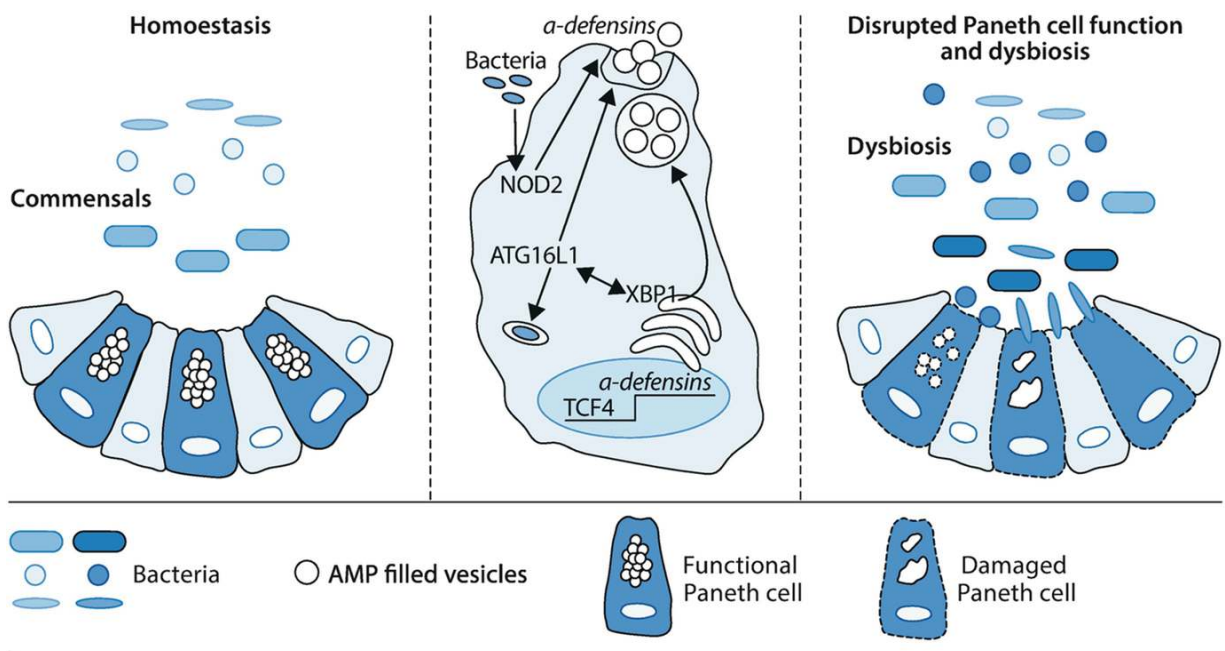


Fig. 7.3 Paneth cell dysfunction. Paneth cells play an essential role in maintaining a homeostatic host-microbial relation by secreting host endogenous antimicrobial peptides (left panel). Paneth cells are subject to damage via diverse environmental stressors and are rendered even more vulnerable by genetic predisposition. NOD2 (Kobayashi et al., [2005](#)), TCF4 (Wehkamp et al., [2007](#)), XBP1 (Adolph et al., [2013](#); Kaser et al., [2008](#)), and ATG16L1 each promote antimicrobial protein expression and secretion by Paneth cells. ATG16L1 (Cadwell et al., [2008](#)) is critical for antibacterial autophagy (middle panel). Polymorphisms in the corresponding genes are associated with Paneth cell dysfunction promoting microbial dysbiosis and increasing the risk for inflammatory bowel disease (right panel)

As described above, impairment in either UPR (*Xbp1*) or autophagy function (*Atg16l*) in intestinal epithelial cells in mouse models both impairs Paneth cell function and promotes severe spontaneous Crohn's disease-like transmural ileitis. It is currently believed that both factors are probably co-dependent and amplify the development of spontaneous inflammation in the small intestine (Adolph et al., [2013](#)). In this scenario, the UPR probably sets the threshold for susceptibility of the host with hypofunctional autophagy to develop inflammation. In summary, Paneth cells with their complex functionality contribute to the maintenance of a beneficial bacterial composition at the mucosal surface and are thus poised to broadly influence host physiology. Thus, it is not surprising that disruption of Paneth cell function is strongly linked to Crohn's disease. Nevertheless, XBP1, NOD2, and ATG16L1 are not specific products of Paneth cells. Thus, their function in other cells may also be relevant for the pathogenesis of Crohn's disease. Until recently also genetic variants in the *LRRK2* gene, which encodes a large serine-threonine kinase, have been linked to Parkinson's disease and Crohn's disease. Zhang et al. reported a Nod2-LRRK2-dependent lysozyme-sorting phenomenon in Paneth cells (Zhang et al., [2015](#), [2024](#)). However, a recent study found no evidence that LRRK2 is expressed in murine or human Paneth cells or that it was required for lysozyme expression in the gut (Tasegian et al., [2024](#)). Thus, while *LRRK2* polymorphisms in Crohn's disease may cause defects in Paneth cell function, these defects are most likely not driven by LRRK2 in a cell-autonomous manner.

7.5.2 Metabolic Injury and Dysbiosis in Chronic Inflammation

A breach in barrier integrity is associated with inflammatory pathologies and requires immediate action to restore barrier function and to induce mucosal healing (Quiros & Nusrat, [2019](#)). Injury-associated stem cells, tissue-related factors, and the microbial milieu are imperative in orchestrating tissue regeneration and barrier restoration. Loss of Lgr5⁺ stem cells in response to severe (often transmural) injury requires additional regenerative capacities in the intestine, involving quiescent slow-cycling cells located at the +4 position in the crypt, de-differentiated epithelial cells, and reserve stem cells (Barker, [2014](#)). Mitochondrial functions are critically involved in epithelial stem cell homeostasis and differentiation, and metabolic reprogramming due to mitochondrial impairment triggers tissue injury and failure to regenerate intestinal wounds. Prohibitin 1 is a mitochondrial chaperone for proteins belonging to the electron transport chain, and prohibitin 1 overexpression in IEC or therapeutic delivery of prohibitin 1 via nanoparticles protected mice from chemically-induced colitis (Jackson et al., [2020](#)). In addition, the conditional deletion of the mitochondrial chaperone heat shock protein 60 (Hsp60) in IEC caused the loss of Lgr5⁺ ISC and emergence of non-granular, dysfunctional Paneth cells, accompanied by tissue aberrations reminiscent of mucosal wound healing (Khaloian et al., [2020](#)). In the absence of immunoregulators and chemoreceptors, such as IL-10 and AhR, mitochondrial dysfunction led to a sustained microbiota-dependent tissue injury associated with accelerated and unresolved inflammation (Urbauer et al., [2024](#)). These findings provide evidence that mitochondrial impairment of the epithelium causes intestinal tissue lesions, described as metabolic injury, and support the hypothesis from Roediger in [1980](#) that ulcerative colitis with chronic inflammation in the colon is an energy-deficient disease. Importantly, mitochondrial perturbations of the intestinal stem cell niche were not only observed in mouse models of intestinal inflammation, but also in Crohn's disease patients. Transcriptional profiling of the metabolically

impaired epithelium identified IBD-relevant gene signatures involving oxidative stress (Nos2 and Duox2) and host tryptophan metabolism (Ido1) (Urbauer et al., [2024](#)). A number of IBD-related genetic risk loci map to mitochondrial function-associated genes, and 5% of IBD susceptibility genes play direct roles in regulating mitochondrial homeostasis (Haberman et al., [2019](#)). These findings support the idea that mitochondrial perturbations and limited metabolic flexibility sensitize the intestinal epithelium to tissue injury and increased risk for recurrent barrier breaches and chronic inflammation.

Intact mitochondrial functions of the intestinal epithelium contribute to epithelial homeostasis and create niches for metabolic cooperation with anaerobic intestinal bacteria. Under normal physiological conditions, the differentiated epithelium performs mitochondrial respiration (oxidative phosphorylation, OXPHOS) to consume oxygen for ATP production, resulting in physiological tissue hypoxia and anaerobic conditions in the intestinal lumen (Fig. [7.4](#)). Infectious bacteria of the family Enterobacteriaceae, such as *Salmonella enterica* serovar *Typhimurium* and *Citrobacter rodentium*, reshape the metabolic niche of the intestinal epithelium by shifting mitochondrial respiration (Byndloss et al., [2017](#)), leading to increased lactate production and oxygen diffusion into the luminal milieu. Increased production of reactive oxygen species (ROS) and nitrogen species (RNS) from infiltrating phagocytes facilitates the formation of respiratory electron acceptors, such as nitrate and tetrathionate, thereby favoring the growth of facultative anaerobes in the intestinal milieu (Lee et al., [2022](#)). Similar to infection-related changes in microbe-metabolic circuits, IBD-related defects in epithelial metabolism induce transcriptional alterations in genes involved in oxidative and nitrosative stress (Nos2 and Duox2), leading to the expansion of IBD-relevant pathobionts such as *Escherichia coli*, *Bacteroides* spp., *Phocaeicola vulgatus*, *Veillonella parvula* (Urbauer et al., [2024](#)). The latter, an anaerobic oral microbe, drives the IBD-related nitrosative host response, creating a metabolic milieu allowing *V. parvula* to perform

anaerobic nitrate respiration, leading to its ectopic colonization of the inflamed colon (Price et al., [2018](#); Rojas-Tapias et al., [2022](#)).

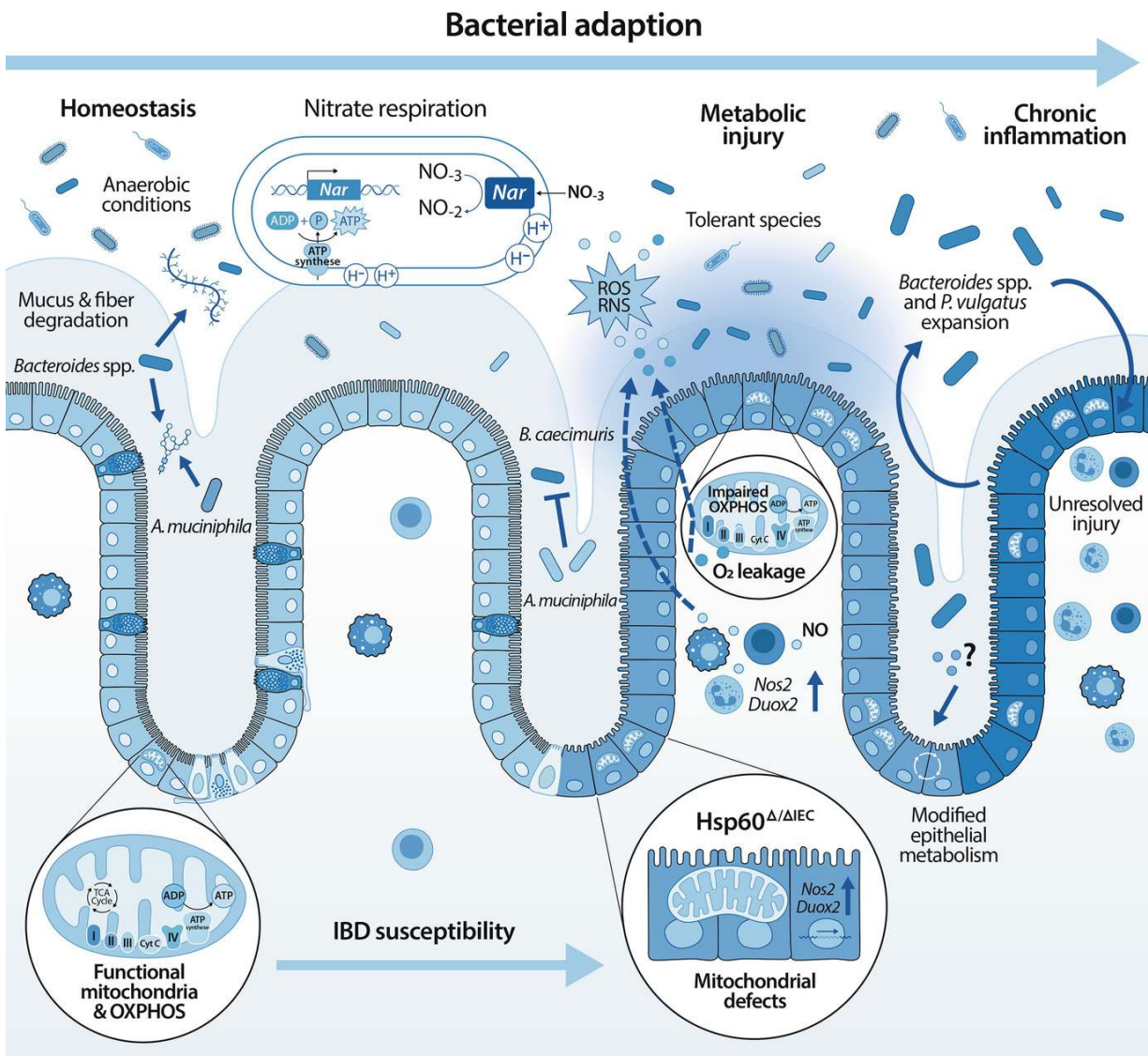


Fig. 7.4 At homeostasis, oxygen consumption by the intestinal epithelium maintains anaerobic conditions in the gut lumen, allowing colonization of anaerobes. Complex plant polysaccharides from dietary fibers and mucus glycans are degraded by bacteria, such as *Akkermansia muciniphila* and *Bacteroides* spp. IBD susceptibility comprises defects in mitochondrial function, leading to a lack of oxygen consumption in the OXPHOS and subsequent leakage in the lumen, favoring aerotolerant bacteria. Additionally, ROS and RNS production by the host contributes to the selection of tolerant species, which can adapt to these environmental stressors. Bacteria capable of using nitrate as a respiratory substrate are favored. Mitochondrial impairment leads to metabolic injury, which is accompanied by increased ROS/RNS transcriptional profiles and expansion of IBD-relevant pathobionts. Under IBD-susceptible conditions, these microbiota changes persist, and chronic inflammation prevents tissue regeneration

Controversy Leaky gut: Fact or Fiction?

The term “leaky gut” has gained significant importance in both the scientific community and clinical practice, yet its precise definition and clinical relevance remain highly debated. Often used to describe increased intestinal permeability, the term lacks clear consensus on its pathological meaning or implications for disease. Several markers are utilized to assess intestinal barrier integrity, including intestinal fatty acid-binding protein (iFABP, cytosolic protein released from injured intestinal epithelial cells), Zonulin or Occludin (regulate paracellular permeability, with altered levels suggesting tight junction disruption), and sugar permeability tests (Lactulose-Mannitol Test) (Camilleri & Vella, [2022](#); Vanuytsel et al., [2021](#); Seethaler et al., [2021](#)). While these tests are commonly used and can provide insights into barrier integrity, their specificity and reliability remain controversially discussed (Wang et al., [2015](#); Camilleri, [2019](#)). It is unclear to what extent these markers accurately reflect true barrier dysfunction or are influenced by unrelated physiological or pathological processes. In diseases like IBD, whether a “leaky gut” corresponds to a concrete disruption of the epithelial barrier that permits bacterial translocation into the lamina propria is not definitively proven (Rath et al., [2023](#)). Although bacterial translocation has been implicated in the inflammatory cascade, direct evidence linking it to measurable permeability defects remains elusive. The clinical and functional consequences of barrier dysfunction may differ significantly between the small and large intestine, yet these regional differences are poorly characterized. The role of the small intestine in nutrient absorption and antigen sampling is quite distinct to the colon with its denser microbial population. These differences highlight the unique challenges to barrier integrity and immune regulation within the two different parts of the gut, which remain inadequately understood. The intestinal epithelium is not a passive barrier but a frontline immune sensor. Pattern recognition receptors (PRRs) enable epithelial cells to detect microbial signals and maintain homeostasis. However, the

dynamics and molecular mechanisms of bacterial interaction within the epithelium and if the luminal compartment is involved is poorly studied. Whether these interactions exacerbate barrier dysfunction or contribute to regulatory processes is an open question. In summary, “leaky gut” remains a widely recognized but scientifically underdefined concept. Significant gaps persist in our understanding of its mechanisms, reliable markers, and clinical relevance, particularly in diseases like IBD. Even less is known about its potential implications in non-mucosal inflammatory conditions (Urbauer et al., [2024](#)).

History

Recent advancements in stem cell biology have enabled the generation of complex 3D organoid structures from the embryonic, neonatal and adult intestinal epithelium, overcoming previous challenges of genetic transformation. Intestinal organoids, derived from species like human, mouse, and pig, replicate in vivo tissue architecture, functionality, and gene expression. These organoids have proven essential for studying stem cell biology, organogenesis, microbiota-host interactions, and diseases such as IBD, infections, and cancer. Their use has revolutionized research into intestinal stem cell behavior and disease mechanisms, with patient-derived organoid models opening doors to personalized medicine and drug screening. Recent developments, such as CRISPR-based genetic manipulation and organoid co-culture systems, have enabled unprecedented insights into host-pathogen interactions, immune responses, and epithelial regeneration. Additionally, innovations like 3D bioprinting and microfluidics have further refined organoid models, creating more physiologically relevant representations of intestinal tissues for both basic and translational research.

A critical advancement that laid the groundwork for studying epithelial development and function was the generation of germ-free (GF) animals. This pioneering approach provided a unique in vivo system to explore the host-microbe interplay, particularly the role of

the microbiota for intestinal epithelial maturation, barrier function, and immune regulation. By introducing specific microbial communities or individual bacterial strains to GF animals, researchers were able to demonstrate how the microbiota influences epithelial differentiation, tight junction integrity, and the host immune response to luminal antigens. This approach also highlighted the dynamic and bidirectional relationship between microbial populations and the host epithelium, offering key insights into the pathogenesis of intestinal and systemic diseases.

Together, GF models and organoid technologies have transformed our ability to investigate intestinal epithelial maturation, barrier integrity, and the complex interactions between microbes and their host.

Highlights

- The intestine is covered by a single layer of epithelial cells. It is organized into crypts (invaginations containing stem cells and transit-amplifying cells) and villi (protrusions that are covered with terminally differentiated cells).
- The intestinal epithelium develops from the embryonic endoderm, which is one of the three primary germ layers derived during gastrulation. In the mouse, the intestinal epithelium originates as a single-layered structure lining the inner surface of the primitive gut (around embryonic day 9.5). The regenerative capacity of the adult epithelium is established between embryonic day 16.5 and postnatal day 7, when mature crypts harboring adult stem cells and progenitor cells develop from a pool of cells located at the base of the embryonic villi (Noah et al., [2011](#)).
- In the adult intestinal epithelium, pluripotent stem cells in the crypt region give rise to a pool of transit-amplifying cells. These cells will (after 2–3 days) terminally differentiate into one of the epithelial cell lineages. This includes absorptive cells called enterocytes, which form the majority, as well as secretory cells,

including Paneth cells, enteroendocrine cells, goblet cells, M (microfold) cells, tuft cells, and cup cells.

- The intestinal epithelium is the most vigorously self-renewing tissue. The *life cycle* of intestinal epithelial cells (4–5 days) is determined by the time span in which these *cells* migrate from their place of origin at the crypt base to the villus tip, from where they are expelled from the epithelial layer through a complex cytoskeletal remodeling process.
- IECs are crucial mediators of intestinal homeostasis. They provide physical barrier functions and enable the establishment of an immunological environment permissive to colonization by commensal bacteria.

References

Adolph, T. E., Tomczak, M. F., Niederreiter, L., Ko, H. J., Bock, J., Martinez-Naves, E., Glickman, J. N., Tschurtschenthaler, M., Hartwig, J., Hosomi, S., et al. (2013). Paneth cells as a site of origin for intestinal inflammation. *Nature*, 503, 272–276.

[<https://doi.org/10.1038/nature12599>][[PubMed](#)]

Alves-Filho, J. C., Sonogo, F., Souto, F. O., Freitas, A., Verri, W. A., Jr., Auxiliadora-Martins, M., Basile-Filho, A., McKenzie, A. N., Xu, D., Cunha, F. Q., & Liew, F. Y. (2010). Interleukin-33 attenuates sepsis by enhancing neutrophil influx to the site of infection. *Nature Medicine*, 16, 708–712.

[[PubMed](#)]

Artis, D. (2008). Epithelial-cell recognition of commensal bacteria and maintenance of immune homeostasis in the gut. *Nature Reviews Immunology*, 8, 411–420.

[[PubMed](#)]

Ayabe, T., Ashida, T., Kohgo, Y., & Kono, T. (2004). The role of Paneth cells and their antimicrobial peptides in innate host defense. *Trends in Microbiology*, 12, 394–398.

[[PubMed](#)]

Baghdadi, M. B., Ayyaz, A., Coquenlorge, S., Chu, B., Kumar, S., Streutker, C., Wrana, J. L., & Kim, T.-H. (2022). Enteric glial cell heterogeneity regulates intestinal stem cell niches. *Cell Stem Cell*, 29, 86–100.e6. <https://doi.org/10.1016/j.stem.2021.10.004>

[[PubMed](#)]

Barker, N. (2014). Adult intestinal stem cells: Critical drivers of epithelial homeostasis and regeneration. *Nature Reviews Molecular Cell Biology*, 15, 19–33. <https://doi.org/10.1038/nrm3721>

[[PubMed](#)]

Barker, N., van de Wetering, M., & Clevers, H. (2008). The intestinal stem cell. *Genes & Development*, 22, 1856–1864.

Belkaid, Y., & Hand, T. W. (2014). Role of the microbiota in immunity and inflammation. *Cell*, 157, 121–141.

[[PubMed](#)]

Berger, E., Rath, E., Yuan, D., Waldschmitt, N., Khaloian, S., Allgäuer, M., Staszewski, O., Lobner, E. M., Schöttl, T., Giesbertz, P., Colemann, O. I., Prinz, M., Weber, A., Gerhard, M., Klingenspor, M., Janssen, K.-P., Heikenwalder, M., & Haller, D. (2016). Mitochondrial function controls intestinal epithelial stemness and proliferation. *Nature Communications*, 7, 13171. <https://doi.org/10.1038/ncomms13171>

[[PubMed](#)]

Bergstrom, K., Liu, X., Zhao, Y., Gao, N., Wu, Q., Song, K., Cui, Y., Li, Y., McDaniel, J. M., McGee, S., Chen, W., Huycke, M. M., Houchen, C. W., Zenewicz, L. A., West, C. M., Chen, H., Braun, J., Fu, J., & Xia, L. (2016). Defective intestinal mucin-type O-glycosylation causes spontaneous colitis-associated cancer in mice. *Gastroenterology*, 151(1), 152–164.e11.

[[PubMed](#)]

Bergstrom, K., Shan, X., Casero, D., Batushansky, A., Lagishetty, V., Jacobs, J. P., Hoover, C., Kondo, Y., Shao, B., Gao, L., Zandberg, W., Noyovitz, B., McDaniel, J. M., Gibson, D. L., Pakpour, S., Kazemian, N., McGee, S., Houchen, C. W., Rao, C. V., Griffin, T. M., Sonnenburg, J. L., McEver, R. P., Braun, J., & Xia, L. (2020). Proximal colon-derived O-glycosylated mucus encapsulates and modulates the microbiota. *Science*, 370(6515), 467–472.

[[PubMed](#)]

Bevins, C. L., & Salzman, N. H. (2011). Paneth cells, antimicrobial peptides and maintenance of intestinal homeostasis. *Nature Reviews Microbiology*, 9, 356–368.

[[PubMed](#)]

Bhattacharya, S., Tie, G., Singh, P. N. P., Malagola, E., Eskiocak, O., He, R., Kraiczy, J., Gu, W., Perlov, Y., Alici-Garipcan, A., et al. (2025). Intestinal secretory differentiation reflects niche-driven phenotypic and epigenetic plasticity of a common signal-responsive terminal cell. *Cell Stem Cell*. <https://doi.org/10.1016/j.stem.2025.03.005>

Biswas, A., Liu, Y. J., Hao, L., Mizoguchi, A., Salzman, N. H., Bevins, C. L., & Kobayashi, K. S. (2010). Induction and rescue of Nod2-dependent Th1-driven granulomatous inflammation of the ileum. *Proceedings of the National Academy of Sciences of the United States of America*, 107, 14739–14744.

[[PubMed](#)]

Bjerknes, M., & Cheng, H. (1981). The stem-cell zone of the small intestinal epithelium. IV. Effects of resecting 30% of the small intestine. *The American Journal of Anatomy*, 160, 93–103.

[[PubMed](#)]

Bjerknes, M., & Cheng, H. (2005). Gastrointestinal stem cells. II. Intestinal stem cells. *American*

Journal of Physiology-Gastrointestinal and Liver Physiology, 289, G381–G387.

[\[PubMed\]](#)

Blazejewski, A. J., Thiemann, S., Schenk, A., Pils, M. C., Galvez, E. J. C., Roy, U., Heise, U., de Zoete, M. R., Flavell, R. A., & Strowig, T. (2017). Microbiota normalization reveals that canonical caspase-1 activation exacerbates chemically induced intestinal inflammation. *Cell Reports*, 19, 2319–2330.

[\[PubMed\]](#)

Buffa, R., Capella, C., Fontana, P., Usellini, L., & Solcia, E. (1978). Types of endocrine cells in the human colon and rectum. *Cell and Tissue Research*, 192, 227–240.

[\[PubMed\]](#)

Buffie, C. G., & Pamer, E. G. (2013). Microbiota-mediated colonization resistance against intestinal pathogens. *Nature Reviews Immunology*, 13, 790–801.

[\[PubMed\]](#)

Bullen, T. F., Forrest, S., Campbell, F., Dodson, A. R., Hershman, M. J., Pritchard, D. M., Turner, J. R., Montrose, M. H., & Watson, A. J. (2006). Characterization of epithelial cell shedding from human small intestine. *Laboratory Investigation*, 86, 1052–1063.

[\[PubMed\]](#)

Byndloss, M. X., Olsan, E. E., Rivera-Chavez, F., Tiffany, C. R., Cevallos, S. A., Lokken, K. L., Torres, T. P., Byndloss, A. J., Faber, F., Gao, Y., Litvak, Y., Lopez, C. A., Xu, G., Napoli, E., Giulivi, C., Tsois, R. M., Revzin, A., Lebrilla, C. B., & Bäumler, A. J. (2017). Microbiota-activated PPAR-gamma signaling inhibits dysbiotic Enterobacteriaceae expansion. *Science*, 357, 570–575. <https://doi.org/10.1126/science.aam9949>

[\[PubMed\]](#)

Cadwell, K., Liu, J. Y., Brown, S. L., Miyoshi, H., Loh, J., Lennerz, J. K., Kishi, C., Kc, W., Carrero, J. A., Hunt, S., et al. (2008). A key role for autophagy and the autophagy gene Atg16l1 in mouse and human intestinal Paneth cells. *Nature*, 456, 259–263.

<https://doi.org/10.1038/nature07416> [\[PubMed\]](#)

Cadwell, K., Patel, K. K., Maloney, N. S., Liu, T. C., Ng, A. C., Storer, C. E., Head, R. D., Xavier, R., Stappenbeck, T. S., & Virgin, H. W. (2010). Virus-plus-susceptibility gene interaction determines Crohn's disease gene Atg16L1 phenotypes in intestine. *Cell*, 141, 1135–1145.

[\[PubMed\]](#)

Camilleri, M. (2019). Leaky gut: Mechanisms, measurement and clinical implications in humans. *Gut*, 68, 1516–1526.

[\[PubMed\]](#)

Camilleri, M., & Vella, A. (2022). What to do about the leaky gut. *Gut*, 71, 424–433.

[\[PubMed\]](#)

Campbell, N., Yio, X. Y., So, L. P., Li, Y., & Mayer, L. (1999). The intestinal epithelial cell: Processing and presentation of antigen to the mucosal immune system. *Immunological Reviews*, 172, 315–324.

[\[PubMed\]](#)

Coleman, O. I., & Haller, D. (2021). Microbe-mucus interface in the pathogenesis of colorectal cancer. *Cancers (Basel)*, 13(4), 616.

[\[PubMed\]](#)

Corr, S. C., Gahan, C. C., & Hill, C. (2008). M-cells: Origin, morphology and role in mucosal immunity and microbial pathogenesis. *FEMS Immunology and Medical Microbiology*, 52, 2–12.

[\[PubMed\]](#)

Crosnier, C., Stamataki, D., & Lewis, J. (2006). Organizing cell renewal in the intestine: Stem cells, signals and combinatorial control. *Nature Reviews Genetics*, 7, 349–359.

[\[PubMed\]](#)

Cuthbert, A. P., Fisher, S. A., Mirza, M. M., King, K., Hampe, J., Croucher, P. J., Mascheretti, S., Sanderson, J., Forbes, A., Mansfield, J., et al. (2002). The contribution of NOD2 gene mutations to the risk and site of disease in inflammatory bowel disease. *Gastroenterology*, 122, 867–874.

[\[PubMed\]](#)

Dahan, S., Roth-Walter, F., Arnaboldi, P., Agarwal, S., & Mayer, L. (2007). Epithelia: Lymphocyte interactions in the gut. *Immunological Reviews*, 215, 243–253.

[\[PubMed\]](#)

de Santa Barbara, P., van den Brink, G. R., & Roberts, D. J. (2003). Development and differentiation of the intestinal epithelium. *Cellular and Molecular Life Sciences*, 60, 1322–1332.

[\[PubMed\]](#)

Eisenhoffer, G. T., Loftus, P. D., Yoshigi, M., Otsuna, H., Chien, C. B., Morcos, P. A., & Rosenblatt, J. (2012). Crowding induces live cell extrusion to maintain homeostatic cell numbers in epithelia. *Nature*, 484, 546–549.

[\[https://doi.org/10.1038/nature10999\]](https://doi.org/10.1038/nature10999)[\[PubMed\]](#)

Elphick, D. A., & Mahida, Y. R. (2005). Paneth cells: Their role in innate immunity and inflammatory disease. *Gut*, 54, 1802–1809.

[\[PubMed\]](#)

Ermund, A., Schütte, A., Johansson, M. E. V., Gustafsson, J. K., & Hansson, G. C. (2013). Studies of mucus in mouse stomach, small intestine, and colon. I. Gastrointestinal mucus layers have different properties depending on location as well as over the Peyer's patches. *American Journal of Physiology-Gastrointestinal and Liver Physiology*, 305(5), G341–G347.

[\[PubMed\]](#)

Farin, H. F., Karthaus, W. R., Kujala, P., Rakhshandehroo, M., Schwank, G., Vries, R. G., Kalkhoven, E., Nieuwenhuis, E. E., & Clevers, H. (2014). Paneth cell extrusion and release of antimicrobial products is directly controlled by immune cell-derived IFN-gamma. *The Journal of Experimental Medicine*, 211, 1393–1405.

[\[PubMed\]](#)

Fothergill, L. J., & Furness, J. B. (2018). Diversity of enteroendocrine cells investigated at cellular and subcellular levels: The need for a new classification scheme. *Histochemistry and Cell Biology*, 150(6), 693–702.

[\[PubMed\]](#)

Furness, J. B., Rivera, L. R., Cho, H. J., Bravo, D. M., & Callaghan, B. (2013). The gut as a sensory organ. *Nature Reviews Gastroenterology & Hepatology*, 10, 729–740.

Gabius, H.-J., & Roth, J. (2017). An introduction to the sugar code. *Histochemistry and Cell Biology*, 147(2), 111–117.

[\[PubMed\]](#)

Gallo, R. L., & Hooper, L. V. (2012). Epithelial antimicrobial defence of the skin and intestine. *Nature Reviews Immunology*, 12, 503–516.

[\[PubMed\]](#)

Gehart, H., van Es, J. H., Hamer, K., Beumer, J., Kretzschmar, K., Dekkers, J. F., Rios, A., & Clevers, H. (2019). Identification of enteroendocrine regulators by real-time single-cell differentiation mapping. *Cell*, 176(5), 1158–1173 e1116.

[\[PubMed\]](#)

Gerbe, F., & Jay, P. (2016). Intestinal tuft cells: Epithelial sentinels linking luminal cues to the immune system. *Mucosal Immunology*, 9, 1353–1359.

[\[PubMed\]](#)

Gerbe, F., Legraverend, C., & Jay, P. (2012). The intestinal epithelium tuft cells: Specification and function. *Cellular and Molecular Life Sciences*, 69, 2907–2917.

[\[PubMed\]](#)

Gerbe, F., Sidot, E., Smyth, D. J., Ohmoto, M., Matsumoto, I., Dardalhon, V., Cesses, P., Garnier, L., Pouzolles, M., Brulin, B., et al. (2016). Intestinal epithelial tuft cells initiate type 2 mucosal immunity to helminth parasites. *Nature*, 529, 226–230.

[\[https://doi.org/10.1038/nature16527\]](https://doi.org/10.1038/nature16527) [\[PubMed\]](#)

Gibbons, D. L., & Spencer, J. (2011). Mouse and human intestinal immunity: Same ballpark, different players; different rules, same score. *Mucosal Immunology*, 4, 148–157.

[\[PubMed\]](#)

Girardin, S. E., Boneca, I. G., Viala, J., Chamaillard, M., Labigne, A., Thomas, G., Philpott, D. J., & Sansonetti, P. J. (2003a). Nod2 is a general sensor of peptidoglycan through muramyl dipeptide (MDP) detection. *The Journal of Biological Chemistry*, 278, 8869–8872.

[\[PubMed\]](#)

Girardin, S. E., Travassos, L. H., Herve, M., Blanot, D., Boneca, I. G., Philpott, D. J., Sansonetti, P. J., & Mengin-Lecreulx, D. (2003b). Peptidoglycan molecular requirements allowing detection by Nod1 and Nod2. *The Journal of Biological Chemistry*, 278, 41702–41708.

[\[PubMed\]](#)

Grencis, R. K., & Worthington, J. J. (2016). Tuft cells: A new flavor in innate epithelial immunity. *Trends in Parasitology*, 32, 583–585.

[\[PubMed\]](#)

Gribble, F. M., & Reimann, F. (2016). Enteroendocrine cells: Chemosensors in the intestinal epithelium. *Annual Review of Physiology*, 78, 277–299.

[[PubMed](#)]

Gronke, K., & Diefenbach, A. (2016). Tuft cell-derived IL-25 activates and maintains ILC2. *Immunology and Cell Biology*, 94, 221–223.

[[PubMed](#)]

Grootjans, J., Kaser, A., Kaufman, R. J., & Blumberg, R. S. (2016). The unfolded protein response in immunity and inflammation. *Nature Reviews Immunology*, 16, 469–484. <https://doi.org/10.1038/nri.2016.62>

[[PubMed](#)]

Gunawardene, A. R., Corfe, B. M., & Staton, C. A. (2011). Classification and functions of enteroendocrine cells of the lower gastrointestinal tract. *International Journal of Experimental Pathology*, 92, 219–231.

[[PubMed](#)]

Gunther, C., Martini, E., Wittkopf, N., Amann, K., Weigmann, B., Neumann, H., Waldner, M. J., Hedrick, S. M., Tenzer, S., Neurath, M. F., & Becker, C. (2011). Caspase-8 regulates TNF- α -induced epithelial necroptosis and terminal ileitis. *Nature*, 477, 335–339.

[<https://doi.org/10.1038/nature10400>][[PubMed](#)]

Gunther, C., Neumann, H., Neurath, M. F., & Becker, C. (2013). Apoptosis, necrosis and necroptosis: Cell death regulation in the intestinal epithelium. *Gut*, 62, 1062–1071.

[[PubMed](#)]

Gunther, C., Josenhans, C., & Wehkamp, J. (2016). Crosstalk between microbiota, pathogens and the innate immune responses. *International Journal of Medical Microbiology*, 306, 257–265.

[[PubMed](#)]

Gustafsson, J. K., & Johansson, M. E. V. (2022). The role of goblet cells and mucus in intestinal homeostasis. *Nature Reviews Gastroenterology & Hepatology*, 19(12), 785–803.

Haberman, Y., Karns, R., Dexheimer, P. J., Schirmer, M., Somekh, J., Jurickova, I., Braun, T., Novak, E., Bauman, L., Collins, M. H., Mo, A., Rosen, M. J., Bonkowski, E., Gotman, N., Marquis, A., Nistel, M., Rufo, P. A., Baker, S. S., Sauer, C. G., Markowitz, J., Pfefferkorn, M. D., Rosh, J. R., Boyle, B. M., Mack, D. R., Baldassano, R. N., Shah, S., Leleiko, N. S., Heyman, M. B., Griffiths, A. M., Patel, A. S., Noe, J. D., Aronow, B. J., Kugathasan, S., Walters, T. D., Gibson, G., Thomas, S. D., Mollen, K., Shen-Orr, S., Huttenhower, C., Xavier, R. J., Hyams, J. S., & Denson, L. A. (2019). Ulcerative colitis mucosal transcriptomes reveal mitochondriopathy and personalized mechanisms underlying disease severity and treatment response. *Nature Communications*, 10, 38. <https://doi.org/10.1038/s41467-018-07841-3>

[[PubMed](#)]

Hadjiyanni, I., Siminovitch, K. A., Danska, J. S., & Drucker, D. J. (2010). Glucagon-like peptide-1 receptor signalling selectively regulates murine lymphocyte proliferation and maintenance of peripheral regulatory T cells. *Diabetologia*, 53(4), 730–740.

[<https://doi.org/10.1007/s00125-009-1643-x>][[PubMed](#)]

Hall, C. A. (1997). Patient management in head injury care: A nursing perspective. *Intensive & Critical Care Nursing*, 13, 329–337.

Haller, D., Russo, M. P., Sartor, R. B., & Jobin, C. (2002). IKK beta and phosphatidylinositol 3-kinase/Akt participate in non-pathogenic Gram-negative enteric bacteria-induced RelA phosphorylation and NF- κ B activation in both primary and intestinal epithelial cell lines. *Journal of Biological Chemistry*, 277, 38168–38178. <https://doi.org/10.1074/jbc.M205737200> [PubMed]

Hansson, G. C. (2020). Mucins and the microbiome. *Annual Review of Biochemistry*, 89, 769–793. [PubMed]

Hasegawa, M., Yada, S., Liu, M. Z., Kamada, N., Munoz-Planillo, R., Do, N., Nunez, G., & Inohara, N. (2014). Interleukin-22 regulates the complement system to promote resistance against pathobionts after pathogen-induced intestinal damage. *Immunity*, 41, 620–632. [PubMed]

Hershberg, R. M., Framson, P. E., Cho, D. H., Lee, L. Y., Kovats, S., Beitz, J., Blum, J. S., & Nepom, G. T. (1997). Intestinal epithelial cells use two distinct pathways for HLA class II antigen processing. *The Journal of Clinical Investigation*, 100, 204–215. [PubMed]

Heuberger, C. E., Janney, A., Ilott, N., Bertocchi, A., Pott, S., Gu, Y., Pohin, M., Friedrich, M., Mann, E. H., Pearson, C., Powrie, F. M., Pott, J., Thornton, E., & Maloy, K. J. (2024). MHC class II antigen presentation by intestinal epithelial cells fine-tunes bacteria-reactive CD4 T-cell responses. *Mucosal Immunology*, 17(3), 416–430. <https://doi.org/10.1016/j.mucimm.2023.05.001> [PubMed]

Hugot, J. P., Chamaillard, M., Zouali, H., Lesage, S., Cezard, J. P., Belaiche, J., Almer, S., Tysk, C., O'Morain, C. A., Gassull, M., et al. (2001). Association of NOD2 leucine-rich repeat variants with susceptibility to Crohn's disease. *Nature*, 411, 599–603. <https://doi.org/10.1038/35079107> [PubMed]

Inohara, N., Ogura, Y., Fontalba, A., Gutierrez, O., Pons, F., Crespo, J., Fukase, K., Inamura, S., Kusumoto, S., Hashimoto, M., et al. (2003). Host recognition of bacterial muramyl dipeptide mediated through NOD2. Implications for Crohn's disease. *Journal of Biological Chemistry*, 278, 5509–5512. [PubMed]

Ireland, H., Houghton, C., Howard, L., & Winton, D. J. (2005). Cellular inheritance of a cre-activated reporter gene to determine Paneth cell longevity in the murine small intestine. *Developmental Dynamics*, 233, 1332–1336. [PubMed]

Jackson, D. N., Panopoulos, M., Neumann, W. L., Turner, K., Cantarel, B. L., Thompson-Snipes, L., Dassopoulos, T., Feagins, L. A., Souza, R. F., Mills, J. C., Blumberg, R. S., Venuprasad, K., Thompson, W. E., & Theiss, L. A. (2020). Mitochondrial dysfunction during loss of prohibitin 1 triggers Paneth cell defects and ileitis. *Gut*, 69, 1928–1938. <https://doi.org/10.1136/gutjnl-2019-319523> [PubMed]

Johansson, M. E. V., Phillipson, M., Petersson, J., Velcich, A., Holm, L., & Hansson, G. C. (2008). The inner of the two Muc2 mucin-dependent mucus layers in colon is devoid of bacteria. *Proceedings of the National Academy of Sciences of the United States of America*, 105(39), 15064–15069.

[[PubMed](#)]

Jung, C., Hugot, J. P., & Barreau, F. (2010). Peyer's patches: The immune sensors of the intestine. *International Journal of Inflammation*, 2010, 823710.

[[PubMed](#)]

Kaiko, G. E., Ryu, S. H., Koues, O. I., Collins, P. L., Solnica-Krezel, L., Pearce, E. J., Pearce, E. L., Oltz, E. M., & Stappenbeck, T. S. (2016). The colonic crypt protects stem cells from microbiota-derived metabolites. *Cell*, 165(7), 1708–1720.

[[PubMed](#)]

Kamada, N., Chen, G. Y., Inohara, N., & Nunez, G. (2013). Control of pathogens and pathobionts by the gut microbiota. *Nature Immunology*, 14, 685–690.

[[PubMed](#)]

Kambayashi, T., & Laufer, T. M. (2014). Atypical MHC class II-expressing antigen-presenting cells: Can anything replace a dendritic cell? *Nature Reviews Immunology*, 14, 719–730.

[[PubMed](#)]

Kaser, A., & Blumberg, R. S. (2008). Paneth cells and inflammation dance together in Crohn's disease. *Cell Research*, 18, 1160–1162.

[[PubMed](#)]

Kaser, A., Lee, A. H., Franke, A., Glickman, J. N., Zeissig, S., Tilg, H., Nieuwenhuis, E. E., Higgins, D. E., Schreiber, S., Glimcher, L. H., & Blumberg, R. S. (2008). XBP1 links ER stress to intestinal inflammation and confers genetic risk for human inflammatory bowel disease. *Cell*, 134, 743–756.

[[PubMed](#)]

Khaloian, S., Rath, E., Hammoudi, N., Gleisinger, E., Blutke, A., Giesbertz, P., Berger, E., Metwaly, A., Waldschmitt, N., Allez, M., & Haller, D. (2020). Mitochondrial impairment drives intestinal stem cell transition into dysfunctional Paneth cells predicting Crohn's disease recurrence. *Gut*, 69, 1939–1951. <https://doi.org/10.1136/gutjnl-2019-319514>

[[PubMed](#)]

Kobayashi, K. S., Chamaillard, M., Ogura, Y., Henegariu, O., Inohara, N., Nunez, G., & Flavell, R. A. (2005). Nod2-dependent regulation of innate and adaptive immunity in the intestinal tract. *Science*, 307, 731–734.

[[PubMed](#)]

Kokrashvili, Z., Rodriguez, D., Yevshayeva, V., Zhou, H., Margolskee, R. F., & Mosinger, B. (2009). Release of endogenous opioids from duodenal enteroendocrine cells requires Trpm5. *Gastroenterology*, 137, 598–606. 606 e591–592.

[[PubMed](#)]

Kvietys, P. R., & Granger, D. N. (2010). Role of intestinal lymphatics in interstitial volume regulation

and transmucosal water transport. *Annals of the New York Academy of Sciences*, 1207(Suppl 1), E29–E43.

[[PubMed](#)]

Lassen, K. G., Kuballa, P., Conway, K. L., Patel, K. K., Becker, C. E., Peloquin, J. M., Villablanca, E. J., Norman, J. M., Liu, T. C., Heath, R. J., et al. (2014). Atg16L1 T300A variant decreases selective autophagy resulting in altered cytokine signaling and decreased antibacterial defense. *Proceedings of the National Academy of Sciences of the United States of America*, 111, 7741–7746.

[[PubMed](#)]

Lee, J. Y., Tsolis, R. M., & Baumler, A. J. (2022). The microbiome and gut homeostasis. *Science*, 377, eabp9960. <https://doi.org/10.1126/science.abp9960>

[[PubMed](#)]

Lin, Q., Zhang, S., Zhang, J., Jin, Y., Chen, T., Lin, R., Lv, R., Xu, W., Wu, T., Tian, S., Ying, L., Li, X., Huang, Z., & Niu, J. (2025). Colonic epithelial-derived FGF1 drives intestinal stem cell commitment toward goblet cells to suppress inflammatory bowel disease. *Nature Communications*, 16, 3264. <https://doi.org/10.1038/s41467-025-58644-2>

[[PubMed](#)]

Mabbott, N. A., Donaldson, D. S., Ohno, H., Williams, I. R., & Mahajan, A. (2013). Microfold (M) cells: Important immunosurveillance posts in the intestinal epithelium. *Mucosal Immunology*, 6, 666–677.

[[PubMed](#)]

Madara, J. L. (1982). Cup cells: Structure and distribution of a unique class of epithelial cells in Guinea pig, rabbit, and monkey small intestine. *Gastroenterology*, 83, 981–994.

[[PubMed](#)]

Mahapatro, M., Foersch, S., Hefele, M., He, G. W., Giner-Ventura, E., McHedlidze, T., Kindermann, M., Vetrano, S., Danese, S., Gunther, C., et al. (2016). Programming of intestinal epithelial differentiation by IL-33 derived from pericryptal fibroblasts in response to systemic infection. *Cell Reports*, 15, 1743–1756.

[[PubMed](#)]

Man, A. L., Prieto-Garcia, M. E., & Nicoletti, C. (2004). Improving M cell mediated transport across mucosal barriers: Do certain bacteria hold the keys? *Immunology*, 113, 15–22.

[[PubMed](#)]

Marchiando, A. M., Shen, L., Graham, W. V., Edelblum, K. L., Duckworth, C. A., Guan, Y., Montrose, M. H., Turner, J. R., & Watson, A. J. (2011). The epithelial barrier is maintained by in vivo tight junction expansion during pathologic intestinal epithelial shedding. *Gastroenterology*, 140, 1208–1218 e1201–1202.

[[PubMed](#)]

Marshman, E., Booth, C., & Potten, C. S. (2002). The intestinal epithelial stem cell. *BioEssays*, 24, 91–98.

[[PubMed](#)]

Martens, E. C., Neumann, M., & Desai, M. S. (2018). Interactions of commensal and pathogenic microorganisms with the intestinal mucosal barrier. *Nature Reviews Microbiology*, 16, 457–470. <https://doi.org/10.1038/s41579-018-0036-x> [PubMed]

McCarthy, N., Tie, G., Madha, S., He, R., Kraiczy, J., Maglieri, A., & Shivdasani, R. A. (2023). Smooth muscle contributes to the development and function of a layered intestinal stem cell niche. *Developmental Cell*, 58, 550–564.e6. <https://doi.org/10.1016/j.devcel.2023.02.012> [PubMed]

McCauley, H. A., Riedman, A. M., Enriquez, J. R., Zhang, X., Watanabe-Chailland, M., Sanchez, J. G., Kechele, D. O., Paul, E. F., Riley, K., Burger, C., Lang, R. A., & Wells, J. M. (2023). Enteroendocrine cells protect the stem cell niche by regulating crypt metabolism in response to nutrients. *Cellular and Molecular Gastroenterology and Hepatology*, 15(6), 1293–1310. [PubMed]

Miller, H., Zhang, J., Kuolee, R., Patel, G. B., & Chen, W. (2007). Intestinal M cells: The fallible sentinels? *World Journal of Gastroenterology*, 13, 1477–1486. [PubMed]

Moran, G. W., Leslie, F. C., Levison, S. E., Worthington, J., & McLaughlin, J. T. (2008). Enteroendocrine cells: Neglected players in gastrointestinal disorders? *Therapeutic Advances in Gastroenterology*, 1(1), 51–60. [PubMed]

Mowat, A. M. (2003). Anatomical basis of tolerance and immunity to intestinal antigens. *Nature Reviews Immunology*, 3, 331–341. [PubMed]

Munoz, M., Eidenschenk, C., Ota, N., Wong, K., Lohmann, U., Kuhl, A. A., Wang, X., Manzanillo, P., Li, Y., Rutz, S., et al. (2015). Interleukin-22 induces interleukin-18 expression from epithelial cells during intestinal infection. *Immunity*, 42, 321–331. [PubMed]

Nguyen, A. T., Mandard, S., Dray, C., Deckert, V., Valet, P., Besnard, P., Drucker, D. J., Lagrost, L., & Grober, J. (2014). Lipopolysaccharides-mediated increase in glucose-stimulated insulin secretion: Involvement of the GLP-1 pathway. *Diabetes*, 63(2), 471–482. [PubMed]

Noah, T. K., Donahue, B., & Shroyer, N. F. (2011). Intestinal development and differentiation. *Experimental Cell Research*, 317, 2702–2710. [PubMed]

Nyström, E. E. L., Martinez-Abad, B., Arike, L., Birchenough, G. M. H., Nonnecke, E. B., Castillo, P. A., Svensson, F., Bevins, C. L., Hansson, G. C., & Johansson, M. E. V. (2021). An intercrypt subpopulation of goblet cells is essential for colonic mucus barrier function. *Science*, 372(6539), eabb1590. [PubMed]

Oboki, K., Ohno, T., Kajiwar, N., Saito, H., & Nakae, S. (2010). IL-33 and IL-33 receptors in host

defense and diseases. *Allergology International*, 59, 143–160.

[[PubMed](#)]

Ogura, Y., Bonen, D. K., Inohara, N., Nicolae, D. L., Chen, F. F., Ramos, R., Britton, H., Moran, T., Karaliuskas, R., Duerr, R. H., et al. (2001). A frameshift mutation in NOD2 associated with susceptibility to Crohn's disease. *Nature*, 411, 603–606.

[<https://doi.org/10.1038/35079114>][[PubMed](#)]

Ogura, Y., Lala, S., Xin, W., Smith, E., Dowds, T. A., Chen, F. F., Zimmermann, E., Tretiakova, M., Cho, J. H., Hart, J., et al. (2003). Expression of NOD2 in Paneth cells: A possible link to Crohn's ileitis. *Gut*, 52, 1591–1597.

[[PubMed](#)]

Okumura, R., & Takeda, K. (2017). Roles of intestinal epithelial cells in the maintenance of gut homeostasis. *Experimental & Molecular Medicine*, 49, e338.

Ouellette, A. J. (2010). Paneth cells and innate mucosal immunity. *Current Opinion in Gastroenterology*, 26, 547–553.

[[PubMed](#)]

Palrasu, M., Kakar, K., Marudamuthu, A., Hamida, H., Thada, S., Zhong, Y., Staley, S., Busbee, P. B., Li, J., Garcia-Buitrago, M., et al. (2025). AhR activation transcriptionally induces anti-microbial peptide alpha-defensin 1 leading to reversal of gut microbiota dysbiosis and colitis. *Gut Microbes*, 17, 2460538. <https://doi.org/10.1080/19490976.2025.2460538>

[[PubMed](#)]

Peterson, L. W., & Artis, D. (2014). Intestinal epithelial cells: Regulators of barrier function and immune homeostasis. *Nature Reviews Immunology*, 14, 141–153.

[[PubMed](#)]

Pickert, G., Neufert, C., Leppkes, M., Zheng, Y., Wittkopf, N., Warntjen, M., Lehr, H. A., Hirth, S., Weigmann, B., Wirtz, S., et al. (2009). STAT3 links IL-22 signaling in intestinal epithelial cells to mucosal wound healing. *The Journal of Experimental Medicine*, 206, 1465–1472.

[[PubMed](#)]

Porter, E. M., Bevins, C. L., Ghosh, D., & Ganz, T. (2002). The multifaceted Paneth cell. *Cellular and Molecular Life Sciences*, 59, 156–170.

[[PubMed](#)]

Price, A. E., Shamardani, K., Lugo, K. A., Deguine, J., Roberts, A. W., Lee, B. L., & Barton, G. M. (2018). A map of Toll-like receptor expression in the intestinal epithelium reveals distinct spatial, cell type-specific, and temporal patterns. *Immunity*, 49, 560–575.e6. <https://doi.org/10.1016/j.immuni.2018.07.016>

[[PubMed](#)]

Quiros, M., & Nusrat, A. (2019). Contribution of wound-associated cells and mediators in orchestrating gastrointestinal mucosal wound repair. *Annual Review of Physiology*, 81, 189–209.

<https://doi.org/10.1146/annurev-physiol-020518-114504>

[[PubMed](#)]

Raetz, M., Hwang, S. H., Wilhelm, C. L., Kirkland, D., Benson, A., Sturge, C. R., Mirpuri, J., Vaishnava, S., Hou, B., Defranco, A. L., et al. (2013). Parasite-induced TH1 cells and intestinal dysbiosis cooperate in IFN-gamma-dependent elimination of Paneth cells. *Nature Immunology*, 14, 136–142.

[\[PubMed\]](#)

Rakoff-Nahoum, S., Hao, L., & Medzhitov, R. (2006). Role of Toll-like receptors in spontaneous commensal-dependent colitis. *Immunity*, 25, 319–329. <https://doi.org/10.1016/j.immuni.2006.06.010>

[\[PubMed\]](#)

Ramanan, D., & Cadwell, K. (2016). Intrinsic defense mechanisms of the intestinal epithelium. *Cell Host & Microbe*, 19, 434–441.

Ramirez, C., & Gebert, A. (2003). Vimentin-positive cells in the epithelium of rabbit ileal villi represent cup cells but not M-cells. *The Journal of Histochemistry and Cytochemistry*, 51, 1533–1544.

[\[PubMed\]](#)

Rath, E., & Haller, D. (2022). Intestinal epithelial cell metabolism at the interface of microbial dysbiosis and tissue injury. *Mucosal Immunology*, 15, 595–604. <https://doi.org/10.1038/s41385-022-00514-x>

[\[PubMed\]](#)

Rath, E., Moschetta, A., & Haller, D. (2018). Mitochondrial function—Gatekeeper of intestinal epithelial cell homeostasis. *Nature Reviews Gastroenterology and Hepatology*, 15, 497–516.

<https://doi.org/10.1038/s41575-018-0021-x>

[\[PubMed\]](#)

Rath, T., Atreya, R., & Neurath, M. F. (2023). A spotlight on intestinal permeability and inflammatory bowel diseases. *Expert Review of Gastroenterology & Hepatology*, 17, 597–610.

Rauch, I., Deets, K. A., Ji, D. X., von Moltke, J., Tenthorey, J. L., Lee, A. Y., Philip, N. H., Ayres, J. S., Brodsky, I. E., Gronert, K., & Vance, R. E. (2017). NAIP-NLRC4 inflammasomes coordinate intestinal epithelial cell expulsion with eicosanoid and IL-18 release via activation of caspase-1 and -8. *Immunity*, 46, 649–659.

[\[PubMed\]](#)

Roediger, W. E. (1980). The colonic epithelium in ulcerative colitis: An energy-deficiency disease? *Lancet*, 2, 712–715. [https://doi.org/10.1016/s0140-6736\(80\)91934-0](https://doi.org/10.1016/s0140-6736(80)91934-0)

[\[PubMed\]](#)

Rojas-Tapias, D. F., Brown, E. M., Temple, E. R., Onyekaba, M. A., Mohamed, A. M. T., Duncan, K., Schirmer, M., Walker, R. L., Mayassi, T., Pierce, K. A., Ávila-Pacheco, J., Clish, C. B., Vlamakis, H., & Xavier, R. J. (2022). Inflammation-associated nitrate facilitates ectopic colonization of oral bacterium *Veillonella parvula* in the intestine. *Nature Microbiology*, 7, 1673–1685. <https://doi.org/10.1038/s41564-022-01224-7>

[\[PubMed\]](#)

Rostan, O., Arshad, M. I., Piquet-Pellorce, C., Robert-Gangneux, F., Gangneux, J. P., & Samson, M.

(2015). Crucial and diverse role of the interleukin-33/ST2 axis in infectious diseases. *Infection and Immunity*, 83, 1738–1748.

[\[PubMed\]](#)

Salzman, N. H. (2010). Paneth cell defensins and the regulation of the microbiome: Detente at mucosal surfaces. *Gut Microbes*, 1, 401–406.

[\[PubMed\]](#)

Sancho, E., Batlle, E., & Clevers, H. (2003). Live and let die in the intestinal epithelium. *Current Opinion in Cell Biology*, 15, 763–770.

[\[PubMed\]](#)

Sato, T., van Es, J. H., Snippert, H. J., Stange, D. E., Vries, R. G., van den Born, M., Barker, N., Shroyer, N. F., van de Wetering, M., & Clevers, H. (2011). Paneth cells constitute the niche for Lgr5 stem cells in intestinal crypts. *Nature*, 469, 415–418.

[\[https://doi.org/10.1038/nature09637\]](https://doi.org/10.1038/nature09637) [\[PubMed\]](#)

Seethaler, B., Basrai, M., Neyrinck, A. M., Rodriguez, J., & Bindels, L. B. (2021). Biomarkers for assessment of intestinal permeability in clinical practice. *American Journal of Physiology-Gastrointestinal and Liver Physiology*, 320, G189–G203.

Sellin, M. E., Maslowski, K. M., Maloy, K. J., & Hardt, W. D. (2015). Inflammasomes of the intestinal epithelium. *Trends in Immunology*, 36, 442–450.

[\[PubMed\]](#)

Shao, L., Kamalu, O., & Mayer, L. (2005). Non-classical MHC class I molecules on intestinal epithelial cells: Mediators of mucosal crosstalk. *Immunological Reviews*, 206, 160–176.

[\[PubMed\]](#)

Snoeck, V., Goddeeris, B., & Cox, E. (2005). The role of enterocytes in the intestinal barrier function and antigen uptake. *Microbes and Infection*, 7, 997–1004.

[\[PubMed\]](#)

Songhet, P., Barthel, M., Stecher, B., Muller, A. J., Kremer, M., Hansson, G. C., & Hardt, W. D. (2011). Stromal IFN-gammaR-signaling modulates goblet cell function during Salmonella Typhimurium infection. *PLoS One*, 6, e22459.

[\[PubMed\]](#)

Sorbara, M. T., & Philpott, D. J. (2011). Peptidoglycan: A critical activator of the mammalian immune system during infection and homeostasis. *Immunological Reviews*, 243, 40–60.

[\[PubMed\]](#)

Sternini, C., & Rozengurt, E. (2025). Bitter taste receptors as sensors of gut luminal contents. *Nature Reviews Gastroenterology & Hepatology*, 22, 39–53. <https://doi.org/10.1038/s41575-024-01005-z>

Stockinger, B., Shah, K., & Wincent, E. (2021). AHR in the intestinal microenvironment: Safeguarding barrier function. *Nature Reviews Gastroenterology & Hepatology*. <https://doi.org/10.1038/s41575-021-00430-8>

Strowig, T., Henao-Mejia, J., Elinav, E., & Flavell, R. (2012). Inflammasomes in health and disease. *Nature*, 481, 278–286.

[<https://doi.org/10.1038/nature10759>][[PubMed](#)]

Tasegian, A., Dikovskaya, D., Scott, M. M., Chawla, A. S., Pemberton, R., Helps, T., Meus, T., McLean, M. H., & Swamy, M. (2024). LRRK2 is not required for lysozyme expression in Paneth cells. *Nature Immunology*, 25, 2037–2039. <https://doi.org/10.1038/s41590-024-01972-0>

[[PubMed](#)]

Thiemann, S., Smit, N., Roy, U., Lesker, T. R., Galvez, E. J. C., Helmecke, J., Basic, M., Bleich, A., Goodman, A. L., Kalinke, U., et al. (2017). Enhancement of IFN γ production by distinct commensals ameliorates salmonella-induced disease. *Cell Host & Microbe*, 21(682–694), e685.

Travassos, L. H., Carneiro, L. A., Ramjeet, M., Hussey, S., Kim, Y. G., Magalhaes, J. G., Yuan, L., Soares, F., Chea, E., Le Bourhis, L., et al. (2010). Nod1 and Nod2 direct autophagy by recruiting ATG16L1 to the plasma membrane at the site of bacterial entry. *Nature Immunology*, 11, 55–62.

[[PubMed](#)]

Urbauer, E., Aguanno, D., Mindermann, N., Omer, H., Metwaly, A., Krammel, T., Faro, T., Remke, M., Reitmeier, S., Bärthel, S., Kersting, J., Huang, Z., Xian, F., Schmidt, M., Saur, D., Huber, S., Stecher, B., List, M., Gómez-Varela, D., Steiger, K., Allez, M., Rath, E., & Haller, D. (2024). Mitochondrial perturbation in the intestine causes microbiota-dependent injury and gene signatures discriminative of inflammatory disease. *Cell Host and Microbe*, 32, 1347–1364.e1310. <https://doi.org/10.1016/j.chom.2024.06.013>

[[PubMed](#)]

van der Flier, L. G., & Clevers, H. (2009). Stem cells, self-renewal, and differentiation in the intestinal epithelium. *Annual Review of Physiology*, 71, 241–260.

[[PubMed](#)]

Van der Sluis, M., De Koning, B. A., De Bruijn, A. C., Velcich, A., Meijerink, J. P., Van Goudoever, J. B., Buller, H. A., Dekker, J., Van Seuningen, I., Renes, I. B., & Einerhand, A. W. (2006). Muc2-deficient mice spontaneously develop colitis, indicating that MUC2 is critical for colonic protection. *Gastroenterology*, 131, 117–129.

[[PubMed](#)]

Vanuytsel, T., Tack, J., & Farre, R. (2021). The role of intestinal permeability in gastrointestinal disorders and current methods of evaluation. *Frontiers in Nutrition*, 8, 717925.

[[PubMed](#)]

Velcich, A., Yang, W., Heyer, J., Fragale, A., Nicholas, C., Viani, S., Kucherlapati, R., Lipkin, M., Yang, K., & Augenlicht, L. (2002). Colorectal cancer in mice genetically deficient in the mucin Muc2. *Science*, 295, 1726–1729.

[[PubMed](#)]

Vitale, S., Picascia, S., & Gianfrani, C. (2016). The cross-talk between enterocytes and intraepithelial lymphocytes. *Molecular and Cellular Pediatrics*, 3, 20.

[[PubMed](#)]

von Moltke, J., Ji, M., Liang, H. E., & Locksley, R. M. (2016). Tuft-cell-derived IL-25 regulates an intestinal ILC2-epithelial response circuit. *Nature*, 529, 221–225.
[<https://doi.org/10.1038/nature16161>]

Wallaey, C., Garcia-Gonzalez, N., Timmermans, S., Vandewalle, J., Vanderhaeghen, T., De Beul, S., Dufoor, H., Eggermont, M., Moens, E., Bosteels, V., et al. (2024). Paneth cell TNF signaling induces gut bacterial translocation and sepsis. *Cell Host & Microbe*, 32, 1725–1743.e7. <https://doi.org/10.1016/j.chom.2024.08.007>

Wang, L., Llorente, C., Hartmann, P., Yang, A. M., & Schnabl, B. (2015). Methods to determine intestinal permeability and bacterial translocation during liver disease. *Journal of Immunological Methods*, 421, 44–53.
[[PubMed](#)]

Wehkamp, J., & Stange, E. F. (2006). Paneth cells and the innate immune response. *Current Opinion in Gastroenterology*, 22, 644–650.
[[PubMed](#)]

Wehkamp, J., & Stange, E. F. (2010). Paneth's disease. *Journal of Crohn's & Colitis*, 4, 523–531.

Wehkamp, J., Wang, G., Kubler, I., Nuding, S., Gregorieff, A., Schnabel, A., Kays, R. J., Fellermann, K., Burk, O., Schwab, M., et al. (2007). The Paneth cell alpha-defensin deficiency of ileal Crohn's disease is linked to Wnt/Tcf-4. *Journal of Immunology*, 179, 3109–3118.

Wilson, C. L., Schmidt, A. P., Pirila, E., Valore, E. V., Ferri, N., Sorsa, T., Ganz, T., & Parks, W. C. (2009). Differential processing of {alpha}- and {beta}-defensin precursors by matrix metalloproteinase-7 (MMP-7). *The Journal of Biological Chemistry*, 284, 8301–8311.
[[PubMed](#)]

Wittkopf, N., Neurath, M. F., & Becker, C. (2014). Immune-epithelial crosstalk at the intestinal surface. *Journal of Gastroenterology*, 49, 375–387.
[[PubMed](#)]

Wittkopf, N., Pickert, G., Billmeier, U., Mahapatro, M., Wirtz, S., Martini, E., Leppkes, M., Neurath, M. F., & Becker, C. (2015). Activation of intestinal epithelial Stat3 orchestrates tissue defense during gastrointestinal infection. *PLoS One*, 10, e0118401.
[[PubMed](#)]

Worthington, J. J., Reimann, F., & Gribble, F. M. (2018). Enteroendocrine cells-sensory sentinels of the intestinal environment and orchestrators of mucosal immunity. *Mucosal Immunology*, 11(1), 3–20.
[[PubMed](#)]

Yusta, B., Baggio, L. L., Koehler, J., Holland, D., Cao, X., Pinnell, L. J., Johnson-Henry, K. C., Yeung, W., Surette, M. G., Bang, K. W., Sherman, P. M., & Drucker, D. J. (2015). GLP-1R agonists modulate enteric immune responses through the intestinal intraepithelial lymphocyte GLP-1R. *Diabetes*, 64(7), 2537–2549.
[[PubMed](#)]

Zhang, Q., Pan, Y., Yan, R., Zeng, B., Wang, H., Zhang, X., Li, W., Wei, H., & Liu, Z. (2015). Commensal bacteria direct selective cargo sorting to promote symbiosis. *Nature Immunology*, 16, 918–926. <https://doi.org/10.1038/ni.3233>
[PubMed]

Zhang, C., Han, L., Dong, K., Zhang, Q., & Liu, Z. (2024). Reply to: LRRK2 is not required for lysozyme expression in Paneth cells. *Nature Immunology*, 25, 2040–2042. <https://doi.org/10.1038/s41590-024-01968-w>
[PubMed]

Zheng, Y., Valdez, P. A., Danilenko, D. M., Hu, Y., Sa, S. M., Gong, Q., Abbas, A. R., Modrusan, Z., Ghilardi, N., de Sauvage, F. J., & Ouyang, W. (2008). Interleukin-22 mediates early host defense against attaching and effacing bacterial pathogens. *Nature Medicine*, 14, 282–289.
[PubMed]

Ziv, E., & Bendayan, M. (2000). Intestinal absorption of peptides through the enterocytes. *Microscopy Research and Technique*, 49, 346–352.
[PubMed]

8. The Interplay of the Microbiome with the Innate Immune System

Till Strowig¹  and Andreas Diefenbach^{2, 3}

- (1) Department of Microbial Immune Regulation, Helmholtz Centre for Infection Research, Braunschweig, Germany
- (2) Institute of Microbiology, Infectious Diseases and Immunology, Charité - Universitätsmedizin Berlin, Berlin, Germany
- (3) Cluster of Excellence ImmunoPreCept, Charité - Universitätsmedizin Berlin, Berlin, Germany

 **Till Strowig**

Email: till.strowig@helmholtz-hzi.de

Abstract

The innate immune system not only functions as the initial line of defense against pathogenic microorganisms but also plays a crucial role in maintaining a balanced interaction with the intestinal microbiota. Various subsets of innate immune cells, including macrophages, dendritic cells, granulocytes, mast cells, and innate lymphoid cells, are distributed throughout the intestinal tissue and organized within tissue-specific lymphoid structures. These cells continuously monitor the intestinal environment for live microbes, preventing their infiltration and strengthening the integrity of the intestinal barrier. Notably, metabolites produced by the commensal microbiota, such as short-chain fatty acids and tryptophan metabolites, have been recognized for their role in sustaining tolerogenic conditions within the intestine, which includes promoting regulatory T cells and suppressing

pro-inflammatory signaling pathways. Conversely, improper recognition and handling of commensal microbes by the innate immune system, along with excessive immune activation following pathogen detection, have been linked to the development of inflammatory conditions, such as inflammatory bowel diseases. Therefore, a comprehensive understanding of the complex interactions between the microbiota and the innate immune system could facilitate the development of innovative therapeutic strategies to enhance human health and prevent auto-inflammatory diseases.

The intestine performs important physiological functions for the organism, such as nutrient resorption, but is also used by many pathogens as an entry site for colonization of the host. To limit local infection and prevent systemic pathogen spread, the innate immune system serves as the first line of defense in the intestinal tract. Yet, in addition to infrequently invading pathogens, the intestinal lumen continuously harbors diverse and dense communities of microorganisms. As a consequence, the tissue-resident immune system is constantly exposed to vast numbers of diverse foreign molecules derived from infectious agents or from harmless sources, such as food or commensal bacteria. Hence, the immune system must avoid mounting strong responses against a broad range of harmless bacteria while inducing an effective protective response against invading pathogens (Thaiss et al. *Nature* 535:65–74, [2016b](#)). This constantly requires the cells of the innate immune system to decide whether to favor tolerance towards foreign molecules and promote intestinal homeostasis or to trigger potent, but potentially harmful and destructive, immune effector mechanisms. In this chapter, the members of the innate immune system in the intestinal tract will be introduced, and their complex interplay with commensal microbes and pathogens will be explored. The specific focus is on the decision-making process between immune homeostasis and inflammation, which is important for preventing aberrant responses to the indigenous intestinal microbiota.

Keywords Microbiome – Innate immunity – Immune recognition – Myeloid cells – Innate lymphoid cells

8.1 Recognition of Microbiota and Pathogens by Innate Immune Receptors

To serve as the first line of defense in the intestine, the innate immune system needs to recognize threats to the host, i.e., the presence of pathogenic microorganisms or other disturbances of homeostatic conditions. In general, this is achieved by direct recognition of conserved structures, so-called microbe-associated molecular patterns (MAMPs), of viruses, bacteria, and fungi via pattern recognition receptors (PRRs) expressed by immune and non-immune cells; or indirectly by recognition of imbalances in the normal function of cells and tissues as a consequence of infection or tissue damage (Magalhaes et al., [2007](#); Palm & Medzhitov, [2009](#)). Germline-encoded PRRs include membrane-bound proteins from the Toll-like receptor (TLR) family and C-type lectin receptors (CLRs), as well as cytoplasmic proteins from the families of RIG-I-like receptors (RLRs), NOD-like receptors (NLRs), AIM2-like receptors (ALRs), and OAS-like receptors (OLRs) (Patten & Collett, [2013](#); Parlato & Yeretssian, [2014](#); Brubaker et al., [2015](#); Thaïss et al., [2016b](#)). These receptors recognize a wide range of molecular structures, including lipopolysaccharides (LPS), lipoproteins, small peptides, and specific types of nucleic acids, which are typically not present in the host. Subsequently, activation of PRRs by distinct cognate ligands leads to the activation of pro-inflammatory pathways through various transcriptional or post-transcriptional regulatory mechanisms, for instance, the induction of pro-inflammatory gene expression, such as interleukin (IL)-6, after sensing of LPS by TLR4. Other immune sensors with important functions in the intestine are inflammasomes, which are multi-protein complexes that recruit and activate caspase-1 (Fig. [8.1](#)) (Strowig et al., [2012](#); Rathinam & Fitzgerald, [2016](#)). They are formed upon detection of diverse activating ligands by multiple sensors from the NLR and ALR families, e.g., NLRP3 or AIM2, respectively,

resulting in activation of caspase-1, which, in turn, cleaves pro-inflammatory cytokines and additional effector pathways, e.g., a gasdermin-dependent form of cell death (Zhu et al., [2024](#)). Strikingly, inflammasomes are an example of shared effector systems induced by many types of foreign- or host-derived molecules, e.g., cytosolic viral dsDNA during viral infections or high extracellular concentrations of potassium ions as a consequence of cell death, being recognized by various receptors. Of note, impairments in inflammasome function are linked to both increased susceptibility to enteric pathogens as well as impaired cross-talk with the microbiota (Elinav et al., [2011](#); Robertson & Girardin, [2013](#); Stewart & Cookson, [2016](#)). Specifically, caspase-1-deficient mice are more susceptible to infection with *Salmonella*, but at the same time, these mice are also characterized by an altered microbiota composition in comparison to wild-type (WT) mice, highlighting that specific immune receptors have important functions in both the hosts' crosstalk with the microbiota as well as with pathogens. Because inflammasome activation is important in various diseases, academic and commercial researchers are currently developing drugs to modulate inflammasome activity, especially the Nlrp3 inflammasome (Chauhan et al., [2020](#)).

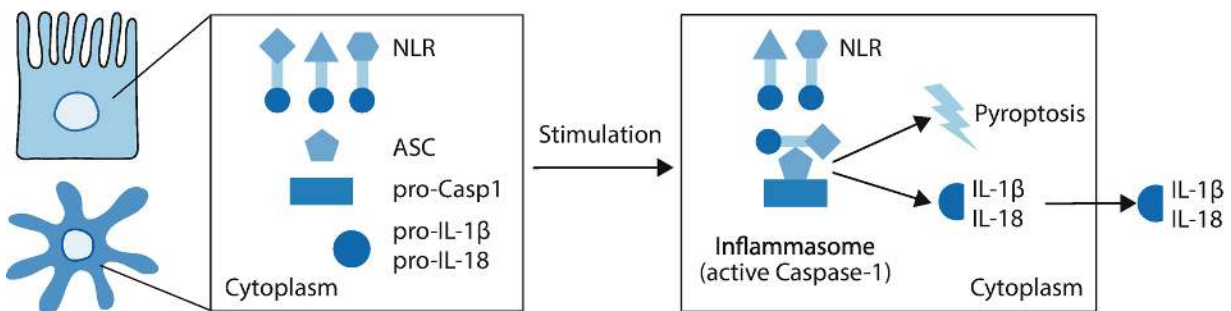


Fig. 8.1 Inflammasomes are modular platforms controlling the activity of Caspase-1. Microbial and host-derived molecules stimulate the formation of inflammasomes, resulting in the activation of the protease caspase-1, which subsequently cleaves pro-IL-1 β and pro-IL-18, which then modulate intestinal immunity, e.g., mucus secretion and helper T cell activation. Different inflammasome sensor proteins are expressed in diverse cell subsets, which determines to which activating signals each cell type can respond

Proper integration of the downstream signaling is important for the type and outcome of immune responses triggered by these receptors (Thaiss et al., [2016a](#)). Importantly, preventing overt inflammation involves recognizing tolerogenic signals and blocking pro-inflammatory pathways. In this regard, sensing of many microbial-derived metabolites produced by the commensal microbiota such as short-chain fatty acids (SCFAs), tryptophan metabolites, and secondary bile acids promotes intestinal homeostasis (Tan et al., [2014](#); Roager & Licht, [2018](#); Collins et al., [2023](#)). SCFAs are produced by bacterial fermentation and are water-soluble, enabling rapid diffusion even across the mucus layer that normally separates luminal bacteria from the host. They are recognized by different G-protein-coupled cell-surface receptors (GPCRs), such as GPR41, GPR43, and GPR109, which are expressed by immune and non-immune cells in the intestine and throughout the organism (Tan et al., [2014](#)). Signaling induced by binding of SCFAs to the cognate receptors, for instance, increases the threshold for the induction of pro-inflammatory signaling in macrophages and epithelial cells, thereby preventing overt immune activation (Park et al., [2007](#)). Additionally, SCFAs are directly and independently of any receptors used as an energy source for intestinal epithelial cells, strengthening the intestinal barrier (Ney et al., [2023](#)). Other important sensors that integrate environmental signals derived from the microbiota and diet into immune cells are the aryl hydrocarbon receptor and farnesoid X receptor, recognizing predominantly tryptophan and bile acid metabolites, respectively (Kiss & Vonarbourg, [2012](#); Jia et al., [2018](#); Roager & Licht, [2018](#); Collins et al., [2023](#)). They generally promote tissue homeostasis and regeneration. Nevertheless, pro-inflammatory roles that depend on cell type and context are also acknowledged, especially concerning bile acid metabolites, which, upon accumulation, can exert direct cytotoxic effects (Roager & Licht, [2018](#); Collins et al., [2023](#)). Together, sensing of commensal bacteria by innate immune pathways as well as their metabolic activity strongly influence the efficient functioning of the

epithelial barrier and the differentiation and maturation of the mucosal immune system.

8.2 Interplay of Innate Immune Cells with the Microbiota

The largest proportion of the immune system, estimated up to 70% of total immune cells, resides in the intestine. These cells are located throughout the gastrointestinal tract in different compartments, either scattered in the epithelial cell layer (intraepithelial cells, IEL) and in the lamina propria (lamina propria leukocytes, LPL), or organized in tissue-specific lymphoid structures such as Peyer's patches (PPs), and solitary intestinal lymphoid tissues (such as isolated lymphoid follicles (ILFs) and cryptopatches (CP)) (Eberl & Lochner, [2009](#)). Together with Goblet cell-associated passages (Kulkarni & Newberry, [2025](#); McDole et al., [2012](#)), these structures facilitate interactions between the microbiota and immune system components. Together, these tissue-specific, organized lymphoid structures in the intestine form the gut-associated lymphoid tissues (GALT) and are responsible for initiating and regulating immune responses in the gut. GALT development during ontogeny and its adaptation during adulthood are profoundly regulated by the presence of the microbiota (Eberl & Lochner, [2009](#)). Specifically, germ-free mice are characterized by an underdeveloped GALT with reduced numbers of IELs and LPLs as well as limited development of PPs and ILFs (Controversy Box). Several bacteria, in particular segmented filamentous bacteria (SFB), have been studied intensely in mice for their ability to modulate GALT and specifically, the expansion of PPs and ILFs in the small intestine resulting in the recruitment of immune cells to the intestine (Cebra et al., [1998](#); Bemark et al., [2024](#)).

Additionally, commensal bacteria influence the proper development and maturation of immune cell populations in the intestine by modulating distinct phases of hematopoiesis, i.e., the differentiation of mature immune cells from pluripotent hematopoietic stem cells (HSCs) in the bone marrow (Gorjifard & Goldszmid, [2016](#)). In a step-wise

differentiation process, those HSCs give rise to common myeloid progenitor cells (CMPs) and common lymphoid progenitor cells (CLPs). While CMPs eventually give rise via distinct progenitor cells to different cells of the innate immune system, such as monocytes, macrophages, dendritic cells, granulocytes, and mast cells, CLPs give rise to cells of the lymphoid lineages, including innate cells such as natural killer (NK) cells and innate lymphoid cells (ILCs). Commensal bacteria modulate this process already at early steps of hematopoiesis, such as intestinal bacteria, which are required for myeloid cell development, i.e., fecal transplantation of a complex microbiota composition into germ-free mice restores defects in global myelopoiesis (Khosravi et al., [2014](#)). Moreover, the microbiota influences the abundance and function of innate immune cells on multiple additional levels, and the effects on specific cell types are discussed in the following.

8.2.1 Granulocytes

Granulocytes belong to the group of myeloid cells and form polymorphic-shaped nuclei, typically looped into three segments (Leiding, [2017](#)). They are also called polymorphonuclear leukocytes. They can be distinguished into distinct subsets (neutrophils, basophils, and eosinophils) and are commonly characterized by the presence of many cytoplasmic granules containing enzymes and potent antimicrobial agents. Upon contact with microbes, these granules are released and are essential for fighting pathogenic invaders, such as extracellular bacteria.

Neutrophils are the most abundant leukocyte population in human blood, from which they are recruited to inflamed tissues through the activity of chemokines secreted, e.g., by epithelial cells and macrophages (Leiding, [2017](#)). In the intestine, neutrophils combat microbes that cross from the lumen into the epithelium and the lamina propria. Moreover, neutrophils can recruit circulating monocytes from the blood by producing chemoattractants. Besides their ability to release pre-stored compounds with antimicrobial activity, neutrophils also release distinct cytokines, such as IL-17 and IL-10, as well as

matrix metalloproteases (MMPs), which cleave a number of chemokines important for the recruitment of additional effector cells, thereby regulating mucosal wound healing of epithelial cells. The microbiota has a crucial role in the development of neutrophils, locally and systemically. In contrast to conventionally colonized animals, germ-free rats, which have never been exposed to microbes, display a neutropenic phenotype with generalized lower phagocytic function (Ohkubo et al., [1999](#)). Peptidoglycans derived from the cell wall of intestinal bacteria are sufficient to restore systemic neutrophil function via the nucleotide oligomerization domain 1 (Nod1) receptor, even in the absence of live bacteria (Clarke et al., [2010](#)), demonstrating that microbiota-derived immune stimulation is required for local and systemic immunity. Interestingly, recent work has shown that neutrophils can be found in tissues such as the intestine also in the absence of infections (Cerezo-Wallis et al., [2026](#); Ballesteros et al., [2020](#)), where they show substantial circadian fluctuations. In tissues, varying neutrophil states have been linked to non-canonical functions of this cell type, including vascular repair and neuro-immune crosstalk (Ballesteros et al., [2020](#)).

Eosinophils develop from eosinophil progenitor cells, and in particular, IL-3, IL-5, and granulocyte-macrophage colony-stimulating factor (GM-CSF) promote eosinophil development in the bone marrow (Mori et al., [2009](#)). After differentiation, eosinophils circulate in the bloodstream and enter mucosal tissues (Weller & Spencer, [2017](#)). Eotaxin-1, released by macrophages in mucosal tissues, strongly influences eosinophil trafficking to the intestinal lamina propria, but it is not known to what degree this process is regulated by the microbiota or by differences in microbiota composition. Tissue homing via eotaxin-1 is highly regulated by the ligation with C-C chemokine-receptor (CCR) 3 (Humbles et al., [2002](#)). Tissue-residing eosinophils only survive in the presence of survival-promoting cytokine signals. In addition to its function in eosinophil development, IL-5 also promotes activation and survival and is induced in the intestine upon colonization with some commensal protozoa or during helminth infection (Gieseck 3rd et al.,

[2018](#)). Under homeostatic conditions, eosinophils have been shown to be involved in the development and maintenance of immunoglobulin (Ig)A-producing cells, which are required for neutralizing intestinal bacteria (Chu et al., [2014](#)). Moreover, eosinophils can release their cytotoxic granule proteins to target bacteria. However, eosinophils have also been demonstrated to play a critical role during pathogenesis, even promoting inflammatory bowel diseases (IBD). Specifically, increased numbers of tissue-resident eosinophils have been demonstrated in patients with ulcerative colitis (Raab et al., [1998](#); Saitoh et al., [1999](#); Gurtner et al., [2023](#)) and eosinophil depletion protects mice from spontaneous ileitis (Masterson et al., [2011](#)). Currently, a monoclonal antibody targeting eotaxin-1, Bertilimumab, is under investigation for the treatment of IBD ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT01671956) identifier: NCT01671956). In contrast to this pathogenic function, microbiota-dependent stimulation of eosinophils by IL-25 has been shown to reduce mortality during *Clostridium difficile* infection (Buonomo et al., [2016](#)).

Basophils are the least common granulocytes in the circulating blood, representing <1% of white blood cells, making it difficult to study their roles, especially during homeostatic conditions. Together with mast cells, basophils are innate effector cells of IgE-dependent allergic inflammation, as both cell types express the high-affinity receptor FcεR1. Basophils can be activated by IL-3 released from CD4⁺ T cells or by binding to IgE. Upon activation, basophils can release histamine, proteases, diverse cytokines, heparin, and other proteoglycans. Release of large quantities of IL-4 and IL-6 by basophils initiates and supports allergic T helper type 2 (Th2) responses (Gomez et al., [2014](#)). Commensal bacteria in the intestine regulate basophil development; therefore, influencing circulating basophil populations. Specifically, it has been shown that disruption of the microbiota upon antibiotic use is associated with elevated IgE levels and augmented basophil population by influencing bone marrow-resident basophil precursors and, in turn, worsening of allergic inflammation (Hill et al., [2012](#); Russell et al., [2013](#)). Moreover, higher amounts of basophils have been detected in the inflamed intestine of IBD patients and have been

hypothesized to contribute to the deterioration of the disease (Chapuy et al., [2014](#)).

8.2.2 Mast Cells

Mast cells are granulated tissue-resident cells that act as first responders to microbes colonizing the gut (Wouters et al., [2016](#)). Mast cell progenitors circulate in the blood and migrate into tissues, such as the intestine, via the CXC chemokine receptor (CXCR) 2 (Kunii et al., [2011](#)). Depending on the microenvironment, those progenitor cells further differentiate within the tissue. In the human gastrointestinal tract, 2–3% of all cells in the lamina propria represent mast cells, as well as 1% of all cells in the submucosa (Bischoff, [2009](#)). Diverse inflammatory mediators are stored in mast cell granules, such as heparin, histamine, and proteases, which can be released via the IgE-mediated pathway, driving allergic reactions, and also in response to other substances, including cytokines and chemokines. Mast cells contribute to initiating and maintaining the inflammatory circuitry in the gut (St John & Abraham, [2013](#)). Intestinal commensal bacterial signals are important for mast cell migration from the blood to the intestine. Specifically, germ-free mice exhibit reduced relative mast cell abundance in the small intestine compared to conventionally housed mice, but elevated mast cell numbers in the blood. This is linked to lower levels of CXCR2 ligands, which control the homing of mast cells into the gut within germ-free mice (Kunii et al., [2011](#)).

8.2.3 Mononuclear Phagocytes

Mononuclear phagocytic cells (MNP) serve as a vital immunoregulatory switch in the intestine, controlling the induction of immune responses and the maintenance of tolerogenic mechanisms (Joeris et al., [2017](#)). MNPs in the intestine comprise both dendritic cells (DCs) and macrophages, which fulfill both overlapping and distinct functions (Cerovic et al., [2014](#)). To fulfill their respective functions, macrophages and DCs express highly specialized machineries, including a diverse repertoire of PRR, to detect microbes and

subsequently release immunomodulatory cytokines with pro- or anti-inflammatory function. The specialization of these MNP subsets for specific tasks allows the innate immune system to fine-tune the immune response to the complex environment of the intestine and the specific requirements of the host.

8.2.3.1 Macrophages

Macrophages are found scattered directly within and underneath the epithelial layer, throughout the lamina propria, as well as in organized lymphoid follicles such as PPs (Fig. [8.2](#)). They are largely resident cells, i.e., they do not leave the intestine upon terminal differentiation. As part of their functions, macrophages in the intestine undergo continuous turnover and are replaced by bone-marrow-derived progenitor cells even in the steady state (Bain et al., [2014](#)). They specifically develop from Ly6C^{high} monocytes circulating in the blood, which enter the intestinal mucosa depending on CCR2. Lineage-tracing experiments have revealed that immigrating monocytes downregulate markers of monocytes, such as Ly6C, and in parallel acquire the expression of markers of intestinal macrophage, such as CXCR1, CD64, and F4/80. The continuous replenishment of macrophages in the lamina propria by Ly6C^{high} monocytes is influenced by commensal gut bacteria via the induction of ligands for CCR2 required for attracting monocytes to the intestine. Notably, this replacement from blood-derived progenitors is rather atypical as tissue-resident macrophages in most other tissues, e.g., the skin and liver, are replaced from tissue-resident progenitors that entered the respective tissue already during fetal development (Lavin et al., [2015](#)).

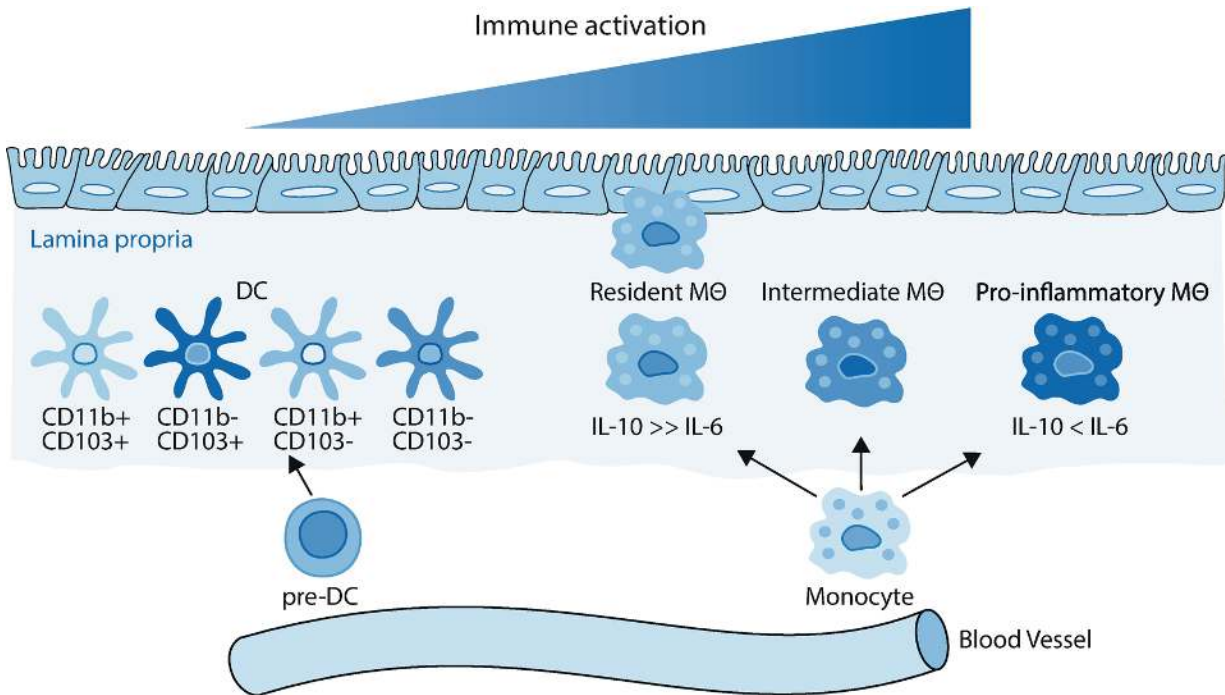


Fig. 8.2 Intestinal mononuclear phagocytic cells terminally differentiate in situ from circulating progenitor cells. Circulating monocytes are recruited via the CCL2-CCR2 axis to the intestinal tissue, where they differentiate into resident macrophages (MΦ). This recruitment is modulated by the microbiota by influencing local CCL2 production. The phenotype and functions of developing macrophages is strongly influenced by the local microenvironment and inflammatory signals. The development of pro-inflammatory macrophages during infections contributes to immune defense but also predisposes the host to intestinal inflammation. Circulating pre-cDCs develop into distinct intestinal dendritic cell (DC) subsets, which are distinguished based on the expression of CD11b and CD103. The abundance and functionality of DC subsets are determined by anatomical site and the local microenvironment, e.g., signals from the microbiota

In the intestinal tissue, mature macrophages serve as innate effector cells, i.e., they are known for their abilities to phagocytose microbes and apoptotic materials, but also promote intestinal homeostasis (Mowat et al., [2017](#)). Specifically, the release of substantial amounts of anti-inflammatory mediators, such as IL-10 and PGE2, is important for maintaining epithelial barrier integrity and for regulatory T cell (Treg) development upon microbial sensing in the steady state (Ueda et al., [2010](#)). IL-10 production by macrophages is modulated by the microbiota, as macrophages from germ-free mice produce less IL-10 after TLR stimulation (Rivollier et al., [2012](#)). Notably, the surprising absence of pro-inflammatory mediators commonly produced by macrophages after microbial stimulation has been attributed to various

factors, including the down-regulation of signaling molecules, such as myeloid differentiation primary response gene 88 (MyD88), which are necessary for PRR signal transduction (Smythies et al., [2010](#)).

Moreover, microbiota-derived SCFAs have been reported to modulate macrophage function in the lamina propria by inhibiting histone deacetylases, rendering macrophages hyporesponsive to intestinal bacteria and limiting the expression of pro-inflammatory cytokines (Chang et al., [2014](#)). Notably, sensing of SCFAs by macrophages in peripheral organs such as the brain influences their maturation and function, demonstrating the long reach of commensal-derived metabolites on innate immune cells (Erny et al., [2015](#)). Similar to DCs (see below), intestinal macrophages present antigens on MHC class II molecules to T cells, but because they perform this function only in intestinal tissue rather than in lymphoid tissues, they primarily interact with effector T cell subsets (Cerovic et al., [2014](#)). Specifically, the microbiota-induced expression of IL-10 favors the expansion of Foxp3-expressing Treg subsets. However, not only is the postnatal microbial colonization crucial for immune cell development, but the maternal microbiota also influences macrophage development in the offspring. In this context, it has recently been shown that maternal microbiota-derived metabolites impact the development of F4/80⁺ mononuclear cells already in utero (Gomez de Agüero et al., [2016](#)).

However, if the strength of pro-inflammatory signals increases or that of anti-inflammatory signals decreases, newly immigrating monocytes acquire potent pro-inflammatory properties, e.g., the ability to secrete IL-6 and TNF- α , rather than becoming anti-inflammatory. Importantly, recruitment of monocytes/macrophages during infections with intestinal bacteria and protozoa is essential for pathogen clearance and prevention of excessive pathology. Their predominantly pro-inflammatory functions are further corroborated by the ability to exacerbate intestinal inflammation after chemically-induced damage to the intestinal barrier during dextran sulfate sodium (DSS)-induced colitis (Platt et al., [2010](#)). Notably, even during inflammation, resident macrophages largely maintain their anti-inflammatory properties, e.g.,

the ability to produce IL-10, which is thought to be important for the resolution of inflammation and subsequent tissue repair (Weber et al., [2011](#)). The dynamic switch between predominantly anti- or pro-inflammatory differentiation has been hypothesized to happen not only during enteric infections, but potentially also in the absence of microbiota-derived SCFAs as a consequence of consumption of diets low in fiber, linking intestinal dysbiosis to impairments of tolerogenic macrophage functions and eventually the development of auto-inflammation in the intestine as observed during human IBD.

8.2.3.2 Dendritic Cells

Similar to macrophages, DCs are scattered throughout the intestinal epithelium, mucosa, and lymphoid structures (Fig. [8.2](#)). They share phagocytic capabilities with macrophages but also possess the exquisite ability to transport antigens to lymphoid organs (Cerovic et al., [2014](#)). There, DCs prime antigen-specific immune responses, including antibody-producing B cells as well as tolerogenic and pro-inflammatory T cell subsets, thereby serving as a relay between innate and adaptive immunity (Merad et al., [2013](#)) (Fig. [8.3](#)). The induction of opposing adaptive immune effector populations is achieved via highly coordinated mechanisms on the level of individual cells, e.g., the ability to integrate multiple signals from the environment, or on the level of the tissue, e.g., the functional specialization of DC subsets. Specifically, intestinal DC subsets are distinguished by the expression of cell adhesion molecules CD11b and CD103, as well as chemokine receptors such as CX3CR1 (Cerovic et al., [2014](#)). The expression patterns of these markers allow the identification of four distinct DC subsets that differ in phenotype and presumably also in functionality. CD103⁺CD11b⁺ DCs have been demonstrated to induce pathogen- and microbiota-specific IgA-producing plasma cells as well as Tregs, Th1, and Th17 cells (Cerovic et al., [2014](#)). They are the most frequent DC subset in the small intestine, but are less abundant in the colon. CD103⁺CD11b⁻ DCs efficiently cross-present soluble antigens to CD8⁺ T cells, but also express the enzyme RALDH that promotes the induction of Foxp3⁺

Tregs. CD103⁺CD11b⁻ DCs are found in the small intestine and colon as well as lymphoid organs such as PPs and mLNs. Together, CD103⁺ DC subsets are considered to induce Tregs by releasing significant amounts of TGFβ and retinoic acid, yet the depletion of neither of the CD103⁺ subsets alone is sufficient to break the induction of tolerance, which only occurs after ablation of all CD103⁺ DCs, suggesting that a redundancy between these subsets exists (Welty et al., [2013](#)). CD103⁻CD11b⁺CX3CR1^{int} DCs resemble, in many respects, intestinal macrophages and have been suggested to take up luminal antigens by extending dendrites into the lumen via transcellular pores in M cells within PPs (Lelouard et al., [2012](#)). Finally, the least abundant subset, identified as CD103⁻CD11b⁻ DCs, is thought to be enriched in ILFs and PPs, as they are absent in mice lacking organized lymphoid structures in the intestine. Due to their low abundance, their functional specialization is less well defined, but it has been suggested that they are capable of inducing Th17 cells (Cerovic et al., [2013](#)). Recently, single-cell transcriptomic approaches have allowed us to analyze at unprecedented depth transcriptional states of conventional DCs residing in the intestine. These studies defined distinct and new DC clusters, among them a population of SILT-associated DCs that express high levels of IL-22 binding protein, a soluble antagonist of the cytokine IL22 (Guendel et al., [2020](#)).

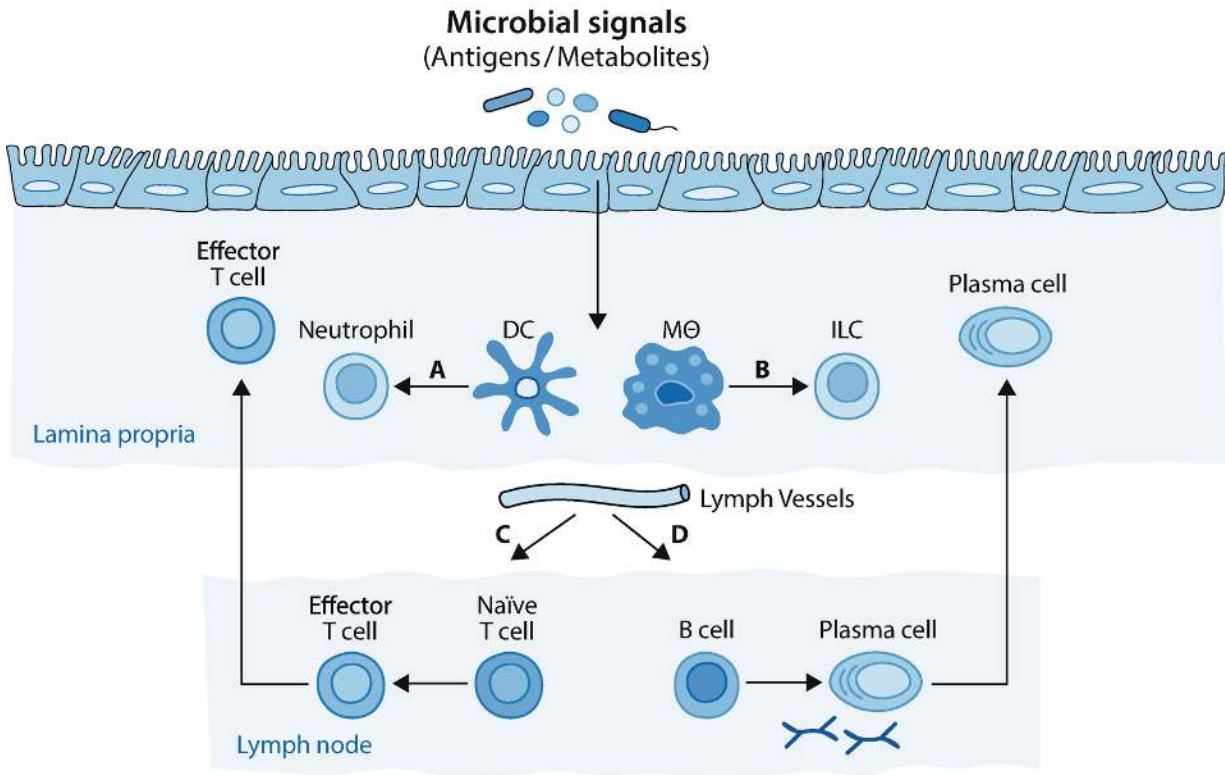


Fig. 8.3 Macrophages and dendritic cells orchestrate intestinal innate and adaptive immune responses. Exposure of intestinal dendritic cells (DC) and macrophages (MΦ) to microbiota-derived molecules results in the coordinated activation of distinct arms of the innate and adaptive immune system. **(a)** Sensing of pro-inflammatory stimuli may result in the recruitment of immune cells, e.g. neutrophils, that are normally largely absent from the intestine to sites of local inflammation. **(b)** Additionally, DCs and MΦ will also activate resident immune cells, e.g., ILCs, which then produce antimicrobial and immunomodulatory compounds. **(c and d)** Upon antigen uptake and immune stimulation, DCs also migrate via the lymph to gut-draining LNs, where they prime naïve adaptive immune cells and direct their differentiation. Depending on the co-stimulatory signals, CD4⁺ T cells may differentiate into regulatory or effector T cell subsets **(c)**. Gut-draining DCs predominantly induce the differentiation of IgA-producing plasma cells, which play an important role in the defense against intestinal pathogens and the spatial segregation of host and microbiota **(d)**

Since the epithelial barrier separating the host and lumen must be tight to prevent water and nutrient loss, the nature of antigen uptake from the lumen by dendritic cells (DCs) has been a major focus of research. In the small intestine, Peyer's patches (PPs) are key sites for both dietary and microbe-derived antigens. Their transfer from the lumen to the tissue occurs through specialized M cells that pinocytose luminal content and then release it to underlying DCs and macrophages on the basolateral side (Ohno, [2016](#)). Additionally, several other

mechanisms have been proposed to aid luminal antigen uptake by DCs in the lamina propria (LP) or lymphoid structures, such as paracellular leaks between epithelial cells, retrograde transport via goblet cells, and the formation of trans-epithelial dendrites (TED) (Niess et al., [2005](#); McDole et al., [2012](#)). The relative importance of each pathway in the uptake of soluble dietary or specific microbial antigens remains debated. Notably, retrograde transport through goblet cells appears to play a significant role in inducing postnatal tolerance to microbial antigens (Knoop et al., [2017](#)). Another source of microbial antigens—whether from pathogens or commensals—is the colonization of host cells in PPs and other intestinal lymphoid structures, which are then transferred intact to the mesenteric lymph nodes (mLNs) (Fung et al., [2016](#)). DC migration from the lamina propria to mLNs occurs via CCR7-dependent processes, where they interact with naïve T and B cells. Signals from the microbiota, either directly or indirectly, influence the type of adaptive immune responses, such as the induction of Th17 cells or Tregs. For example, the microbiota is essential for DCs in the mLN to acquire the ability to induce tolerogenic T cells (Cording et al., [2013](#)). Furthermore, the microbiota affects DC abundance and activity at multiple levels; for instance, germ-free mice have significantly fewer intestinal DCs in their mLNs (Walton et al., [2006](#)). Additionally, the microbiota modulates the egress of monocytes and plasmacytoid DCs from the bone marrow via induction of CCL2, facilitating CCR2-dependent migration and demonstrating systemic microbiota influence on myeloid cell trafficking (Swiecki et al., [2017](#)). Finally, microbiota-driven pre-activation of DCs is necessary for their functional licensing in the periphery (spleen, lymph nodes, etc.), enabling them to efficiently prime immune responses systemically (Ganal et al., [2012](#)). Systemic priming of conventional (c)DCs depends on microbiota-controlled tonic (constitutive) type I IFN expression by plasmacytoid DCs. Tonicly produced type I IFNs instruct a specific epigenomic and metabolic basal state that poises cDCs for future pathogen combat. However, such beneficial biological function comes with a trade-off. Instructed cDCs can prime T-cell responses against harmless peripheral

antigens, suggesting evolutionary trade-offs that come with successful adaptation of vertebrates to their microbial environment (Schaupp et al., [2020](#)).

The importance of microbiota-dendritic cell communication goes beyond the steady state to include emerging immune-based treatments such as checkpoint inhibitor therapies. Early studies from smaller groups showed that differences in microbiome composition could be linked to how well immunotherapy works, a finding later supported by meta-analyses and the discovery of common microbiota patterns in responders versus non-responders. However, the functional connection was less well understood. Notably, a recent study showed that a specific commensal strain, isolated from a human patient responding to checkpoint inhibitor therapy, could boost treatment outcomes by influencing dendritic cell migration (Lin et al., [2025](#)).

In summary, intestinal macrophages and DCs play an important part in the mutualism between host and microbiota. They induce and preserve tolerance to harmless microbes, while activating powerful antimicrobial responses when encountering potentially harmful pathogens. Signals derived from the microbiota, including metabolites such as SCFA and structural components such as LPS, are essential for fine-tuning the balance between these opposing types of immunity. Imbalances in the microbiota have been associated with modulation of macrophage and DC function, resulting in the development of host pathologies, including IBD. Nevertheless, targeted use of commensal bacteria may be exploited via DCs to boost immunotherapies in the future.

8.2.4 Innate Lymphoid Cells

Innate lymphoid cells (ILCs) are the lymphoid branch of the innate immune system. In analogy to the bifurcation in the T cell lineage (CD4⁺ helper T cells and CD8⁺ cytotoxic T cells), two major ILC lineages are recognized, cytotoxic ILCs (i.e., conventional NK cells, cNK cells) and helper-like ILCs (i.e., ILC1, ILC2, ILC3, including lymphoid tissue inducer (LTi) cells) (Fig. [8.4](#)). The three helper-like ILC subsets roughly

resemble the various CD4⁺ T helper cell effector states (i.e., Th1, Th2, Th17). While an ILCreg population was recognized that seems developmentally distinct from the other ILC subsets (Wang et al., [2017](#)), these cells did not express the Treg master transcription factor Foxp3. It remains an ongoing discussion whether a bona fide ILCreg population exists or if ILC with immunoregulatory functions are discrete states of the existing ILC groups, such as IL-10-producing ILC2 (Bando et al., [2020](#); Jegatheeswaran et al., [2021](#)).

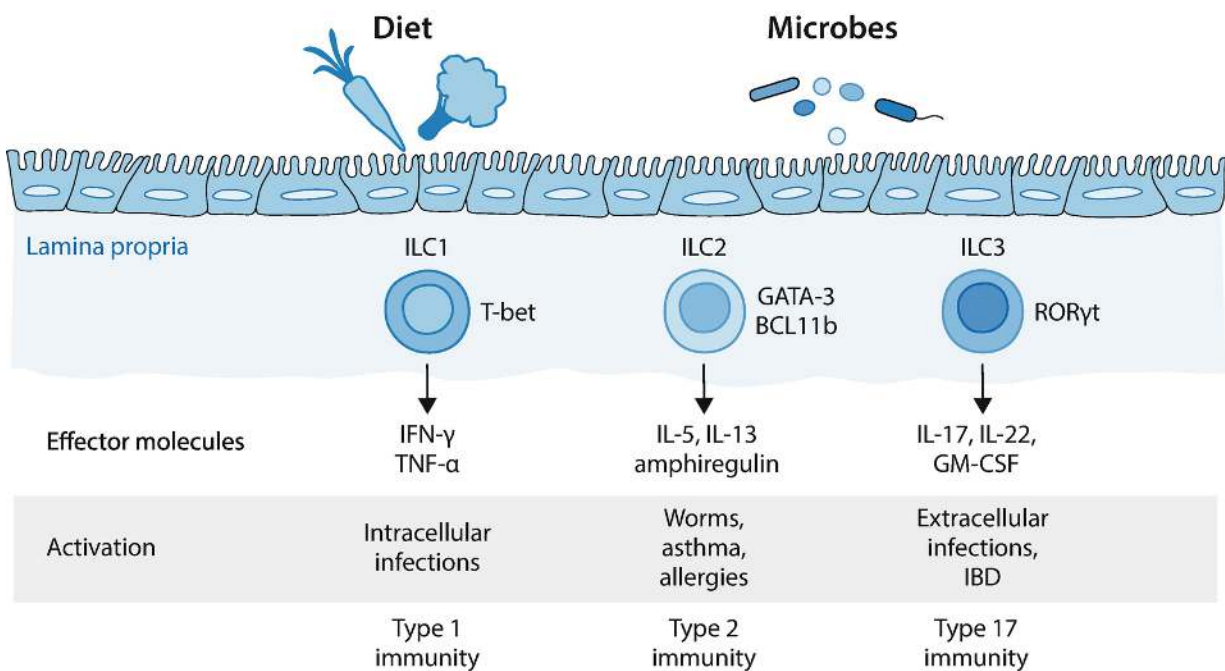


Fig. 8.4 Innate lymphoid cells can be phenotypically and functionally divided into three subsets. Dietary compounds and metabolites of the intestinal microbiota influence the recruitment, development, and functions of ILCs in the intestine. Subsets of ILCs are distinguished by the expression of specific transcription factors and effector molecules such as cytokines. These subsets are activated during different infections and contribute to properly shaping the immune system

8.2.4.1 Cytotoxic ILC: Conventional NK Cells

NK cells are cytotoxic lymphocytes belonging to the innate immune system. In contrast to the myeloid cell types described above, they derive from CLPs in the bone marrow. The early ILC progenitor (EILP) marks the branching off of the T and B cell lineage and has the potential to develop into all ILC lineages (NK cells and helper-like ILCs but not B

and T cells) (Yang et al., [2015](#)). Downstream of the EILP is a precursor referred to as CHILP (Klose et al., [2014](#); Constantinides et al., [2014](#)) (characterized by the expression of Id2 (CHILP-A) and Id2 plus PLZF (CHILP-B or ILCP), respectively). CHILP can differentiate into all helper-like ILC subsets but only very inefficiently into NK cells (or any adaptive lymphoid lineage). Thus, while NK cells and helper-like ILCs share a common progenitor (the EILP), cNK cells and helper-like ILCs form separate branches of the ILC lineage (Diefenbach et al., [2014](#); Das et al., [2026](#)).

NK cells are systemically present in the bone marrow, blood, and secondary lymphoid organs but also in non-lymphoid tissues such as the liver, lung, and intestine, where NK cells acquire organ-specific functions (Bjorkstrom et al., [2016](#)). Using transcriptional profiling of single NK cells allowed to appreciate the diversity of NK cells but also to identify common states that could form a framework to classify NK cells (Rebuffet et al., [2024](#); Tang et al., [2023](#)). In their mature state, NK cells have granules in their cytoplasm containing perforin, granzymes, and proteases, which allow NK cells to kill transformed or infected cells. Target cell recognition by NK cells is determined by various inhibitory (NKG2A in humans and mice, KIR molecules in humans, and Ly49 receptors in mice) and activating receptors (e.g., NKG2D, NKp30, NKp44, NKp46, DNAM1). Inhibitory receptors recognize either directly (Ly49 and KIR) or indirectly (NKG2A) MHC class I molecules (Raulet et al., [2001](#)). As all healthy cells express MHC class I, NK cell inhibition by MHC class I is an important mechanism to keep NK cells tolerant against normal self-cells. In the context of viral infection and tumor transformation MHC class I is often downregulated, allowing for NK cell activation (“missing self-recognition”) (Karre et al., [1986](#)). While the “missing self-recognition” mode for NK cell activation has been recognized for a long time, the activating mode has only been investigated more recently. Initial work identified ligands for the activating receptor NKG2D, MICA/B in humans (Bauer et al., [1999](#)) and the Rae1 family of ligands in humans and mice (Cerwenka et al., [2000](#); Diefenbach et al., [2000](#)). Importantly, these MHC class I-related NKG2D

ligands are not expressed on healthy cells, but are up-regulated after cellular insults such as tumor transformation, infection, DNA damage, and senescence (Diefenbach & Raulet, [2003](#)). Once cells acquire NKG2D ligand expression, they become targets for NK cells, and the NKG2D receptor/ligand system is a substantial barrier against tumor development (Guerra et al., [2008](#)). It is believed that there is a certain hierarchy for NK cell activation, with some receptors being very potent when triggered in isolation (such as CD16, a component of the Fc receptor) whereas others were not able to trigger resting NK cells in the absence of priming signals (see below) (Bryceson et al., [2006](#)). The activation of NK cells through activating receptors or through type I interferons (IFN), IL-12, and IL-18 leads to the production of other cytokines and chemokines such as tumor necrosis factor (TNF), IFN γ , and granulocyte-macrophage colony-stimulating factor (GM-CSF) important to act as immunoregulators.

It has recently become clear that there is considerable diversity between NK cells in the circulation and those located in tissues. The expression of CD69 as well as CD103 and CD49a is characteristic of NK cells residing in the tissue and distinguish them from circulating cNK cells (Bjorkstrom et al., [2016](#)). It remains to be seen if the various tissue-resident NK cell subsets are indeed tissue-resident NK cells or belong to separate ILC lineages.

Although NK cells are considered “naturally active,” there is a substantial cell extrinsic component for priming of NK cell function. Indeed, freshly isolated NK cells from SPF mice show very poor cytotoxic activity and do not produce cytokines. However, after injections of microbial compounds such as poly(I:C), LPS, and CpG, NK cells very effectively recognize target cells. This process is often referred to as “NK cell priming” and requires the presence of mononuclear phagocytes, in particular DCs (Lucas et al., [2007](#)). Mice lacking DCs cannot prime NK cell function after injection of PAMP (Ganal et al., [2012](#)). On a molecular level, various cytokines (such as IL-2, IL-12 or IL-15) produced by mononuclear phagocytes have been implicated in the priming process. More recently, it became clear that

NK cell priming by components of the myeloid system requires the presence of the microbiota. It has been demonstrated that germ-free and antibiotic-treated mice show reduced NK cell activity, due to the inability of mononuclear phagocytes to release cytokines required for NK cell priming (Luu et al., [2016](#)). On a mechanistic level, stimulation of mononuclear phagocytes resulted in normal initiation of the signal transduction cascade downstream of PRR. However, the activated transcription factors (i.e., the NF- κ B subunit p65 and IRF3) could not bind to the promoter regions of cytokine genes because of an inappropriately resolved chromatin barrier. These data indicate that signals controlled by the microbiota calibrate mononuclear phagocytes involving epigenetic processes that prepare these cells to respond with a powerful cytokine response upon pathogen encounter (Ganal et al., [2012](#)). It is unknown if this microbiota-controlled program is mediated by distinct microbes, distinct metabolites or by soluble factors produced by cells “sensing” commensal microbiota.

8.2.4.2 Helper-Like ILCs

Helper-like ILCs are a recently identified group of innate lymphocytes, which are deposited in tissues during ontogeny and then are maintained in tissues either by self-renewal or by differentiation from tissue-resident progenitors without appreciable renewal from circulating cells, as demonstrated by parabiosis experiments (Gasteiger et al., [2015](#)). This extreme sedentary lifestyle indicates close links of ILCs to organ function and organ homeostasis (Diefenbach et al., [2020](#)). In the intestine, direct or indirect stimulation through microbes induces distinct subsets of ILCs to release diverse sets of cytokines themselves, some of which directly act on IEC, leading, for example, to enhanced production of antimicrobial peptides and proteins, impacting the composition of the gut microbiota.

ILC1 express the T-box transcription factor T-bet but not Eomes (characteristic for NK cells), produce IFN γ and TNF, and are involved in the early immune response against intracellular pathogens (Daussy et al., [2014](#); Klose et al., [2014](#)). ILC2 express high levels of GATA-3 and

developmentally depend on the transcription factor BCL11b (Hoyler et al., [2012](#); Mjosberg et al., [2012](#); Walker et al., [2015](#)). They produce IL-5 and IL-13 and play important roles in type 2 immunity against worms. If inappropriately activated, ILC2s contribute to the pathogenesis of allergic diseases. Finally, ILC3 represent the “type 17 module” of ILCs. ILC3 are characterized by the expression of the transcription factor ROR γ t, and they are strictly required for the formation of secondary lymphoid organs such as lymph nodes, PPs, CPs, and ILFs. ILC3 produce IL-22, IL-17A, and IL-17F, and are required for immunity to certain types of bacterial infections (i.e., “attaching-and-effacing” intestinal pathogens) and viral infections (e.g., rotavirus, norovirus). The complex crosstalk between the microbiota and ILCs has been reviewed in detail (Sonnenberg & Artis, [2012](#); Britanova & Diefenbach, [2017](#)).

In contrast to the corresponding T cell populations, ILCs tonically produce cytokines at steady-state (i.e., in the absence of infections or inflammation). It is becoming increasingly clear that ILCs are deeply integrated into the regulation of tissue function. Changes in tonic cytokine expression in response to microbial or dietary signals are integrated by ILCs and adapt tissue function to environmental changes. For example, ILC3s regulate epithelial cell function in response to dietary changes (Gronke et al., [2019](#); Talbot et al., [2020](#); Sullivan et al., [2021](#)). The activity of ILCs is tuned by components of the microbiota. Germ-free mice show reduced ILC3 activity (Sanos et al., [2009](#); Satoh-Takayama et al., [2008](#)). ILC2 function is controlled by cytokines such as IL-25 produced by chemosensory, intestinal Tuft cells. Tuft cell function is driven by succinate contained in diets or released from intestinal protists (Nadsombati et al., [2018](#); Schneider et al., [2018](#)). Such a microbial metabolic sensing circuit may have evolved to facilitate mutualistic responses to luminal pathosymbionts.

8.3 Summary and Outlook

The cells of the innate immune system play a crucial role in maintaining a balanced interaction with the intestinal microbiota. During health,

different subsets of innate immune cells originating from the myeloid and lymphoid lineages help in sensing the normal microbiota and integrate multiple signals, such as MAMPs and metabolites, to promote tolerogenic conditions. Disruption of this balance, due to infections or impaired barrier function, can lead to excessive immune activation, which causes local inflammatory conditions like inflammatory bowel diseases and has also been linked to systemic diseases such as metabolic syndrome. Going forward, it will be very important to develop a detailed molecular understanding of the crosstalk between the innate immune system and microbiota, including identifying microbial metabolites and the corresponding host receptors and effectors, to enable new therapies that promote health and specifically prevent auto-inflammatory diseases. Moreover, the role of the imbalance between innate immunity and the microbiota in other complex diseases, such as neurodegeneration, remains to be explored. Understanding their potential involvement in these diseases, which carry high socio-economic costs, is likely to increase awareness of the importance of this evolutionarily ancient component of the immune system for human health.

Controversy *Limitations of laboratory mice—a bias introduced by the microbiota?*

Many of our recent insights into the immune system have come from analyzing laboratory mice. Yet, distinct mouse models of diseases have been criticized because they do not always reflect relevant aspects of the human immune system and/or show a high degree of variability between different laboratories. Potential explanations for this limitation have been recently identified. Laboratory mice are housed under increasingly hygienic conditions (so called SPF status), but these are highly variable between animal facilities (Rausch et al., [2016](#)). Additionally, recent data has shown that the cellular and genetic signatures of the immune system in laboratory mouse strains is more reminiscent of human newborns but not of adult humans (Beura et al., [2016](#); Rosshart et al., [2017](#)). For example, laboratory

mice lack effector-differentiated and mucosally distributed T cells and ILCs. However, such populations were found in feral mice or in pet shop mice, whose microbiota composition and exposure to pathogens was profoundly different. Interestingly co-housing of laboratory mice with pet shop mice or transfer of fecal material from wildling to SPF mice introduced wide-ranging changes so that immune cell signatures were now resembling adult humans, whereas gene expression signatures found in newborns were suppressed (Beura et al., [2016](#); Rosshart et al., [2017](#); Runge et al., [2025](#)). Such changes had wide-ranging functional consequences. Laboratory mice exposed to a “dirty” microbiome were more resistant to bacterial and viral infections and developed less inflammation-induced cancer. These data underscore that exposure to diverse microbial factors has a profound impact on the constitution of our immune system.

History

The discovery of dendritic cells

The ability of specific subsets of host cells to phagocytose microbes and to thereby kill them was first described by Ilya Ilyich Metchnikov. He specifically observed the ability of distinct cells in starfish larvae to engulf and degrade bacteria and hypothesized that this instinctive response was an important part of the immune system. In 1908, he received the Nobel Prize for his discoveries that were the foundation of what is known today as innate immune responses. Large advances were made in the following decades, describing the cellular and biochemical processes, including the discovery of phagosome acidification and the production of reactive oxygen species that explained the exquisite properties of macrophages to contribute to the innate immune response. The discovery of the link between innate and adaptive immunity, i.e., the ability of phagocytes to present microbial antigens to adaptive immune cells for the eventual formation of memory responses, was made by Ralph Steinman and colleagues. Before his groundbreaking studies, scientists were already aware of the need for accessory cells in the priming of adaptive

immune responses, but their exact identity was not known, since these cells were relatively scarce. Ralph Steinman then developed new methods to isolate and visualize these critical cells in the 1970s and coined the term dendritic cells for them (Steinman & Cohn, [1973](#)). These cells differed from classical macrophages in their ability to phagocytose microbes and protein antigens for subsequent presentation of peptide antigens on major histocompatibility complex class I and II molecules. Many researchers initially disbelieved this discovery due to their difficulties in isolating pure populations of dendritic cells, which in turn also contaminated macrophage preparations. However, advances in the methodology to isolate dendritic cells and study their functions in vivo have proven Steinman's initial hypothesis of the distinct functionality of this cell population. The underlying model of antigen presentation and priming of adaptive immunity involving a central role for dendritic cells in this process has been widely accepted since and culminated in the Nobel prize for the late Ralph Steinman in 2011. It is important to note that this model does not preclude a role of antigen presentation in macrophages, which occurs in both lymphoid organs and peripheral tissues such as the gastrointestinal tract, but to a much lower level and without the exquisite co-stimulatory abilities of dendritic cells.

Highlights

1. The innate immune system is central to recognizing and responding not only to pathogens but also to commensals in the intestine.
2. The innate immune system senses microbes via a variety of MAMPs, such as bacterial cell wall components and microbe-specific metabolites.
3. Resident mononuclear phagocytes, i.e., macrophages and dendritic cells, are essential for immunosurveillance in the tissue as well as for transmitting information to gut-draining

- lymphoid organs.
4. Specialized subsets of innate lymphoid cells are an immediate source of immunoregulatory mediators contributing to the balanced interplay between microbiota and host.
 5. Impaired or dysregulated innate immunity promotes the development of imbalanced intestinal ecosystems and dysbiosis-associated diseases.

Acknowledgement

We want to recognize Sophie Thiemann's contribution to the original version of this chapter and Daniela Somplatzki for support in editing this version.

References

Bain, C. C., Bravo-Blas, A., Scott, C. L., et al. (2014). Constant replenishment from circulating monocytes maintains the macrophage pool in the intestine of adult mice. *Nature Immunology*, 15, 929–937.

[\[PubMedCentral\]](#)

Ballesteros, I., Rubio-Ponce, A., Genua, M., et al. (2020). Co-option of neutrophil fates by tissue environments. *Cell*, 183, 1282–1297.

Bando, J. K., Gilfillan, S., Di Luccia, B., et al. (2020). ILC2s are the predominant source of intestinal ILC-derived IL-10. *The Journal of Experimental Medicine*, 217.

Bauer, S., Groh, V., Wu, J., et al. (1999). Activation of NK cells and T cells by NKG2D, a receptor for stress-inducible MICA. *Science*, 285, 727–729.

Bemark, M., Pitcher, M. J., Dionisi, C., et al. (2024). Gut-associated lymphoid tissue: A microbiota-driven hub of B cell immunity. *Trends in Immunology*, 45, 211–223.

[\[PubMedCentral\]](#)

Beura, L. K., Hamilton, S. E., Bi, K., et al. (2016). Normalizing the environment recapitulates adult human immune traits in laboratory mice. *Nature*, 532, 512–516.

[\[https://doi.org/10.1038/nature17655\]](https://doi.org/10.1038/nature17655)[\[PubMedCentral\]](#)

Bischoff, S. C. (2009). Physiological and pathophysiological functions of intestinal mast cells. *Seminars in Immunopathology*, 31, 185–205.

Bjorkstrom, N. K., Ljunggren, H. G., & Michaelsson, J. (2016). Emerging insights into natural killer cells in human peripheral tissues. *Nature Reviews. Immunology*, 16, 310–320.

Britanova, L., & Diefenbach, A. (2017). Interplay of innate lymphoid cells and the microbiota. *Immunological Reviews*, 279, 36–51.

Brubaker, S. W., Bonham, K. S., Zanoni, I., et al. (2015). Innate immune pattern recognition: a cell biological perspective. *Annual Review of Immunology*, 33, 257–290.
[[PubMedCentral](#)]

Bryceson, Y. T., March, M. E., Ljunggren, H. G., et al. (2006). Synergy among receptors on resting NK cells for the activation of natural cytotoxicity and cytokine secretion. *Blood*, 107, 159–166.

Buonomo, E. L., Cowardin, C. A., Wilson, M. G., et al. (2016). Microbiota-regulated IL-25 increases eosinophil number to provide protection during clostridium difficile infection. *Cell Reports*, 16, 432–443.
[[PubMedCentral](#)]

Cebra, J. J., Periwal, S. B., Lee, G., et al. (1998). Development and maintenance of the gut-associated lymphoid tissue (GALT): The roles of enteric bacteria and viruses. *Developmental Immunology*, 6, 13–18.
[[PubMedCentral](#)]

Cerezo-Wallis, D., Rubio-Ponce, A., Richter, M., et al. (2026). Architecture of the neutrophil compartment. *Nature*, 649, 1003–1012.
[<https://doi.org/10.1038/s41586-025-09807-0>]

Cerovic, V., Houston, S. A., Scott, C. L., et al. (2013). Intestinal CD103(–) dendritic cells migrate in lymph and prime effector T cells. *Mucosal Immunology*, 6, 104–113.

Cerovic, V., Bain, C. C., Mowat, A. M., et al. (2014). Intestinal macrophages and dendritic cells: What's the difference? *Trends in Immunology*, 35, 270–277.

Cerwenka, A., Bakker, A. B., McClanahan, T., et al. (2000). Retinoic acid early inducible genes define a ligand family for the activating NKG2D receptor in mice. *Immunity*, 12, 721–727.

Chang, P. V., Hao, L., Offermanns, S., et al. (2014). The microbial metabolite butyrate regulates intestinal macrophage function via histone deacetylase inhibition. *Proceedings of the National Academy of Sciences of the United States of America*, 111, 2247–2252.
[[PubMedCentral](#)]

Chapuy, L., Bsai, M., Mehta, H., et al. (2014). Basophils increase in Crohn disease and ulcerative colitis and favor mesenteric lymph node memory TH17/TH1 response. *The Journal of Allergy and Clinical Immunology*, 134, 978–981.

Chauhan, D., Vande Walle, L., & Lamkanfi, M. (2020). Therapeutic modulation of inflammasome pathways. *Immunological Reviews*, 297, 123–138.
[[PubMedCentral](#)]

Chu, V. T., Beller, A., Rausch, S., et al. (2014). Eosinophils promote generation and maintenance of immunoglobulin-A-expressing plasma cells and contribute to gut immune homeostasis. *Immunity*, 40, 582–593.

Clarke, T. B., Davis, K. M., Lysenko, E. S., et al. (2010). Recognition of peptidoglycan from the microbiota by Nod1 enhances systemic innate immunity. *Nature Medicine*, 16, 228–231.
[[PubMedCentral](#)]

Collins, S. L., Stine, J. G., Bisanz, J. E., et al. (2023). Bile acids and the gut microbiota: Metabolic interactions and impacts on disease. *Nature Reviews. Microbiology*, 21, 236–247.

Constantinides, M. G., McDonald, B. D., Verhoef, P. A., et al. (2014). A committed precursor to innate lymphoid cells. *Nature*, 508, 397–401.
[<https://doi.org/10.1038/nature13047>][[PubMedCentral](#)]

Cording, S., Fleissner, D., Heimesaat, M. M., et al. (2013). Commensal microbiota drive proliferation of conventional and Foxp3(+) regulatory CD4(+) T cells in mesenteric lymph nodes and Peyer's patches. *Eur J Microbiol Immunol (Bp)*, 3, 1–10.
[[PubMedCentral](#)]

Das, A., Ding, Y., Harly, C., et al. (2026). The development of innate lymphoid cells. *Nature Immunology*.

Daussy, C., Faure, F., Mayol, K., et al. (2014). T-bet and Eomes instruct the development of two distinct natural killer cell lineages in the liver and in the bone marrow. *The Journal of Experimental Medicine*, 211, 563–577.
[[PubMedCentral](#)]

Diefenbach, A., & Raulet, D. H. (2003). Innate immune recognition by stimulatory immunoreceptors. *Current Opinion in Immunology*, 15, 37–44.

Diefenbach, A., Jamieson, A. M., Liu, S. D., et al. (2000). Ligands for the murine NKG2D receptor: Expression by tumor cells and activation of NK cells and macrophages. *Nature Immunology*, 1, 119–126.

Diefenbach, A., Colonna, M., & Koyasu, S. (2014). Development, differentiation, and diversity of innate lymphoid cells. *Immunity*, 41, 354–365.
[[PubMedCentral](#)]

Diefenbach, A., Gnafakis, S., & Shomrat, O. (2020). Innate lymphoid cell-epithelial cell modules sustain intestinal homeostasis. *Immunity*, 52, 452–463.

Eberl, G., & Lochner, M. (2009). The development of intestinal lymphoid tissues at the interface of self and microbiota. *Mucosal Immunology*, 2, 478–485.

Elinav, E., Strowig, T., Kau, A. L., et al. (2011). NLRP6 inflammasome regulates colonic microbial ecology and risk for colitis. *Cell*, 145, 745–757.
[[PubMedCentral](#)]

Erny, D., Hrabec de Angelis, A. L., Jaitin, D., et al. (2015). Host microbiota constantly control

maturation and function of microglia in the CNS. *Nature Neuroscience*, 18, 965–977.

[\[PubMedCentral\]](#)

Fung, T. C., Bessman, N. J., Hepworth, M. R., et al. (2016). Lymphoid-tissue-resident commensal bacteria promote members of the IL-10 cytokine family to establish mutualism. *Immunity*, 44, 634–646.

[\[PubMedCentral\]](#)

Ganal, S. C., Sanos, S. L., Kallfass, C., et al. (2012). Priming of natural killer cells by nonmucosal mononuclear phagocytes requires instructive signals from commensal microbiota. *Immunity*, 37, 171–186.

Gasteiger, G., Fan, X., Dikiy, S., et al. (2015). Tissue residency of innate lymphoid cells in lymphoid and nonlymphoid organs. *Science*, 350, 981–985.

[\[PubMedCentral\]](#)

Gieseck, R. L., 3rd, Wilson, M. S., & Wynn, T. A. (2018). Type 2 immunity in tissue repair and fibrosis. *Nature Reviews. Immunology*, 18, 62–76.

Gomez de Agüero, M., Ganal-Vonarburg, S. C., Fuhrer, T., et al. (2016). The maternal microbiota drives early postnatal innate immune development. *Science*, 351, 1296–1302.

Gomez, M. R., Talke, Y., Hofmann, C., et al. (2014). Basophils control T-cell responses and limit disease activity in experimental murine colitis. *Mucosal Immunology*, 7, 188–199.

Gorjifard, S., & Goldszmid, R. S. (2016). Microbiota-myeloid cell crosstalk beyond the gut. *Journal of Leukocyte Biology*, 100, 865–879.

[\[PubMedCentral\]](#)

Gronke, K., Hernandez, P. P., Zimmermann, J., et al. (2019). Interleukin-22 protects intestinal stem cells against genotoxic stress. *Nature*, 566, 249–253.

[\[https://doi.org/10.1038/s41586-019-0899-7\]](https://doi.org/10.1038/s41586-019-0899-7) [\[PubMedCentral\]](#)

Guendel, F., Kofoed-Branzk, M., Gronke, K., et al. (2020). Group 3 innate lymphoid cells program a distinct subset of IL-22BP-producing dendritic cells demarcating solitary intestinal lymphoid tissues. *Immunity*, 53, 1015–1032.

Guerra, N., Tan, Y. X., Joncker, N. T., et al. (2008). NKG2D-deficient mice are defective in tumor surveillance in models of spontaneous malignancy. *Immunity*, 28, 571–580.

[\[PubMedCentral\]](#)

Gurtner, A., Borrelli, C., Gonzalez-Perez, I., et al. (2023). Active eosinophils regulate host defence and immune responses in colitis. *Nature*, 615, 151–157.

[\[https://doi.org/10.1038/s41586-022-05628-7\]](https://doi.org/10.1038/s41586-022-05628-7)

Hill, D. A., Siracusa, M. C., Abt, M. C., et al. (2012). Commensal bacteria-derived signals regulate basophil hematopoiesis and allergic inflammation. *Nature Medicine*, 18, 538–546.

[\[PubMedCentral\]](#)

Hoyler, T., Klose, C. S., Souabni, A., et al. (2012). The transcription factor GATA-3 controls cell fate

and maintenance of type 2 innate lymphoid cells. *Immunity*, 37, 634–648.

[[PubMedCentral](#)]

Humbles, A. A., Lu, B., Friend, D. S., et al. (2002). The murine CCR3 receptor regulates both the role of eosinophils and mast cells in allergen-induced airway inflammation and hyperresponsiveness. *Proceedings of the National Academy of Sciences of the United States of America*, 99, 1479–1484.

[[PubMedCentral](#)]

Jegatheeswaran, S., Mathews, J. A., & Crome, S. Q. (2021). Searching for the elusive regulatory innate lymphoid cell. *Journal of Immunology*, 207, 1949–1957.

Jia, W., Xie, G., & Jia, W. (2018). Bile acid-microbiota crosstalk in gastrointestinal inflammation and carcinogenesis. *Nature Reviews. Gastroenterology & Hepatology*, 15, 111–128.

Joeris, T., Muller-Luda, K., Agace, W. W., et al. (2017). Diversity and functions of intestinal mononuclear phagocytes. *Mucosal Immunology*, 10, 845–864.

Karre, K., Ljunggren, H. G., Piontek, G., et al. (1986). Selective rejection of H-2-deficient lymphoma variants suggests alternative immune defence strategy. *Nature*, 319, 675–678.

[<https://doi.org/10.1038/319675a0>]

Khosravi, A., Yanez, A., Price, J. G., et al. (2014). Gut microbiota promote hematopoiesis to control bacterial infection. *Cell Host & Microbe*, 15, 374–381.

Kiss, E. A., & Vonarbourg, C. (2012). Aryl hydrocarbon receptor: A molecular link between postnatal lymphoid follicle formation and diet. *Gut Microbes*, 3, 577–582.

[[PubMedCentral](#)]

Klose, C. S. N., Flach, M., Mohle, L., et al. (2014). Differentiation of type 1 ILCs from a common progenitor to all helper-like innate lymphoid cell lineages. *Cell*, 157, 340–356.

Knoop, K. A., Gustafsson, J. K., McDonald, K. G., et al. (2017). Microbial antigen encounter during a preweaning interval is critical for tolerance to gut bacteria. *Science Immunology*, 2(18).

Kulkarni, D. H., & Newberry, R. D. (2025). Antigen uptake in the gut: An underappreciated piece to the puzzle? *Annual Review of Immunology*, 43, 571–588.

[[PubMedCentral](#)]

Kunii, J., Takahashi, K., Kasakura, K., et al. (2011). Commensal bacteria promote migration of mast cells into the intestine. *Immunobiology*, 216, 692–697.

Lavin, Y., Mortha, A., Rahman, A., et al. (2015). Regulation of macrophage development and function in peripheral tissues. *Nature Reviews. Immunology*, 15, 731–744.

[[PubMedCentral](#)]

Leiding, J. W. (2017). Neutrophil evolution and their diseases in humans. *Frontiers in Immunology*, 8, 1009.

[[PubMedCentral](#)]

Lelouard, H., Fallet, M., de Bovis, B., et al. (2012). Peyer's patch dendritic cells sample antigens by

extending dendrites through M cell-specific transcellular pores. *Gastroenterology*, 142, 592–601.

Lin, N. Y., Fukuoka, S., Koyama, S., et al. (2025). Microbiota-driven antitumour immunity mediated by dendritic cell migration. *Nature*, 644, 1058–1068.

[<https://doi.org/10.1038/s41586-025-09249-8>] [[PubMedCentral](#)]

Lucas, M., Schachterle, W., Oberle, K., et al. (2007). Dendritic cells prime natural killer cells by trans-presenting interleukin 15. *Immunity*, 26, 503–517.

[[PubMedCentral](#)]

Luu, T. T., Ganesan, S., Wagner, A. K., et al. (2016). Independent control of natural killer cell responsiveness and homeostasis at steady-state by CD11c+ dendritic cells. *Scientific Reports*, 6, 37996.

[[PubMedCentral](#)]

Magalhaes, J. G., Tattoli, I., & Girardin, S. E. (2007). The intestinal epithelial barrier: How to distinguish between the microbial flora and pathogens. *Seminars in Immunology*, 19, 106–115.

Masterson, J. C., McNamee, E. N., Jedlicka, P., et al. (2011). CCR3 blockade attenuates eosinophilic ileitis and associated remodeling. *The American Journal of Pathology*, 179, 2302–2314.

[[PubMedCentral](#)]

McDole, J. R., Wheeler, L. W., McDonald, K. G., et al. (2012). Goblet cells deliver luminal antigen to CD103+ dendritic cells in the small intestine. *Nature*, 483, 345–349.

[<https://doi.org/10.1038/nature10863>] [[PubMedCentral](#)]

Merad, M., Sathe, P., Helft, J., et al. (2013). The dendritic cell lineage: Ontogeny and function of dendritic cells and their subsets in the steady state and the inflamed setting. *Annual Review of Immunology*, 31, 563–604.

[[PubMedCentral](#)]

Mjosberg, J., Bernink, J., Golebski, K., et al. (2012). The transcription factor GATA3 is essential for the function of human type 2 innate lymphoid cells. *Immunity*, 37, 649–659.

Mori, Y., Iwasaki, H., Kohno, K., et al. (2009). Identification of the human eosinophil lineage-committed progenitor: Revision of phenotypic definition of the human common myeloid progenitor. *The Journal of Experimental Medicine*, 206, 183–193.

Mowat, A. M., Scott, C. L., & Bain, C. C. (2017). Barrier-tissue macrophages: Functional adaptation to environmental challenges. *Nature Medicine*, 23, 1258–1270.

Nadsombati, M. S., McGinty, J. W., Lyons-Cohen, M. R., et al. (2018). Detection of succinate by intestinal tuft cells triggers a type 2 innate immune circuit. *Immunity*, 49, 33–41.

[[PubMedCentral](#)]

Ney, L. M., Wipplinger, M., Grossmann, M., et al. (2023). Short chain fatty acids: Key regulators of the local and systemic immune response in inflammatory diseases and infections. *Open Biology*, 13, 230014.

[[PubMedCentral](#)]

Niess, J. H., Brand, S., Gu, X., et al. (2005). CX3CR1-mediated dendritic cell access to the intestinal lumen and bacterial clearance. *Science*, 307, 254–258.

Ohkubo, T., Tsuda, M., Suzuki, S., et al. (1999). Peripheral blood neutrophils of germ-free rats modified by in vivo granulocyte-colony-stimulating factor and exposure to natural environment. *Scandinavian Journal of Immunology*, 49, 73–77.

Ohno, H. (2016). Intestinal M cells. *Journal of Biochemistry*, 159, 151–160.

Palm, N. W., & Medzhitov, R. (2009). Pattern recognition receptors and control of adaptive immunity. *Immunological Reviews*, 227, 221–233.

Park, J. S., Lee, E. J., Lee, J. C., et al. (2007). Anti-inflammatory effects of short chain fatty acids in IFN-gamma-stimulated RAW 264.7 murine macrophage cells: Involvement of NF-kappaB and ERK signaling pathways. *International Immunopharmacology*, 7, 70–77.

Parlato, M., & Yeretssian, G. (2014). NOD-like receptors in intestinal homeostasis and epithelial tissue repair. *International Journal of Molecular Sciences*, 15, 9594–9627.

[\[PubMedCentral\]](#)

Patten, D. A., & Collett, A. (2013). Exploring the immunomodulatory potential of microbial-associated molecular patterns derived from the enteric bacterial microbiota. *Microbiology (Reading)*, 159, 1535–1544.

Platt, A. M., Bain, C. C., Bordon, Y., et al. (2010). An independent subset of TLR expressing CCR2-dependent macrophages promotes colonic inflammation. *Journal of Immunology*, 184, 6843–6854.

Raab, Y., Fredens, K., Gerdin, B., et al. (1998). Eosinophil activation in ulcerative colitis: Studies on mucosal release and localization of eosinophil granule constituents. *Digestive Diseases and Sciences*, 43, 1061–1070.

Rathinam, V. A., & Fitzgerald, K. A. (2016). Inflammasome complexes: Emerging mechanisms and effector functions. *Cell*, 165, 792–800.

[\[PubMedCentral\]](#)

Raulet, D. H., Vance, R. E., & McMahon, C. W. (2001). Regulation of the natural killer cell receptor repertoire. *Annual Review of Immunology*, 19, 291–330.

Rausch, P., Basic, M., Batra, A., et al. (2016). Analysis of factors contributing to variation in the C57BL/6J fecal microbiota across German animal facilities. *International Journal of Medical Microbiology*, 306, 343–355.

Rebuffet, L., Melsen, J. E., Escaliere, B., et al. (2024). High-dimensional single-cell analysis of human natural killer cell heterogeneity. *Nature Immunology*, 25, 1474–1488.

[\[PubMedCentral\]](#)

Rivollier, A., He, J., Kole, A., et al. (2012). Inflammation switches the differentiation program of Ly6Chi monocytes from antiinflammatory macrophages to inflammatory dendritic cells in the colon. *The Journal of Experimental Medicine*, 209, 139–155.

[\[PubMedCentral\]](#)

Roager, H. M., & Licht, T. R. (2018). Microbial tryptophan catabolites in health and disease. *Nature Communications*, 9, 3294.

[\[PubMedCentral\]](#)

Robertson, S. J., & Girardin, S. E. (2013). Nod-like receptors in intestinal host defense: Controlling pathogens, the microbiota, or both? *Current Opinion in Gastroenterology*, 29, 15–22.

Rosshart, S. P., Vassallo, B. G., Angeletti, D., et al. (2017). Wild mouse gut microbiota promotes host fitness and improves disease resistance. *Cell*, 171, 1015–1028.

[\[PubMedCentral\]](#)

Runge, S., von Zedtwitz, S., Maucher, A. M., et al. (2025). Laboratory mice engrafted with natural gut microbiota possess a wildling-like phenotype. *Nature Communications*, 16, 5301.

[\[PubMedCentral\]](#)

Russell, S. L., Gold, M. J., Willing, B. P., et al. (2013). Perinatal antibiotic treatment affects murine microbiota, immune responses and allergic asthma. *Gut Microbes*, 4, 158–164.

[\[PubMedCentral\]](#)

Saitoh, O., Kojima, K., Sugi, K., et al. (1999). Fecal eosinophil granule-derived proteins reflect disease activity in inflammatory bowel disease. *The American Journal of Gastroenterology*, 94, 3513–3520.

Sanos, S. L., Bui, V. L., Mortha, A., et al. (2009). ROR γ and commensal microflora are required for the differentiation of mucosal interleukin 22-producing NKp46⁺ cells. *Nature Immunology*, 10, 83–91.

Satoh-Takayama, N., Vosshenrich, C. A., Lesjean-Pottier, S., et al. (2008). Microbial flora drives interleukin 22 production in intestinal NKp46⁺ cells that provide innate mucosal immune defense. *Immunity*, 29, 958–970.

Schaupp, L., Muth, S., Rogell, L., et al. (2020). Microbiota-induced type I interferons instruct a poised basal state of dendritic cells. *Cell*, 181, 1080–1096.

Schneider, C., O’Leary, C. E., von Moltke, J., et al. (2018). A metabolite-triggered tuft cell-ILC2 circuit drives small intestinal remodeling. *Cell*, 174, 271–284.

[\[PubMedCentral\]](#)

Smythies, L. E., Shen, R., Bimczok, D., et al. (2010). Inflammation anergy in human intestinal macrophages is due to Smad-induced IkappaB α expression and NF-kappaB inactivation. *The Journal of Biological Chemistry*, 285, 19593–19604.

[\[PubMedCentral\]](#)

Sonnenberg, G. F., & Artis, D. (2012). Innate lymphoid cell interactions with microbiota: Implications for intestinal health and disease. *Immunity*, 37, 601–610.

[\[PubMedCentral\]](#)

St John, A. L., & Abraham, S. N. (2013). Innate immunity and its regulation by mast cells. *Journal of*

Immunology, 190, 4458–4463.

Steinman, R. M., & Cohn, Z. A. (1973). Identification of a novel cell type in peripheral lymphoid organs of mice. I. Morphology, quantitation, tissue distribution. *The Journal of Experimental Medicine*, 137, 1142–1162.

[[PubMedCentral](#)]

Stewart, M. K., & Cookson, B. T. (2016). Evasion and interference: Intracellular pathogens modulate caspase-dependent inflammatory responses. *Nature Reviews. Microbiology*, 14, 346–359.

Strowig, T., Henao-Mejia, J., Elinav, E., et al. (2012). Inflammasomes in health and disease. *Nature*, 481, 278–286.

[<https://doi.org/10.1038/nature10759>]

Sullivan, Z. A., Khoury-Hanold, W., Lim, J., et al. (2021). gammadelta T cells regulate the intestinal response to nutrient sensing. *Science*, 371(6535).

Swiecki, M., Miller, H. L., Sesti-Costa, R., et al. (2017). Microbiota induces tonic CCL2 systemic levels that control pDC trafficking in steady state. *Mucosal Immunology*, 10, 936–945.

Talbot, J., Hahn, P., Kroehling, L., et al. (2020). Feeding-dependent VIP neuron-ILC3 circuit regulates the intestinal barrier. *Nature*, 579, 575–580.

[<https://doi.org/10.1038/s41586-020-2039-9>][[PubMedCentral](#)]

Tan, J., McKenzie, C., Potamitis, M., et al. (2014). The role of short-chain fatty acids in health and disease. *Advances in Immunology*, 121, 91–119.

Tang, F., Li, J., Qi, L., et al. (2023). A pan-cancer single-cell panorama of human natural killer cells. *Cell*, 186, 4235–4251.

Thaiss, C. A., Levy, M., Itav, S., et al. (2016a). Integration of innate immune signaling. *Trends in Immunology*, 37, 84–101.

Thaiss, C. A., Zmora, N., Levy, M., et al. (2016b). The microbiome and innate immunity. *Nature*, 535, 65–74.

[<https://doi.org/10.1038/nature18847>]

Ueda, Y., Kayama, H., Jeon, S. G., et al. (2010). Commensal microbiota induce LPS hyporesponsiveness in colonic macrophages via the production of IL-10. *International Immunology*, 22, 953–962.

Walker, J. A., Oliphant, C. J., Englezakis, A., et al. (2015). Bcl11b is essential for group 2 innate lymphoid cell development. *The Journal of Experimental Medicine*, 212, 875–882.

[[PubMedCentral](#)]

Walton, K. L., He, J., Kelsall, B. L., et al. (2006). Dendritic cells in germ-free and specific pathogen-free mice have similar phenotypes and in vitro antigen presenting function. *Immunology Letters*, 102, 16–24.

Wang, S., Xia, P., Chen, Y., et al. (2017). Regulatory innate lymphoid cells control innate intestinal inflammation. *Cell*, 171, 201–216.

Weber, B., Saurer, L., Schenk, M., et al. (2011). CX3CR1 defines functionally distinct intestinal mononuclear phagocyte subsets which maintain their respective functions during homeostatic and inflammatory conditions. *European Journal of Immunology*, 41, 773–779.

Weller, P. F., & Spencer, L. A. (2017). Functions of tissue-resident eosinophils. *Nature Reviews. Immunology*, 17, 746–760.
[\[PubMedCentral\]](#)

Welty, N. E., Staley, C., Ghilardi, N., et al. (2013). Intestinal lamina propria dendritic cells maintain T cell homeostasis but do not affect commensalism. *The Journal of Experimental Medicine*, 210, 2011–2024.
[\[PubMedCentral\]](#)

Wouters, M. M., Vicario, M., & Santos, J. (2016). The role of mast cells in functional GI disorders. *Gut*, 65, 155–168.

Yang, Q., Li, F., Harly, C., et al. (2015). TCF-1 upregulation identifies early innate lymphoid progenitors in the bone marrow. *Nature Immunology*, 16, 1044–1050.
[\[PubMedCentral\]](#)

Zhu, C., Xu, S., Jiang, R., et al. (2024). The gasdermin family: Emerging therapeutic targets in diseases. *Signal Transduction and Targeted Therapy*, 9, 87.
[\[PubMedCentral\]](#)

9. Microbiome and Gut Immunity: T Cells

Martina Palatella¹, Sabrina Wagner², Dirk H. Busch² and Jochen Huehn^{1,3} 

- (1) Department of Experimental Immunology, Helmholtz Centre for Infection Research, Braunschweig, Germany
- (2) Institute for Medical Microbiology, Immunology and Hygiene, Technical University of Munich, Munich, Germany
- (3) Cluster of Excellence RESIST (EXC 2155), Hannover Medical School, Hannover, Germany

 **Jochen Huehn**
Email: jochen.huehn@helmholtz-hzi.de

Abstract

T cells are central mediators of immune responses, balancing protective immunity with tolerance to self and environmental antigens. Their development begins in the thymus, where selection processes shape a diverse, self-tolerant repertoire. In the periphery, CD4⁺ T helper cells, CD8⁺ cytotoxic T cells, and regulatory T (T_{reg}) cells differentiate into specialized subsets to meet distinct immunological challenges.

Nowhere is this balance more critical than in the intestinal environment, where T cells must regulate immune responses amidst continuous exposure to microbial and dietary antigens. Gut-draining lymph nodes (gLNs) orchestrate these responses through interactions between antigen-presenting cells (APCs), stromal cells, and T cells, thereby maintaining homeostasis. This review explores T cell

development, differentiation, and intestinal immune regulation, integrating recent advances to highlight unresolved questions and emerging concepts that may reshape our understanding of mucosal immunity and tolerance.

Keywords Intestinal homeostasis – Regulatory T cells (Treg cells) – Mucosal immunity – Gut-draining lymph nodes – ROR γ t⁺ APCs – Gut microbiota – Mucosal tolerance

Highlights

- The gut represents an immunological challenge, as it is a dynamic environment requiring finely tuned immune responses.
- Gut-draining lymph nodes function as immune hubs, coordinating intestinal T cell responses through interactions with antigen-presenting cells (APCs) and stromal cells.
- The gut microbiota and its metabolites have been shown to influence T cell development and function.
- A reevaluation of tissue-resident T_{reg} cells reveals that they are not static within one tissue but rather continuously recirculate between tissues.
- Emerging roles of ROR γ t⁺ APCs: expanding our understanding of intestinal immune regulation.

9.1 Introduction: T Cell Development and Differentiation

9.1.1 T Cell Development Within the Thymus

Adaptive immunity provides a highly specific, yet slower response compared to innate immunity, as it requires the clonal expansion of antigen-specific lymphocytes to effectively combat pathogens. This system, mediated by B and T lymphocytes, establishes immunological memory for rapid and robust responses to future exposures. Both B and

T cells derive from multipotent hematopoietic stem cells in the bone marrow, expressing specialized receptors, B cell receptor (BCR) or T cell receptor (TCR), respectively. B cells develop within the bone marrow. Instead, T cell development begins with bone marrow-derived thymic seeding progenitors (TSPs), which migrate to the thymus, where immature CD4⁻CD8⁻ double-negative (DN) thymocytes undergo a highly organized process of differentiation and selection, involving TCR specificity, cytokine signaling, and interactions with thymic antigen-presenting cells (APCs) (Hosokawa & Rothenberg, [2018](#); Klein et al., [2019](#); Hosokawa & Rothenberg, [2021](#); Klein & Petrozziello, [2024](#)).

During early thymic development, DN thymocytes progress from DN1 to DN4 maturational stages, which can be distinguished based on the expression of CD44 and CD25, ultimately becoming CD4⁺CD8⁺ double-positive (DP) thymocytes (Yui & Rothenberg, [2014](#); Kurd & Robey, [2016](#)). These DP thymocytes undergo stochastic somatic rearrangement of their TCR gene loci through V(D)J recombination, followed by development into either $\gamma:\delta$ or $\alpha:\beta$ T cells (Bhandoola et al., [2007](#); Ciofani & Zúñiga-Pflücker, [2010](#); Vantourout & Hayday, [2013](#)). V(D)J recombination is a process that involves the assembly of variable (V), diversity (D), and joining (J) gene segments. This process is facilitated by lymphocyte-specific recombinase proteins RAG1 and RAG2. These proteins identify recombination signal sequences flanking V, D, and J segments, generate double-strand breaks at these sites, and enable nonhomologous end joining proteins to rejoin the coding ends, completing the variable genes (Carico & Krangel, [2015](#)). This process imparts immense diversity through the random selection of V, D, and J segments and additional variability at their junctions due to heterogeneous coding end processing (Carico & Krangel, [2015](#)).

The stochastic nature of TCR generation inevitably produces T cells with either “useless” or “harmful” receptors. The former fail to interact with major histocompatibility complex (MHC) molecules, while the latter possess specificity for self-antigens, posing a risk of autoimmunity (Klein et al., [2019](#); Sun et al., [2023](#); Klein & Petrozziello, [2024](#)). To eliminate these undesired cells, the nascent T cell repertoire

undergoes positive and negative selection. Positive selection takes place in the thymic cortex, ensuring that thymocytes produce TCRs capable of recognizing self-MHC molecules. Following positive selection, $\alpha:\beta$ thymocytes differentiate within the thymic medulla into either CD8⁺ or CD4⁺ single-positive (SP) thymocytes, based on their TCR specificity for MHC class I or II molecules (Klein et al., [2019](#); Klein & Petrozziello, [2024](#)). Negative selection, on the other hand, occurs in the medulla, where thymocytes displaying a TCR with high affinity for self-antigenic peptides ectopically expressed in the thymus are negatively selected and undergo apoptosis (Klein et al., [2019](#); Klein & Petrozziello, [2024](#)). Conversely, thymocytes expressing TCRs with moderate affinity for self-antigens are diverted to the thymus-derived regulatory T (tT_{reg}) cell lineage, thereby contributing to immune tolerance (Klein et al., [2019](#); Klein & Petrozziello, [2024](#)).

Finally, mature CD4⁺ T cells, CD8⁺ T cells, and T_{reg} cells exit the thymus and travel through the bloodstream to secondary lymphoid organs (SLOs), such as the spleen and lymph nodes (LNs), where they form the Tnaïve cell pool (Sun et al., [2023](#)) (Fig. [9.1](#)).

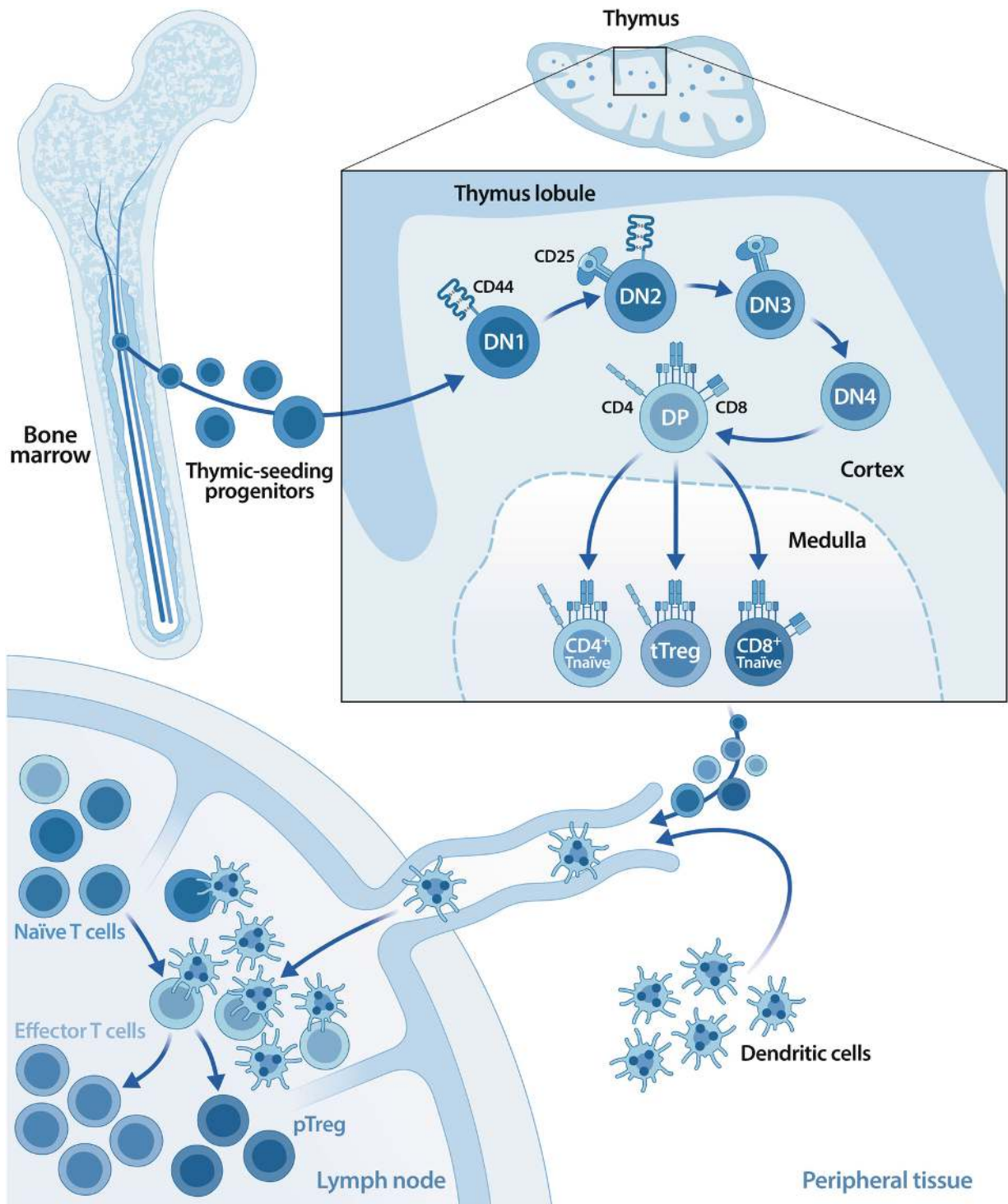


Fig. 9.1 T cell development: from thymus to periphery. Naïve CD4⁺, Foxp3⁺ T_{reg}, and CD8⁺ T cells originate from common bone marrow-derived lymphoid precursors through thymic development. Within the thymus, T cells undergo sequential maturation stages from TSPs to DN and DP. After undergoing positive selection in the thymic cortex, and negative selection in the medulla, DP cells differentiate into either CD4⁺ or CD8⁺ SP Tnaïve or tT_{reg} cells. These cells exit

the thymus via the bloodstream and migrate to secondary lymphoid organs (SLOs), including the spleen and LNs. There, they can further mature into effector T cells or contribute to the formation of peripherally induced T_{reg} (pT_{reg}) cells. (Figure created in <https://BioRender.com>)

9.1.2 Peripheral T Helper Cell Subset Differentiation

The pool of Tnaïve cells continuously patrols SLOs, surveying APCs for their cognate antigens. APCs, including dendritic cells (DCs), macrophages, and B cells, capture antigens in peripheral tissues and migrate to local LNs, where they present extracellular antigens, acquired via the endocytic pathway, on MHC class II molecules to naïve CD4⁺ T cells (Eisenbarth, [2019](#)). These cells become activated upon recognizing the antigen-MHCII complex through their TCR and receiving costimulatory signals from APCs. Then, they undergo clonal expansion and differentiate into specific T helper (T_H) cell subsets influenced by the surrounding cytokine and costimulatory milieu (Zhu et al., [2010](#); Sun et al., [2023](#)). These subsets include T_{H1}, T_{H2}, T_{H17}, T follicular helper (T_{FH}), T_{H9}, and T_{H22} cells, each defined by their transcriptional programs and cytokine profiles (Luckheeram et al., [2012](#); Geginat et al., [2014](#)). Once differentiated, effector T cells migrate to peripheral tissues to perform immune surveillance and combat pathogens.

T_{H1} cells are induced by interferon- γ (IFN- γ) and interleukin (IL)-12, through activation of the transcription factor T-bet via STAT1 and STAT4 signaling (Meitei & Lal, [2023](#); Sun et al., [2023](#)). These cells secrete IFN- γ , IL-2, and tumor necrosis factor- α (TNF- α), with IFN- γ being critical for defense against intracellular bacteria (e.g., *Listeria monocytogenes*) and viruses (Sun et al., [2023](#)).

T_{H2} cells differentiate in response to TCR-stimulated TCF-1 activation, IL-2 and IL-4 signaling (Sun et al., [2023](#)). These cells, defined by the expression of the transcription factor GATA-binding protein 3 (GATA3), produce IL-4, IL-5, and IL-13, which support humoral immunity, enhance B cell responses, and activate eosinophils and mast cells to defend against extracellular pathogens like helminths (Walker & McKenzie, [2018](#); Sun et al., [2023](#)). Additionally, T_{H2} cells contribute to

tissue repair and are implicated in chronic inflammatory conditions, such as asthma and allergy (Walker & McKenzie, [2018](#); Sun et al., [2023](#)).

T_{H17} cells are characterized by the transcription factor retinoic acid receptor-related orphan receptor gamma t (ROR γ t) and production of IL-17A, IL-17F, IL-21, IL-22, and IL-23. T_{H17} cell differentiation is driven by IL-6 and transforming growth factor β (TGF- β), while IL-21 and IL-23 stabilize the Th17 lineage by inducing ROR γ t expression (Sun et al., [2023](#)). These cells provide protection against extracellular bacteria (e.g., *Klebsiella pneumoniae*, *Citrobacter rodentium*) and fungi (e.g., *Candida albicans*), especially in mucosal tissues, where they contribute to barrier defense (He et al., [2017](#); Schnell et al., [2023](#); Sun et al., [2023](#)). However, T_{H17} cells have also been implicated in chronic inflammation and autoimmune diseases (Schnell et al., [2023](#)).

T_{FH} cells, distinguished by the transcription factor Bcl-6 and the surface markers CXC-chemokine receptor 5 (CXCR5) and programmed cell death protein 1 (PD1), are critical for humoral immunity (Crotty, [2019](#); Eisenbarth et al., [2021](#); Walker, [2022](#)). T_{FH} differentiation is promoted by IL-6 and IL-21, while it is inhibited by IL-2 (Sun et al., [2023](#)). T_{FH} cells reside in germinal centers (GCs), where they provide essential signals such as IL-21 and CD40L to support B cell proliferation, differentiation, and high-affinity antibody production (Crotty, [2019](#); Eisenbarth et al., [2021](#); Walker, [2022](#)). As a result, they play pivotal roles in regulating antibody responses in contexts such as infectious diseases, allergies, autoimmune disorders, and vaccination (Crotty, [2019](#); Eisenbarth et al., [2021](#); Yu et al., [2022](#); Walker, [2022](#)). Although primarily located in SLOs, circulating T_{FH} cells are found in the blood, with altered frequencies observed in autoimmune diseases (Walker, [2022](#)).

T_{H9} cells, characterized by the transcription factor PU.1, are notable for their robust production of IL-9 (Sun et al., [2023](#)), along with IL-10 and IL-21 (You et al., [2017](#)). These cells participate in host defense against parasitic infections (e.g., helminths), and support anti-tumor immunity by facilitating the recruitment of cytotoxic CD8⁺ T

lymphocytes (CTLs). Additionally, T_{H9} cells contribute to the pathogenesis of various autoimmune disorders, where dysregulated IL-9 contributes to inflammatory processes (Vyas & Goswami, [2018](#); Chen et al., [2020](#); Angkasekwinai & Dong, [2021](#)). Their differentiation is induced by TCR stimulation in the presence of IL-4 and TGF- β (Sun et al., [2023](#)).

Finally, T_{H22} cells, a less well-defined subset, are characterized by the production of IL-22, IL-13, IL-26, TNF- α , and granzyme B. T_{H22} differentiation is driven by the transcription factor aryl hydrocarbon receptor (AhR) (Jiang et al., [2021](#)), and these cells are abundant in human skin, where they contribute to wound healing and exhibit anti-inflammatory, antibacterial, and antiviral functions (Eyerich et al., [2009](#)). They are increasingly recognized for their roles in skin-related inflammatory diseases, such as psoriasis, as well as allergies, autoimmune diseases, and intestinal disorders (e.g., ulcerative colitis) (Nogralles et al., [2009](#); Zhang et al., [2012](#); Gittler et al., [2012](#); Leung et al., [2014](#); Jiang et al., [2021](#)).

In summary, the differentiation of $CD4^+$ Tnaïve cells into specialized effector subsets ensures a tailored immune response to diverse pathogens, while maintaining barrier function and tissue integrity. This complex system highlights the versatility and precision of the adaptive immune response in health and disease.

9.1.3 $CD8^+$ T Cell Subset Differentiation

Naïve $CD8^+$ T cells exit the thymus after successfully undergoing positive and negative selection, carrying a highly diverse repertoire of distinct TCRs. These cells encounter antigens within SLOs, presented on MHC-I complexes on the surface of APCs. The antigens presented on MHC-I molecules originate from the degradation of cytosolic proteins, including endogenous self-proteins or foreign proteins. Professional APCs, such as DCs and macrophages, can also present exogenous antigens on MHC-I through a mechanism known as cross-presentation (Stemberger et al., [2007](#); Klein & Petrozziello, [2024](#)).

The initial activation (priming) of naïve CD8⁺ T cells requires the engagement of the TCR with the epitope-MHC class I complex on the surface of specialized APCs, along with co-stimulatory signals, such as those from CD28 and 4-1BB. Additionally, cytokines, particularly IL-2, are critical for successful priming. IL-2 can be supplied by CD8⁺ T cells themselves or provided by CD4⁺ T_H cells (Stemberger et al., [2007](#); Klein & Petrozziello, [2024](#)). Upon successful priming, naïve CD8⁺ T cells differentiate into effector T cells, acquiring cytotoxic functions and the ability to secrete key effector molecules such as perforin, granzymes, and pro-inflammatory cytokines, including IFN- γ and TNF- α . This differentiation is accompanied by rapid clonal expansion, allowing a small pool of precursor cells to generate a robust immune response.

However, maintaining the metabolic demands of effector T cells is highly resource-intensive due to their elevated functional requirements. To prevent unnecessary energy expenditure and minimize the risk of chronic inflammation or immunopathology, most effector T cells undergo apoptosis once the pathogen has been cleared. A small subset, however, survives beyond the contraction phase and transitions into memory T cells, which persist in the absence of antigen. Unlike their naïve counterparts, memory T cells exhibit enhanced recall immunity, enabling more efficient immune surveillance and a rapid response upon re-infection (Laidlaw et al., [2016](#); Buchholz et al., [2016](#); Konjar et al., [2017](#)).

A common feature shared by all memory T cells is the expression of IL-7 receptor alpha chain (CD127) in both mice and humans (Kaech et al., [2003](#); Huster et al., [2004](#)). Nevertheless, CD8⁺ memory T cells are a heterogeneous population comprising several subtypes with distinct phenotypes, effector functions, proliferative capacity, plasticity, and migratory behaviors. One major subset, central memory T (T_{CM}) cells, circulate continuously between blood, lymph, and SLOs, in a manner similar to naïve T cells. T_{CM} cells express CD62L and CCR7, both of which are essential for extravasation and homing to SLOs. In contrast, effector memory T (T_{EM}) cells are predominantly localized in non-

lymphoid tissues (NLTs), but can also be detected in the spleen and bloodstream. T_{EM} cells lack the expression of CD62L and CCR7 but express tissue-specific homing receptors that enable their migration to peripheral sites (Springer, [1994](#); Sallusto et al., [1999](#); Masopust et al., [2001](#); Stemberger et al., [2007](#); Stemberger et al., [2009](#); Buchholz et al., [2016](#); Konjar et al., [2017](#)). While T_{EM} cells can rapidly execute effector functions upon antigen re-encounter, they have a more limited proliferative capacity. In contrast, T_{CM} cells exhibit robust proliferative responses upon antigen re-exposure, but have less immediate effector activity compared to T_{EM} cells (Sarkar et al., [2008](#); Stemberger et al., [2009](#)).

9.1.4 Regulatory T Cells: Development and Subsets

T_{reg} cells, first identified by Sakaguchi et al. in 1995, represent a distinct subset of $CD4^+$ T cells characterized by constitutive expression of CD25. These cells play a crucial role in maintaining immune tolerance to self-antigens, dietary components, and commensal microbes, ensuring protection against autoimmune and inflammatory diseases throughout life (Sakaguchi et al., [1995](#); Kim et al., [2007](#); Vignali et al., [2008](#); Sumida et al., [2024](#)). Additionally, T_{reg} cells regulate excessive immune responses, facilitate inflammation resolution, and promote tissue repair. The critical nature of T_{reg} cells is exemplified by patients with immune dysregulation polyendocrinopathy enteropathy X-linked (IPEX) syndrome and scurfy mice, both of which lack functional Foxp3 and suffer from severe autoimmune conditions due to uncontrolled T cell activity (Bennett et al., [2001](#); Brunkow et al., [2001](#); Wildin et al., [2001](#)).

Although CD25 is not exclusive to T_{reg} cells, the transcription factor Foxp3, identified in 2003, is a definitive marker essential for their function (Hori et al., [2003](#); Fontenot et al., [2003](#); Khattri et al., [2003](#)). Ectopic expression of Foxp3 can trigger a suppressive phenotype in conventional T cells (Hori et al., [2003](#)). Consequently, precise regulation of Foxp3 expression is essential for preserving the balance of T-cell-

mediated immune responses. Extensive research over the years has focused on understanding the mechanisms controlling the *Foxp3* locus, making it one of the most intensely investigated genes in recent times (Lee & Lee, [2018](#)). Four conserved non-coding sequences (CNS) have been identified at the *Foxp3* locus: CNS-0, which is situated on an intron of the neighboring gene 5' of the *Foxp3* locus; CNS-1 and CNS-2 located within the first intron of the *Foxp3* locus; and CNS-3 located downstream of exon 1 (Lee & Lee, [2018](#)). The stability of the T_{reg} lineage is supported by selective demethylation of the CpG-rich CNS-2, also termed the T_{reg}-specific demethylated region (TSDR) (Floess et al., [2007](#); Kim & Leonard, [2007](#); Polansky et al., [2008](#); Zheng et al., [2010](#); Toker et al., [2013](#)). Interestingly, the TSDR is fully demethylated in ex vivo isolated T_{reg} cells, but remains methylated in naïve and effector T cells (Floess et al., [2007](#)). In vitro induced T_{reg} (iT_{reg}) cells exhibit only partial TSDR demethylation, despite robust *Foxp3* expression (Floess et al., [2007](#)). This incomplete epigenetic modification compromises iT_{reg} cells' stability, as they lose *Foxp3* expression and suppressive function upon restimulation in the absence of TGF-β. These findings underscore the importance of epigenetic regulation for maintaining a stable T_{reg} phenotype (Floess et al., [2007](#)).

Most T_{reg} cells are generated in the thymus during thymic T cell development (Hsieh et al., [2012](#)). During negative selection, thymocytes with moderately high TCR affinity for self-antigens presented by thymic APCs are directed to differentiate into tT_{reg} cells (Burchill et al., [2008](#); Lio & Hsieh, [2008](#); Hemmers et al., [2019](#)). Consequently, tT_{reg} cells are thought to primarily recognize self-antigens. Cytokines such as IL-2 and IL-15 are critical for tT_{reg} development and maintenance, with IL-2 playing a key role in upregulating *Foxp3* expression and promoting the maturation of Foxp3⁺CD4⁺ T_{reg} cells (Hsieh et al., [2012](#)). tT_{reg} cells are typically identified by the expression of the transcription factor Helios and the surface receptor neuropilin 1 (Nrp1) (Thornton et al., [2010](#); Singh et al., [2015](#)). However, these markers are not exclusive to tT_{reg} cells, since they can also be expressed by other T cells under

inflammatory conditions or induced in peripherally-derived Treg (pT_{reg}) cells under specific conditions (Thornton et al., [2010](#); Weiss et al., [2012](#)).

After leaving the thymus, tT_{reg} cells circulate via the bloodstream. A fraction of these cells, termed tissue-resident T_{reg} cells, establishes local immunotolerance in peripheral NLTs, including the intestine (Panduro et al., [2016](#)). These cells differ from their SLO-derived counterparts due to their residence within NLTs, reduced TCR repertoire diversity, and completely distinct transcriptomes and functional properties (Panduro et al., [2016](#)). Tissue-resident T_{reg} cells are characterized by expression of canonical tissue-resident memory (T_{RM}) markers, such as CD69, CD103, CD11a, and PD-1, in addition to other markers such as ST2 and KLRG1 (Muñoz-Rojas & Mathis, [2021](#)). Tissue-resident T_{reg} cells not only contribute to immunological tolerance within the tissue but also exert non-canonical functions. For instance, adipose tissue T_{reg} cells aid insulin sensitivity and lipolysis, while controlling inflammation by suppressing pro-inflammatory macrophages and secreting IL-10 (Feuerer et al., [2009](#); Cipolletta et al., [2012](#); Cipolletta et al., [2015](#); Kolodin et al., [2015](#)). Skeletal muscle T_{reg} cells contribute to tissue repair by secreting amphiregulin, which promotes myogenesis and suppresses local inflammation (Burzyn et al., [2013](#); Kuswanto et al., [2016](#)). Brain T_{reg} cells modulate neuroinflammation, support myelin repair, and facilitate recovery in neurodegenerative conditions, driving oligodendrocyte differentiation (Dombrowski et al., [2017](#)). Skin T_{reg} cells maintain immune tolerance to commensal microbes and prevent excessive inflammation and fibrosis. They also support hair follicle regeneration by producing growth factors (Ali et al., [2017](#); Kalekar et al., [2019](#)). Uterine T_{reg} cells promote feto-maternal tolerance by suppressing effector T cell responses to fetal antigens and ensuring successful pregnancy outcomes (Samstein et al., [2012](#); Rowe et al., [2012](#); Miller et al., [2020](#)).

In addition, T_{reg} cells can also develop in the periphery, as a result of the conversion of mature Foxp3⁻ conventional CD4⁺ T cells, and are

named pT_{reg} cells (Curotto de Lafaille et al., [2008](#); Curotto de Lafaille & Lafaille, [2009](#); Kerdiles et al., [2010](#); Haribhai et al., [2011](#); Lathrop et al., [2011](#); Bilate & Lafaille, [2012](#); Cording et al., [2014](#)). This de novo induction from naïve CD4⁺ T cells very efficiently takes place in gut-draining LNs (gLNs) in the presence of TGF-β-rich conditions (Chen et al., [2003](#); Fantini et al., [2004](#)). Analogously to the development of T_H cells, the generation of pT_{reg} cells is also a stepwise process. After activation, T_{reg} cells downregulate CD62L expression and simultaneously upregulate the expression of CCR9, as well as integrin α₄β₇ (Capece & Kim, [2016](#)), enabling them to migrate to the intestine after having left the gLNs. There, pT_{reg} cells undergo local expansion while retaining Foxp3 expression, and are believed to recognize harmless foreign antigens and mainly mediate tolerance towards dietary and commensal-derived antigens (Curotto de Lafaille & Lafaille, [2009](#)). It has been proposed that pT_{reg} cells are required to broaden the TCR repertoire of tT_{reg} cells to innocuous non-self-antigens, like commensal microbiota, food, and fetal antigens, to promote respectively mucosal, oral, and feto-maternal tolerance (Hadis et al., [2011](#); Haribhai et al., [2011](#); Josefowicz et al., [2012](#); Samstein et al., [2012](#)).

9.1.5 Suppressive Mechanisms of T_{reg} Cells

T_{reg} cells utilize diverse mechanisms to regulate immune responses and maintain immune homeostasis. These mechanisms involve the release of immunosuppressive cytokines, such as IL-10, TGF-β, and IL-35 (Shevach, [2009](#); Banchereau et al., [2012](#)). Moreover, T_{reg} cells express high levels of the IL-2 receptor alpha chain (IL-2Rα, CD25), enabling them to outcompete effector T cells for IL-2, depleting this molecule and suppressing effector T cell survival and proliferation (Pandiyani et al., [2007](#)). T_{reg} cells also mediate suppression through enzymatic activity. Surface ecto-5'-nucleotidase enzymes CD39 and CD73 convert ATP to adenosine, which accumulates in the microenvironment and inhibits antigen presentation by DCs, while suppressing effector T cell

proliferation via adenosine receptor 2A activation (Kobie et al., [2006](#); Dwyer et al., [2007](#); Deaglio et al., [2007](#); Smyth et al., [2013](#)). Additionally, T_{reg} cells interact directly with effector T cells and/or APCs through surface molecules such as CTLA-4, GITR, Nrp1, PD-1, lymphocyte activation gene 3 (LAG-3), fibrinogen-like protein-2 (FGL-2), and TNFR superfamily member 4 (OX40) (Wing et al., [2008](#); Shevach, [2009](#); Park et al., [2015](#)). For instance, CTLA-4 binds to CD80 and CD86 on APCs, functioning as a negative regulator to suppress T cell activation, in contrast to CD28, which enhances activation (Walker & Sansom, [2011](#)). Similarly, LAG3 inhibits MHC-II expression on DCs, further dampening immune responses (Shevach, [2009](#)). Besides, T_{reg} cells can also employ cytolytic and metabolic mechanisms. They can secrete granzyme A, which triggers apoptosis in target immune cells (Gondek et al., [2005](#); Cao et al., [2007](#)), and transfer inhibitory second messenger cyclic AMP (cAMP) into effector T cells through membrane gap junctions, thereby suppressing their activity (Bopp et al., [2007](#)).

Controversy: Revisiting the Paradigm of Tissue-Resident T_{reg} Cells

Since their first discovery, tissue-resident T_{reg} cells were thought to exhibit a behavior like T_{RM} cells, stably residing within NLTs, including the intestine, where they maintain local immune tolerance and execute tissue-specific functions. These cells have always been described as possessing unique transcriptomic and functional properties compared to their SLO-derived counterparts. For instance, Areg was believed to be expressed uniquely by muscle T_{reg} cells (Burzyn et al., [2013](#)), and PPAR γ only by adipose tissue T_{reg} cells (Cipolletta et al., [2012](#)).

However, these long-held assumptions have been recently challenged by Burton et al., who proposed a new “pan-tissue T_{reg} cell” model (Burton et al., [2024](#)). According to this paradigm, tissue-resident T_{reg} cells do not stably remain in a single tissue, but rather recirculate across multiple tissues (Burton et al., [2024](#)). This novel model shifts the understanding of these cells as transient inhabitants

of various tissues, with short residency periods of approximately 3 weeks before migrating to new sites (Burton et al., [2024](#)). A core finding supporting the “pan-tissue” hypothesis is the discovery that tissue-resident T_{reg} cells share a conserved phenotype across NLTs, marked by elevated expression of molecules such as CD69, ST2, Areg, KLRG1, IL-10, and CTLA-4 (Panduro et al., [2016](#); Burton et al., [2024](#)). Similar findings were detected in DNA methylation and chromatin accessibility profiling studies when tissue-resident T_{reg} cells were compared across NLTs (Delacher et al., [2017](#), [2020](#); Spath et al., [2022](#)). The conservation of this phenotype, not only across tissue T_{reg} cells from multiple NLTs, but also in T_{RM} cells, underscores the importance of these signature genes for the tissue adaptation of lymphocytes.

One of the most striking observations in this study was the tissue-agnostic nature of tissue T_{reg} cells upon re-entry (Burton et al., [2024](#)). Tissue T_{reg} cells displayed the ability to migrate across multiple non-gut NLTs, driven by shared TCR specificity (Burton et al., [2024](#)). This finding suggests that T_{reg} cells capable of recognizing broadly distributed antigens migrate through various tissues, optimizing their distribution. By contrast, tissue T_{reg} clones with restricted tissue-specific antigen recognition were a minority and exhibited more localized residency patterns (Burton et al., [2024](#)).

Interestingly, a notable exception was the gut. Intestinal T_{reg} cells were distinct from their counterparts in other NLTs (Burton et al., [2024](#)). They exhibited unique phenotypic and TCR characteristics, suggesting a higher degree of tissue specificity (Burton et al., [2024](#)). This differentiation likely reflects the unique antigenic landscape of the gut, heavily influenced by the microbiome. The gut-resident T_{reg} cells’ enhanced antigen specificity aligns with their specialized role in maintaining tolerance to dietary antigens and commensal microbes, which sets them apart from the broadly adaptive T_{reg} populations observed in other tissues.

The authors also suggest that a key distinction between tissue T_{reg} cells and T_{RM} cells is how they respond to antigens (Burton et al.,

[2024](#)). T_{RM} cells, activated during infections, encounter antigens at specific times and locations, which requires them to establish a fixed residency program to remain in the same tissue, without needing ongoing stimulation (Burton et al., [2024](#)). In contrast, tissue T_{reg} cells engage with antigens continuously and across various locations, allowing them to migrate between tissues and adapt their function dynamically (Burton et al., [2024](#)). Rather than relying on a permanent genetic program, T_{reg} cells maintain their tissue-specific roles through continuous signaling, enabling efficient distribution throughout the body.

This study raises significant questions about the old tissue-residency dogma for T_{reg} cells. If these cells are indeed transient and capable of recirculating broadly, the paradigm of tissue-specific adaptation may need to be re-evaluated. Additionally, the distinct behavior of intestinal T_{reg} cells invites further investigation into how the unique environment of the intestine influences T_{reg} cell biology and contributes to tissue-specific immune regulation. Future studies are essential to determine how these findings translate to human tissue-resident T_{reg} cells, and how inflammation or disease states might modify their behavior.

9.2 Intestinal T Cells and Immune Regulation in the Gut

The gastrointestinal (GI) tract represents a uniquely challenging environment, where immune tolerance to dietary antigens and commensal bacteria must coexist with the capacity to defend against pathogens. This delicate balance is orchestrated by diverse populations of intestinal T cells, which include T_{reg} cells, intraepithelial lymphocytes (IELs), as well as conventional $CD4^+$ and $CD8^+$ T cells. These T cell subsets are uniquely adapted to the gut's antigen-rich and microbiota-dense environment, displaying distinct phenotypic and functional characteristics that enable both immune surveillance and tolerance (Ma

et al., [2019](#)). Unlike T cells in other NLTs, intestinal T cells are highly specialized to function within the lamina propria (LP) and intraepithelial compartments, where they dynamically interact with dietary antigens, microbial metabolites, and epithelial cells (Ma et al., [2019](#); Burton et al., [2024](#)). CD4⁺ effector T cells contribute to mucosal immunity by promoting pathogen clearance, while CD8⁺ T cells and IELs provide frontline cytotoxic defense against infected or damaged epithelial cells. At the same time, intestinal T_{reg} cells play a central role in maintaining immune homeostasis, actively suppressing excessive inflammation and preventing immune-mediated damage to gut tissues. Notably, intestinal T_{reg} cells have an extended residency period, persisting for 7–8 weeks, significantly longer than the 3-week dwell time observed in other tissues, underscoring their importance in long-term immune regulation (Burton et al., [2024](#)).

In this review, we explore the specialized functions of intestinal T cells, focusing on their development, migration, and interactions within gLNs. We further examine the impact of the microbiota and microbial-derived metabolites on the induction, maintenance, and functional specialization of intestinal T cells, highlighting their pivotal role in shaping gut immunity and maintaining homeostasis.

9.2.1 Gut-Draining Lymph Nodes as Orchestrators of Intestinal T Cell Responses

The GI tract is a complex system of structural layers designed not only for digestion and nutrient absorption but also as a formidable line of immune defense. The innermost mucus layer, enriched with mucins, secretory IgA (SIgA), and antimicrobial peptides, serves as a critical barrier against pathogens. Its composition is tailored to the microbial load of each region, with the colon possessing a thicker double-layered mucus compared to the small intestine (Song et al., [2023](#)). Beneath this protective layer lies the intestinal epithelial monolayer, which absorbs nutrients and interacts with microbial components through pattern-recognition receptors (PRRs). This layer is supported by specialized epithelial cells, including goblet, Paneth, and enterochromaffin cells,

each contributing to the maintenance of intestinal homeostasis (Allaire et al., [2018](#)). Below the epithelium, the LP houses a rich array of immune cells, blood vessels, and lymphatic structures, including mucosal-associated lymphoid tissues (MALT), such as Peyer's patches (PPs) and isolated lymphoid follicles (ILFs) (Mörbe et al., [2021](#)). PPs develop in the fetal small intestine independently of microbial flora (Mowat, [2003](#)), while ILFs are microbiota-responsive structures that increase during chronic inflammation (Lorenz et al., [2003](#)).

In addition to these local lymphoid tissues, there are other gut-associated lymphoid tissues (GALT), including mesenteric lymph nodes (mLNs), which drain the small and large intestine, and the liver-draining celiac lymph nodes (cLNs). These gLNs are pivotal sites for initiating adaptive immune responses to luminal antigens (Cording et al., [2014](#); da Fonseca et al., [2015](#); Randolph et al., [2017](#)) (Fig. [9.2](#)). Recent findings highlighted that gLNs support either tolerogenic or inflammatory immune responses, depending on the segment of the gut they drain (Esterházy et al., [2019](#)). Proximal gLNs, associated with the duodenum and jejunum, favor the induction of tolerogenic responses, such as pT_{reg} generation, contributing to oral tolerance, thereby preventing inappropriate reactions to dietary antigens (Esterházy et al., [2019](#)). In contrast, distal gLNs, which drain the ileum and colon, are more prone to supporting pro-inflammatory T cell responses, such as T_{H17} differentiation, crucial for defending against pathogenic insults in these microbe-rich environments (Esterházy et al., [2019](#)). This functional specialization has profound implications. Proximal gLNs play a central role in preventing food allergies by promoting tolerance to dietary antigens. However, perturbations, such as duodenal infections or dysbiosis, can alter this tolerogenic balance, potentially exacerbating allergic or inflammatory outcomes. Conversely, distal gLNs, with their inflammatory propensity, may be ideal therapeutic targets for intestinal vaccinations (Esterházy et al., [2019](#)). Instead, leveraging the dietary duodenal route could offer a novel strategy for inducing tolerance to inaccessible pro-inflammatory antigen sources, such as those derived from dysbiotic bacteria in inflammatory bowel disease (IBD)

(Esterházy et al., [2019](#)). Interestingly, a defining feature of gLNs is their exceptional capacity to induce T_{reg} cells. This property is attributed to their specialized stromal cells and DC populations, which create a microenvironment conducive to T_{reg} differentiation (Cording et al., [2014](#); Pezoldt et al., [2018](#); Wiechers et al., [2021](#)). Unlike skin-draining LNs, which have limited T_{reg} -inducing abilities, gLNs are uniquely equipped to generate tolerogenic responses under homeostatic conditions. This capacity ensures the maintenance of intestinal immune homeostasis and prevents unnecessary immune activation against food-borne and microbiota-derived antigens.

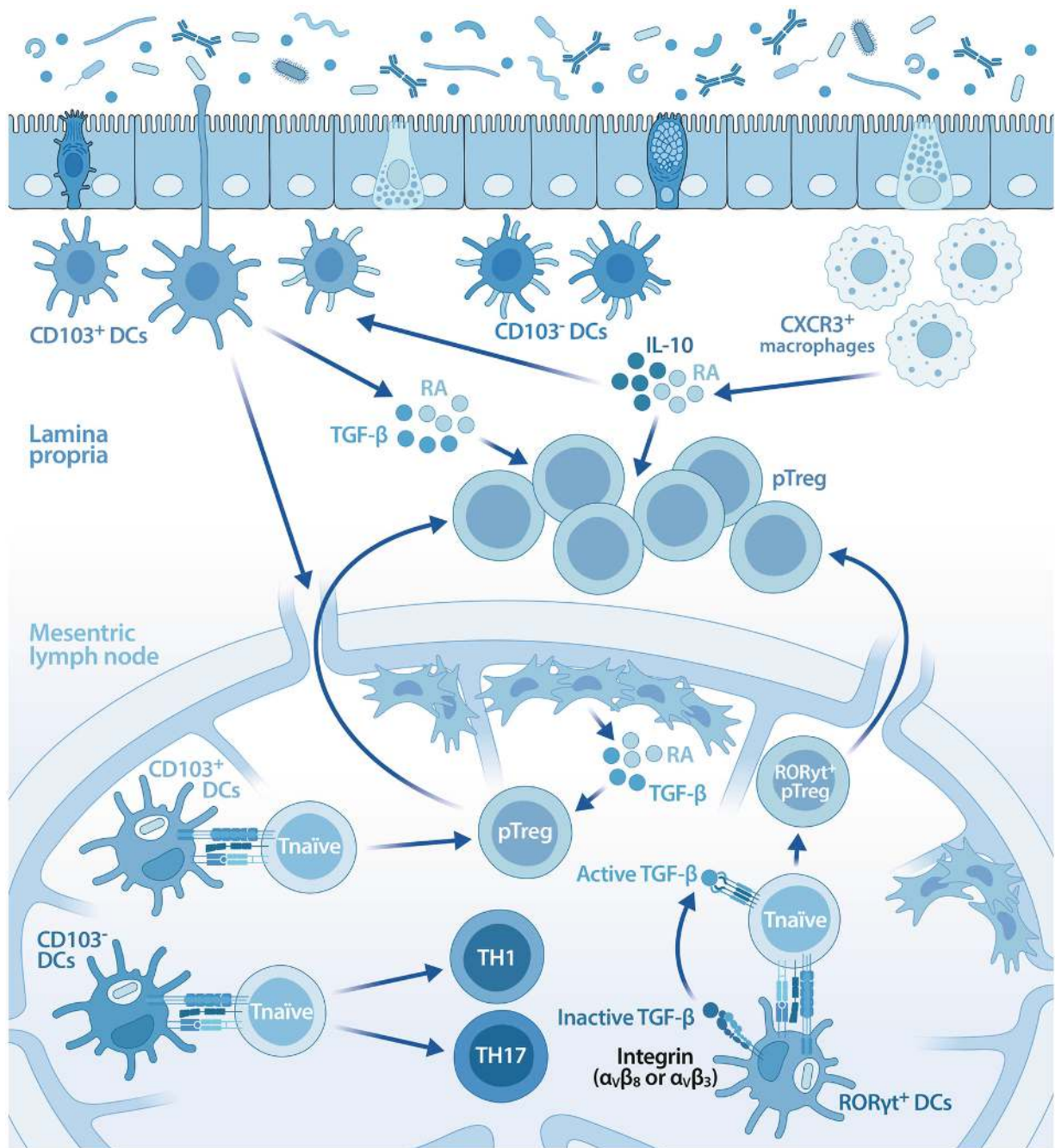


Fig. 9.2 CD4⁺ T cell differentiation in the gut. T cell differentiation is a dynamic, multistep process heavily influenced by the local intestinal microenvironment and the microbiota. Naïve T cells require interaction with professional APCs presenting their cognate antigen to initiate differentiation. Depending on the functional characteristics of the APC, either tolerogenic CD103⁺ DCs or non-tolerogenic CD103⁻ DCs, the process leads to the generation of pT_{reg} cells or effector TH1/TH17 cells, respectively. Primary differentiation occurs in gLNs, where LN stromal cells contribute to the tolerogenic environment by producing RA and TGF-β. Within gLNs, RORγt-expressing APCs activate TGF-β through integrins (α_vβ₈ or α_vβ₃ subunits), enabling its interaction with TGF-β receptors on Tnaïve cells. This signaling induces the differentiation of Tnaïve cells into

pT_{reg} cells, expressing both Foxp3 and ROR γ t, which are critical for suppressing immune responses against commensal bacteria. Regardless of the T cell subtype, the mLN microenvironment facilitates the induction of gut-homing markers $\alpha_4\beta_7$ and CCR9, enabling efficient T cell migration to intestinal tissues. Once in the gut, APCs continue to present antigens, supporting the expansion and final differentiation of pT_{reg} and effector T cells. This final differentiation stage is shaped by the local environment, including IL-10 production by CX3CR1⁺ macrophages. APCs antigen-presenting cell, DCs dendritic cell, gLNs gut-draining lymph nodes, pT_{reg} peripherally-induced Treg, RA retinoic acid. (Figure created in <https://BioRender.com>)

9.2.2 Interplay of APCs and Stromal Cells in Balancing Tolerogenic and Effector T Cell Responses

A finely tuned interplay between APCs and stromal cells orchestrates the balance between tolerogenic and effector T cell responses in the intestine (Esterházy et al., [2019](#)). This compartmentalized system leverages unique APC populations and the specialized microenvironment within gLNs, to maintain intestinal immune homeostasis. APCs, including DCs and macrophages, are pivotal in initiating immune responses by presenting antigens both in intestinal tissues and gLNs. pT_{reg} cells and effector T_H cells are generated through a sequential process, involving the migration of DCs from the LP to the mLNs (Pabst & Bernhardt, [2013](#)). This migration is CCR7-dependent (Worbs et al., [2006](#)). In the LP and gLNs, CD103⁺ DCs are specialized in promoting pT_{reg} induction through TGF- β , retinoic acid (RA), indoleamine 2,3-dioxygenase (IDO), and thymic stromal lymphopoietin (TSLP)-dependent mechanisms (Coombes et al., [2007](#); Matteoli et al., [2010](#); Spadoni et al., [2012](#)) (Fig. [9.2](#)). RA supports T_{reg} induction, while antagonizing IL-6-driven T_{H17} differentiation (Mucida et al., [2007](#)). These tolerogenic DCs also prime Tnaïve cells with gut-homing chemokine receptors CCR9 and integrin $\alpha_4\beta_7$, facilitating their migration to the intestine (Agace, [2006](#); Siewert et al., [2007](#)). Notably, depletion of DCs diminishes pT_{reg} induction, Foxp3 expression, and overall T_{reg} homeostasis (Darrasse-Jèze et al., [2009](#); Cording et al., [2014](#)), thus suggesting that DCs are essential for inducing pT_{reg} cells. Conversely, CD103⁻ DCs drive effector T cell differentiation by

secreting, for instance, IL-23, which promotes T_{H17} responses, and IL-22, which supports epithelial barrier integrity (Guo et al., [2014](#); Parks et al., [2015](#)) (Fig. [9.2](#)).

Intestinal macrophages rarely prime Tnaïve cells, but they rather modulate the differentiation of T_{reg} and T_{H17} cells subsequent to the priming in the gLNs, based on the intestinal environment. Under homeostatic conditions, $CD11b^+F4/80^+CD11c^-CX3CR1^+$ macrophages enhance the expansion of LN-primed T_{reg} cells through IL-10 secretion (Murai et al., [2009](#); Hadis et al., [2011](#)) (Fig. [9.2](#)). Instead, under inflammatory conditions, $Ly6C^+$ monocytes are recruited to the LP, where they produce pro-inflammatory cytokines, such as TNF- α , IL-6, and IL-23, contributing to a T_{H17} -dominated immune response (Weber et al., [2011](#); Bain & Mowat, [2014](#)).

Hot Topic: Emerging Roles of ROR γ t⁺ APCs Recently, three back-to-back seminal studies in *Nature* (Akagbosu et al., [2022](#); Kedmi et al., [2022](#); Lyu et al., [2022](#)) have unveiled the critical roles of ROR γ t-expressing APCs in maintaining intestinal immune homeostasis. This diverse population of APCs includes innate lymphoid cells 3 (ILC3s) and novel subsets of both Aire⁺ and Aire⁻ APCs (Akagbosu et al., [2022](#); Kedmi et al., [2022](#); Lyu et al., [2022](#)). ROR γ t⁺ APCs constitute a unique subset with specialized functions in the gut, primarily inducing microbiota-specific ROR γ t⁺ T_{reg} cells by presenting antigens on MHC-II molecules, and converting TGF- β in its active form via binding with integrins, such as $\alpha_v\beta_8$ or $\alpha_v\beta_3$ (Akagbosu et al., [2022](#); Kedmi et al., [2022](#); Lyu et al., [2022](#)) (Fig. [9.2](#)). ROR γ t⁺ APCs are critical to establish tolerance to commensal bacteria (Akagbosu et al., [2022](#); Kedmi et al., [2022](#); Lyu et al., [2022](#); Travis & Romagnani, [2022](#)), since their deficiency leads to reduced ROR γ t⁺ T_{reg} cell populations and a concomitant increase in pro-inflammatory T_{H17} cells, potentially resulting in intestinal inflammation (Travis & Romagnani, [2022](#)).

Despite significant advancements, discrepancies exist in the conclusions and nomenclature across these recent studies. Lyu et al.

identified RORyt⁺ APCs as including ILC3s and two clusters of RORyt⁺ extrathymic Aire-expressing cells (also known as Janus cells), attributing pT_{reg} induction exclusively to ILC3s (Lyu et al., [2022](#)). Kedmi et al. also noted ILC3s, but highlighted a population of RORyt⁺Aire⁺ Janus cells as central players (Kedmi et al., [2022](#)). Meanwhile, Akagbosu et al. described four subsets of Thetis cells (TC I-IV), with Aire-negative TC IV cells driving pT_{reg} induction, dismissing ILC3 involvement (Akagbosu et al., [2022](#)). Differences in experimental approaches, such as the reporter mice used (Aire-GFP, RORyt-GFP, RORyt-Venus), mouse age, and LN origin, might affect cell type identification and abundance. However, despite these discrepancies, sufficient overlap in the findings allows for meaningful comparisons between datasets.

In contrast to their tolerogenic role, a recent study highlighted a novel and unexpected function of Aire⁺RORyt⁺ APCs in adaptive immunity against fungal commensals (Dobeš et al., [2022](#)). Using RORyt-Cre Aire-flox mice, researchers demonstrated that the absence of Aire in RORyt-lineage cells impaired T cell responses in an immunization model, involving heat-killed *Candida albicans* conjugated to ovalbumin (OVA) (Dobeš et al., [2022](#)). Aire deficiency resulted in a significant reduction in OVA-specific T cell responses, which was mirrored in the polyclonal T cell population targeting *C. albicans* (Dobeš et al., [2022](#)). This impaired immunization response translated into weaker protection against live *Candida* infections (Dobeš et al., [2022](#)).

Overall, these groundbreaking studies collectively illuminate a new dimension of RORyt-expressing APC biology, emphasizing their dual functionality in balancing immune tolerance and inflammation (Travis & Romagnani, [2022](#); Gardner & Liston, [2022](#)). However, to fully harness their therapeutic potential, future research must aim to reconcile the discordant nomenclature of these cell subsets and deepen our understanding of their specialized roles in shaping gut immunity.

Besides APCs, stromal cells within gLNs, including fibroblastic reticular cells (FRCs), lymphatic endothelial cells (LECs), and blood endothelial cells (BECs), actively shape tissue-specific immune responses (Hammerschmidt et al., [2008](#); Malhotra et al., [2012](#)) (Fig. [9.2](#)). These cells create a stable microenvironment for T cell activation and differentiation. Unlike DCs, which are short-lived, stromal cells persist under homeostatic conditions, providing consistent immunoregulatory support (Molenaar et al., [2009](#)). Once regarded as passive structural components, they are now recognized as active regulators of tissue-specific immune responses (Zou et al., [2021](#)). Stromal cells play a critical role in fostering tolerogenic T cell responses within gLNs. FRCs in mLNs express enzymes like *Aldh1a2* and *Aldh1a3*, which generate high levels of RA and enhance T_{reg} induction, while suppressing T_{H17} differentiation by reducing IL-6 production (Malhotra et al., [2012](#)). These tolerogenic properties are imprinted early in life and persist throughout adulthood, as evidenced by LNs transplantation experiments, showing that mLNs and cLNs maintain their capacity to induce T_{reg} cells, even when transplanted to non-gut sites (Cording et al., [2014](#)). The tolerogenic function of stromal cells is influenced by tissue-specific signals and the microbiota. Interestingly, the microbiota is not essential for the initial stages of T_{reg} induction in mLNs, which rely predominantly on intrinsic stromal and DCs-driven mechanisms (Wiechers et al., [2021](#)). Germ-free (GF) mice retain the ability to induce T_{reg} cells in mLNs, indicating that the microbiota is more critical for later stages of T_{reg} maintenance and expansion in the LP (Wiechers et al., [2021](#)). However, both vitamin A in cLNs, and intestinal microbiota in mLNs are important for sustaining T_{reg} induction, as deficiencies in either reduce T_{reg} induction capacities (Cording et al., [2014](#)). Stromal cells not only modulate DC behavior, but also stabilize an environment that supports antigen-specific T cell responses to dietary and microbial antigens. Interestingly, neonatal gastrointestinal infections can disrupt the tolerogenic properties of cLNs, by altering stromal cell subsets and impairing their ability to support T_{reg} induction (Zou et al., [2024](#)). This

disruption can have long-term effects; however, early-life supplementation with vitamin A has been shown to restore the tolerogenic functionality of stromal cells (Zou et al., [2024](#)). These findings highlight the importance of stromal cells in shaping and maintaining intestinal immune tolerance across the lifespan.

9.2.3 Impact of Microbiota and Microbiota-Derived Metabolites on Intestinal T_{reg} Cells

The gut harbors a vast and diverse microbiota, dominated by the phyla Firmicutes, Bacteroidetes, Proteobacteria, and Actinobacteria (Macpherson & Harris, [2004](#); Gensollen et al., [2016](#); Fan & Pedersen, [2021](#); Suriano et al., [2022](#)). Microbial density increases along the gastrointestinal tract, peaking in the colon with its highly diverse community (Macpherson & Harris, [2004](#)). Under homeostatic conditions, this microbiota supports nutrient metabolism, vitamin synthesis, short-chain fatty acids (SCFA) production and pathogen defense (Kau et al., [2011](#); Topping & Clifton, [2001](#); McDermott & Huffnagle, [2014](#)). In addition, the gut microbiota profoundly influences the immune system, by balancing protective effector T cell responses with tolerogenic responses. This dynamic balance is crucial for a successful host-microbiota symbiosis. However, disruptions of the microbial composition and diversity, known as dysbiosis, alter the delicate balance between the microbiota and immune system, potentially leading to pathological conditions, including intestinal inflammatory disorders, autoimmune or metabolic diseases, allergy, and cancer (Fan & Pedersen, [2021](#); Hou et al., [2022](#); Suriano et al., [2022](#)). Initial evidence for the role of microbiota in immune regulation came from studies in germ-free (GF) mice, which exhibit a reduced proportion of Helios⁺ pT_{reg} cells, particularly in the colon (Kullberg et al., [2002](#); Ostman et al., [2006](#); Ishikawa et al., [2008](#)). These mice also show compromised production of IL-17 and IFN- γ , with an increased proportion of T_{H2} cells (Mazmanian et al., [2005](#); Hall et al., [2008](#); Ivanov et al., [2009](#)). Upon microbial colonization, pT_{reg} levels rise, non-

pathogenic T_{H1}/T_{H17} responses stabilize, and T_{H2} responses decrease, underscoring the microbiota's role in balancing T cell populations (Round & Mazmanian, [2010](#); Atarashi et al., [2011](#); Geuking et al., [2011](#); Atarashi et al., [2013](#); Faith et al., [2014](#); Ohnmacht et al., [2015](#); Sefik et al., [2015](#); Geva-Zatorsky et al., [2017](#); Xu et al., [2018](#); Abdel-Gadir et al., [2019](#)).

Further studies have revealed direct and indirect mechanisms through which gut microbes influence T cell responses. Microbes can directly modulate T cell responses through their molecular structures that interact with innate immune sensors or adaptive immune receptors. For example, *Bacteroides fragilis* produces capsular polysaccharide A, which binds Toll-like receptor 2 (TLR2) on plasmacytoid DCs to promote pT_{reg} differentiation (Mazmanian et al., [2008](#); Round & Mazmanian, [2010](#); Round et al., [2011](#); Dasgupta et al., [2014](#)). Similarly, the capsules of *Bifidobacterium bifidum* and *Escherichia coli* Nissle drive $ROR\gamma^+ T_{reg}$ differentiation via distinct mechanisms (Verma et al., [2018](#); Sassone-Corsi et al., [2022](#)). For instance, capsule components of *E. coli* Nissle promote both $ROR\gamma^+ T_{reg}$ and T_{H17} cell induction (Sassone-Corsi et al., [2022](#)), by activating a signaling cascade through intestinal epithelial cells (IECs). This cascade influences T cell activation by DCs and resembles the mechanisms triggered by Segmented Filamentous Bacteria (SFB) in the epithelium (Goto et al., [2014](#); Panea et al., [2015](#); Sano et al., [2015](#)). Bacteria can also indirectly support the induction of intestinal T_{reg} cells through the production of microbial metabolites. For instance, it has been reported that the murine gastrointestinal nematode parasite *Heligmosomoides polygyrus* expands T_{reg} cells by secreting a molecule that mimics TGF- β (Finney et al., [2007](#); Johnston et al., [2017](#)). Instead, species from *Clostridia* clusters IV and XIVa effectively induce pT_{reg} cells in the colon by producing SCFAs and creating an environment enriched with TGF- β and IDO, both of which positively regulate T_{reg} cells (Atarashi et al., [2011](#), [2013](#); Smith et al., [2013](#); Furusawa et al., [2013](#)). Colonization of mice with a mixture of 17 human-derived *Clostridia* strains results in

the accumulation of *Clostridia*-specific ROR γ ⁺Helios⁻ pT_{reg} cells, along with increased levels of immunosuppressive cytokines such as IL-10 and TGF- β , which are crucial for maintaining gut immune tolerance (Atarashi et al., [2011](#); Atarashi et al., [2013](#)).

One of the gut microbiota's essential contributions to host physiology is the generation of a diverse range of metabolites and bioactive compounds, which play a pivotal role in shaping the immune system by modulating both local and systemic immune responses through various mechanisms. SCFAs, such as butyrate, succinate, and propionate, are a notable group of microbial metabolites that foster intestinal T_{reg} induction. They are derivatives of the breakdown of indigestible dietary fibers, and serve multiple functions: they provide energy for gut microbes and colonocytes, regulate microbial and immunological activity, combat pathogens, and preserve gut integrity (Tao et al., [2007](#); Maslowski et al., [2009](#); Kim et al., [2013b](#); Atarashi et al., [2013](#); Smith et al., [2013](#); Arpaia et al., [2013](#); Furusawa et al., [2013](#); Ragonnaud & Biragyn, [2021](#)). The highest concentrations of SCFAs are found in the cecum and colon, where fermenting bacteria, particularly from the *Bacteroidetes* phylum, are highly abundant (Kuhn & Stappenbeck, [2013](#); Furusawa et al., [2013](#); Mowat & Agace, [2014](#)). SCFAs influence T cell differentiation at several levels. They modulate the activity of intestinal DCs, regulate T_{reg} migration, and induce epigenetic modifications in T cells. For example, butyrate binds to the receptor Gpr109a expressed on colonic immune cells, and its absence in Gpr109a-deficient mice leads to increased levels of pro-inflammatory cytokines IL-17 and IL-6, reduced levels of IL-10 and Aldh1a1, and a decline in T_{reg} frequencies in the colonic LP (Singh et al., [2014](#)). Similarly, propionate affects the homing properties of T_{reg} cells by engaging the receptor Gpr43 and upregulating Gpr15, a chemoattractant receptor essential for T_{reg} migration to the colonic LP (Smith et al., [2013](#); Schaper et al., [2014](#); Kim et al., [2016](#)). SCFAs also play a critical role in stabilizing the T_{reg} lineage (Mann et al., [2024](#)). They create an environment that allows T cells to differentiate

appropriately based on the immune context. During infection and inflammation, SCFAs suppress effector T cell responses, while under homeostatic conditions, they support pT_{reg} induction (Kim, Kang, et al., [2013](#)). This effect is particularly evident in the permissive histone acetylation of CNS-1 and CNS-3 regions in the *Foxp3* locus, observed under SCFA treatment or colonization with SCFA-producing microbiota. This process promotes increased *Foxp3* expression, without affecting the specification of other T_H cell lineages (Tao et al., [2007](#); Arpaia et al., [2013](#); Furusawa et al., [2013](#)).

Another important class of microbial-derived metabolites is the secondary bile acids (BAs). The gut microbiota plays a crucial role in BA metabolism. Primary BAs are synthesized in the liver and secreted into the duodenum, where they aid in emulsifying dietary lipids. Most are reabsorbed in the ileum and returned to the liver, but a portion escapes reabsorption and undergoes transformation by colonic bacteria into secondary BAs, through de-hydroxylation and de-conjugation reactions. Beyond their role in lipid digestion and absorption, BAs contribute to microbiota homeostasis and gut mucosal immune balance, by interacting with specific BA receptors, including nuclear receptors (VDR, PPAR, and FXR) and G protein-coupled receptors (TGR5 and S1PR2) (Hang et al., [2019](#); Song et al., [2020](#); Campbell et al., [2020](#); Sun et al., [2021](#); Li et al., [2021](#)). Certain BAs, such as glycocholic acid (GCA) and deoxycholic acid (DCA), exhibit anti-inflammatory properties. GCA strengthens intestinal barrier integrity, while DCA promotes colonization resistance, both limiting bacterial translocation and maintaining gut homeostasis (Sun et al., [2021](#)). Conversely, taurocholic acid (TCA) has been shown to enhance spore germination, outgrowth, and toxin activity in pathogenic strains of *Clostridium difficile* (Sun et al., [2021](#)). Moreover, innate immune cells also express BA receptors, and GCA shifts their phenotype from pro-inflammatory to anti-inflammatory. In T cells, GCA promotes T_{reg} differentiation, particularly of RORγt⁺ T_{reg} cells (Song et al., [2020](#)), and suppresses T_{H17} differentiation, thereby exerting a broad anti-inflammatory effect (Hang et al., [2019](#)).

Although several microbes and microbiota-derived constituents have been shown to modulate T_{reg} differentiation, stabilization, and expansion, many questions remain unanswered. While it is clear that the microbiota profoundly influences intestinal T_{reg} development and function, the exact cellular players and molecular pathways mediating this interaction are not fully understood. With over a thousand microbial species coexisting in a single adult gut, it remains unclear how T_{reg} cells integrate these diverse signals and what benefit the microbiota derives from inducing T_{reg} cells. This led Ramanan et al. to raise some provocative questions (Ramanan et al., [2023](#)). Are T_{reg} cells primarily a host mechanism to tolerate commensals, or are they, in part, manipulated by the microbiota to serve microbial interests? Are T_{reg} cells acting as generic regulators, controlling excessive immune responses indiscriminately to prevent tissue damage, or do they respond specifically to microbial antigens to direct tolerance and immunity in a microorganism-specific manner? Addressing these fundamental questions will provide critical insights into the nuanced relationship between the microbiota, microbiota-derived metabolites, and the immune system, potentially uncovering new therapeutic opportunities to modulate immune responses in health and disease.

9.2.4 CD8⁺ T Cell Subsets in the Intestinal Environment

Beside circulating memory CD8⁺ T cells, tissue-resident memory CD8⁺ T cells (T_{RM}) cells remain at the initial site of infection without recirculating, playing a critical role in local immune surveillance and rapid protection against reinfection (Gebhardt et al., [2009](#); Masopust et al., [2010](#); Gebhardt et al., [2011](#); Cheng & Becattini, [2022](#)) (Fig. [9.3](#)). CD8⁺ T_{RM} cells share certain features with T_{EM} cells, such as high CD44 expression and low CD62L expression. However, they are uniquely characterized by the expression of CD69 and CD103, as well as transcription factors such as Hobit, Blimp1 and Runx3 (Schön et al., [1999](#); Masopust et al., [2010](#); Mackay et al., [2013](#), [2016](#); Konjar et al., [2017](#); Milner et al., [2017](#); Bartolomé-Casado et al., [2019](#); Cheng &

Becattini, [2022](#)). Unlike T_{CM} cells, T_{RM} cells exhibit a certain degree of plasticity and reversible differentiation. Upon reinfection, some T_{RM} cells can proliferate and generate progeny that contribute to the circulating T_{EM} population, a subset referred to as “ex- T_{RM} ” cells (Fonseca et al., [2020](#); Barreto de Albuquerque et al., [2021](#); Liu et al., [2024](#)).

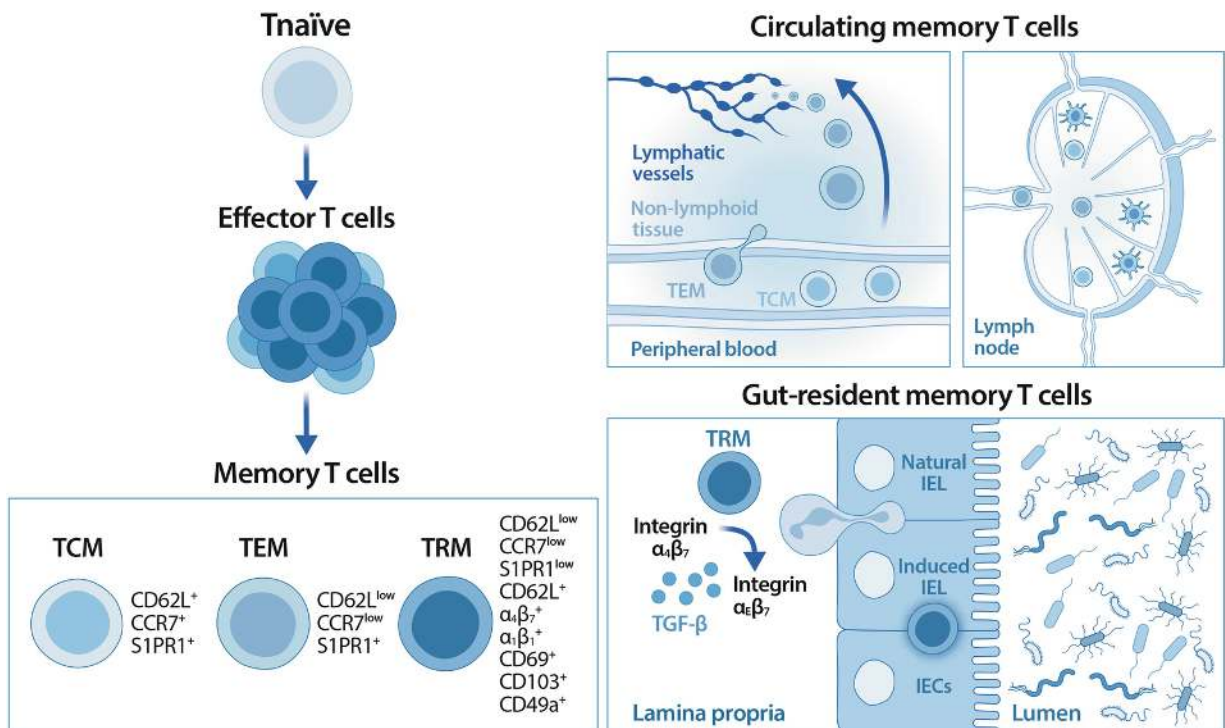


Fig. 9.3 CD8⁺ memory T cell formation. Upon antigen priming, naïve CD8⁺ T cells differentiate into effector T cells and undergo clonal expansion to mount a robust immune response. Once the pathogen is cleared, most effector T cells undergo apoptosis, while some differentiate into memory CD8⁺ T cells, including T_{CM} , T_{EM} , and T_{RM} subsets. These subsets differ in gene and surface marker expression, influencing their migratory behavior. T_{CM} cells circulate through blood, lymph, and SLOs, expressing CD62L and CCR7, which mediate extravasation and homing to SLOs. T_{EM} cells downregulate these markers, home to NLTs, and circulate through the spleen and bloodstream. T_{RM} cells remain at the initial site of infection without recirculating, mediated by homing receptors like CCR9 and α₄β₇, which facilitate migration to the GI tract. The local intestinal microenvironment further drives their differentiation into T_{RM} cells. TGF-β signaling induces CD103 expression in T_{RM} cells, replacing α₄ to form the α_Eβ₇ complex, a receptor for E-cadherin on IECs. T_{RM} cells can infiltrate the intestinal epithelium as induced IELs, alongside innate and innate-like natural IELs. (Figure created in <https://BioRender.com>)

CD8⁺ T_{RM} cells are predominantly localized at epithelial barrier sites, including the skin, lungs, reproductive organs, and GI tract. In the GI tract, T_{RM} cells migrate between the LP and IECs, but do not circulate between the small and large intestine (Thompson et al., [2019](#); Cheng & Becattini, [2022](#)) (Fig. [9.3](#)). The mechanisms governing the induction of gut T_{RM} cells remain incompletely understood, though evidence suggests that local DC populations play a key role. For example, DCs in PPs promote T_{RM} cell differentiation by inducing the tissue-specific chemokine receptors CCR9 and $\alpha_4\beta_7$ on T cells. The ligand for CCR9, CCL25, is predominantly produced by IECs in the small intestine, aligning with the strong CCR9-inducing potential of small intestinal DCs through their production of vitamin-A-derived retinoic acid. In contrast, colonic DCs induce higher expression of $\alpha_4\beta_7$, potentially due to lower expression of retinal dehydrogenase (*Raldh*), the enzyme involved in the conversion of vitamin A into retinoic acid. Integrin $\alpha_4\beta_7$ facilitates adhesion to mucosal addressin cell adhesion molecule-1 (MAdCAM-1), which is constitutively expressed in mLNs and a subset of vascular endothelial cells in both small and large intestinal LP. Once T cells acquire CCR9 and $\alpha_4\beta_7$, they home to the original antigen site within the GI tract, where local intestinal microenvironment, including cytokines such as TGF- β , IL-12, and type-I interferons, further drive their differentiation into T_{RM} cells (Berlin et al., [1993](#); Briskin et al., [1997](#); Mora et al., [2003](#); Iwata et al., [2004](#); Barreto de Albuquerque et al., [2021](#); Cheng & Becattini, [2022](#); Qiu et al., [2023](#)).

A defining feature of T_{RM} cells is the upregulation of retention factors alongside the downregulation of receptors involved in lymph node entry and circulation. TGF- β signaling induces integrin α_E (CD103) expression in T_{RM} cells, which replaces α_4 in the $\alpha_4\beta_7$ complex. The $\alpha_E\beta_7$ heterodimer binds to E-cadherin, a molecule expressed on IECs and subsets of DCs (Cepek et al., [1994](#); Borges da Silva et al., [2020](#); Barreto de Albuquerque et al., [2021](#); Cheng & Becattini, [2022](#)). However, the exact role of CD103 in T_{RM} establishment remains a topic

of debate. While initial studies suggested that CD103 is essential for T_{RM} maintenance in the intestinal tissue, but not for their accumulation, more recent findings indicate that CD103 enhances T_{RM} recruitment, but is dispensable for their retention in the tissue (Schön et al., [1999](#); Masopust et al., [2010](#); Casey et al., [2012](#); Borges da Silva et al., [2020](#)). Despite these discrepancies, CD103 remains a widely recognized hallmark of T_{RM} cells. Some $CD8^+$ T_{RM} cells also express $\alpha_1\beta_1$, which interacts with CD49a to bind collagen I and IV, further supporting T_{RM} adherence within intestinal tissues (Cheuk et al., [2017](#); Bromley et al., [2020](#); Barreto de Albuquerque et al., [2021](#)). In addition to integrin-mediated retention, T_{RM} cells downregulate key receptors involved in recirculation. S1PR1, an antagonist of CD69, is downregulated in T_{RM} cells and regulated by KLF2, a transcription factor that governs recirculation based on S1P gradients. Similarly, CCR7, a chemokine receptor essential for lymphoid homing and migration through afferent lymphatics, is also downregulated, thereby reinforcing tissue residency. The suppression of these migratory pathways prevents T_{RM} cells from leaving the intestine, ensuring their long-term retention within tissues (Bankovich et al., [2010](#); Cyster & Schwab, [2012](#); Skon et al., [2013](#); Mackay et al., [2015](#); Cheng & Becattini, [2022](#)). Although CD69 is widely used as a signature marker for identifying T_{RM} cells, emerging evidence suggests that it may be dispensable for T_{RM} function. In mice, CD69 was found to have no effect on $CD4^+$ T_{RM} differentiation, but negatively impacted $CD8^+$ T_{RM} development. Moreover, the influence of CD69 may vary depending on the organ, as studies indicate that CD69 is a reliable marker for T_{RM} residency in the human intestine (Walsh et al., [2019](#); Barreto de Albuquerque et al., [2021](#)).

Beyond T_{RM} cells residing in the LP, a subset of activated T_{RM} cells infiltrates in the intestinal epithelium, differentiating into IELs. Within this microenvironment, IELs interact with innate and innate-like lymphocytes, which include both TCR^- and TCR^+ subsets. Induced IELs are conventional $CD4^+$ or $CD8\alpha\beta^+$ $TCR\alpha\beta^+$ T cells, whereas natural IELs

lack CD4 or CD8 $\alpha\beta$ and instead express a CD8 $\alpha\alpha$ homodimer, paired with either TCR $\alpha\beta$ or TCR $\gamma\delta$. The developmental origins of natural IELs remain hitherto uncertain, though prevailing evidence suggests that the majority of TCR $\alpha\beta^+$ CD8 $\alpha\alpha^+$ IELs originate in the thymus, with an alternative extrathymic developmental pathway potentially contributing to their generation. Within the thymus, unconventional TCR $\alpha\beta^+$ IELs follow a distinct developmental trajectory that grants them innate-like properties (Konjar et al., [2017](#)). Unlike conventional T cells, natural IEL precursors do not undergo negative selection and instead exhibit high-affinity interactions with self-peptides presented on classical and nonclassical MHC-I molecules. Normally, such strong TCR signaling would induce apoptosis during negative selection; however, natural IELs escape clonal deletion through alternative mechanisms that remain incompletely understood (Kurd et al., [2021](#); Gui et al., [2022](#)).

Current data suggest two potential pathways for IEL development. One model proposes that DP thymocytes evade negative selection by downregulating CD8 $\alpha\beta$ while upregulating CD8 $\alpha\alpha$, thereby initiating an altered gene expression signature. Another hypothesis suggests that CD8 $\alpha\alpha^+$ IELs arise from TCR $\alpha\beta^+$ DP thymocytes receiving weak or altered selection signals, allowing them to differentiate into innate-like T cells. Following thymic selection, IELs express S1PR1, $\alpha_4\beta_7$, CD103, and CCR9, facilitating their migration to the intestinal epithelium, where they undergo further adaptation. Within the intestinal epithelium, IELs contribute to immune homeostasis, regulate epithelial integrity, and respond to microbial challenges, highlighting their essential role in mucosal immunity (Cheroutre et al., [2011](#); Ruscher et al., [2017](#); Gui et al., [2022](#)).

9.2.5 The Diverse Subsets of T_{reg} Cells in the Intestinal Environment

In the intestine, T_{reg} cells play a pivotal role in maintaining tolerance to self, dietary, and microbial antigens. While Foxp3-dependent epigenetic

mechanisms ensure T_{reg} lineage commitment and suppressive functionality, the intestinal microenvironment is crucial for T_{reg} accumulation and differentiation. Both tT_{reg} and pT_{reg} cells are present in the GI tract, with pT_{reg} cells representing the majority. Nevertheless, tT_{reg} cells make a significant contribution to the colonic T_{reg} pool, as evidenced by the striking similarity in TCR repertoires between thymus- and colon-derived T_{reg} cells (Cebula et al., [2013](#)). The generation of pT_{reg} depends on specific elements within the *Foxp3* locus, particularly the CNS-1, an enhancer located within the first intron (Lee & Lee, [2018](#)). This element is necessary for pT_{reg} development, but is largely dispensable for tT_{reg} generation (Tone et al., [2008](#)). BAC-transgenic Δ CNS-1 reporter mice enable the distinction between tT_{reg} cells and pT_{reg} cells based on CNS-1 dependency (Zhang et al., [2017](#)). The critical role of pT_{reg} cells in intestinal immunity is highlighted by CNS-1-deficient mice, which exhibit intestine-specific autoimmune diseases (Josefowicz et al., [2012](#)) and colitis in the absence of pT_{reg} cells (Haribhai et al., [2011](#)). Environmental antigens also influence intestinal T_{reg} dynamics. GF mice and those on antigen-free diets display significantly reduced T_{reg} frequencies in the intestine compared to specific pathogen-free (SPF) counterparts, underscoring the essential role of microbiota- and food-derived antigens in the establishment and maintenance of intestinal T_{reg} cells (Kim et al., [2016](#)). Notably, the T_{reg} -to-effector T cell ratio increases along the intestine, from the small intestine to the colon, driven by elevated levels of TGF- β and RA, which promote pT_{reg} generation (Lathrop et al., [2011](#)). Additionally, high levels of RA and microbial metabolites upregulate homing markers such as $\alpha_4\beta_7$ integrin, CCR9, and GPR15, facilitating T_{reg} migration to the gut (Siewert et al., [2007](#); Coombes et al., [2007](#); Sun et al., [2007](#); Smith et al., [2013](#)). Remarkably, tT_{reg} cells migrate to the gut early in ontogeny and occupy a niche that is independent of IL-2 and MHC-II antigen presentation (Korn et al., [2014](#); Torow et al., [2015](#)). These tT_{reg} cells contribute to the suppressive environment, without

requiring recognition of foreign antigens. Meanwhile, pT_{reg} proportions vary with environmental factors: food-derived antigens influence pT_{reg} cells in the small intestine, whereas microbial colonization determines their levels in the colon (Kim et al., [2016](#)). tT_{reg} cells, however, remain unaffected by diet or colonization status (Sefik et al., [2015](#); Kim et al., [2016](#)).

Beyond their origins, T_{reg} subsets are further defined by the expression of additional lineage-specifying transcription factors. Among intestinal T_{reg} cells, Gata3 and RORγt expression delineate three dominant subpopulations: Gata3⁺, RORγt⁻ and RORγt⁺ T_{reg} cells. Gata3⁺ T_{reg} cells, comprising up to 20% of the intestinal T_{reg} cells (Wohlfert et al., [2011](#); Schiering et al., [2014](#); Ohnmacht et al., [2015](#); Tanoue et al., [2016](#)), predominantly originate from the thymus, as indicated by their expression of Helios (Wohlfert et al., [2011](#)). These cells rely on IL-33 signaling for their induction and accumulation, especially during inflammation (Schiering et al., [2014](#)). Gata3⁺ T_{reg} cells play critical roles in tissue repair, responding to IL-33 by upregulating amphiregulin (AREG), a key repair factor (Wohlfert et al., [2011](#); Schiering et al., [2014](#); Hegazy & Powrie, [2015](#)). IL-33R activation, together with TCR signaling and IL-2, triggers *Gata3* expression, which enhances T_{reg}-mediated suppression (Wohlfert et al., [2011](#)). Loss of *Gata3* expression leads to reduced T_{reg} accumulation in the small intestine, especially during inflammation, where Gata3⁺ T_{reg} cells are essential for protecting from inflammation caused by pathogens (Wohlfert et al., [2011](#)). Despite broad-spectrum antibiotic treatments reducing overall colonic T_{reg} numbers, Gata3⁺ T_{reg} cells persist, suggesting independence from microbiota-derived cues (Geuking et al., [2011](#); Sefik et al., [2015](#)). The small intestine mostly harbors RORγt⁻Helios⁻ T_{reg} cells, a subset influenced by food-derived antigens that support oral tolerance (Kim et al., [2016](#)). In line with the decreasing concentration of dietary antigens from the small intestine to the colon, the frequency of these cells also decreases. In contrast, RORγt⁺Helios⁻ T_{reg} cells represent the largest

intestinal T_{reg} subset, comprising 35% of T_{reg} cells in the small intestine and 65% in the colon (Ohnmacht et al., [2015](#); Sefik et al., [2015](#); Yang et al., [2016](#)). This subset of T_{reg} cells promotes tolerance to the gut microbiota, as discussed above, and regulates distinct effector cells, including T_{H17} and ILC3s (Ohnmacht et al., [2015](#); Sefik et al., [2015](#); Bapat et al., [2015](#); Yang et al., [2016](#)). RORγt⁺ T_{reg} cells exhibit an activated phenotype, characterized by markers such as CD44, ICOS, and CTLA-4 (Ohnmacht et al., [2015](#); Cosovanu & Neumann, [2020](#)), and produce IL-10, a key anti-inflammatory cytokine (Rubtsov et al., [2008](#)). Importantly, mice deficient in RORγt⁺ T_{reg} cells in the intestine display microbial dysbiosis, exacerbated colitis, increased susceptibility to food allergies, autoimmune diseases, and cancer (Tartar et al., [2010](#); Ohnmacht et al., [2015](#); Sefik et al., [2015](#); Yang et al., [2016](#); Chellappa et al., [2016](#); Ye et al., [2017](#); Xu et al., [2018](#); Neumann et al., [2019](#); Al Nabhani et al., [2019](#); Abdel-Gadir et al., [2019](#)). The dependence of RORγt⁺ T_{reg} cells on microbiota is highlighted by their absence in GF or antibiotic-treated mice (Ohnmacht et al., [2015](#); Sefik et al., [2015](#); Whibley et al., [2019](#)). Notably, *Clostridiales* are well-known for their ability to foster a tolerogenic environment by inducing RORγt⁺ T_{reg} cells (Zhou et al., [2008](#); Voo et al., [2009](#); Ayyoub et al., [2009](#); Ohnmacht et al., [2015](#); Sefik et al., [2015](#)). Besides gut microbiota, dietary factors also influence T_{reg} dynamics in the gut. For instance, dietary tryptophan is metabolized by gut microbes into microbial metabolites, which act as AhR ligands and regulate RORγt⁺ T_{reg} proliferation in the gut. In contrast, tryptophan deficiency results in RORγt⁺ T_{reg} expansion and parallel reduction of the Gata3⁺ T_{reg} population, thus highlighting the interplay between diet, microbial metabolism, and T_{reg} subset balance (Rankin et al., [2023](#)). Interestingly, the development of colonic T_{reg} subsets is also temporally regulated (Pratama et al., [2020](#)). Gata3⁺Helios⁺ T_{reg} cells dominate early in life due to limited bacterial presence in the gut. However, at weaning, the dietary shift from milk to solid food, as well as rapid microbial expansion, drives the development

of other T_{reg} populations. Altogether, these intestinal T_{reg} subsets exemplify the intricate interplay between host and environmental cues, with distinct roles in inflammation resolution, tissue repair, and immune suppression. Understanding these subsets and their regulation offers critical insights into the mechanisms underlying intestinal immunity and provides a foundation for therapeutic strategies targeting immune-mediated gut disorders.

9.2.6 Origin and Migration of RORyt⁺ T_{reg} Cells

Recent years have seen significant progress in the understanding of the development and functional dynamics of RORyt⁺ T_{reg} cells. The induction of these cells in vivo is dependent on the canonical T_{H17}-polarizing IL-6-/IL-23 signaling pathway (Ohnmacht et al., [2015](#); Sefik et al., [2015](#)). The process of induction is modulated by intestinal CX3CR1⁺ APCs, which integrate microbiota-derived stimuli in a MyD88-independent manner, while CD103⁺ DCs can suppress RORyt⁺ T_{reg} induction via the CD40-CD40L pathway (Solomon & Hsieh, [2016](#); Barthels et al., [2017](#)). The site of initial induction for RORyt⁺ T_{reg} cells, whether in mLNs or directly in the colonic LP, remains unknown. Evidence suggests that these cells primarily derive from pT_{reg} cells, given their reliance on microbiota for their generation and the lack of tT_{reg} markers like Helios. In line with this, adoptive transfer studies confirm that CD4⁺ Tnaïve cells preferentially differentiate into RORyt⁺ T_{reg} cells (Solomon & Hsieh, [2016](#)). However, TCR repertoire analyses of colonic T_{reg} cells revealed contrasting findings: Lathrop et al. demonstrated that colonic T_{reg} cells and tT_{reg} cells exhibit distinct TCR repertoires (Lathrop et al., [2011](#)), while Cebula et al. observed, using TCR repertoire profiling at the single-cell level, that tT_{reg} cells predominantly mediate tolerance to microbiota-derived antigens (Cebula et al., [2013](#)). Therefore, it is reasonable to think that tT_{reg} cells upregulate RORyt expression in the intestine. Consistent with this hypothesis, preliminary observations have revealed that tT_{reg} cells are

fully capable of upregulating ROR γ t both in vivo and in vitro (Yang et al., [2016](#)). However, due to the lack of methods to unambiguously distinguish pT $_{\text{reg}}$ from tT $_{\text{reg}}$ cells, uncertainty persists regarding their differentiation mechanisms and physiological roles.

Beyond their origin, another challenge concerning ROR γ t⁺ T $_{\text{reg}}$ cells lies in understanding their function. These cells exhibit diverse functionalities, ranging from beneficial to detrimental to the host in a variety of different pathological contexts. For instance, they are fully suppressive in colon to partly pro-inflammatory in crescentic glomerulonephritis and pancreatic cancer (Tartar et al., [2010](#); Ohnmacht et al., [2015](#); Sefik et al., [2015](#); Chellappa et al., [2016](#)). Recent studies indicate developmental and functional parallels between ROR γ t⁺ T $_{\text{reg}}$ cells and homeostatic T $_{\text{H17}}$ cells (van Veen et al., [2022](#)). Van der Veen et al. utilized genetic tracing models to selectively label newly induced pT $_{\text{reg}}$ cells to better investigate the role of Foxp3 induction in these cells. Single-cell RNA sequencing (scRNA-seq) analysis revealed that numerous transcriptional features of newly differentiated pT $_{\text{reg}}$ cells in mLNs are induced independently of Foxp3. Moreover, these features are shared with T $_{\text{H17}}$ cells, suggesting that pT $_{\text{reg}}$ and T $_{\text{H17}}$ cells share an early developmental transcriptional program prior to Foxp3 induction. To evaluate Foxp3-independent features of newly differentiated pT $_{\text{reg}}$ cells, colonic Foxp3-deficient reporter-null cells were analyzed. These cells exhibited characteristics similar to T $_{\text{H17}}$ cells, but maintained their commitment to the T $_{\text{reg}}$ lineage, as demonstrated by the stable expression of the Foxp3 reporter-null allele. Moreover, even in the absence of Foxp3 protein, these cells maintained a tolerogenic functional behavior, rather than an inflammatory one, as evidenced by their capacity to suppress T cell expansion in the colon (van Veen et al., [2022](#)). This study challenges the conventional perspective that pT $_{\text{reg}}$ and T $_{\text{H17}}$ cells are strictly antagonistic. Instead, it posits that pT $_{\text{reg}}$ cells constitute a subset of T $_{\text{H17}}$ -like cells that progressively acquire Foxp3 expression and tolerogenic functions during differentiation. This gradual process

refines the immunomodulatory and tissue-supportive functions of homeostatic T_{H17} cells, highlighting a continuum in the acquisition of tolerogenic properties (van Veecken et al., [2022](#)). This assertion is further corroborated by analogous observations derived from other studies. For instance, it was discovered that nearly 15% of colonic RORγt⁺ T_{reg} cells have a history of *Il17a* expression, indicating that some IL-17-producing cells retain the ability to subsequently induce Foxp3. Additionally, the absence of the CNS-1 in the *Foxp3* locus was shown to hinder, but not entirely prevent, peripheral Foxp3 induction in a TCR transgenic system (Nutsch et al., [2016](#); Pratama et al., [2020](#)). These findings collectively underscore that, even in the absence of Foxp3 protein, Foxp3 expression in colonic pT_{reg} cells is persistently enforced by cell-intrinsic or extrinsic stimuli. While the exact nature of these signals remains unclear, potential candidates include TGF-β, STAT3-activating cytokines, c-Maf, and other transcription factors implicated in pT_{reg} differentiation. It is also of interest that RORγt⁺ T_{reg} cells might consist of varying subpopulations originating from different T_{reg} progenitors, a notion supported by the identification of two distinct RORγt⁺ T_{reg} populations in humans (Halim et al., [2017](#)). Nevertheless, to gain a more nuanced understanding of the modulatory composition of T_{reg} functionality within the intestinal environment, a more thorough examination of T_{reg} heterogeneity and the contribution of tT_{reg} to the pool of intestinal T_{reg} cells is necessary.

Notably, while increasing proportions of RORγt⁺ T_{reg} cells may help prevent inflammation, their levels in mice are homeostatically regulated, with microbial manipulations unable to surpass a pre-defined threshold (Geva-Zatorsky et al., [2017](#)). A seminal study by Al Nabhani et al. revealed that around weaning, the host mounts the first, strong, and broad immune reactivity to the expanding microbiota. This phenomenon, known as the weaning reaction, occurs only in a specific time window of opportunity and plays a pivotal role in the ontogenesis of the immune system, programming it towards a homeostatic imprinting rather than a pathological one (Al Nabhani et al., [2019](#)). The

authors attributed the protective effect of the weaning reaction to the induction of RORyt⁺ T_{reg} cells. Transient depletion of T_{reg} cells during this critical period eliminated the protective benefits of the weaning reaction later in life. Remarkably, these animals exhibited increased susceptibility to inflammation despite having normal T_{reg} numbers at the time of the challenge. This finding underscores the essential role of RORyt⁺ T_{reg} cells generated during the weaning reaction in preventing excessive immune responses and reducing vulnerability to various inflammatory pathologies in later life (Al Nabhani et al., [2019](#)). Goblet-cell-associated antigen passages (GAPs) facilitate antigen delivery during this period, inducing microbiota-specific RORyt⁺ T_{reg} cells that maintain long-term tolerance to analogous bacteria in later life. It is hypothesized that GAPs open once the epidermal growth factor level in the maternal milk recedes below a threshold, and close by yet unknown mechanisms (Al Nabhani et al., [2019](#)).

Similarly, another study revealed the importance of maternal milk in the early development of RORyt⁺ T_{reg} cells. Ramanan et al. demonstrated that colonic RORyt⁺ T_{reg} proportions are maternally transmitted during a brief postnatal window and remain stable throughout life. This transmission, linked to secretory IgA (SIgA) production and coating of gut commensals, operates via a double-negative feedback loop involving the entero-mammary axis. Maternal SIgA levels transferred via milk in early life influence microbial coating in the neonatal gut, modifying their stimulatory properties and their ability to induce RORyt⁺ T_{reg} cells (Ramanan et al., [2020](#)). Specifically, in the maternal colon, RORyt⁺ T_{reg} cells suppress IgA-producing plasma cells, reducing SIgA levels in the gut lumen. SIgA is transported to the mammary glands and secreted in milk, reaching the neonatal colon. IgA-coating of bacteria dampens RORyt⁺ T_{reg} induction in the neonatal gut (Ramanan et al., [2020](#)). When the female mouse herself becomes pregnant, this low or high IgA trait is passed to offspring via milk, perpetuating the cycle across generations. This immunological mechanism of multi-generational transmission may provide

adaptability and fine-tune gut immune responses, influencing susceptibility to inflammatory diseases (Ramanan et al., [2020](#)).

Beyond their role in mucosal health, ROR γ t⁺ T_{reg} cells act as “gut-trained emissaries,” promoting tissue regeneration at extra-intestinal sites, such as injured skeletal muscle and fatty liver (Hanna et al., [2023](#)). For instance, in injured muscles, local inflammatory mediators, in combination with MHC-II-dependent T cell activation, drive the accumulation of gut-derived ROR γ t⁺ T_{reg} cells. Once having migrated into the muscles, these cells suppress IL-17A-producing T cells while enhancing muscle stem cell differentiation, facilitating inflammation resolution and tissue repair (Hanna et al., [2023](#)). This study highlights the far-reaching impact of colonic ROR γ t⁺ T_{reg} cells beyond their site of origin. As ROR γ t⁺ T_{reg} cells primarily respond to commensal antigens, their TCR-mediated proliferation and retention in regenerating muscle raise intriguing questions about the role of local antigens in triggering this response. The authors propose cross-reactivity between microbial antigens and autoantigens, facilitated by epitope mimicry, as a potential explanation, particularly given that tissue damage and inflammation could enhance autoantigen presentation. The concept that enteric microbes can drive effector T cell responses to extra-gut challenges is well established (Mazmanian et al., [2008](#); Wu et al., [2010](#); Ichinohe et al., [2011](#); Kim et al., [2017](#); Mager et al., [2020](#)). Extending this paradigm to T_{reg} cells raises the possibility of a self-regulating system, where gut-trained effector and T_{reg} cells coordinate rapid homeostasis restoration, while preventing excessive microbiota-induced inflammatory responses (Hanna et al., [2023](#)). Notably, a fraction of ROR γ t⁺ T_{reg} cells constantly migrates from the colon to extra-gut sites at steady-state conditions. This raises the question of whether ROR γ t⁺ T_{reg} cells in injured muscles originate directly from the colon or transit through intermediary tissues. To formally address this point, sophisticated cell tracking experiments to delineate their migration pathways and mechanisms will be required.

Outlook

Although the intestinal barrier is designed to minimize direct contact between bacteria and immune components, a vast array of T cell responses is continuously initiated. The necessary permeability of the gut, essential for nutrient absorption, requires the immune system to maintain a delicate balance between effector T cells and tolerogenic T_{reg} responses. As the largest compartment of T cells in the body, the gut represents an energy-intensive immune niche, where maintaining homeostasis prevents excessive inflammation, while allowing controlled immune responses against microbial threats.

Disruption of the intestinal barrier and dysregulation of T cell responses can lead to chronic inflammatory diseases such as IBD and gastrointestinal graft-versus-host disease (GvHD). Despite differences in pathophysiology, both conditions share common hallmarks, including loss of barrier integrity, microbial dysbiosis characterized by reduced microbial diversity and loss of beneficial species, and excessive activation of effector T cells (Haring et al., [2021](#)). In murine models of GvHD, inflammation-induced upregulation of MHC-II on IECs has been shown to drive clonal expansion of microbiota-specific CD4⁺ T cells, correlating with disease severity and lethality (Yeh et al., [2024](#)). Studies of human gastrointestinal biopsies could show that GvHD severity correlated with clonal expansion of cytotoxic CD8⁺ T cells. The suppressive capacity of gastrointestinal T_{reg} cells was dependent on microbiota diversity and the presence of SCFA-producing bacteria (Jarosch et al., [2023](#)). Similarly, IBD is associated with an imbalance between pro-inflammatory T_{H17} cells and regulatory T cells, a process significantly influenced by gut microbiota (Bleich & Mahler, [2005](#); Seiderer et al., [2008](#); Kobayashi et al., [2008](#); Britton et al., [2019](#); Haring et al., [2021](#)). Microbiome analyses from IBD patients reveal a reduction in beneficial bacteria, such as *Faecalibacterium prausnitzii* (Sokol et al., [2009](#); Cao et al., [2014](#); Zhao et al., [2021](#); Touch et al., [2022](#)) and *Bifidobacterium* (Yao et al., [2021](#); Bosselaar et al., [2024](#)), alongside an increase in pro-inflammatory species like *E. coli* (Martin et al., [2004](#);

Darfeuille-Michaud et al., [2004](#); Zhang et al., [2022](#); Khorsand et al., [2022](#); Zheng et al., [2022](#)) and *Fusobacterium* (Strauss et al., [2011](#); Liu et al., [2019](#), [2020](#); Zhang et al., [2022](#)).

Therapeutic strategies for both GvHD and IBD aim to restore immune homeostasis and reduce inflammation. Microbiota-centered interventions, including anti-inflammatory diets, probiotics, and fecal microbiota transplantation (FMT), have demonstrated promising effects in both diseases (Spindelboeck et al., [2017](#); Guandalini & Sansotta, [2019](#); Darb Emamie et al., [2021](#); Sasson et al., [2021](#); Cusimano & Damas, [2022](#); Boicean et al., [2023](#); Feng et al., [2023](#); Weber et al., [2024](#); DeFilipp et al., [2024](#)). However, challenges such as infection risks and variability in clinical efficacy highlight the need for further research to optimize bacterial engraftment and identify suitable FMT donors (Weber et al., [2024](#); Yadegar et al., [2024](#)). Conventional approaches include immunosuppressive agents such as corticosteroids, the JAK 1/2 inhibitor Ruxolitinib (Rudra et al., [2022](#); DeFilipp et al., [2024](#); Denk et al., [2024](#)), and anti-TNF α therapies (van Dullemen et al., [1995](#); Yalniz et al., [2017](#)). Additionally, targeted strategies blocking gut-homing integrins, such as Vedolizumab targeting $\alpha_4\beta_7$, have demonstrated efficacy in modulating gut-specific T cell migration (Fløisand et al., [2017](#); Scribano, [2018](#); Danylesko et al., [2019](#); Crooks et al., [2020](#)). The therapeutic potential of T_{reg} cells is becoming increasingly apparent in addressing a spectrum of immune-mediated disorders, including autoimmune diseases, transplant rejection, and inflammatory conditions such as IBD (Raffin et al., [2020](#)). For instance, approaches such as Smad7 inhibition (Monteleone et al., [2001](#), [2023](#)) and adoptive T_{reg} transfer showed therapeutic potential in IBD (Desreumaux et al., [2012](#); Voskens et al., [2021](#); Laukova & Glatman Zaretsky, [2023](#)), and enhancing T_{reg} induction in donor grafts before transplantation has proven beneficial (Di Ianni et al., [2011](#); MacMillan et al., [2021](#); Guo et al., [2021](#)). Over 50 clinical trials have explored T_{reg}-based adoptive cell therapy, demonstrating its excellent safety profile and feasibility for clinical use (Raffin et al., [2020](#)). Innovative strategies to enhance T_{reg}

therapies include the development of antigen-specific T_{reg} cells, which offer superior efficiency and localized action compared to polyclonal T_{reg} cells. Advancements such as CRISPR-based gene editing and chimeric antigen receptor (CAR) engineering are expanding the horizons of T_{reg} therapies, enabling precise modulation of specificity, stability, and persistence. For instance, engineered T_{reg} cells have shown promise in reducing pathological immune responses without generalized immunosuppression by leveraging their site-specific activity (Raffin et al., [2020](#)). More recently, CAR T_{reg} cells engineered to target microbial antigens, such as flagellin, have shown promise in IBD by promoting intestinal epithelial integrity and exerting potent suppressive effects (Raffin et al., [2020](#); Boardman et al., [2023](#)). Furthermore, the development of “off-the-shelf” universal donor T_{reg} cells through genome editing is an emerging frontier, potentially overcoming the logistical challenges of autologous T_{reg} therapies (Raffin et al., [2020](#)).

Despite these advancements, key challenges remain, particularly in ensuring the long-term stability of engineered T_{reg} cells and preventing their pathogenic conversion under inflammatory conditions. Future research should focus on refining cell engineering, improving scalability, and elucidating the mechanisms by which T_{reg} cells modulate inflammation and tissue repair. Combining T_{reg}-based therapies with adjunctive strategies, such as low-dose IL-2 or targeted chemokine receptor expression, may further optimize their therapeutic potential. As our understanding of gut T cell dynamics and microbiota interactions deepens, novel strategies will continue to emerge, offering promising avenues for the treatment of immune-mediated diseases in the gut and beyond.

Acknowledgments

This project has received funding from the European Union’s Horizon 2020 research and innovation program under the Marie Skłodowska-Curie grant agreement No 955321 and from the Deutsche

Forschungsgemeinschaft (DFG, German Research Foundation)
(SFB/TRR 355/1, project number: 490846870 and SFB 1371/P04,
project number: 395357507).

References

Abdel-Gadir, A., Stephen-Victor, E., Gerber, G. K., Noval Rivas, M., Wang, S., Harb, H., Wang, L., Li, N., Crestani, E., Spielman, S., Secor, W., Biehl, H., DiBenedetto, N., Dong, X., Umetsu, D. T., Bry, L., Rachid, R., & Chatila, T. A. (2019). Microbiota therapy acts via a regulatory T cell MyD88/ROR γ t pathway to suppress food allergy. *Nature Medicine*, 25(7), 1164–1174.

[\[PubMed\]](#)

Agace, W. W. (2006). Tissue-tropic effector T cells: Generation and targeting opportunities. *Nature Reviews. Immunology*, 6(9), 682–692.

[\[PubMed\]](#)

Akagbosu, B., Tayyebi, Z., Shibu, G., Paucar Iza, Y. A., Deep, D., Parisotto, Y. F., Fisher, L., Pasolli, H. A., Thevin, V., Elmentaite, R., Knott, M., Hemmers, S., Jahn, L., Friedrich, C., Verter, J., Wang, Z.-M., van den Brink, M., Gasteiger, G., Grünewald, T. G. P., Marie, J. C., Leslie, C., Rudensky, A. Y., & Brown, C. C. (2022). Novel antigen-presenting cell imparts Treg-dependent tolerance to gut microbiota. *Nature*, 610(7933), 752–760.

[\[https://doi.org/10.1038/s41586-022-05309-5\]](https://doi.org/10.1038/s41586-022-05309-5)[\[PubMed\]](#)

Al Nabhani, Z., Dulauroy, S., Marques, R., Cousu, C., Al Bounny, S., Déjardin, F., Sparwasser, T., Bérard, M., Cerf-Bensussan, N., & Eberl, G. (2019). A weaning reaction to microbiota is required for resistance to immunopathologies in the adult. *Immunity*, 50(5), 1276–1288.e5.

[\[PubMed\]](#)

Ali, N., Zirak, B., Rodriguez, R. S., Pauli, M. L., Truong, H.-A., Lai, K., Ahn, R., Corbin, K., Lowe, M. M., Scharschmidt, T. C., Taravati, K., Tan, M. R., Ricardo-Gonzalez, R. R., Nosbaum, A., Bertolini, M., Liao, W., Nestle, F. O., Paus, R., Cotsarelis, G., Abbas, A. K., & Rosenblum, M. D. (2017). Regulatory T cells in skin facilitate epithelial stem cell differentiation. *Cell*, 169(6), 1119–1129.e11.

[\[PubMed\]](#)

Allaire, J. M., Crowley, S. M., Law, H. T., Chang, S.-Y., Ko, H.-J., & Vallance, B. A. (2018). The intestinal epithelium: Central coordinator of mucosal immunity. *Trends in Immunology*, 39(9), 677–696.

[\[PubMed\]](#)

Angkasekwinai, P., & Dong, C. (2021). IL-9-producing T cells: Potential players in allergy and cancer. *Nature Reviews. Immunology*, 21(1), 37–48.

[\[PubMed\]](#)

Arpaia, N., Campbell, C., Fan, X., Dikiy, S., van der Veen, J., deRoos, P., Liu, H., Cross, J. R., Pfeffer, K., Coffey, P. J., & Rudensky, A. Y. (2013). Metabolites produced by commensal bacteria promote peripheral regulatory T-cell generation. *Nature*, 504(7480), 451–455.

[\[https://doi.org/10.1038/nature12726\]](https://doi.org/10.1038/nature12726)[\[PubMed\]](#)

Atarashi, K., Tanoue, T., Shima, T., Imaoka, A., Kuwahara, T., Momose, Y., Cheng, G., Yamasaki, S., Saito, T., Ohba, Y., Taniguchi, T., Takeda, K., Hori, S., Ivanov, I. I., Umesaki, Y., Itoh, K., & Honda, K. (2011). Induction of colonic regulatory T cells by indigenous *Clostridium* species. *Science*, 331(6015), 337–341.

[\[PubMed\]](#)

Atarashi, K., Tanoue, T., Oshima, K., Suda, W., Nagano, Y., Nishikawa, H., Fukuda, S., Saito, T., Narushima, S., Hase, K., Kim, S., Fritz, J. V., Wilmes, P., Ueha, S., Matsushima, K., Ohno, H., Olle, B., Sakaguchi, S., Taniguchi, T., Morita, H., Hattori, M., & Honda, K. (2013). Treg induction by a rationally selected mixture of *Clostridia* strains from the human microbiota. *Nature*, 500(7461), 232–236.

[\[https://doi.org/10.1038/nature12331\]](https://doi.org/10.1038/nature12331) [\[PubMed\]](#)

Ayyoub, M., Deknuydt, F., Raimbaud, I., Dousset, C., Leveque, L., Bioley, G., & Valmori, D. (2009). Human memory FOXP3⁺ Tregs secrete IL-17 ex vivo and constitutively express the T(H)17 lineage-specific transcription factor RORgamma t. *Proceedings of the National Academy of Sciences of the United States of America*, 106(21), 8635–8640.

[\[PubMed\]](#)

Bain, C. C., & Mowat, A. M. (2014). Macrophages in intestinal homeostasis and inflammation. *Immunological Reviews*, 260(1), 102–117.

[\[PubMed\]](#)

Banchereau, J., Pascual, V., & O'Garra, A. (2012). From IL-2 to IL-37: The expanding spectrum of anti-inflammatory cytokines. *Nature Immunology*, 13(10), 925–931.

[\[PubMed\]](#)

Bankovich, A. J., Shiow, L. R., & Cyster, J. G. (2010). CD69 suppresses Sphingosine 1-Phosphate Receptor-1 (S1P1) function through interaction with membrane helix 4. *The Journal of Biological Chemistry*, 285(29), 22328–22337.

[\[PubMed\]](#)

Bapat, S. P., Myoung Suh, J., Fang, S., Liu, S., Zhang, Y., Cheng, A., Zhou, C., Liang, Y., LeBlanc, M., Liddle, C., Atkins, A. R., Yu, R. T., Downes, M., Evans, R. M., & Zheng, Y. (2015). Depletion of fat-resident Treg cells prevents age-associated insulin resistance. *Nature*, 528(7580), 137–141.

[\[https://doi.org/10.1038/nature16151\]](https://doi.org/10.1038/nature16151) [\[PubMed\]](#)

Barreto de Albuquerque, J., Mueller, C., & Gungor, B. (2021). Tissue-resident T cells in chronic relapsing-remitting intestinal disorders. *Cells*, 10(8), 1882.

[\[PubMed\]](#)

Barthels, C., Ogrinc, A., Steyer, V., Meier, S., Simon, F., Wimmer, M., Blutke, A., Straub, T., Zimmer-Strobl, U., Lutgens, E., Marconi, P., Ohnmacht, C., Garzetti, D., Stecher, B., & Brocker, T. (2017).

CD40-signalling abrogates induction of RORγt⁺ Treg cells by intestinal CD103⁺ DCs and causes fatal colitis. *Nature Communications*, 8(1), 14715.

[\[PubMed\]](#)

Bartolomé-Casado, R., Landsverk, O. J. B., Chauhan, S. K., Richter, L., Phung, D., Greiff, V., Risnes, L. F.,

Yao, Y., Neumann, R. S., Yaqub, S., Øyen, O., Horneland, R., Aandahl, E. M., Paulsen, V., Sollid, L. M., Qiao, S.-W., Baekkevold, E. S., & Jahnsen, F. L. (2019). Resident memory CD8 T cells persist for years in human small intestine. *The Journal of Experimental Medicine*, 216(10), 2412–2426.
[\[PubMed\]](#)

Bennett, C. L., Christie, J., Ramsdell, F., Brunkow, M. E., Ferguson, P. J., Whitesell, L., Kelly, T. E., Saulsbury, F. T., Chance, P. F., & Ochs, H. D. (2001). The immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX) is caused by mutations of FOXP3. *Nature Genetics*, 27(1), 20–21.
[\[PubMed\]](#)

Berlin, C., Berg, E. L., Briskin, M. J., Andrew, D. P., Kilshaw, P. J., Holzmann, B., Weissman, I. L., Hamann, A., & Butcher, E. C. (1993). $\alpha 4\beta 7$ integrin mediates lymphocyte binding to the mucosal vascular addressin MAdCAM-1. *Cell*, 74(1), 185–195.
[\[PubMed\]](#)

Bhandoola, A., Von Boehmer, H., Petrie, H. T., & Zúñiga-Pflücker, J. C. (2007). Commitment and developmental potential of extrathymic and intrathymic T cell precursors: Plenty to choose from. *Immunity*, 26(6), 678–689.
[\[PubMed\]](#)

Bilate, A. M., & Lafaille, J. J. (2012). Induced CD4⁺Foxp3⁺ regulatory T cells in immune tolerance. *Annual Review of Immunology*, 30, 733–758.
[\[PubMed\]](#)

Bleich, A., & Mahler, M. (2005). Environment as a critical factor for the pathogenesis and outcome of gastrointestinal disease: Experimental and human inflammatory bowel disease and helicobacter-induced gastritis. *Pathobiology: Journal of Immunopathology and Molecular & Cellular Biology*, 72(6), 293–307.

Boardman, D. A., Wong, M. Q., Rees, W. D., Wu, D., Himmel, M. E., Orban, P. C., Vent-Schmidt, J., Zachos, N. C., Steiner, T. S., & Levings, M. K. (2023). Flagellin-specific human CAR Tregs for immune regulation in IBD. *Journal of Autoimmunity*, 134, 102961.
[\[PubMed\]](#)

Boicean, A., Birlutiu, V., Ichim, C., Anderco, P., & Birsan, S. (2023). Fecal microbiota transplantation in inflammatory bowel disease. *Biomedicine*, 11(4), 1016.

Bopp, T., Becker, C., Klein, M., Klein-Heßling, S., Palmetshofer, A., Serfling, E., Heib, V., Becker, M., Kubach, J., Schmitt, S., Stoll, S., Schild, H., Staeger, M. S., Stassen, M., Jonuleit, H., & Schmitt, E. (2007). Cyclic adenosine monophosphate is a key component of regulatory T cell-mediated suppression. *The Journal of Experimental Medicine*, 204(6), 1303–1310.
[\[PubMed\]](#)

Borges da Silva, H., Peng, C., Wang, H., Wanhainen, K. M., Ma, C., Lopez, S., Khoruts, A., Zhang, N., & Jameson, S. C. (2020). Sensing of ATP via the purinergic receptor P2RX7 promotes CD8⁺ Trm cell generation by enhancing their sensitivity to the cytokine TGF- β . *Immunity*, 53(1), 158–171.e6.
[\[PubMed\]](#)

Bosselaar, S., Dhelin, L., Dautel, E., Titecat, M., Duthoy, S., Stelmaszczyk, M., Delory, N., De Sousa, V. M., Machuron, F., Ait-Abderrahim, H., Desreumaux, P., Foligné, B., & Monnet, C. (2024). Taxonomic and phenotypic analysis of bifidobacteria isolated from IBD patients as potential probiotic strains. *BMC Microbiology*, 24(1), 233.

[\[PubMed\]](#)

Briskin, M., Winsor-Hines, D., Shyjan, A., Cochran, N., Bloom, S., Wilson, J., McEvoy, L. M., Butcher, E. C., Kassam, N., Mackay, C. R., Newman, W., & Ringler, D. J. (1997). Human mucosal addressin cell adhesion molecule-1 is preferentially expressed in intestinal tract and associated lymphoid tissue. *The American Journal of Pathology*, 151(1), 97–110.

[\[PubMed\]](#)

Britton, G. J., Contijoch, E. J., Mogno, I., Vennaro, O. H., Llewellyn, S. R., Ng, R., Li, Z., Mortha, A., Merad, M., Das, A., Gevers, D., McGovern, D. P. B., Singh, N., Braun, J., Jacobs, J. P., Clemente, J. C., Grinspan, A., Sands, B. E., Colombel, J.-F., Dubinsky, M. C., & Faith, J. J. (2019). Microbiotas from humans with inflammatory bowel disease alter the balance of gut Th17 and ROR γ ⁺ regulatory T cells and exacerbate colitis in mice. *Immunity*, 50(1), 212–224.e4.

[\[PubMed\]](#)

Bromley, S. K., Akbaba, H., Mani, V., Mora-Buch, R., Chasse, A. Y., Sama, A., & Luster, A. D. (2020). CD49a regulates cutaneous resident memory CD8⁺ T cell persistence and response. *Cell Reports*, 32(9), 108085.

[\[PubMed\]](#)

Brunkow, M. E., Jeffery, E. W., Hjerrild, K. A., Paepers, B., Clark, L. B., Yasayko, S.-A., Wilkinson, J. E., Galas, D., Ziegler, S. F., & Ramsdell, F. (2001). Disruption of a new forkhead/winged-helix protein, scurf, results in the fatal lymphoproliferative disorder of the scurfy mouse. *Nature Genetics*, 27(1), 68–73.

[\[PubMed\]](#)

Buchholz, V. R., Schumacher, T. N. M., & Busch, D. H. (2016). T cell fate at the single-cell level. *Annual Review of Immunology*, 34(1), 65–92.

[\[PubMed\]](#)

Burchill, M. A., Yang, J., Vang, K. B., Moon, J. J., Chu, H. H., Lio, C.-W. J., Vegoe, A. L., Hsieh, C.-S., Jenkins, M. K., & Farrar, M. A. (2008). Linked T cell receptor and cytokine signaling govern the development of the regulatory T cell repertoire. *Immunity*, 28(1), 112–121.

[\[PubMed\]](#)

Burton, O. T., Bricard, O., Tareen, S., Gergelits, V., Andrews, S., Biggins, L., Roca, C. P., Whyte, C., Junius, S., Brajic, A., Pasciuto, E., Ali, M., Lemaitre, P., Schlenner, S. M., Ishigame, H., Brown, B. D., Dooley, J., & Liston, A. (2024). The tissue-resident regulatory T cell pool is shaped by transient multi-tissue migration and a conserved residency program. *Immunity*, 57(7), 1586–1602.e10.

[\[PubMed\]](#)

Burzyn, D., Kuswanto, W., Kolodin, D., Shadrach, J. L., Cerletti, M., Jang, Y., Sefik, E., Tan, T. G., Wagers, A. J., Benoist, C., & Mathis, D. (2013). A special population of regulatory T cells potentiates muscle repair. *Cell*, 155(6), 1282–1295.

[\[PubMed\]](#)

Campbell, C., McKenney, P. T., Konstantinovskiy, D., Isaeva, O. I., Schizas, M., Verter, J., Mai, C., Jin, W.-B., Guo, C.-J., Violante, S., Ramos, R. J., Cross, J. R., Kadaveru, K., Hambor, J., & Rudensky, A. Y. (2020). Bacterial metabolism of bile acids promotes generation of peripheral regulatory T cells. *Nature*, 581(7809), 475–479.

[\[https://doi.org/10.1038/s41586-020-2193-0\]](https://doi.org/10.1038/s41586-020-2193-0)[\[PubMed\]](#)

Cao, X., Cai, S. F., Fehniger, T. A., Song, J., Collins, L. I., Piwnicka-Worms, D. R., & Ley, T. J. (2007). Granzyme B and perforin are important for regulatory T cell-mediated suppression of tumor clearance. *Immunity*, 27(4), 635–646.

[\[PubMed\]](#)

Cao, Y., Shen, J., & Ran, Z. H. (2014). Association between *Faecalibacterium prausnitzii* reduction and inflammatory bowel disease: A meta-analysis and systematic review of the literature. *Gastroenterology Research and Practice*, 2014, 872725.

[\[PubMed\]](#)

Capece, T., & Kim, M. (2016). The role of lymphatic niches in T cell differentiation. *Molecules and Cells*, 39(7), 515–523.

[\[PubMed\]](#)

Carico, Z., & Krangel, M. S. (2015). Chapter Seven - Chromatin dynamics and the development of the TCR α and TCR δ repertoires. In C. Murre (Ed.), *Advances in immunology* (pp. 307–361). Academic Press.

Casey, K. A., Fraser, K. A., Schenkel, J. M., Moran, A., Abt, M. C., Beura, L. K., Lucas, P. J., Artis, D., Wherry, E. J., Hogquist, K., Vezys, V., & Masopust, D. (2012). Antigen-independent differentiation and maintenance of effector-like resident memory T cells in tissues. *Journal of Immunology*, 188(10), 4866–4875.

Cebula, A., Seweryn, M., Rempala, G. A., Pabla, S. S., McIndoe, R. A., Denning, T. L., Bry, L., Kraj, P., Kisielow, P., & Ignatowicz, L. (2013). Thymus-derived regulatory T cells contribute to tolerance to commensal microbiota. *Nature*, 497(7448), 258–262.

[\[https://doi.org/10.1038/nature12079\]](https://doi.org/10.1038/nature12079)[\[PubMed\]](#)

Cepek, K. L., Shaw, S. K., Parker, C. M., Russell, G. J., Morrow, J. S., Rimm, D. L., & Brenner, M. B. (1994). Adhesion between epithelial cells and T lymphocytes mediated by E-cadherin and the α E β 7 integrin. *Nature*, 372(6502), 190–193.

[\[https://doi.org/10.1038/372190a0\]](https://doi.org/10.1038/372190a0)[\[PubMed\]](#)

Chellappa, S., Hugenschmidt, H., Hagness, M., Line, P. D., Labori, K. J., Wiedswang, G., Taskén, K., & Aandahl, E. M. (2016). Regulatory T cells that co-express ROR γ t and FOXP3 are pro-inflammatory and immunosuppressive and expand in human pancreatic cancer. *Oncoimmunology*, 5(4), e1102828.

[\[PubMed\]](#)

Chen, W., Jin, W., Hardegen, N., Lei, K., Li, L., Marinos, N., McGrady, G., & Wahl, S. M. (2003).

Conversion of peripheral CD4⁺CD25[−] naive T cells to CD4⁺CD25⁺ regulatory T cells by TGF- β

induction of transcription factor Foxp3. *The Journal of Experimental Medicine*, 198(12), 1875–1886.

[\[PubMed\]](#)

Chen, T., Guo, J., Cai, Z., Li, B., Sun, L., Shen, Y., Wang, S., Wang, Z., Wang, Z., Wang, Y., Zhou, H., Cai, Z., & Ye, Z. (2020). Th9 cell differentiation and its dual effects in tumor development. *Frontiers in Immunology*, 11, 1026.

[\[PubMed\]](#)

Cheng, L., & Becattini, S. (2022). Intestinal CD8⁺ tissue-resident memory T cells: From generation to function. *European Journal of Immunology*, 52(10), 1547–1560.

[\[PubMed\]](#)

Cheroutre, H., Lambolez, F., & Mucida, D. (2011). The light and dark sides of intestinal intraepithelial lymphocytes. *Nature Reviews. Immunology*, 11(7), 445–456.

[\[PubMed\]](#)

Cheuk, S., Schlums, H., Gallais S  r  zal, I., Martini, E., Chiang, S. C., Marquardt, N., Gibbs, A., Detlofsson, E., Introini, A., Forkel, M., H   g, C., Tjernlund, A., Micha  lsson, J., Folkersen, L., Mj  sberg, J., Blomqvist, L., Ehrstr  m, M., St  hle, M., Bryceson, Y. T., & Eidsmo, L. (2017). CD49a expression defines tissue-resident CD8⁺ T cells poised for cytotoxic function in human skin. *Immunity*, 46(2), 287–300.

[\[PubMed\]](#)

Ciofani, M., & Z   niga-Pfl  cker, J. C. (2010). Determining $\gamma\delta$ versus $\alpha\beta$ T cell development. *Nature Reviews. Immunology*, 10(9), 657–663.

[\[PubMed\]](#)

Cipolletta, D., Feuerer, M., Li, A., Kamei, N., Lee, J., Shoelson, S. E., Benoist, C., & Mathis, D. (2012). PPAR- γ is a major driver of the accumulation and phenotype of adipose tissue Treg cells. *Nature*, 486(7404), 549–553.

[\[https://doi.org/10.1038/nature11132\]](https://doi.org/10.1038/nature11132) [\[PubMed\]](#)

Cipolletta, D., Cohen, P., Spiegelman, B. M., Benoist, C., & Mathis, D. (2015). Appearance and disappearance of the mRNA signature characteristic of Treg cells in visceral adipose tissue: Age, diet, and PPAR γ effects. *Proceedings of the National Academy of Sciences*, 112(2), 482–487.

Coombes, J. L., Siddiqui, K. R. R., Arancibia-C  rcamo, C. V., Hall, J., Sun, C.-M., Belkaid, Y., & Powrie, F. (2007). A functionally specialized population of mucosal CD103⁺ DCs induces Foxp3⁺ regulatory T cells via a TGF- β and retinoic acid-dependent mechanism. *The Journal of Experimental Medicine*, 204(8), 1757–1764.

[\[PubMed\]](#)

Cording, S., Wahl, B., Kulkarni, D., Chopra, H., Pezoldt, J., Buettner, M., Dummer, A., Hadis, U., Heimesaat, M., Bereswill, S., Falk, C., Bode, U., Hamann, A., Fleissner, D., Huehn, J., & Pabst, O. (2014). The intestinal micro-environment imprints stromal cells to promote efficient Treg induction in gut-draining lymph nodes. *Mucosal Immunology*, 7(2), 359–368.

[\[PubMed\]](#)

Cosovanu, C., & Neumann, C. (2020). The many functions of Foxp3⁺ regulatory T cells in the intestine. *Frontiers in Immunology*, 11, 600973.

[\[PubMed\]](#)

Crooks, B., Barnes, T., & Limdi, J. K. (2020). Vedolizumab in the treatment of inflammatory bowel disease: Evolving paradigms. *Drugs Context*, 9, 2019-10-2.

[\[PubMed\]](#)

Crotty, S. (2019). T follicular helper cell biology: A decade of discovery and diseases. *Immunity*, 50(5), 1132–1148.

[\[PubMed\]](#)

Curotto de Lafaille, M. A., & Lafaille, J. J. (2009). Natural and adaptive Foxp3⁺ regulatory T cells: More of the same or a division of labor? *Immunity*, 30(5), 626–635.

[\[PubMed\]](#)

Curotto de Lafaille, M. A., Kutchukhidze, N., Shen, S., Ding, Y., Yee, H., & Lafaille, J. J. (2008). Adaptive Foxp3⁺ regulatory T cell-dependent and -independent control of allergic inflammation. *Immunity*, 29(1), 114–126.

[\[PubMed\]](#)

Cusimano, F. A., & Damas, O. M. (2022). Diet as a treatment for inflammatory bowel disease: Is it ready for prime time? *Current Opinion in Gastroenterology*, 38(4), 358–372.

[\[PubMed\]](#)

Cyster, J. G., & Schwab, S. R. (2012). Sphingosine-1-phosphate and lymphocyte egress from lymphoid organs. *Annual Review of Immunology*, 30, 69–94.

[\[PubMed\]](#)

da Fonseca, D. M., Hand, T. W., Han, S.-J., Gerner, M. Y., Glatman Zaretsky, A., Byrd, A. L., Harrison, O. J., Ortiz, A. M., Quinones, M., Trinchieri, G., Brenchley, J. M., Brodsky, I. E., Germain, R. N., Randolph, G. J., & Belkaid, Y. (2015). Microbiota-dependent sequelae of acute infection compromise tissue-specific immunity. *Cell*, 163(2), 354–366.

[\[PubMed\]](#)

Danylesko, I., Bukauskas, A., Paulson, M., Peceliunas, V., Gedde-Dahldy, T., Shimoni, A., Shouval, R., Griskevicius, L., Floisand, Y., & Nagler, A. (2019). Anti- $\alpha 4\beta 7$ integrin monoclonal antibody (vedolizumab) for the treatment of steroid-resistant severe intestinal acute graft-versus-host disease. *Bone Marrow Transplantation*, 54(7), 987–993.

[\[PubMed\]](#)

Darb Emamie, A., Rajabpour, M., Ghanavati, R., Asadolahi, P., Farzi, S., Sobouti, B., & Darbandi, A. (2021). The effects of probiotics, prebiotics and synbiotics on the reduction of IBD complications, a periodic review during 2009–2020. *Journal of Applied Microbiology*, 130(6), 1823–1838.

[\[PubMed\]](#)

Darfeuille-Michaud, A., Boudeau, J., Bulois, P., Neut, C., Glasser, A.-L., Barnich, N., Bringer, M.-A., Swidsinski, A., Beaugerie, L., & Colombel, J.-F. (2004). High prevalence of adherent-invasive

Escherichia coli associated with ileal mucosa in Crohn's disease. *Gastroenterology*, 127(2), 412–421.

[\[PubMed\]](#)

Darrasse-Jèze, G., Deroubaix, S., Mouquet, H., Victora, G. D., Eisenreich, T., Yao, K., Masilamani, R. F., Dustin, M. L., Rudensky, A., Liu, K., & Nussenzweig, M. C. (2009). Feedback control of regulatory T cell homeostasis by dendritic cells in vivo. *The Journal of Experimental Medicine*, 206(9), 1853–1862.

[\[PubMed\]](#)

Dasgupta, S., Erturk-Hasdemir, D., Ochoa-Reparaz, J., Reinecker, H.-C., & Kasper, D. L. (2014). Plasmacytoid dendritic cells mediate anti-inflammatory responses to a gut commensal molecule via both innate and adaptive mechanisms. *Cell Host & Microbe*, 15(4), 413–423.

Deaglio, S., Dwyer, K. M., Gao, W., Friedman, D., Usheva, A., Erat, A., Chen, J.-F., Enjyoji, K., Linden, J., Oukka, M., Kuchroo, V. K., Strom, T. B., & Robson, S. C. (2007). Adenosine generation catalyzed by CD39 and CD73 expressed on regulatory T cells mediates immune suppression. *The Journal of Experimental Medicine*, 204(6), 1257–1265.

[\[PubMed\]](#)

DeFilipp, Z., Kim, H. T., Spyrou, N., Katsivelos, N., Kowalyk, S., Eng, G., Kasikis, S., Beheshti, R., Baez, J., Akahoshi, Y., Ayuk, F., Choe, H., Etra, A., Grupp, S. A., Hexner, E. O., Hogan, W. J., Kitko, C. L., Qayed, M., Reshef, R., Vasova, I., Zeiser, R., Young, R., Holler, E., Ferrara, J. L. M., Nakamura, R., Levine, J. E., & Chen, Y.-B. (2024). The MAGIC algorithm probability predicts treatment response and long-term outcomes to second-line therapy for acute GVHD. *Blood Advances*, 8(13), 3488–3496.

[\[PubMed\]](#)

Delacher, M., Imbusch, C. D., Weichenhan, D., Breiling, A., Hotz-Wagenblatt, A., Träger, U., Hofer, A.-C., Kägebein, D., Wang, Q., Frauhammer, F., Mallm, J.-P., Bauer, K., Herrmann, C., Lang, P. A., Brors, B., Plass, C., & Feuerer, M. (2017). Genome-wide DNA-methylation landscape defines specialization of regulatory T cells in tissues. *Nature Immunology*, 18(10), 1160–1172.

[\[PubMed\]](#)

Delacher, M., Imbusch, C. D., Hotz-Wagenblatt, A., Mallm, J.-P., Bauer, K., Simon, M., Riegel, D., Rendeiro, A. F., Bittner, S., Sanderink, L., Pant, A., Schmidleithner, L., Braband, K. L., Echtenachter, B., Fischer, A., Giunchiglia, V., Hoffmann, P., Edinger, M., Bock, C., Rehli, M., Brors, B., Schmidl, C., & Feuerer, M. (2020). Precursors for nonlymphoid-tissue Treg cells reside in secondary lymphoid organs and are programmed by the transcription factor BATF. *Immunity*, 52(2), 295–312.e11.

[\[PubMed\]](#)

Denk, A., Edinger, M., Weber, D., Holler, E., Fante, M., Meedt, E., Gunes, S., Poeck, H., Mittermaier, C., Herr, W., & Wolff, D. (2024). Ruxolitinib for the treatment of acute graft-versus-host disease: A retrospective analysis. *Annals of Hematology*, 103(8), 3071–3081.

[\[PubMed\]](#)

Desreumaux, P., Foussat, A., Allez, M., Beaugerie, L., Hébuterne, X., Bouhnik, Y., Nachury, M., Brun, V., Bastian, H., Belmonte, N., Ticchioni, M., Duchange, A., Morel-Mandrino, P., Neveu, V., Clerget-Chossat, N., Forte, M., & Colombel, J.-F. (2012). Safety and efficacy of antigen-specific regulatory T-cell therapy for patients with refractory Crohn's disease. *Gastroenterology*, 143(5), 1207–1217.e2.

[\[PubMed\]](#)

Di Ianni, M., Falzetti, F., Carotti, A., Terenzi, A., Castellino, F., Bonifacio, E., Del Papa, B., Zei, T., Ostini, R. I., Cecchini, D., Aloisi, T., Perruccio, K., Ruggeri, L., Balucani, C., Pierini, A., Sportoletti, P., Aristei, C., Falini, B., Reisner, Y., Velardi, A., Aversa, F., & Martelli, M. F. (2011). Tregs prevent GVHD and promote immune reconstitution in HLA-haploidentical transplantation. *Blood*, *117*(14), 3921–3928.

[\[PubMed\]](#)

Dobeš, J., Ben-Nun, O., Binyamin, A., Stoler-Barak, L., Oftedal, B. E., Goldfarb, Y., Kadouri, N., Gruper, Y., Givony, T., Zelayat, I., Kováčová, K., Böhmová, H., Valter, E., Shulman, Z., Filipp, D., Husebye, E. S., & Abramson, J. (2022). Extrathymic expression of Aire controls the induction of effective TH17 cell-mediated immune response to *Candida albicans*. *Nature Immunology*, *23*(7), 1098–1108.

[\[PubMed\]](#)

Dombrowski, Y., O'Hagan, T., Dittmer, M., Penalva, R., Mayoral, S. R., Bankhead, P., Fleville, S., Eleftheriadis, G., Zhao, C., Naughton, M., Hassan, R., Moffat, J., Falconer, J., Boyd, A., Hamilton, P., Allen, I. V., Kissenpfennig, A., Moynagh, P. N., Evergren, E., Perbal, B., Williams, A. C., Ingram, R. J., Chan, J. R., Franklin, R. J. M., & Fitzgerald, D. C. (2017). Regulatory T cells promote myelin regeneration in the central nervous system. *Nature Neuroscience*, *20*(5), 674–680.

[\[PubMed\]](#)

Dwyer, K. M., Deaglio, S., Gao, W., Friedman, D., Strom, T. B., & Robson, S. C. (2007). CD39 and control of cellular immune responses. *Purinergic Signal*, *3*(1–2), 171–180.

[\[PubMed\]](#)

Eisenbarth, S. C. (2019). Dendritic cell subsets in T cell programming: Location dictates function. *Nature Reviews. Immunology*, *19*(2), 89–103.

[\[PubMed\]](#)

Eisenbarth, S. C., Baumjohann, D., Craft, J., Fazilleau, N., Ma, C. S., Tangye, S. G., Vinuesa, C. G., & Linterman, M. A. (2021). CD4⁺ T cells that help B cells - a proposal for uniform nomenclature. *Trends in Immunology*, *42*(8), 658–669.

[\[PubMed\]](#)

Esterházy, D., Canesso, M. C. C., Mesin, L., Muller, P. A., de Castro, T. B. R., Lockhart, A., ElJalby, M., Faria, A. M. C., & Mucida, D. (2019). Compartmentalized gut lymph node drainage dictates adaptive immune responses. *Nature*, *569*(7754), 126–130.

[\[https://doi.org/10.1038/s41586-019-1125-3\]](https://doi.org/10.1038/s41586-019-1125-3) [\[PubMed\]](#)

Eyerich, S., Eyerich, K., Pennino, D., Carbone, T., Nasorri, F., Pallotta, S., Cianfarani, F., Odorisio, T., Traidl-Hoffmann, C., Behrendt, H., Durham, S. R., Schmidt-Weber, C. B., & Cavani, A. (2009). Th22 cells represent a distinct human T cell subset involved in epidermal immunity and remodeling. *The Journal of Clinical Investigation*, *119*(12), 3573–3585.

[\[PubMed\]](#)

Faith, J. J., Ahern, P. P., Ridaaura, V. K., Cheng, J., & Gordon, J. I. (2014). Identifying gut microbe-host phenotype relationships using combinatorial communities in gnotobiotic mice. *Science Translational Medicine*, *6*(220), 220ra11.

[\[PubMed\]](#)

Fan, Y., & Pedersen, O. (2021). Gut microbiota in human metabolic health and disease. *Nature Reviews. Microbiology*, 19(1), 55–71.

[\[PubMed\]](#)

Fantini, M. C., Becker, C., Monteleone, G., Pallone, F., Galle, P. R., & Neurath, M. F. (2004). Cutting edge: TGF-beta induces a regulatory phenotype in CD4⁺CD25⁻ T cells through Foxp3 induction and down-regulation of Smad7. *Journal of Immunology (Baltimore, Md.: 1950)*, 172(9), 5149–5153.

[\[PubMed\]](#)

Feng, J., Chen, Y., Liu, Y., Lin, L., Lin, X., Gong, W., Xia, R., He, J., Sheng, J., Cai, H., & Xiao, C. (2023). Efficacy and safety of fecal microbiota transplantation in the treatment of ulcerative colitis: A systematic review and meta-analysis. *Scientific Reports*, 13(1), 14494.

[\[PubMed\]](#)

Feuerer, M., Herrero, L., Cipolletta, D., Naaz, A., Wong, J., Nayer, A., Lee, J., Goldfine, A. B., Benoist, C., Shoelson, S., & Mathis, D. (2009). Lean, but not obese, fat is enriched for a unique population of regulatory T cells that affect metabolic parameters. *Nature Medicine*, 15(8), 930–939.

[\[PubMed\]](#)

Finney, C. A. M., Taylor, M. D., Wilson, M. S., & Maizels, R. M. (2007). Expansion and activation of CD4⁺CD25⁺ regulatory T cells in Heligmosomoides polygyrus infection. *European Journal of Immunology*, 37(7), 1874–1886.

[\[PubMed\]](#)

Floess, S., Freyer, J., Siewert, C., Baron, U., Olek, S., Polansky, J., Schlawe, K., Chang, H.-D., Bopp, T., Schmitt, E., Klein-Hessling, S., Serfling, E., Hamann, A., & Huehn, J. (2007). Epigenetic control of the foxp3 locus in regulatory T cells. *PLoS Biology*, 5(2), e38.

[\[PubMed\]](#)

Fløisand, Y., Lundin, K. E. A., Lazarevic, V., Kristiansen, J. D., Osnes, L. T. N., Tjønnfjord, G. E., Reims, H. M., & Gedde-Dahl, T. (2017). Targeting integrin $\alpha 4\beta 7$ in steroid-refractory intestinal graft-versus-host disease. *Biology of Blood and Marrow Transplantation*, 23(1), 172–175.

[\[PubMed\]](#)

Fonseca, R., Beura, L. K., Quarnstrom, C. F., Ghoneim, H. E., Fan, Y., Zebley, C. C., Scott, M. C., Fares-Frederickson, N. J., Wijeyesinghe, S., Thompson, E. A., Borges da Silva, H., Vezys, V., Youngblood, B., & Masopust, D. (2020). Developmental plasticity allows outside-in immune responses by resident memory T cells. *Nature Immunology*, 21(4), 412–421.

[\[PubMed\]](#)

Fontenot, J. D., Gavin, M. A., & Rudensky, A. Y. (2003). Foxp3 programs the development and function of CD4⁺CD25⁺ regulatory T cells. *Nature Immunology*, 4(4), 330–336.

[\[PubMed\]](#)

Furusawa, Y., Obata, Y., Fukuda, S., Endo, T. A., Nakato, G., Takahashi, D., Nakanishi, Y., Uetake, C.,

Kato, K., Kato, T., Takahashi, M., Fukuda, N. N., Murakami, S., Miyauchi, E., Hino, S., Atarashi, K., Onawa, S., Fujimura, Y., Lockett, T., Clarke, J. M., Topping, D. L., Tomita, M., Hori, S., Ohara, O., Morita, T., Koseki, H., Kikuchi, J., Honda, K., Hase, K., & Ohno, H. (2013). Commensal microbe-derived butyrate induces the differentiation of colonic regulatory T cells. *Nature*, *504*(7480), 446–450.

[<https://doi.org/10.1038/nature12721>][[PubMed](#)]

Gardner, J. M., & Liston, A. (2022). ROR γ t-lineage APCs: The Aire apparent. *Science Immunology*, *7*(78), eade9240.

[[PubMed](#)]

Gebhardt, T., Wakim, L. M., Eidsmo, L., Reading, P. C., Heath, W. R., & Carbone, F. R. (2009). Memory T cells in nonlymphoid tissue that provide enhanced local immunity during infection with herpes simplex virus. *Nature Immunology*, *10*(5), 524–530.

[[PubMed](#)]

Gebhardt, T., Whitney, P. G., Zaid, A., Mackay, L. K., Brooks, A. G., Heath, W. R., Carbone, F. R., & Mueller, S. N. (2011). Different patterns of peripheral migration by memory CD4⁺ and CD8⁺ T cells. *Nature*, *477*(7363), 216–219.

[<https://doi.org/10.1038/nature10339>][[PubMed](#)]

Geginat, J., Paroni, M., Maglie, S., Alfen, J. S., Kastirr, I., Gruarin, P., De Simone, M., Pagani, M., & Abrignani, S. (2014). Plasticity of human CD4 T cell subsets. *Frontiers in Immunology*, *5*, 630.

[[PubMed](#)]

Gensollen, T., Iyer, S. S., Kasper, D. L., & Blumberg, R. S. (2016). How colonization by microbiota in early life shapes the immune system. *Science*, *352*(6285), 539–544.

[[PubMed](#)]

Geuking, M. B., Cahenzli, J., Lawson, M. A. E., Ng, D. C. K., Slack, E., Hapfelmeier, S., McCoy, K. D., & Macpherson, A. J. (2011). Intestinal bacterial colonization induces mutualistic regulatory T cell responses. *Immunity*, *34*(5), 794–806.

[[PubMed](#)]

Geva-Zatorsky, N., Sefik, E., Kua, L., Pasman, L., Tan, T. G., Ortiz-Lopez, A., Yanortsang, T. B., Yang, L., Jupp, R., Mathis, D., Benoist, C., & Kasper, D. L. (2017). Mining the human gut microbiota for immunomodulatory organisms. *Cell*, *168*(5), 928–943.e11.

[[PubMed](#)]

Gittler, J. K., Shemer, A., Suárez-Fariñas, M., Fuentes-Duculan, J., Gulewicz, K. J., Wang, C. Q. F., Mitsui, H., Cardinale, I., de Strong, C. G., Krueger, J. G., & Guttman-Yassky, E. (2012). Progressive activation of TH2/TH22 cytokines and selective epidermal proteins characterizes acute and chronic atopic dermatitis. *The Journal of Allergy and Clinical Immunology*, *130*(6), 1344–1354.

[[PubMed](#)]

Gondek, D. C., Lu, L.-F., Quezada, S. A., Sakaguchi, S., & Noelle, R. J. (2005). Cutting edge: Contact-mediated suppression by CD4⁺CD25⁺ regulatory cells involves a granzyme B-dependent, perforin-independent mechanism. *Journal of Immunology (Baltimore, Md.: 1950)*, *174*(4), 1783–1786.

[\[PubMed\]](#)

Goto, Y., Panea, C., Nakato, G., Cebula, A., Lee, C., Diez, M. G., Laufer, T. M., Ignatowicz, L., & Ivanov, I. I. (2014). Segmented filamentous bacteria antigens presented by intestinal dendritic cells drive mucosal Th17 cell differentiation. *Immunity*, 40(4), 594–607.

[\[PubMed\]](#)

Guandalini, S., & Sansotta, N. (2019). Probiotics in the treatment of inflammatory bowel disease. *Advances in Experimental Medicine and Biology*, 1125, 101–107.

[\[PubMed\]](#)

Gui, Y., Cheng, H., Zhou, J., Xu, H., Han, J., & Zhang, D. (2022). Development and function of natural TCR⁺ CD8α⁺ intraepithelial lymphocytes. *Frontiers in Immunology*, 13, 1059042.

[\[PubMed\]](#)

Guo, X., Qiu, J., Tu, T., Yang, X., Deng, L., Anders, R. A., Zhou, L., & Fu, Y.-X. (2014). Induction of innate lymphoid cell-derived interleukin-22 by the transcription factor STAT3 mediates protection against intestinal infection. *Immunity*, 40(1), 25–39.

[\[PubMed\]](#)

Guo, W., Su, X., Wang, M., Han, M., Feng, X., & Jiang, E. (2021). Regulatory T cells in GVHD therapy. *Frontiers in Immunology*, 12, 697854.

[\[PubMed\]](#)

Hadis, U., Wahl, B., Schulz, O., Hardtke-Wolenski, M., Schippers, A., Wagner, N., Müller, W., Sparwasser, T., Förster, R., & Pabst, O. (2011). Intestinal tolerance requires gut homing and expansion of FoxP3⁺ regulatory T cells in the lamina propria. *Immunity*, 34(2), 237–246.

[\[PubMed\]](#)

Halim, L., Romano, M., McGregor, R., Correa, I., Pavlidis, P., Grageda, N., Hoong, S.-J., Yuksel, M., Jassem, W., Hannen, R. F., Ong, M., Mckinney, O., Hayee, B., Karagiannis, S. N., Powell, N., Lechler, R. I., Nova-Lamperti, E., & Lombardi, G. (2017). An atlas of human regulatory T helper-like cells reveals features of Th2-like Tregs that support a tumorigenic environment. *Cell Reports*, 20(3), 757–770.

[\[PubMed\]](#)

Hall, J. A., Bouladoux, N., Sun, C. M., Wohlfert, E. A., Blank, R. B., Zhu, Q., Grigg, M. E., Berzofsky, J. A., & Belkaid, Y. (2008). Commensal DNA limits regulatory T cell conversion and is a natural adjuvant of intestinal immune responses. *Immunity*, 29(4), 637–649.

[\[PubMed\]](#)

Hammerschmidt, S. I., Ahrendt, M., Bode, U., Wahl, B., Kremmer, E., Förster, R., & Pabst, O. (2008). Stromal mesenteric lymph node cells are essential for the generation of gut-homing T cells in vivo. *The Journal of Experimental Medicine*, 205(11), 2483–2490.

[\[PubMed\]](#)

Hang, S., Paik, D., Yao, L., Kim, E., Trinath, J., Lu, J., Ha, S., Nelson, B. N., Kelly, S. P., Wu, L., Zheng, Y., Longman, R. S., Rastinejad, F., Devlin, A. S., Krout, M. R., Fischbach, M. A., Littman, D. R., & Huh, J. R.

(2019). Bile acid metabolites control TH17 and Treg cell differentiation. *Nature*, 576(7785), 143–148.

[\[https://doi.org/10.1038/s41586-019-1785-z\]](https://doi.org/10.1038/s41586-019-1785-z)[\[PubMed\]](#)

Hanna, B. S., Wang, G., Galván-Peña, S., Mann, A. O., Ramirez, R. N., Muñoz-Rojas, A. R., Smith, K., Wan, M., Benoist, C., & Mathis, D. (2023). The gut microbiota promotes distal tissue regeneration via ROR γ^+ regulatory T cell emissaries. *Immunity*, 56(4), 829–846.e8.

[\[PubMed\]](#)

Haribhai, D., Williams, J. B., Jia, S., Nickerson, D., Schmitt, E. G., Edwards, B., Ziegelbauer, J., Yassai, M., Li, S.-H., Relland, L. M., Wise, P. M., Chen, A., Zheng, Y.-Q., Simpson, P. M., Gorski, J., Salzman, N. H., Hessner, M. J., Chatila, T. A., & Williams, C. B. (2011). A requisite role for induced regulatory T cells in tolerance based on expanding antigen receptor diversity. *Immunity*, 35(1), 109–122.

[\[PubMed\]](#)

Haring, E., Zeiser, R., & Apostolova, P. (2021). Interfering with inflammation: Heterogeneous effects of interferons in graft-versus-host disease of the gastrointestinal tract and inflammatory bowel disease. *Frontiers in Immunology*, 12, 705342.

[\[PubMed\]](#)

He, Z., Ma, J., Wang, R., Zhang, J., Huang, Z., Wang, F., Sen, S., Rothenberg, E. V., & Sun, Z. (2017). A two-amino-acid substitution in the transcription factor ROR γ t disrupts its function in TH17 differentiation but not in thymocyte development. *Nature Immunology*, 18(10), 1128–1138.

[\[PubMed\]](#)

Hegazy, A. N., & Powrie, F. (2015). MICROBIOME. Microbiota ROR γ ulates intestinal suppressor T cells. *Science*, 349(6251), 929–930.

[\[PubMed\]](#)

Hemmers, S., Schizas, M., Azizi, E., Dikiy, S., Zhong, Y., Feng, Y., Altan-Bonnet, G., & Rudensky, A. Y. (2019). IL-2 production by self-reactive CD4 thymocytes scales regulatory T cell generation in the thymus. *The Journal of Experimental Medicine*, 216(11), 2466–2478.

[\[PubMed\]](#)

Hori, S., Nomura, T., & Sakaguchi, S. (2003). Control of regulatory T cell development by the transcription factor Foxp3. *Science*, 299(5609), 1057–1061.

[\[PubMed\]](#)

Hosokawa, H., & Rothenberg, E. V. (2018). Cytokines, transcription factors, and the initiation of T-cell development. *Cold Spring Harbor Perspectives in Biology*, 10(5), a028621.

[\[PubMed\]](#)

Hosokawa, H., & Rothenberg, E. V. (2021). How transcription factors drive choice of the T cell fate. *Nature Reviews. Immunology*, 21(3), 162–176.

[\[PubMed\]](#)

Hou, K., Wu, Z.-X., Chen, X.-Y., Wang, J.-Q., Zhang, D., Xiao, C., Zhu, D., Koya, J. B., Wei, L., Li, J., & Chen, Z.-S. (2022). Microbiota in health and diseases. *Signal Transduction and Targeted Therapy*, 7(1), 1–28.

Hsieh, C.-S., Lee, H.-M., & Lio, C.-W. J. (2012). Selection of regulatory T cells in the thymus. *Nature Reviews. Immunology*, 12(3), 157–167.

[\[PubMed\]](#)

Huster, K. M., Busch, V., Schiemann, M., Linkemann, K., Kerksiek, K. M., Wagner, H., & Busch, D. H. (2004). Selective expression of IL-7 receptor on memory T cells identifies early CD40L-dependent generation of distinct CD8⁺ memory T cell subsets. *Proceedings of the National Academy of Sciences of the United States of America*, 101(15), 5610–5615.

[\[PubMed\]](#)

Ichinohe, T., Pang, I. K., Kumamoto, Y., Peaper, D. R., Ho, J. H., Murray, T. S., & Iwasaki, A. (2011). Microbiota regulates immune defense against respiratory tract influenza A virus infection. *Proceedings of the National Academy of Sciences*, 108(13), 5354–5359.

Ishikawa, H., Tanaka, K., Maeda, Y., Aiba, Y., Hata, A., Tsuji, N. M., Koga, Y., & Matsumoto, T. (2008). Effect of intestinal microbiota on the induction of regulatory CD25⁺ CD4⁺ T cells. *Clinical and Experimental Immunology*, 153(1), 127–135.

[\[PubMed\]](#)

Ivanov, I. I., Atarashi, K., Manel, N., Brodie, E. L., Shima, T., Karaoz, U., Wei, D., Goldfarb, K. C., Santee, C. A., Lynch, S. V., Tanoue, T., Imaoka, A., Itoh, K., Takeda, K., Umesaki, Y., Honda, K., & Littman, D. R. (2009). Induction of intestinal Th17 cells by segmented filamentous bacteria. *Cell*, 139(3), 485–498.

[\[PubMed\]](#)

Iwata, M., Hirakiyama, A., Eshima, Y., Kagechika, H., Kato, C., & Song, S.-Y. (2004). Retinoic acid imprints gut-homing specificity on T cells. *Immunity*, 21(4), 527–538.

[\[PubMed\]](#)

Jarosch, S., Köhlen, J., Ghimire, S., Orberg, E. T., Hammel, M., Gaag, D., Evert, M., Janssen, K.-P., Hiergeist, A., Gessner, A., Weber, D., Meedt, E., Poeck, H., D'Ippolito, E., Holler, E., Busch, D. H. (2023). Multimodal immune cell phenotyping in GI biopsies reveals microbiome-related T cell modulations in human GvHD. *Cell Reports Medicine*, 4(7), 101125. <https://doi.org/10.1016/j.xcrm.2023.101125>

Jiang, Q., Yang, G., Xiao, F., Xie, J., Wang, S., Lu, L., & Cui, D. (2021). Role of Th22 cells in the pathogenesis of autoimmune diseases. *Frontiers in Immunology*, 12, 688066.

[\[PubMed\]](#)

Johnston, C. J. C., Smyth, D. J., Kodali, R. B., White, M. P. J., Harcus, Y., Filbey, K. J., Hewitson, J. P., Hinck, C. S., Ivens, A., Kemter, A. M., Kildemoes, A. O., Le Bihan, T., Soares, D. C., Anderton, S. M., Brenn, T., Wigmore, S. J., Woodcock, H. V., Chambers, R. C., Hinck, A. P., McSorley, H. J., & Maizels, R. M. (2017). A structurally distinct TGF- β mimic from an intestinal helminth parasite potently induces regulatory T cells. *Nature Communications*, 8(1), 1741.

[\[PubMed\]](#)

Josefowicz, S. Z., Niec, R. E., Kim, H. Y., Treuting, P., Chinen, T., Zheng, Y., Umetsu, D. T., & Rudensky, A. Y. (2012). Extrathymically generated regulatory T cells control mucosal TH2 inflammation.

Nature, 482(7385), 395–399.

[\[https://doi.org/10.1038/nature10772\]](https://doi.org/10.1038/nature10772) [\[PubMed\]](#)

Kaech, S. M., Tan, J. T., Wherry, E. J., Konieczny, B. T., Surh, C. D., & Ahmed, R. (2003). Selective expression of the interleukin 7 receptor identifies effector CD8 T cells that give rise to long-lived memory cells. *Nature Immunology*, 4(12), 1191–1198.

[\[PubMed\]](#)

Kalekar, L. A., Cohen, J. N., Prevel, N., Sandoval, P. M., Mathur, A. N., Moreau, J. M., Lowe, M. M., Nosbaum, A., Wolters, P. J., Haemel, A., Boin, F., & Rosenblum, M. D. (2019). Regulatory T cells in skin are uniquely poised to suppress profibrotic immune responses. *Science Immunology*, 4(39), eaaw2910.

[\[PubMed\]](#)

Kau, A. L., Ahern, P. P., Griffin, N. W., Goodman, A. L., & Gordon, J. I. (2011). Human nutrition, the gut microbiome and the immune system. *Nature*, 474(7351), 327–336.

[\[https://doi.org/10.1038/nature10213\]](https://doi.org/10.1038/nature10213) [\[PubMed\]](#)

Kedmi, R., Najar, T. A., Mesa, K. R., Grayson, A., Kroehling, L., Hao, Y., Hao, S., Pokrovskii, M., Xu, M., Talbot, J., Wang, J., Germino, J., Lareau, C. A., Satpathy, A. T., Anderson, M. S., Laufer, T. M., Aifantis, I., Bartleson, J. M., Allen, P. M., Paidassi, H., Gardner, J. M., Stoeckius, M., & Littman, D. R. (2022). A ROR γ ⁺ cell instructs gut microbiota-specific Treg cell differentiation. *Nature*, 610(7933), 737–743.

[\[https://doi.org/10.1038/s41586-022-05089-y\]](https://doi.org/10.1038/s41586-022-05089-y) [\[PubMed\]](#)

Kerdiles, Y. M., Stone, E. L., Beisner, D. L., McGargill, M. A., Ch'en, I. L., Stockmann, C., Katayama, C. D., & Hedrick, S. M. (2010). Foxo transcription factors control regulatory T cell development and function. *Immunity*, 33(6), 890–904.

[\[PubMed\]](#)

Khattari, R., Cox, T., Yasayko, S.-A., & Ramsdell, F. (2003). An essential role for Scurfin in CD4⁺CD25⁺ T regulatory cells. *Nature Immunology*, 4(4), 337–342.

[\[PubMed\]](#)

Khorsand, B., Asadzadeh Aghdaei, H., Nazemalhosseini-Mojarad, E., Nadalian, B., Nadalian, B., & Hour, H. (2022). Overrepresentation of Enterobacteriaceae and Escherichia coli is the major gut microbiome signature in Crohn's disease and ulcerative colitis; a comprehensive metagenomic analysis of IBDMDB datasets. *Frontiers in Cellular and Infection Microbiology*, 12, 1015890.

[\[PubMed\]](#)

Kim, H.-P., & Leonard, W. J. (2007). CREB/ATF-dependent T cell receptor-induced FoxP3 gene expression: A role for DNA methylation. *The Journal of Experimental Medicine*, 204(7), 1543–1551.

[\[PubMed\]](#)

Kim, J. M., Rasmussen, J. P., & Rudensky, A. Y. (2007). Regulatory T cells prevent catastrophic autoimmunity throughout the lifespan of mice. *Nature Immunology*, 8(2), 191–197.

[\[PubMed\]](#)

Kim, M. H., Kang, S. G., Park, J. H., Yanagisawa, M., & Kim, C. H. (2013a). Short-chain fatty acids activate GPR41 and GPR43 on intestinal epithelial cells to promote inflammatory responses in mice. *Gastroenterology*, 145(2), 396-406.e1–10.

[\[PubMed\]](#)

Kim, S. V., Xiang, W. V., Kwak, C., Yang, Y., Lin, X. W., Ota, M., Sarpel, U., Rifkin, D. B., Xu, R., & Littman, D. R. (2013b). GPR15-mediated homing controls immune homeostasis in the large intestine mucosa. *Science*, 340(6139), 1456–1459.

[\[PubMed\]](#)

Kim, K. S., Hong, S.-W., Han, D., Yi, J., Jung, J., Yang, B.-G., Lee, J. Y., Lee, M., & Surh, C. D. (2016). Dietary antigens limit mucosal immunity by inducing regulatory T cells in the small intestine. *Science*, 351(6275), 858–863.

[\[PubMed\]](#)

Kim, S., Kim, H., Yim, Y. S., Ha, S., Atarashi, K., Tan, T. G., Longman, R. S., Honda, K., Littman, D. R., Choi, G. B., & Huh, J. R. (2017). Maternal gut bacteria promote neurodevelopmental abnormalities in mouse offspring. *Nature*, 549(7673), 528–532.

[\[https://doi.org/10.1038/nature23910\]](https://doi.org/10.1038/nature23910) [\[PubMed\]](#)

Klein, L., & Petrozziello, E. (2024). Antigen presentation for central tolerance induction. *Nature Reviews. Immunology*, 25, 1–16.

Klein, L., Robey, E. A., & Hsieh, C.-S. (2019). Central CD4⁺ T cell tolerance: deletion versus regulatory T cell differentiation. *Nature Reviews. Immunology*, 19(1), 7–18.

[\[PubMed\]](#)

Kobayashi, T., Okamoto, S., Hisamatsu, T., Kamada, N., Chinen, H., Saito, R., Kitazume, M. T., Nakazawa, A., Sugita, A., Koganei, K., Isobe, K., & Hibi, T. (2008). IL23 differentially regulates the Th1/Th17 balance in ulcerative colitis and Crohn's disease. *Gut*, 57(12), 1682–1689.

[\[PubMed\]](#)

Kobie, J. J., Shah, P. R., Yang, L., Rebhahn, J. A., Fowell, D. J., & Mosmann, T. R. (2006). T regulatory and primed uncommitted CD4 T cells express CD73, which suppresses effector CD4 T cells by converting 5'-adenosine monophosphate to adenosine. *Journal of Immunology*, 177(10), 6780–6786.

Kolodin, D., van Panhuys, N., Li, C., Magnuson, A. M., Cipolletta, D., Miller, C. M., Wagers, A., Germain, R. N., Benoist, C., & Mathis, D. (2015). Antigen- and cytokine-driven accumulation of regulatory T cells in visceral adipose tissue of lean mice. *Cell Metabolism*, 21(4), 543–557.

[\[PubMed\]](#)

Konjar, Š., Ferreira, C., Blankenhaus, B., & Veldhoen, M. (2017). Intestinal barrier interactions with specialized CD8 T cells. *Frontiers in Immunology*, 8, 1281.

[\[PubMed\]](#)

Korn, L. L., Hubbeling, H. G., Porrett, P. M., Yang, Q., Barnett, L. G., & Laufer, T. M. (2014). Regulatory T cells occupy an isolated niche in the intestine that is antigen independent. *Cell Reports*, 9(5), 1567–1573.

[\[PubMed\]](#)

Kuhn, K. A., & Stappenbeck, T. S. (2013). Peripheral education of the immune system by the colonic microbiota. *Seminars in Immunology*, 25(5). <https://doi.org/10.1016/j.smim.2013.10.002>

Kullberg, M. C., Jankovic, D., Gorelick, P. L., Caspar, P., Letterio, J. J., Cheever, A. W., & Sher, A. (2002). Bacteria-triggered CD4⁺ T regulatory cells suppress *Helicobacter hepaticus*-induced colitis. *The Journal of Experimental Medicine*, 196(4), 505–515.

[\[PubMed\]](#)

Kurd, N., & Robey, E. A. (2016). T-cell selection in the thymus: A spatial and temporal perspective. *Immunological Reviews*, 271(1), 114–126.

[\[PubMed\]](#)

Kurd, N. S., Hoover, A., Yoon, J., Weist, B. M., Lutes, L., Chan, S. W., & Robey, E. A. (2021). Factors that influence the thymic selection of CD8 $\alpha\alpha$ intraepithelial lymphocytes. *Mucosal Immunology*, 14(1), 68–79.

[\[PubMed\]](#)

Kuswanto, W., Burzyn, D., Panduro, M., Wang, K. K., Jang, Y. C., Wagers, A. J., Benoist, C., & Mathis, D. (2016). Poor repair of skeletal muscle in aging mice reflects a defect in local, Interleukin-33-dependent accumulation of regulatory T cells. *Immunity*, 44(2), 355–367.

[\[PubMed\]](#)

Laidlaw, B. J., Craft, J. E., & Kaech, S. M. (2016). The multifaceted role of CD4⁺ T cells in CD8⁺ T cell memory. *Nature Reviews. Immunology*, 16(2), 102–111.

[\[PubMed\]](#)

Lathrop, S. K., Bloom, S. M., Rao, S. M., Nutsch, K., Lio, C.-W., Santacruz, N., Peterson, D. A., Stappenbeck, T. S., & Hsieh, C.-S. (2011). Peripheral education of the immune system by colonic commensal microbiota. *Nature*, 478(7368), 250–254.

[\[https://doi.org/10.1038/nature10434\]](https://doi.org/10.1038/nature10434)[\[PubMed\]](#)

Laukova, M., & Glatman Zaretsky, A. (2023). Regulatory T cells as a therapeutic approach for inflammatory bowel disease. *European Journal of Immunology*, 53(2), 2250007.

[\[PubMed\]](#)

Lee, W., & Lee, G. R. (2018). Transcriptional regulation and development of regulatory T cells. *Experimental & Molecular Medicine*, 50(3), e456–e456.

Leung, J. M., Davenport, M., Wolff, M. J., Wiens, K. E., Abidi, W. M., Poles, M. A., Cho, I., Ullman, T., Mayer, L., & Loke, P. (2014). IL-22-producing CD4⁺ cells are depleted in actively inflamed colitis tissue. *Mucosal Immunology*, 7(1), 124–133.

[\[PubMed\]](#)

Li, W., Hang, S., Fang, Y., Bae, S., Zhang, Y., Zhang, M., Wang, G., McCurry, M. D., Bae, M., Paik, D., Franzosa, E. A., Rastinejad, F., Huttenhower, C., Yao, L., Devlin, A. S., & Huh, J. R. (2021). A bacterial

bile acid metabolite modulates Treg activity through the nuclear hormone receptor NR4A1. *Cell Host & Microbe*, 29(9), 1366–1377.e9.

Lio, C.-W. J., & Hsieh, C.-S. (2008). A two-step process for thymic regulatory T cell development. *Immunity*, 28(1), 100–111.

[\[PubMed\]](#)

Liu, L., Liang, L., Liang, H., Wang, M., Lu, B., Xue, M., Deng, J., & Chen, Y. (2019). *Fusobacterium nucleatum* aggravates the progression of colitis by regulating M1 macrophage polarization via AKT2 pathway. *Frontiers in Immunology*, 10, 1324.

[\[PubMed\]](#)

Liu, H., Hong, X. L., Sun, T. T., Huang, X. W., Wang, J. L., & Xiong, H. (2020). *Fusobacterium nucleatum* exacerbates colitis by damaging epithelial barriers and inducing aberrant inflammation. *Journal of Digestive Diseases*, 21(7), 385–398.

[\[PubMed\]](#)

Liu, G., Wang, Z., & Li, S. (2024). Heterogeneity and plasticity of tissue-resident memory T cells in skin diseases and homeostasis: A review. *Frontiers in Immunology*, 15, 1378359.

[\[PubMed\]](#)

Lorenz, R. G., Chaplin, D. D., McDonald, K. G., McDonough, J. S., & Newberry, R. D. (2003). Isolated lymphoid follicle formation is inducible and dependent upon lymphotoxin-sufficient B lymphocytes, lymphotoxin beta receptor, and TNF receptor I function. *Journal of Immunology (Baltimore, Md.: 1950)*, 170(11), 5475–5482.

[\[PubMed\]](#)

Luckheeram, R. V., Zhou, R., Verma, A. D., & Xia, B. (2012). CD4⁺ T cells: Differentiation and functions. *Clinical & Developmental Immunology*, 2012, 925135.

Lyu, M., Suzuki, H., Kang, L., Gaspal, F., Zhou, W., Goc, J., Zhou, L., Zhou, J., Zhang, W., Shen, Z., Fox, J. G., Sockolow, R. E., Laufer, T. M., Fan, Y., Eberl, G., Withers, D. R., & Sonnenberg, G. F. (2022). ILC3s select microbiota-specific regulatory T cells to establish tolerance in the gut. *Nature*, 610(7933), 744–751.

[\[https://doi.org/10.1038/s41586-022-05141-x\]](https://doi.org/10.1038/s41586-022-05141-x) [\[PubMed\]](#)

Ma, H., Tao, W., & Zhu, S. (2019). T lymphocytes in the intestinal mucosa: Defense and tolerance. *Cellular & Molecular Immunology*, 16(3), 216–224.

Mackay, L. K., Rahimpour, A., Ma, J. Z., Collins, N., Stock, A. T., Hafon, M.-L., Vega-Ramos, J., Lauzurica, P., Mueller, S. N., Stefanovic, T., Tschärke, D. C., Heath, W. R., Inouye, M., Carbone, F. R., & Gebhardt, T. (2013). The developmental pathway for CD103⁺CD8⁺ tissue-resident memory T cells of skin. *Nature Immunology*, 14(12), 1294–1301.

[\[PubMed\]](#)

Mackay, L. K., Braun, A., Macleod, B. L., Collins, N., Tebartz, C., Bedoui, S., Carbone, F. R., & Gebhardt, T. (2015). Cutting Edge: CD69 interference with sphingosine-1-phosphate receptor function regulates peripheral T cell retention. *Journal of Immunology*, 194(5), 2059–2063.

Mackay, L. K., Minnich, M., Kragten, N. A. M., Liao, Y., Nota, B., Seillet, C., Zaid, A., Man, K., Preston, S., Freestone, D., Braun, A., Wynne-Jones, E., Behr, F. M., Stark, R., Pellicci, D. G., Godfrey, D. I., Belz, G. T., Pellegrini, M., Gebhardt, T., Busslinger, M., Shi, W., Carbone, F. R., van Lier, R. A. W., Kallies, A., & van Gisbergen, K. P. J. M. (2016). Hobit and Blimp1 instruct a universal transcriptional program of tissue residency in lymphocytes. *Science*, 352(6284), 459–463.

[\[PubMed\]](#)

MacMillan, M. L., Hippen, K. L., McKenna, D. H., Kadidlo, D., Sumstad, D., DeFor, T. E., Brunstein, C. G., Holtan, S. G., Miller, J. S., Warlick, E. D., Weisdorf, D. J., Wagner, J. E., & Blazar, B. R. (2021). First-in-human phase 1 trial of induced regulatory T cells for graft-versus-host disease prophylaxis in HLA-matched siblings. *Blood Advances*, 5(5), 1425–1436.

[\[PubMed\]](#)

Macpherson, A. J., & Harris, N. L. (2004). Interactions between commensal intestinal bacteria and the immune system. *Nature Reviews. Immunology*, 4(6), 478–485.

[\[PubMed\]](#)

Mager, L. F., Burkhard, R., Pett, N., Cooke, N. C. A., Brown, K., Ramay, H., Paik, S., Stagg, J., Groves, R. A., Gallo, M., Lewis, I. A., Geuking, M. B., & McCoy, K. D. (2020). Microbiome-derived inosine modulates response to checkpoint inhibitor immunotherapy. *Science*, 369(6510), 1481–1489.

[\[PubMed\]](#)

Malhotra, D., Fletcher, A. L., Astarita, J., Lukacs-Kornek, V., Tayalia, P., Gonzalez, S. F., Elpek, K. G., Chang, S. K., Knoblich, K., Hemler, M. E., Brenner, M. B., Carroll, M. C., Mooney, D. J., Turley, S. J., & Immunological Genome Project Consortium. (2012). Transcriptional profiling of stroma from inflamed and resting lymph nodes defines immunological hallmarks. *Nature Immunology*, 13(5), 499–510.

[\[PubMed\]](#)

Mann, E. R., Lam, Y. K., & Uhlig, H. H. (2024). Short-chain fatty acids: Linking diet, the microbiome and immunity. *Nature Reviews. Immunology*, 24(8), 577–595.

[\[PubMed\]](#)

Martin, H. M., Campbell, B. J., Hart, C. A., Mpofu, C., Nayar, M., Singh, R., Englyst, H., Williams, H. F., & Rhodes, J. M. (2004). Enhanced *Escherichia coli* adherence and invasion in Crohn's disease and colon cancer. *Gastroenterology*, 127(1), 80–93.

[\[PubMed\]](#)

Maslowski, K. M., Vieira, A. T., Ng, A., Kranich, J., Sierro, F., Di, Y., Schilter, H. C., Rolph, M. S., Mackay, F., Artis, D., Xavier, R. J., Teixeira, M. M., & Mackay, C. R. (2009). Regulation of inflammatory responses by gut microbiota and chemoattractant receptor GPR43. *Nature*, 461(7268), 1282–1286.

[\[https://doi.org/10.1038/nature08530\]](https://doi.org/10.1038/nature08530) [\[PubMed\]](#)

Masopust, D., Vezys, V., Marzo, A. L., & Lefrançois, L. (2001). Preferential localization of effector memory cells in nonlymphoid tissue. *Science*, 291(5512), 2413–2417.

[\[PubMed\]](#)

Masopust, D., Choo, D., Vezys, V., Wherry, E. J., Duraiswamy, J., Akondy, R., Wang, J., Casey, K. A.,

Barber, D. L., Kawamura, K. S., Fraser, K. A., Webby, R. J., Brinkmann, V., Butcher, E. C., Newell, K. A., & Ahmed, R. (2010). Dynamic T cell migration program provides resident memory within intestinal epithelium. *The Journal of Experimental Medicine*, 207(3), 553–564.

[\[PubMed\]](#)

Matteoli, G., Mazzini, E., Iliev, I. D., Mileti, E., Fallarino, F., Puccetti, P., Chieppa, M., & Rescigno, M. (2010). Gut CD103⁺ dendritic cells express indoleamine 2,3-dioxygenase which influences T regulatory/T effector cell balance and oral tolerance induction. *Gut*, 59(5), 595–604.

[\[PubMed\]](#)

Mazmanian, S. K., Liu, C. H., Tzianabos, A. O., & Kasper, D. L. (2005). An immunomodulatory molecule of symbiotic bacteria directs maturation of the host immune system. *Cell*, 122(1), 107–118.

[\[PubMed\]](#)

Mazmanian, S. K., Round, J. L., & Kasper, D. L. (2008). A microbial symbiosis factor prevents intestinal inflammatory disease. *Nature*, 453(7195), 620–625.

[\[https://doi.org/10.1038/nature07008\]](https://doi.org/10.1038/nature07008) [\[PubMed\]](#)

McDermott, A. J., & Huffnagle, G. B. (2014). The microbiome and regulation of mucosal immunity. *Immunology*, 142(1), 24–31.

[\[PubMed\]](#)

Meitei, H. T., & Lal, G. (2023). T cell receptor signaling in the differentiation and plasticity of CD4⁺ T cells. *Cytokine & Growth Factor Reviews*, 69, 14–27.

Miller, D., Gershater, M., Slutsky, R., Romero, R., & Gomez-Lopez, N. (2020). Maternal and fetal T cells in term pregnancy and preterm labor. *Cellular & Molecular Immunology*, 17(7), 693–704.

Milner, J. J., Toma, C., Yu, B., Zhang, K., Omilusik, K., Phan, A. T., Wang, D., Getzler, A. J., Nguyen, T., Crotty, S., Wang, W., Pipkin, M. E., & Goldrath, A. W. (2017). Runx3 programs CD8⁺ T cell residency in non-lymphoid tissues and tumours. *Nature*, 552(7684), 253–257.

[\[https://doi.org/10.1038/nature24993\]](https://doi.org/10.1038/nature24993) [\[PubMed\]](#)

Molenaar, R., Greuter, M., van der Marel, A. P. J., Roozendaal, R., Martin, S. F., Edele, F., Huehn, J., Förster, R., O'Toole, T., Jansen, W., Eestermans, I. L., Kraal, G., & Mebius, R. E. (2009). Lymph node stromal cells support dendritic cell-induced gut-homing of T cells. *Journal of Immunology (Baltimore, Md.: 1950)*, 183(10), 6395–6402.

[\[PubMed\]](#)

Monteleone, G., Kumberova, A., Croft, N. M., McKenzie, C., Steer, H. W., & MacDonald, T. T. (2001). Blocking Smad7 restores TGF- β 1 signaling in chronic inflammatory bowel disease. *The Journal of Clinical Investigation*, 108(4), 601–609.

[\[PubMed\]](#)

Monteleone, G., Laudisi, F., & Stolfi, C. (2023). Smad7 as a positive regulator of intestinal inflammatory diseases. *Current Research in Immunology*, 4, 100055.

[\[PubMed\]](#)

Mora, J. R., Bono, M. R., Manjunath, N., Weninger, W., Cavanagh, L. L., Roseblatt, M., & von Andrian, U. H. (2003). Selective imprinting of gut-homing T cells by Peyer's patch dendritic cells. *Nature*, 424(6944), 88–93.

[<https://doi.org/10.1038/nature01726>][[PubMed](#)]

Mörbe, U. M., Jørgensen, P. B., Fenton, T. M., von Burg, N., Riis, L. B., Spencer, J., & Agace, W. W. (2021). Human gut-associated lymphoid tissues (GALT); diversity, structure, and function. *Mucosal Immunology*, 14(4), 793–802.

[[PubMed](#)]

Mowat, A. M. (2003). Anatomical basis of tolerance and immunity to intestinal antigens. *Nature Reviews. Immunology*, 3(4), 331–341.

[[PubMed](#)]

Mowat, A. M., & Agace, W. W. (2014). Regional specialization within the intestinal immune system. *Nature Reviews. Immunology*, 14(10), 667–685.

[[PubMed](#)]

Mucida, D., Park, Y., Kim, G., Turovskaya, O., Scott, I., Kronenberg, M., & Cheroutre, H. (2007). Reciprocal TH17 and regulatory T cell differentiation mediated by retinoic acid. *Science*, 317(5835), 256–260.

[[PubMed](#)]

Muñoz-Rojas, A. R., & Mathis, D. (2021). Tissue regulatory T cells: Regulatory chameleons. *Nature Reviews. Immunology*, 21(9), 597–611.

[[PubMed](#)]

Murai, M., Turovskaya, O., Kim, G., Madan, R., Karp, C. L., Cheroutre, H., & Kronenberg, M. (2009). Interleukin 10 acts on regulatory T cells to maintain expression of the transcription factor Foxp3 and suppressive function in mice with colitis. *Nature Immunology*, 10(11), 1178–1184.

[[PubMed](#)]

Neumann, C., Blume, J., Roy, U., Teh, P. P., Vasanthakumar, A., Beller, A., Liao, Y., Heinrich, F., Arenzana, T. L., Hackney, J. A., Eidenschenk, C., Gálvez, E. J. C., Stehle, C., Heinz, G. A., Maschmeyer, P., Sidwell, T., Hu, Y., Amsen, D., Romagnani, C., Chang, H.-D., Kruglov, A., Mashreghi, M.-F., Shi, W., Strowig, T., Rutz, S., Kallies, A., & Scheffold, A. (2019). c-Maf-dependent Treg cell control of intestinal TH17 cells and IgA establishes host-microbiota homeostasis. *Nature Immunology*, 20(4), 471–481.

[[PubMed](#)]

Nogralles, K. E., Zaba, L. C., Shemer, A., Fuentes-Duculan, J., Cardinale, I., Kikuchi, T., Ramon, M., Bergman, R., Krueger, J. G., & Guttman-Yassky, E. (2009). IL-22-producing “T22” T cells account for upregulated IL-22 in atopic dermatitis despite reduced IL-17-producing TH17 T cells. *The Journal of Allergy and Clinical Immunology*, 123(6), 1244–1252.e2.

[[PubMed](#)]

Nutsch, K., Chai, J. N., Ai, T. L., Russler-Germain, E., Feehley, T., Nagler, C. R., & Hsieh, C.-S. (2016). Rapid and efficient generation of regulatory T cells to commensal antigens in the periphery. *Cell Reports*, 17(1), 206–220.

[\[PubMed\]](#)

Ohnmacht, C., Park, J.-H., Cording, S., Wing, J. B., Atarashi, K., Obata, Y., Gaboriau-Routhiau, V., Marques, R., Dulauroy, S., Fedoseeva, M., Busslinger, M., Cerf-Bensussan, N., Boneca, I. G., Voehringer, D., Hase, K., Honda, K., Sakaguchi, S., & Eberl, G. (2015). MUCOSAL IMMUNOLOGY. The microbiota regulates type 2 immunity through ROR γ ⁺ T cells. *Science*, 349(6251), 989–993.

[\[PubMed\]](#)

Ostman, S., Rask, C., Wold, A. E., Hultkrantz, S., & Teleme, E. (2006). Impaired regulatory T cell function in germ-free mice. *European Journal of Immunology*, 36(9), 2336–2346.

[\[PubMed\]](#)

Pabst, O., & Bernhardt, G. (2013). On the road to tolerance — Generation and migration of gut regulatory T cells. *European Journal of Immunology*, 43(6), 1422–1425.

[\[PubMed\]](#)

Pandiyan, P., Zheng, L., Ishihara, S., Reed, J., & Lenardo, M. J. (2007). CD4⁺CD25⁺Foxp3⁺ regulatory T cells induce cytokine deprivation-mediated apoptosis of effector CD4⁺ T cells. *Nature Immunology*, 8(12), 1353–1362.

[\[PubMed\]](#)

Panduro, M., Benoist, C., & Mathis, D. (2016). TISSUE-Tregs. *Annual Review of Immunology*, 34, 609–633.

[\[PubMed\]](#)

Panea, C., Farkas, A. M., Goto, Y., Abdollahi-Roodsaz, S., Lee, C., Koscsó, B., Gowda, K., Hohl, T. M., Bogunovic, M., & Ivanov, I. I. (2015). Intestinal monocyte-derived macrophages control commensal-specific Th17 responses. *Cell Reports*, 12(8), 1314–1324.

[\[PubMed\]](#)

Park, H. J., Park, J. S., Jeong, Y. H., Son, J., Ban, Y. H., Lee, B.-H., Chen, L., Chang, J., Chung, D. H., Choi, I., & Ha S.-J. (2015). PD-1 upregulated on regulatory T cells during chronic virus infection enhances the suppression of CD8⁺ T cell immune response via the interaction with PD-L1 expressed on CD8⁺ T cells. *Journal of Immunology (Baltimore, Md.: 1950)*, 194(12), 5801–5811.

[\[PubMed\]](#)

Parks, O. B., Pociask, D. A., Hodzic, Z., Kolls, J. K., & Good, M. (2015). Interleukin-22 signaling in the regulation of intestinal health and disease. *Frontiers in Cell and Development Biology*, 3, 85.

Pezoldt, J., Pasztoi, M., Zou, M., Wiechers, C., Beckstette, M., Thierry, G. R., Vafadarnejad, E., Floess, S., Arampatzi, P., Buettner, M., Schweer, J., Fleissner, D., Vital, M., Pieper, D. H., Basic, M., Dersch, P., Strowig, T., Hornef, M., Bleich, A., Bode, U., Pabst, O., Bajénoff, M., Saliba, A.-E., & Huehn, J. (2018). Neonatally imprinted stromal cell subsets induce tolerogenic dendritic cells in mesenteric lymph nodes. *Nature Communications*, 9(1), 3903.

[\[PubMed\]](#)

Polansky, J. K., Kretschmer, K., Freyer, J., Floess, S., Garbe, A., Baron, U., Olek, S., Hamann, A., von

Boehmer, H., & Huehn, J. (2008). DNA methylation controls Foxp3 gene expression. *European Journal of Immunology*, 38(6), 1654–1663.

[\[PubMed\]](#)

Pratama, A., Schnell, A., Mathis, D., & Benoist, C. (2020). Developmental and cellular age direct conversion of CD4⁺ T cells into RORγ⁺ or Helios⁺ colon Treg cells. *The Journal of Experimental Medicine*, 217(1), e20190428.

[\[PubMed\]](#)

Qiu, Z., Khairallah, C., Chu, T. H., Imperato, J. N., Lei, X., Romanov, G., Atakilit, A., Puddington, L., & Sheridan, B. S. (2023). Retinoic acid signaling during priming licenses intestinal CD103⁺ CD8 TRM cell differentiation. *The Journal of Experimental Medicine*, 220(5), e20210923.

[\[PubMed\]](#)

Raffin, C., Vo, L. T., & Bluestone, J. A. (2020). Treg cell-based therapies: Challenges and perspectives. *Nature Reviews. Immunology*, 20(3), 158–172.

[\[PubMed\]](#)

Ragonnaud, E., & Biragyn, A. (2021). Gut microbiota as the key controllers of “healthy” aging of elderly people. *Immunity & Ageing*, 18(1), 2.

Ramanan, D., Sefik, E., Galván-Peña, S., Wu, M., Yang, L., Yang, Z., Kostic, A., Golovkina, T. V., Kasper, D. L., Mathis, D., & Benoist, C. (2020). An immunologic mode of multigenerational transmission governs a gut Treg setpoint. *Cell*, 181(6), 1276–1290.e13.

[\[PubMed\]](#)

Ramanan, D., Pratama, A., Zhu, Y., Venezia, O., Sassone-Corsi, M., Chowdhary, K., Galván-Peña, S., Sefik, E., Brown, C., Gélinau, A., Mathis, D., & Benoist, C. (2023). Regulatory T cells in the face of the intestinal microbiota. *Nature Reviews. Immunology*, 23(11), 749–762.

[\[PubMed\]](#)

Randolph, G. J., Ivanov, S., Zinselmeyer, B. H., & Scallan, J. P. (2017). The lymphatic system: Integral roles in immunity. *Annual Review of Immunology*, 35, 31–52.

[\[PubMed\]](#)

Rankin, L. C., Kaiser, K. A., de los Santos-Alexis, K., Park, H., Uhlemann, A.-C., Gray, D. H. D., & Arpaia, N. (2023). Dietary tryptophan deficiency promotes gut RORγt⁺ Treg cells at the expense of Gata3⁺ Treg cells and alters commensal microbiota metabolism. *Cell Reports*, 42(3), 112135.

[\[PubMed\]](#)

Round, J. L., & Mazmanian, S. K. (2010). Inducible Foxp3⁺ regulatory T-cell development by a commensal bacterium of the intestinal microbiota. *Proceedings of the National Academy of Sciences of the United States of America*, 107(27), 12204–12209.

[\[PubMed\]](#)

Round, J. L., Lee, S. M., Li, J., Tran, G., Jabri, B., Chatila, T. A., & Mazmanian, S. K. (2011). The Toll-like receptor 2 pathway establishes colonization by a commensal of the human microbiota. *Science*, 332(6032), 974–977.

[[PubMed](#)]

Rowe, J. H., Ertelt, J. M., Xin, L., & Way, S. S. (2012). Pregnancy imprints regulatory memory that sustains anergy to fetal antigen. *Nature*, 490(7418), 102–106.

[<https://doi.org/10.1038/nature11462>][[PubMed](#)]

Rubtsov, Y. P., Rasmussen, J. P., Chi, E. Y., Fontenot, J., Castelli, L., Ye, X., Treuting, P., Siewe, L., Roers, A., Henderson, W. R., Muller, W., & Rudensky, A. Y. (2008). Regulatory T cell-derived interleukin-10 limits inflammation at environmental interfaces. *Immunity*, 28(4), 546–558.

[[PubMed](#)]

Rudra, S., Shaul, E., Conrad, M., Patel, T., Moore, A., Dawany, N., Canavan, M. C., Sullivan, K. E., Behrens, E., & Kelsen, J. R. (2022). Ruxolitinib: Targeted approach for treatment of autoinflammatory very early onset inflammatory bowel disease. *Clinical Gastroenterology and Hepatology*, 20(6), 1408–1410.e2.

[[PubMed](#)]

Ruscher, R., Kummer, R. L., Lee, Y. J., Jameson, S. C., & Hogquist, K. A. (2017). CD8 α intraepithelial lymphocytes arise from two main thymic precursors. *Nature Immunology*, 18(7), 771–779.

[[PubMed](#)]

Sakaguchi, S., Sakaguchi, N., Asano, M., Itoh, M., & Toda, M. (1995). Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor α -chains (CD25). Breakdown of a single mechanism of self-tolerance causes various autoimmune diseases. *Journal of Immunology*, 155(3), 1151–1164.

Sallusto, F., Lenig, D., Förster, R., Lipp, M., & Lanzavecchia, A. (1999). Two subsets of memory T lymphocytes with distinct homing potentials and effector functions. *Nature*, 401(6754), 708–712.

[<https://doi.org/10.1038/44385>][[PubMed](#)]

Samstein, R. M., Josefowicz, S. Z., Arvey, A., Treuting, P. M., & Rudensky, A. Y. (2012). Extrathymic generation of regulatory T cells in placental mammals mitigates maternal-fetal conflict. *Cell*, 150(1), 29–38.

[[PubMed](#)]

Sano, T., Huang, W., Hall, J. A., Yang, Y., Chen, A., Gavzy, S. J., Lee, J.-Y., Ziel, J. W., Miraldi, E. R., Domingos, A. I., Bonneau, R., & Littman, D. R. (2015). An IL-23R/IL-22 circuit regulates epithelial serum amyloid A to promote local effector Th17 responses. *Cell*, 163(2), 381–393.

[[PubMed](#)]

Sarkar, S., Kalia, V., Haining, W. N., Konieczny, B. T., Subramaniam, S., & Ahmed, R. (2008). Functional and genomic profiling of effector CD8 T cell subsets with distinct memory fates. *The Journal of Experimental Medicine*, 205(3), 625–640.

[[PubMed](#)]

Sasson, A. N., Ananthakrishnan, A. N., & Raman, M. (2021). Diet in treatment of inflammatory bowel diseases. *Clinical Gastroenterology and Hepatology*, 19(3), 425–435.e3.

[[PubMed](#)]

Sassone-Corsi, M., Azriel, S., Simon, A., Ramanan, D., Ortiz-Lopez, A., Chen, F., Yissachar, N., Mathis, D., & Benoist, C. (2022). Sequestration of gut pathobionts in intraluminal casts, a mechanism to avoid dysregulated T cell activation by pathobionts. *Proceedings of the National Academy of Sciences*, 119(41), e2209624119.

Schaper, K., Kietzmann, M., & Bäumer, W. (2014). Sphingosine-1-phosphate differently regulates the cytokine production of IL-12, IL-23 and IL-27 in activated murine bone marrow derived dendritic cells. *Molecular Immunology*, 59(1), 10–18.

[[PubMed](#)]

Schiering, C., Krausgruber, T., Chomka, A., Fröhlich, A., Adelmann, K., Wohlfert, E. A., Pott, J., Griseri, T., Bollrath, J., Hegazy, A. N., Harrison, O. J., Owens, B. M. J., Löhning, M., Belkaid, Y., Fallon, P. G., & Powrie, F. (2014). The alarmin IL-33 promotes regulatory T-cell function in the intestine. *Nature*, 513(7519), 564–568.

[<https://doi.org/10.1038/nature13577>][[PubMed](#)]

Schnell, A., Littman, D. R., & Kuchroo, V. K. (2023). TH17 cell heterogeneity and its role in tissue inflammation. *Nature Immunology*, 24(1), 19–29.

[[PubMed](#)]

Schön, M. P., Arya, A., Murphy, E. A., Adams, C. M., Strauch, U. G., Agace, W. W., Marsal, J., Donohue, J. P., Her, H., Beier, D. R., Olson, S., Lefrancois, L., Brenner, M. B., Grusby, M. J., & Parker, C. M. (1999). Mucosal T lymphocyte numbers are selectively reduced in integrin alpha E (CD103)-deficient mice. *Journal of Immunology (Baltimore, Md.: 1950)*, 162(11), 6641–6649.

[[PubMed](#)]

Scribano, M. L. (2018). Vedolizumab for inflammatory bowel disease: From randomized controlled trials to real-life evidence. *World Journal of Gastroenterology*, 24(23), 2457–2467.

[[PubMed](#)]

Sefik, E., Geva-Zatorsky, N., Oh, S., Konnikova, L., Zemmour, D., McGuire, A. M., Burzyn, D., Ortiz-Lopez, A., Lobera, M., Yang, J., Ghosh, S., Earl, A., Snapper, S. B., Jupp, R., Kasper, D., Mathis, D., & Benoist, C. (2015). Individual intestinal symbionts induce a distinct population of ROR γ^+ regulatory T cells. *Science*, 349(6251), 993–997.

[[PubMed](#)]

Seiderer, J., Elben, I., Diegelmann, J., Glas, J., Stallhofer, J., Tillack, C., Pfennig, S., Jürgens, M., Schmechel, S., Konrad, A., Göke, B., Ochsenkühn, T., Müller-Myhsok, B., Lohse, P., & Brand, S. (2008). Role of the novel Th17 cytokine IL-17F in inflammatory bowel disease (IBD): Upregulated colonic IL-17F expression in active Crohn's disease and analysis of the IL17F p.His161Arg polymorphism in IBD. *Inflammatory Bowel Diseases*, 14(4), 437–445.

[[PubMed](#)]

Shevach, E. M. (2009). Mechanisms of foxp3 $^+$ T regulatory cell-mediated suppression. *Immunity*, 30(5), 636–645.

[[PubMed](#)]

Siewert, C., Menning, A., Dudda, J., Siegmund, K., Lauer, U., Floess, S., Campbell, D. J., Hamann, A., &

Huehn, J. (2007). Induction of organ-selective CD4⁺ regulatory T cell homing. *European Journal of Immunology*, 37(4), 978–989.

[\[PubMed\]](#)

Singh, N., Gurav, A., Sivaprakasam, S., Brady, E., Padia, R., Shi, H., Thangaraju, M., Prasad, P. D., Manicassamy, S., Munn, D. H., Lee, J. R., Offermanns, S., & Ganapathy, V. (2014). Activation of Gpr109a, receptor for niacin and the commensal metabolite butyrate, suppresses colonic inflammation and carcinogenesis. *Immunity*, 40(1), 128–139.

[\[PubMed\]](#)

Singh, K., Hjort, M., Thorvaldson, L., & Sandler, S. (2015). Concomitant analysis of Helios and Neuropilin-1 as a marker to detect thymic derived regulatory T cells in naïve mice. *Scientific Reports*, 5(1), 7767.

[\[PubMed\]](#)

Skon, C. N., Lee, J.-Y., Anderson, K. G., Masopust, D., Hogquist, K. A., & Jameson, S. C. (2013). Transcriptional downregulation of S1pr1 is required for the establishment of resident memory CD8⁺ T cells. *Nature Immunology*, 14(12), 1285–1293.

[\[PubMed\]](#)

Smith, P. M., Howitt, M. R., Panikov, N., Michaud, M., Gallini, C. A., Bohlooly-Y, M., Glickman, J. N., & Garrett, W. S. (2013). The microbial metabolites, short-chain fatty acids, regulate colonic Treg cell homeostasis. *Science*, 341(6145), 569–573.

[\[PubMed\]](#)

Smyth, L. A., Ratnasothy, K., Tsang, J. Y. S., Boardman, D., Warley, A., Lechler, R., & Lombardi, G. (2013). CD73 expression on extracellular vesicles derived from CD4⁺ CD25⁺ Foxp3⁺ T cells contributes to their regulatory function. *European Journal of Immunology*, 43(9), 2430–2440.

[\[PubMed\]](#)

Sokol, H., Seksik, P., Furet, J. P., Firmesse, O., Nion-Larmurier, I., Beaugerie, L., Cosnes, J., Corthier, G., Marteau, P., & Doré, J. (2009). Low counts of *Faecalibacterium prausnitzii* in colitis microbiota. *Inflammatory Bowel Diseases*, 15(8), 1183–1189.

[\[PubMed\]](#)

Solomon, B. D., & Hsieh, C.-S. (2016). Antigen-specific development of mucosal Foxp3⁺RORγt⁺ T cells from regulatory T cell precursors. *Journal of Immunology*, 197(9), 3512–3519.

Song, X., Sun, X., Oh, S. F., Wu, M., Zhang, Y., Zheng, W., Geva-Zatorsky, N., Jupp, R., Mathis, D., Benoist, C., & Kasper, D. L. (2020). Microbial bile acid metabolites modulate gut RORγ⁺ regulatory T cell homeostasis. *Nature*, 577(7790), 410–415.

[\[https://doi.org/10.1038/s41586-019-1865-0\]](https://doi.org/10.1038/s41586-019-1865-0)[\[PubMed\]](#)

Song, C., Chai, Z., Chen, S., Zhang, H., Zhang, X., & Zhou, Y. (2023). Intestinal mucus components and secretion mechanisms: What we do and do not know. *Experimental & Molecular Medicine*, 55(4), 681–691.

Spadoni, I., Iliev, I. D., Rossi, G., & Rescigno, M. (2012). Dendritic cells produce TSLP that limits the differentiation of Th17 cells, fosters Treg development, and protects against colitis. *Mucosal Immunology*, 5(2), 184–193.

[\[PubMed\]](#)

Spath, S., Roan, F., Presnell, S. R., Höllbacher, B., & Ziegler, S. F. (2022). Profiling of Tregs across tissues reveals plasticity in ST2 expression and hierarchies in tissue-specific phenotypes. *iScience*, 25(9), 104998.

[\[PubMed\]](#)

Spindelboeck, W., Schulz, E., Uhl, B., Kashofer, K., Aigelsreiter, A., Zinke-Cerwenka, W., Mulabecirovic, A., Kump, P. K., Halwachs, B., Gorkiewicz, G., Sill, H., Greinix, H., Högenauer, C., & Neumeister, P. (2017). Repeated fecal microbiota transplantations attenuate diarrhea and lead to sustained changes in the fecal microbiota in acute, refractory gastrointestinal graft-versus-host-disease. *Haematologica*, 102(5), e210–e213.

[\[PubMed\]](#)

Springer, T. A. (1994). Traffic signals for lymphocyte recirculation and leukocyte emigration: The multistep paradigm. *Cell*, 76(2), 301–314.

[\[PubMed\]](#)

Stemberger, C., Neuenhahn, M., Buchholz, V. R., & Busch, D. H. (2007). Origin of CD8⁺ effector and memory T cell subsets, 4(6), 399.

Stemberger, C., Neuenhahn, M., Gebhardt, F. E., Schiemann, M., Buchholz, V. R., & Busch, D. H. (2009). Stem cell-like plasticity of naïve and distinct memory CD8⁺ T cell subsets. *Seminars in Immunology*, 21(2), 62–68.

[\[PubMed\]](#)

Strauss, J., Kaplan, G. G., Beck, P. L., Rioux, K., Panaccione, R., DeVinney, R., Lynch, T., & Allen-Vercoe, E. (2011). Invasive potential of gut mucosa-derived fusobacterium nucleatum positively correlates with IBD status of the host. *Inflammatory Bowel Diseases*, 17(9), 1971–1978.

[\[PubMed\]](#)

Sumida, T. S., Cheru, N. T., & Hafler, D. A. (2024). The regulation and differentiation of regulatory T cells and their dysfunction in autoimmune diseases. *Nature Reviews. Immunology*, 24(7), 503–517.

[\[PubMed\]](#)

Sun, C.-M., Hall, J. A., Blank, R. B., Bouladoux, N., Oukka, M., Mora, J. R., & Belkaid, Y. (2007). Small intestine lamina propria dendritic cells promote de novo generation of Foxp3 T reg cells via retinoic acid. *The Journal of Experimental Medicine*, 204(8), 1775–1785.

[\[PubMed\]](#)

Sun, R., Xu, C., Feng, B., Gao, X., & Liu, Z. (2021). Critical roles of bile acids in regulating intestinal mucosal immune responses. *Therapeutic Advances in Gastroenterology*, 14, 17562848211018098.

[\[PubMed\]](#)

Sun, L., Su, Y., Jiao, A., Wang, X., & Zhang, B. (2023). T cells in health and disease. *Signal Transduction and Targeted Therapy*, 8(1), 1–50.

Suriano, F., Nyström, E. E. L., Sergi, D., & Gustafsson, J. K. (2022). Diet, microbiota, and the mucus layer: The guardians of our health. *Frontiers in Immunology*, 13, 953196.

[\[PubMed\]](#)

Tanoue, T., Atarashi, K., & Honda, K. (2016). Development and maintenance of intestinal regulatory T cells. *Nature Reviews. Immunology*, 16(5), 295–309.

[\[PubMed\]](#)

Tao, R., de Zoeten, E. F., Özkaynak, E., Chen, C., Wang, L., Porrett, P. M., Li, B., Turka, L. A., Olson, E. N., Greene, M. I., Wells, A. D., & Hancock, W. W. (2007). Deacetylase inhibition promotes the generation and function of regulatory T cells. *Nature Medicine*, 13(11), 1299–1307.

[\[PubMed\]](#)

Tartar, D. M., VanMorlan, A. M., Wan, X., Guloglu, F. B., Jain, R., Haymaker, C. L., Ellis, J. S., Hoeman, C. M., Cascio, J. A., Dhakal, M., Oukka, M., & Zaghoulani, H. (2010). FoxP3⁺RORγt⁺ T helper intermediates display suppressive function against autoimmune diabetes. *Journal of Immunology*, 184(7), 3377–3385.

Thompson, E. A., Mitchell, J. S., Beura, L. K., Torres, D. J., Mrass, P., Pierson, M. J., Cannon, J. L., Masopust, D., Fife, B. T., & Vezys, V. (2019). Interstitial migration of CD8αβ T cells in the small intestine is dynamic and is dictated by environmental cues. *Cell Reports*, 26(11), 2859–2867.e4.

[\[PubMed\]](#)

Thornton, A. M., Korty, P. E., Tran, D. Q., Wohlfert, E. A., Murray, P. E., Belkaid, Y., & Shevach, E. M. (2010). Expression of Helios, an Ikaros transcription factor family member, differentiates thymic-derived from peripherally induced Foxp3⁺ T regulatory cells. *Journal of Immunology*, 184(7), 3433–3441.

Toker, A., Engelbert, D., Garg, G., Polansky, J. K., Floess, S., Miyao, T., Baron, U., Düber, S., Geffers, R., Giehr, P., Schallenberg, S., Kretschmer, K., Olek, S., Walter, J., Weiss, S., Hori, S., Hamann, A., & Huehn, J. (2013). Active demethylation of the Foxp3 locus leads to the generation of stable regulatory T cells within the thymus. *Journal of Immunology (Baltimore, Md.: 1950)*, 190(7), 3180–3188.

[\[PubMed\]](#)

Tone, Y., Furuuchi, K., Kojima, Y., Tykocinski, M. L., Greene, M. I., & Tone, M. (2008). Smad3 and NFAT cooperate to induce Foxp3 expression through its enhancer. *Nature Immunology*, 9(2), 194–202.

[\[PubMed\]](#)

Topping, D. L., & Clifton, P. M. (2001). Short-chain fatty acids and human colonic function: Roles of resistant starch and nonstarch polysaccharides. *Physiological Reviews*, 81(3), 1031–1064.

[\[PubMed\]](#)

Torow, N., Yu, K., Hassani, K., Freitag, J., Schulz, O., Basic, M., Brennecke, A., Sparwasser, T., Wagner, N., Bleich, A., Lochner, M., Weiss, S., Förster, R., Pabst, O., & Hornef, M. W. (2015). Active

suppression of intestinal CD4⁺TCRαβ⁺ T-lymphocyte maturation during the postnatal period. *Nature Communications*, 6, 7725.

[\[PubMed\]](#)

Touch, S., Godefroy, E., Rolhion, N., Danne, C., Oeuvray, C., Straube, M., Galbert, C., Brot, L., Salgueiro, I. A., Chadi, S., Ledent, T., Chatel, J.-M., Langella, P., Jotereau, F., Altare, F., & Sokol, H. (2022). Human CD4⁺CD8α⁺ Tregs induced by *Faecalibacterium prausnitzii* protect against intestinal inflammation. *JCI Insight*, 7(12), e154722.

[\[PubMed\]](#)

Travis, M. A., & Romagnani, C. (2022). How regulatory T cells are primed to aid tolerance of gut bacteria. *Nature*, 610(7933), 638–640.

[\[https://doi.org/10.1038/d41586-022-03368-2\]](https://doi.org/10.1038/d41586-022-03368-2) [\[PubMed\]](#)

van Dullemen, H. M., van Deventer, S. J. H., Hommes, D. W., Bijl, H. A., Jansen, J., Tytgat, G. N. J., & Woody, J. (1995). Treatment of Crohn's disease with anti-tumor necrosis factor chimeric monoclonal antibody (cA2). *Gastroenterology*, 109(1), 129–135.

[\[PubMed\]](#)

van der Veeken, J., Campbell, C., Pritykin, Y., Schizas, M., Verter, J., Hu, W., Wang, Z.-M., Matheis, F., Mucida, D., Charbonnier, L.-M., Chatila, T. A., & Rudensky, A. Y. (2022). Genetic tracing reveals transcription factor Foxp3-dependent and Foxp3-independent functionality of peripherally induced Treg cells. *Immunity*, 55(7), 1173–1184.e7.

[\[PubMed\]](#)

Vantourout, P., & Hayday, A. (2013). Six-of-the-best: Unique contributions of γδ T cells to immunology. *Nature Reviews. Immunology*, 13(2), 88–100.

[\[PubMed\]](#)

Verma, R., Lee, C., Jeun, E.-J., Yi, J., Kim, K. S., Ghosh, A., Byun, S., Lee, C.-G., Kang, H.-J., Kim, G.-C., Jun, C.-D., Jan, G., Suh, C.-H., Jung, J.-Y., Sprent, J., Rudra, D., De Castro, C., Molinaro, A., Surh, C. D., & Im, S.-H. (2018). Cell surface polysaccharides of *Bifidobacterium bifidum* induce the generation of Foxp3⁺ regulatory T cells. *Science Immunology*, 3(28), eaat6975.

[\[PubMed\]](#)

Vignali, D. A. A., Collison, L. W., & Workman, C. J. (2008). How regulatory T cells work. *Nature Reviews. Immunology*, 8(7), 523–532.

[\[PubMed\]](#)

Voo, K. S., Wang, Y.-H., Santori, F. R., Boggiano, C., Wang, Y.-H., Arima, K., Bover, L., Hanabuchi, S., Khalili, J., Marinova, E., Zheng, B., Littman, D. R., & Liu, Y.-J. (2009). Identification of IL-17-producing FOXP3⁺ regulatory T cells in humans. *Proceedings of the National Academy of Sciences of the United States of America*, 106(12), 4793–4798.

[\[PubMed\]](#)

Voskens, C. J., Stoica, D., Roessner, S., Vitali, F., Zundler, S., Rosenberg, M., Wiesinger, M., Wunder, J., Siegmund, B., Schuler-Thurner, B., Schuler, G., Berking, C., Atreya, R., & Neurath, M. F. (2021). Safety and tolerability of a single infusion of autologous ex vivo expanded regulatory T cells in adults

with ulcerative colitis (ER-TREG 01): Protocol of a phase 1, open-label, fast-track dose-escalation clinical trial. *BMJ Open*, 11(12), e049208.

[\[PubMed\]](#)

Vyas, S. P., & Goswami, R. (2018). A decade of Th9 cells: Role of Th9 cells in inflammatory bowel disease. *Frontiers in Immunology*, 9, 1139.

[\[PubMed\]](#)

Walker, L. S. K. (2022). The link between circulating follicular helper T cells and autoimmunity. *Nature Reviews. Immunology*, 22(9), 567–575.

[\[PubMed\]](#)

Walker, J. A., & McKenzie, A. N. J. (2018). TH2 cell development and function. *Nature Reviews. Immunology*, 18(2), 121–133.

[\[PubMed\]](#)

Walker, L. S. K., & Sansom, D. M. (2011). The emerging role of CTLA4 as a cell-extrinsic regulator of T cell responses. *Nature Reviews. Immunology*, 11(12), 852–863.

[\[PubMed\]](#)

Walsh, D. A., Borges da Silva, H., Beura, L. K., Peng, C., Hamilton, S. E., Masopust, D., & Jameson, S. C. (2019). The functional requirement for CD69 in establishment of resident memory CD8⁺ T cells varies with tissue location. *Journal of Immunology (Baltimore, Md.: 1950)*, 203(4), 946–955.

[\[PubMed\]](#)

Weber, B., Saurer, L., Schenk, M., Dickgreber, N., & Mueller, C. (2011). CX3CR1 defines functionally distinct intestinal mononuclear phagocyte subsets which maintain their respective functions during homeostatic and inflammatory conditions. *European Journal of Immunology*, 41(3), 773–779.

[\[PubMed\]](#)

Weber, D., Meedt, E., Poeck, H., Thiele-Orberg, E., Hiergeist, A., Gessner, A., & Holler, E. (2024). Fecal microbiota transfer in acute graft-versus-host disease following allogeneic stem cell transplantation. *Visceral Medicine*, 40(4), 1–6.

[\[PubMed\]](#)

Weiss, J. M., Bilate, A. M., Gobert, M., Ding, Y., Curotto de Lafaille, M. A., Parkhurst, C. N., Xiong, H., Dolpady, J., Frey, A. B., Ruocco, M. G., Yang, Y., Floess, S., Huehn, J., Oh, S., Li, M. O., Niec, R. E., Rudensky, A. Y., Dustin, M. L., Littman, D. R., & Lafaille, J. J. (2012). Neuropilin 1 is expressed on thymus-derived natural regulatory T cells, but not mucosa-generated induced Foxp3⁺ T reg cells. *The Journal of Experimental Medicine*, 209(10), 1723–1742.

[\[PubMed\]](#)

Whibley, N., Tucci, A., & Powrie, F. (2019). Regulatory T cell adaptation in the intestine and skin. *Nature Immunology*, 20(4), 386–396.

[\[PubMed\]](#)

Wiechers, C., Zou, M., Galvez, E., Beckstette, M., Ebel, M., Strowig, T., Huehn, J., & Pezoldt, J. (2021).

The microbiota is dispensable for the early stages of peripheral regulatory T cell induction within mesenteric lymph nodes. *Cellular & Molecular Immunology*, 18(5), 1211–1221.

Wildin, R. S., Ramsdell, F., Peake, J., Faravelli, F., Casanova, J.-L., Buist, N., Levy-Lahad, E., Mazzella, M., Goulet, O., Perroni, L., Dagna Bricarelli, F., Byrne, G., McEuen, M., Proll, S., Appleby, M., & Brunkow, M. E. (2001). X-linked neonatal diabetes mellitus, enteropathy and endocrinopathy syndrome is the human equivalent of mouse scurfy. *Nature Genetics*, 27(1), 18–20.
[\[PubMed\]](#)

Wing, K., Onishi, Y., Prieto-Martin, P., Yamaguchi, T., Miyara, M., Fehervari, Z., Nomura, T., & Sakaguchi, S. (2008). CTLA-4 control over Foxp3⁺ regulatory T cell function. *Science*, 322(5899), 271–275.
[\[PubMed\]](#)

Wohlfert, E. A., Grainger, J. R., Bouladoux, N., Konkel, J. E., Oldenhove, G., Ribeiro, C. H., Hall, J. A., Yagi, R., Naik, S., Bhairavabhotla, R., Paul, W. E., Bosselut, R., Wei, G., Zhao, K., Oukka, M., Zhu, J., & Belkaid, Y. (2011). GATA3 controls Foxp3⁺ regulatory T cell fate during inflammation in mice. *The Journal of Clinical Investigation*, 121(11), 4503–4515.
[\[PubMed\]](#)

Worbs, T., Bode, U., Yan, S., Hoffmann, M. W., Hintzen, G., Bernhardt, G., Förster, R., & Pabst, O. (2006). Oral tolerance originates in the intestinal immune system and relies on antigen carriage by dendritic cells. *The Journal of Experimental Medicine*, 203(3), 519–527.
[\[PubMed\]](#)

Wu, H.-J., Ivanov, I. I., Darce, J., Hattori, K., Shima, T., Umesaki, Y., Littman, D. R., Benoist, C., & Mathis, D. (2010). Gut-residing segmented filamentous bacteria drive autoimmune arthritis via T helper 17 cells. *Immunity*, 32(6), 815–827.
[\[PubMed\]](#)

Xu, M., Pokrovskii, M., Ding, Y., Yi, R., Au, C., Harrison, O. J., Galan, C., Belkaid, Y., Bonneau, R., & Littman, D. R. (2018). c-MAF-dependent regulatory T cells mediate immunological tolerance to a gut pathobiont. *Nature*, 554(7692), 373–377.
[\[https://doi.org/10.1038/nature25500\]](https://doi.org/10.1038/nature25500)[\[PubMed\]](#)

Yadegar, A., Bar-Yoseph, H., Monaghan, T. M., Pakpour, S., Severino, A., Kuijper, E. J., Smits, W. K., Terveer, E. M., Neupane, S., Nabavi-Rad, A., Sadeghi, J., Cammarota, G., Ianiro, G., Nap-Hill, E., Leung, D., Wong, K., & Kao, D. (2024). Fecal microbiota transplantation: Current challenges and future landscapes. *Clinical Microbiology Reviews*, 37(2), e00060–e00022.
[\[PubMed\]](#)

Yalniz, F. F., Hefazi, M., McCullough, K., Litzow, M. R., Hogan, W. J., Wolf, R., Alkhateeb, H., Kansagra, A., Damla, M., & Patnaik, M. M. (2017). Safety and efficacy of Infliximab therapy in the setting of steroid-refractory acute graft-versus-host disease. *Biology of Blood and Marrow Transplantation*, 23(9), 1478–1484.
[\[PubMed\]](#)

Yang, B.-H., Hagemann, S., Mamareli, P., Lauer, U., Hoffmann, U., Beckstette, M., Föhse, L., Prinz, I.,

Pezoldt, J., Suerbaum, S., Sparwasser, T., Hamann, A., Floess, S., Huehn, J., & Lochner, M. (2016).

Foxp3⁺ T cells expressing ROR γ t represent a stable regulatory T-cell effector lineage with enhanced suppressive capacity during intestinal inflammation. *Mucosal Immunology*, 9(2), 444–457.

[\[PubMed\]](#)

Yao, S., Zhao, Z., Wang, W., & Liu, X. (2021). Bifidobacterium longum: Protection against inflammatory bowel disease. *J Immunol Res*, 2021, 8030297.

[\[PubMed\]](#)

Ye, J., Qiu, J., Bostick, J. W., Ueda, A., Schjerven, H., Li, S., Jobin, C., Chen, Z. E., & Zhou, L. (2017). The aryl hydrocarbon receptor preferentially marks and promotes gut regulatory T cells. *Cell Reports*, 21(8), 2277–2290.

[\[PubMed\]](#)

Yeh, A. C., Koyama, M., Waltner, O. G., Minnie, S. A., Boiko, J. R., Shabaneh, T. B., Takahashi, S., Zhang, P., Ensbey, K. S., Schmidt, C. R., Legg, S. R. W., Sekiguchi, T., Nelson, E., Bhise, S. S., Stevens, A. R., Goodpaster, T., Chakka, S., Furlan, S. N., Markey, K. A., Bleakley, M. E., Elson, C. O., Bradley, P. H., & Hill, G. R. (2024). Microbiota dictate T cell clonal selection to augment graft-versus-host disease after stem cell transplantation. *Immunity*, 57(7), 1648–1664.e9.

[\[PubMed\]](#)

You, F.-P., Zhang, J., Cui, T., Zhu, R., Lv, C.-Q., Tang, H.-T., & Sun, D.-W. (2017). Th9 cells promote antitumor immunity via IL-9 and IL-21 and demonstrate atypical cytokine expression in breast cancer. *International Immunopharmacology*, 52, 163–167.

[\[PubMed\]](#)

Yu, D., Walker, L. S. K., Liu, Z., Linterman, M. A., & Li, Z. (2022). Targeting TFH cells in human diseases and vaccination: Rationale and practice. *Nature Immunology*, 23(8), 1157–1168.

[\[PubMed\]](#)

Yui, M. A., & Rothenberg, E. V. (2014). Developmental gene networks: A triathlon on the course to T cell identity. *Nature Reviews. Immunology*, 14(8), 529–545.

[\[PubMed\]](#)

Zhang, L., Li, Y., Li, Y., Qi, L., Liu, X., Yuan, C., Hu, N., Ma, D., Li, Z., Yang, Q., Li, W., & Li, J. (2012). Increased frequencies of Th22 cells as well as Th17 cells in the peripheral blood of patients with ankylosing spondylitis and rheumatoid arthritis. *PLoS One*, 7(4), e31000.

[\[PubMed\]](#)

Zhang, Z., Zhang, W., Guo, J., Gu, Q., Zhu, X., & Zhou, X. (2017). Activation and functional specialization of regulatory T cells lead to the generation of Foxp3 instability. *Journal of Immunology (Baltimore, Md.: 1950)*, 198(7), 2612–2625.

[\[PubMed\]](#)

Zhang, L., Liu, F., Xue, J., Lee, S. A., Liu, L., & Riordan, S. M. (2022). Bacterial species associated with human inflammatory bowel disease and their pathogenic mechanisms. *Frontiers in Microbiology*, 13, 801892.

[\[PubMed\]](#)

Zhao, H., Xu, H., Chen, S., He, J., Zhou, Y., & Nie, Y. (2021). Systematic review and meta-analysis of the role of alteration in inflammatory bowel disease. *Journal of Gastroenterology and Hepatology*, 36(2), 320–328.

[\[PubMed\]](#)

Zheng, Y., Josefowicz, S., Chaudhry, A., Peng, X. P., Forbush, K., & Rudensky, A. Y. (2010). Role of conserved non-coding DNA elements in the Foxp3 gene in regulatory T-cell fate. *Nature*, 463(7282), 808–812.

[\[https://doi.org/10.1038/nature08750\]](https://doi.org/10.1038/nature08750) [\[PubMed\]](#)

Zheng, L., Duan, S.-L., Dai, Y.-C., & Wu, S.-C. (2022). Role of adherent invasive Escherichia coli in pathogenesis of inflammatory bowel disease. *World Journal of Clinical Cases*, 10(32), 11671–11689.

[\[PubMed\]](#)

Zhou, L., Lopes, J. E., Chong, M. M. W., Ivanov, I. I., Min, R., Victora, G. D., Shen, Y., Du, J., Rubtsov, Y. P., Rudensky, A. Y., Ziegler, S. F., & Littman, D. R. (2008). TGF- β -induced Foxp3 inhibits TH17 cell differentiation by antagonizing ROR γ t function. *Nature*, 453(7192), 236–240.

[\[https://doi.org/10.1038/nature06878\]](https://doi.org/10.1038/nature06878) [\[PubMed\]](#)

Zhu, J., Yamane, H., & Paul, W. E. (2010). Differentiation of effector CD4 T cell populations. *Annual Review of Immunology*, 28, 445–489.

[\[PubMed\]](#)

Zou, M., Wiechers, C., & Huehn, J. (2021). Lymph node stromal cell subsets—Emerging specialists for tailored tissue-specific immune responses. *International Journal of Medical Microbiology*, 311(3), 151492.

[\[PubMed\]](#)

Zou, M., Pezoldt, J., Mohr, J., Philipsen, L., Leufgen, A., Cerovic, V., Wiechers, C., Pils, M., Ortiz, D., Hao, L., Yang, J., Beckstette, M., Dupont, A., Hornef, M., Dersch, P., Strowig, T., Müller, A. J., Raila, J., & Huehn, J. (2024). Early-life vitamin A treatment rescues neonatal infection-induced durably impaired tolerogenic properties of celiac lymph nodes. *Cell Reports*, 43(5), 114153.

[\[PubMed\]](#)

10. Microbiome and Gut Immunity: B Cells

Oliver Pabst¹ 

(¹) Institute of Molecular Medicine, RWTH Aachen University, Aachen, Germany

 **Oliver Pabst**

Email: opabst@ukaachen.de

Abstract

The intestinal mucosa is protected by secretory immunoglobulins (SIg). SIgs are produced by the combined function of plasma cells and epithelial cells, and constitute the main type of antibody produced in mice and humans. After their release into the gut lumen, SIgs regulate the interaction with the microbiome, neutralize toxins, and prevent infections. In this chapter, the organization of the intestinal B cell compartment, pathways of SIg generation, and the function of SIg in regulating the intestinal microbiota will be discussed.

Key words B cells – GALT – IgA – Secretory antibodies

10.1 Organization of the Intestinal B Cell Compartment

The intestinal immune system can be subdivided into inductive and effector compartments. Inductive compartments comprise the gut-associated lymphoid tissue (GALT) and the gut-draining mesenteric

lymph nodes (mLN), whereas the gut lamina propria forms the major effector site (Fig. [10.1](#)). With respect to the B cell compartment, naïve B cells and activated B cells undergoing somatic hypermutation and class switch recombination in germinal centers are characteristic of the inductive sites. Human B cells in GALT typically co-express CD19 and CD20. In contrast, fully mature plasma cells producing antibodies are the major B cell population in the lamina propria. Human plasma cells and their precursors do not express CD20 but may retain some expression of CD19 (Spencer & Bemark, [2023](#)).

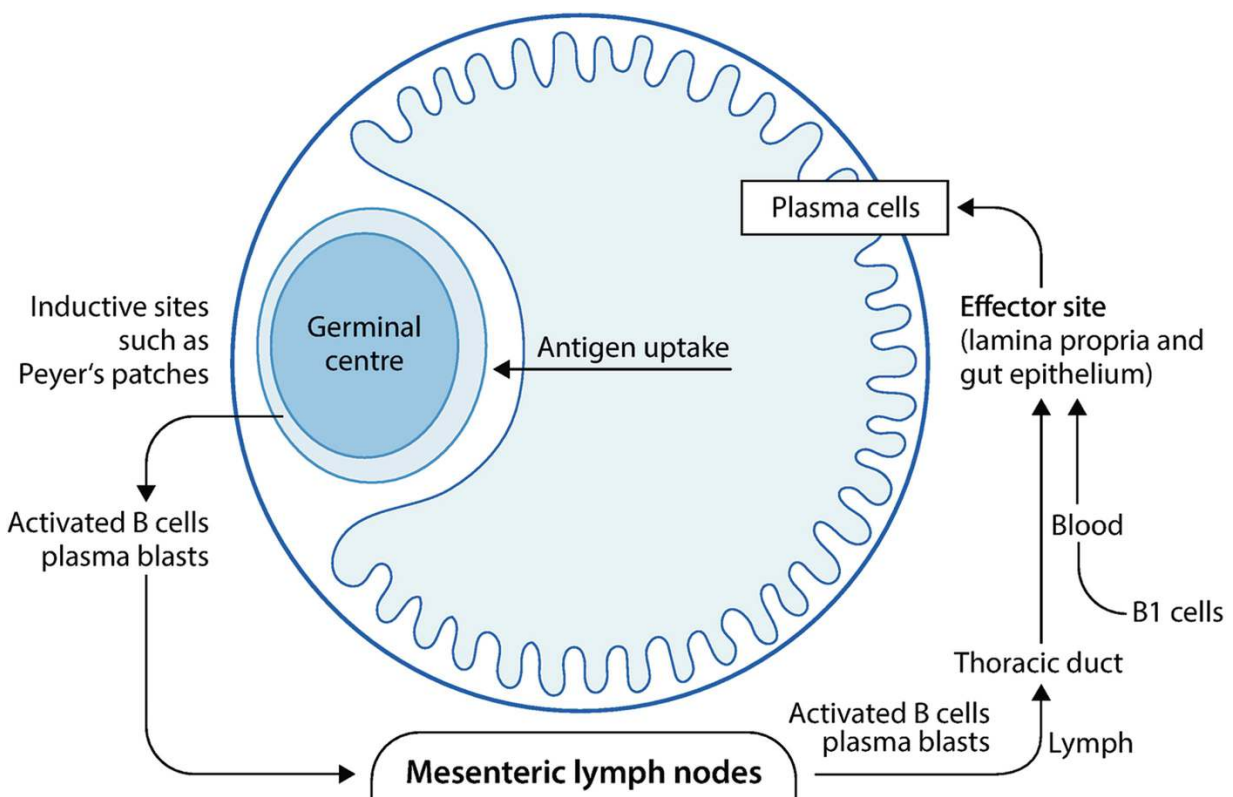


Fig. 10.1 Organization of the intestinal B cell immune system. Peyer's patches constitute a major inductive compartment of the gut immune system. PP constantly receives antigen from the gut lumen. Antigen presentation, lymphocyte activation, cognate interaction between B and T cells, and cytokine networks generate activated B cells and plasma blasts. These activated cells leave the PP, enter the mesenteric lymph nodes, and via the thoracic duct, they enter the central circulation. From blood plasma, blasts enter the intestinal lamina propria. Alternative pathways, including homing of B1 cells from circulation directly into the lamina propria, have been suggested

GALT shows important differences compared to peripheral lymph nodes or mLN. Whereas lymph nodes are encapsulated structures and

receive antigen via afferent lymphatics and blood, GALT structures lack a capsule, lack afferent lymphatics, and are separated from the intestinal lumen by a single epithelial layer, the follicle-associated epithelium (FAE). Within the FAE, a unique type of epithelial cells, the M cells, together with myeloid cells (Lelouard et al., [2012](#)) embedded in the FAE, confer the constitutive uptake of luminal antigen into the underlying lymphoid tissue.

The archetypal representatives of GALT are Peyer's patches (PP), secondary lymphoid organs of the small intestine named after the German anatomist Johann Conrad Peyer. PP form during gestation and consists of a number of aggregated lymphoid follicles, each covered by FAE and characterized by the constitutive presence of large germinal centers. In mice, PP are macroscopically visible and distributed along the length of the small intestine. A single PP-like structure, the cecal patch, is found in the murine cecum, and 3–5 large lymphoid follicles are present in the colon (also referred to as colonic patches (Baptista, [2013](#)). Besides PP, cecal and colonic patches, small-sized lymphoid clusters named isolated lymphoid follicles (ILF) are dispersed throughout the murine small intestine. ILF share key features of PP, including the presence of FAE and a characteristic B cell follicle, but in contrast to PP are much smaller and form only after birth (Baptista, [2013](#)). Notably, the presence and function of GALT and the composition of the microbiota are intrinsically linked: GALT serves the induction of B cell responses against the microbiota, and conversely, the microbiota shapes GALT development and function and leads to its chronic activation.

Similar to the situation in mice, in humans PP form during gestation, but compared to mice show a more accentuated aggregation in the ileum. The ontogeny of human ILF has not yet been fully established, but similar to the situation in mice, small-sized lymphoid clusters have also been described in the human intestine (Bemark et al., [2024](#)).

GALT does not have a distinct border and diffuses gradually into the surrounding lamina propria. In consequence, cells isolated from the

lamina propria may easily be contaminated by cells isolated from adjacent GALT, in particular from ILF. Therefore, some controversy exists concerning the presence of non-plasma cell B cells in the lamina propria. Irrespective of this subtlety, the main population of B cells found in the lamina propria is fully matured antibody-producing plasma cells. Intestinal plasma cells are estimated to make up to 80% of all plasma cells in humans and to produce several grams of antibody per day (Tomasi et al., [1965](#)). This makes the intestinal mucosa the most active site of antibody production in the whole body.

10.2 Origin of Antibodies in the Gut Lumen

With respect to structure and generation, intestinal antibodies show important differences between mice and humans. In mice, by far the dominating isotype in the intestinal lamina propria is polymeric immunoglobulin A (pIgA). In contrast, humans have two IgA isotypes, IgA1 and IgA2, encoded by distinct constant regions as well as IgM-expressing plasma cells, which are more numerous compared to mice (Magri et al., [2017](#)). This picture is characteristic of the healthy gut mucosa but dramatically changes during inflammation when, in particular, IgG-expressing plasma cells accumulate besides IgA- and IgM-secreting cells in the inflamed gut tissue (Spencer & Bemerk, [2023](#)).

IgA1 and IgA2 show unique biological properties. All SIgs are relatively resistant to proteolytic cleavage. However, IgA1 possesses a hinge region lacking in IgA2, making IgA1 more prone to proteolytic breakdown in the gut (Woof & Russell, [2011](#)). Consistently, the IgA2 isotype dominates in distal gut segments that are densely colonized by gut microbiota, whereas the proximal small intestine harbors about equal numbers of IgA1- and IgA2-producing plasma cells.

Polymeric Ig (pIg) complexes are tied together by the “joining” chain (J chain), a 15 kDa polypeptide not to be confused with the J gene segment encoded in the Ig loci. The J chain enables binding to the polymeric Ig receptor (pIgR) and thus the active transepithelial

transport and secretion of SIg into the gut lumen. J chain containing pIg binds to the pIgR present on the basolateral side of gut epithelial cells. Subsequently, the complex of pIgR and pIg is internalized by receptor-mediated endocytosis and transported to the apical side of the epithelial cells. At the apical side, proteolytic cleavage of pIgR generates the secretory component, a fragment of the pIgR, and the complex of secretory component and pIg is released into the gut lumen (Fig. 10.2; Tomasi et al., 1965). Thus, secretory antibodies are effectively produced by the combined function of plasma cells and pIgR-expressing epithelial cells.

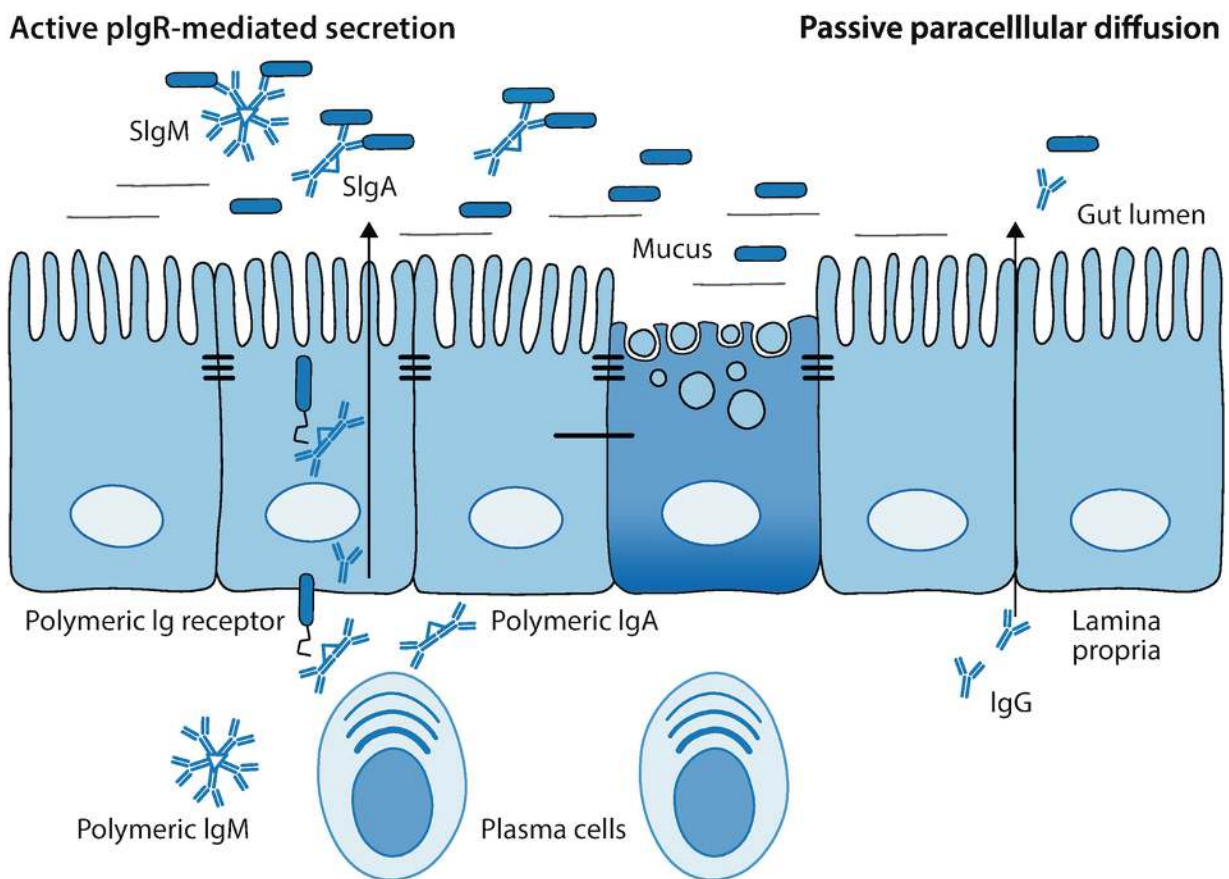


Fig. 10.2 Sources of intraluminal antibodies. Plasma cells in the lamina propria produce polymeric immunoglobulin, mostly dimeric IgA and pentameric IgM, both of which comprise a short polypeptide called the J chain. J chain-assisted binding of polymeric Ig to the polymeric Ig receptor enables transepithelial transport of the complex. At the apical side of epithelial cells, the complex of polymeric Ig and a fragment of the pIgR is released as secretory Ig (SIgA and SIgM). IgG present in the blood or produced by IgG-secreting plasma cells in the inflamed mucosa enters the gut lumen by paracellular diffusion

Besides the active pIgR-mediated transport by gut epithelial cells, additional mechanisms can facilitate the presence of antibodies in the gut lumen. Murine hepatocytes express pIgR and secrete SIgA into bile. Similarly, human bile also contains SIgA because of pIgR expression in cholangiocytes. SIgA in bile enters the proximal intestine by biliary secretion. The contribution of this process to overall intestinal SIgA, however, is not well understood. Another pathway of IgA transport is provided by breastfeeding. SIgA is a crucial component of breast milk; hence, breastfeeding introduces a highly concentrated cocktail of antibodies into the neonate's intestine. Finally, under some circumstances, antibodies can passively enter the gut lumen. While the healthy gut mucosa does not permit passive diffusion of antibodies, the situation is fundamentally different during inflammation and impaired barrier integrity. During inflammation, IgG-expressing plasma cells accumulate in the gut lamina propria, and IgG produced locally as well as derived from circulation can enter the gut lumen.

10.3 Pathways of B Cell Activation and IgA Plasma Cell Differentiation

Pathways of how intestinal IgA-producing plasma cells are generated are controversially debated. Class switch to IgA can occur at different anatomical sites: within GALT germinal centers, inside GALT but independently of germinal centers, and in situ in the intestinal lamina propria. Moreover, T cell-dependent and T cell-independent pathways of IgA induction, as well as an alternative origin of IgA-secreting plasma cells from B1 cells and B2 cells, have been reported. I will firstly describe the archetypal pathway of T cell-dependent B cell activation in germinal centers before discussing alternative concepts of B cell activation and particular features of GALT.

There is a broad consensus that GALT serves the generation of IgA responses directed against the microbiota and pathogens. As described above, PP even in germ-free mice constitutively harbor germinal centers. These germinal centers form early after birth when luminal

antigen is entering the “nascent” PP via the FAE. Dendritic cells (DC) take up and present processed antigen to activate T cells that subsequently differentiate into effector T cells and provide B cell help.

In adult PP, the germinal center dominates the overall compartment and comprises newly activated B cells and memory B cell populations of different clonal origins that, in parallel, undergo selection and differentiation and class switch recombination. Germinal centers provide an environment supporting class switch recombination and somatic hypermutation. Class switch recombination enables a change of Ig isotype, which, in PP, typically, but not exclusively, entails a switch from IgM to IgA. In fact, memory B cells expressing IgG are typically present in GALT, even though no or very few IgG-secreting plasma cells are present in the healthy human gut lamina propria. During somatic hypermutation, B cells undergo successive rounds of mutations, consequently yielding selected B cells with increased antigen affinity. Both of these processes rely on the activity of activation-induced cytidine deaminase (AID), an enzyme that catalyzes the mutation of DNA and is transiently expressed in activated B cells.

The local environment in PP favors class switch to IgA and expression of the gut-homing factors CCR9 and $\alpha 4\beta 7$ -integrin. IgA class switch is supported by the cytokine transforming growth factor β (TGF β). Deficiency of the TGF β receptor in mice results in almost complete lack of IgA, suggesting a critical role of this cytokine. However, the cellular sources of active TGF β in PP still need to be defined. Moreover, DC can produce nitric oxide, the cytokine BAFF (B cell-activating factor, a member of the tumor necrosis factor family), and APRIL (a proliferation-inducing ligand), which all support IgA class switch recombination. Expression of gut-homing molecules can be induced by retinoic acid, and indeed, DC in PP express high levels of retinoic acid-producing enzymes (Mora et al., [2006](#)). However, in vivo, the situation appears more complex, and various cell types, including DC, stroma cells, and potentially epithelial cells, cooperatively shape an environment in PP that facilitates the generation of intestinal IgA responses.

While there is broad consensus that the mode described above is highly efficient at generating intestinal plasma cells, the relevance of alternative pathways of IgA induction remains more controversial. The number of IgA-secreting plasma cells is unchanged in mice lacking germinal centers (Bemark et al., [2012](#)), and also humans with genetically impaired ability to raise germinal centers have IgA. Indeed, AID expression has been reported outside of germinal centers in PP of mice and humans. Moreover, IgA-secreting plasma cells are strongly reduced but still detectable in T cell-deficient mice. Such T cell-independent class switch has been linked to the activity of BAFF and APRIL and might induce AID-mediated class switch recombination inside the gut lamina propria. Some of this controversy of germinal center- and T cell-dependent as compared to germinal center- and T cell-independent responses may be resolved when considering the chronicity of gut B cell responses and the nature of gut microbial antigen. Many gut antigens can be present together, allowing for non-cognate T-B interaction and the evolution of B cell responses against classical T cell-independent antigens such as glycans. Encounters outside the germinal centres, particularly in the sub-epithelial dome, i.e., the space between the FAE and the germinal center, can generate activated B cells with sufficient affinity to enter the germinal centres. This suggests that T cell-dependent and independent pathways may not necessarily be mutually exclusive pathways, but rather develop as intertwined responses.

Moreover, besides B2 cells, the “conventional” B cell population populating lymphoid organs, B1 cells, have been proposed to generate IgA-secreting plasma cells. Pathways of B1 cell-derived gut plasma cell generation have been linked to early life B cell responses: Fate tracking of murine B cells revealed that early life responses generated a wave of plasma cells that persisted into adulthood (Vergani et al., [2022](#)). The relevance of these pathways has not yet been resolved and may differ between species, with different developmental ages and the particular type of antigen considered (Macpherson & McCoy, [2015](#)).

10.4 Plasma Blast Dissemination from GALT and Migration into the Lamina Propria

The germinal center response generates plasma cell precursors, also named plasma blasts. Plasma blasts exit the germinal center via efferent lymph or blood, disseminate to local effector sites, and finally differentiate into fully mature plasma cells. Migration from the circulation into the gut lamina propria is guided by gut-homing factors and their ligands present on intestinal venules. Accordingly, mice with defective $\beta 7$ -integrin, $\beta 7$ -integrin ligand, mucosal addressin cell adhesion molecule-1 (MadCAM-1), chemokine receptor CCR9, or its ligand CCL25 have impaired intestinal plasma cell populations. In the lamina propria, plasma cells can be maintained for prolonged time to secrete Ig. In humans long-lived plasma cell population have been described as CD45/CD19 double negative population (Lindeman et al., [2021](#)).

Plasma blasts are thought to have similar homing properties with respect to different segments of the small but not the large intestine. Sequencing of the Ig-encoding gene loci identified clonally related plasma cells in the proximal and distal small intestine. In contrast, fewer clonally related sequences were observed between plasma cells of the small and large intestine (Lindner et al., [2012](#)). Yet, migration of GALT-derived plasma blasts is not limited to the gut, and clonally related populations of plasma cells have also been observed in the gut and mammary glands (Lindner et al., [2015](#)). Indirect evidence suggests that such a notion may extend to the liver (Moro-Sibilot et al., [2016](#)).

The occurrence of different plasma cell clones in the small and large intestine indicates that distinct homing cues may allow the differential recruitment and/or retention of plasma cells in both compartments. One factor potentially involved in this is chemokine receptor CCR10. Plasma blasts activated in the cecal patch show higher expression of CCR10 compared to plasma blasts present in the small intestinal PP. Additionally, B cells present in the cecal patch are enriched in the colon compared to the small intestine (Masahata et al., [2014](#)).

10.5 The Role of SIg in Maintaining Host-Microbiota Interactions

Various mechanisms of SIg-mediated mucosal protection have been described. For instance, SIg neutralizes toxins in the gut lumen and limits infection with rotavirus or *Salmonella*. Yet, the precise functions of SIg in host-microbiota interaction are still emerging. Microbiota comprise a collection of eubacteria, archaea, viruses, fungi, and eukaryotes. After a highly dynamic early life phase, in adults, the composition of the microbiota stabilizes and shows a characteristic species composition that enables gut homeostasis and is essential for our well-being. A major deviation from this beneficial state of the microbiota is characteristic of various disease states and referred to as dysbiosis. SIg responses are by far the best studied against bacteria. However, they also include fungi and other putative components of the microbiota (Ost, [2021](#)).

Global consequences of impaired SIg production have been studied in pIgR-deficient mice that fail to transport polymeric IgA into the gut lumen. pIgR-deficient mice show no overt immunopathology but instead significant differences in the composition of their microbiota compared to littermate controls. Similarly, B cell-deficient mice show reduced diversity of their gut microbiota compared to wild-type controls (Kawamoto et al., [2014](#)).

Accordingly, AID deficiency, marked by a defective class switch recombination and somatic hypermutation, results in intestinal dysbiosis, in particular in the overgrowth of *Firmicutes*. Interestingly, a related observation has been made in mice expressing a hypomorphic AID variant that preserves class switching but does not enable hypermutation (Wei et al., [2011](#)). This indicates that not only the total amount but also the quality of IgA is relevant to sustain unperturbed host-microbiota interaction. In line with this concept, mice lacking PD-1 showed impaired effector T cell populations in PP that translated into a SIg-mediated intestinal dysbiosis (Kawamoto et al., [2012](#)).

Consequences of IgA deficiency have also been studied in humans. Individuals with selective IgA deficiency (SIgAD) show increased risk, e.g., to develop allergies, but generally remain healthy, presumably because of SIgM compensating for the lack of IgA. Yet, SIgAD, in particular in individuals also lacking SIgA in feces, was linked to increased translocation of specific bacterial taxa, increased titers of systemic IgG directed against these taxa, and immune dysregulation (Donaldson, [2018](#)). Altogether, these observations support a general function of SIg in maintaining host-microbiota interactions in the intestine and shielding the systemic compartment from gut microbiota.

Exciting insights into the interplay between SIgA and the microbiota came from recent studies that investigated functional differences between bacteria targeted or bacteria non-targeted by IgA (Bunker et al., [2015](#); Kau et al., [2015](#); Palm et al., [2014](#)). These studies relied on the purification of IgA-coated and non-coated bacteria from the microbiota and their subsequent identification by 16S rRNA gene sequencing. In common to all these studies, differential IgA coating was observed for distinct bacterial taxa (reviewed in Macpherson et al., [2015](#)). SIg coating of gut bacteria was higher in the proximal small intestine compared to the colon. Moreover, bacteria that showed SIgA coating in the colon were typically also observed in the small intestine, whereas typical members of the colonic microbiota showed no detectable IgA coat (Bunker et al., [2015](#)). This may indicate that small intestinal GALT plays a dominant role in generating microbiota-directed antibodies.

Notably, SIgA coating was preserved for some taxa in T cell-deficient mice, suggesting that T cell-independent Ig responses can generate antibodies binding to gut bacteria. Accordingly, antibodies cloned from single murine plasma cells bound microbiota independent of somatic mutations (Bunker et al., [2017](#)), a finding that contrasts with our own observations: We found that monoclonal antibodies derived from single human plasma cells typically lose their microbiota binding when these antibodies are expressed as germ-line variants (Kabbert, [2020](#)). This, in turn, indicates that T cell-dependent somatic mutations might contribute to the generation of antibodies with efficient microbiota

reactivity in humans. The situation might be even more complex in vivo, and the endogenous SIgA coat of single bacteria might comprise several antibodies of different specificities and affinities. Moreover, the secretory component in itself, as well as the glycosylated Fc part of IgA, can confer reactivity to microbiota (Mathias & Corthesy, [2011](#)). Therefore, the nature of the SIgA coat has to be explored in more detail as different pathways of IgA induction may predominate depending of the bacterial taxa looked at, the genetic layout of the host, and age.

10.6 Molecular Mechanism of SIg-Mediated Control of the Microbiota

How does SIgA coating of intestinal bacteria affect their function? Peterson and colleagues have studied this question in mice carrying single monoclonal antibodies directed against *Bacteroides thetaiotaomicron* (Peterson et al., [2007](#), [2015](#)). One such antibody reduced proinflammatory responses and colonization levels of bacteria recognized by the respective antibody (Peterson et al., [2007](#)). However, a second antibody did not show such an effect, and no reduction of bacterial fitness was observed (Peterson et al., [2015](#)). Thus, the precise mode of action, of how an antibody modulates the microbiota, might depend on the particular antibody. Related observations have been named as “parallelism of IgA” to describe that the intestinal IgA response to a single microbe comprises parallel components with distinct effects on microbial functions (Rollenske, [2021](#)) .

In the following paragraph, I will dissect different mechanisms of SIgA-mediated modulation of the microbiota. I will refer to these mechanisms as “immune exclusion,” “enchainment,” “fitness,” “anchoring,” and “sampling” (Fig. [10.3](#)).

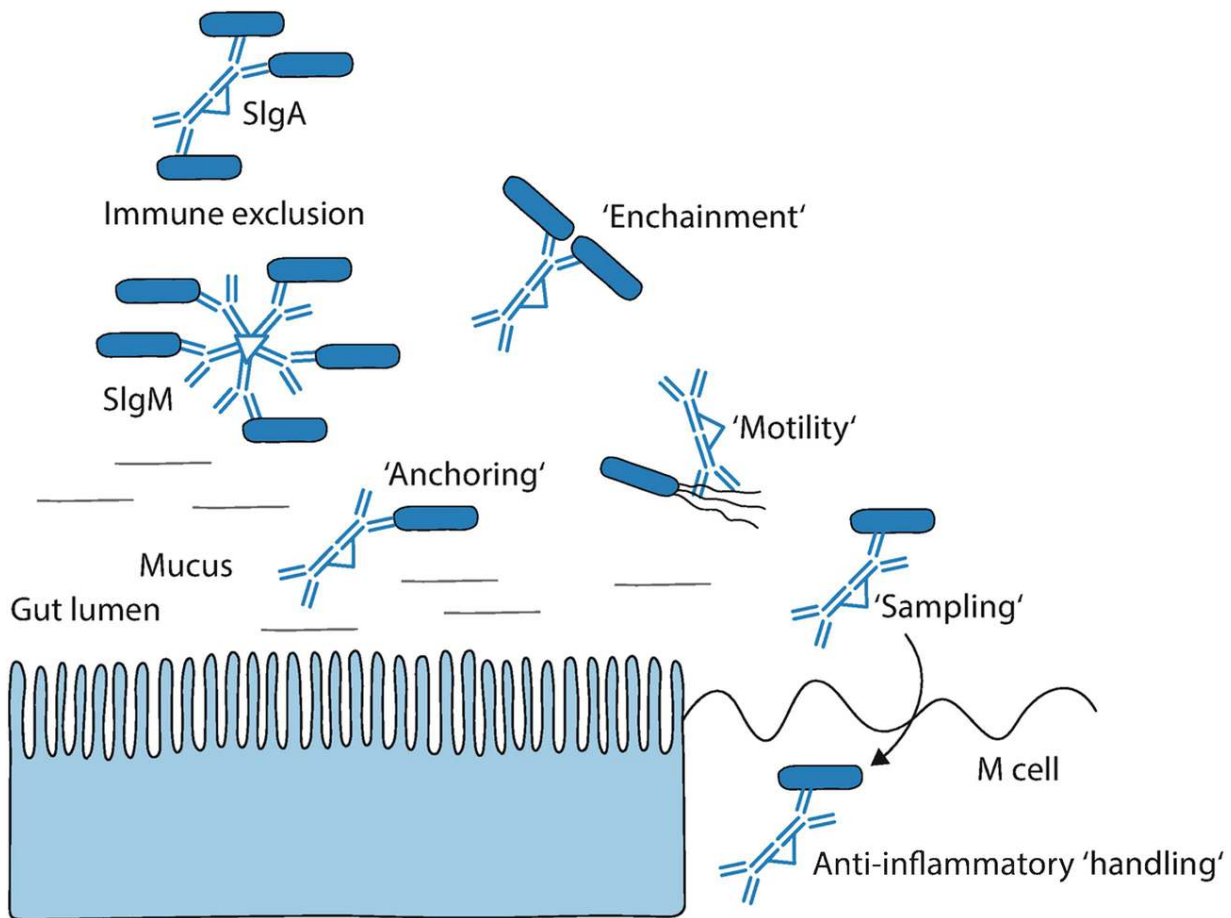


Fig. 10.3 Mechanisms of SIg-mediated modulation of the microbiota. SIgA and SIgM can agglutinate bacteria to form clumps that become secreted by gut peristalsis in a process called immune exclusion. “Enchainment” prevents the separation of dividing bacteria and thereby leads to growth-dependent formation of bacterial clumps. Binding of bacteria by antibodies may affect their behavior directly, such as antibodies can reduce bacterial motility by binding to flagella, affect bacterial metabolism and membrane integrity, and increase susceptibility to bacteriophages and bile salt toxicity. Finally, antibody-coated bacteria may be anchored to the mucus, affecting carbon-source availability and allowing for more efficient sampling by M cells compared to non-coated bacteria

“Immune Exclusion” and “Enchainment”

Antibody-mediated crosslinking of bacteria leads to agglutination and clump formation. Clumps of bacteria are thought to be excreted more efficiently compared to single bacteria by intestinal peristalsis in a process called immune exclusion (Fig. 10.3). Agglutination and immune exclusion are particularly efficient for multivalent antibodies such as SIgA and SIgM and are considered a major mechanism of SIg-mediated control of the microbiota.

A variation of “immune exclusion” has recently been suggested to target growing bacteria (Moor et al., [2017](#)). In a situation of low bacterial density, e.g., during the early phase of an infection, agglutination may be very inefficient. Nevertheless, SIgA has the ability to enchain dividing bacteria, thereby preventing their separation after division, resulting in growth-dependent clump formation. Additionally, clumping of bacteria and immune exclusion may be supported by antibodies binding to different species, e.g., because of binding to an antigen shared between different species.

“Fitness”

The binding of antibodies to bacteria can directly modify their function and host-microbe interactions. Examples include (1) flagella-binding antibodies that impair motility and potentially the virulence of *Salmonella*. (2) O-antigen directed antibodies immobilizing the bacterial outer membrane at the nanoscale (Hockenberry, [2023](#)) (3) IgA directed against outer membrane pores affecting bacterial metabolism and (4) less specific effects of IgA coating on bile salt toxicity and susceptibility for bacteriophage infection (Rollenske, [2021](#)).

“Anchoring” and “Sampling”

SIgA loosely binds to mucus (Phalipon et al., [2002](#)). Thus, SIgA coating of microbiota may anchor the bacteria within the mucus layer and thereby help to generate a niche that increases the competitive fitness of the bound bacteria. For instance, specific strains modulate their surface to enhance IgA binding and thus to open niches close to the gut epithelium for their colonization (Donaldson, [2018](#)).

Such close SIgA-mediated interaction with the host also enhances their M cell-dependent sampling into PP (Boullier et al., [2009](#); Kadaoui & Cortes, [2007](#)). For instance, coating of *Salmonella* with IgA facilitated its uptake and resulted in stronger adaptive immune responses (Fransen et al., [2015](#)). Thus, coating with SIgA may drive a positive

feedback loop that sustains antibody responses to certain bacterial species.

Cooperatively, the abovementioned SIgA-mediated mechanisms might create niches for colonization and foster diversity of the microbiota. Agglutination might be particularly efficient for highly abundant bacteria, favoring colonization by minorities and enhancing microbial diversity. “Anchoring” in the mucus might retain bacteria within certain “habitats,” and differential concentrations of SIgA along the crypt-villus axis may affect the competitive fitness of bacteria. Consequently, SIgA plays an important role in regulating beneficial and stable host–microbe interactions in the intestine.

Controversy *What determines whether or not bacteria become SIgA-coated?* IgA coating has been suggested to target “inflammatory commensals that preferentially drive intestinal disease” (Palm et al., [2014](#)). Additionally, the anatomical location (of the respective microbes, but also the site of IgA induction) has been suggested to contribute. I speculate that the propensity of bacteria to acquire an IgA coat might primarily reflect their anatomical localization. Bacteria that occupy niches close to the gut epithelium are more likely to be sampled by the immune system and to induce antibody responses. At the same time, proximity might favor detrimental effects, such as the induction of colitogenic immune responses, but also beneficial effects (such as the stimulation of lymphoid organogenesis).

What is the specificity of SIg? Different members of the microbiota show a differential propensity to be coated with IgA, but the molecular structures recognized by SIg are largely unknown. Clearly, SIg responses can be generated against classical T cell-dependent antigens, such as proteins that provide conjugated T and B cell epitopes. However, SIg responses also include classical T cell-independent antigens and glycans, which in the gut, in contrast to non-mucosal tissues, generate somatically mutated variable regions. I speculate that co-processing of T and B cell epitopes, non-cognate

interactions and cytokine networks may drive the evolution of microbiota-directed B cell responses.

History

In 1965, “an immunological system which is characteristic of certain external secretions” was described, and it was suggested that this system might “play a significant role in the body’s defense mechanisms against allergens and microorganisms” (Tomasi et al., [1965](#)). Subsequently, intensive research established the pathways that enable transepithelial transport and secretion of polymeric immunoglobulin, polymeric IgA and IgM, into body fluids (Brandtzaeg & Prydz, [1984](#)), and the concept of compartmentalized mucosal immunity that was thought to structurally and functionally differ from systemic immunity was developed (a concept which based on current knowledge seems to be less strict as compared to its initial formulation). During these years, research on secretory immunoglobulin dominated the field of mucosal immunology. However, eventually other aspects of mucosal immunity, such as immune regulation, dendritic cells in mucosal tissues, and most recently innate lymphocyte functions, moved into the limelight, and the IgA system received less attention. Now this situation seems to change again, and with the growing interest in microbiota, research on mucosal B cells and their function is on the rise again. It will be exciting to follow the field that with the availability of new technologies and the integration of research on microbiota and mucosal immunity, might rapidly move forward.

Highlights

- Immunoglobulin A is the main antibody isotype produced in humans and mice.
- IgA induction comprises different inductive pathways as well as anatomical sites.
- Secretory antibodies protect mucosal surfaces from pathogen invasion and toxins.

- Secretory IgA coats distinct members of the microbiota and regulates their interaction with the host.

References

Baptista, A.P., Olivier, B.J., Goverse, G., Greuter, M., Knippenberg, M., Kusser, K., Domingues, R.G., Veiga-Fernandes, H., Luster, A.D., Lugering, A., et al. (2013). Colonic patch and colonic SILT development are independent and differentially regulated events. *Mucosal Immunol* 6, 511–521. <https://doi.org/10.1038/mi.2012.90>.

Bemark, M., Boysen, P., & Lycke, N. Y. (2012). Induction of gut IgA production through T cell-dependent and T cell-independent pathways. *Annals of the New York Academy of Sciences*, 1247, 97–116.

[<https://doi.org/10.1111/j.1749-6632.2011.06378.x>][PubMed]

Bemark, M., Pitcher, M.J., Dionisi, C., & Spencer, J. (2024). Gut-associated lymphoid tissue: a microbiota-driven hub of B cell immunity. *Trends Immunol* 45, 211–223. <https://doi.org/10.1016/j.it.2024.01.006>.

Boullier, S., Tanguy, M., Kadaoui, K. A., Caubet, C., Sansonetti, P., Corthesy, B., & Phalipon, A. (2009). Secretory IgA-mediated neutralization of *Shigella flexneri* prevents intestinal tissue destruction by down-regulating inflammatory circuits. *Journal of Immunology*, 183, 5879–5885.

[<https://doi.org/10.4049/jimmunol.0901838>]

Brandtzaeg, P., & Prydz, H. (1984). Direct evidence for an integrated function of J chain and secretory component in epithelial transport of immunoglobulins. *Nature*, 311, 71–73.

[<https://doi.org/10.1038/311071a0>][PubMed]

Bunker, J. J., Flynn, T. M., Koval, J. C., Shaw, D. G., Meisel, M., McDonald, B. D., Ishizuka, I. E., Dent, A. L., Wilson, P. C., Jabri, B., et al. (2015). Innate and adaptive humoral responses coat distinct commensal bacteria with immunoglobulin A. *Immunity*, 43, 541–553.

[<https://doi.org/10.1016/j.immuni.2015.08.007>][PubMed][PubMedCentral]

Bunker, J. J., Erickson, S. A., Flynn, T. M., Henry, C., Koval, J. C., Meisel, M., Jabri, B., Antonopoulos, D. A., Wilson, P. C., & Bendelac, A. (2017). Natural polyreactive IgA antibodies coat the intestinal microbiota. *Science*, 358. <https://doi.org/10.1126/science.aan6619>

Donaldson, G.P., Ladinsky, M.S., Yu, K.B., Sanders, J.G., Yoo, B.B., Chou, W.C., Conner, M.E., Earl, A.M., Knight, R., Bjorkman, P.J., & Mazmanian, S.K. (2018). Gut microbiota utilize immunoglobulin A for mucosal colonization. *Science* 360, 795–800. <https://doi.org/10.1126/science.aag0926>.

Fransen, F., Zagato, E., Mazzini, E., Fosso, B., Manzari, C., El Aidy, S., Chiavelli, A., D’Erchia, A. M., Sethi, M. K., Pabst, O., et al. (2015). BALB/c and C57BL/6 mice differ in polyreactive IgA abundance, which impacts the generation of antigen-specific IgA and microbiota diversity. *Immunity*, 43, 527–540.

[<https://doi.org/10.1016/j.immuni.2015.08.011>][PubMed]

Hockenberry, A., Radiom, M., Arnoldini, M., Turgay, Y., Dunne, M., Adamcik, J., Stadtmueller, B., Mezzenga, R., Ackermann, M., & Slack, E. (2023). Nanoscale clustering by O-antigen-Secretory Immunoglobulin-A binding limits outer membrane diffusion by encaging individual Salmonella cells. *bioRxiv*. <https://doi.org/10.1101/2023.07.13.548943>.

Kabbert, J., Benckert, J., Rollenske, T., Hitch, T.C.A., Clavel, T., Cerovic, V., Wardemann, H., & Pabst, O. (2020). High microbiota reactivity of adult human intestinal IgA requires somatic mutations. *J Exp Med* 217. <https://doi.org/10.1084/jem.20200275>.

Kadaoui, K. A., & Corthesy, B. (2007). Secretory IgA mediates bacterial translocation to dendritic cells in mouse Peyer's patches with restriction to mucosal compartment. *Journal of Immunology*, 179, 7751–7757. <https://doi.org/10.4049/jimmunol.179.11.7751>

Kau, A. L., Planer, J. D., Liu, J., Rao, S., Yatsunenkov, T., Trehan, I., Manary, M. J., Liu, T. C., Stappenbeck, T. S., Maleta, K. M., et al. (2015). Functional characterization of IgA-targeted bacterial taxa from undernourished Malawian children that produce diet-dependent enteropathy. *Science Translational Medicine*, 7, 276ra224. <https://doi.org/10.1126/scitranslmed.aaa4877>

Kawamoto, S., Tran, T. H., Maruya, M., Suzuki, K., Doi, Y., Tsutsui, Y., Kato, L. M., & Fagarasan, S. (2012). The inhibitory receptor PD-1 regulates IgA selection and bacterial composition in the gut. *Science*, 336, 485–489. <https://doi.org/10.1126/science.1217718>][PubMed]

Kawamoto, S., Maruya, M., Kato, L. M., Suda, W., Atarashi, K., Doi, Y., Tsutsui, Y., Qin, H., Honda, K., Okada, T., et al. (2014). Foxp3(+) T cells regulate immunoglobulin a selection and facilitate diversification of bacterial species responsible for immune homeostasis. *Immunity*, 41, 152–165. <https://doi.org/10.1016/j.immuni.2014.05.016>][PubMed]

Lelouard, H., Fallet, M., de Bovis, B., Meresse, S., & Gorvel, J. P. (2012). Peyer's patch dendritic cells sample antigens by extending dendrites through M cell-specific transcellular pores. *Gastroenterology*, 142, 592–601 e593. <https://doi.org/10.1053/j.gastro.2011.11.039>

Lindeman, I., Zhou, C., Eggesbo, L.M., Miao, Z., Polak, J., Lundin, K.E.A., Jahnsen, J., Qiao, S.W., Iversen, R., & Sollid, L.M. (2021). Longevity, clonal relationship, and transcriptional program of celiac disease-specific plasma cells. *J Exp Med*, 218. <https://doi.org/10.1084/jem.20200852>.

Lindner, C., Wahl, B., Fohse, L., Suerbaum, S., Macpherson, A. J., Prinz, I., & Pabst, O. (2012). Age, microbiota, and T cells shape diverse individual IgA repertoires in the intestine. *The Journal of Experimental Medicine*, 209, 365–377. <https://doi.org/10.1084/jem.20111980>][PubMed][PubMedCentral]

Lindner, C., Thomsen, I., Wahl, B., Ugur, M., Sethi, M. K., Friedrichsen, M., Smoczek, A., Ott, S., Baumann, U., Suerbaum, S., et al. (2015). Diversification of memory B cells drives the continuous adaptation of secretory antibodies to gut microbiota. *Nature Immunology*, 16, 880–888.

[<https://doi.org/10.1038/ni.3213>][[PubMed](#)]

Macpherson, A. J., & McCoy, K. D. (2015). Independence day for IgA. *Immunity*, 43, 416–418.
[<https://doi.org/10.1016/j.immuni.2015.08.024>][[PubMed](#)]

Macpherson, A. J., Koller, Y., & McCoy, K. D. (2015). The bilateral responsiveness between intestinal microbes and IgA. *Trends in Immunology*, 36, 460–470.
[<https://doi.org/10.1016/j.it.2015.06.006>][[PubMed](#)]

Magri, G., Comerma, L., Pybus, M., Sintes, J., Llige, D., Segura-Garzon, D., Bascones, S., Yeste, A., Grasset, E. K., Gutzeit, C., et al. (2017). Human secretory IgM emerges from plasma cells clonally related to gut memory B cells and targets highly diverse commensals. *Immunity*, 47, 118–134 e118.
[<https://doi.org/10.1016/j.immuni.2017.06.013>]

Masahata, K., Umemoto, E., Kayama, H., Kotani, M., Nakamura, S., Kurakawa, T., Kikuta, J., Gotoh, K., Motooka, D., Sato, S., et al. (2014). Generation of colonic IgA-secreting cells in the caecal patch. *Nature Communications*, 5, 3704.
[<https://doi.org/10.1038/ncomms4704>][[PubMed](#)]

Mathias, A., & Corthesy, B. (2011). Recognition of gram-positive intestinal bacteria by hybridoma- and colostrum-derived secretory immunoglobulin A is mediated by carbohydrates. *The Journal of Biological Chemistry*, 286, 17239–17247.
[<https://doi.org/10.1074/jbc.M110.209015>][[PubMed](#)][[PubMedCentral](#)]

Moor, K., Diard, M., Sellin, M. E., Felmy, B., Wotzka, S. Y., Toska, A., Bakkeren, E., Arnoldini, M., Bansept, F., Co, A. D., et al. (2017). High-avidity IgA protects the intestine by enchainning growing bacteria. *Nature*, 544, 498–502.
[<https://doi.org/10.1038/nature22058>][[PubMed](#)]

Mora, J.R., Iwata, M., Eksteen, B., Song, S.Y., Junt, T., Senman, B., Otipoby, K.L., Yokota, A., Takeuchi, H., Ricciardi-Castagnoli, P., et al. (2006). Generation of gut-homing IgA-secreting B cells by intestinal dendritic cells. *Science* 314, 1157–1160. <https://doi.org/10.1126/science.1132742>.

Moro-Sibilot, L., Blanc, P., Taillardet, M., Bardel, E., Couillault, C., Boschetti, G., Traverse-Glehen, A., Defrance, T., Kaiserlian, D., & Dubois, B. (2016). Mouse and human liver contain immunoglobulin A-secreting cells originating from Peyer's patches and directed against intestinal antigens. *Gastroenterology*, 151, 311–323.
[<https://doi.org/10.1053/j.gastro.2016.04.014>][[PubMed](#)]

Ost, K.S., O'Meara, T.R., Stephens, W.Z., Chiaro, T., Zhou, H., Penman, J., Bell, R., Catanzaro, J.R., Song, D., Singh, S., et al. (2021). Adaptive immunity induces mutualism between commensal eukaryotes. *Nature* 596, 114–118. <https://doi.org/10.1038/s41586-021-03722-w>.

Palm, N. W., de Zoete, M. R., Cullen, T. W., Barry, N. A., Stefanowski, J., Hao, L., Degnan, P. H., Hu, J., Peter, I., Zhang, W., et al. (2014). Immunoglobulin A coating identifies colitogenic bacteria in inflammatory bowel disease. *Cell*, 158, 1000–1010.
[<https://doi.org/10.1016/j.cell.2014.08.006>][[PubMed](#)][[PubMedCentral](#)]

Peterson, D. A., McNulty, N. P., Guruge, J. L., & Gordon, J. I. (2007). IgA response to symbiotic bacteria as a mediator of gut homeostasis. *Cell Host & Microbe*, 2, 328–339. [\[https://doi.org/10.1016/j.chom.2007.09.013\]](https://doi.org/10.1016/j.chom.2007.09.013)

Peterson, D. A., Planer, J. D., Guruge, J. L., Xue, L., Downey-Virgin, W., Goodman, A. L., Seedorf, H., & Gordon, J. I. (2015). Characterizing the interactions between a naturally primed immunoglobulin A and its conserved *Bacteroides thetaiotaomicron* species-specific epitope in gnotobiotic mice. *The Journal of Biological Chemistry*, 290, 12630–12649. [\[https://doi.org/10.1074/jbc.M114.633800\]](https://doi.org/10.1074/jbc.M114.633800) [\[PubMed\]](#) [\[PubMedCentral\]](#)

Phalipon, A., Cardona, A., Kraehenbuhl, J. P., Edelman, L., Sansonetti, P. J., & Corthesy, B. (2002). Secretory component: A new role in secretory IgA-mediated immune exclusion in vivo. *Immunity*, 17, 107–115. [\[https://doi.org/10.1016/S1074-7613\(02\)00341-2\]](https://doi.org/10.1016/S1074-7613(02)00341-2) [\[PubMed\]](#)

Rollenske, T., Burkhalter, S., Muerner, L., von Gunten, S., Lukasiewicz, J., Wardemann, H., & Macpherson, A. J. (2021). Parallelism of intestinal secretory IgA shapes functional microbial fitness. *Nature* 598, 657–661. <https://doi.org/10.1038/s41586-021-03973-7>.

Spencer, J., and Bemark, M. (2023). Human intestinal B cells in inflammatory diseases. *Nat Rev Gastroenterol Hepatol* 20, 254–265. <https://doi.org/10.1038/s41575-023-00755-6>.

Tomasi, T. B., Jr., Tan, E. M., Solomon, A., & Prendergast, R. A. (1965). Characteristics of an immune system common to certain external secretions. *The Journal of Experimental Medicine*, 121, 101–124. [\[https://doi.org/10.1084/jem.121.1.101\]](https://doi.org/10.1084/jem.121.1.101) [\[PubMed\]](#) [\[PubMedCentral\]](#)

Vergani, S., Muleta, K. G., Da Silva, C., Doyle, A., Kristiansen, T. A., Sodini, S., Krausse, N., Montano, G., Kotarsky, K., Nakawesi, J., et al. (2022). A self-sustaining layer of early-life-origin B cells drives steady-state IgA responses in the adult gut. *Immunity* 55, 1829–1842 e1826. <https://doi.org/10.1016/j.immuni.2022.08.018>.

Wei, M., Shinkura, R., Doi, Y., Maruya, M., Fagarasan, S., & Honjo, T. (2011). Mice carrying a knock-in mutation of *Aicda* resulting in a defect in somatic hypermutation have impaired gut homeostasis and compromised mucosal defense. *Nature Immunology*, 12, 264–270. [\[https://doi.org/10.1038/ni.1991\]](https://doi.org/10.1038/ni.1991) [\[PubMed\]](#)

Woof, J. M., & Russell, M. W. (2011). Structure and function relationships in IgA. *Mucosal Immunology*, 4, 590–597. [\[https://doi.org/10.1038/mi.2011.39\]](https://doi.org/10.1038/mi.2011.39) [\[PubMed\]](#)

11. Microbiome and Diseases: Inflammatory Bowel Diseases

Kai Hildner¹✉ and Dirk Haller²✉

(1) Department of Medicine 1, University Hospital Erlangen,
University of Erlangen-Nuremberg, Erlangen, Germany

(2) Chair of Nutrition and Immunology, School of Life Sciences,
Technical University of Munich, Freising, Germany

✉ **Kai Hildner**

Email: Kai.Hildner@uk-erlangen.de

✉ **Dirk Haller (Corresponding author)**

Email: dirk.haller@tum.de

Abstract

Inflammatory bowel diseases, such as Crohn's disease and ulcerative colitis, are chronically relapsing, immune-mediated disorders of the gastrointestinal tract that have been steadily increasing over the past decades. The hallmark of IBD is an uncontrolled manifestation of intestinal and extraintestinal inflammation within genetically susceptible individuals. Herein, compelling research on host genetics paves the way to a better understanding of disease pathogenesis. Currently, more than 300 genetic risk factors have been identified, showing a disturbed cross-talk of the immune-epithelial cell-microbiota axis. Additionally, epidemiologic studies have pointed toward Western lifestyle and habits as part of the central environmental factors contributing to both the development and

maintenance of intestinal inflammation. In this regard, the intestinal microbiota is thought to play a decisive role in disease progression. The intestinal microbiota was unequivocally shown to be indispensable in orchestrating the development and functionality of the immune system, further having a critical impact on both intestinal homeostasis and inflammation in preclinical models. Even though profound changes in the composition of the intestinal microbiota have been frequently observed in human IBD, unraveling the causes and consequences of intestinal dysbiosis needs further understanding of the interaction between host genetics, microbial ecosystems, and environmental triggers. In this chapter, we will discuss different aspects of the etiology of intestinal inflammation and particularly address the role of host-microbe interaction in disease development, progression, and intervention.

Keywords Crohn's disease (CD) – Host-microbe interactions in IBD – Inflammatory bowel diseases (IBD) – Intestinal microbiota – Ulcerative colitis (UC)

11.1 Introduction

Inflammatory bowel diseases (IBD) represent an umbrella term for a number of conditions characterized by chronically active and/or relapsing inflammatory episodes and largely consist of the two main subtypes, Crohn's disease (CD) and ulcerative colitis (UC) (Chang, [2020](#)). Gastrointestinal tract (GIT) manifestations in IBD are typically restricted to selected anatomic regions: In UC, the intestinal manifestation is mainly restricted to the colon, except for 10–20% of the patients with additional ileal manifestations (so-called backwash ileitis). In CD, however, intestinal inflammation can occur throughout the entire GIT from the mouth to the anus in a discontinuous, i.e., segmental, manner, while the terminal ileum and the colon are most frequently affected. Disease initiation, progression, and persistence are assumed to be triggered by environmental factors in genetically

susceptible individuals (Renz et al., [2011](#)). Both IBD entities are often complicated by extraintestinal manifestations, most frequently involving the skin, eyes, and joints, and the occurrence of a cholestatic liver disease, primary sclerosing cholangitis (PSC), is still under debate whether it represents an extraintestinal manifestation or rather an independent disease entity that is often associated with IBD (van Munster et al., [2024](#)). Genome-wide association studies (GWAS) and animal data provide evidence for an interrelated role of the intestinal microbiota, epithelial interface, and immune system driving the pathogenesis of IBD. This chapter provides an overview of the current state of research on the role of the intestinal microbiota in CD and UC. In addition, approaches to the treatment of IBD by the modulation of the intestinal microbiota are discussed.

11.2 Epidemiology of IBD

IBD frequently occurs in populations of the northern hemisphere. Here, the highest IBD rates are found in Northern and Western Europe, respectively, and North America. Published data on IBD incidences range from 5 to 20 IBD cases within 100,000 individuals (Kaplan, [2015](#)). Among those, CD cases have continued to rise over the past decades due to so far poorly understood reasons, while the prevalence of UC seems to have reached a plateau or has even decreased. However, estimations of an overall prevalence are methodically compromised due to the challenge of a nonhomogenous regional distribution of IBD, including countries with enhanced IBD rates (Kaplan & Windsor, [2021](#)).

The increased risk of IBD in populations with a Western lifestyle nurtured the hypothesis that common environmental signals, such as dietary habits (e.g., high-fat, high-salt, and high-sugar contents) and/or consumption of industrialized food (e.g., highly processed food products), confer a higher susceptibility to develop IBD. Consistent with these findings, economically developing countries (e.g., China, India) that progressively change to Western lifestyle habits are noticing a steady increase in IBD cases, thus providing further evidence for the

impact of environmental signals in disease progression (Kaplan, [2015](#)). The idea that environmental triggers could abet IBD manifestation gained further attention by the observation that individuals moving from low- to high-incidence countries and vice versa, especially before or during adolescence, adapt to the incidence rates of their new environment.

In this regard, the enhanced susceptibility could be driven by direct or indirect effects, such as the modulation of immune cell functionality and changes of the intestinal microbiota compositions and/or functionality, respectively, thereby promoting an overall pro-inflammatory micro-milieu in genetically predisposed individuals. Such a mechanistic concept nurtured the induction of an increasing number of clinical studies seeking to identify environmental cues and modifiers acting in a pre-clinical phase as putative co-factors and triggers of IBD manifestation (Rudbaek et al., [2024](#)).

The onset of clinical manifestations of IBD usually peaks during young adulthood, i.e., between the second and fourth decade of life, while 10–15% of the patients represent children. However, adults beyond 65 years have been recognized to represent the fastest-growing IBD subpopulation, while the age at which children are diagnosed is steadily decreasing overall, underscoring a change of epidemiologic paradigms in IBD beyond geographical aspects (Vieujean et al., [2022](#); Khan et al., [2023](#)). In UC, male individuals were described to numerically prevail over females. In contrast, female patients preferentially suffer from CD, especially in countries with a high socioeconomic standard, while males appear to outnumber female patients in low-incidence countries. In contrast to adults, pediatric CD patients are predominantly boys, while the majority of pediatric UC patients are females.

Heritability of IBD has been suspected since and first described at the beginning of the twentieth century. However, details on the complex genetic traits associated with IBD could not be identified and confirmed until modern times with the invention and broad application of GWAS and *next-generation sequencing* (NGS) technologies. Here, studies

analyzing familial occurrence of IBD indicated that familial cases of IBD confer the highest risk for developing IBD (Orholm et al., [1991](#)). While there is a pronounced interfamilial heterogeneity of the clinical presentation of IBD cases, intrafamilial IBD manifestations are usually surprisingly homogenous, further supporting the interpretation that genetic cues underlie the manifestation of specific familial IBD phenotypes (Colombel et al., [1996](#)). The importance of genetics gained further attention in the light of study cohorts that compared both IBD incidences and phenotypic manifestations among mono- and dizygotic twins. Here, numerous studies with the first published in the late 1980s showed a higher concordance rate in monozygotic compared to dizygotic twins, especially in CD (Tysk et al., [1988](#)). One conclusion of these studies is that genetic traits are more affecting CD than UC pathology. Nevertheless, concluding twin studies is difficult to interpret since results are confined by the fact that, besides the common genetic pool, twins usually share similar environmental signals acquired both during pregnancy and/or during infancy, which might critically impact IBD susceptibility. Regardless, however, population-centered genetic IBD research over the last five decades built the basis for more in-depth genetics-based approaches that have been performed over the past 10–20 years.

11.3 Genetic Susceptibility in IBD

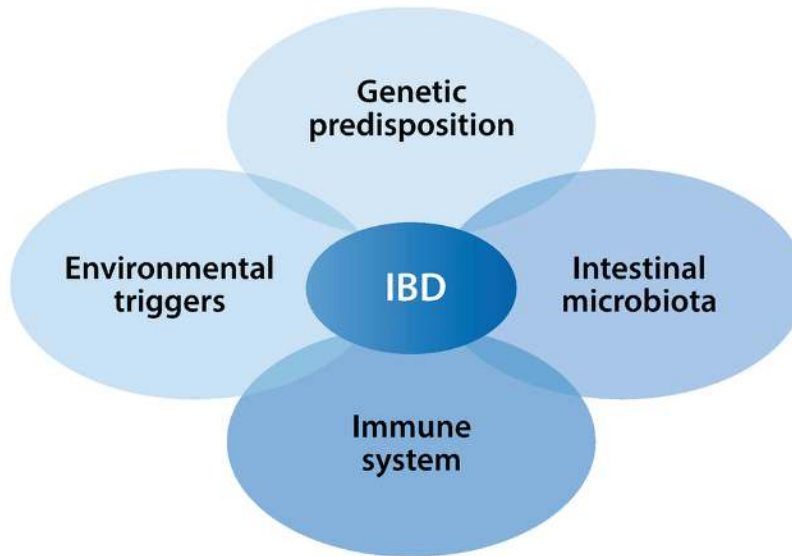
Genetic linkage studies generally aim at identifying abnormal chromosomal regions that are enriched in populations suffering from complex diseases such as IBD when compared to matched healthy individuals. Hence, this approach represents a largely hypothesis-free, however valuable tool to decipher the genetic basis of complex, non-Mendelian diseases. The identification of disease-associated chromosomal regions usually reflects the first step, which is followed by a more detailed characterization of the precise nature of the mutation within the allelic variant. Finally, the description of the affected gene or genetic region should be complemented by attempts to

experimentally prove that the variant functionally contributes to an increased risk for the disease of choice. Historically, HLA class II genes were among the first described genetic alterations that had been associated with an increased risk for IBD and other immune-mediated disorders (Stokkers et al., [1999](#)). However, the first specific associations between distinct chromosomal loci and IBD development resulted from first-generation genetic linkage studies in the mid- to late 1990s, which identified a total of nine IBD (IBD1–9) loci (Ahmad et al., [2001](#)).

One of these genes, located within the IBD1 locus, initially found by GWAS, is the protein *nucleotide-binding oligomerization domain-containing protein 2* (NOD2), an intracellular sensor for microbial-derived components (Hugot et al., [2001](#); Ogura et al., [2001](#)). NOD2 is specifically linked to CD but also to acute graft versus host disease in patients undergoing allogeneic stem cell transplantation, whereas associations to UC were not found. Consecutive studies helped to molecularly define a number of distinct single-nucleotide polymorphisms (SNPs) that were shown to increase disease susceptibility, including allele variants of the *NOD2* gene or within the *NOD2* locus, respectively. Notably, carriage of one risk-conferring allele leads to a two- to threefold increased risk of CD, while homozygosity as well as the heterozygous presence of two risk alleles particularly enhances the risk up to 20- to 40-fold (Lesage et al., [2002](#)). Studies on NOD2 biology in the context of IBD revealed important mechanisms that putatively contribute to IBD pathology and paved the way to better understand gene-environment interactions linking microbial triggers to the immune system (Fig. [11.1a](#)). NOD2 is a pattern recognition receptor that is broadly expressed within both immune and epithelial cells. Three of the most abundant SNPs were shown to result in functionally hampered NOD2 activity, subsequently leading to diminished nuclear factor κ B (NF- κ B) activation upon the presence of the NOD2 ligand *muramyl dipeptide* (MDP), which displays a cell wall component of most bacteria. In mice, however, *Nod2* deficiency leads to changes in the microbial composition in the intestine under physiological conditions, while bacterial clearance was compromised in the case of an intestinal

infection (Petnicki-Ocwieja et al., [2009](#)). Hence, one hypothesis deduced from these preclinical data is that impaired microbial-host interactions in patients carrying NOD2-associated risk factors for CD could result in a harmful modulation of the composition and/or location of the intestinal microbiota. In the occasional case of a disruption of the intestinal epithelial barrier (e.g., in the course of intestinal infection), increased bacterial translocation and/or hampered elimination of those bacteria might result in an overshooting, inadequate immune stimulation that is frequently observed in IBD patients. However, *NOD2* alleles that confer an increased risk of CD are common variants found in >5% of the population. Additionally, *NOD2* mutations are predominantly found in CD sub-cohorts displaying an ileal manifestation and are almost exclusively detected in Caucasian but not in Asian populations. Conclusively, accumulating knowledge on NOD2 genetics and biology is valuable in the understanding of host-microbe interactions, but can only be applied to subsets of CD patients, vividly illustrating the shortcomings of genetics-based approaches to decipher complex disease mechanisms in the pathogenesis of the vast majority of adult-onset IBD forms.

A



B

Inflammatory bowel diseases (IBD)		
	Ulcerative Colitis (UC)	Crohn's Disease (CD)
Epidemiology	Diseases of the Northern hemisphere - highest annual incidences (about 10-15 / 100.000) in North America & Europe - increasing incidences in selected Asian countries are paralleling western life-style adaptations Peak incidence between <i>second and fourth decade of life</i> m ≥ f	
Clinical presentation & anatomic distribution	Chronic relapsing large bowel inflammation Small bowel rarely affected (backwash-ileitis) Obligatory involving the procto-rectal region with ascending dissemination	Chronic inflammation putatively of the entire GIT Small bowel (terminal ileum) > 80% affected About 30% of patients have perianal disease while in 20% of patients the disease is limited to the colon
Pathology - macroscopic - microscopic	Inflammatory affections limited to the mucosa Usually circumferential & continuous lesions Crypt abscesses (UC >> CD)	Transmural inflammation with fistula formation Usually discontinuous ("skip") lesion Granulomas (CD >> UC)
Clinical manifestations - intestinal	frequently bloody, hemorrhagy less typical rarely fistulas absent	<u>Diarrhoe</u> more commonly non-bloody, watery <u>Abdominal pain</u> typically observed frequently fistulas -> abscess <u>Upper GIT lesions</u> e.g. oral aphthous ulcers in 5-15%
- extraintestinal	Joint and connective tissue affections (e.g. ankylosing spondylitis) Eye involvement (e.g. uveitis) Skin lesions (e.g. erythema nodosum or pyoderma gangrenosum) Cholangiopathies (e.g. primary sclerosing cholangitis [PSC]) Hypercoagulability (e.g. venous thrombembolism) Kidney lesions (e.g. renal stones) Pulmonary manifestations (e.g. Bronchiolitis obliterans with organizing pneumonia, BOOP)	
Complications	Intestinal Hemorrhagy Intestinal fibrosis, strictures, short bowel syndrom Colorectal cancer (CU > CD), cholangiocarcinoma	Phlegmona or abscesses with sepsis -> loss of enteral functionality, malabsorption (esp. if PSC+); small bowel cancer (CD)

Fig. 11.1 Etiologic, epidemiologic, and clinical features of IBD. **(a)** IBD describe a number of disease states that manifest in genetically susceptible individuals. IBD are characterized by recurrent episodes of intestinal inflammation resulting from an uncontrolled immune response that is considered to be initiated and/or promoted by both environmental and microbial signals. **(b)** The two major subtypes of IBD, ulcerative colitis (UC) and Crohn's disease (CD), are characterized by chronic or frequently relapsing episodes of intestinal inflammation. Many disease-related features are shared by both forms of IBD, while only very few epidemiological, pathological, or clinical aspects are indicative of the presence of a certain IBD subset. GIT, gastro-intestinal tract; PSC, primary sclerosing cholangitis; BOOP, bronchiolitis obliterans with organizing pneumonia

Another clinically significant gene locus conferring depending on the allelic subtype either predisposes or protects in both CD and UC cohorts, affects the IL23R locus (Duerr et al., [2006](#)). Mechanistically, a plethora of data across many preclinical immune-mediated inflammatory disease models unraveled that IL23/IL23R interaction is critical to promote T cell-driven tissue pathology, presumably via driving Th17 cell differentiation and/or maintenance knowingly broadly abundant in the intestinal mucosa of IBD patients (Neurath et al., [1995](#); von Boyen et al., [2006](#)). Specifically, microbial signals derived from IBD-enriched consortia were shown to induce IL23 expression in mucosal-resident myeloid cells, linking microbiota-regulated IL23 expression to IL23R+ T cell pathogenicity (Sakuraba et al., [2009](#)). Interestingly, systemic IL17A blockage via Secukinumab in CD patients rather deteriorated than improved outcome parameters, suggesting that putatively epithelial barrier-protective effects by IL17 may prevail over its assumed proinflammatory effects downstream of IL23 and/ or that the IL23/IL23R axis employs an IL17A-independent mechanism to promote intestinal tissue inflammation (Hueber et al., [2012](#)). However, importantly, recent studies assessing IL23 inhibitors, e.g., Risankizumab or Mirikizumab, for their clinical efficacy provided critical evidence for the pathomechanistic and functional relevance of the IL23/IL23R axis for intestinal inflammation in both major subtypes of IBD, CD and UC (D'Haens et al., [2023](#); D'Haens et al., [2022](#)).

Recent evidence points toward altered metabolic functions in IBD (Adolph et al., [2022](#)), in particular on the high-energy demand of the inflamed mucosa during healing processes, which was long ago

proposed under the “energy deficiency” hypothesis in UC (Roediger, [1980](#)). Mitochondria are a cornerstone in intestinal homeostasis and are critically involved in intestinal epithelial cell (IEC) metabolism (Rath et al., [2018](#); Haque et al., [2024](#)). Transcriptional profiling identified gene targets involved in mitochondrial dysfunction in IBD (Haberman et al., [2019](#)). We demonstrated that the mitochondrial metabolism of the intestinal stem cell (ISC) niche leads to aberrant Paneth cell fate in IBD patients (Khaloian et al., [2020](#)). Mitochondrial dysfunction of intestinal epithelial cells impairs regenerative capacities of this barrier interface, and contributes to dysbiotic adaptation of the intestinal microbiota (Urbauer et al., [2024](#)). However, as applying to almost all genetic alterations identified so far, its impact on environmental signals, microbiota, and overall gene-host interaction remains to be fully elucidated.

Up to now, more than 300 genetic loci associated with an increased risk for IBD have been identified by GWAS, considering cohorts both from European and East Asian populations (Liu et al., [2023](#); Jostins et al., [2012](#)). Within these risk loci, candidate genes coding for proteins were identified that crucially impact the innate (CARD9, IL18RAP, NOD2, OSMR etc.) and adaptive immune response (IL-23R, Stat3, Jak2, CCR6, RORC, IRF4, NFkB1, IL-21, PRDM1, Bach2, SMAD3 etc.), the epithelial barrier function, integrity, and repair (NKX2-3, Stat3, NOD2, Rel, MUC19, etc.) as well as the endoplasmic reticulum stress (ER) and autophagy response (IRGM, XBP1, ATG16L1, etc.). Strikingly, these results particularly illustrate to what extent a largely hypothesis-free approach can unravel numerous disease-associated pathways, thus providing enormous insight into the diversity of affected cellular compartments and important signaling mechanisms in IBD (Liu & Stappenbeck, [2016](#)). Nevertheless, these studies further showed that the majority of the identified loci are not specifically associated with CD and/or UC, respectively, but are further linked to the occurrence of other immune-mediated disorders, such as psoriasis and arthritis.

In summary, genetic approaches, such as GWAS, exponentially enlarged the knowledge of the mechanisms and pathways underlying

IBD. As a matter of fact, though, the causal genomic regions or even the involved genes have remained unknown for the majority of the identified genomic risk loci. However, in line with a non-Mendelian inheritance at least in adult-onset IBD, an allelic variant alone, i.e., a single IBD susceptibility locus, on average merely affects the individual probability to develop IBD but of note can specifically entail a high risk with regard to a homozygous carriage of one allelic variant, such as the NOD2 risk allele. Therefore, the currently known susceptibility loci for IBD are predicted to only account for ~13% of the CD and ~8% of the UC cases, again underscoring the multifactorial pathogenesis and especially the impact of environmental aspects for IBD manifestation. Hence, recent studies taking advantage of novel technologies, such as *whole genome* and/or *whole exome sequencing* (WES), respectively, are instrumental to uncover uncommon alleles and molecularly pinpoint genetic alteration within single IBD patients to microbial traits (Agrawal et al., [2021](#)). These approaches shed critical light on the gene-environment interaction taking place in IBD patients, going beyond the level of resolution that is reached by either GWAS or microbiota-based analyses alone. However, the impact of host genetics on microbiota communities is detectable in a population-based cohort (e.g., *C. aerofaciens* at the ABO locus) but is subtle, difficult to decipher, and hence will require large study cohorts (Lopera-Maya et al., [2022](#)). Together, major challenges remain, and, as *a conditio sine qua non*, functional studies need to flank genetics-based approaches, including NGS-derived data sets, to evaluate the pathogenetic relevance of mutations in IBD (Gibson et al., [2025](#)).

In contrast to the non-Mendelian inheritance of adult-onset IBD, very early- and early-onset forms of IBD that are observed in pediatric patients often represent monogenic Mendelian disorders. They are frequently associated with a positive family history of IBD-like disorders (Kelsen & Baldassano, [2008](#)). Rare, monogenic diseases are usually missed by classical GWAS. However, as NGS technologies (e.g., WES) emerged, a series of single mutants could be identified and functionally validated to be causatively involved in these early

manifesting pediatric IBD forms. Mutations identified by this approach include genes that usually account for the integrity of the epithelial barrier function (dysregulated upon deletion of, e.g., *ADAM17*) (Blaydon et al., [2011](#)) and prevent hyper-inflammatory states due to the proper regulation of apoptosis (dysregulated upon deletion of, e.g., *XIAP*) (Worthey et al., [2011](#)) or due to the uncompromised functionality of regulatory T cells (e.g., dysregulated upon mutations of *FOXP3*) (Okou et al., [2014](#)). Loss of immunosuppressive responses as a result of Mendelian inheritance of the IL10 and IL10RA gene variants leads to an impaired barrier function and the development of very early-onset disease phenotypes. Colitogenic mechanisms of IL10R deficiency involve a complex interplay of pro-inflammatory macrophages, IL23-IL22 signaling, Th17, and type 3 innate lymphoid cells (ILC3s) as well as epithelial responses skewed towards antimicrobial activities (Pazmandi et al., [2019](#)). Interestingly, the development of very early-onset IBD has particularly highlighted common cellular functions shared between immune and epithelial cells. These include NOX1 and DUOX2 genes, suggesting defective oxidative responses and mitochondrial involvement (Hayes et al., [2015](#)).

In summary, genetic findings, i.e., allelic variants which are detectable within adult-onset IBD patient cohorts, have strongly informed and inspired the field of IBD research since these studies confirmed previous findings but further uncovered so far less recognized pathways (e.g., autophagy, cell stress, mitochondrial dysfunction) to be relevant in IBD pathogenesis. However, the overall power of individual chromosomal aberrations is increasingly seen to be limited and superseded by the impact of environmental signals. In contrast, however, genetics-based approaches are a preserve to particularly evaluate rare forms, but not exclusively in pediatric IBD patients with early-onset forms. Here, a monogenic etiology often results in the manifestation of immune-mediated disorders, including an IBD-like disease. Hence, NGS-based approaches to individual IBD cases will be the method of choice within the near future, hereby simultaneously integrating both host-derived genetic and

environmental (e.g., from intestinal microbiota) triggers and behavioral patterns (e.g., diet).

11.4 Impact of Environmental Triggers on IBD Manifestation and Progression

Both epidemiologic data and genomic studies suggest that only a few genomic alterations are sufficient to account for IBD manifestation but that environmental signals are crucial to set pathogenic mechanisms in motion in individuals at-risk ultimately leading to clinical symptoms (Fig. [11.2](#)). Hence, the so-called pre-clinical phase of IBD has recently moved in the center of attention as it also provides the possibility to ideally apply a primary prevention approach by specifically interfering with signals driving pathways underlying IBD manifestation (Rudbaek et al., [2024](#)). So far, several critical insults within the first 5 years of life have been identified in epidemiologic studies and include, besides moving from low- to high-IBD incidence communities, whether breastfeeding was applied, if and how frequently antibiotics intake was performed, the rate of infections, and the exposure to passive smoking (Agrawal et al., [2021](#)). Exposure to one or a combination of triggers was associated with substantial aberrations of the composition of the microbiota, thereby or directly impacting both innate and adaptive immune response patterns in this early instructive phase, overall raising the risk of developing IBD or other immune-mediated inflammatory disease entities (Agrawal et al., [2023](#); Torres et al., [2020](#)). However, also later in adult life, exposition to certain compounds (antibiotics, non-steroidal anti-inflammatory drugs), lifestyle drugs (e.g., smoking), ultra-processed food and living in urban areas (e.g., exposure to air pollution) significantly enhanced the likelihood of developing IBD (Berger et al., [2016](#); Rudbaek et al., [2024](#)). Striking evidence for a causal interaction of nutrition on microbiome functions is provided for the therapeutic impact of exclusive enteral nutrition in pediatric CD (Häcker et al., [2024](#)). Here, we demonstrated that medium-chain fatty acids of the liquid formula directly interact with

disease-relevant bacterial taxa, supporting the functional relevance of diet in IBD etiology. In fact, within this preclinical phase, adult individuals at risk displayed enhanced intestinal permeability characteristics for IBD before future manifestation of IBD (Turpin et al., [2020](#)). Moreover, gut microbiome signatures have been associated with the future onset of CD overall, suggesting that alteration of intestinal microbiota by environmental insults in the preclinical phase seems to critically contribute to CD pathogenesis (Raygoza Garay et al., [2023](#)).

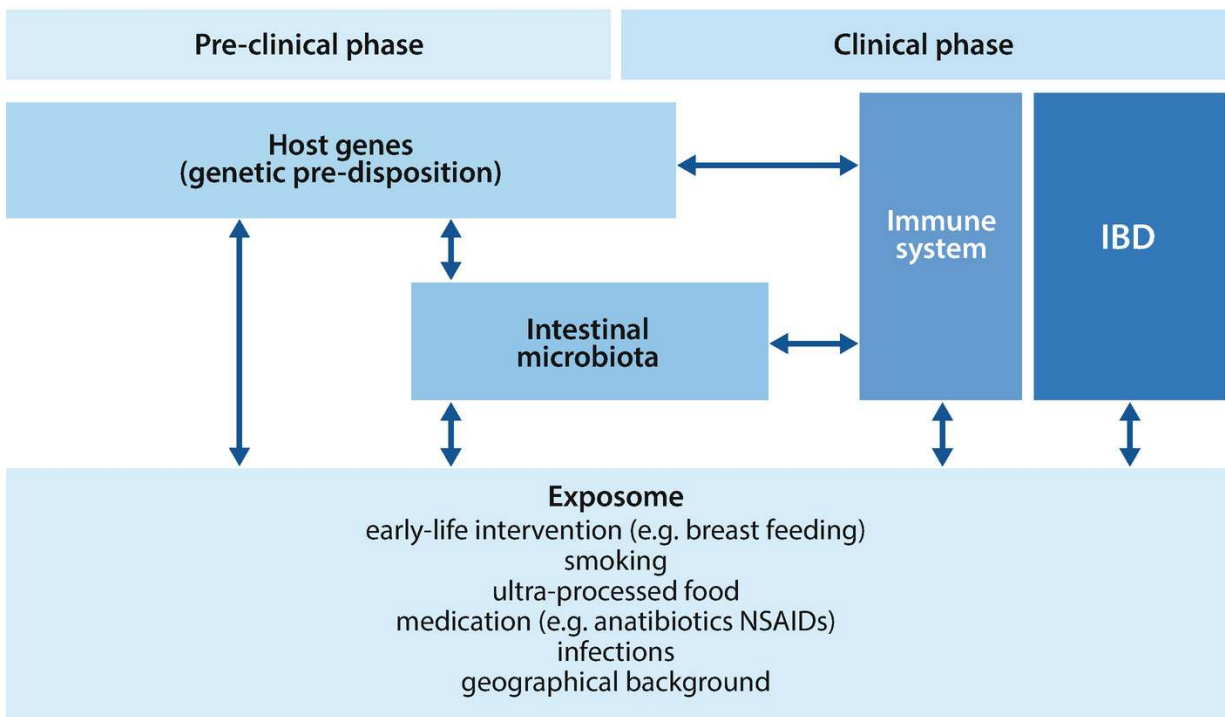


Fig. 11.2 Current pathogenetic concepts consider IBD to consist of both pre-clinical and clinical disease stages. After experiencing so far only partially deciphered environmental insults, commonly termed exposome, and undergoing compositional and putatively functional changes of the intestinal microbiota in a pre-clinical phase of unknown duration, genetically-predisposed individuals clinically manifest immune-driven IBD. Thereafter, both immune, environmental and microbial signals within the defined genetic context continuously impact IBD manifestation resulting in distinct, highly patient-specific disease phenotypes

11.5 Pathology and Clinical Manifestation

The clinical presentation of IBD patients depends on the obligatory presence of inflammatory manifestations within the gastrointestinal

tract, while extraintestinal manifestations are frequently but variably observed. Intestinal manifestations of UC are characterized by chronically persisting and/or relapsing ulcers within the large bowel that are morphologically superficial, i.e., limited to the intestinal mucosa. In 10–20% of the cases, however, inflammatory lesions can also be found in the terminal ileum, which is described by the so-called backwash ileitis. In general, the rectum is obligatorily afflicted in UC. From there, the colitis manifestation continuously ascends in a retrograde circumferential and in terms of longitudinal extent variable fashion.

In contrast to UC, inflammation in CD occurs within the entire gastrointestinal tract, i.e., from the oral cavity to the anus. Here, more than 80% of CD patients show terminal ileitis. CD lesions are characterized by a segmental, discontinuous distribution of typically aphthous or confluent deep linear ulcers. These lesions are also termed “skip lesions” due to the patchy, adjacent coexistence of healthy and highly inflamed areas within the same anatomic location, giving the macroscopic impression of a cobblestone pattern. Another critical feature of CD-associated lesions is the transmural extent of the inflammatory affection of the bowel wall. Transmural inflammation is assumed to represent the preexisting condition that facilitates and precedes the development of fistulas, which are especially found in the small bowel and the perianal region. Here, transmural inflammatory lesions rather randomly inflict adjacent tissues and organs, resulting in the formation of penetrating fistulas. Both interenteric (i.e., between two intestinal loops), entero-genital, entero-vesical, and entero-cutaneous fistulas are frequently observed and often accompanied by the formation of abscesses and septic complications. Another complication associated with chronic intestinal inflammation represents the development of intestinal fibrosis and—if fibrosis is restricted to a distinct anatomic location—of strictures overall hampering the functionality of the gut, which further increases the risk for additional complications such as, e.g., ileus formation. Altogether, macroscopic pathological manifestations often guide the way to

diagnose IBD and to identify the subtype based on the endoscopic and imaging-based (e.g., ultrasound or MRI of the small intestine) distribution pattern of intestinal inflammation. However, none of the visible signs of intestinal inflammation is sufficient to unequivocally diagnose IBD and/or discriminate between IBD subtypes (Fig. [11.1b](#)).

In this regard, histopathological assessments of samples that are obtained by endoscopy-guided biopsies or surgical resection are considered to be potentially helpful in characterizing intestinal tissue manifestations and subtyping IBD but are not sufficient to unequivocally diagnose IBD. Histopathological correlates typical for UC are represented by widespread crypt architectural distortion and depletion of mucin as well as by a diffuse transmucosal inflammatory infiltrate with basal plasmacytosis that putatively triggers cryptitis and the formation of crypt abscesses. In delineation from UC, focal chronic inflammation together with crypt irregularities and granulomas that are not associated with crypt injuries is assumed to reflect CD-associated colonic and less accurately ileal manifestations. Overall, however, discrimination of CD and UC entities that are based on detailed histopathological criteria has not been reliably established and reproducibly achieved, even among experts in the field of gastrointestinal pathology.

While the onset of IBD is assumed to represent the starting point of a lifelong and per se incurable disease state, the type, characteristics, and severity of clinical presentations among IBD patients are highly variable. Moreover, even within the same IBD patient, the distribution pattern and the longitudinal dynamics of the clinical manifestation and symptomatology are highly volatile, thereby indicating a high degree of intraindividual phenotypical adaptations over time, which is presumably due to so far poorly understood changes in immunological processes underlying IBD. Despite these limitations, all IBD patients usually show at least one of the two hallmark symptoms reflecting intestinal manifestations: (a) irregularities/changes of the stool habits (e.g., mostly diarrhea but also obstipation, an increase of stool frequency, bloody feces, hemorrhage) and/or (b) abdominal pain

sensations. Any of these symptoms justifies the initiation of further diagnostic testing (e.g., microbiological stool analyses, endoscopy of the upper and lower gastrointestinal tract with histopathologic evaluation of biopsies, ultrasound of the small and large bowel) that aims to confirm or dismiss the diagnosis of IBD. From a clinical point of view, UC patients typically develop more frequent bloody diarrhea, while abdominal pain episodes are less prevalent in UC. However, the most abundant symptom in CD represents abdominal pain episodes, which are putatively due to and/or indicative of the affection of the small bowel. The increased burden of abdominal pain among CD patients is also partly dependent on the appearance of penetrating fistulas and abscesses. Furthermore, CD patients who are describing either upper abdominal and chest pain, respectively, or seem to have difficulties chewing or swallowing might be screened for the presence of upper gastrointestinal tract lesions, especially in the oral cavity, the esophagus, stomach, or small intestine.

In the majority of IBD patients, the disease course is characterized by the occurrence of extraintestinal disease manifestations over time. Here, similar to intestinal inflammation, the underlying pathomechanisms are largely unknown. However, it is assumed that extraintestinal manifestations are directly or indirectly affected by the pro-inflammatory micro-milieu. Although extraintestinal manifestations can be virtually found in all organ systems, the predominately affected sites represent the musculoskeletal (e.g., arthritis), the dermal (e.g., erythema nodosum, pyoderma gangrenosum), the ocular (e.g., uveitis), the hepatopancreatobiliary (e.g., primary sclerosing cholangitis, PSC), the pulmonary (e.g., obstructive lung disease), and the renal systems (e.g., calcium oxalate kidney stones), or the vasculature (e.g., venous thromboembolism). In contrast to the clinical typology of intestinal manifestations, there is a less stringent assignment of extraintestinal disease manifestations to either IBD subtypes but its occurrence and dynamics often reflect uncontrolled activity of the underlying intestinal disease.

Finally, both intestinal and extraintestinal disease manifestations promote the development of long-term complications, such as intestinal dysfunction due to fibrosis, strictures, or short bowel syndrome in resected patients, cancer development within the large (UC and CD patients with colon involvement) and less frequently, small bowel (in CD), occurrence of carcinomas arising from fistulas, and cholangiocarcinoma. Together with the multifaceted spectrum of clinical symptoms directly elicited by chronic intestinal and extraintestinal inflammatory processes, long-term complications contribute to and add another level of morbidity to the preexisting disease burden prevalent in IBD patients.

11.6 Microbiome Changes in Human IBD

The human gastrointestinal (GI) tract represents the most densely colonized organ of the body, with the highest microbial load of 10^{11} bacteria/mL content in the colon (Sender et al., [2016](#)). Bacteria dominate the microbial ecosystem in the GI tract, with more than 90% belonging to the phyla Bacteroidota (former Bacteroidetes) and the Bacillota (former Firmicutes), but archaea, viruses, yeasts, and protozoa contribute to the diversity of this microbial ecosystem. At the species level, high-throughput 16S rRNA gene sequencing and metagenomic analysis allow the detection of approximately 300–500 operational taxonomic units (OTU) per individual, reaching an overall taxonomic diversity of more than 4600 different bacterial species with a functional capacity of estimated 170 million proteins (Almeida et al., [2021](#)). Nevertheless, interindividual variation of microbiota composition and spatial differences that occur along the proximal to the distal part of the intestine need to be considered in the evaluation of microbiome signatures relevant to the pathogenesis of IBD (Schirmer et al., [2019](#)). Predictive signatures are able to classify population but not individual risk (Wirbel et al., [2021](#)), with substantial overlap across different types of chronic diseases (Metwally et al., [2022](#)). Thus, a profound functional understanding of the microbiome is crucial to

explain the complex intestinal ecosystem and its interaction with the host. The evidence that many chronic disorders, such as allergies and autoimmune and metabolic diseases, are associated with compositional shifts of the gut microbiota in clinical and/or animal studies underlies the therapeutic importance of this field of research.

The identification of genetic risk factors in IBD paved the way for a better mechanistic understanding of the disease pathogenesis, and bacterial triggers are considered to play an important role in the initiation and progression of chronic intestinal inflammation (Jostins et al., [2012](#)). Firstly, fecal stream diversion in subsets of patients with active Crohn's disease provided the first clinical evidence for the central role of bacteria in the pathogenesis of this chronic inflammatory disorder (Rutgeerts et al., [1991](#); D'Haens et al., [1998](#)). Secondly, fecal microbiota transfer (FMT) has been established as a reasonable therapeutic application for a number of different pathologies, including IBD (Sorbara & Pamer, [2022](#)). A meta-analysis of controlled FMT trials identified significant therapeutic efficacy in patients with ulcerative colitis, reaching an average 30.4% response rate in FMT-treated patients compared to 9.8% in the placebo group (El Hage Chehade et al., [2023](#)). Finally, the colonization of germ-free rodent models with disease-relevant bacteria (Rath et al., [1996](#)) and fecal material from patients re-established chronic intestinal inflammation (Metwaly et al., [2020](#)), supporting a causal role of the microbiome in the pathogenesis of IBD.

High-throughput sequencing analysis of microbial communities was used to identify risk patterns associated with distinct IBD phenotypes (Frank et al., [2007](#)), first-degree relatives (Joossens et al., [2011](#)), and geographic distribution of disease manifestation (Rehman et al., [2016](#)). The bacterial diversity and composition of mucosa-associated and fecal samples substantially differed between CD phenotypes, and the most pronounced effects were shown for patients with ileal compared to colonic disease involvement (Willing et al., [2010](#)). Analysis of 447 treatment-naïve new-onset pediatric CD and 221 non-IBD control samples confirmed the association between disease severity and low

species richness (alpha diversity) (Gevers et al., [2014](#)). Together, a number of cross-sectional and longitudinal cohort studies investigated microbiota alterations in patients with IBD by conducting microbial profiling of luminal and mucosal microbial communities. Overall, active IBD is associated with increased relative abundance of Enterobacteriaceae, *Fusobacterium*, *Ruminococcus gnavus*, *Streptococcus anginosus*, *Klebsiella pneumoniae*, Enterococcus, Megasphaera, Campylobacter, and sulfate-reducing Gammaproteobacteria and Deltaproteobacteria. In contrast, beneficial taxa, including *Faecalibacterium prausnitzii*, Christensenellaceae, Collinsella, Roseburia, Ruminococcus, and other butyrate-producing bacteria, are diminished in IBD (Metwaly et al., [2022](#)). In addition, Bacteroides and Phocaeicola spp. colonize the human gut after birth (Heppner et al., [2024](#)), reaching high abundances in the human adult microbiota (Gacesa et al., [2022](#)). They are members of the healthy microbiota, but increased abundances of Bacteroides and Phocaeicola spp. in IBD patients are frequently reported (Yilmaz et al., [2019](#); Lloyd-Price et al., [2019](#); Urbauer et al., [2024](#)). Due to the inter-individual variations and dynamic changes of microbiota profiles across the variable disease courses, it seems unlikely that in the absence of a mechanistic understanding, the sole description of microbial communities will identify IBD-relevant phylotypes across a broader range of patients. This is even more evident when considering the dynamics of bacterial strain variation between patients and along the disease course (Häcker et al., [2024](#)). Nevertheless, some bacterial species seem to be consistently associated with protective effects, such as *Faecalibacterium prausnitzii* (Sokol et al., [2008](#)), while others are linked to adverse clinical situations, including *Escherichia coli*, and specifically adherent-invasive E. coli (AIEC) (Darfeuille-Michaud, [2002](#)). AIEC and *Mycobacterium avium* subsp. *paratuberculosis* are frequently associated with the pathogenesis of inflammation in Crohn's disease patients, leading to the speculation that transient infection or specific pathobionts rather than complex microbial signatures are causally linked to IBD pathogenesis.

Dysbiosis is considered as an alteration in microbiota community structure and/or function, capable of causing/driving a detrimental distortion of microbe-host homeostasis that specifically initiates or propagates disease. Loss of species richness correlates with disease activity in various studies and subsets of IBD patients; however, the “egg or hen” question related to the cause or consequence in the context of inflammation-driven changes in the microbiota remains unanswered. Thus, the need to define the functional relevance and specificity of single bacteria (pathobiont) or bacterial networks (dysbiosis) is most important for the understanding of microbe-host interactions in the pathogenesis of IBD.

11.7 Pathogenesis of Microbe–Host Interactions

Intestinal tissue homeostasis requires the active engagement of multifaceted microbial (Burgueno & Abreu, [2020](#)) and chemical sensors (Lavelle & Sokol, [2020](#)). A pleiotropic network of immune, metabolic, and trophic functions contributes to microbiome-host symbiosis, and the disruption of these functionally mutual interactions contributes to the initiation and progression of diseases. Considering the fact that up to 70% of the intestinal bacteria are not yet culturable and 50% of the metagenomic information relates to unknown function, it seems understandable that the precise mechanistic contribution of the microbiome in the IBD etiology is still largely unknown. Studies in germ-free mouse models using synthetic mice (Afrizal et al., [2022](#)) and human communities (Cheng et al., [2022](#)) play an essential role in understanding the functional role of the intestinal microbiome in health and chronic inflammation (Aguanno et al., [2022](#)).

11.7.1 IBD Mouse Models

A substantial number of different mouse models develop IBD-like pathologies (Fig. [11.3](#)). Although none of them completely replicate the human disease phenotype, at least 74 genetically engineered mice

spontaneously develop intestinal inflammation, and 20 of these experimental models carry IBD-relevant susceptibility genes (Mizoguchi et al., [2016](#)). One important way to investigate the role of the (intestinal) microbiota in the context of specific host functions or complex diseases is the use of germ-free experimental disease models. Mono-association studies or studies using selected consortia of microorganisms allow important mechanistic insights into specific microbe-microbe and microbe-host interactions under highly controlled and standardized conditions regarding genotype, diet, and microbial colonization (Hörmannspurger et al., [2015](#)). As introduced above, *NOD2* is an important susceptibility gene for Crohn's disease associated with a loss-of-function mutation; however, *Nod2*^{-/-} mice fail to spontaneously develop inflammation but show increased susceptibility to *Listeria monocytogenes* infection (Kobayashi et al., [2005](#)). Most IBD animal models show reduced or a lack of disease under germ-free conditions, indicating that the intestinal microbiota is involved in the development of most complex diseases. At present, the majority of IBD-related animal models are suitable for studying chronic inflammation in the colon, but only a few models develop Crohn's disease-like pathology, including transmural inflammation in the ileum, cobblestone lesions in the mucosa, as well as extraintestinal disease manifestations such as arthritis. SAMP1/YitFc and *Tnf*^{ΔARE/+} mice spontaneously develop chronic inflammation in the ileum and combine most of the Crohn's disease-like pathological manifestations (Metwaly & Haller, [2024](#)). In genetically susceptible *Tnf*^{ΔARE/+} mice, inflammation-associated changes in the microbiota caused transmissible disease in the genetically susceptible host (TNF^{ΔARE} mice) but not in wild-type (WT) recipients, while germ-free mice remain disease-free, demonstrating that microbial communities, although developed under the same experimental conditions, may undergo progressive changes in their microbial environment, leading to a more aggressive phenotype over time (Schaubeck et al., [2016](#)). Interestingly, later studies identified segmented filamentous bacteria as a key pathobiont in *Tnf*^{ΔARE/+} mice (Metwaly et al., [2023](#)), suggesting

that dysbiotic changes in the microbiota allow the expansion of disease-causing taxa. Interestingly, ZAP-70^{W163C}-mutant (SKG) mice, which develop spondyloarthritis and ileitis upon intraperitoneal injection of β -1,3-glucan (curdlan) remain completely disease-free under germ-free conditions (Rehaume et al., 2014), suggesting a role for the microbiota also in joint inflammation. Additional Crohn's disease models in mice, such as *Caspase8* ^{Δ IEC} (Gunther et al., 2011), *Xbp1* ^{Δ IEC} (*X Atg16l1* ^{Δ IEC} or *Atg7* ^{Δ IEC}) (Adolph et al., 2013), *Hsp60* ^{Δ IEC} (Berger et al., 2016), *Prohibitin1* ^{Δ IEC} (Jackson et al., 2020), *Opa1* (Bao et al., 2025) and pigs (*Tnf* ^{Δ ARE}) (Winogrodzki et al., 2023) have been recently developed.

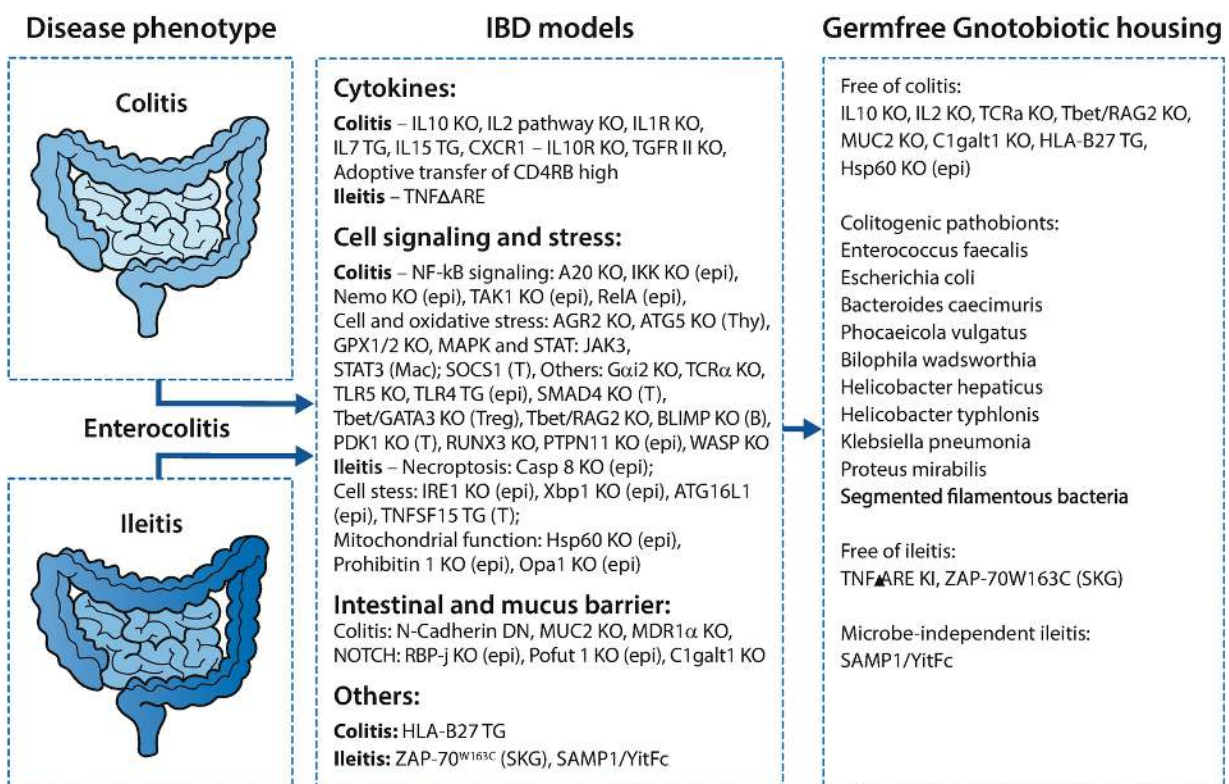


Fig. 11.3 Overview of IBD-relevant animal models. Complete- and tissue-specific (epi, intestinal epithelium) genetic manipulation (KO, knockout; KI, knock-in; TG, transgenic) mouse and rat (e.g., HLA-B27 TG) models are summarized according to the anatomical site of inflammation (ileitis, colitis) and the cellular/molecular targets of genetic manipulation (cytokine expression, signaling molecules, barrier integrity). Most of the IBD models are disease-free under germ-free housing conditions. Gnotobiotic mice colonized with selective pathobionts reestablish disease

The genetically engineered interleukin-10-deficient mouse (*Il10*^{-/-}) provides a model of spontaneous colonic intestinal inflammation (Kuhn et al., [1993](#)) and has been extensively used to dissect IBD etiology. Evidence for the requirement of resident enteric bacteria for the development of colitis in *Il10*^{-/-} mice stemmed from studies in germ-free animals, where colitis development was not observed (Sellon et al., [1998](#)). It has been shown that the Gram-positive intestinal bacterium *E. faecalis* drives distal colonic inflammation in *Il10*^{-/-} mice following mono-association (Balish & Warner, [2002](#)). Findings from our group identified that the virulence factor gelatinase E (GelE) partially impairs intestinal epithelial barrier integrity in *Il10*^{-/-} mice (Steck et al., [2011](#)) and that the colitogenic activity of *E. faecalis* was partially and almost completely abrogated when deficient for the enterococcal polysaccharide antigen (Δ *epaB*) and lipoproteins (Δ *lgt*) envelope structures, respectively (Ocvirk et al., [2015](#)). Such results invite the hypothesis that particular microbial effectors, or a combination of effectors from different commensal bacteria, are required to elicit pathogenesis or maintain the necessary barrier function for intestinal homeostasis. Identifying bacterial gene products that drive protective rather than pathogenic inflammation in the intestine is crucial to rebalance homeostasis in inflammatory diseases and malignancies. *Lactobacillus* species, such as *L. acidophilus*, are normal inhabitants of the intestinal microbiota and have received considerable attention as beneficial ecosystem members. *L. acidophilus* stimulates DCs through TLR2 via lipoteichoic acid (LTA) to trigger the production of inflammatory and regulatory cytokines. Deletion of the phosphoglycerol transferase gene (LBA0447) that synthesizes LTA generated an *L. acidophilus* derivative (NCK2025) that diminishes colitis when administered orally in a murine colitis model (Mohamadzaheh et al., [2011](#)), confirming the role of LTA in inducing inflammation. In another example, *L. paracasei* was found to secrete the prtP-encoded protease lactocepin with anti-inflammatory effects via the degradation of pro-inflammatory chemokines (Von Schillde et al., [2012](#)). Collectively, the above findings support the notion that the

colitogenic activity can be assigned to specific bacterial structures and that such characterizations are indispensable in understanding host-microbe interactions relevant for the development of intestinal inflammation.

11.7.2 Innate Immune Functions in Sensing Commensalism at the Intestinal Interface

Loss of epithelial barrier function and innate immunity are fundamental to the pathogenesis of inflammatory and infectious diseases. The intestinal immune system has the challenge of responding to pathogens while remaining tolerant to food antigens and the commensal microbiota. The intestinal epithelium executes a compartmentalization between the lumen and the host, simultaneously acting as a selectively permeable first line of defense to fulfill its function of absorption while maintaining an effective barrier against the intestinal microbiota, antigens, and toxins. Intestinal epithelial cells (IECs) express pro-inflammatory cytokines in response to infectious invasive bacteria but largely ignore nonpathogenic commensals. Certain intestinal pathogens and opportunistic commensals, however, can evade this first line of defense and enter IECs, suggesting that the existence of IEC-intrinsic immune mechanisms for bacterial detection and limitation is essential. One key cell-autonomous mechanism of antibacterial defense is IEC autophagy, shown to be activated following bacterial invasion through adaptor protein myeloid differentiation primary response gene 88 (MyD88) cell-intrinsic signaling, with autophagy deficiency in mice causing increased dissemination of invasive bacteria (Benjamin et al., [2013](#)), indicating that autophagy could have a broader role in inflammatory diseases. IECs and innate immune cells of the lamina propria are able to differentiate self from nonself through a selective spatial and cellular expression of pattern recognition receptors (PRRs). Classically, the detection of pathogen-associated molecular patterns (PAMPs) allows the intestinal epithelium to activate signaling pathways that induce the early host response to infection. The role of microbe-associated molecular patterns (MAMPs)

in mediating innate recognition of the commensal “noninfectious” microbiota remains controversial. Paradoxically, recent progress in understanding the IBD pathogenesis suggests that a defective innate immune system predisposes the host toward chronic inflammation, supporting a protective role of PRR signaling in maintaining intestinal tissue homeostasis. Early work related to the activation of inflammation-related transcription factors, such as NF- κ B, suggested a hormetic adaptation of the epithelium in response to commensal bacteria (Haller et al., [2000](#), [2002](#)), with elegant studies related to IEC-specific inhibition of NF- κ B activation validating the importance of this signaling pathway in maintaining tissue homeostasis (Nenci et al., [2007](#)). This paradigm shift was supported by Medzhitov and colleagues, demonstrating that microbiota-derived signals via the toll-like receptor (TLR)-related adaptor protein MyD88 protect mice from the development of colitis (Rakoff-Nahoum et al., [2004](#)) and intestinal tumor formation (Rakoff-Nahoum & Medzhitov, [2007](#)).

11.7.3 Metabolic Circuits of Microbe–Host Interaction

An intriguing new concept suggests that metabolic circuits and specific metabolites of the microbiome and its host contribute to intestinal homeostasis. In line with the high energy demand of the inflamed mucosa, loss of metabolic circuits between microbiome and host, including butyrate (Roediger & Nance, [1986](#)), carnitine (Smith et al., [2021](#)), purine (Cader et al., [2020](#)) and tryptophan metabolism (Lamas et al., [2016](#)), are involved in the development of chronic inflammation, supporting the idea that microbial-metabolic circuits contributes to functional perturbation in IBD (Rath & Haller, [2022](#)). Among the multifaceted mechanisms of metabolic signal integration, and despite the complex nature of chemical ligands present in the microbial milieu, the aryl hydrocarbon receptor (AhR) plays a pivotal role in regulating tissue homeostasis and intestinal inflammation (Stockinger et al., [2021](#)). The epithelium is at the frontline of the intricate intersection of microbiome, immune system and metabolism, and mitochondria are emerging as intriguing interceptors of milieu signals in the intestinal

tissue (Rath et al., [2018](#); Jackson & Theiss, [2020](#)). Under normal physiological conditions, the differentiated epithelium performs mitochondrial respiration to consume oxygen for ATP production, resulting in physiological tissue hypoxia and anaerobic conditions in the intestinal lumen. Infectious bacteria of the family Enterobacteriaceae, such as *Salmonella enterica* serovar *Thyphimurium* and *Citrobacter rodentium*, reconstruct the metabolic niche of the intestinal epithelium from mitochondrial respiration, leading to increased lactate production and oxygen diffusion into the luminal milieu. Increased production of reactive oxygen species (ROS) and nitrogen species (RNS) from infiltrating phagocytes facilitates the formation of respiratory electron acceptors, such as nitrate and tetrathionate, thereby favoring the growth of facultative anaerobes in the intestinal milieu. The asaccharolytic, anaerobic, oral microbe *Veillonella parvula* illustrates that host-driven intestinal inflammation, in particular the nitrosative host response, creates a metabolic milieu allowing *V. parvula* to perform anaerobic nitrate respiration, leading to its ectopic colonization of the inflamed colon (Rojas-Tapias et al., [2022](#)). We recently demonstrated that mitochondrial perturbation contributes to inflammation-associated tissue injury in mouse models and IBD patients (Khaloian et al., [2020](#)). Failure to sustain mitochondrial functions leads to chronic tissue wounding, which in turn poses a substantial threat to susceptible individuals to evolve into chronically recurring disease flares. Mitochondrial impairment of the epithelium is also involved in shaping dysbiotic adaptations of microbial communities, favoring the growth of *Bacteroides* and *Phocaeicola* (Urbauer et al., [2024](#)) (Fig. [11.4](#)).

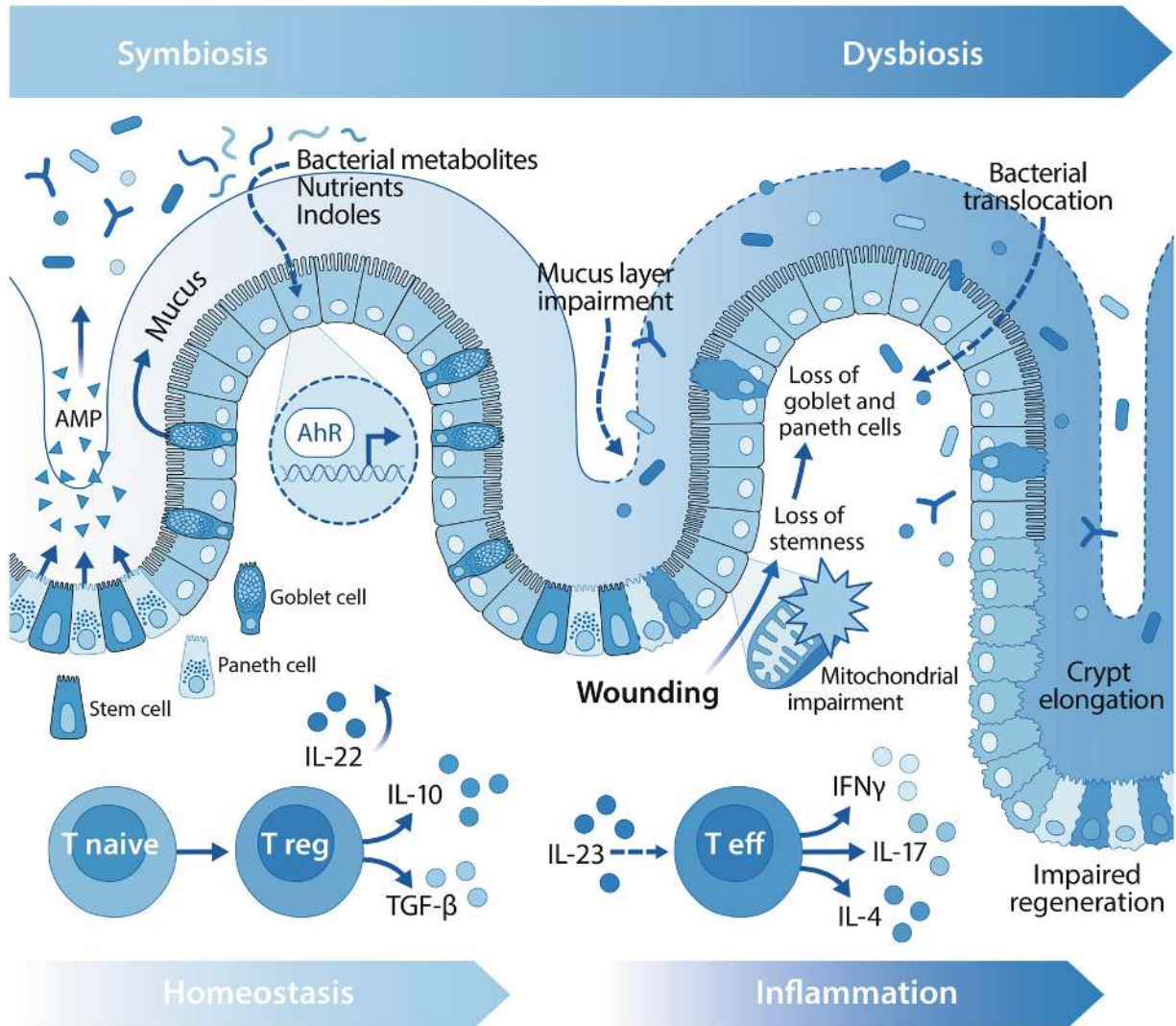


Fig. 11.4 Microbiome–host interactions contribute to gut mucosal tissue homeostasis and the disruption of this symbiotic relationship contributes to the development of chronic inflammation. Nutritional and bacterial ligands of the chemoreceptor AhR as well as anti-inflammatory and tissue homeostatic cytokines (IL-10, TGF- β , IL-22) maintain epithelial barrier integrity and support immune tolerance. Dysbiotic changes in the microbiome contribute to impaired tissue regeneration and loss of barrier integrity, leading to pro-inflammatory immune activation and progressive disease evolution in patients with IBD

11.7.4 Mucus Barrier Disruption Associated with Dysbiosis and Spatial Changes in Bacteria Localization

As the first line of defense protecting the intestinal epithelium, mucus contributes to the maintenance of epithelial homeostasis and protects against mechanical, chemical, and microbial assaults. Along with the high microbial density, the colon presents the thickest mucus layer,

measuring around 830 μm . The stratified inner mucus layer forms a physical barrier acting as a size-exclusion filter that separates the intestinal microbiota from the epithelial cell layer, overlaid by the outer loose layer that forms a habitat for bacteria (Hansson, [2020](#)). Goblet cells of the intestinal epithelium constitutively produce and secrete mucus. In the ER of Goblet cells, MUC2 monomers dimerize and subsequently trimerize via C-terminal and N-terminal disulfide bridges. Secreted oligomers are anchored at the glycocalyx of the intestinal epithelium and subsequently become highly hydrated to form expanded sheets that comprise the three-dimensional mucus layer. MUC2 is a highly O-glycosylated mucin, with more than 80% of its total molecular weight (2.7 MDa) consisting of oligosaccharide side chains that form a crucial part of microbe-mucus interactions (Coleman & Haller, [2021](#)). The permanently renewing intestinal mucus layer represents an important ecological niche rich in nutrients, providing a particularly beneficial environment for metabolically flexible commensal bacteria that can sequentially degrade mucin O-glycans for utilization as carbon and energy sources. Although the degradation of mucin was initially associated with pathogenicity, however commensal bacteria, including *Bacteroides thetaiotaomicron*, *Barnesiella intestinihominis*, *Ruminococcus gnavus*, and *Akkermansia muciniphila*, are functionally capable of degrading the complex carbohydrates. Interestingly, *B. thetaiotaomicron* contains polysaccharide utilization loci (PULs) that are required for successful colonization (Martens et al., [2008](#)). Another specialist for the degradation of mucin is *A. muciniphila*, which is an abundant member of the intestinal microbiota, and a systematic review of clinical trials linked the increased abundance of *A. muciniphila* to dietary modulation, such as caloric restriction (Verhoog et al., [2019](#)). It has been shown that *A. muciniphila* is able to prevent or treat metabolic disorders, reduce metabolic inflammation, and restore the gut barrier (Cani et al., [2022](#)). In accordance with these findings, Desai et al. showed that the deficiency in dietary plant fibers promotes expansion and activity of the mucin-degrading bacteria *A. muciniphila* and *B. caccae* in a synthetic human gut microbiota assembled in a

gnotobiotic mouse model (Desai et al., [2016](#)). This shift in mucin-degrading bacteria impaired the colonic mucus barrier and increased the colitis susceptibility to *Citrobacter rodentium*. Muc2 knockout mice provided direct evidence that mucus layer deficiency results in the development of spontaneous colitis (Van der Sluis et al., [2006](#)), supporting the clinical evidence for the loss of Goblet cell function and mucus structure in UC patients (Johansson et al., [2014](#)).

Concluding Remarks

Compelling evidence from human and mouse studies supports the view that the microbiome plays an important role in the pathogenesis of IBD. However, the multifactorial nature of gene-environment interactions and the existence of a variety of confounding factors in human studies (e.g., drugs, disease location, and behavior) hamper a clear conclusion on how microbiome risk profiles should look to be clinically implemented for prognostic and therapeutic purposes. Despite recently gained novel insight into the epidemiological, immunological, and microbiota-related characteristics of the preclinical phase of IBD, it is still unclear to what extent disease risk is predicably based on characteristic changes in bacterial community structure and/or function and whether microbiome changes have a causal role in disease onset. The development of standards is essential to ensure reproducibility and comparability between human studies. The scientific field needs to validate the specificity and selectivity of microbiome signatures, considering different mechanisms in the onset and progression of Crohn's disease and ulcerative colitis. To reach this goal, research strategies need to go beyond metagenomics sequencing, aiming at the identification of functional mediators and, most importantly, addressing mechanistic links between microbiota-derived signals and host sensors and finally, leading to evidence-based intervention strategies selectively targeted to specific human diseases.

Controversy A wealth of experimental and clinical evidence suggests that microbiome-related factors contribute to the etiology of

IBD. However, the clinical translation towards microbiome-based therapies is suffering from highly variable disease signatures in individual IBD patients along their complex disease courses. At the compositional and functional level, IBD-related dysbiosis is poorly defined and universal targets for microbiome-based therapeutic intervention are lacking. While a variety of intervention strategies have been applied to modulate the intestinal microbiome including the administration of antibiotics, prebiotics (e.g., dietary components that promote the growth and metabolic activity of beneficial bacteria), probiotics (i.e., beneficial bacteria), or fecal microbiota transfer (FMT), the clinical relevance in IBD is still not conclusive (Zhang et al., [2025](#)). Two initial double-blind randomized controlled trials showed the opposite efficacy of FMT in UC patients. While the study from Moayyedi et al. reported secondary efficacy endpoints of FMT in active UC (Moayyedi et al., [2015](#)), the trial carried out by the Ponsioen group showed no benefits for IBD patients by FMT (Rossen et al., [2015](#)). The contrasting results may be explained by the different approaches adopted by the two research teams, including the mode of administration, the composition, and the dose of the donor microbiota used. A subsequent multi-donor and multi-dosing FMT strategy was successful in inducing steroid-free clinical and endoscopic remission in active UC (Paramsothy et al., [2017](#)), and a meta-analysis of controlled FMT trials confirmed a reasonably significant therapeutic efficacy in UC (El Hage Chehade et al., [2023](#)). The therapeutic efficacy of FMT in CD patients remains inconclusive and clearly requires more controlled intervention trials (Zhou et al., [2023](#)). The combination of therapeutic interventions provides an interesting new concept in defined IBD sub-cohorts, including the implementation of FMT as maintenance therapy after EEN-induced remission in pediatric CD patients (Hoelz et al., [2023](#)). Targeted suppression of IBD-related bacterial taxa is a promising new approach (Federici et al., [2022](#)), however, these first experimental data require validation in controlled human interventions.

History

Due to the incomplete understanding of the underlying mechanisms inducing, promoting, resolving, and/or failing to resolve intestinal inflammation, until now, a precise definition of IBD subtypes, accurate diagnostic tools of discrimination, and curative approaches and therapeutics of either IBD manifestation are lacking. Although disease states associated with diarrhea as the most important symptom of IBD were mentioned in historic documents dating back to the Greek age (Baillie, [1793](#)), it was not until 1859 that the physician Sir Samuel Wilks coined the term “ulcerative colitis” (UC). In 1909, a larger cohort of UC patients was broadly discussed in the scientific community (Wilks, [1859](#)), presumably for the first time. CD was first described as a distinct, separate IBD entity (“regional ileitis”) by Burril Bernhard Crohn (Crohn et al., [1932](#)) although several previous case reports may have unintentionally described clinical cases of CD falsely as UC cases given the presence of “regional ileitis” (Crohn et al., [1932](#)).

Ever since, microbial cues (“germs”) have been implicated as a putatively critical component contributing to the IBD pathogenesis. This hypothesis was presumably further promoted at the end of the nineteenth and beginning of the twentieth century by the perception that infections frequently represent the underlying trigger of diarrhea (Paulson, [1928](#)). Consequently, upon the invention of antibiotics in the late 1930s, IBD patients were broadly treated with antibiotics. However, these treatment regimens yielded expectedly variable results and failed to induce stable clinical long-term remission.

This phase was followed by the steroid era in the 1950s, yielding for the first time dramatically positive therapeutic effects that established a sustainable success of steroids in IBD treatment (Truelove & Witts, [1955](#)). Consequently, the scientific focus steadily shifted to the immune system as a worthwhile module to be targeted in IBD patients. Hence the era of anti-inflammatory (e.g., sulfasalazine) (Svartz, [1968](#)) and immunosuppressive (e.g., 6-mercaptopurine) (Bean, [1962](#)) drugs started its successful march

through the clinic and represents still a mainstay in IBD treatment. However, the dissection of the underlying immunologic mechanisms with modern technologies resulted in the identification of cytokines as putative therapeutic targets. As first in class, TNF-alpha antibodies proved to be safe and effective in CD (van Dullemen et al., [1995](#)) and consecutively also in UC patients (Rutgeerts et al., [2005](#)). Consequently, to date additional cytokines and their resp. signaling pathways are specifically (e.g., IL12/IL23) or more generally (e.g. Jak inhibitors) targeted in IBD to combat intestinal inflammation (Neurath, [2024](#)). Until now, TNF-alpha targeting has been one of the main therapeutic stents to induce and maintain remission in IBD patients.

The genomic era generated awareness for the heritability of IBD, while especially genome-wide association studies managed to identify a plethora of disease risk susceptibility loci. These loci contain genes like *NOD2* that are assumed to provide a critical link between bacterial, immunological, and epithelial abnormalities observed in IBD. However, novel effective therapeutic inventions derived from these data sets are still awaited.

Highlights

- An increased risk of IBD is observed in populations with a Western lifestyle.
- The association of *NOD2* mutations with CD gave the first evidence of disease-relevant implications of the intestinal microbiota.
- GWAS on IBD patient cohorts identified over 300 genetic factors showing a genetically driven disruption of distinct host-microbe interactions.
- Fecal stream diversion in subsets of IBD patients provided the first clinical evidence for the central role of bacteria in the pathogenesis of Crohn's disease.

- Germ-free animal models are mostly disease-free, indicating a fundamental relevance for microbial triggers in IBD pathogenesis.

References

Adolph, T. E., Tomczak, M. F., Niederreiter, L., Ko, H. J., Bock, J., Martinez-Naves, E., Glickman, J. N., Tschurtschenthaler, M., Hartwig, J., Hosomi, S., Flak, M. B., Cusick, J. L., Kohno, K., Iwawaki, T., Billmann-Born, S., Raine, T., Bharti, R., Lucius, R., Kweon, M. N., Marciniak, S. J., Choi, A., Hagen, S. J., Schreiber, S., Rosenstiel, P., Kaser, A., & Blumberg, R. S. (2013). Paneth cells as a site of origin for intestinal inflammation. *Nature*, *503*, 272–276.

[\[https://doi.org/10.1038/nature12599\]](https://doi.org/10.1038/nature12599)[\[PubMed\]](#)

Adolph, T. E., Meyer, M., Schwarzler, J., Mayr, L., Grabherr, F., & Tilg, H. (2022). The metabolic nature of inflammatory bowel diseases. *Nature Reviews. Gastroenterology & Hepatology*, *19*, 753–767.

Afrizal, A., Jennings, S. A. V., Hitch, T. C. A., Riedel, T., Basic, M., Panyot, A., Treichel, N., Hager, F. T., Wong, E. O., Wolter, B., Viehof, A., von Stempel, A., Eberl, C., Buhl, E. M., Abt, B., Bleich, A., Tolba, R., Blank, L. M., Navarre, W. W., Kiessling, F., Horz, H. P., Torow, N., Cerovic, V., Stecher, B., Strowig, T., Overmann, J., & Clavel, T. (2022). Enhanced cultured diversity of the mouse gut microbiota enables custom-made synthetic communities. *Cell Host & Microbe*, *30*, 1630–45 e25.

Agrawal, M., Sabino, J., Frias-Gomes, C., Hillenbrand, C. M., Soudant, C., Axelrad, J. E., Shah, S. C., Ribeiro-Mourao, F., Lambin, T., Peter, I., Colombel, J. F., Narula, N., & Torres, J. (2021). Early life exposures and the risk of inflammatory bowel disease: Systematic review and meta-analyses. *EClinicalMedicine*, *36*, 100884.

[\[PubMed\]](#)

Agrawal, M., Poulsen, G., Colombel, J. F., Allin, K. H., & Jess, T. (2023). Maternal antibiotic exposure during pregnancy and risk of IBD in offspring: A population-based cohort study. *Gut*, *72*, 804–805.

[\[PubMed\]](#)

Aguanno, D., Metwally, A., Coleman, O. I., & Haller, D. (2022). Modeling microbiota-associated human diseases: From minimal models to complex systems. *Microbiome Research Reports*, *1*, 17.

[\[PubMed\]](#)

Ahmad, T., Satsangi, J., McGovern, D., Bunce, M., & Jewell, D. P. (2001). Review article: The genetics of inflammatory bowel disease. *Alimentary Pharmacology & Therapeutics*, *15*, 731–748.

Almeida, A., Nayfach, S., Boland, M., Strozzi, F., Beracochea, M., Shi, Z. J., Pollard, K. S., Sakharova, E., Parks, D. H., Hugenholtz, P., Segata, N., Kyrpides, N. C., & Finn, R. D. (2021). A unified catalog of 204,938 reference genomes from the human gut microbiome. *Nature Biotechnology*, *39*, 105–114.

[\[PubMed\]](#)

Baillie, M. (1793). *The morbid anatomy of some of the most important parts of the human body*. J. Johnson and G. Nicol.

Balish, E., & Warner, T. (2002). Enterococcus faecalis induces inflammatory bowel disease in interleukin-10 knockout mice. *The American Journal of Pathology*, 160, 2253–2257.

[\[PubMed\]](#)

Bao, L. L., Yu, Y. Q., Gonzalez-Acera, M., Patankar, J. V., Giessler, A., Sturm, G., Kuhl, A. A., Atreya, R., Erkert, L., Gamez-Belmonte, R., Krug, S. M., Schmid, B., Tripal, P., Chiriac, M. T., Hildner, K., Siegmund, B., Wirtz, S., Sturzl, M., Abdou, M. M., Trajanoski, Z., T. R. R. IBDome Consortium, Neurath, M. F., Zorzano, A., & Becker, C. (2025). Epithelial OPA1 links mitochondrial fusion to inflammatory bowel disease. *Science Translational Medicine*, 17, eadn8699.

[\[PubMed\]](#)

Bean, R. H. (1962). The treatment of chronic ulcerative colitis with 6-mercaptopurine. *The Medical Journal of Australia*, 49(2), 592–593.

Benjamin, J. L., Sumpter, R., Jr., Levine, B., & Hooper, L. V. (2013). Intestinal epithelial autophagy is essential for host defense against invasive bacteria. *Cell Host & Microbe*, 13, 723–734.

Berger, E., Rath, E., Yuan, D., Waldschmitt, N., Khaloian, S., Allgauer, M., Staszewski, O., Lobner, E. M., Schottl, T., Giesbertz, P., Coleman, O. I., Prinz, M., Weber, A., Gerhard, M., Klingenspor, M., Janssen, K. P., Heikenwalder, M., & Haller, D. (2016). Mitochondrial function controls intestinal epithelial stemness and proliferation. *Nature Communications*, 7, 13171.

[\[PubMed\]](#)

Blaydon, D. C., Biancheri, P., Di, W. L., Plagnol, V., Cabral, R. M., Brooke, M. A., van Heel, D. A., Ruschendorf, F., Toynbee, M., Walne, A., O'Toole, E. A., Martin, J. E., Lindley, K., Vulliamy, T., Abrams, D. J., MacDonald, T. T., Harper, J. I., & Kelsell, D. P. (2011). Inflammatory skin and bowel disease linked to ADAM17 deletion. *The New England Journal of Medicine*, 365, 1502–1508.

[\[PubMed\]](#)

Burgueno, J. F., & Abreu, M. T. (2020). Epithelial Toll-like receptors and their role in gut homeostasis and disease. *Nature Reviews. Gastroenterology & Hepatology*, 17, 263–278.

Cader, M. Z., de Almeida Rodrigues, R. P., West, J. A., Sewell, G. W., Md-Ibrahim, M. N., Reikine, S., Sirago, G., Unger, L. W., Iglesias-Romero, A. B., Ramshorn, K., Haag, L. M., Saveljeva, S., Ebel, J. F., Rosenstiel, P., Kaneider, N. C., Lee, J. C., Lawley, T. D., Bradley, A., Dougan, G., Modis, Y., Griffin, J. L., & Kaser, A. (2020). FAMIN is a multifunctional purine enzyme enabling the Purine Nucleotide cycle. *Cell*, 180, 278–95 e23.

Cani, P. D., Depommier, C., Derrien, M., Everard, A., & de Vos, W. M. (2022). Akkermansia muciniphila: Paradigm for next-generation beneficial microorganisms. *Nature Reviews. Gastroenterology & Hepatology*, 19, 625–637.

Chang, J. T. (2020). Pathophysiology of inflammatory bowel diseases. *The New England Journal of Medicine*, 383, 2652–2664.

[\[PubMed\]](#)

Cheng, A. G., Ho, P. Y., Aranda-Diaz, A., Jain, S., Yu, F. B., Meng, X., Wang, M., Iakiviak, M., Nagashima, K., Zhao, A., Murugkar, P., Patil, A., Atabakhsh, K., Weakley, A., Yan, J., Brumbaugh, A. R., Higginbottom, S., Dimas, A., Shiver, A. L., Deutschbauer, A., Neff, N., Sonnenburg, J. L., Huang, K. C., &

Fischbach, M. A. (2022). Design, construction, and in vivo augmentation of a complex gut microbiome. *Cell*, 185, 3617–36 e19.

Coleman, O. I., & Haller, D. (2021). Microbe-Mucus interface in the pathogenesis of colorectal cancer. *Cancers (Basel)*, 13, 616.

[\[PubMed\]](#)

Colombel, J. F., Grandbastien, B., Gower-Rousseau, C., Plegat, S., Evrard, J. P., Dupas, J. L., Gendre, J. P., Modigliani, R., Belaiche, J., Hostein, J., Hugot, J. P., van Kruiningen, H., & Cortot, A. (1996). Clinical characteristics of Crohn's disease in 72 families. *Gastroenterology*, 111, 604–607.

[\[PubMed\]](#)

Crohn, B. B., Ginzburg, L., & Oppenheimer, G. D. (1932). Regional ileitis: A pathologic and clinical entity. *JAMA*, 99(16), 1323–1329.

Darfeuille-Michaud, A. (2002). Adherent-invasive Escherichia coli: A putative new E. coli pathotype associated with Crohn's disease. *International Journal of Medical Microbiology*, 292, 185–193.

[\[PubMed\]](#)

Desai, M. S., Seekatz, A. M., Koropatkin, N. M., Kamada, N., Hickey, C. A., Wolter, M., Pudlo, N. A., Kitamoto, S., Terrapon, N., Muller, A., Young, V. B., Henrissat, B., Wilmes, P., Stappenbeck, T. S., Nunez, G., & Martens, E. C. (2016). A dietary fiber-deprived gut microbiota degrades the Colonic Mucus barrier and enhances pathogen susceptibility. *Cell*, 167, 1339–53 e21.

D'Haens, G. R., Geboes, K., Peeters, M., Baert, F., Penninckx, F., & Rutgeerts, P. (1998). Early lesions of recurrent Crohn's disease caused by infusion of intestinal contents in excluded ileum. *The Gastroenterologist*, 114, 262–267.

D'Haens, G., Panaccione, R., Baert, F., Bossuyt, P., Colombel, J. F., Danese, S., Dubinsky, M., Feagan, B. G., Hisamatsu, T., Lim, A., Lindsay, J. O., Loftus, E. V., Jr., Panes, J., Peyrin-Biroulet, L., Ran, Z., Rubin, D. T., Sandborn, W. J., Schreiber, S., Neimark, E., Song, A., Kligys, K., Pang, Y., Pivorunas, V., Berg, S., Duan, W. R., Huang, B., Kalabic, J., Liao, X., Robinson, A., Wallace, K., & Ferrante, M. (2022). Risankizumab as induction therapy for Crohn's disease: Results from the phase 3 ADVANCE and MOTIVATE induction trials. *Lancet*, 399, 2015–2030.

[\[PubMed\]](#)

D'Haens, G., Dubinsky, M., Kobayashi, T., Irving, P. M., Howaldt, S., Pokrotnieks, J., Krueger, K., Laskowski, J., Li, X., Lissos, T., Milata, J., Morris, N., Arora, V., Milch, C., Sandborn, W., Sands, B. E., & Lucent Study Group. (2023). Mirikizumab as induction and maintenance therapy for ulcerative colitis. *The New England Journal of Medicine*, 388, 2444–2455.

[\[PubMed\]](#)

Duerr, R. H., Taylor, K. D., Brant, S. R., Rioux, J. D., Silverberg, M. S., Daly, M. J., Steinhart, A. H., Abraham, C., Regueiro, M., Griffiths, A., Dassopoulos, T., Bitton, A., Yang, H., Targan, S., Datta, L. W., Kistner, E. O., Schumm, L. P., Lee, A. T., Gregersen, P. K., Barmada, M. M., Rotter, J. I., Nicolae, D. L., & Cho, J. H. (2006). A genome-wide association study identifies IL23R as an inflammatory bowel disease gene. *Science*, 314, 1461–1463.

[\[PubMed\]](#)

El Hage Chehade, N., Ghoneim, S., Shah, S., Chahine, A., Mourad, F. H., Francis, F. F., Binion, D. G., Farraye, F. A., & Hashash, J. G. (2023). Efficacy of fecal microbiota transplantation in the treatment of active ulcerative colitis: A systematic review and meta-analysis of double-blind randomized controlled trials. *Inflammatory Bowel Diseases*, 29, 808–817.

[[PubMed](#)]

Federici, S., Kredo-Russo, S., Valdes-Mas, R., Kviatcovsky, D., Weinstock, E., Matiuhin, Y., Silberberg, Y., Atarashi, K., Furuichi, M., Oka, A., Liu, B., Fibelman, M., Weiner, I. N., Khabra, E., Cullin, N., Ben-Yishai, N., Inbar, D., Ben-David, H., Nicenboim, J., Kowalsman, N., Lieb, W., Kario, E., Cohen, T., Geffen, Y. F., Zelcbuch, L., Cohen, A., Rappo, U., Gahali-Sass, I., Golembo, M., Lev, V., Dori-Bachash, M., Shapiro, H., Moresi, C., Cuevas-Sierra, A., Mohapatra, G., Kern, L., Zheng, D., Nobs, S. P., Suez, J., Stettner, N., Harmelin, A., Zak, N., Puttagunta, S., Bassan, M., Honda, K., Sokol, H., Bang, C., Franke, A., Schramm, C., Maharshak, N., Sartor, R. B., Sorek, R., & Elinav, E. (2022). Targeted suppression of human IBD-associated gut microbiota commensals by phage consortia for treatment of intestinal inflammation. *Cell*, 185, 2879–98 e24.

Frank, D. N., St Amand, A. L., Feldman, R. A., Boedeker, E. C., Harpaz, N., & Pace, N. R. (2007). Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases. *Proceedings of the National Academy of Sciences of the United States of America*, 104, 13780–13785.

[[PubMed](#)]

Gacesa, R., Kurilshikov, A., Vich Vila, A., Sinha, T., Klaassen, M. A. Y., Bolte, L. A., Andreu-Sanchez, S., Chen, L., Collij, V., Hu, S., Dekens, J. A. M., Lenters, V. C., Bjork, J. R., Swarte, J. C., Swertz, M. A., Jansen, B. H., Gelderloos-Arends, J., Jankipersadsing, S., Hofker, M., Vermeulen, R. C. H., Sanna, S., Harmsen, H. J. M., Wijmenga, C., Fu, J., Zhernakova, A., & Weersma, R. K. (2022). Environmental factors shaping the gut microbiome in a Dutch population. *Nature*, 604, 732–739.

[<https://doi.org/10.1038/s41586-022-04567-7>][[PubMed](#)]

Gevers, D., Kugathasan, S., Denson, L. A., Vazquez-Baeza, Y., Van Treuren, W., Ren, B., Schwager, E., Knights, D., Song, S. J., Yassour, M., Morgan, X. C., Kostic, A. D., Luo, C., Gonzalez, A., McDonald, D., Haberman, Y., Walters, T., Baker, S., Rosh, J., Stephens, M., Heyman, M., Markowitz, J., Baldassano, R., Griffiths, A., Sylvester, F., Mack, D., Kim, S., Crandall, W., Hyams, J., Huttenhower, C., Knight, R., & Xavier, R. J. (2014). The treatment-naïve microbiome in new-onset Crohn's disease. *Cell Host & Microbe*, 15, 382–392.

Gibson, G., Rioux, J. D., Cho, J. H., Haritunians, T., Thoutam, A., Abreu, M. T., Brant, S. R., Kugathasan, S., McCauley, J. L., Silverberg, M., & McGovern, D. (2025). Eleven grand challenges for inflammatory bowel disease genetics and genomics. *Inflammatory Bowel Diseases*, 31, 272–284.

[[PubMed](#)]

Gunther, C., Martini, E., Wittkopf, N., Amann, K., Weigmann, B., Neumann, H., Waldner, M. J., Hedrick, S. M., Tenzer, S., Neurath, M. F., & Becker, C. (2011). Caspase-8 regulates TNF-alpha-induced epithelial necroptosis and terminal ileitis. *Nature*, 477, 335–339.

[<https://doi.org/10.1038/nature10400>][[PubMed](#)]

Haberman, Y., Karns, R., Dexheimer, P. J., Schirmer, M., Somekh, J., Jurickova, I., Braun, T., Novak, E., Bauman, L., Collins, M. H., Mo, A., Rosen, M. J., Bonkowski, E., Gotman, N., Marquis, A., Nistel, M.,

Rufo, P. A., Baker, S. S., Sauer, C. G., Markowitz, J., Pfefferkorn, M. D., Rosh, J. R., Boyle, B. M., Mack, D. R., Baldassano, R. N., Shah, S., Leleiko, N. S., Heyman, M. B., Griffiths, A. M., Patel, A. S., Noe, J. D., Aronow, B. J., Kugathasan, S., Walters, T. D., Gibson, G., Thomas, S. D., Mollen, K., Shen-Orr, S., Huttenhower, C., Xavier, R. J., Hyams, J. S., & Denson, L. A. (2019). Ulcerative colitis mucosal transcriptomes reveal mitochondriopathy and personalized mechanisms underlying disease severity and treatment response. *Nature Communications*, 10, 38.

[\[PubMed\]](#)

Häcker, D., Siebert, K., Smith, B. J., Köhler, N., Riva, A., Mahapatra, A., Heimes, H., Nie, J., Metwaly, A., Hölz, H., Manz, Q., De Zen, F., Heetmeyer, J., Socas, K., Le Thi, G., Meng, C., Kleigrew, K., Pauling, J. K., Neuhaus, K., List, M., Pollard, K. S., Schwerd, T., & Haller, D. (2024). Exclusive enteral nutrition initiates individual protective microbiome changes to induce remission in pediatric Crohn's disease. *Cell Host and Microbe*, 32, 2019–34.e8.

[\[PubMed\]](#)

Haller, D., Blum, S., Bode, C., Hammes, W. P., & Schiffrin, E. J. (2000). Activation of human peripheral blood mononuclear cells by nonpathogenic bacteria in vitro: Evidence of NK cells as primary targets. *Infection and Immunity*, 68, 752–759.

[\[PubMed\]](#)

Haller, D., Russo, M. P., Sartor, R. B., & Jobin, C. (2002). IKK beta and phosphatidylinositol 3-kinase/Akt participate in non-pathogenic Gram-negative enteric bacteria-induced RelA phosphorylation and NF-kappa B activation in both primary and intestinal epithelial cell lines. *The Journal of Biological Chemistry*, 277, 38168–38178.

[\[PubMed\]](#)

Hansson, G. C. (2020). Mucins and the microbiome. *Annual Review of Biochemistry*, 89, 769–793.

[\[PubMed\]](#)

Haque, P. S., Kapur, N., Barrett, T. A., & Theiss, A. L. (2024). Mitochondrial function and gastrointestinal diseases. *Nature Reviews. Gastroenterology & Hepatology*, 21, 537–555.

Hayes, P., Dhillon, S., O'Neill, K., Thoeni, C., Hui, K. Y., Elkadri, A., Guo, C. H., Kovacic, L., Aviello, G., Alvarez, L. A., Griffiths, A. M., Snapper, S. B., Brant, S. R., Doroshov, J. H., Silverberg, M. S., Peter, I., McGovern, D. P., Cho, J., Brumell, J. H., Uhlig, H. H., Bourke, B., Muise, A. A., & Knaus, U. G. (2015). Defects in NADPH oxidase genes NOX1 and DUOX2 in very early onset inflammatory bowel disease. *Cellular and Molecular Gastroenterology and Hepatology*, 1, 489–502.

[\[PubMed\]](#)

Heppner, N., Reitmeier, S., Heddes, M., Merino, M. V., Schwartz, L., Dietrich, A., List, M., Gigl, M., Meng, C., van der Veen, D. R., Schirmer, M., Kleigrew, K., Omer, H., Kiessling, S., & Haller, D. (2024). Diurnal rhythmicity of infant fecal microbiota and metabolites: A randomized controlled interventional trial with infant formula. *Cell Host and Microbe*, 32, 573–87.e5.

[\[PubMed\]](#)

Hoelz, H., Heetmeyer, J., Tsakmaklis, A., Hiergeist, A., Siebert, K., De Zen, F., Hacker, D., Metwaly, A., Neuhaus, K., Gessner, A., Mjgt Vehreschild, D., & Haller, and T. Schwerd. (2023). Is autologous fecal microbiota transfer after exclusive enteral nutrition in pediatric Crohn's disease patients rational and feasible? Data from a feasibility test. *Nutrients*, 15, 1742.

[\[PubMed\]](#)

Hörmannspurger, G., Schauback, M., & Haller, D. (2015). Intestinal microbiota in animal models of inflammatory diseases. *ILAR Journal*, 56, 179–191.

[\[PubMed\]](#)

Hueber, W., Sands, B. E., Lewitzky, S., Vandemeulebroecke, M., Reinisch, W., Higgins, P. D., Wehkamp, J., Feagan, B. G., Yao, M. D., Karczewski, M., Karczewski, J., Pezous, N., Bek, S., Bruin, G., Mellgard, B., Berger, C., Londei, M., Bertolino, A. P., Tougas, G., Travis, S. P., & Group Secukinumab in Crohn's Disease Study. (2012). Secukinumab, a human anti-IL-17A monoclonal antibody, for moderate to severe Crohn's disease: Unexpected results of a randomised, double-blind placebo-controlled trial. *Gut*, 61, 1693–1700.

[\[PubMed\]](#)

Hugot, J. P., Chamaillard, M., Zouali, H., Lesage, S., Cezard, J. P., Belaiche, J., Almer, S., Tysk, C., O'Morain, C. A., Gassull, M., Binder, V., Finkel, Y., Cortot, A., Modigliani, R., Laurent-Puig, P., Gower-Rousseau, C., Macry, J., Colombel, J. F., Sahbatou, M., & Thomas, G. (2001). Association of NOD2 leucine-rich repeat variants with susceptibility to Crohn's disease. *Nature*, 411, 599–603.

[\[https://doi.org/10.1038/35079107\]](https://doi.org/10.1038/35079107) [\[PubMed\]](#)

Jackson, D. N., & Theiss, A. L. (2020). Gut bacteria signaling to mitochondria in intestinal inflammation and cancer. *Gut Microbes*, 11, 285–304.

[\[PubMed\]](#)

Jackson, D. N., Panopoulos, M., Neumann, W. L., Turner, K., Cantarel, B. L., Thompson-Snipes, L., Dassopoulos, T., Feagins, L. A., Souza, R. F., Mills, J. C., Blumberg, R. S., Venuprasad, K., Thompson, W. E., & Theiss, A. L. (2020). Mitochondrial dysfunction during loss of prohibitin 1 triggers Paneth cell defects and ileitis. *Gut*, 69, 1928–1938.

[\[PubMed\]](#)

Johansson, M. E., Gustafsson, J. K., Holmen-Larsson, J., Jabbar, K. S., Xia, L., Xu, H., Ghishan, F. K., Carvalho, F. A., Gewirtz, A. T., Sjövall, H., & Hansson, G. C. (2014). Bacteria penetrate the normally impenetrable inner colon mucus layer in both murine colitis models and patients with ulcerative colitis. *Gut*, 63, 281–291.

[\[PubMed\]](#)

Joossens, M., Huys, G., Cnockaert, M., De Preter, V., Verbeke, K., Rutgeerts, P., Vandamme, P., & Vermeire, S. (2011). Dysbiosis of the faecal microbiota in patients with Crohn's disease and their unaffected relatives. *Gut*, 60, 631–637.

[\[PubMed\]](#)

Jostins, L., Ripke, S., Weersma, R. K., Duerr, R. H., McGovern, D. P., Hui, K. Y., Lee, J. C., Schumm, L. P., Sharma, Y., Anderson, C. A., Essers, J., Mitrovic, M., Ning, K., Cleynen, I., Theatre, E., Spain, S. L., Raychaudhuri, S., Goyette, P., Wei, Z., Abraham, C., Achkar, J. P., Ahmad, T., Amininejad, L., Ananthakrishnan, A. N., Andersen, V., Andrews, J. M., Baidoo, L., Balschun, T., Bampton, P. A., Bitton, A., Boucher, G., Brand, S., Buning, C., Cohain, A., Cichon, S., D'Amato, M., De Jong, D., Devaney, K. L., Dubinsky, M., Edwards, C., Ellinghaus, D., Ferguson, L. R., Franchimont, D., Fransen, K., Gearry, R., Georges, M., Gieger, C., Glas, J., Haritunians, T., Hart, A., Hawkey, C., Hedl, M., Hu, X., Karlsen, T. H., Kucpinksas, L., Kugathasan, S., Latiano, A., Laukens, D., Lawrance, I. C., Lees, C. W., Louis, E., Mahy,

G., Mansfield, J., Morgan, A. R., Mowat, C., Newman, W., Palmieri, O., Ponsioen, C. Y., Potocnik, U., Prescott, N. J., Regueiro, M., Rotter, J. I., Russell, R. K., Sanderson, J. D., Sans, M., Satsangi, J., Schreiber, S., Simms, L. A., Sventoraityte, J., Targan, S. R., Taylor, K. D., Tremelling, M., Verspaget, H. W., De Vos, M., Wijmenga, C., Wilson, D. C., Winkelmann, J., Xavier, R. J., Zeissig, S., Zhang, B., Zhang, C. K., Zhao, H., I. B. D. Genetics Consortium International, Silverberg, M. S., Annesse, V., Hakonarson, H., Brant, S. R., Radford-Smith, G., Mathew, C. G., Rioux, J. D., Schadt, E. E., Daly, M. J., Franke, A., Parkes, M., Vermeire, S., Barrett, J. C., & Cho, J. H. (2012). Host-microbe interactions have shaped the genetic architecture of inflammatory bowel disease. *Nature*, 491, 119–124.
[\[https://doi.org/10.1038/nature11582\]](https://doi.org/10.1038/nature11582) [\[PubMed\]](#)

Kaplan, G. G. (2015). The global burden of IBD: From 2015 to 2025. *Nature Reviews. Gastroenterology & Hepatology*, 12, 720–727.

Kaplan, G. G., & Windsor, J. W. (2021). The four epidemiological stages in the global evolution of inflammatory bowel disease. *Nature Reviews. Gastroenterology & Hepatology*, 18, 56–66.

Kelsen, J., & Baldassano, R. N. (2008). Inflammatory bowel disease: The difference between children and adults. *Inflammatory Bowel Diseases*, 14(Suppl 2), S9–11.
[\[PubMed\]](#)

Khaloian, S., Rath, E., Hammoudi, N., Gleisinger, E., Blutke, A., Giesbertz, P., Berger, E., Metwaly, A., Waldschmitt, N., Allez, M., & Haller, D. (2020). Mitochondrial impairment drives intestinal stem cell transition into dysfunctional Paneth cells predicting Crohn's disease recurrence. *Gut*, 69, 1939–1951.
[\[PubMed\]](#)

Khan, R., Kuenzig, M. E., & Benchimol, E. I. (2023). Epidemiology of pediatric inflammatory bowel disease. *Gastroenterology Clinics of North America*, 52, 483–496.
[\[PubMed\]](#)

Kobayashi, K. S., Chamaillard, M., Ogura, Y., Henegariu, O., Inohara, N., Nunez, G., & Flavell, R. A. (2005). Nod2-dependent regulation of innate and adaptive immunity in the intestinal tract. *Science*, 307, 731–734.
[\[PubMed\]](#)

Kuhn, R., Lohler, J., Rennick, D., Rajewsky, K., & Muller, W. (1993). Interleukin-10-deficient mice develop chronic enterocolitis. *Cell*, 75, 263–274.
[\[PubMed\]](#)

Lamas, B., Richard, M. L., Leducq, V., Pham, H. P., Michel, M. L., Da Costa, G., Bridonneau, C., Jegou, S., Hoffmann, T. W., Natividad, J. M., Brot, L., Taleb, S., Couturier-Maillard, A., Nion-Larmurier, I., Merabtene, F., Seksik, P., Bourrier, A., Cosnes, J., Ryffel, B., Beaugerie, L., Launay, J. M., Langella, P., Xavier, R. J., & Sokol, H. (2016). CARD9 impacts colitis by altering gut microbiota metabolism of tryptophan into aryl hydrocarbon receptor ligands. *Nature Medicine*, 22, 598–605.
[\[PubMed\]](#)

Lavelle, A., & Sokol, H. (2020). Gut microbiota-derived metabolites as key actors in inflammatory bowel disease. *Nature Reviews. Gastroenterology & Hepatology*, 17, 223–237.

Lesage, S., Zouali, H., Cezard, J. P., Colombel, J. F., Belaiche, J., Almer, S., Tysk, C., O'Morain, C., Gassull, M., Binder, V., Finkel, Y., Modigliani, R., Gower-Rousseau, C., Macry, J., Merlin, F., Chamaillard, M., Jannot, A. S., Thomas, G., Hugot, J. P., & Epwg-Ibd Group, Epimad Group, and Getaid Group. (2002). CARD15/NOD2 mutational analysis and genotype-phenotype correlation in 612 patients with inflammatory bowel disease. *American Journal of Human Genetics*, 70, 845–857.

[\[PubMed\]](#)

Liu, T. C., & Stappenbeck, T. S. (2016). Genetics and pathogenesis of inflammatory bowel disease. *Annual Review of Pathology*, 11, 127–148.

[\[PubMed\]](#)

Liu, Z., Liu, R., Gao, H., Jung, S., Gao, X., Sun, R., Liu, X., Kim, Y., Lee, H. S., Kawai, Y., Nagasaki, M., Umeno, J., Tokunaga, K., Kinouchi, Y., Masamune, A., Shi, W., Shen, C., Guo, Z., Yuan, K., FinnGen, Consortium International Inflammatory Bowel Disease Genetics, Consortium Chinese Inflammatory Bowel Disease Genetics, Zhu, S., Li, D., Liu, J., Ge, T., Cho, J., Daly, M. J., McGovern, D. P. B., Ye, B. D., Song, K., Kakuta, Y., Li, M., & Huang, H. (2023). Genetic architecture of the inflammatory bowel diseases across East Asian and European ancestries. *Nature Genetics*, 55, 796–806.

[\[PubMed\]](#)

Lloyd-Price, J., Arze, C., Ananthakrishnan, A. N., Schirmer, M., Avila-Pacheco, J., Poon, T. W., Andrews, E., Ajami, N. J., Bonham, K. S., Brislawn, C. J., Casero, D., Courtney, H., Gonzalez, A., Graeber, T. G., Hall, A. B., Lake, K., Landers, C. J., Mallick, H., Plichta, D. R., Prasad, M., Rahnavard, G., Sauk, J., Shungin, D., Vazquez-Baeza, Y., White, R. A., 3rd, Ibdmdb Investigators, J., Braun, L. A., Denson, J. K., Jansson, R., Knight, S., Kugathasan, D. P. B., McGovern, J. F., Petrosino, T. S., Stappenbeck, H. S., Winter, C. B., Clish, E. A., Franzosa, H., Vlamakis, R. J., & Xavier, and C. Huttenhower. (2019). Multi-omics of the gut microbial ecosystem in inflammatory bowel diseases. *Nature*, 569, 655–662.

[\[https://doi.org/10.1038/s41586-019-1237-9\]](https://doi.org/10.1038/s41586-019-1237-9) [\[PubMed\]](#)

Lopera-Maya, E. A., Kurilshikov, A., van der Graaf, A., Hu, S., Andreu-Sanchez, S., Chen, L., Vila, A. V., Gacesa, R., Sinha, T., Collij, V., Klaassen, M. A. Y., Bolte, L. A., Gois, M. F. B., Neerincx, P. B. T., Swertz, M. A., Cohort, S. L. L., Harmsen, H. J. M., Wijmenga, C., Fu, J., Weersma, R. K., Zhernakova, A., & Sanna, S. (2022). Effect of host genetics on the gut microbiome in 7,738 participants of the Dutch Microbiome Project. *Nature Genetics*, 54, 143–151.

[\[PubMed\]](#)

Martens, E. C., Chiang, H. C., & Gordon, J. I. (2008). Mucosal glycan foraging enhances fitness and transmission of a saccharolytic human gut bacterial symbiont. *Cell Host & Microbe*, 4, 447–457.

Metwaly, A., & Haller, D. (2024). TheTNFΔARE model of Crohn's disease-like Ileitis. *Inflammatory Bowel Diseases*, 30, 132–145.

[\[PubMed\]](#)

Metwaly, A., Dunkel, A., Waldschmitt, N., Raj, A. C. D., Lagkouvardos, I., Corraliza, A. M., Mayorgas, A., Martinez-Medina, M., Reiter, S., Schlöter, M., Hofmann, T., Allez, M., Panes, J., Salas, A., & Haller, D. (2020). Integrated microbiota and metabolite profiles link Crohn's disease to sulfur metabolism. *Nature Communications*, 11, 4322.

[\[PubMed\]](#)

Metwaly, A., Reitmeier, S., & Haller, D. (2022). Microbiome risk profiles as biomarkers for inflammatory and metabolic disorders. *Nature Reviews Gastroenterology and Hepatology*, 19, 383–397.

[\[PubMed\]](#)

Metwaly, A., Jovic, J., Waldschmitt, N., Khaloian, S., Heimes, H., Häcker, D., Ahmed, M., Hammoudi, N., Le Bourhis, L., Mayorgas, A., Siebert, K., Basic, M., Schwerd, T., Allez, M., Panes, J., Salas, A., Bleich, A., Zeissig, S., Schnupf, P., Cominelli, F., & Haller, D. (2023). Diet prevents the expansion of segmented filamentous bacteria and ileo-colonic inflammation in a model of Crohn's disease. *Microbiome*, 11, 66.

[\[https://doi.org/10.1186/s40168-023-01508-y\]](https://doi.org/10.1186/s40168-023-01508-y) [\[PubMed\]](#)

Mizoguchi, A., Takeuchi, T., Himuro, H., Okada, T., & Mizoguchi, E. (2016). Genetically engineered mouse models for studying inflammatory bowel disease. *The Journal of Pathology*, 238, 205–219.

[\[PubMed\]](#)

Moayyedi, P., Surette, M. G., Kim, P. T., Libertucci, J., Wolfe, M., Onischi, C., Armstrong, D., Marshall, J. K., Kassam, Z., Reinisch, W., & Lee, C. H. (2015). Fecal microbiota transplantation induces remission in patients with active ulcerative colitis in a randomized, controlled trial. *Gastroenterology*, 149, 102.

[\[PubMed\]](#)

Mohamadzadeh, M., Pfeiler, E. A., Brown, J. B., Zadeh, M., Gramarossa, M., Managlia, E., Bere, P., Sarraj, B., Khan, M. W., Pakanati, K. C., Ansari, M. J., O'Flaherty, S., Barrett, T., & Klaenhammer, T. R. (2011). Regulation of induced colonic inflammation by *Lactobacillus acidophilus* deficient in lipoteichoic acid. *Proceedings of the National Academy of Sciences of the United States of America*, 108(Suppl 1), 4623–4630.

[\[PubMed\]](#)

Nenci, A., Becker, C., Wullaert, A., Gareus, R., van Loo, G., Danese, S., Huth, M., Nikolaev, A., Neufert, C., Madison, B., Gumucio, D., Neurath, M. F., & Pasparakis, M. (2007). Epithelial NEMO links innate immunity to chronic intestinal inflammation. *Nature*, 446, 557–561.

[\[https://doi.org/10.1038/nature05698\]](https://doi.org/10.1038/nature05698) [\[PubMed\]](#)

Neurath, M. F. (2024). Strategies for targeting cytokines in inflammatory bowel disease. *Nature Reviews. Immunology*, 24, 559–576.

[\[PubMed\]](#)

Neurath, M. F., Fuss, I., Kelsall, B. L., Stuber, E., & Strober, W. (1995). Antibodies to interleukin 12 abrogate established experimental colitis in mice. *The Journal of Experimental Medicine*, 182, 1281–1290.

[\[PubMed\]](#)

Ocvirk, S., Sava, I. G., Lengfelder, I., Lagkouvardos, I., Steck, N., Roh, J. H., Tchaptchet, S., Bao, Y., Hansen, J. J., Huebner, J., Carroll, I. M., Murray, B. E., Sartor, R. B., & Haller, D. (2015). Surface-associated lipoproteins link *Enterococcus Faecalis* Virulence to Colitogenic activity in IL-10-deficient mice independent of their expression levels. *PLoS Pathogens*, 11, e1004911.

[\[PubMed\]](#)

Ogura, Y., Bonen, D. K., Inohara, N., Nicolae, D. L., Chen, F. F., Ramos, R., Britton, H., Moran, T., Karaliuskas, R., Duerr, R. H., Achkar, J. P., Brant, S. R., Bayless, T. M., Kirschner, B. S., Hanauer, S. B., Nunez, G., & Cho, J. H. (2001). A frameshift mutation in NOD2 associated with susceptibility to Crohn's disease. *Nature*, *411*, 603–606.

[<https://doi.org/10.1038/35079114>][[PubMed](#)]

Okou, D. T., Mondal, K., Faubion, W. A., Kobrynski, L. J., Denson, L. A., Mulle, J. G., Ramachandran, D., Xiong, Y., Svingen, P., Patel, V., Bose, P., Waters, J. P., Prahalad, S., Cutler, D. J., Zwick, M. E., & Kugathasan, S. (2014). Exome sequencing identifies a novel FOXP3 mutation in a 2-generation family with inflammatory bowel disease. *Journal of Pediatric Gastroenterology and Nutrition*, *58*, 561–568.

[[PubMed](#)]

Orholm, M., Munkholm, P., Langholz, E., Nielsen, O. H., Sorensen, T. I., & Binder, V. (1991). Familial occurrence of inflammatory bowel disease. *The New England Journal of Medicine*, *324*, 84–88.

[[PubMed](#)]

Paramsothy, S., Kamm, M. A., Kaakoush, N. O., Walsh, A. J., van den Bogaerde, J., Samuel, D., Leong, R. W. L., Connor, S., Ng, W., Paramsothy, R., Xuan, W., Lin, E., Mitchell, H. M., & Borody, T. J. (2017). Multidonor intensive faecal microbiota transplantation for active ulcerative colitis: A randomised placebo-controlled trial. *Lancet*, *389*, 1218–1228.

[[PubMed](#)]

Paulson, M. (1928). Chronic ulcerative colitis with reference to a bacterial etiology. Experimental studies. *Archives of Internal Medicine*, *41*(1), 75–96.

Pazmandi, J., Kalinichenko, A., Ardy, R. C., & Boztug, K. (2019). Early-onset inflammatory bowel disease as a model disease to identify key regulators of immune homeostasis mechanisms.

Immunological Reviews, *287*, 162–185.

[[PubMed](#)]

Petnicki-Ocwieja, T., Hrnčir, T., Liu, Y. J., Biswas, A., Hudcovic, T., Tlaskalova-Hogenova, H., & Kobayashi, K. S. (2009). Nod2 is required for the regulation of commensal microbiota in the intestine. *Proceedings of the National Academy of Sciences of the United States of America*, *106*, 15813–15818.

[[PubMed](#)]

Rakoff-Nahoum, S., & Medzhitov, R. (2007). Regulation of spontaneous intestinal tumorigenesis through the adaptor protein MyD88. *Science*, *317*, 124–127.

[[PubMed](#)]

Rakoff-Nahoum, S., Paglino, J., Eslami-Varzaneh, F., Edberg, S., & Medzhitov, R. (2004). Recognition of commensal microflora by toll-like receptors is required for intestinal homeostasis. *Cell*, *118*, 229–241.

[[PubMed](#)]

Rath, E., & Haller, D. (2022). Intestinal epithelial cell metabolism at the interface of microbial dysbiosis and tissue injury. *Mucosal Immunology*, *15*, 595–604.

[[PubMed](#)]

Rath, H. C., Herfarth, H. H., Ikeda, J. S., Grenther, W. B., Hamm, T. E., Jr., Balish, E., Taurog, J. D., Hammer, R. E., Wilson, K. H., & Sartor, R. B. (1996). Normal luminal bacteria, especially *Bacteroides* species, mediate chronic colitis, gastritis, and arthritis in HLA-B27/human beta2 microglobulin transgenic rats. *The Journal of Clinical Investigation*, 98, 945–953.

[\[PubMed\]](#)

Rath, E., Moschetta, A., & Haller, D. (2018). Mitochondrial function — Gatekeeper of intestinal epithelial cell homeostasis. *Nature Reviews Gastroenterology and Hepatology*, 15, 497–516.

[\[PubMed\]](#)

Raygoza Garay, J. A., Turpin, W., Lee, S. H., Smith, M. I., Goethel, A., Griffiths, A. M., Moayyedi, P., Espin-Garcia, O., Abreu, M., Aumais, G. L., Bernstein, C. N., Biron, I. A., Cino, M., Deslandres, C., Dotan, I., El-Matary, W., Feagan, B., Guttman, D. S., Huynh, H., Dieleman, L. A., Hyams, J. S., Jacobson, K., Mack, D., Marshall, J. K., Otley, A., Panaccione, R., Ropeleski, M., Silverberg, M. S., Steinhart, A. H., Turner, D., Yerushalmi, B., Paterson, A. D., Xu, W., & Ccc Gem Project Research Consortium, and K. Croitoru. (2023). Gut microbiome composition is associated with future onset of Crohn's disease in healthy first-degree relatives. *Gastroenterology*, 165, 670–681.

[\[PubMed\]](#)

Rehaume, L. M., Mondot, S., Aguirre de Carcer, D., Velasco, J., Benham, H., Hasnain, S. Z., Bowman, J., Ruutu, M., Hansbro, P. M., McGuckin, M. A., Morrison, M., & Thomas, R. (2014). ZAP-70 genotype disrupts the relationship between microbiota and host, leading to spondyloarthritis and ileitis in SKG mice. *Arthritis & Rheumatology*, 66, 2780–2792.

Rehman, A., Rausch, P., Wang, J., Skieceviciene, J., Kiudelis, G., Bhagalia, K., Amarapurkar, D., Kupcinskis, L., Schreiber, S., Rosenstiel, P., Baines, J. F., & Ott, S. (2016). Geographical patterns of the standing and active human gut microbiome in health and IBD. *Gut*, 65, 238–248.

[\[PubMed\]](#)

Renz, H., Von Mutius, E., Brandtzaeg, P., Cookson, W. O., Autenrieth, I. B., & Haller, D. (2011). Gene-environment interactions in chronic inflammatory disease. *Nature Immunology*, 12, 273–277.

[\[PubMed\]](#)

Roediger, W. E. (1980). The colonic epithelium in ulcerative colitis: An energy-deficiency disease? *Lancet*, 2, 712–715.

[\[PubMed\]](#)

Roediger, W. E., & Nance, S. (1986). Metabolic induction of experimental ulcerative colitis by inhibition of fatty acid oxidation. *British Journal of Experimental Pathology*, 67, 773–782.

[\[PubMed\]](#)

Rojas-Tapias, D. F., Brown, E. M., Temple, E. R., Onyekaba, M. A., Mohamed, A. M. T., Duncan, K., Schirmer, M., Walker, R. L., Mayassi, T., Pierce, K. A., Avila-Pacheco, J., Clish, C. B., Vlamakis, H., & Xavier, R. J. (2022). Inflammation-associated nitrate facilitates ectopic colonization of oral bacterium *Veillonella parvula* in the intestine. *Nature Microbiology*, 7, 1673–1685.

[\[PubMed\]](#)

Rossen, N. G., Fuentes, S., van der Spek, M. J., Tijssen, J. G., Hartman, J. H., Duflou, A., Lowenberg, M.,

van den Brink, G. R., Mathus-Vliegen, E. M., de Vos, W. M., Zoetendal, E. G., D'Haens, G. R., & Ponsioen, C. Y. (2015). Findings from a randomized controlled trial of fecal transplantation for patients with ulcerative colitis. *Gastroenterology*, 149, 110–118 e4.

Rudbaek, J. J., Agrawal, M., Torres, J., Mehandru, S., Colombel, J. F., & Jess, T. (2024). Deciphering the different phases of preclinical inflammatory bowel disease. *Nature Reviews. Gastroenterology & Hepatology*, 21, 86–100.

Rutgeerts, P., Goboos, K., Peeters, M., Hiele, M., Penninckx, F., Aerts, R., Kerremans, R., & Vantrappen, G. (1991). Effect of faecal stream diversion on recurrence of Crohn's disease in the neoterminal ileum. *Lancet*, 338, 771–774.

[\[PubMed\]](#)

Rutgeerts, P., Sandborn, W. J., Feagan, B. G., Reinisch, W., Olson, A., Johanns, J., Travers, S., Rachmilewitz, D., Hanauer, S. B., Lichtenstein, G. R., de Villiers, W. J., Present, D., Sands, B. E., Colombel, J. F. (2005). Infliximab for induction and maintenance therapy for ulcerative colitis. *New England Journal of Medicine*, 353(23), 2462–2476.

Sakuraba, A., Sato, T., Kamada, N., Kitazume, M., Sugita, A., & Hibi, T. (2009). Th1/Th17 immune response is induced by mesenteric lymph node dendritic cells in Crohn's disease. *Gastroenterology*, 137, 1736–1745.

[\[PubMed\]](#)

Schaubek, M., Clavel, T., Calasan, J., Lagkouravdos, I., Haange, S. B., Jehmlich, N., Basic, M., Dupont, A., Hornef, M., Von Bergen, M., Bleich, A., & Haller, D. (2016). Dysbiotic gut microbiota causes transmissible Crohn's disease-like ileitis independent of failure in antimicrobial defence. *Gut*, 65, 225–237.

[\[PubMed\]](#)

Schirmer, M., Garner, A., Vlamakis, H., & Xavier, R. J. (2019). Microbial genes and pathways in inflammatory bowel disease. *Nature Reviews. Microbiology*, 17, 497–511.

[\[PubMed\]](#)

Sellon, R. K., Tonkonogy, S., Schultz, M., Dieleman, L. A., Grenther, W., Balish, E., Rennick, D. M., & Sartor, R. B. (1998). Resident enteric bacteria are necessary for development of spontaneous colitis and immune system activation in interleukin-10-deficient mice. *Infection and Immunity*, 66, 5224–5231.

[\[PubMed\]](#)

Sender, R., Fuchs, S., & Milo, R. (2016). Are we really vastly outnumbered? Revisiting the ratio of bacterial to host cells in humans. *Cell*, 164, 337–340.

[\[PubMed\]](#)

Smith, S. A., Ogawa, S. A., Chau, L., Whelan, K. A., Hamilton, K. E., Chen, J., Tan, L., Chen, E. Z., Keilbaugh, S., Fogt, F., Bewtra, M., Braun, J., Xavier, R. J., Clish, C. B., Slaff, B., Weljie, A. M., Bushman, F. D., Lewis, J. D., Li, H., Master, S. R., Bennett, M. J., Nakagawa, H., & Wu, G. D. (2021). Mitochondrial dysfunction in inflammatory bowel disease alters intestinal epithelial metabolism of hepatic acylcarnitines. *The Journal of Clinical Investigation*, 131, e133371.

[\[PubMed\]](#)

Sokol, H., Pigneur, B., Watterlot, L., Lakhdari, O., Bermudez-Humaran, L. G., Gratadoux, J. J., Blugeon, S., Bridonneau, C., Furet, J. P., Corthier, G., Grangette, C., Vasquez, N., Pochart, P., Trugnan, G., Thomas, G., Blottiere, H. M., Dore, J., Marteau, P., Seksik, P., & Langella, P. (2008). *Faecalibacterium prausnitzii* is an anti-inflammatory commensal bacterium identified by gut microbiota analysis of Crohn disease patients. *Proceedings of the National Academy of Sciences of the United States of America*, 105, 16731–16736.

[\[PubMed\]](#)

Sorbara, M. T., & Pamer, E. G. (2022). Microbiome-based therapeutics. *Nature Reviews. Microbiology*, 20, 365–380.

[\[PubMed\]](#)

Steck, N., Hoffmann, M., Sava, I. G., Kim, S. C., Hahne, H., Tonkonogy, S. L., Mair, K., Krueger, D., Pruteanu, M., Shanahan, F., Vogelmann, R., Schemann, M., Kuster, B., Sartor, R. B., & Haller, D. (2011). *Enterococcus faecalis* metalloprotease compromises epithelial barrier and contributes to intestinal inflammation. *Gastroenterology*, 141, 959–971.

[\[PubMed\]](#)

Stockinger, B., Shah, K., & Wincent, E. (2021). AHR in the intestinal microenvironment: Safeguarding barrier function. *Nature Reviews. Gastroenterology & Hepatology*, 18, 559.

Stokkers, P. C., Reitsma, P. H., Tytgat, G. N., & van Deventer, S. J. (1999). HLA-DR and -DQ phenotypes in inflammatory bowel disease: A meta-analysis. *Gut*, 45, 395–401.

[\[PubMed\]](#)

Svartz, M. (1968). Treatment of ulcerative colitis with salazopyrin. *International Surgery*, 50(5), 421–427.

Torres, J., Hu, J., Seki, A., Eisele, C., Nair, N., Huang, R., Tarassishin, L., Jharap, B., Cote-Daigneault, J., Mao, Q., Mogno, I., Britton, G. J., Uzzan, M., Chen, C. L., Kornbluth, A., George, J., Legnani, P., Maser, E., Loudon, H., Stone, J., Dubinsky, M., Faith, J. J., Clemente, J. C., Mehandru, S., Colombel, J. F., & Peter, I. (2020). Infants born to mothers with IBD present with altered gut microbiome that transfers abnormalities of the adaptive immune system to germ-free mice. *Gut*, 69, 42–51.

[\[PubMed\]](#)

Truelove, S. C., & Witts, L. J. (1955). Cortisone in ulcerative colitis; final report on a therapeutic trial. *British Medical Journal*, 2, 1041–1048.

[\[PubMed\]](#)

Turpin, W., Lee, S. H., Garay, J. A. R., Madsen, K. L., Meddings, J. B., Bedrani, L., Power, N., Espin-Garcia, O., Xu, W., Smith, M. I., Griffiths, A. M., Moayyedi, P., Turner, D., Seidman, E. G., Steinhart, A. H., Marshall, J. K., Jacobson, K., Mack, D., Huynh, H., Bernstein, C. N., Paterson, A. D., & Crohn's Consortium Colitis Canada Genetic Environmental Microbial Project Research, Ccc Gem Project recruitment site directors include Maria Abreu, and K. Croitoru. (2020). Increased intestinal permeability is associated with later development of Crohn's disease. *Gastroenterology*, 159, 2092–100 e5.

Tysk, C., Lindberg, E., Jarnerot, G., & Floderus-Myrhed, B. (1988). Ulcerative colitis and Crohn's disease in an unselected population of monozygotic and dizygotic twins. A study of heritability

and the influence of smoking. *Gut*, 29, 990–996.

[\[PubMed\]](#)

Urbauer, E., Aguanno, D., Mindermann, N., Omer, H., Metwaly, A., Krammel, T., Faro, T., Remke, M., Reitmeier, S., Bärthel, S., Kersting, J., Huang, Z., Xian, F., Schmidt, M., Saur, D., Huber, S., Stecher, B., List, M., Gómez-Varela, D., Steiger, K., Allez, M., Rath, E., & Haller, D. (2024). Mitochondrial perturbation in the intestine causes microbiota-dependent injury and gene signatures discriminative of inflammatory disease. *Cell Host and Microbe*, 32, 1347–64.e10.

[\[PubMed\]](#)

Van der Sluis, M., De Koning, B. A., De Bruijn, A. C., Velcich, A., Meijerink, J. P., Van Goudoever, J. B., Buller, H. A., Dekker, J., Van Seuningen, I., Renes, I. B., & Einerhand, A. W. (2006). Muc2-deficient mice spontaneously develop colitis, indicating that MUC2 is critical for colonic protection. *Gastroenterology*, 131, 117–129.

[\[PubMed\]](#)

van Dullemen, H. M., van Deventer, S. J., Hommes, D. W., Bijl, H. A., Jansen, J., Tytgat, G. N., Woody, J. (1995). Treatment of Crohn's disease with anti-tumor necrosis factor chimeric monoclonal antibody (cA2). *Gastroenterology*, 109(1), 129–135.

van Munster, K. N., Bergquist, A., & Ponsioen, C. Y. (2024). Inflammatory bowel disease and primary sclerosing cholangitis: One disease or two? *Journal of Hepatology*, 80, 155–168.

[\[PubMed\]](#)

Verhoog, S., Taneri, P. E., Roa Diaz, Z. M., Marques-Vidal, P., Troup, J. P., Bally, L., Franco, O. H., Glisic, M., & Muka, T. (2019). Dietary factors and modulation of bacteria strains of Akkermansia muciniphila and Faecalibacterium prausnitzii: A systematic review. *Nutrients*, 11, 1565.

[\[PubMed\]](#)

Vieujean, S., Caron, B., Jairath, V., Benetos, A., Danese, S., Louis, E., & Peyrin-Biroulet, L. (2022). Is it time to include older adults in inflammatory bowel disease trials? A call for action. *The Lancet Healthy Longevity*, 3, e356–ee66.

[\[PubMed\]](#)

von Boyen, G. B., Steinkamp, M., Geerling, I., Reinshagen, M., Schafer, K. H., Adler, G., & Kirsch, J. (2006). Proinflammatory cytokines induce neurotrophic factor expression in enteric glia: A key to the regulation of epithelial apoptosis in Crohn's disease. *Inflammatory Bowel Diseases*, 12, 346–354.

Von Schillde, M. A., Hörmannspurger, G., Weiher, M., Alpert, C. A., Hahne, H., Bäuerl, C., Van Huynegem, K., Steidler, L., Hrnčir, T., Pérez-Martínez, G., Kuster, B., & Haller, D. (2012). Lactocepin secreted by Lactobacillus exerts anti-inflammatory effects by selectively degrading proinflammatory chemokines. *Cell Host and Microbe*, 11, 387–396.

Wilks, S. (1859). Morbid appearances in the intestines of Miss Banks. *London Medical Gazette*, 2, 264–265.

Willing, B. P., Dicksved, J., Halfvarson, J., Andersson, A. F., Lucio, M., Zheng, Z., Jarnerot, G., Tysk, C., Jansson, J. K., & Engstrand, L. (2010). A pyrosequencing study in twins shows that gastrointestinal

microbial profiles vary with inflammatory bowel disease phenotypes. *Gastroenterology*, 139, 1844–54 e1.

Winogrodzki, T., Metwaly, A., Grodziecki, A., Liang, W., Klinger, B., Flisikowska, T., Fischer, K., Flisikowski, K., Steiger, K., Haller, D., & Schnieke, A. (2023). TNF Δ AREPigs: A translational Crohn's disease model. *Journal of Crohn's and Colitis*, 17, 1128–1138.

[\[PubMed\]](#)

Wirbel, J., Zych, K., Essex, M., Karcher, N., Kartal, E., Salazar, G., Bork, P., Sunagawa, S., & Zeller, G. (2021). Microbiome meta-analysis and cross-disease comparison enabled by the SIAMCAT machine learning toolbox. *Genome Biology*, 22, 93.

[\[PubMed\]](#)

Worthey, E. A., Mayer, A. N., Syverson, G. D., Helbling, D., Bonacci, B. B., Decker, B., Serpe, J. M., Dasu, T., Tschannen, M. R., Veith, R. L., Basehore, M. J., Broeckel, U., Tomita-Mitchell, A., Arca, M. J., Casper, J. T., Margolis, D. A., Bick, D. P., Hessner, M. J., Routes, J. M., Verbsky, J. W., Jacob, H. J., & Dimmock, D. P. (2011). Making a definitive diagnosis: Successful clinical application of whole exome sequencing in a child with intractable inflammatory bowel disease. *Genetics in Medicine*, 13, 255–262.

[\[PubMed\]](#)

Yilmaz, B., Juillerat, P., Oyas, O., Ramon, C., Bravo, F. D., Franc, Y., Fournier, N., Michetti, P., Mueller, C., Geuking, M., Pittet, V. E. H., Maillard, M. H., Rogler, G., I. B. D. Cohort Investigators Swiss, Wiest, R., Stelling, J., & Macpherson, A. J. (2019). Microbial network disturbances in relapsing refractory Crohn's disease. *Nature Medicine*, 25, 323–336.

[\[PubMed\]](#)

Zhang, T., Li, X., Li, J., Sun, F., & Duan, L. (2025). Gut microbiome-targeted therapies as adjuvant treatments in inflammatory bowel diseases: A systematic review and network meta-analysis. *Journal of Gastroenterology and Hepatology*, 40, 78–88.

[\[PubMed\]](#)

Zhou, S., Cui, Y., Zhang, Y., Zhao, T., & Cong, J. (2023). Fecal microbiota transplantation for induction of remission in Crohn's disease: A systematic review and meta-analysis. *International Journal of Colorectal Disease*, 38, 62.

12. Microbiome and Diseases: Allergy

Yacine Amar¹, Valentina Faihs¹, Miriam Hils¹, Martin Köberle¹ and
Tilo Biedermann¹✉

(¹) Department for Dermatology and Allergy, School of Medicine and
Health, Technical University of Munich, Munich, Germany

✉ **Tilo Biedermann**

Email: tilo.biedermann@tum.de

Abstract

The increasing prevalence of allergies and atopic diseases is contrasted by a yet unmet goal of prevention and treatment of allergic diseases. Allergies and atopic diseases develop and manifest in surface organs, which are inhabited by a specific composition of a microbiome. Consequently, the interdependence of microbial colonization of surface organs and the development of allergic/ atopic diseases, orchestrating either immune tolerance or immune hyper-reactivity, is a focus of research. This chapter will focus on what is known about the gut microbiota regulating atopic/allergic diseases and shortly allude to a possible role of microbiomes beyond the gut in regulating these diseases. Three prototypic diseases will be covered: Food allergy, atopic dermatitis, and allergic asthma. As many functional analyses were performed in the murine system, these will be discussed and complemented with studies in humans, in some cases with appropriate interventional trials.

Keywords Asthma – Atopic dermatitis – Food allergy – Skin microbiome – Allergic sensitization – Gut-lung axis

12.1 Introduction

An ongoing increase in the prevalence of allergies and atopic diseases is well documented, and therefore, these diseases are often referred to as the epidemic of developed countries (Platts-Mills, [2015](#)). They are associated with a significant loss of quality of life. Permanent medication and frequent sick leaves burden public health budgets. Preventing the onset or progression of disease is the unmet goal for the treatment of allergic diseases. However, an in-depth understanding of the underlying mechanisms is the basis for the development of novel strategies for prevention and treatment. Of note, allergies and atopic diseases are illnesses that develop and manifest at surface organs. Consequently, among the new frontiers explored to better understand and intervene with allergies and atopic diseases, the role and influence of the composition of the microbiome on different body surfaces is increasingly studied (Belkaid & Harrison, [2017](#)).

Allergic reactions can be sub-grouped into different variants based on the immune cells and the reaction pattern involved (Coombs & Gells, [1963](#)). Environmental allergens can elicit type I allergic reactions, and those will be the focus of this chapter. The majority of the other variants develop in response to drugs, topical medication, and stimuli ranging from cosmetics to occupational substances and are less likely to be fundamentally influenced by microbiomes. Type I allergic reactions develop on the basis of allergen-specific T helper (Th) 2 cells and T follicular helper (Tfh) cells that orchestrate the switch of B cells to produce allergen-specific IgE antibodies (Kay, [2001](#); Akiyama et al., [2022](#)). Allergen-specific antibodies bind to the high-affinity receptor FcεRI on mast cells and basophils. Upon subsequent contact to the allergen, crosslinking of FcεRI-bound IgE by these allergens leads to the so-called immediate or type I immune reaction that is the degranulation of mast cells or basophils (Fig. [12.1](#)). This degranulation sets free various mediators, among them histamine, inducing symptoms within minutes. Typical elicitors of type I allergic reactions are proteins derived from pollen, from house dust mites, or from animal

fur (Traidl-Hoffmann et al., [2009](#)). Importantly, type I allergies can develop in patients with and without atopy. Atopy describes the predisposition to develop atopic diseases, which are, e.g., atopic dermatitis, allergic asthma, allergic rhinoconjunctivitis, and food allergy. Prototypic type I allergies that can be found in patients independent of being atopic are type I allergies to bee or wasp venom. The most severe type I allergic reaction is anaphylaxis (Wölbing & Biedermann, [2013](#)). It can be classified into four levels of severity of symptoms that include urticaria of the skin, asthmatic reactions, diarrhea, and the drop of blood pressure that can be followed by unconsciousness and even cardiac arrest (Ring et al., [2004](#)). Importantly, many of the presentations of the atopic diseases cannot be solely explained by IgE-mediated degranulation of mast cells and basophils, but rather involve the cellular compartment of the adaptive immune system as well as type 2 prone innate immune cells. Especially Th2 cells—together with type 2 innate lymphoid cells (ILC2)—are the basis of cutaneous inflammation in atopic dermatitis and are dominant contributors to inflammation in allergic asthma (Biedermann et al., [2004](#); Cohn et al., [2004](#); Imai et al., [2021](#); Gurram & Zhu, [2019](#)).

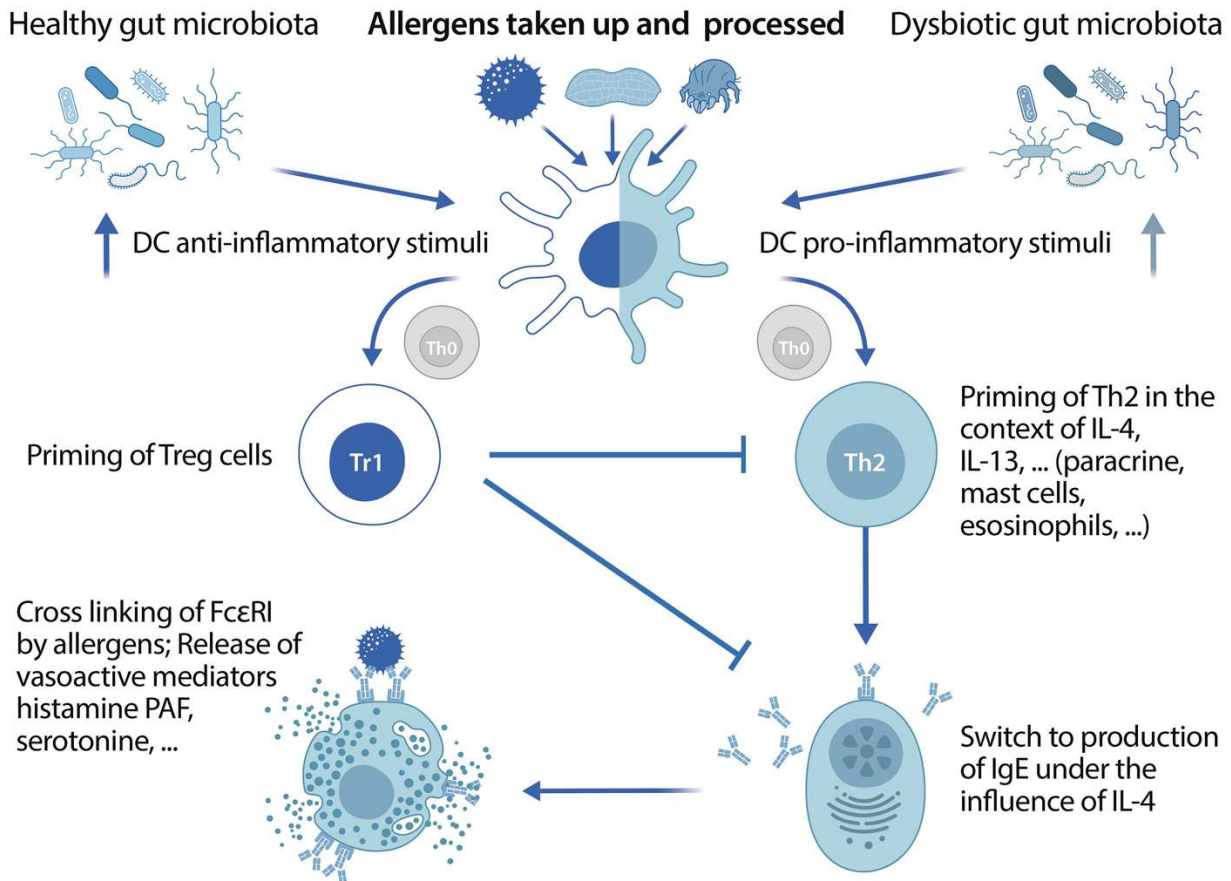


Fig. 12.1 Gut microbiota shape the context of allergen presentation. Gut antigens are presented to T cells by dendritic cells. The microbiota regulate the behavior and immune profile of dendritic cells and thus contribute to skewing the differentiation of naïve Th0 cells primed to gut antigens. The result may be a pro-allergic Th2 or a tolerogenic Treg response with local and possibly systemic immune consequences

It is evident that allergic/atopic inflammatory diseases develop predominantly on surface organs of the body, such as the gut, the skin, and the respiratory system. As these surfaces are inhabited by a specifically composed microbiota, the interdependence of microbial colonization of surface organs and the development of allergic/ atopic diseases has gained tremendous interest (Belkaid & Harrison, [2017](#); Biedermann et al., [2015](#)). Especially the immune system of the gut is acknowledged to be orchestrating tolerance to, e.g., food antigens (Fig. [12.1](#)). Thus, disturbance of the interface between the microbes, allergens such as food antigens, and the intestinal barrier and its immune system is a focus of research to better understand also

systemic immune responses. Today we understand that a similar concept is also functional at the respiratory surfaces and the skin, orchestrating immune tolerance as well as immune responses to antigenic constituents they are exposed to (Fig. 12.2). This chapter will focus on what is known about the gut microbiota regulating atopic/ allergic diseases and shortly allude to a possible role of microbiomes beyond the gut in regulating allergic/ atopic diseases in effector organs (Fig. 12.3). The most obvious interdependence between the intestinal microbiome and allergies may be anticipated for food allergy (Plunkett & Nagler, 2017).

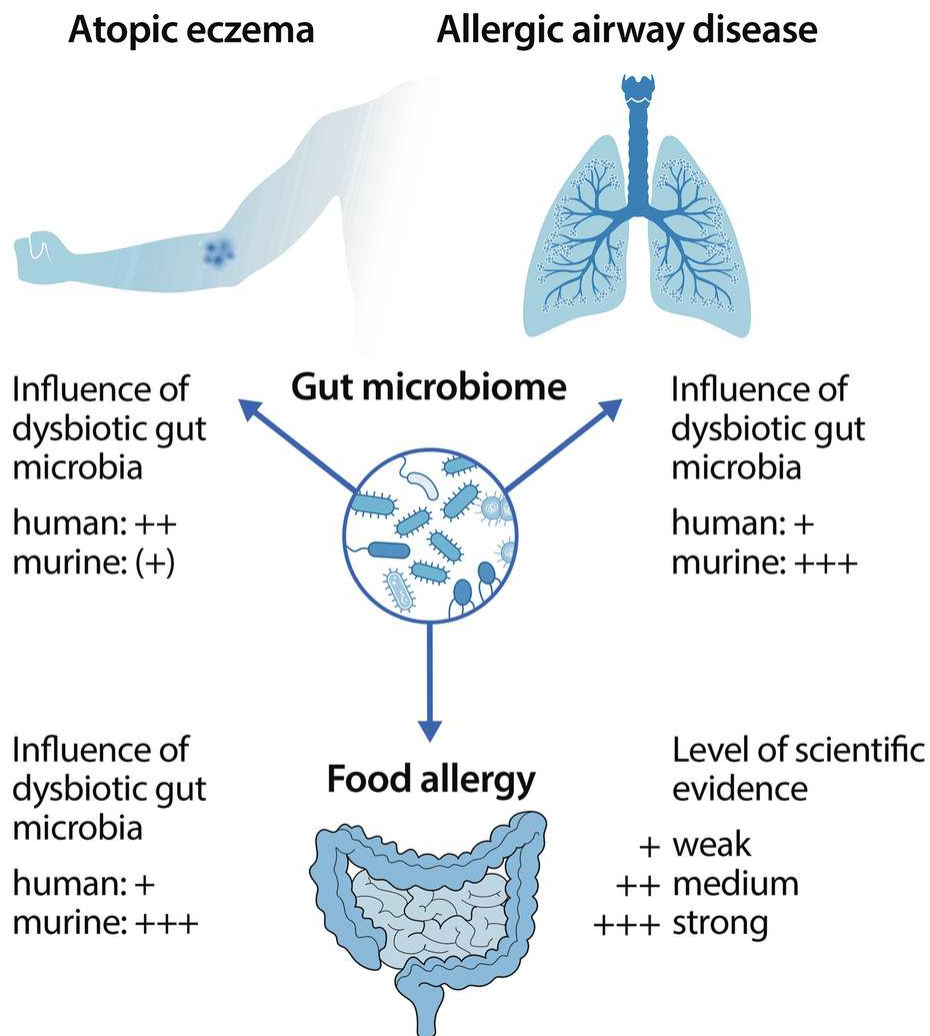


Fig. 12.2 Gut microbiota influence sensitization and allergic diseases at interface organs. The gut microbiota is believed to shape immune responses at interface organs. There is accumulating evidence that dysbiotic gut microbiota can promote sensitization to environmental

antigens and development of allergic/atopic diseases at the skin, the airways and the gut. The level of scientific evidence of these processes varies between the different interface organs and the human and murine immune system and is therefore indicated (+ to +++)

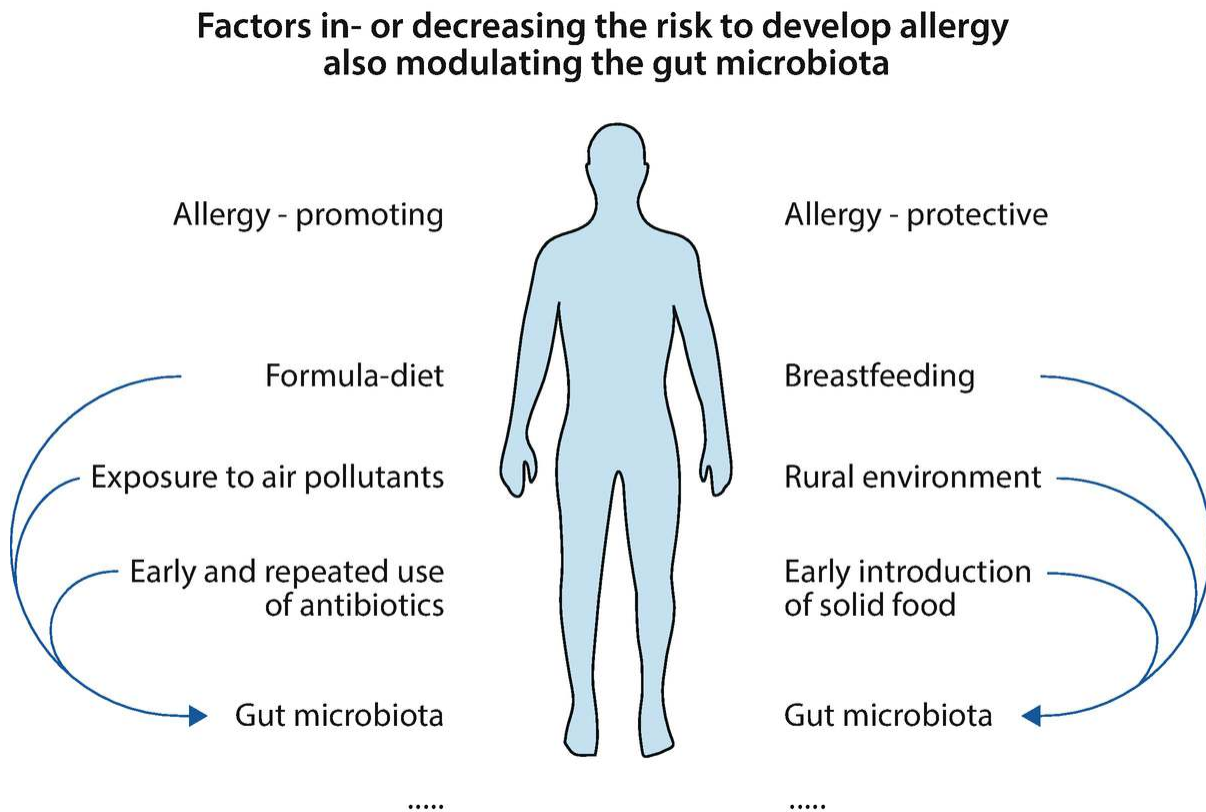


Fig. 12.3 Environmental conditions modulating the risk to develop allergic diseases and their interplay. There is a high evidence level that certain environmental conditions can either increase or decrease the risk to develop allergic diseases. All these factors can also modulate the gut microbiota, which, dependent on its composition, maybe crucially involved in regulating tolerance development. However, general mechanisms still need to be identified

12.2 Gut Microbiome and Food Allergy

12.2.1 Allergies to Food and Tolerance Induction in the Intestine

The incidence of food allergies has substantially increased in the last 2 decades, with up to 8% of children and 10% of adults in westernized countries reported to suffer from adverse reactions to food (Gupta et al., [2018](#), [2019](#)). However, therapeutic options are limited (Sicherer et al., [2020](#)) and emergency department visits caused by food-allergy related anaphylaxis are reported to be approximately 100.000/year in

the USA, demonstrating the socioeconomic and personal burden of this disease (Clark et al., [2011](#)).

Under physiological circumstances, ingested antigens are tolerated by the intestinal immune system, for which the term “oral tolerance” has been coined (Wells, [1911](#); Tordesillas & Berin, [2018](#)). In recent years, it has been demonstrated that food antigens are not neglected by the immune system but rather induce tolerogenic immune responses (Tordesillas & Berin, [2018](#)). In mouse models of food allergy, oral tolerance is achieved by feeding the antigen of interest before sensitization, resulting in the expansion of FoxP3⁺ regulatory T cells (Tregs) and the secretion of immunosuppressive cytokines such as TGFβ in the intestine (Kim et al., [2016](#); Frossard et al., [2004](#)). Differentiation of these intestinal peripheral Tregs (pTregs) is induced by specialized tolerogenic CD103⁺ dendritic cells, which take up antigens in the lamina propria and subsequently migrate to the mesenteric lymph nodes to instruct naïve T cells (Coombes et al., [2007](#); Schulz et al., [2009](#)). In a third step, pTregs home back to the lamina propria and are expanded depending on IL-10-producing CX3CR1⁺ macrophages (Hadis et al., [2011](#)). In addition, other pathways for induction of tolerance in the gut exist, e.g., by induction of regulatory Tr1 cells or regulatory innate lymphoid cells that may team up with FoxP3⁺ pTregs (Weiner et al., [2011](#); Morita et al., [2019](#); Palomares et al., [2021](#)). The breakdown of oral tolerance has been suggested to account for the development of sensitization to food, resulting in food allergy. The detailed mechanisms are unknown yet; however, there is evidence for a breakdown of tolerance via cutaneous sensitization (Suprun et al., [2023](#); Brough et al., [2020](#); Tran et al., [2018](#)). Studies in peanut-allergic cohorts could show that the majority of peanut-specific T cells in the peripheral blood of allergic individuals expressed the skin-homing receptor CLA. These T cells were Th2-skewed, indicating that sensitization to the food antigen is initiated in skin-draining lymph nodes (Weissler et al., [2018](#); Chan et al., [2012](#)). Cutaneous type 2 inflammation even results in alterations in intestinal homeostasis and immunity, the so-called ‘skin-gut axis’, such as the expansion of tuft and

goblet cells, resulting in altered epithelial barrier function, a type 2 environment, and expansion of mast cells (Leyva-Castillo et al., [2019](#); Wang et al., [2023](#)). A crucial role for FoxP3⁺ Tregs in establishing and maintaining oral tolerance was demonstrated in murine models either by depletion of Tregs or by transfer of CD4⁺ CD25⁺ Tregs to sensitized hosts (Curotto de Lafaille et al., [2008](#); Yamashita et al., [2012](#)), and both IgE levels and the susceptibility to allergic diseases have been linked to the presence of Treg cells in mice and humans (Lin et al., [2005](#); Wildin et al., [2002](#)).

12.2.2 Role of Intestinal Microbiota Orchestrating Food Allergy or Tolerance

Functional analyses from murine studies demonstrated that intestinal microbiota induce a subset of regulatory T cells expressing the transcription factor ROR γ t, controlling Th2 responses (Ohnmacht et al., [2015](#); Sefik et al., [2015](#); Abdel-Gadir et al., [2019](#)). As development of the intestinal immune system in early life is paralleled by microbial colonization of the gut, perturbations of the gut microbiota may directly influence the shaping of tolerogenic immune responses, hampering the induction of oral tolerance (Maynard et al., [2012](#); Plunkett & Nagler, [2017](#); Renz et al., [2018](#)). Indeed, intestinal ROR γ t⁺ Tregs are drastically reduced both in germ-free and antibiotic-treated mice and are restored upon recolonization of germ-free mice with microbiota of specific pathogen-free mice, indicating that microbial colonization of the intestine is driving the differentiation of intestinal pTregs (Ohnmacht et al., [2015](#); Geuking et al., [2011](#)). In line with ROR γ t⁺ pTregs playing an essential role in maintaining oral tolerance, mice develop aggravated Th2 and Th17 pathologies in the intestine if this cell type is missing (Ohnmacht et al., [2015](#); Sefik et al., [2015](#)).

Children who develop food allergies during childhood show an altered gut microbiome already in their first months of life (Kalliomaki et al., [2001](#)). In line with an essential role of the microbiome in establishing oral tolerance to food antigens, treatments with broad-spectrum antibiotics in young infants result in an increased risk of

developing food allergies (Kummeling et al., [2007](#); Marra et al., [2009](#)). Using murine models, the critical role of an intact commensal gut microbiome community could be confirmed. An early report could already show that oral administration of the antibiotic kanamycin, targeting mainly Gram-negative bacteria, to 3-week-old mice resulted in increased IgE and IgG1 serum levels as well as predominant production of the Th2 hallmark cytokine IL-4 while suppressing the Th1 cytokine IFN- γ in splenic lymphocytes (Oyama et al., [2001](#)). In line with this finding, mice devoid of TLR4 were shown to be prone to evolving predominant Th2 responses and enhanced IgE levels after intragastral sensitization to peanut extract (Bashir et al., [2004](#)). Depletion of the intestinal microbiota by antibiotics in wild-type animals mimicked the results obtained with TLR4-deficient mice, demonstrating a pivotal role for the gut microbiota in maintaining oral tolerance and inhibiting food allergy (Bashir et al., [2004](#)). Interestingly, the susceptibility to develop food allergy in this model was restricted to specific genetic backgrounds, such as BALB/c mice, whereas C3H or C57Bl/6 mice did not develop anaphylaxis (Berin et al., [2006](#)). More recently, analysis of germ-free mice revealed significantly elevated IgE levels in the absence of commensal microbiota compared to conventionally reared animals (Cahenzli et al., [2013](#); Rodriguez et al., [2011](#); Hill et al., [2012](#)). Moreover, after sensitization, germ-free mice also showed more severe anaphylaxis compared to control animals with normal microbiome (Cahenzli et al., [2013](#); Rodriguez et al., [2011](#); Stefka et al., [2014](#)). Strikingly, administration of a low complexity microbiota harboring only 40 phylotypes completely protected germ-free mice from aberrant IgE production (Cahenzli et al., [2013](#)). These observations clearly highlight the important role of the commensal intestinal microbiota in maintaining oral tolerance and preventing a detrimental type 2 immune response to orally ingested antigens.

Food allergy-prone mice with a gain-of-function mutation in the IL-4 receptor α -chain (*Il4ra*F709) displayed a severely altered microbiome compared to their wild-type littermates (Noval Rivas et al., [2013](#)). A significant decrease in the relative abundance of the bacterial family

Erysipelotrichaceae, which belongs to the *Firmicutes* phylum as well as the *Enterobacteriaceae* family (*Proteobacteria* phylum) was observed in sensitized mice (Noval Rivas et al., [2013](#)). By transfer of the microbiota of *IL4raF709* mice to germ-free mice, food-induced anaphylaxis after oral sensitization could be elicited, demonstrating a crucial role for a dysbiotic microbiota in triggering food allergy.

In line with an increased risk of allergic sensitizations in children with perturbations of the gut microbiome in early life, there is a clear association between the composition of the gut microbiome and sensitization to food-derived allergens. Microbiome composition can even differ between individual food allergies (Fazlollahi et al., [2018a](#)). Comparing the intestinal microbiome of food-allergic and non-allergic children, a small but pioneering study found increased levels of the genera *Clostridium* sensu stricto spp. and *Anaerobacter* spp. and decreased levels of *Bacteroides* and *Clostridium* cluster XVIII in infants with IgE-mediated food allergy (Ling et al., [2014](#)). These findings are of utmost importance as it could be shown that certain *Clostridium* species devoid of toxin expression, including *Clostridium* cluster XVIII species, induce FoxP3⁺ regulatory T cells, thereby dampening IgE production and severity in an ovalbumin-induced allergic diarrhea model (Atarashi et al., [2013](#); Atarashi et al., [2011](#)). These findings even laid the foundation for an ongoing phase I clinical trial for the treatment of peanut allergy using fecal microbiota transplantation (FMT) of defined strains (NCT03936998). Moreover, colonization of germ-free mice with *Clostridia* attenuated anaphylactic reactions in orally sensitized mice, highlighting a specific role for *Clostridia* in establishing oral tolerance and preventing food allergy (Stefka et al., [2014](#)). Two other studies could confirm a beneficial role of *Clostridiales*. FMT of stool from allergic or healthy human donors into GF mice transmitted the respective phenotype upon sensitization with a food allergen (Abdel-Gadir et al., [2019](#); Feehley et al., [2019](#)). Abdel-Gadhir and colleagues further evaluated the microbiota composition of healthy and food-allergic donor infants and found significant differences in 77 OTUs, including defined species of *Clostridiales*. Transfer of seven

defined specimens into IL4raF709 mice was sufficient to either ameliorate or exacerbate food allergy in these mice (Abdel-Gadir et al., [2019](#)). Interestingly, protection from food allergy was associated with an expansion of RORyt⁺ Tregs in the intestine, further demonstrating that this Treg subset is directly affected by the presence and the composition of intestinal microbiota.

A study in infants with food sensitization in early life demonstrated an altered fecal microbiota with lower microbiota diversity compared to healthy controls (Chen et al., [2016](#)). Children with food sensitization had significantly increased numbers of *Sphingomonas* spp., *Sutterella* spp., *Bifidobacterium* spp., *Collinsella* spp., *Clostridium sensu stricto* spp., *Clostridium* IV spp., *Enterococcus* spp., *Lactobacillus* spp., *Roseburia* spp., *Faecalibacterium* spp., *Ruminococcus* spp., *Subdoligranulum* spp. and *Akkermansia* spp., whereas *Bacteroides* spp., *Parabacteroides* spp., *Prevotella* spp., *Alistipes* spp., *Streptococcus* spp., and *Veillonella* spp. were decreased in children with sensitization to food (Chen et al., [2016](#)). Comparability of the results reported by Ling et al. and Chen et al. is limited as the latter reported on a food-sensitized cohort of children on the basis of specific IgE levels. In contrast, Ling et al. investigated infants with challenge-proven food allergy. A specific signature in the microbiome of children with cow's milk allergy during infancy predictive for resolution at the age of 8 years was also reported (Bunyavanich et al., [2016](#)). Resolution of cow's milk allergy until the age of 8 years was associated with an enrichment of *Clostridia* and other *Firmicutes* in the microbiome at the age of 3–6 months (Bunyavanich et al., [2016](#)). Two studies in USA and Japanese infants confirmed an association between early life gut microbiome composition and food allergen sensitization in childhood (Savage et al., [2018](#); Tanaka et al., [2017](#)). Tanaka et al. showed that an underrepresentation of the genera *Leuconostoc*, *Weissella*, and *Veillonella* at age 2 months is connected to the development of self-reported food allergy at age 2 years (Tanaka et al., [2017](#)). A lower relative abundance of *Haemophilus*, *Dialister*, *Dorea*, and *Clostridium* at

age 3 and 6 months was linked to sensitization to at least one food allergen among milk, egg, peanut, soy, or wheat (Savage et al., [2018](#)).

Taken together, results from murine and human investigations show that the development of food allergy is associated with a dysbiotic microbiome. It still needs to be studied in more detail how diets based on food avoidance interfere with these results. In addition, further research is needed to characterize more precisely the impact of specific bacterial species or strains on the initiation or resolution of food allergy.

12.2.3 Prevention and Therapy of Food Allergy by Modulating the Microbiota

Given the association of a dysbiotic gut microbiome with the development of food allergy, probiotic bacteria have been evaluated for therapy or prevention of food allergy (Aitoro et al., [2017](#); du Toit et al., [2016](#)). In a meta-analysis on the beneficial effects of probiotics to prevent food allergy, these were only observed when given pre- and postnatally to mother and child. Intervention limited either to prenatal administration to the pregnant mother or to postnatal application to the child displayed no significant effects (Zhang et al., [2016](#)).

Studies investigating the therapeutic efficacy of probiotics in the resolution of food allergy have obtained conflicting results. Administration of *Lactobacillus casei* CRL 431 and *Bifidobacterium lactis* Bb12 to children with cow's milk allergy failed to induce significantly enhanced allergy resolution and acquisition of oral tolerance to milk (Hol et al., [2008](#)). In contrast, supplementation of a highly hydrolyzed casein formula diet with the popular and well-studied probiotic *Lactobacillus rhamnosus* GG revealed significantly augmented acquisition of tolerance to cow's milk protein after a 12-month period (Berni Canani et al., [2012](#)). In a follow-up study, the same authors showed that feeding the formula diet plus *L. rhamnosus* GG for 3 years could further enhance the incidence of tolerance induction (Berni Canani et al., [2017](#)). In addition, it has been shown that administration of probiotic *Lactobacillus rhamnosus* CGMCC 1.3724

together with peanut in a placebo-controlled randomized clinical trial induced tolerance in >80% of peanut-allergic children (Tang et al., [2015](#)) and 67% of children still tolerated peanut 4 years after treatment cessation, compared to 4% of children only receiving peanut without probiotic as oral immunotherapy (Hsiao et al., [2017](#)). Tolerance was accompanied by a higher IgG4 to IgE ratio and a smaller peanut skin prick test response size in the treatment versus control group. Taken together, prebiotics might be a promising tool to treat or prevent food allergy; however, data is thus far still insufficient to recommend probiotic supplementation for food allergy (Osborn & Sinn, [2007](#); Cuello-Garcia et al., [2015](#); Fox et al., [2019](#); Fiocchi et al., [2015](#)).

Gut microbiome composition can additionally be manipulated by certain metabolites generated by distinct microbes through processing the diet. Short-chain fatty acids (SCFA), including butyrate, which are generated by fermentation of dietary fiber are well-accepted for their potential to promote oral tolerance to food antigens (Kim, [2023](#); Tan et al., [2016](#)), in part by supporting Treg polarization and expansion (Roduit et al., [2019](#); Ohnmacht et al., [2015](#); Tan et al., [2016](#)). Moreover, an abnormal production of SCFA has been reported in children with food allergy or atopic dermatitis (Goldberg et al., [2020](#)). Consumption of fiber-rich food, such as vegetables and fruits, within the first year of life was associated with increased butyrate levels in stool samples and reduced sensitization to food allergens in 3- to 6-year-old children (Roduit et al., [2019](#)). However, so far, no clinical trials of fiber and/or SCFA supplementation for treatment of food allergy have been undertaken (for the role of SCFA in asthma, see 12.5.4).

12.3 Microbiomes Beyond the Gut

12.3.1 The Vaginal Microbiome

The vaginal microbiome is dynamically shaped by gestational status, use of contraceptives, menstrual cycle, and sexual activity. It is typically dominated by one or two of the *Lactobacillus* species *L. iners*, *L. crispatus*, *L. gasseri*, *L. jensenii*, *L. planatum*, or *L. rhamnosus*, but more

diverse communities with higher numbers of anaerobic bacteria also can be found. Vaginal microbial profiles vary between different world regions and probably between ethnic groups. Pregnancy typically results in reduced diversity and *Lactobacilli* dominance, whereas low *Lactobacilli* numbers predispose to infections and excessive inflammation (Ravel et al., [2011](#); Saraf et al., [2021](#)).

Vaginal *Lactobacilli* abundance is postulated to depend on vaginal glycogen production stimulated by estrogen. Since *Lactobacilli* cannot directly metabolize glycogen, it needs to be pre-metabolized by human amylases or other microorganisms (Nunn & Forney, [2016](#)).

The production of lactic acid by vaginal bacteria causes the low vaginal pH. In recent years, attention to the vaginal microbiome was boosted by studies suggesting it had a prominent role in shaping the microbiomes of the prior sterile newborn, but improved evidence suggests that differences in the microbiomes correlating with the mode of delivery may be rather caused by the medical conditions leading to the caesarean section (Chu et al., [2017](#)).

12.3.2 Respiratory Microbiome

Nasal, lung, and sometimes oropharyngeal microbial communities are summarized as the respiratory microbiome. Traditionally, the healthy lung has been regarded as a sterile compartment. But recent vigorously controlled NGS-based approaches identified *Prevotella*, *Streptococcus*, *Veillonella*, *Fusobacterium*, and *Haemophilus* as the main lung bacterial genera (Wypych et al., [2019](#)). Airways are colonized by bacteria immigrating from the upper airways immediately after birth. Lung microbiomes of vaginally born babies are dominated by *Ureaplasmas*, while pre-term babies delivered by cesarean sections start with a Staphylococci-dominated microbiome before microbial diversification begins. Microbes are removed from the lung by phagocytes and mucus clearance. Ineffective microbial clearance, resulting in higher microbial loads, e.g., in cystic fibrosis, leads to more frequent infections (Wypych et al., [2019](#); Natalini et al., [2023](#)).

The nasal microbiome was found to be dominated by the genera *Moraxella*, *Staphylococcus*, *Corynebacterium*, *Haemophilus*, and *Streptococcus* (Natalini et al., [2023](#)). It is mostly determined by the environment, but also by gender and genetics. Nasal colonization by specific taxa, including lactic acid bacteria, can affect *S. aureus* colonization (Liu et al., [2015](#)). Butyric acid-producing bacteria in the nares correlate with decreased olfactory sensitivity (Koskinen et al., [2018](#)).

The respiratory microbiome receives increasing attention in regard to its possible role in the development of atopic asthma, especially in neutrophilic type asthma (Fazlollahi et al., [2018b](#); Natalini et al., [2023](#)) (see also Sect. [12.5](#)).

12.3.3 Skin Microbiome

Our skin harbors an abundant resident microbiota comprising bacteria, viruses, archaea, fungi, and mites that play key roles in human health and disease (Chen et al., [2022](#)). Host factors such as age, gender, ethnicity, and lifestyle, in addition to cutaneous pH, moisture, and sebum secretions, exert a significant impact on the microbiome composition (Oh et al., [2014](#)). Remarkably, the cutaneous microbiome remains relatively stable over time despite the constant environmental perturbations (Zhou et al., [2024](#); Oh et al., [2016](#)). Microbial characterization of skin swabs revealed the presence of four dominant phyla, namely *Actinobacteria*, *Firmicutes*, *Proteobacteria*, and *Bacteroidetes*, and over 200 genera largely represented by *Propionibacterium*, *Corynebacterium*, and *Staphylococcus*. The abundance of each group can fluctuate depending on the ecological characteristics of the anatomical niche (Balato et al., [2019](#)). *Cutibacteria* and *Staphylococci* predominate in sebaceous sites (forehead, chest, and back) while *Corynebacteria* prevail in moist sites (armpit, inner elbow, and inguinal crease), although *Staphylococci* species are also represented. A mixed and more diverse population is observed in dry skin sites (volar forearm, hypothenar palm, and buttock) (Smythe & Wilkinson, [2023](#)). Several inflammatory skin diseases, such as atopic

dermatitis, psoriasis, Darier's disease, polymorphic light eruption, or acne inversa, are characterized by a dysbalanced microbiome, and the correction of this dysbiosis may help to prevent or even treat the disease (Ito & Amagai, [2022](#); Amar et al., [2023](#), [2024](#); Paller et al., [2019](#); Chang et al., [2018](#)).

12.4 Microbiome Shaping of Atopic Dermatitis

12.4.1 Introduction to Atopic Dermatitis

Atopic eczema is the most frequent chronic inflammatory skin condition, affecting 10–20% of children and 2–5% of adults in industrialized countries (Yamazaki et al., [2017](#)). Primarily affected sites are the flexures of the limbs, and the dominant symptoms are highly irritable, itching skin with erythematous-squamous plaques, papulovesicles, and lichenification (Werfel, [2015](#)). Various factors are involved in AD development, mostly attributed to two main mechanisms: (i) a primary imbalance of the immune system leading to IgE sensitization and impairment of the skin barrier, and (ii) defects in the epithelial barrier leading to immune dysregulation and inflammation (Weidinger et al., [2018](#)). This disease is characterized by a type 2 immune response where T helper-2 (Th2) cells and ILC2s expressing IL-4, IL-5, IL-9, and IL-13 cytokines are known to play a key role (Ogulur et al., [2021](#)).

Skin barrier defects in AD can be based on mutations of central proteins of the cutaneous barrier in affected patients. Findings include reduced levels of tight junction protein claudin 1 (Tokumasu et al., [2016](#)), enhanced levels of kallikrein protease, or changes in the composition of lipids (Egawa & Kabashima, [2016](#)). The most prominent genetic predisposition is loss-of-function mutations of the structural protein filaggrin, determining the quality of the outermost layer of the skin, the stratum corneum, representing a key protein of the skin barrier. The aforementioned barrier defects result in increased epidermal water loss and decreased physical protection against microbes (Yoshida et al., [2022](#)). They also reduce protection against sensitization to allergens, leading to a correlation of AD with food

allergies and atopic asthma (atopic march). A significant association between Toll-like receptor-2 (TLR2) gene polymorphisms and AD has been reported, indicating a possible role in compromising the microbial sensing (Zhou et al., [2023](#)).

Skin migration or function of cells of the innate immune system, especially neutrophils, is often impaired, and type 2 cytokines suppress the IL-23-IL-17 axis by type 2 immune responses and/ or microbial dysbiosis (Guenova et al., [2015](#)).

On the interface between skin barrier and skin immunity, antimicrobial peptides (AMPs) play an important role in the early defense of microbial pathogens and the orchestration of the microbiome composition. Lesional skin of AD patients has been shown to express significantly lower amounts of antimicrobial peptides like human β -defensins (hBDs)-2 and 3, LL-37, and dermcidin, possibly due to inhibition by Th2 cytokines, whereas psoriasin and RNase 7 showed a noticeable increase (Suwanchote et al., [2022](#)). Taken together, these alterations associated with AD not only boost allergic inflammation and facilitate sensitization to allergens but also hamper proper antimicrobial immune responses and facilitate microbial dysbiosis.

12.4.2 The Intestinal Microbiome in Atopic Dermatitis

In some older studies, the intestine of atopic infants was found to be colonized less with *Bacteroides* and *Bifidobacterium* species, as well as having a lower ratio of Bifidobacteria to Clostridia when developing AD (Kalliomaki et al., [2001](#); Watanabe et al., [2003](#)). However, many of the subsequent studies examining larger collectives have not been able to fully reproduce these findings (Abrahamsson et al., [2012](#)) or to show alterations of the intestinal microbiome in AD at all (Bisgaard et al., [2011](#); Palmer et al., [2006](#); Adlerberth et al., [2007](#)). Relatively recent systematic reviews also differ in their conclusions. If differences in gut microbiomes are found, the AD microbiomes are often less diverse or contain higher numbers of *Clostridia*. The effectiveness of probiotics is unclear as well (Petersen et al., [2019](#); Liu et al., [2022](#)). Notably, patient collectives differed between these studies, and many included studies

were of limited phylogenetic resolution. Some studies suggest that differences in single bacterial species like *Ruminococcus gnavus* (Lee et al., [2024](#)) or subspecies, e.g., of *Faecalibacterium prausnitzii* may correlate with AD (Song et al., [2016](#)).

Because of the heterogeneous results of human epidemiological studies and the lack of evidence created by mouse model research, it is hard to decide whether risk factors for atopic dermatitis may act via the gut microbiome (Figs. [12.2](#) and [12.3](#)).

12.4.3 The Skin Microbiome in Atopic Dermatitis

An imbalanced skin microbiota can lead to aberrant immune responses that compromise the integrity of the epithelial barrier and allow further penetration of microbial antigens, which can eventually lead to chronic inflammation (Balato et al., [2019](#)). A reduced microbiome diversity and skin colonization by *Staphylococcus aureus* are observed in over 90% of patients with AD (Grice & Segre, [2011](#)). It has also been noticed that other *Staphylococci*, such as *S. epidermidis*, were increased on AD lesions while a decrease was reported for other genera including *Cutibacterium*, *Corynebacterium*, and *Streptococcus* (Kong et al., [2012](#); Gonzalez et al., [2016](#)). These key characteristics of human AD are also present in an oxazolone-treatment mouse model of AD (Amar et al., [2022](#)).

Inflamed AD skin shows reduced quantities of antimicrobial peptides and the epidermal barrier protein filaggrin, which makes it more prone to *S. aureus* colonizations and even infections. It has been shown that this pathogen, penetrating into the epidermis, induces the release of several cytokines such as IL-4, IL-13, and IL-22, and the secretion of serine proteases like Kallikreins, able to degrade filaggrin and desmoglein-1, thus further compromising skin barrier integrity (Nakatsuji et al., [2016](#); Williams et al., [2017](#); Pothmann et al., [2019](#)). Key mechanisms by which *Staphylococci* increase and prolong AD inflammation are thought to be staphylococcal superantigens, host TLR2 and NOD/CARD pathogen sensing, IL-36-driven T-cell activation, and the appearance of IL-17 and associated transcription signatures

(Biedermann, [2006](#); Volz et al., [2010](#); Liu et al., [2017](#); Eyerich et al., [2009](#)). We were able to show that exposure to TLR2 ligands such as those from *Staphylococcus aureus* from the inflamed skin, leads to significant inhibition of the IL-10 response and subsequently to exaggerated and tremendously prolonged cutaneous inflammation, due to Th2 cell activation (Kaesler et al., [2014](#); Volz et al., [2014](#)). The fungal opportunistically pathogenic *Malassezia* species can also contribute to AD inflammation, both by stimulating IgE production and Th2 cells and by delivering ligands for immune cell activation (Glatz et al., [2015](#); Lee et al., [2023b](#)).

Microbial diversity can be effectively reinforced by anti-inflammatory treatment, including IL-4R α blockade by dupilumab or IL-13 neutralization by tralokinumab, but also by treatment with emollients alone (Kong et al., [2012](#); Seite et al., [2014](#); Beck et al., [2023](#); Callewaert et al., [2020](#)). Similar to the intestinal microbiome, grafting beneficial bacteria to the skin could ameliorate AD symptoms. In a pilot study on children with AD, a treatment with *Roseomonas mucosa* improved the disease severity and epithelial barrier function while reducing *Staphylococcus aureus* burden as well as the need for topical steroids (Myles et al., [2020](#)). Similarly, autologously transferred commensal *Staphylococci* decreased *S. aureus* burden by secretion of antimicrobial lantibiotics and other mechanisms (Nakatsuji et al., [2017](#)). However, the phase II placebo-controlled clinical trial (ClinicalTrials.gov ID: NCT04936113) combining three strains of *R. mucosa* for a live therapeutic product (FB-401) in AD patients was terminated, because it failed to demonstrate any significant differences in patients for achieving the primary outcome of reducing the disease score by 50% (EASI50). In addition, for the “targeted investigation of microbiome elimination” trial (TIME-1; NCT05177328), following the pilot studies to reduce *S. aureus* published in 2017 and 2020 (Nakatsuji et al., [2017](#); Myles et al., [2020](#)), enrollment (planned to be at least 20 participants) was terminated early without giving further details. Thus, it has to be acknowledged that AD is not primarily caused by *S. aureus*. Even though initiation of AD can be facilitated by *S. aureus* and AD is

accepted to be amplified and transformed into chronic dermatitis by *S. aureus*, suppression of *S. aureus* alone is not sufficient to treat ongoing cutaneous type 2 inflammation. Other anti-inflammatory or immune modulatory interventions seem to be needed in addition.

In this respect, it is interesting that application of lysates of the nonpathogenic bacterium *Vitreoscilla filiformis* in a cream significantly ameliorated mild atopic dermatitis compared to placebo (Gueniche et al., [2008](#)). These lysates were not selected based on their capability to shape the microbiome but rather to supplement its impact on cutaneous immunity. Indeed, subsequent mechanistic analyses displayed that TLR2 ligands within these lysates upregulate IL-10 production in innate immune cells, such as dendritic cells, and subsequently in adaptive immune cells (Tr1 cells) (Volz et al., [2014](#)). Thus, the loss of IL-10 caused by concomitant signaling through the IL-4 receptor and TLR2 can be reconstituted by appropriate signals from beneficial bacteria. Importantly, targeting type 2 immunity by dupilumab treatment noticeably improved the clinical outcomes in moderate to severe AD by reducing the inflammatory signatures and *S. aureus* loads in the affected skin (Simpson et al., [2023](#); Callewaert et al., [2020](#)).

12.5 Asthma and Gut Microbiota

12.5.1 Introduction to Asthma

Asthma is a chronic inflammatory condition affecting the lower airways, marked by bronchial hyper-responsiveness and variable airflow obstruction (Lommatzsch et al., [2023](#)). It is one of the most relevant chronic diseases worldwide, leading to a high disease burden and considerable economic costs (Accordini et al., [2013](#)). Asthma prevalence surged globally during the twentieth century, particularly among children and adolescents. While its prevalence seems to have stabilized in many Western countries over the last few years, it continues to rise in other parts of the world (Douwes et al., [2011](#)). It can be classified as allergic, non-allergic, or a combination of both and

by severity or by the type of predominant cell types infiltrating the airways as “eosinophilic” or “neutrophilic” asthma.

Allergic asthma, the most common form, is triggered by inhaled allergens that promote Th2-mediated chronic inflammation. The Th2 cytokines IL-4, IL-5, and IL-13 trigger a class switch in plasma cells from the production of allergen-specific IgG to IgE, which binds to FcεRI on effector cells, such as mast cells and basophils, and mediates their activation in case of allergen contact (Fig. [12.1](#)). Recurrent inflammation results in airway remodelling and allergen-independent bronchial hyperreactivity. The onset of allergic asthma arises from a complex interaction between pro-inflammatory factors, particularly Th2-dominant responses, and tolerance-promoting elements, such as regulatory T cells (Tregs). Additionally, local factors, including epithelial barrier integrity and airway remodeling, play a critical role in shaping the disease’s progression.

Although studying the microbiome in the lower airways poses significant methodological challenges, the local microbiome seems to be disturbed in patients suffering from asthma. Lung microbiota showed a shift to *Proteobacteria* in adult as well as childhood asthma (Hilty et al., [2010](#)), especially in patients with neutrophilic asthma, where the abundance of *Moraxella* and *Haemophilus* was increased (Taylor et al., [2018](#)).

12.5.2 The Gut–Lung Axis

While asthma primarily manifests with lower airway symptoms, it is increasingly recognized as a systemic disease (Smulders et al., [2024](#)). The direct mechanistic connections between the lower airways and other anatomical regions support this perspective. Based on these observations and the knowledge about the role of the gut microbiota in shaping patterns of immune responses, the current model of asthma pathogenesis includes the concept of a bidirectional cross-talk between mucosal immune compartments, often described as “gut–lung axis” (Fig. [12.2](#)) (Song et al., [2024](#)). Understanding the gut–lung axis is

crucial, as it provides a systemic perspective on asthma, potentially unlocking innovative therapeutic approaches.

12.5.3 Intestinal Microbiota and the Development of Allergic Sensitization and Asthma

There is increasing evidence that the composition of the gut microbiota very early in life helps shape the development of the immune system.

Numerous studies investigating the infants' gut microbiome and its potential link to asthma development have identified a correlation between a reduced relative abundance of the genera *Roseburia*, *Ruminococcus*, *Faecalibacterium*, *Clostridium*, and *Lachnospira* and an increased risk of developing asthma (Smulders et al., [2024](#); Aslam et al., [2024](#); Aldriwesh et al., [2023](#)). However, findings regarding other genera remain inconsistent and require further investigation (Aslam et al., [2024](#); Smulders et al., [2024](#)).

Interestingly, most conditions known to reduce type I allergy and asthma risk share the common factor of increased microbial exposure, likely influencing gut microbiota diversity and composition during infancy. Well-known protective factors include vaginal birth (Kolokotroni et al., [2012](#)), breastfeeding (Oddy, [2009](#)), close contact with farm animals, multiple older siblings (Ball et al., [2000](#)), raw milk consumption (Waser et al., [2007](#)), early day-care attendance (Ball et al., [2000](#)), or growing up in more rural and less industrialized countries (ISAAC, [1998](#)) (Fig. [12.3](#)).

The initial microbial colonization of the gut directly after birth by the so-called pioneer microbiome has lifelong implications (Ozcam & Lynch, [2024](#)). In addition to its impact on the developing immune system, it also determines the conditions for subsequent colonization by other microbes (Sarkar et al., [2021](#)). It is, therefore, shaping the developing gut microbiome that becomes relatively stable in adulthood.

The route of delivery at birth provides the first contact of the gut to microbes. Despite some conflicting evidence, data generally indicate that cesarean delivery, whether elective or as an emergency, is linked to an elevated risk of asthma (Huang et al., [2015](#)). Research indicates that

maternal microbial transmission during childbirth influences the newborn's microbiome, and cesarean delivery may increase the risk of childhood asthma by altering gut microbial colonization patterns (Gurdeniz et al., [2022](#)). In a prospective birth cohort study, it was observed that increased asthma risk was only present in children delivered by cesarean section whose gut microbiota at 1 year of age still retained a cesarean section microbial signature, suggesting that appropriate maturation of the gut microbiota could mitigate the increased asthma risk (Stokholm et al., [2020](#)).

Another potential explanation for the link between the risk of asthma development and the mode of delivery is the routine administration of antibiotics before cesarean section, leading to disruptions in the gut microbiome. In a recent study by Lee et al., prenatal antibiotic exposure and cesarean-section delivery were both associated with childhood asthma. However, even more interestingly, a synergistic effect of these factors was observed (Lee et al., [2023a](#)). Similar results supporting a link between early-life antibiotic use and asthma were found in a Finnish study, where early macrolide use led to persistent dysbiosis characterised by a significant reduction in *Actinobacteria* and an increased risk of asthma development between the ages of 2 and 7 years (Korpela et al., [2016](#)). Interestingly, the antibiotic type and administration timing may influence the outcome. For instance, early-life vancomycin administration in mice enhanced future susceptibility to allergic asthma, while streptomycin did not show this effect (Russell et al., [2012](#)). However, these effects could not be observed in similarly treated adult mice (Russell et al., [2012](#)). In a study from Australia, exposure to any second-generation cephalosporin was associated with a 2.7-fold increase in the risk of early-persistent asthma, while exposure to any β -lactam antibiotic other than cephalosporins or macrolides doubled the risk (Lu et al., [2023](#)).

Breastfeeding plays a central role in shaping the gut microbiome during early life, significantly influencing the colonization and composition of an infant's intestinal microbiota (Davis et al., [2022](#)). A systematic review and meta-analysis suggested that the duration and

exclusivity of breastfeeding are associated with a lower risk of asthma in children under the age of 7 years (Xue et al., [2021](#)). In a recent study by Dai et al., children who received antibiotics without breastfeeding had a threefold increase in asthma risk. In contrast, this was not the case in those breastfed, benefiting from a “re-balancing” of the gut microbiome and protective colonization by *Bifidobacterium longum subsp. infantis* (Dai et al., [2023](#)).

However, the early dietary habits after food introduction may also be relevant. A study in a Finnish birth cohort found that by the age of 12 months, a lower diversity of food intake was linked to a higher risk of asthma development (Nwaru et al., [2014](#)).

12.5.4 Mechanisms of the Crosstalk in the Gut–Lung Axis

Various elements, such as systemic dissemination of microbial-derived immunomodulatory metabolites, extracellular vesicles, and epigenetic modification of immune cells, have demonstrated their influence along the gut–lung axis (Ozcam & Lynch, [2024](#)).

SCFA control epithelial barrier function and mucosal and systemic immunity through evolutionarily conserved mechanisms, including G protein-coupled receptor signalling and histone deacetylase activity (Mann et al., [2024](#)). They have a well-established role in mediating tolerance to food allergens (see Sect. [12.2.3](#)). Butyrate represents the best-studied SCFA so far. The lung microbiota produces minimal butyrate locally; however, butyrate derived from the intestine can indirectly influence lung immunity by impacting bone marrow precursor cells and blood-derived cells (phagocytes, B cells, plasma cells, and regulatory and effector T cells) before they migrate to the lung mucosa (Mann et al., [2024](#)). Butyrate has the potential to epigenetically alter cell fates, meaning that the biological effects of butyrate exposure might be observed long after the initial exposure (Yip et al., [2021](#)). This could explain the observed “window of opportunity” in early life, during which commensal bacteria and their metabolites can potentially influence lifelong allergic susceptibility and severity (Yip et al., [2021](#)).

Trompette et al. found that mice consuming a high-fiber diet displayed increased levels of SCFA and showed protection against allergic inflammation in the lungs. In contrast, mice on a low-fiber diet with decreased SCFA levels demonstrated greater susceptibility to allergic airway disease (Trompette et al., [2014](#)). A study on a birth cohort found significant correlations between SCFA levels in fecal samples and infants' diets (consumption of yoghurt, fish, fruit, and vegetables) (Roduit et al., [2019](#)). Even more interestingly, children with the highest faecal concentrations of butyrate and propionate at 1 year of age exhibited significantly lower rates of atopic sensitization and were less likely to develop asthma between the ages of 3 and 6 (Roduit et al., [2019](#)).

SCFA may also be a relevant factor in the protective effect of breastfeeding against asthma development. One proposed mechanism suggests that breastfeeding promotes the growth of *Bifidobacterium infantis* (as described above) in the child's gut microbiome, a species highly efficient at metabolising breastmilk into SCFA (Chichlowski et al., [2020](#)). Regarding the negative effect of antibiotics, Cait et al. showed that mice treated with vancomycin showed increased susceptibility to airway inflammation. However, this effect could be inhibited by dietary supplementation of mice with SCFA (Cait et al., [2018](#)).

In addition to the potential role of SCFA, lipopolysaccharide (LPS), a component of bacterial cell walls, is also discussed as a potential disease modifier in asthma (Smulders et al., [2024](#); Song et al., [2024](#)).

Additionally, research is ongoing about the role of other bacterial metabolites in the gut-lung axis. These include desamino-tyrosine, histamine released by gut microbiota, quorum-sensing molecules, linoleic acid metabolites, and microbial extracellular vesicles (Ozcam & Lynch, [2024](#); Smulders et al., [2024](#); Song et al., [2024](#)).

12.5.5 Interventional Studies on Intestinal Microbiota and the Development and Management of Asthma

Currently, no microbiome-based therapeutics have been approved for asthma prevention or management, although these interventions could

hold significant potential in the future.

Probiotics

Probiotic interventions are considered a promising potential therapeutic strategy to modify gut microbiota and prevent asthma development or alleviate asthma symptoms. Currently, most research focuses on bacteria of the genus *Lactobacillus* and *Bifidobacterium* (Kleniewska & Pawliczak, [2024](#)).

The evidence regarding the effectiveness of probiotic treatment during pregnancy or early childhood in reducing the risk of asthma in children is mixed and inconclusive. In a systematic review by Colquitt et al. that assessed randomized controlled trials, probiotic supplementation in pregnant women was found to have no protective effect against asthma or wheezing (Colquitt et al., [2022](#)).

In an early study by Arrieta et al., the bacterial genera *Lachnospira*, *Veillonella*, *Faecalibacterium*, and *Rothia*, which were found to be significantly decreased in children at risk of asthma, were inoculated to germ-free mice, which led to significantly reduced airway inflammation in adult mice (Arrieta et al., [2015](#)). However, several randomized controlled trials investigating specific probiotic strains for asthma prevention in early childhood have demonstrated no beneficial effect (Fiocchi et al., [2022](#)). In a meta-analysis by Wei et al., no significant association between probiotic supplementation in infants and a reduced risk of asthma or wheezing was found compared to a placebo. However, a subgroup analysis revealed that probiotics significantly lowered the incidence of wheezing in infants with atopic disease (Wei et al., [2020](#)). On the other side, in a more recent meta-analysis, probiotics supplementation in the first months after birth did not decrease the risk of asthma development in high-risk children (Wawryk-Gawda et al., [2021](#)). The efficacy of probiotics is most probably strain-specific, underscoring the need for targeted research. Further research with careful selection of probiotic strains and more

extended follow-up periods may be needed to clarify the potential role of probiotics in asthma prevention.

Regarding a potential therapeutic effect in asthma, animal studies suggest that probiotic microorganisms exhibit anti-inflammatory effects, reduce airway hyperresponsiveness, and decrease mucus secretion in the airways (Kleniewska & Pawliczak, [2024](#)). In human research, a large randomized controlled trial in Italy found that specific probiotic strains (*Ligilactobacillus salivarius* LS01 and *Bifidobacterium breve* B632) significantly reduced asthma exacerbations by more than a third in children (Drago et al., [2022](#)). In single studies, combining multi-strain probiotics with prebiotics has yielded promising outcomes in treating asthma's clinical manifestations, including reduced exacerbation frequency and improved lung function parameters (Kleniewska & Pawliczak, [2024](#)). However, findings remain inconsistent due to variations in probiotic strains, dosages, and study designs used across different trials.

In conclusion, the in vivo interventions mentioned only appear to affect asthma symptoms in older children and adults without preventing asthma development when administered during pregnancy and to newborns (Smulders et al., [2024](#)). This contrasts with studies in germ-free mice, where it is easier for a microbiome to colonize an unoccupied gut than to compete with an existing microbiome, as in human experiments (Smulders et al., [2024](#)). Mechanistic studies in humans and mice show that the existing gut microbiome largely dictates the response to dietary or microbial interventions (Ozcam & Lynch, [2024](#)). For the future, developing robust platforms to identify patients who could benefit from specific microbiome-based therapeutics that offer protective effects with minimal side effects remains a significant challenge in the field, given that “one-size-fits-all” interventions are unlikely to succeed (Kau et al., [2024](#)).

Other Potential Interventions: High-Fiber Diet, Prebiotics and Fecal Microbiota Transplantation

Commensal gut bacteria can ferment fiber to produce SCFA, as explained above. Although studies in mice have shown that oral administration of SCFA could reduce allergic inflammation, human studies are missing (Yip et al., [2021](#)). Research on high-fiber diets in mouse models of asthma has produced mixed results, reflecting the intricate relationship between diet and immune responses. While some studies suggest that a high-fiber diet can alleviate lung inflammation and asthma symptoms, others indicate that excessive fiber intake may worsen allergic reactions (Aslam et al., [2024](#)). In humans, large cross-sectional studies suggested that higher dietary fiber intake was associated with fewer asthma symptoms and greater asthma control (Andrianasolo et al., [2019](#); Saeed et al., [2020](#)).

Prebiotics are non-digestible fibers such as fructooligosaccharide, galactooligosaccharide, or human milk oligosaccharide that may show a therapeutic benefit (Fiocchi et al., [2022](#)). A systematic review and meta-analysis found that prebiotic supplementation in early childhood may reduce the risk of asthma development in high-risk children (Wawryk-Gawda et al., [2021](#)). However, the research on pediatric prebiotic interventions is limited, and their mechanisms of action are not well-defined. Additionally, the effectiveness of prebiotics may vary depending on factors such as the specific type of prebiotic, dosage, and timing of administration. While a few studies have shown positive results, further confirmatory studies are necessary before specific clinical recommendations can be made (Fiocchi et al., [2022](#)).

Faecal microbiota transplantation (FMT), which transfers microbiota from a healthy donor to a recipient, has shown promising results in treating various gastrointestinal disorders, but research regarding asthma is lacking. This method could potentially represent an interesting treatment option in the future, but more research in animal and human models is needed (Aslam et al., [2024](#)).

12.5.6 Outlook

Emerging evidence highlights the critical role of early-life microbial colonization in shaping immune responses and influencing asthma susceptibility. Factors such as delivery mode, antibiotic use, breastfeeding, and diet significantly impact gut microbial diversity and composition, with implications for asthma development. Microbial metabolites, particularly SCFA, seem to play a key role in modulating systemic immunity and airway inflammation. Future research on the gut–lung axis holds promise for advancing asthma prevention and management. Understanding individual microbiome variability and its immune pathways will be critical for creating effective, patient-specific treatments like personalized diets, prebiotics, and probiotics.

12.6 Conclusion

In recent years, it has become evident that microbes have an enormous capacity to educate and fine-tune our immune system, and allergic diseases are no exception. Diversity of the human microbiomes has been recognized as an important factor associated with reduced manifestation of allergic diseases. This is most evident in food allergy. Conversely, dysbiotic microbial colonization boosts inflammation and thus symptom severity of allergic diseases. Further studies providing high-resolution microbiome data will lead to a better understanding of the contribution of specific microbes to the incidence or severity of different allergic diseases (Fig. [12.2](#)).

History

- Allergies and atopic diseases are not new to mankind and have been described since ancient times. But their etiology has been poorly understood before the concept of an immune system was introduced in the late nineteenth century. Therefore, earlier reports can hardly be related to modern disease classifications (Bergmann & Ring, [2014](#)).
- 1873: Charles Blackley found hay-fever to be less frequent among farmers' children (no consideration of microbes at the time,

though)

- Beginning of the twentieth century: Several physicians independently recognized that symptoms during infectious diseases did not necessarily have to be caused by the pathogens. Instead, they were largely the result of the pro-inflammatory immune reactions (because symptoms and incubation times were similar to infections by various pathogens). Furthermore, impressive, sometimes fatal consequences were noted with patients repeatedly treated with the recently developed antisera (Igea, [2013](#)).
- 1902: Portier und Richet coined the term “anaphylaxis”
- 1906: von Pirquet coined the term “Allergie”
- 1923: Coca and Cooke (Coca & Cooke, [1923](#)) published a new classification for the various forms of hypersensitivity reactions and for the first time summarized the group of hay fever and asthma disorders under the term atopy.
- 1933: Wise and Sulzberger proposed the term “atopic dermatitis” for a recurrent eczema with family history and added this disease to the group of atopic disorders as coined by Coca and Cooke. Scientific discussion about the influence of food allergy on AD begins.
- 1974: Leyden et al. found *Staphylococcus aureus* to be the dominant microbe on atopic dermatitis lesions (Leyden et al., [1974](#)).
- 1989: Strachan introduced the “hygiene hypothesis” as a study inversely correlating asthma incidence with big, unhygienic households (Strachan, [1989](#)).
- 1997: Majamaa and Isolauri reported the first successful use of a probiotic (*Lactobacillus rhamnosus* GG) on children with cow’s milk allergy and AD after lactobacilli were reported to strengthen the intestinal barrier (Majamaa & Isolauri, [1997](#)).
- 2000: Kalliomäki et al. demonstrated distinct microbiomes in healthy children and children with atopic dermatitis by non-culture methods. Bacterial culture was shown to be not sensitive enough,

hinting at the potential of NGS investigations (Kalliomaki et al., [2001](#)).

- 2012: Kong et al. provide a high-resolution NGS investigation of microbiome shifts in AD (Kong et al., [2012](#)).
- 2014: Trompette et al. show that a high-fiber diet increased SCFA levels and protected mice from allergic lung inflammation, while a low-fiber diet increased susceptibility (Trompette et al., [2014](#)). Similar findings on food allergy and atopic dermatitis follow.
- 2020: Callewaert et al. show resolution of skin microbiome dysbiosis by type 2 immunity suppressing dupilumab treatment (Callewaert et al., [2020](#)).

Controversy Atopic diseases and allergies develop at organ surfaces colonized by a multitude of microorganisms, the microbiome. Thus, it is obvious that ‘the microbiome’ somehow interferes with inflammation in atopic diseases and allergic reactions.

However, controversies regarding specific interactions and consequences remain:

- How much can microbiomes orchestrate immune reactions and barrier function across surfaces? It is well accepted that the gut microbiome governs immune reactions functional within the gut and that the skin microbiome conditions cutaneous immunity towards effective defense and specific reactions of immune modulation and tolerance. The constantly increasing understanding of tissue-resident immune memory (T_{RM} cells) further strengthens the belief that compartmentalized immunity is a default pathway in immune orchestration and regulation. Consequently, local microbiomes will directly influence local immunity and may drive systemic immune profiles and reactions only under exceptional circumstances. Contrariwise, the gastrointestinal barrier function and the development of the intestinal immune system especially early in life impacts systemic immune cell development and reactivity, arguing for a role of the gut microbiome also for immune reactions at other surfaces than the intestinal mucosa. Recent

research increasingly acknowledges the importance of the gut-lung axis in asthma, while potential effects of a gut-skin axis in AD are less clear.

- How can we distinguish between epiphenomena and causal findings regarding the microbiome and atopic diseases and allergies? As pointed out, atopic inflammation and allergies develop at surfaces, which are colonized by “the microbiome.” We understand that atopic diseases and allergies develop based on a complex genetic trait, including genes responsible for barrier functions, innate/adaptive immune responses, or non-immune cells “inflammation.” Microbial constituents can therefore function as triggers, but changes in microbial composition will also be a consequence of a dysfunctional barrier, innate or adaptive immune reactions, or inflammatory pathways. The latter may result in microbial dysbiosis based on “surface instability” tempting us to call this an epiphenomenon of the susceptibility for atopic diseases or allergies. However, this dysbiosis may well act as a switch to transform initial immune dysregulation into overt inflammation and disease. In addition, dysbiosis may result in subtle changes in surface response patterns, which then impact disease development directly, representing a primary cause rather than an epiphenomenon. As the latter also implies that intervention at an early stage of disease development/pre-disposition may be feasible, further research is highly desirable.

In addition, controversies remain regarding specific aspects of investigation:

- Human studies by nature deliver less causal insights compared to animal experiments and can remain speculative in regard to mechanistic interpretations. For example, there is controversial evidence regarding the role of caesarean sections for changes in the composition of the gut microbiota and the risk of developing allergy, atopic dermatitis or asthma, which cannot be resolved with animal experiments.

- Loss of microbial diversity due to a westernized lifestyle with higher hygiene standards, less infectious diseases, smaller family sizes, and less contact with rural environments (biodiversity hypothesis) has been well shown to be associated with allergic sensitization and asthma, not so well with atopic dermatitis or food allergy. Compared to the rise of allergic asthma and atopic dermatitis, the increase of food allergy in the last two decades can only in part be explained by the biodiversity hypothesis, as its rise occurred approximately >30 years later. Thus, additional yet unknown susceptibility factors with direct effects on the induction of type 2 immunity in the gut or with indirect effects shaping the composition of the intestinal microbiota are likely to exist.

Highlights

- Investigations in the murine and human system using 16S rRNA gene phylotyping and metagenomics have demonstrated an association of food allergy with an altered (dysbiotic) intestinal microbiota (Chen et al., [2016](#); Ling et al., [2014](#); Noval Rivas et al., [2013](#)).
- Research in the last decade has elucidated cellular and molecular mechanisms of oral tolerance and the role of the intestinal microbiota in shaping tolerogenic immune responses in regard to orally ingested antigens (Pabst & Mowat, [2012](#); Cahenzli et al., [2013](#); Hadis et al., [2011](#)).
- Early-life gut microbiota composition plays a critical role in shaping immune responses, with reduced genera such as *Roseburia*, *Ruminococcus*, *Faecalibacterium*, *Clostridium*, and *Lachnospira* in the infant gut microbiome associated with an increased risk of asthma development (Simpson et al., [2023](#); Smulders et al., [2024](#); Aldriwesh et al., [2023](#)).
- High-fiber diets in early life, promoting SCFA production like butyrate and propionate, have shown protective effects against atopic diseases (Trompette et al., [2014](#); Roduit et al., [2019](#)).

References

Abdel-Gadir, A., Stephen-Victor, E., Gerber, G. K., Noval Rivas, M., Wang, S., Harb, H., Wang, L., Li, N., Crestani, E., Spielman, S., Secor, W., Biehl, H., DiBenedetto, N., Dong, X., Umetsu, D. T., Bry, L., Rachid, R., & Chatila, T. A. (2019). Microbiota therapy acts via a regulatory T cell MyD88/RORgammat pathway to suppress food allergy. *Nature Medicine*, 25, 1164–1174.

[\[PubMed\]](#)

Abrahamsson, T. R., Jakobsson, H. E., Andersson, A. F., Bjorksten, B., Engstrand, L., & Jenmalm, M. C. (2012). Low diversity of the gut microbiota in infants with atopic eczema. *The Journal of Allergy and Clinical Immunology*, 129, 434–40, 40 e1–2.

Accordini, S., Corsico, A. G., Braggion, M., Gerbase, M. W., Gislason, D., Gulsvik, A., Heinrich, J., Janson, C., Jarvis, D., Jogi, R., Pin, I., Schoefer, Y., Bugiani, M., Cazzoletti, L., Cerveri, I., Marcon, A., & de Marco, R. (2013). The cost of persistent asthma in Europe: An international population-based study in adults. *International Archives of Allergy and Immunology*, 160, 93–101.

[\[PubMed\]](#)

Adlerberth, I., Strachan, D. P., Matricardi, P. M., Ahrne, S., Orfei, L., Aberg, N., Perkin, M. R., Tripodi, S., Hesselmar, B., Saalman, R., Coates, A. R., Bonanno, C. L., Panetta, V., & Wold, A. E. (2007). Gut microbiota and development of atopic eczema in 3 European birth cohorts. *The Journal of Allergy and Clinical Immunology*, 120, 343–350.

[\[PubMed\]](#)

Aitoro, R., Paparo, L., Amoroso, A., Di Costanzo, M., Cosenza, L., Granata, V., Di Scala, C., Nocerino, R., Trinchese, G., Montella, M., Ercolini, D., & Berni Canani, R. (2017). Gut microbiota as a target for preventive and therapeutic intervention against food allergy. *Nutrients*, 9, 672.

[\[PubMed\]](#)

Akiyama, M., Kaneko, Y., & Takeuchi, T. (2022). T follicular helper cells mediate local production of allergen-specific IgE and IgG4. *Journal of Allergy and Clinical Immunology*, 150, 1045–1047.

[\[PubMed\]](#)

Aldriwesh, M. G., Al-Mutairi, A. M., Alharbi, A. S., Aljohani, H. Y., Alzahrani, N. A., Ajina, R., & Alanazi, A. M. (2023). Paediatric asthma and the microbiome: A systematic review. *Microorganisms*, 11, 939.

[\[PubMed\]](#)

Amar, Y., Schneider, E., Koberle, M., Seeholzer, T., Musiol, S., Holge, I. M., Gschwendtner, S., Krappmann, D., Steiger, K., Biedermann, T., Schmidt-Weber, C. B., & Alessandrini, F. (2022). Microbial dysbiosis in a mouse model of atopic dermatitis mimics shifts in human microbiome and correlates with the key pro-inflammatory cytokines IL-4, IL-33 and TSLP. *Journal of the European Academy of Dermatology and Venereology*, 36, 705–716.

[\[PubMed\]](#)

Amar, Y., Rogner, D., Silva, R. L., Foessel, B. U., Ud-Dean, M., Lagkouvardos, I., Steimle-Grauer, S. A., Niedermeier, S., Kublik, S., Jargosch, M., Heinig, M., Thomas, J., Eyerich, S., Wikström, J. D., Schlöter,

M., Eyerich, K., Biedermann, T., & Köberle, M. (2023). Darier's disease exhibits a unique cutaneous microbial dysbiosis associated with inflammation and body malodour. *Microbiome*, 11, 162. [\[https://doi.org/10.1186/s40168-023-01587-x\]](https://doi.org/10.1186/s40168-023-01587-x) [\[PubMed\]](#)

Amar, Y., Niedermeier, S., Silva, R., Kublik, S., Schloter, M., Biedermann, T., Köberle, M., & Eberlein, B. (2024). Skin microbiome dynamics in patients with polymorphic light eruption in response to UV radiations. *The British Journal of Dermatology*, 192, 684.

Andrianasolo, R. M., Hercberg, S., Kesse-Guyot, E., Druesne-Pecollo, N., Touvier, M., Galan, P., & Varraso, R. (2019). Association between dietary fibre intake and asthma (symptoms and control): Results from the French national e-cohort NutriNet-Sante. *The British Journal of Nutrition*, 122, 1040–1051. [\[PubMed\]](#)

Arrieta, M. C., Stiemsma, L. T., Dimitriu, P. A., Thorson, L., Russell, S., Yurist-Doutsch, S., Kuzeljevic, B., Gold, M. J., Britton, H. M., Lefebvre, D. L., Subbarao, P., Mandhane, P., Becker, A., McNagny, K. M., Sears, M. R., Kollmann, T., Mohn, W. W., Turvey, S. E., & Finlay, B. B. (2015). Early infancy microbial and metabolic alterations affect risk of childhood asthma. *Science Translational Medicine*, 7, 307ra152. [\[PubMed\]](#)

Aslam, R., Herrles, L., Aoun, R., Pioskowik, A., & Pietrzyk, A. (2024). Link between gut microbiota dysbiosis and childhood asthma: Insights from a systematic review. *J Allergy Clin Immunol Glob*, 3, 100289. [\[PubMed\]](#)

Atarashi, K., Tanoue, T., Shima, T., Imaoka, A., Kuwahara, T., Momose, Y., Cheng, G., Yamasaki, S., Saito, T., Ohba, Y., Taniguchi, T., Takeda, K., Hori, S., Ivanov, Y., Il, Umesaki, K. I., & Honda, K. (2011). Induction of colonic regulatory T cells by indigenous *Clostridium* species. *Science*, 331, 337–341. [\[PubMed\]](#)

Atarashi, K., Tanoue, T., Oshima, K., Suda, W., Nagano, Y., Nishikawa, H., Fukuda, S., Saito, T., Narushima, S., Hase, K., Kim, S., Fritz, J. V., Wilmes, P., Ueha, S., Matsushima, K., Ohno, H., Olle, B., Sakaguchi, S., Taniguchi, T., Morita, H., Hattori, M., & Honda, K. (2013). Treg induction by a rationally selected mixture of *Clostridia* strains from the human microbiota. *Nature*, 500, 232–236. [\[https://doi.org/10.1038/nature12331\]](https://doi.org/10.1038/nature12331) [\[PubMed\]](#)

Balato, A., Cacciapuoti, S., Di Caprio, R., Marasca, C., Masarà, A., Raimondo, A., & Fabbrocini, G. (2019). Human microbiome: Composition and role in inflammatory skin diseases. *Archivum Immunologiae et Therapiae Experimentalis (Warsz)*, 67, 1–18.

Ball, T. M., Castro-Rodriguez, J. A., Griffith, K. A., Holberg, C. J., Martinez, F. D., & Wright, A. L. (2000). Siblings, day-care attendance, and the risk of asthma and wheezing during childhood. *The New England Journal of Medicine*, 343, 538–543. [\[PubMed\]](#)

Bashir, M. E., Louie, S., Shi, H. N., & Nagler-Anderson, C. (2004). Toll-like receptor 4 signaling by intestinal microbes influences susceptibility to food allergy. *Journal of Immunology*, 172, 6978–6987.

Beck, L. A., Bieber, T., Weidinger, S., Tauber, M., Saeki, H., Irvine, A. D., Eichenfield, L. F., Werfel, T., Arlert, P., Jiang, L., Røpke, M., & Paller, A. S. (2023). Tralokinumab treatment improves the skin microbiota by increasing the microbial diversity in adults with moderate-to-severe atopic dermatitis: Analysis of microbial diversity in ECZTRA 1, a randomized controlled trial. *Journal of the American Academy of Dermatology*, 88, 816–823.

[\[PubMed\]](#)

Belkaid, Y., & Harrison, O. J. (2017). Homeostatic immunity and the microbiota. *Immunity*, 46, 562–576.

[\[PubMed\]](#)

Bergmann, K.-C., & Ring, J. (2014). History of allergy. *Chemical Immunology and Allergy*, 100, 426.

Berin, M. C., Zheng, Y., Domaradzki, M., Li, X. M., & Sampson, H. A. (2006). Role of TLR4 in allergic sensitization to food proteins in mice. *Allergy*, 61, 64–71.

[\[PubMed\]](#)

Berni Canani, R., Nocerino, R., Terrin, G., Coruzzo, A., Cosenza, L., Leone, L., & Troncone, R. (2012). Effect of Lactobacillus GG on tolerance acquisition in infants with cow's milk allergy: A randomized trial. *The Journal of Allergy and Clinical Immunology*, 129, 580–2, 82 e1–5.

Berni Canani, R., Di Costanzo, M., Bedogni, G., Amoroso, A., Cosenza, L., Di Scala, C., Granata, V., & Nocerino, R. (2017). Extensively hydrolyzed casein formula containing Lactobacillus rhamnosus GG reduces the occurrence of other allergic manifestations in children with cow's milk allergy: 3-year randomized controlled trial. *The Journal of Allergy and Clinical Immunology*, 139, 1906–13 e4.

Biedermann, T. (2006). Dissecting the role of infections in atopic dermatitis. *Acta Dermato-Venereologica*, 86(2), 99–109.

Biedermann, T., Rocken, M., & Carballido, J. M. (2004). TH1 and TH2 lymphocyte development and regulation of TH cell-mediated immune responses of the skin. *The Journal of Investigative Dermatology. Symposium Proceedings*, 9, 5–14.

[\[PubMed\]](#)

Biedermann, T., Skabytska, Y., Kaesler, S., & Volz, T. (2015). Regulation of T cell immunity in atopic dermatitis by microbes: The Yin and Yang of cutaneous inflammation. *Frontiers in Immunology*, 6, 353.

[\[PubMed\]](#)

Bisgaard, H., Li, N., Bonnelykke, K., Chawes, B. L., Skov, T., Paludan-Muller, G., Stokholm, J., Smith, B., & Krogfelt, K. A. (2011). Reduced diversity of the intestinal microbiota during infancy is associated with increased risk of allergic disease at school age. *The Journal of Allergy and Clinical Immunology*, 128, 646–52 e1–5.

[\[PubMed\]](#)

Brough, H. A., Nadeau, K. C., Sindher, S. B., Alkotob, S. S., Chan, S., Bahnson, H. T., Leung, D. Y. M., & Lack, G. (2020). Epicutaneous sensitization in the development of food allergy: What is the evidence and how can this be prevented? *Allergy*, 75, 2185–2205.

[\[PubMed\]](#)

Bunyavanich, S., Shen, N., Grishin, A., Wood, R., Burks, W., Dawson, P., Jones, S. M., Leung, D. Y. M., Sampson, H., Sicherer, S., & Clemente, J. C. (2016). Early-life gut microbiome composition and milk allergy resolution. *The Journal of Allergy and Clinical Immunology*, 138, 1122–1130.

[\[PubMed\]](#)

Cahenzli, J., Koller, Y., Wyss, M., Geuking, M. B., & McCoy, K. D. (2013). Intestinal microbial diversity during early-life colonization shapes long-term IgE levels. *Cell Host & Microbe*, 14, 559–570.

Cait, A., Hughes, M. R., Antignano, F., Cait, J., Dimitriu, P. A., Maas, K. R., Reynolds, L. A., Hacker, L., Mohr, J., Finlay, B. B., Zaph, C., McNagny, K. M., & Mohn, W. W. (2018). Microbiome-driven allergic lung inflammation is ameliorated by short-chain fatty acids. *Mucosal Immunology*, 11, 785–795.

[\[PubMed\]](#)

Callewaert, C., Nakatsuji, T., Knight, R., Kosciulek, T., Vrbanc, A., Kotol, P., Ardeleanu, M., Hultsch, T., Guttman-Yassky, E., Bissonnette, R., Silverberg, J. I., Krueger, J., Menter, A., Graham, N. M. H., Pirozzi, G., Hamilton, J. D., & Gallo, R. L. (2020). IL-4R α Blockade by Dupilumab decreases *Staphylococcus aureus* colonization and increases microbial diversity in atopic dermatitis. *Journal of Investigative Dermatology*, 140, 191–202.e7.

[\[PubMed\]](#)

Chan, S. M., Turcanu, V., Stephens, A. C., Fox, A. T., Grieve, A. P., & Lack, G. (2012). Cutaneous lymphocyte antigen and alpha4beta7 T-lymphocyte responses are associated with peanut allergy and tolerance in children. *Allergy*, 67, 336–342.

[\[PubMed\]](#)

Chang, H. W., Yan, D., Singh, R., Liu, J., Lu, X., Ucmak, D., Lee, K., Afifi, L., Fadrosh, D., Leech, J., Vasquez, K. S., Lowe, M. M., Rosenblum, M. D., Scharschmidt, T. C., Lynch, S. V., & Liao, W. (2018). Alteration of the cutaneous microbiome in psoriasis and potential role in Th17 polarization. *Microbiome*, 6, 154.

[\[https://doi.org/10.1186/s40168-018-0533-1\]](https://doi.org/10.1186/s40168-018-0533-1)[\[PubMed\]](#)

Chen, C. C., Chen, K. J., Kong, M. S., Chang, H. J., & Huang, J. L. (2016). Alterations in the gut microbiotas of children with food sensitization in early life. *Pediatric Allergy and Immunology*, 27, 254–262.

[\[PubMed\]](#)

Chen, H., Zhao, Q., Zhong, Q., Duan, C., Krutmann, J., Wang, J., & Xia, J. (2022). Skin microbiome, metabolome and skin phenome, from the perspectives of skin as an ecosystem. *Phenomics*, 2, 363–382.

[\[https://doi.org/10.1007/s43657-022-00073-y\]](https://doi.org/10.1007/s43657-022-00073-y)[\[PubMed\]](#)

Chichlowski, M., Shah, N., Wampler, J. L., Wu, S. S., & Vanderhoof, J. A. (2020). *Bifidobacterium longum* subspecies *infantis* (*B. infantis*) in pediatric nutrition: Current state of knowledge. *Nutrients*, 12, 1581.

[\[PubMed\]](#)

Chu, D. M., Ma, J., Prince, A. L., Antony, K. M., Seferovic, M. D., & Aagaard, K. M. (2017). Maturation

of the infant microbiome community structure and function across multiple body sites and in relation to mode of delivery. *Nature Medicine*, 23, 314–326.

[\[PubMed\]](#)

Clark, S., Espinola, J., Rudders, S. A., Banerji, A., & Camargo, C. A., Jr. (2011). Frequency of US emergency department visits for food-related acute allergic reactions. *The Journal of Allergy and Clinical Immunology*, 127, 682–683.

[\[PubMed\]](#)

Coca, A. F., & Cooke, R. A. (1923). On the classification of the phenomena of hypersensitiveness. *The Journal of Immunology*, 8, 163–182.

Cohn, L., Elias, J. A., & Chupp, G. L. (2004). Asthma: Mechanisms of disease persistence and progression. *Annual Review of Immunology*, 22, 789–815.

[\[PubMed\]](#)

Colquitt, A. S., Miles, E. A., & Calder, P. C. (2022). Do probiotics in pregnancy reduce allergies and asthma in infancy and childhood? A systematic review. *Nutrients*, 14, 1852.

[\[PubMed\]](#)

Coombes, J. L., Siddiqui, K. R., Arancibia-Carcamo, C. V., Hall, J., Sun, C. M., Belkaid, Y., & Powrie, F. (2007). A functionally specialized population of mucosal CD103+ DCs induces Foxp3+ regulatory T cells via a TGF-beta and retinoic acid-dependent mechanism. *The Journal of Experimental Medicine*, 204, 1757–1764.

[\[PubMed\]](#)

Coombs, R. R. A., & Gells, P. G. H. (1963). The classification of allergic reactions underlying disease. In R. R. A. Coombs & P. G. H. Gells (Eds.), *Clinical aspects of immunology*. Blackwell Science.

Cuello-Garcia, C. A., Brozek, J. L., Fiocchi, A., Pawankar, R., Yepes-Nunez, J. J., Terracciano, L., Gandhi, S., Agarwal, A., Zhang, Y., & Schunemann, H. J. (2015). Probiotics for the prevention of allergy: A systematic review and meta-analysis of randomized controlled trials. *The Journal of Allergy and Clinical Immunology*, 136, 952–961.

[\[PubMed\]](#)

Curotto de Lafaille, M. A., Kutchukhidze, N., Shen, S., Ding, Y., Yee, H., & Lafaille, J. J. (2008). Adaptive Foxp3+ regulatory T cell-dependent and -independent control of allergic inflammation. *Immunity*, 29, 114–126.

[\[PubMed\]](#)

Dai, D. L. Y., Petersen, C., Hoskinson, C., Del Bel, K. L., Becker, A. B., Moraes, T. J., Mandhane, P. J., Finlay, B. B., Simons, E., Kozyrskyj, A. L., Patrick, D. M., Subbarao, P., Bode, L., Azad, M. B., & Turvey, S. E. (2023). Breastfeeding enrichment of *B. longum* subsp. *infantis* mitigates the effect of antibiotics on the microbiota and childhood asthma risk. *The Medicus*, 4, 92–112 e5.

Davis, E. C., Castagna, V. P., Sela, D. A., Hillard, M. A., Lindberg, S., Mantis, N. J., Seppo, A. E., & Jarvinen, K. M. (2022). Gut microbiome and breast-feeding: Implications for early immune development. *The Journal of Allergy and Clinical Immunology*, 150, 523–534.

[\[PubMed\]](#)

Douwes, J., Brooks, C., van Dalen, C., & Pearce, N. (2011). Importance of allergy in asthma: An epidemiologic perspective. *Current Allergy and Asthma Reports*, 11, 434–444.

[\[PubMed\]](#)

Drago, L., Cioffi, L., Giuliano, M., Pane, M., Amoroso, A., Schiavetti, I., Reid, G., Ciprandi, G., & Group Propam Study. (2022). The Probiotics in Pediatric Asthma Management (PROPAM) study in the primary care setting: A randomized, controlled, double-blind trial with *Ligilactobacillus salivarius* LS01 (DSM 22775) and *Bifidobacterium breve* B632 (DSM 24706). *Journal of Immunology Research*, 2022, 3837418.

[\[PubMed\]](#)

du Toit, G., Tsakok, T., Lack, S., & Lack, G. (2016). Prevention of food allergy. *The Journal of Allergy and Clinical Immunology*, 137, 998–1010.

[\[PubMed\]](#)

Egawa, G., & Kabashima, K. (2016). Multifactorial skin barrier deficiency and atopic dermatitis: Essential topics to prevent the atopic march. *The Journal of Allergy and Clinical Immunology*, 138, 350–58.e1.

[\[PubMed\]](#)

Eyerich, K., Pennino, D., Scarponi, C., Foerster, S., Nasorri, F., Behrendt, H., Ring, J., Traidl-Hoffmann, C., Albanesi, C., & Cavani, A. (2009). IL-17 in atopic eczema: linking allergen-specific adaptive and microbial-triggered innate immune response. *Journal of Allergy and Clinical Immunology*, 123, 59–66.

Fazlollahi, M., Chun, Y., Grishin, A., Wood, R. A., Burks, A. W., Dawson, P., Jones, S. M., Leung, D. Y. M., Sampson, H. A., Sicherer, S. H., & Bunyavanich, S. (2018a). Early-life gut microbiome and egg allergy. *Allergy*, 73, 1515–1524.

[\[PubMed\]](#)

Fazlollahi, M., Lee, T. D., Andrade, J., Oguntuyo, K., Chun, Y., Grishina, G., Grishin, A., & Bunyavanich, S. (2018b). The nasal microbiome in asthma. *The Journal of Allergy and Clinical Immunology*, 142, 834–43 e2.

Feehley, T., Plunkett, C. H., Bao, R., Choi Hong, S. M., Cullen, E., Belda-Ferre, P., Campbell, E., Aitoro, R., Nocerino, R., Paparo, L., Andrade, J., Antonopoulos, D. A., Berni Canani, R., & Nagler, C. R. (2019). Healthy infants harbor intestinal bacteria that protect against food allergy. *Nature Medicine*, 25, 448–453.

[\[PubMed\]](#)

Fiocchi, A., Pawankar, R., Cuello-Garcia, C., Ahn, K., Al-Hammadi, S., Agarwal, A., Beyer, K., Burks, W., Canonica, G. W., Ebisawa, M., Gandhi, S., Kamenwa, R., Lee, B. W., Li, H., Prescott, S., Riva, J. J., Rosenwasser, L., Sampson, H., Spigler, M., Terracciano, L., Vereda-Ortiz, A., Wasserman, S., Yepes-Nunez, J. J., Brozek, J. L., & Schunemann, H. J. (2015). World Allergy Organization-McMaster University guidelines for allergic disease prevention (GLAD-P): Probiotics. *World Allergy Organization Journal*, 8, 4.

[\[PubMed\]](#)

Fiocchi, A., Cabana, M. D., & Mennini, M. (2022). Current use of probiotics and prebiotics in allergy.

The Journal of Allergy and Clinical Immunology. In Practice, 10, 2219–2242.

[\[PubMed\]](#)

Fox, A., Bird, J. A., Fiocchi, A., Knol, J., Meyer, R., Salminen, S., Sitang, G., Szajewska, H., & Papadopoulos, N. (2019). The potential for pre-, pro- and synbiotics in the management of infants at risk of cow's milk allergy or with cow's milk allergy: An exploration of the rationale, available evidence and remaining questions. *World Allergy Organization Journal*, 12, 100034.

[\[PubMed\]](#)

Frossard, C. P., Hauser, C., & Eigenmann, P. A. (2004). Antigen-specific secretory IgA antibodies in the gut are decreased in a mouse model of food allergy. *The Journal of Allergy and Clinical Immunology*, 114, 377–382.

[\[PubMed\]](#)

Geuking, M. B., Cahenzli, J., Lawson, M. A., Ng, D. C., Slack, E., Hapfelmeier, S., McCoy, K. D., & Macpherson, A. J. (2011). Intestinal bacterial colonization induces mutualistic regulatory T cell responses. *Immunity*, 34, 794–806.

[\[PubMed\]](#)

Glatz, M., Bosshard, P. P., Hoetzenecker, W., & Schmid-Grendelmeier, P. (2015). The role of *Malassezia* spp. in atopic dermatitis. *Journal of Clinical Medicine*, 4, 1217–1228.

[\[PubMed\]](#)

Goldberg, M. R., Mor, H., Magid Neriya, D., Magzal, F., Muller, E., Appel, M. Y., Nachshon, L., Borenstein, E., Tamir, S., Louzoun, Y., Youngster, I., Elizur, A., & Koren, O. (2020). Microbial signature in IgE-mediated food allergies. *Genome Medicine*, 12, 92.

[\[PubMed\]](#)

Gonzalez, M. E., Schaffer, J. V., Orlov, S. J., Gao, Z., Li, H., Alekseyenko, A. V., & Blaser, M. J. (2016). Cutaneous microbiome effects of fluticasone propionate cream and adjunctive bleach baths in childhood atopic dermatitis. *Journal of the American Academy of Dermatology*, 75, 481–93.e8.

[\[PubMed\]](#)

Grice, E. A., & Segre, J. A. (2011). The skin microbiome. *Nature Reviews. Microbiology*, 9, 244–253.

[\[PubMed\]](#)

Gueniche, A., Knaudt, B., Schuck, E., Volz, T., Bastien, P., Martin, R., Röcken, M., Breton, L., & Biedermann, T. (2008). Effects of nonpathogenic gram-negative bacterium *Vitreoscilla filiformis* lysate on atopic dermatitis: a prospective, randomized, double-blind, placebo-controlled clinical study. *British Journal of Dermatology*, 159, 1357–1363.

Guenova, E., Skabytska, Y., Hoetzenecker, W., Weindl, G., Sauer, K., Tham, M., Kim, K. W., Park, J. H., Seo, J. H., Ignatova, D., Cozzio, A., Levesque, M. P., Volz, T., Köberle, M., Kaesler, S., Thomas, P., Mailhammer, R., Ghoreschi, K., Schakel, K., Amarov, B., Eichner, M., Schaller, M., Clark, R. A., Röcken, M., & Biedermann, T. (2015). IL-4 abrogates T(H)17 cell-mediated inflammation by selective silencing of IL-23 in antigen-presenting cells. *Proceedings of the National Academy of Sciences of the United States of America*, 112, 2163–2168.

[\[PubMed\]](#)

Gupta, R. S., Warren, C. M., Smith, B. M., Blumenstock, J. A., Jiang, J., Davis, M. M., & Nadeau, K. C. (2018). The public health impact of parent-reported childhood food allergies in the United States. *Pediatrics*, 142, e20181235.

[\[PubMed\]](#)

Gupta, R. S., Warren, C. M., Smith, B. M., Jiang, J., Blumenstock, J. A., Davis, M. M., Schleimer, R. P., & Nadeau, K. C. (2019). Prevalence and severity of food allergies among US adults. *JAMA Network Open*, 2, e185630.

[\[PubMed\]](#)

Gurdeniz, G., Ernst, M., Rago, D., Kim, M., Courraud, J., Stokholm, J., Bonnelykke, K., Bjorkbom, A., Trivedi, U., Sorensen, S. J., Brix, S., Hougaard, D., Rasmussen, M., Cohen, A. S., Bisgaard, H., & Chawes, B. (2022). Neonatal metabolome of caesarean section and risk of childhood asthma. *The European Respiratory Journal*, 59, 2102406.

[\[PubMed\]](#)

Gurram, R. K., & Zhu, J. (2019). Orchestration between ILC2s and Th2 cells in shaping type 2 immune responses. *Cellular & Molecular Immunology*, 16, 225–235.

Hadis, U., Wahl, B., Schulz, O., Hardtke-Wolenski, M., Schippers, A., Wagner, N., Muller, W., Sparwasser, T., Forster, R., & Pabst, O. (2011). Intestinal tolerance requires gut homing and expansion of FoxP3⁺ regulatory T cells in the lamina propria. *Immunity*, 34, 237–246.

[\[PubMed\]](#)

Hill, D. A., Siracusa, M. C., Abt, M. C., Kim, B. S., Kobuley, D., Kubo, M., Kambayashi, T., Larosa, D. F., Renner, E. D., Orange, J. S., Bushman, F. D., & Artis, D. (2012). Commensal bacteria-derived signals regulate basophil hematopoiesis and allergic inflammation. *Nature Medicine*, 18, 538–546.

[\[PubMed\]](#)

Hilty, M., Burke, C., Pedro, H., Cardenas, P., Bush, A., Bossley, C., Davies, J., Ervine, A., Poulter, L., Pachter, L., Moffatt, M. F., & Cookson, W. O. C. (2010). Disordered microbial communities in asthmatic airways. *PLoS One*, 5, e8578.

[\[PubMed\]](#)

Hol, J., van Leer, E. H., Elink Schuurman, B. E., de Ruiter, L. F., Samsom, J. N., Hop, W., Neijens, H. J., de Jongste, J. C., Nieuwenhuis, E. E., & Elimination Cow's Milk Allergy Modified by, and group Lactobacilli study. (2008). The acquisition of tolerance toward cow's milk through probiotic supplementation: A randomized, controlled trial. *The Journal of Allergy and Clinical Immunology*, 121, 1448–1454.

[\[PubMed\]](#)

Hsiao, K. C., Ponsonby, A. L., Axelrad, C., Pitkin, S., Tang, M. L. K., & Ppoit Study Team. (2017). Long-term clinical and immunological effects of probiotic and peanut oral immunotherapy after treatment cessation: 4-year follow-up of a randomised, double-blind, placebo-controlled trial. *Lancet Child & Adolescent Health*, 1, 97–105.

Huang, L., Chen, Q., Zhao, Y., Wang, W., Fang, F., & Bao, Y. (2015). Is elective cesarean section associated with a higher risk of asthma? A meta-analysis. *The Journal of Asthma*, 52, 16–25.

[\[PubMed\]](#)

Igea, J. M. (2013). The history of the idea of allergy. *Allergy*, 68, 966–973.

[\[PubMed\]](#)

Imai, Y., Kusakabe, M., Nagai, M., Yasuda, K., & Yamanishi, K. (2021). Dupilumab effects on innate lymphoid cell and helper T cell populations in patients with atopic dermatitis. *JID Innovations*, 1, 100003.

[\[PubMed\]](#)

ISAAC. (1998). Worldwide variation in prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and atopic eczema: ISAAC. The International Study of Asthma and Allergies in Childhood (ISAAC) Steering Committee. *Lancet*, 351, 1225–1232.

Ito, Y., & Amagai, M. (2022). Controlling skin microbiome as a new bacteriotherapy for inflammatory skin diseases. *Inflammation and Regeneration*, 42, 26.

[\[PubMed\]](#)

Kaesler, S., Volz, T., Skabytska, Y., Köberle, M., Hein, U., Chen, K. M., Guenova, E., Wölbing, F., Röcken, M., & Biedermann, T. (2014). Toll-like receptor 2 ligands promote chronic atopic dermatitis through IL-4-mediated suppression of IL-10. *Journal of Allergy and Clinical Immunology*, 134, 92–99.

Kalliomaki, M., Kirjavainen, P., Eerola, E., Kero, P., Salminen, S., & Isolauri, E. (2001). Distinct patterns of neonatal gut microflora in infants in whom atopy was and was not developing. *The Journal of Allergy and Clinical Immunology*, 107, 129–134.

[\[PubMed\]](#)

Kau, A. L., Rosen, A. L., & Rosas-Salazar, C. (2024). Can therapeutic targeting of the human microbiome influence asthma management? A Pro/Con debate. *The Journal of Allergy and Clinical Immunology. In Practice*, 12, 863–869.

[\[PubMed\]](#)

Kay, A. B. (2001). Allergy and allergic diseases. First of two parts. *The New England Journal of Medicine*, 344, 30–37.

[\[PubMed\]](#)

Kim, C. H. (2023). Complex regulatory effects of gut microbial short-chain fatty acids on immune tolerance and autoimmunity. *Cellular & Molecular Immunology*, 20, 341–350.

Kim, K. S., Hong, S. W., Han, D., Yi, J., Jung, J., Yang, B. G., Lee, J. Y., Lee, M., & Surh, C. D. (2016). Dietary antigens limit mucosal immunity by inducing regulatory T cells in the small intestine. *Science*, 351, 858–863.

[\[PubMed\]](#)

Kleniewska, P., & Pawliczak, R. (2024). Can probiotics be used in the prevention and treatment of bronchial asthma? *Pharmacological Reports*, 76, 740–753.

[\[PubMed\]](#)

Kolokotroni, O., Middleton, N., Gavatha, M., Lamnisos, D., Priftis, K. N., & Yiallourous, P. K. (2012). Asthma and atopy in children born by caesarean section: Effect modification by family history of

allergies - a population based cross-sectional study. *BMC Pediatrics*, 12, 179.

[\[PubMed\]](#)

Kong, H. H., Oh, J., Deming, C., Conlan, S., Grice, E. A., Beatson, M. A., Nomicos, E., Polley, E. C., Komarow, H. D., Murray, P. R., Turner, M. L., & Segre, J. A. (2012). Temporal shifts in the skin microbiome associated with disease flares and treatment in children with atopic dermatitis. *Genome Research*, 22, 850–859.

[\[PubMed\]](#)

Korpela, K., Salonen, A., Virta, L. J., Kekkonen, R. A., Forslund, K., Bork, P., & de Vos, W. M. (2016). Intestinal microbiome is related to lifetime antibiotic use in Finnish pre-school children. *Nature Communications*, 7, 10410.

[\[PubMed\]](#)

Koskinen, K., Reichert, J. L., Hoier, S., Schachenreiter, J., Duller, S., Moissl-Eichinger, C., & Schopf, V. (2018). The nasal microbiome mirrors and potentially shapes olfactory function. *Scientific Reports*, 8, 1296.

[\[PubMed\]](#)

Kummeling, I., Stelma, F. F., Dagnelie, P. C., Snijders, B. E., Penders, J., Huber, M., van Ree, R., van den Brandt, P. A., & Thijs, C. (2007). Early life exposure to antibiotics and the subsequent development of eczema, wheeze, and allergic sensitization in the first 2 years of life: The KOALA Birth Cohort Study. *Pediatrics*, 119, e225–e231.

[\[PubMed\]](#)

Lee, E., Park, Y. M., Lee, S. Y., Lee, S. H., Park, M. J., Ahn, K., Kim, K. W., Shin, Y. H., Suh, D. I., & Hong, S. J. (2023a). Associations of prenatal antibiotic exposure and delivery mode on childhood asthma inception. *Annals of Allergy, Asthma & Immunology*, 131, 52–58 e1.

Lee, Y.-J., Yassa, C., Park, S.-H., Song, S. W., Jung, W. H., Lee, Y. W., Kang, H., & Kim, J.-E. (2023b). Interactions between *Malassezia* and new therapeutic agents in atopic dermatitis affecting skin barrier and inflammation in recombinant human epidermis model. *International Journal of Molecular Sciences*, 24, 6171.

[\[PubMed\]](#)

Lee, E., Kim, J. H., Lee, S. Y., Lee, S. H., Park, Y. M., Oh, H. Y., Yeom, J., Ahn, H. S., Yoo, H. J., Kim, B. S., Yun, S. M., Choi, E. J., Song, K. B., Park, M. J., Ahn, K., Kim, K. W., Shin, Y. H., Suh, D. I., Song, J. Y., & Hong, S. J. (2024). Developmental trajectories of atopic dermatitis with multiomics approaches in the infant gut: COCOA birth cohort. *The Journal of Allergy and Clinical Immunology*, 155, 557.

[\[PubMed\]](#)

Leyden, J. J., Marples, R. R., & Kligman, A. M. (1974). *Staphylococcus aureus* in the lesions of atopic dermatitis. *The British Journal of Dermatology*, 90, 525–530.

[\[PubMed\]](#)

Leyva-Castillo, J. M., Galand, C., Kam, C., Burton, O., Gurish, M., Musser, M. A., Goldsmith, J. D., Hait, E., Nurko, S., Brombacher, F., Dong, C., Finkelman, F. D., Lee, R. T., Ziegler, S., Chiu, I., Austen, K. F., & Geha, R. S. (2019). Mechanical skin injury promotes food anaphylaxis by driving intestinal mast cell expansion. *Immunity*, 50, 1262–75 e4.

Lin, W., Truong, N., Grossman, W. J., Haribhai, D., Williams, C. B., Wang, J., Martin, M. G., & Chatila, T. A. (2005). Allergic dysregulation and hyperimmunoglobulinemia E in Foxp3 mutant mice. *The Journal of Allergy and Clinical Immunology*, 116, 1106–1115.

[\[PubMed\]](#)

Ling, Z., Li, Z., Liu, X., Cheng, Y., Luo, Y., Tong, X., Yuan, L., Wang, Y., Sun, J., Li, L., & Xiang, C. (2014). Altered fecal microbiota composition associated with food allergy in infants. *Applied and Environmental Microbiology*, 80, 2546–2554.

[\[PubMed\]](#)

Liu, C. M., Price, L. B., Hungate, B. A., Abraham, A. G., Larsen, L. A., Christensen, K., Stegger, M., Skov, R., & Andersen, P. S. (2015). Staphylococcus aureus and the ecology of the nasal microbiome. *Science Advances*, 1, e1400216.

[\[PubMed\]](#)

Liu, H., Archer, N. K., Dillen, C. A., Wang, Y., Ashbaugh, A. G., Ortines, R. V., Kao, T., Lee, S. K., Cai, S. S., Miller, R. J., Marchitto, M. C., Zhang, E., Riggins, D. P., Plaut, R. D., Stibitz, S., Geha, R. S., & Miller, L. S. (2017). Staphylococcus aureus epicutaneous exposure drives skin inflammation via IL-36-mediated T cell responses. *Cell Host Microbe*, 22, 653–666.

Liu, Y., Du, X., Zhai, S., Tang, X., Liu, C., & Li, W. (2022). Gut microbiota and atopic dermatitis in children: A scoping review. *BMC Pediatrics*, 22, 323.

[\[PubMed\]](#)

Lommatzsch, M., Brusselle, G. G., Levy, M. L., Canonica, G. W., Pavord, I. D., Schatz, M., & Virchow, J. C. (2023). A(2)BCD: a concise guide for asthma management. *The Lancet Respiratory Medicine*, 11, 573–576.

[\[PubMed\]](#)

Lu, Y., Wang, Y., Wang, J., Lowe, A. J., Grzeskowiak, L. E., & Hu, Y. J. (2023). Early-life antibiotic exposure and childhood asthma trajectories: A National Population-Based Birth Cohort. *Antibiotics (Basel)*, 12, 314.

[\[PubMed\]](#)

Majamaa, H., & Isolauri, E. (1997). Probiotics: A novel approach in the management of food allergy. *The Journal of Allergy and Clinical Immunology*, 99, 179–185.

[\[PubMed\]](#)

Mann, E. R., Lam, Y. K., & Uhlig, H. H. (2024). Short-chain fatty acids: Linking diet, the microbiome and immunity. *Nature Reviews. Immunology*, 24, 577–595.

[\[PubMed\]](#)

Marra, F., Marra, C. A., Richardson, K., Lynd, L. D., Kozyrskyj, A., Patrick, D. M., Bowie, W. R., & Fitzgerald, J. M. (2009). Antibiotic use in children is associated with increased risk of asthma. *Pediatrics*, 123, 1003–1010.

[\[PubMed\]](#)

Maynard, C. L., Elson, C. O., Hatton, R. D., & Weaver, C. T. (2012). Reciprocal interactions of the intestinal microbiota and immune system. *Nature*, 489, 231–241.

[\[https://doi.org/10.1038/nature11551\]](https://doi.org/10.1038/nature11551) [\[PubMed\]](#)

Morita, H., Kubo, T., Ruckert, B., Ravindran, A., Soyka, M. B., Rinaldi, A. O., Sugita, K., Wawrzyniak, M., Wawrzyniak, P., Motomura, K., Tamari, M., Orimo, K., Okada, N., Arae, K., Saito, K., Altunbulakli, C., Castro-Giner, F., Tan, G., Neumann, A., Sudo, K., O'Mahony, L., Honda, K., Nakae, S., Saito, H., Mjosberg, J., Nilsson, G., Matsumoto, K., Akdis, M., & Akdis, C. A. (2019). Induction of human regulatory innate lymphoid cells from group 2 innate lymphoid cells by retinoic acid. *The Journal of Allergy and Clinical Immunology*, 143, 2190–201 e9.

Myles, I. A., Castillo, C. R., Barbican, K. D., Kanakabandi, K., Virtaneva, K., Fitzmeyer, E., Paneru, M., Otaizo-Carrasquero, F., Myers, T. G., Markowitz, T. E., Moore, I. N., Liu, X., Ferrer, M., Sakamachi, Y., Garantziotis, S., Swamydas, M., Lionakis, M. S., Anderson, E. D., Earland, N. J., Ganesan, S., Sun, A. A., Bergerson, J. R. E., Silverman, R. A., Petersen, M., Martens, C. A., & Datta, S. K. (2020). Therapeutic responses to *Roseomonas mucosa* in atopic dermatitis may involve lipid-mediated TNF-related epithelial repair. *Science Translational Medicine*, 12, eaaz8631.
[\[PubMed\]](#)

Nakatsuji, T., Chen, T. H., Two, A. M., Chun, K. A., Narala, S., Geha, R. S., Hata, T. R., & Gallo, R. L. (2016). *Staphylococcus aureus* exploits epidermal barrier defects in atopic dermatitis to trigger cytokine expression. *The Journal of Investigative Dermatology*, 136, 2192–2200.
[\[PubMed\]](#)

Nakatsuji, T., Chen, T. H., Narala, S., Chun, K. A., Two, A. M., Yun, T., Shafiq, F., Kotol, P. F., Bouslimani, A., Melnik, A. V., Latif, H., Kim, J. N., Lockhart, A., Artis, K., David, G., Taylor, P., Streib, J., Dorrestein, P. C., Grier, A., Gill, S. R., Zengler, K., Hata, T. R., Leung, D. Y., & Gallo, R. L. (2017). Antimicrobials from human skin commensal bacteria protect against *Staphylococcus aureus* and are deficient in atopic dermatitis. *Science Translational Medicine*, 9, eaah4680.
[\[PubMed\]](#)

Natalini, J. G., Singh, S., & Segal, L. N. (2023). The dynamic lung microbiome in health and disease. *Nature Reviews. Microbiology*, 21, 222–235.
[\[PubMed\]](#)

Noval Rivas, M., Burton, O. T., Wise, P., Zhang, Y. Q., Hobson, S. A., Garcia Lloret, M., Chehoud, C., Kuczynski, J., DeSantis, T., Warrington, J., Hyde, E. R., Petrosino, J. F., Gerber, G. K., Bry, L., Oettgen, H. C., Mazmanian, S. K., & Chatila, T. A. (2013). A microbiota signature associated with experimental food allergy promotes allergic sensitization and anaphylaxis. *The Journal of Allergy and Clinical Immunology*, 131, 201–212.
[\[PubMed\]](#)

Nunn, K. L., & Forney, L. J. (2016). Unraveling the dynamics of the human vaginal microbiome. *The Yale Journal of Biology and Medicine*, 89, 331–337.
[\[PubMed\]](#)

Nwaru, B. I., Takkinen, H. M., Kaila, M., Erkkola, M., Ahonen, S., Pekkanen, J., Simell, O., Veijola, R., Ilonen, J., Hyoty, H., Knip, M., & Virtanen, S. M. (2014). Food diversity in infancy and the risk of childhood asthma and allergies. *The Journal of Allergy and Clinical Immunology*, 133, 1084–1091.
[\[PubMed\]](#)

Oddy, W. H. (2009). The long-term effects of breastfeeding on asthma and atopic disease. *Advances in Experimental Medicine and Biology*, 639, 237–251.

[\[PubMed\]](#)

Ogurlur, I., Pat, Y., Ardicli, O., Barletta, E., Cevhertas, L., Fernandez-Santamaria, R., Huang, M., Bel Imam, M., Koch, J., Ma, S., Maurer, D. J., Mitamura, Y., Peng, Y., Radzikowska, U., Rinaldi, A. O., Rodriguez-Coira, J., Satitsuksanoa, P., Schneider, S. R., Wallimann, A., Zhakparov, D., Ziadlou, R., Brügggen, M. C., van de Veen, W., Sokolowska, M., Baerenfaller, K., Zhang, L., Akdis, M., & Akdis, C. A. (2021). Advances and highlights in biomarkers of allergic diseases. *Allergy*, 76, 3659–3686.

[\[PubMed\]](#)

Oh, J., Byrd, A. L., Deming, C., Conlan, S., Kong, H. H., & Segre, J. A. (2014). Biogeography and individuality shape function in the human skin metagenome. *Nature*, 514, 59–64.

[\[https://doi.org/10.1038/nature13786\]](https://doi.org/10.1038/nature13786) [\[PubMed\]](#)

Oh, J., Byrd, A. L., Park, M., NISC Comparative Sequencing Program, Kong, H. H., & Segre, J. A. (2016). Temporal stability of the human skin microbiome. *Cell*, 165, 854–866.

[\[PubMed\]](#)

Ohnmacht, C., Park, J. H., Cording, S., Wing, J. B., Atarashi, K., Obata, Y., Gaboriau-Routhiau, V., Marques, R., Dulauroy, S., Fedoseeva, M., Busslinger, M., Cerf-Bensussan, N., Boneca, I. G., Voehringer, D., Hase, K., Honda, K., Sakaguchi, S., & Eberl, G. (2015). MUCOSAL IMMUNOLOGY. The microbiota regulates type 2 immunity through ROR γ mat(+) T cells. *Science*, 349, 989–993.

[\[PubMed\]](#)

Osborn, D. A., & Sinn, J. K. (2007). Probiotics in infants for prevention of allergic disease and food hypersensitivity. *Cochrane Database of Systematic Reviews*, 4, CD006475.

Oyama, N., Sudo, N., Sogawa, H., & Kubo, C. (2001). Antibiotic use during infancy promotes a shift in the T(H)1/T(H)2 balance toward T(H)2-dominant immunity in mice. *The Journal of Allergy and Clinical Immunology*, 107, 153–159.

[\[PubMed\]](#)

Ozcam, M., & Lynch, S. V. (2024). The gut-airway microbiome axis in health and respiratory diseases. *Nature Reviews. Microbiology*, 22, 492–506.

[\[PubMed\]](#)

Pabst, O., & Mowat, A. M. (2012). Oral tolerance to food protein. *Mucosal Immunology*, 5, 232–239.

[\[PubMed\]](#)

Paller, A. S., Kong, H. H., Seed, P., Naik, S., Scharschmidt, T. C., Gallo, R. L., Luger, T., & Irvine, A. D. (2019). The microbiome in patients with atopic dermatitis. *The Journal of Allergy and Clinical Immunology*, 143, 26–35.

[\[PubMed\]](#)

Palmer, C. N., Irvine, A. D., Terron-Kwiatkowski, A., Zhao, Y., Liao, H., Lee, S. P., Goudie, D. R., Sandilands, A., Campbell, L. E., Smith, F. J., O'Regan, G. M., Watson, R. M., Cecil, J. E., Bale, S. J., Compton, J. G., DiGiovanna, J. J., Fleckman, P., Lewis-Jones, S., Arseculeratne, G., Sergeant, A., Munro, C. S., El Houate, B., McElreavey, K., Halkjaer, L. B., Bisgaard, H., Mukhopadhyay, S., &

McLean, W. H. (2006). Common loss-of-function variants of the epidermal barrier protein filaggrin are a major predisposing factor for atopic dermatitis. *Nature Genetics*, 38, 441–446.

[\[PubMed\]](#)

Palomares, F., Gomez, F., Bogas, G., Maggi, L., Cosmi, L., Annunziato, F., Nunez, R., Perez, N., Munoz-Cano, R., Torres, M. J., & Mayorga, C. (2021). Innate lymphoid cells type 2 in LTP-allergic patients and their modulation during sublingual immunotherapy. *Allergy*, 76, 2253–2256.

[\[PubMed\]](#)

Petersen, E. B. M., Skov, L., Thyssen, J. P., & Jensen, P. (2019). Role of the gut microbiota in atopic dermatitis: A systematic review. *Acta Dermato-Venereologica*, 99, 5–11.

[\[PubMed\]](#)

Platts-Mills, T. A. (2015). The allergy epidemics: 1870-2010. *The Journal of Allergy and Clinical Immunology*, 136, 3–13.

[\[PubMed\]](#)

Plunkett, C. H., & Nagler, C. R. (2017). The influence of the microbiome on allergic sensitization to food. *Journal of Immunology*, 198, 581–589.

Pothmann, A., Illing, T., Wiegand, C., Hartmann, A. A., & Elsner, P. (2019). The microbiome and atopic dermatitis: A review. *American Journal of Clinical Dermatology*, 20, 749–761.

[\[PubMed\]](#)

Ravel, J., Gajer, P., Abdo, Z., Schneider, G. M., Koenig, S. S., McCulle, S. L., Karlebach, S., Gorle, R., Russell, J., Tacket, C. O., Brotman, R. M., Davis, C. C., Ault, K., Peralta, L., & Forney, L. J. (2011). Vaginal microbiome of reproductive-age women. *Proceedings of the National Academy of Sciences of the United States of America*, 108(Suppl 1), 4680–4687.

[\[PubMed\]](#)

Renz, H., Allen, K. J., Sicherer, S. H., Sampson, H. A., Lack, G., Beyer, K., & Oettgen, H. C. (2018). Food allergy. *Nature Reviews. Disease Primers*, 4, 17098.

[\[PubMed\]](#)

Ring, J., Brockow, K., & Behrendt, H. (2004). History and classification of anaphylaxis. *Novartis Foundation Symposium*, 257, 6–16; discussion 16–24, 45–50, 276–85.

[\[PubMed\]](#)

Rodriguez, B., Prioult, G., Bibiloni, R., Nicolis, I., Mercenier, A., Butel, M. J., & Waligora-Dupriet, A. J. (2011). Germ-free status and altered caecal subdominant microbiota are associated with a high susceptibility to cow's milk allergy in mice. *FEMS Microbiology Ecology*, 76, 133–144.

[\[PubMed\]](#)

Roduit, C., Frei, R., Ferstl, R., Loeliger, S., Westermann, P., Rhyner, C., Schiavi, E., Barcik, W., Rodriguez-Perez, N., Wawrzyniak, M., Chassard, C., Lacroix, C., Schmausser-Hechfellner, E., Depner, M., von Mutius, E., Braun-Fahrlander, C., Karvonen, A. M., Kirjavainen, P. V., Pekkanen, J., Dalphin, J. C., Riedler, J., Akdis, C., Lauener, R., O'Mahony, L., Efraim, P., & study group. (2019). High levels of butyrate and propionate in early life are associated with protection against atopy. *Allergy*, 74, 799–809.

[\[PubMed\]](#)

Russell, S. L., Gold, M. J., Hartmann, M., Willing, B. P., Thorson, L., Wlodarska, M., Gill, N., Blanchet, M. R., Mohn, W. W., McNagny, K. M., & Finlay, B. B. (2012). Early life antibiotic-driven changes in microbiota enhance susceptibility to allergic asthma. *EMBO Reports*, 13, 440–447.

[\[PubMed\]](#)

Saeed, M. A., Gribben, K. C., Alam, M., Lyden, E. R., Hanson, C. K., & LeVan, T. D. (2020). Association of Dietary Fiber on asthma, respiratory symptoms, and inflammation in the adult National Health and Nutrition Examination Survey Population. *Annals of the American Thoracic Society*, 17, 1062–1068.

[\[PubMed\]](#)

Saraf, V. S., Sheikh, S. A., Ahmad, A., Gillevet, P. M., Bokhari, H., & Javed, S. (2021). Vaginal microbiome: normalcy vs dysbiosis. *Archives of Microbiology*, 203, 3793–3802.

[\[PubMed\]](#)

Sarkar, A., Yoo, J. Y., Dutra, S. V. O., Morgan, K. H., & Groer, M. (2021). The association between early-life gut microbiota and long-term health and diseases. *Journal of Clinical Medicine*, 10, 459.

[\[PubMed\]](#)

Savage, J. H., Lee-Sarwar, K. A., Sordillo, J., Bunyavanich, S., Zhou, Y., O'Connor, G., Sandel, M., Bacharier, L. B., Zeiger, R., Sodergren, E., Weinstock, G. M., Gold, D. R., Weiss, S. T., & Litonjua, A. A. (2018). A prospective microbiome-wide association study of food sensitization and food allergy in early childhood. *Allergy*, 73, 145–152.

[\[PubMed\]](#)

Schulz, O., Jaensson, E., Persson, E. K., Liu, X., Worbs, T., Agace, W. W., & Pabst, O. (2009). Intestinal CD103+, but not CX3CR1+, antigen sampling cells migrate in lymph and serve classical dendritic cell functions. *The Journal of Experimental Medicine*, 206, 3101–3114.

[\[PubMed\]](#)

Sefik, E., Geva-Zatorsky, N., Oh, S., Konnikova, L., Zemmour, D., McGuire, A. M., Burzyn, D., Ortiz-Lopez, A., Lobera, M., Yang, J., Ghosh, S., Earl, A., Snapper, S. B., Jupp, R., Kasper, D., Mathis, D., & Benoist, C. (2015). MUCOSAL IMMUNOLOGY. Individual intestinal symbionts induce a distinct population of RORgamma(+) regulatory T cells. *Science*, 349, 993–997.

[\[PubMed\]](#)

Seite, S., Flores, G. E., Henley, J. B., Martin, R., Zelenkova, H., Aguilar, L., & Fierer, N. (2014). Microbiome of affected and unaffected skin of patients with atopic dermatitis before and after emollient treatment. *Journal of Drugs in Dermatology*, 13, 1365–1372.

[\[PubMed\]](#)

Sicherer, S. H., Warren, C. M., Dant, C., Gupta, R. S., & Nadeau, K. C. (2020). Food allergy from infancy through adulthood. *The Journal of Allergy and Clinical Immunology. In Practice*, 8, 1854–1864.

[\[PubMed\]](#)

Simpson, E. L., Schlievert, P. M., Yoshida, T., Lussier, S., Boguniewicz, M., Hata, T., Fuxench, Z., De Benedetto, A., Ong, P. Y., Ko, J., Calatroni, A., Rudman Spengel, A. K., Plaut, M., Quataert, S. A., Kilgore,

S. H., Peterson, L., Gill, A. L., David, G., Mosmann, T., Gill, S. R., Leung, D. Y. M., & Beck, L. A. (2023). Rapid reduction in *Staphylococcus aureus* in atopic dermatitis subjects following dupilumab treatment. *The Journal of Allergy and Clinical Immunology*, 152, 1179–1195.

[\[PubMed\]](#)

Smulders, T., Van Der Schee, M. P., Maitland-Van Der Zee, A. H., Dijkers, F. G., & Van Drunen, C. M. (2024). Influence of the gut and airway microbiome on asthma development and disease. *Pediatric Allergy and Immunology*, 35, e14095.

[\[PubMed\]](#)

Smythe, P., & Wilkinson, H. N. (2023). The skin microbiome: Current landscape and future opportunities. *International Journal of Molecular Sciences*, 24, 3950.

[\[PubMed\]](#)

Song, H., Yoo, Y., Hwang, J., Na, Y. C., & Kim, H. S. (2016). *Faecalibacterium prausnitzii* subspecies-level dysbiosis in the human gut microbiome underlying atopic dermatitis. *The Journal of Allergy and Clinical Immunology*, 137, 852–860.

[\[PubMed\]](#)

Song, X. L., Liang, J., Lin, S. Z., Xie, Y. W., Ke, C. H., Ao, D., Lu, J., Chen, X. M., He, Y. Z., Liu, X. H., & Li, W. (2024). Gut-lung axis and asthma: A historical review on mechanism and future perspective. *Clinical and Translational Allergy*, 14, e12356.

[\[PubMed\]](#)

Stefka, A. T., Feehley, T., Tripathi, P., Qiu, J., McCoy, K., Mazmanian, S. K., Tjota, M. Y., Seo, G. Y., Cao, S., Theriault, B. R., Antonopoulos, D. A., Zhou, L., Chang, E. B., Fu, Y. X., & Nagler, C. R. (2014). Commensal bacteria protect against food allergen sensitization. *Proceedings of the National Academy of Sciences of the United States of America*, 111, 13145–13150.

[\[PubMed\]](#)

Stokholm, J., Thorsen, J., Blaser, M. J., Rasmussen, M. A., Hjelmsø, M., Shah, S., Christensen, E. D., Chawes, B. L., Bonnelykke, K., Brix, S., Mortensen, M. S., Brejnrod, A., Vestergaard, G., Trivedi, U., Sørensen, S. J., & Bisgaard, H. (2020). Delivery mode and gut microbial changes correlate with an increased risk of childhood asthma. *Science Translational Medicine*, 12, eaax9929.

[\[PubMed\]](#)

Strachan, D. P. (1989). Hay fever, hygiene, and household size. *BMJ*, 299, 1259–1260.

[\[PubMed\]](#)

Suprun, M., Bahnson, H. T., du Toit, G., Lack, G., Suarez-Farinas, M., & Sampson, H. A. (2023). In children with eczema, expansion of epitope-specific IgE is associated with peanut allergy at 5 years of age. *Allergy*, 78, 586–589.

[\[PubMed\]](#)

Suwanchote, S., Waitayangkoon, P., Chancheewa, B., Inthanachai, T., Niwetbowornchai, N., Edwards, S. W., Virakul, S., Thammahong, A., Kiatsurayanon, C., Rerknimitr, P., & Chiewchengchol, D. (2022). Role of antimicrobial peptides in atopic dermatitis. *International Journal of Dermatology*, 61, 532–540.

[\[PubMed\]](#)

Tan, J., McKenzie, C., Vuillermin, P. J., Goverse, G., Vinuesa, C. G., Mebius, R. E., Macia, L., & Mackay, C. R. (2016). Dietary Fiber and bacterial SCFA enhance oral tolerance and protect against food allergy through diverse cellular pathways. *Cell Reports*, 15, 2809–2824.

[\[PubMed\]](#)

Tanaka, M., Korenori, Y., Washio, M., Kobayashi, T., Momoda, R., Kiyohara, C., Kuroda, A., Saito, Y., Sonomoto, K., & Nakayama, J. (2017). Signatures in the gut microbiota of Japanese infants who developed food allergies in early childhood. *FEMS Microbiology Ecology*, 93, fix099.

Tang, M. L., Ponsonby, A. L., Orsini, F., Tey, D., Robinson, M., Su, E. L., Licciardi, P., Burks, W., & Donath, S. (2015). Administration of a probiotic with peanut oral immunotherapy: A randomized trial. *The Journal of Allergy and Clinical Immunology*, 135, 737–44 e8.

[\[PubMed\]](#)

Taylor, S. L., Leong, L. E. X., Choo, J. M., Wesselingh, S., Yang, I. A., Upham, J. W., Reynolds, P. N., Hodge, S., James, A. L., Jenkins, C., Peters, M. J., Baraket, M., Marks, G. B., Gibson, P. G., Simpson, J. L., & Rogers, G. B. (2018). Inflammatory phenotypes in patients with severe asthma are associated with distinct airway microbiology. *Journal of Allergy and Clinical Immunology*, 141, 94–103.e15.

[\[PubMed\]](#)

Tokumasu, R., Yamaga, K., Yamazaki, Y., Murota, H., Suzuki, K., Tamura, A., Bando, K., Furuta, Y., Katayama, I., & Tsukita, S. (2016). Dose-dependent role of claudin-1 in vivo in orchestrating features of atopic dermatitis. *Proceedings of the National Academy of Sciences of the United States of America*, 113, E4061–E4068.

[\[PubMed\]](#)

Tordesillas, L., & Berin, M. C. (2018). Mechanisms of oral tolerance. *Clinical Reviews in Allergy and Immunology*, 55, 107–117.

[\[PubMed\]](#)

Traidl-Hoffmann, C., Jakob, T., & Behrendt, H. (2009). Determinants of allergenicity. *The Journal of Allergy and Clinical Immunology*, 123, 558–566.

[\[PubMed\]](#)

Tran, M. M., Lefebvre, D. L., Dharma, C., Dai, D., Lou, W. Y. W., Subbarao, P., Becker, A. B., Mandhane, P. J., Turvey, S. E., Sears, M. R., & Investigators Canadian Healthy Infant Longitudinal Development Study. (2018). Predicting the atopic march: Results from the Canadian Healthy Infant Longitudinal Development Study. *The Journal of Allergy and Clinical Immunology*, 141, 601–07 e8.

Trompette, A., Gollwitzer, E. S., Yadava, K., Sichelstiel, A. K., Sprenger, N., Ngom-Bru, C., Blanchard, C., Junt, T., Nicod, L. P., Harris, N. L., & Marsland, B. J. (2014). Gut microbiota metabolism of dietary fiber influences allergic airway disease and hematopoiesis. *Nature Medicine*, 20, 159–166.

[\[PubMed\]](#)

Volz, T., Nega, M., Buschmann, J., Kaesler, S., Guenova, E., Peschel, A., Röcken, M., Götz, F., & Biedermann, T. (2010). Natural *Staphylococcus aureus*-derived peptidoglycan fragments activate NOD2 and act as potent costimulators of the innate immune system exclusively in the presence of TLR signals. *The FASEB Journal*, 24, 4089–4102.

Volz, T., Skabytska, Y., Guenova, E., Chen, K. M., Frick, J. S., Kirschning, C. J., Kaesler, S., Rocken, M., & Biedermann, T. (2014). Nonpathogenic bacteria alleviating atopic dermatitis inflammation induce IL-10-producing dendritic cells and regulatory Tr1 cells. *The Journal of Investigative Dermatology*, 134, 96–104.

[\[PubMed\]](#)

Wang, S., Liu, B., Huang, J., He, H., Zhou, L., He, Y., Yan, J., & Tao, A. (2023). Succinate and mitochondrial DNA trigger atopic march from atopic dermatitis to intestinal inflammation. *The Journal of Allergy and Clinical Immunology*, 151, 1050–66 e7.

Waser, M., Michels, K. B., Bieli, C., Floistrup, H., Pershagen, G., von Mutius, E., Ege, M., Riedler, J., Schram-Bijkerk, D., Brunekreef, B., van Hage, M., Lauener, R., & Braun-Fahrlander, C. (2007). Inverse association of farm milk consumption with asthma and allergy in rural and suburban populations across Europe. *Clinical and Experimental Allergy*, 37, 661–670.

[\[PubMed\]](#)

Watanabe, S., Narisawa, Y., Arase, S., Okamatsu, H., Ikenaga, T., Tajiri, Y., & Kumemura, M. (2003). Differences in fecal microflora between patients with atopic dermatitis and healthy control subjects. *The Journal of Allergy and Clinical Immunology*, 111, 587–591.

[\[PubMed\]](#)

Wawryk-Gawda, E., Markut-Miotla, E., & Emeryk, A. (2021). Postnatal probiotics administration does not prevent asthma in children, but using prebiotics or synbiotics may be the effective potential strategies to decrease the frequency of asthma in high-risk children - a meta-analysis of clinical trials. *Allergol Immunopathol (Madr)*, 49, 4–14.

[\[PubMed\]](#)

Wei, X., Jiang, P., Liu, J., Sun, R., & Zhu, L. (2020). Association between probiotic supplementation and asthma incidence in infants: A meta-analysis of randomized controlled trials. *The Journal of Asthma*, 57, 167–178.

[\[PubMed\]](#)

Weidinger, S., Beck, L. A., Bieber, T., Kabashima, K., & Irvine, A. D. (2018). Atopic dermatitis. *Nature Reviews Disease Primers*, 4, 1.

[\[PubMed\]](#)

Weiner, H. L., da Cunha, A. P., Quintana, F., & Wu, H. (2011). Oral tolerance. *Immunological Reviews*, 241, 241–259.

[\[PubMed\]](#)

Weissler, K. A., Rasooly, M., DiMaggio, T., Bolan, H., Cantave, D., Martino, D., Neeland, M. R., Tang, M. L. K., Dang, T. D., Allen, K. J., & Frischmeyer-Guerrerio, P. A. (2018). Identification and analysis of peanut-specific effector T and regulatory T cells in children allergic and tolerant to peanut. *The Journal of Allergy and Clinical Immunology*, 141, 1699–710 e7.

Wells, H. G. (1911). Studies on the chemistry of Anaphylaxis (III). Experiments with isolated proteins, especially those of the hen's egg. *The Journal of Infectious Diseases*, 9, 147–171.

Werfel, T. (2015). Atopische dermatitis. In T. Biedermann, W. Heppt, H. Renz, & M. Röcken (Eds.),

Allergologie. Springer-Verlag.

Wildin, R. S., Smyk-Pearson, S., & Filipovich, A. H. (2002). Clinical and molecular features of the immunodysregulation, polyendocrinopathy, enteropathy, X linked (IPEX) syndrome. *Journal of Medical Genetics*, 39, 537–545.

[\[PubMed\]](#)

Williams, M. R., Nakatsuji, T., Sanford, J. A., Vrbanc, A. F., & Gallo, R. L. (2017). Staphylococcus aureus induces increased serine protease activity in Keratinocytes. *The Journal of Investigative Dermatology*, 137, 377–384.

[\[PubMed\]](#)

Wölbing, F., & Biedermann, T. (2013). Anaphylaxis: Opportunities of stratified medicine for diagnosis and risk assessment. *Allergy*, 68, 1499–1508.

[\[PubMed\]](#)

Wypych, T. P., Wickramasinghe, L. C., & Marsland, B. J. (2019). The influence of the microbiome on respiratory health. *Nature Immunology*, 20, 1279–1290.

[\[PubMed\]](#)

Xue, M., Dehaas, E., Chaudhary, N., O'Byrne, P., Satia, I., & Kurmi, O. P. (2021). Breastfeeding and risk of childhood asthma: A systematic review and meta-analysis. *ERJ Open Research*, 7, 00504.

[\[PubMed\]](#)

Yamashita, H., Takahashi, K., Tanaka, H., Nagai, H., & Inagaki, N. (2012). Overcoming food allergy through acquired tolerance conferred by transfer of Tregs in a murine model. *Allergy*, 67, 201–209.

[\[PubMed\]](#)

Yamazaki, Y., Nakamura, Y., & Núñez, G. (2017). Role of the microbiota in skin immunity and atopic dermatitis. *Allergology International*, 66, 539–544.

[\[PubMed\]](#)

Yip, W., Hughes, M. R., Li, Y., Cait, A., Hirst, M., Mohn, W. W., & McNagny, K. M. (2021). Butyrate shapes immune cell fate and function in allergic asthma. *Frontiers in Immunology*, 12, 628453.

[\[PubMed\]](#)

Yoshida, T., Beck, L. A., & De Benedetto, A. (2022). Skin barrier defects in atopic dermatitis: From old idea to new opportunity. *Allergology International*, 71, 3–13.

[\[PubMed\]](#)

Zhang, G. Q., Hu, H. J., Liu, C. Y., Zhang, Q., Shakya, S., & Li, Z. Y. (2016). Probiotics for prevention of atopy and food hypersensitivity in early childhood: A PRISMA-compliant systematic review and meta-analysis of randomized controlled trials. *Medicine (Baltimore)*, 95, e2562.

[\[PubMed\]](#)

Zhou, B., Liang, S., Shang, S., & Li, L. (2023). Association of TLR2 and TLR9 gene polymorphisms with atopic dermatitis: A systematic review and meta-analysis with trial sequential analysis. *Immunological Medicine*, 46, 32–44.

[\[PubMed\]](#)

Zhou, X., Shen, X., Johnson, J. S., Spakowicz, D. J., Agnello, M., Zhou, W., Avina, M., Honkala, A., Chleilat, F., Chen, S. J., Cha, K., Leopold, S., Zhu, C., Chen, L., Lyu, L., Hornburg, D., Wu, S., Zhang, X., Jiang, C., Jiang, L., Jiang, L., Jian, R., Brooks, A. W., Wang, M., Contrepois, K., Gao, P., Rose, S. M. S., Tran, T. D. B., Nguyen, H., Celli, A., Hong, B. Y., Bautista, E. J., Dorsett, Y., Kavathas, P. B., Zhou, Y., Sodergren, E., Weinstock, G. M., & Snyder, M. P. (2024). Longitudinal profiling of the microbiome at four body sites reveals core stability and individualized dynamics during health and disease. *Cell Host & Microbe*, 32, 506–26 e9.

13. Microbiome and Diseases: Graft-Versus-Host Disease

D. Weber¹✉, H. Poeck¹✉ and E. Holler¹✉

(1) Experimentelle allogene KMT – Innere Medizin III,
Universitätsklinikum Regensburg, Regensburg, Germany

✉ **D. Weber (Corresponding author)**

Email: daniela.weber@ukr.de

✉ **H. Poeck**

Email: hendrik.poeck@ukr.de

✉ **E. Holler**

Email: ernst.holler@ukr.de

Abstract

Acute graft-versus-host disease (GvHD) of the gastrointestinal (GI) tract remains a major cause of severe morbidity and mortality following allogeneic stem cell transplantation (ASCT). The intestinal “microbiome” has been a focus of research in the pathophysiology of acute GI GvHD for many years. In 1974, van Bekkum and colleagues reported a possible role of microbiota, observing that mice kept under germ-free conditions did not develop acute GI GvHD. Clinical studies showed that total or selective gut decontamination and isolation reduced the severity of GvHD, which became standard practice for years. However, with the advent of new molecular techniques such as 16S rRNA sequencing, our understanding of the relationship between

microbiota and GI GvHD has evolved. During ASCT, an early loss of intestinal microbiome diversity—particularly concerning commensal *Clostridiales*—and a shift toward an enteropathogenic flora have been observed, which are associated with increased GvHD-related mortality. A major risk factor for the loss of commensal bacteria is the use of systemic broad-spectrum antibiotics for prophylaxis and treatment of neutropenic infections, which are common in ASCT patients. Both the type of antibiotics and the timing of their administration influence patient outcomes after ASCT. Furthermore, damage and destruction of Paneth cells by GvHD itself reduce the production of antimicrobial peptides, further aggravating intestinal dysbiosis during GvHD. Although the exact underlying mechanisms still need to be fully elucidated, these findings suggest that microbiota manipulation could be a promising approach in the future—not only for the prophylaxis and treatment of acute GI GvHD but also for eradicating colonization with multiple antibiotic-resistant pathogens to prevent infections or even relapse.

Keywords Graft-versus-host Disease – Multiple Antibiotic-resistant Pathogens – Severe GvHD – Dysbiosis – GvHD-related Mortality.

13.1 Introduction

Acute and chronic graft-versus-host disease (GvHD), along with associated immunodeficiencies, remain the primary causes of transplant-related mortality (TRM) and long-term morbidity in patients undergoing allogeneic stem cell transplantation (ASCT) for hematological malignancies such as acute leukemias (Ferrara et al., [2009](#)). Although advances in supportive care and toxicity reduction have generally improved ASCT outcomes, approximately 15–20% of patients still experience 1-year TRM, and over 50% require long-term immunosuppression, which carries all associated side effects and long-term complications (Gooley et al., [2010](#)). The activation of donor T lymphocytes—co-transplanted with the stem cell graft—by major and

minor HLA differences is a critical step in the pathophysiology of GvHD. However, this activation is heavily regulated by the tissue microenvironment. The main target organs of acute GvHD are the skin, gut, and liver, while chronic GvHD additionally involves the eyes, mouth, lungs, and urogenital system. A common feature across all these target organs is their exposure to and extensive interaction with the respective microbiota, which already suggests a significant role of microbiota in the pathophysiology of GvHD and other ASCT-related complications.

13.2 The Early Times: van Bekkum and the Clinical Impact of Decontamination

The role of microbiota in intestinal GvHD was described early in experimental ASCT. Van Bekkum reported that mice receiving bone marrow did not experience GvHD at all if they were housed under germ-free conditions for at least 40 days after transplantation. A stepwise reduction of the germ-free period resulted in a corresponding increase in GvHD-related mortality, with nearly more than 90% of mice transplanted under conventional housing dying from GvHD. This effect of the “intestinal flora” was also observed when gut tissue from germ-free versus conventionally housed mice was transplanted into recipients, followed by GvHD induction: again, the histological GvHD score was significantly higher in the gut of mice exposed to a conventional flora (van Bekkum et al., [1974](#); van Bekkum & Knaan, [1977](#)). These observations prompted experiments in dogs and monkeys, confirming that maximal decontamination could help prevent GvHD. Early reports of patients with aplastic anemia and children transplanted under maximal decontamination in protective environments supported these findings (Vriesendorp et al., [1982](#); Storb et al., [1983](#); Schmeiser et al., [1985](#)). Later, protective environments and decontamination were combined with prophylactic antibiotics, such as quinolones, to reduce the incidence and mortality from neutropenic infections (Gaftor-Gvili et al., [2005](#)). The Essen group conducted the

only randomized study comparing prophylactic ciprofloxacin alone with a combination of ciprofloxacin and metronidazole; the combined approach was associated with significantly less acute GvHD and improved outcomes (Beelen et al., [1999](#)). To this day, different centers follow varying standards of decontamination and prophylactic antibiotic use, ranging from maximal to almost no prophylaxis, as further studies are lacking or have yielded inconclusive results (Whangbo et al., [2017](#)).

13.3 Indirect Evidence for a More Complex Role of Microbiota

In the 1990s, experimental and clinical studies indicated that T cell activation by antigen-presenting cells required inflammatory signals to induce antigen expression. Proinflammatory cytokines such as TNF (Piguet et al., [1987](#); Holler et al., [1990](#)) and IL-1 (Ferrara et al., [2009](#)) were shown to be involved both in the initiation and effector phases of GvHD, ultimately resulting in a cytokine storm. Translocated LPS via the damaged intestinal mucosa was recognized as a major trigger of inflammation, as confirmed by experiments using LPS-sensitive and LPS-resistant mice (Cooke et al., [2001](#), [2002](#)).

With the discovery of Toll-like receptors (TLRs) and NOD-like receptors (NLRs) and their respective ligands, it became clear that multiple ligands—either derived from pathogens such as bacteria and viruses (so-called PAMPs) or endogenous ligands from damaged tissue, known as DAMPs (Zeiser et al., [2011](#); Apostolova & Zeiser, [2016](#))—can activate and initiate this inflammatory response, subsequently leading to GvHD. Parallels to the pathophysiology of inflammatory bowel diseases (IBD) were also described: NOD2/CARD15 and ATG16L1 gene polymorphisms, which are known risk factors for IBD, have been identified as markers for a higher risk of GvHD. A direct role of NOD2/CARD15 in GvHD has been demonstrated in murine knockout models (Holler et al., [2006](#); Penack et al., [2009](#)).

Since NOD2/CARD15 is involved in regulating the production of antibacterial peptides by Paneth cells (Bevins et al., [2009](#)), these findings suggest a strong interaction between GvHD-related inflammation and antibacterial defense mechanisms in target tissues such as the gastrointestinal tract and intestinal microbiota. Interestingly, the prognostic significance of NOD2/CARD15 polymorphisms varies considerably between different transplant centers, and the type of gastrointestinal decontamination employed appears to partially explain this heterogeneity (Holler et al., [2006](#)). Subsequent studies have reported heterogeneous results regarding the prognostic value of NOD2/CARD15, further supporting the idea that these associations are highly dependent on specific inflammatory contexts.

13.4 Microbiome Analysis Reveals Severe Dysbiosis Early After SCT

While early data on decontamination primarily relied on culture-based analyses of intestinal and stool bacteria, and these culture-dependent studies have shown some benefit of decontamination (Vossen et al., [2014](#)), they may have overlooked a large proportion of bacteria, especially commensal bacteria. The advent of 16S rRNA sequencing within the Human Microbiome Project has revolutionized our understanding, including in the context of ASCT. The Memorial Sloan Kettering group, led by Eric Pamer and Marcel van den Brink, was the first (Jenq et al., [2012](#)) to report microbiota sequencing in both murine models of GvHD and human patients. They found a strong association between dysbiosis, reduced microbial diversity, and GvHD. In murine models, modulation of the microbiota significantly impacted survival: elimination of *Lactobacillales* worsened GvHD, while restoring this genus improved outcomes. Similar microbial changes were observed in patients with GvHD. Our group applied 16S rRNA sequencing to serial samples from 32 patients. Consistent with Jenq's findings, we observed a marked loss of diversity early after SCT, with a notable increase in

enterococci, particularly in patients developing acute gastrointestinal GvHD (Holler et al., [2014](#)). At the same time, Taur reported a direct association between early microbiota diversity post-engraftment and mortality (Taur et al., [2014](#)). Subsequently, we confirmed the link between early microbiota disruption, reduced diversity, and increased transplant-related mortality (TRM) and GvHD by using an indirect approach: analyzing urine levels of indoxyl sulfate (IS) in a large patient cohort. As shown in 2014 and 2015, urinary IS results from bacterial indoles produced by commensal gut bacteria and conjugated in the liver. Patients with low IS levels within the first 10 days after ASCT had worse outcomes, and low IS was an independent risk factor for non-relapse mortality (NRM) (Weber et al., [2015](#)). The protective role of commensal anaerobic bacteria, especially *Blautia* spp., was further confirmed by Jenq et al. ([2015](#)). Patients with abundant *Blautia* at the time of engraftment had a low TRM of around 5%, whereas those with a loss of *Blautia* faced nearly 30% TRM. Several single-center studies have also supported these findings, linking early dysbiosis to increased GvHD incidence and poorer outcomes (Golob et al., [2017](#); Doki et al., [2017](#); Kaysen et al., [2017](#); Bilinski et al., [2016](#)).

In the meantime, Peled and colleagues demonstrated in a first multicenter study comprising 8767 fecal samples obtained from 1362 patients undergoing ASCT at four different centers across three continents that patterns of microbiota disruption during ASCT were similar across transplantation centers and geographic locations. Microbial patterns characterized by a loss of diversity and domination by single taxa such as *Enterococci* were associated with high transplant-related mortality, primarily due to acute GvHD. Conversely, higher diversity of the intestinal microbiota at the time of neutrophil engraftment was linked to lower mortality (Peled et al., [2020](#); Malard et al., [2023](#)). Additionally, Stein-Thoeringer et al. confirmed that *Enterococcus* predomination in both humans and murine models of ASCT was associated with the development of acute gastrointestinal GvHD and increased mortality. In gnotobiotic models, *Enterococcus* overgrowth was able to exacerbate disease severity, and its growth was

dependent on the disaccharide lactose. Therefore, dietary lactose depletion was able to attenuate *Enterococcus* overgrowth and reduce the severity of GvHD in mice (Stein-Thoeringer et al., [2019](#)). The role of *Enterococci* in the pathophysiology of acute GI GvHD warrants further investigation. In particular, future research will focus on whether *Enterococci* act as true aggravators of GVHD or merely serve as indirect indicators of microbiome diversity loss.

The correlation between gut microbiota diversity and survival, which has been studied in adult ASCT recipients, has recently been confirmed in pediatric patients. Gut microbiota diversity prior to ASCT serves as a predictor of mortality in children undergoing the procedure: high diversity, characterized by high relative abundances of potentially health-associated microbial families such as *Ruminococcaceae* and *Oscillospiraceae*, was linked to improved survival. In contrast, low diversity, with an overrepresentation of *Enterococcaceae* and *Enterobacteriaceae*—particularly *Enterococcus*, *Escherichia-Shigella*, and *Enterobacter*—was associated with increased transplant-related mortality and a higher likelihood of developing acute GvHD (Masetti et al., [2023](#)).

Furthermore, Margolis et al. demonstrated that microbial patterns evaluated at two distinct timepoints during ASCT—preconditioning and neutrophil recovery—could predict the development of infections and acute GvHD (Margolis et al., [2023](#)).

13.5 Causes of Early Dysbiosis

13.5.1 Antibiotic Prophylaxis and Treatment

In all experimental and clinical analyses reported so far, antibiotic prophylaxis used for infection prevention and decontamination, as well as broad-spectrum antibiotics administered for neutropenic fever and infections in ASCT patients, have been identified as key factors driving early dysbiosis (Jenq et al., [2012](#); Holler et al., [2014](#); Weber et al., [2015](#)). Shono and colleagues clearly demonstrated that antibiotics strongly suppressing commensal bacteria—such as carbapenems and

piperacillin/tazobactam—induce more GvHD in both experimental and clinical SCT. Conversely, antibiotics with some sparing effect on commensal and anaerobic bacteria, like cefepime or aztreonam, appear to be more protective (Shono et al., [2016](#)).

In an analysis of over 600 patients transplanted at MSKCC in New York and in Regensburg, we recently showed that not only the use of these broad-spectrum antibiotics in general but also the timing of their administration is critical. Patients who started these antibiotics prior to the day of transplantation had increased non-relapse mortality (NRM) compared to those who initiated treatment later. The best survival rates were observed in the small subgroup—about 8% of patients—who never required systemic antibiotics beyond prophylaxis (Weber et al., [2017b](#)).

Early initiation of systemic antibiotics resulted in an earlier loss of *Clostridia* and a deeper suppression of early intestinal microbiota (IM) levels, again linking early suppression of commensals with increased GvHD risk. Surprisingly, the early use of broad-spectrum antibiotics emerged as an independent risk factor for outcome, as even low-risk patients—who would otherwise be expected to have a superior prognosis—showed this strong negative effect (Fig. [13.1](#)).

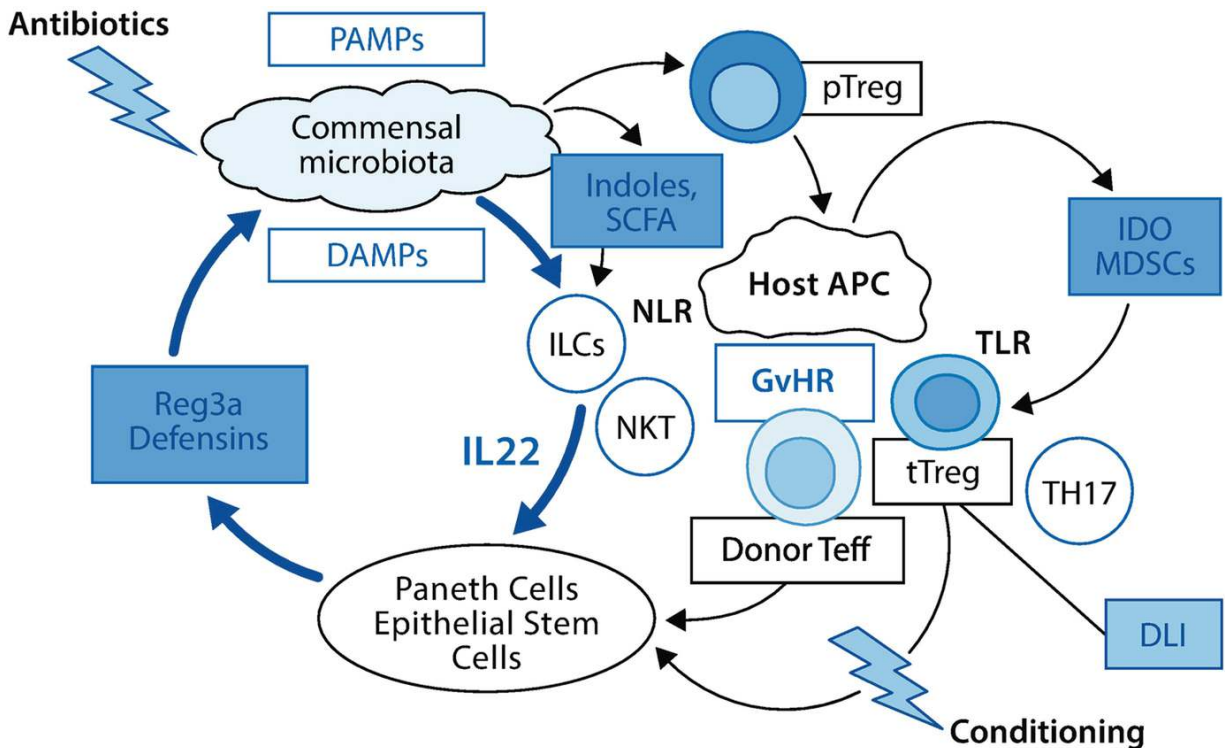


Fig. 13.1 Regulation of graft-versus-host reactions by microbiota

Given the negative impact of early antibiotic use on transplant outcomes, our transplant unit at the university hospital in Regensburg has revised its antibiotic strategy. We have shifted from a permissive approach—initiating antibiotics in all patients with neutropenic fever—to a more restrictive use, applying antibiotics only in cases with a high likelihood of cytokine release syndrome (e.g., after anti-thymocyte globulin therapy). This restrictive approach delayed the start of antibiotic administration and significantly reduced the duration of antibiotic treatment without an increase in infectious complications. Additionally, we observed beneficial effects of this strategy on microbiome diversity, as well as a positive trend toward a reduced incidence of severe gastrointestinal GvHD (Weber et al., [2023a](#)).

Randomized prospective studies on antibiotic prophylaxis are currently lacking, and the question of whether antibiotic prophylaxis during ASCT is beneficial or potentially harmful remains unresolved. Our group discontinued the prophylactic use of ciprofloxacin and metronidazole after observing a high abundance of *Enterococci*, along

with a significant prevalence of vancomycin-resistant *Enterococcus* (VRE) in patients. We transitioned to prophylaxis with low doses of the non-absorbable antibiotic rifaximin, which is commonly used for the treatment of travelers' diarrhea.

In a retrospective analysis, patients receiving rifaximin prophylaxis exhibited higher urinary IS levels and a decreased incidence of severe graft-versus-host disease (GvHD) (Weber et al., [2016](#)). Supporting the notion of a negative impact of antibiotic prophylaxis, a study from Canadian centers (Routy et al., [2016](#)) found that patients on antibiotic prophylaxis had nearly double the incidence of severe gastrointestinal GvHD (21% vs. 11%, $p < 0.001$) and reduced overall survival compared to those not receiving any prophylaxis. Furthermore, recent data from an Australian research group indicate that rifaximin, which has been used prophylactically in patients with liver disease to prevent hepatic encephalopathy, may cause cross-resistance to daptomycin, a last-resort antibiotic for vancomycin-resistant *Enterococcus faecium*. Therefore, the use of rifaximin for prophylaxis in ASCT should be reconsidered due to its potential to induce daptomycin resistance (Turner et al., [2024](#)).

In summary, there is no doubt that long-term antibiotic use induces luminal and mucosal dysbiosis, which negatively affects transplant outcomes. However, earlier studies showing a reduced incidence of graft-versus-host disease (GvHD) in germ-free mice, along with recent findings, suggest that microbiota suppression and antibiotics can also positively influence bacterial translocation. This translocation is a hallmark of GvHD initiation, as bacterial pathogen-associated molecular patterns (PAMPs) trigger T cell stimulation and activation (Hülsdünker et al., [2018](#), [2019](#)).

Furthermore, in the context of immunotherapy for hematological and other malignancies using immune checkpoint inhibitors, there is evidence that antibiotics administered, particularly before immunotherapy, negatively impact tumor response and survival. This effect cannot be solely attributed to intestinal dysbiosis; rather, it is more closely linked to bacterial translocation into tumors and

lymphatic organs, as well as the suppression of T cell costimulation caused by antibiotic therapy (Routy et al., [2018](#); Thomas et al., [2023](#)).

Thus, a more nuanced understanding of the mechanisms and modes of action of antibiotic interference can help explain some of the reported paradoxical effects. These mechanisms must be taken into account when developing future intervention strategies. In the context of ASCT, more sophisticated timing of antibiotic administration may enhance overall antibiotic stewardship. Additionally, strategies that preserve luminal and mucosal eubiosis while preventing translocation may be necessary.

13.5.2 Paneth Cell Damage in GvHD

In an effort to identify biomarkers of GvHD, a proteomic approach has highlighted several candidates, including Reg3 α , the human homologue of murine Reg3y, as a biomarker for intestinal GvHD (Paczesny et al., [2009](#)). The specificity of Reg3 α was confirmed in a study involving samples from Ann Arbor, Regensburg, and Kyushu (Ferrara et al., [2011](#)). Interestingly, Reg3 α is an antimicrobial peptide produced by Paneth cells. In a subsequent study analyzing biopsies from patients with intestinal GvHD, we demonstrated that damage and destruction of Paneth cells serve as sensitive histological markers of human GvHD (Levine et al., [2013](#)). Paneth cells are located in the crypts beneath epithelial stem cells, which are also vulnerable targets in intestinal GvHD. This aligns with findings of Paneth cell damage and subsequent dysbiosis observed in murine models of GvHD (Eriguchi et al., [2012](#), [2013](#), [2015](#)).

We reported that in patients with severe GvHD, there was a loss of expression of all major defensins (HBD5, HBD6, and Reg3 α) in ileal biopsies, which correlated with increased systemic levels of Reg3 α (Weber et al., [2017a](#)). More recently, we discovered that Reg3 α levels are already elevated early in the course of ASCT, even on the day of stem cell infusion, when acute GvHD cannot yet be the cause of Paneth cell destruction. Interestingly, this early increase in Reg3 α concentrations was predictive of the risk of TRM. Since elevated Reg3 α levels on the

day of ASCT correlated with intestinal dysbiosis induced by early antibiotic treatment, we hypothesize that Reg3 α may serve not only as a biomarker for acute GI GvHD but also as an early indicator of intestinal mucosal damage following antibiotic-induced dysbiosis and conditioning therapy (Weber et al., [2023b](#)).

Beyond Paneth cells, other protective enteroendocrine epithelial L cells have been described as playing a role in supporting intestinal stem cells and modulating immune responses through antimicrobial peptides. Additionally, glucagon-like peptide-2 (GLP-2) agonists have been shown to repair L cells and Paneth cells and are currently being explored in clinical trials for GvHD (Norona et al., [2020](#)).

13.6 Potential Mechanisms Explaining a Pathophysiological Role of Dysbiosis in GvHD

So far, the damaging mechanisms associated with dysbiosis in GvHD are not different from those reported for microbiota-dependent diseases in general. Therefore, we will focus here on specific findings in the context of SCT.

13.6.1 Microbial Metabolites and GvHD

Based on the observation that histone deacetylase inhibitors modulate the immunosuppressive enzyme indoleamine dioxygenase and subsequently influence GvHD (Reddy et al., [2008](#)), the group of Pavan Reddy became interested in short-chain fatty acids, especially butyrate, as natural HDAC inhibitors derived from bacterial metabolites. They recently demonstrated a reduction of butyrate levels in the intestinal walls of mice suffering from GvHD; restoring commensal *Clostridia* in a BMT model not only increased butyrate levels but also improved GvHD-related mortality. Similar effects were observed with butyrate gavage (Mathewson et al., [2016](#)). Reddy and colleagues recently summarized the potential impact of bacterial metabolites on GvHD (Riwes & Reddy, [2017](#)), indicating that bacterial metabolites—such as short-chain fatty acids like butyrate, bile acids, polyamines, and indoles binding to the

aryl hydrocarbon receptor (AHR)—may play a beneficial role in epithelial and immune regulation during GvHD.

In line with this, Orberg and colleagues recently identified a microbiome signature involving *Lachnospiraceae* and *Oscillospiraceae* and their associated bacteriophages, which correlated with the production of immunomodulatory metabolites (IMMs) such as short-chain and branched-chain fatty acids, as well as interferon-1-inducing metabolites. The production of IMMs strongly correlated with outcomes in ASCT recipients and was significantly suppressed by broad-spectrum antibiotics but could be restored through fecal microbiota transfer. Based on these findings, they developed the IMM risk index (IMM-RI), a combination of five microbiota-derived metabolites that could predict survival rates and relapse risk in ASCT patients (Orberg et al., [2024](#)). However, this correlation still needs to be validated in larger, prospective cohorts of ASCT patients.

In our own research, we used the microbiome biomarker and metabolite indoxyl sulfate, which is derived from commensal bacteria and hydroxylated in the liver, in in vitro cultures of dendritic cells and mixed lymphocyte reactions. Indeed, physiologically relevant concentrations of indoxyl sulfate induced an anti-inflammatory cytokine profile in dendritic cells, characterized by low IL-12 and IL-6 levels and high IL-10 levels. This translated into diminished IFN- γ release in mixed lymphocyte reactions (Ghimire et al., [2018](#)).

13.6.2 Mucus Layer and GvHD

Antibiotics have repeatedly been shown to worsen dysbiosis and subsequent GvHD. Shono et al. ([2016](#)) reported a higher incidence of GvHD and related mortality in both patients and mice treated with imipenem compared to those treated with fourth-generation cephalosporins. They did not find differences in regulatory or effector T cells in imipenem-treated mice but observed increased expression of IL-23 and neutrophil influx in the colon. Notably, treatment with imipenem in mice with GvHD led to the loss of the protective mucus lining of the colon and compromised intestinal barrier function, as

evidenced by increased FITC-dextran uptake. Sequencing of mouse stool samples showed an increase in *Akkermansia muciniphila*, a gut bacterium known for mucus-degrading capabilities, raising the possibility that mucus degradation may contribute to GvHD in mice. More recently, the aggravation of GvHD by *Bacteroides* was reported in a murine model and was attributed to the high levels of mucolytic enzymes produced by this bacterium (Hayase et al., [2022](#)).

13.6.3 Microbiota-Dependent Effector Cells

Interleukin-22 (IL-22), produced by recipient innate lymphoid cells (ILCs), has been reported to prevent epithelial stem cell apoptosis in the GI tract and to protect mice from lethal GvHD (Hanash et al., [2012](#)). The release of IL-22 by ILCs is strongly regulated by the binding of metabolites—such as indoles—from commensal bacteria to the AHR. This mechanism may further contribute to epithelial instability in GvHD. Yang and colleagues demonstrated that microbiota-derived short-chain fatty acids (SCFAs) promote IL-22 production by ILCs and T cells through the G-protein-coupled receptor 41 (GPR41) by inhibiting histone deacetylases (Yang et al., [2020](#)). Additionally, Chun et al. showed that GPR43 regulates the expansion of ILC3 cells and IL-22 expression via the AKT and STAT3 signaling pathways, further supporting the role of microbiota in modulating IL-22 production (Chun et al., [2019](#)). In this context, Ghimire et al. reported, for the first time, clinical data on tissue expression of IL-22 in adult patients who developed acute GvHD after ASCT. The expression of IL-22 was significantly regulated by the expression of SCFA receptors GPR41 and GPR43. Lower IL-22 levels were associated with a higher probability of TRM after ASCT and appeared to be microbiota-dependent, as the use of broad-spectrum antibiotics significantly reduced IL-22 expression. These findings highlight an essential role for IL-22 in the prognosis of patients undergoing ASCT (Ghimire et al., [2022](#)).

Besides ILCs, neutrophils are increasingly recognized as amplifiers of GvHD-related inflammation. The group of R. Zeiser observed increased accumulation of neutrophils in the ileum of GvHD mice,

which was not seen in germ-free mice. Depleting neutrophils with antibodies reduced GvHD severity and mortality, indicating a microbiota-neutrophil recruitment pathway of tissue damage in GvHD (Schwab et al., [2014](#)). This can be explained by increased inflammation through reactive oxygen species (ROS).

IL-17, produced by certain T cell populations, may worsen GvHD but can also modulate inflammation depending on the involved cellular subtypes. Recently, Hill et al. reported that IL-17 has a protective role, as mice unable to signal via the IL-17 receptor developed hyperactive GvHD. Interestingly, susceptibility to GvHD could be transferred via the microbiota, since wild-type mice cohoused with IL-17 receptor A/C (IL17RA/RC) deficient mice also developed accelerated and more severe GvHD (Varelias et al., [2017](#)). The authors speculate that host-derived IL-17 contributes to increased expression of antimicrobial peptides, such as Reg3 γ , in these mice.

13.6.4 Specific Immune Responses: Effector and Regulatory T Cells and B Cells

Specific immune responses within the adaptive immune system are at the core of GvHD. A recent study in mice showed that antibiotic treatment has profound effects on nearly all specific immune cell populations, including regulatory T cells and B cells. These effects can be partially reversed through experimental FMT. Functionally, these immune cells significantly alter their cytokine profiles, especially those cytokines important for intestinal homeostasis, such as IL-17 and IL-22 (Ekmekciu et al., [2017](#)). However, the study did not address antigen specificity.

Recent data from healthy individuals and patients with IBD indicate a broad and polyclonal T cell response against commensal bacteria, supporting this hypothesis. The immunological situation in ASCT is even more complex due to the exchange of recipient immune cells with donor immune cells. An unanswered question is whether the different T cell and B cell repertoires of the stem cell donor against microbiota contribute to the development and progression of GvHD.

Recent experimental data indicate that the intestinal microbiota have a strong impact on the activation of alloreactive T cells. The group of G. Hill demonstrated that the pre-transplant gut microbiome plays a major role in the development and severity of GvHD by influencing MHC II expression on intestinal epithelial cells, which allows communication with immune cells. Mice with higher levels of gut MHCII developed more severe GvHD, and the level of MHCII expression in the gut epithelium was closely dependent on the microbiome composition. Interestingly, mice raised in different locations showed different microbiomes and varying levels of MHCII expression in their gut epithelium. Using genetic sequencing, the researchers were able to analyze the correlations between bacterial groups and MHCII expression, distinguishing between bacterial species that induce or suppress MHCII. They also demonstrated that modulating these bacterial groups could influence MHCII levels. Additionally, administering antibiotics prior to transplant, as well as daily dosing with bacteria that suppress MHCII-inducing species, reduced the presence of these bacteria, leading to decreased MHCII expression and mitigated GvHD. These findings highlight potential therapeutic mechanisms by which pre-transplant microbial taxa may influence GvHD outcomes (Koyama et al., [2019](#), [2023](#)).

Even more recently, the same group demonstrated in CD4-dependent GvHD models that microbiota-specific CD4 T cells directly enhance donor-specific T cell-induced damage in GvHD (Yeh et al., [2024](#)). Conversely, GvHD-induced inflammation and MHC expression in the intestinal epithelium lead to the expansion of bacterial-specific CD4 clones, which in turn cause additional tissue damage.

Beyond the direct role of bacterial antigen-specific T cell immunity, it is likely that GvHD-specific alloreactive T cells are strongly regulated by the microbiota. Several studies highlight a connection between Tregs and the microbiome: microbiota metabolites significantly contribute to maintaining Treg homeostasis and to the generation of peripherally induced Tregs (pTregs), which are key mediators of gut tolerance (Meedt et al., [2022](#); Arpaia et al., [2013](#)). Our recent work analyzing

tissue interactions between clones of cytotoxic CD8+ cells and regulatory T cells in gastrointestinal biopsies from GvHD patients further supports this hypothesis (Jarosch et al., [2023](#)). Using 10x Genomics to characterize Treg function through genetic signatures, we found that Treg activity was strongly dependent on the presence of commensal microbiota. FoxP3+ T cells from individuals exposed to antibiotics exhibited a less immunosuppressive phenotype and lower gene expression levels. Additionally, transcriptional analysis within the FoxP3+ compartment revealed a differentiation trajectory from cells with gene signatures typical of effector T cells to cells with a strong Treg phenotype, suggesting that antibiotic exposure may disrupt pTreg differentiation. We also observed that taxa producing SCFAs, especially from the *Clostridia* and *Bacteroides* classes, were associated with more differentiated and suppressive Treg phenotypes. Conversely, biopsies from patients who received antibiotics at the time of sampling showed suppressed regulatory T cell gene expression, while effector T cell genes were highly stimulated. In line with these findings, increased Treg infiltration was observed in a single steroid-refractory GvHD case treated with FMT, supporting the idea that greater microbiome diversity and abundance of SCFA-producing species can aid in reconstituting a functional gut Treg niche.

Given the important role of IgA in distinguishing between commensals and pathogens, a role for specific B cell immunity is likely, but has not yet been thoroughly investigated. To date, only one experimental study has examined the effects of microbiota from germ-free, conventional, and SPF donor mice on T cell reactivity in a murine BMT model, reporting no significant impact (Tawara et al., [2013](#)). A clinical study by Liu et al. ([2017](#)), which compared microbiota diversity between recipients and donors, confirmed increased mortality in patients with low microbial diversity. Interestingly, it also reported a lower incidence of GvHD in patients who received stem cells from donors with high microbiota diversity—possibly reflecting a more optimized T cell repertoire capable of regulating intestinal microbiota (Liu et al., [2017](#)).

13.7 Microbiota Manipulation as a Prophylactic or Therapeutic Approach

Experimental studies utilizing lactobacilli to modulate GvHD in mice (Gerbitz et al., [2004](#)), as well as studies involving mice pretreated with antibiotics (Jenq et al., [2012](#)) and consortia of *Clostridia* to enhance intestinal butyrate production (Mathewson et al., [2016](#)), clearly indicate that microbiota modulation can significantly impact outcomes following ASCT. Numerous retrospective analyses on antibiotic prophylaxis and treatment in patients further support this concept; however, prospective studies are currently underway, and results are pending. Potential candidates for microbiota modulation, including prebiotics, probiotics, and postbiotics, have been summarized (Peled et al., [2016](#)). To date, only one prospective randomized trial involving lactobacilli in patients has been published, showing minimal effect. Similar to a previous prophylaxis trial with *Lactobacillus rhamnosus* conducted in our unit, probiotics were administered only after engraftment (Gorshein et al., [2017](#)). Given that nearly all analyses indicated dysbiosis occurring prior to engraftment as a critical factor exacerbating GvHD, these findings are not surprising; probiotic intervention may have commenced too late. Concerns exist regarding the use of probiotics during the neutropenic period, as *Lactobacillus* spp. septicemias have been reported in leukemia patients. However, a recent safety trial involving lactobacilli administered from day -8 pre-transplant until recovery from neutropenia in children found no instances of *Lactobacillus* bacteremia among 30 patients (Ladas et al., [2016](#)). Currently, there are no general recommendations regarding the use of pre- and probiotics in immunocompromised patients undergoing ASCT. It appears that nutritional prebiotic supplements rich in fiber support the growth of commensal organisms and may be the best option at this time for enhancing intestinal microbiota diversity (Spencer & Wargo, [2021](#); Andermann et al., [2016](#)). A recent report on the use of resistant potato starch in ASCT recipients demonstrated an

increased production of protective SCFAs in these patients (Riwe et al., [2023](#)).

Concerns regarding infectious risks have also been raised about performing FMT in patients undergoing ASCT. However, FMT for *Clostridium difficile* has been reported to be safe for immunosuppressed patients (Mandalia et al., [2016](#)) and has also been successfully administered to seven patients after ASCT (Webb et al., [2016](#)), five of whom were on immunosuppressive therapy. In 2016, two research groups took the next step by using FMT to treat steroid-refractory GvHD. In Kakihana's study ([2016](#)), four patients were treated, resulting in three complete responses and one partial response, all without major side effects. Additionally, a potential modulation of circulating regulatory T cells was observed. Similarly, Spindelboeck and colleagues treated three patients with refractory GvHD using repeated FMT and noted comparable responses ([2017](#)). Following these pioneering pilot studies involving FMT in this vulnerable and high-risk patient population, several subsequent studies have demonstrated the efficacy of FMT without an increased risk of complications in ASCT patients. To date, FMT has been utilized to eliminate antibiotic-resistant bacteria, restore microbiota diversity after hematopoietic recovery, and, in most cases, to treat steroid-resistant GvHD.

Given these promising initial studies in GvHD, multiple small reports as well as more extensive studies have confirmed responses in about half of the patients, showing mitigation of GvHD symptoms and improved survival rates (van Lier et al., [2020](#); Goeser et al., [2021](#)). The largest group of FMT-treated refractory acute GvHD patients was included in the prospective Heracles trial, a European multicenter phase II study published by Malard et al. in 2023 (Malard et al., [2023](#)). In this trial, patients were treated with MAAT013, a highly diverse multidonor FMT product manufactured by Maat Pharma. Among the 24 patients included, the overall GI response rate was 38%. Additionally, in a further 52 patients treated through a French early access program, the overall response rate (ORR) was even 58%, with about one-third of FMT-treated patients surviving at 12 months. However, severe

infectious complications occurred in 5 of the treated patients, and it could not be excluded that these were related to the study intervention. The extreme susceptibility to bacterial infections in severely immunosuppressed patients, such as those undergoing ASCT, remains a major concern. The toxicity of conditioning therapy and the effects of acute GvHD can make the GI tract highly vulnerable to bacterial translocation. This, combined with severe neutropenia, makes ASCT patients particularly prone to bacteremia and sepsis. For this reason, all reported applications of FMT in ASCT patients have been administered after neutrophil recovery. Although infectious complications during FMT appear to be rare, increased vigilance and careful monitoring of these vulnerable patients are essential.

Additionally, prophylactic trials using either pretransplant autologous FMT or third-party donor FMT have been conducted to prevent and treat dysbiosis following ASCT. In 2018, Taur et al. used autologous fecal material collected prior to ASCT to restore the microbiota in patients who showed reduced *Bacteroidetes* PCR copy numbers after engraftment. Although the beneficial effect of FMT was highly significant, the lack of suitable autologous FMT products limited its use in many patients, often due to preexisting microbiota damage caused by antibiotic exposure during previous antileukemic treatments (Taur et al., [2018](#)). In a single-arm study, DeFilipp et al. applied third-party donor FMT capsules in patients recovering from neutropenia after ASCT. The results indicated that empiric third-party FMT after ASCT was feasible and safe during the period immediately following neutrophil engraftment. Moreover, it was associated with a significant expansion of the recipient's microbiome diversity (DeFilipp et al., [2019](#)). The first randomized study on FMT in ASCT patients, presented in 2023, used oral encapsulated FMT to restore microbiota diversity after recovery from neutropenia. Patients who received prophylactic FMT showed improved recovery from dysbiosis and a reduced abundance of expanded pathobionts like *Enterococci*, suggesting potential benefits of FMT. Notably, low microbial diversity was associated with more severe acute GvHD (Rashidi et al., [2023](#)).

According to current knowledge, the use of FMT in the setting of ASCT is strongly supported, as a balanced microbiome composition influences the threshold for T-cell activation and affects tissue tolerance and regeneration. However, FMT may be less effective in the treatment of steroid-resistant GvHD, which often involves advanced and potentially irreversible tissue damage. Therefore, current approaches aim to apply FMT earlier in the course of GvHD. In 2024, DeFilipp et al. reported a pilot study using third-party, single-donor FMT in combination with systemic corticosteroids in treatment-naïve patients with high-risk acute gastrointestinal GvHD. The study met its primary endpoint, with 9 of 10 participants completing all eligible doses of encapsulated FMT, and showed an organ-specific GI complete response rate of 70% at day 28. Exploratory analyses indicated an increase in α -diversity, expansion of donor-derived species, and rises in tryptophan metabolites and short-chain fatty acids within the first 7 days after FMT (DeFilipp et al., [2024](#)).

Another promising approach is the prevention of intestinal dysbiosis even before conditioning damage occurs and neutropenia develops, in addition to microbiome restoration after the critical phase of neutropenia. Additionally, the application of FMT based on biomarker levels to prophylactically treat patients at high risk of poor outcomes should be considered in future studies.

Diet and nutrition are also important factors for maintaining microbiota diversity. To date, only one randomized study has compared the impact of enteric versus parenteral nutrition on GvHD, reporting nearly a 50% reduction in severe GvHD in the group receiving enteral nutrition (Gonzales et al., [2017](#)). Several groups have observed a reduced incidence of GvHD in patients undergoing outpatient or homecare transplantation. An interesting, yet not fully analyzed, hypothesis for this observation is that better enteral nutrition and continuous exposure to the patients' home microbiota may play a role. Of course, other factors such as selection bias—favoring patients with lower risk and less antibiotic use—might also explain these findings (Svahn et al., [2002](#)).

13.8 Impact of Dysbiosis on Further Complications

13.8.1 Role in Infections

A major finding in ASCT recipients, as well as in leukemia patients, is the rapid replacement of a diverse microbiota by an overabundance of one or very few species, leading to extensive colonization. This dominance is a significant risk factor for subsequent bloodstream infections and septicemia, as these bacteria can translocate through the damaged intestinal barrier. For example, enterococcal domination has been shown to increase the risk of enterococcal bloodstream infection (BSI) ninefold, while domination by *Proteobacteria* confers a fivefold increased risk (Taur et al., [2012](#); Lee et al., [2017](#)). A French research group even developed a BSI-risk index based on pretreatment microbiota composition, which can predict infections with 90% specificity (Montassier et al., [2016](#)). The abundance of gram-negative bacteria, documented through 16S sequencing, has been shown to predict bloodstream infections in further studies (Stoma et al., [2021](#)). Conversely, a predominance of the genus *Blautia* appears to be protective against such infections, both in adults (Rashidi et al., [2022](#)) and children (Margolis et al., [2023](#)). The lung is another critical organ concerning infections following ASCT. So far, one small study reported an association between shifts in the oral microbiome and subsequent pulmonary infections (Ames et al., [2012](#)). However, further analysis of these interactions is both worthwhile and necessary. As generally discussed, it is also possible that pulmonary infections are triggered by alterations in the intestinal microbiota. This is supported by a recent study from Harris (Harris et al., [2016](#)), which found that domination of the intestinal microbiome by gamma-Proteobacteria predicted pulmonary complications and increased mortality.

Colonization with multiple antibiotic-resistant pathogens is an increasing threat to hematological patients. Recent reports on the use of healthy donor FMT to eliminate these bacteria rely on the natural ability of commensals to restore colonization resistance and have

shown an unexpectedly high success rate of nearly 80% in leukemia and ASCT patients (Bilinski et al., [2017](#)). Battipaglia et al. reported decolonization of carbapenem-resistant bacteria and VRE using FMT before or after ASCT, without major side effects (Battipaglia et al., [2019](#)). Several additional studies have confirmed these results, making FMT an interesting and widely used approach for patients with multidrug-resistant bacteria after repeated antibiotic treatments (Innes et al., [2021](#); Su et al., [2021](#)). Interestingly, in a trial on prophylactic autologous FMT in acute leukemia patients—aimed at restoring microbiome diversity after chemotherapy and treatment of neutropenic infections—the rapid increase of antibiotic resistance (ABR) genes during neutropenia could be corrected by FMT in the majority of patients (Malard et al., [2021](#)). Given the lack of new antibiotics and the potential risk of further induction of resistance genes, FMT may become an attractive alternative to restore a diverse microbiome in these patients (Manges et al., [2016](#)).

13.8.2 Relapse and Microbiota

The opposing and interacting principles of ASCT, GvHD, and graft-versus-leukemia (GvL) effects suggest that any intervention affecting GvHD may also influence GvL effects. So far, only one large analysis has addressed this question in detail: Interestingly, Peled et al. were able to identify a single bacterial species, *Eubacterium limosum*, which conferred protection against relapse in a larger series of patients ([2017](#)). This observation suggests more specific mechanisms beyond simply modulating alloreactivity and indicates that certain bacteria may promote antileukemic activity through yet-unknown immunological pathways. Furthermore, Orberg et al. made an unexpected observation: they found that the IMM-RI—a combination of five immunomodulatory and bacteria-derived metabolites—not only correlates with GvHD-related mortality but also with relapse rates after ASCT. A high abundance of short-chain fatty acids was detected in IMM-RI low-risk patients and was associated with improved survival and reduced relapse (Orberg et al., [2024](#)). These findings are also in line

with recent experimental and clinical observations that the antitumoral and antileukemic activity of specific cancer treatments is influenced by the microbiota (Pflug et al., [2016](#); Routy et al., [2017](#); Zitvogel et al., [2017](#)).

13.9 Future Aspects and Unsolved Issues

- As with the vast majority of dysbiosis-associated diseases, the precise impact of microbiota modulation on the prevention and treatment of GvHD remains largely speculative at this point. Since almost all major organs affected by GvHD—both in acute and chronic forms—are organs with epithelia exposed to their respective microbiota, we can anticipate a significant expansion of knowledge regarding the interactions within these organs and their potential therapeutic applications. Given the profound influence of commensal microbiota on the function of both innate and adaptive immune cells, it is likely that in nearly all targets of GvHD epithelial tissue, recipient and donor immune cells and the respective microbiota have to be considered as an interacting “menage a trois.”
- Given that the negative impact of early antibiotic use on transplant outcomes has raised questions about whether antibiotic prophylaxis for gut decontamination is still beneficial or potentially harmful, this remains an unresolved issue. Prospective studies so far have been inconclusive or are still ongoing. As a result, transplant centers have adopted varying standards for decontamination and prophylactic antibiotics, ranging from aggressive to almost no prophylaxis at all. For children undergoing ASCT, clinical practice guidelines updated in 2019 provided weak initial recommendations against the routine use of systemic antibacterial prophylaxis, while also noting that data on long-term effectiveness should be carefully considered (Lehrnbecher et al., [2020](#)).
- A still larger unknown world is the role of the virome and mycobiome in the setting of transplantation. While the mycobiome might be less heterogenous and future research might help to

understand why certain patients are more susceptible to fungal infections (Shelburne et al., [2015](#)), a recent longitudinal analysis of the virome in 44 ASCT patients revealed an exciting burst of vertebrate viral genomes in the period of T cell deficiency early after SCT: Especially, Picobirnaviruses correlated highly with more severe gastrointestinal GvHD (Legoff et al., [2017](#)). In 78 patients receiving ASCT at two German transplant centers, Orberg et al. demonstrated recently a simultaneous loss of diversity in bacterial and viral communities, which was lowest at day +14, whereas fungal diversity did not decline during the peri-engraftment period. Virome alterations during ASCT occurred at the level of bacteriophages, which shifted in response to factors affecting their host bacteria. Combining amplicon sequencing, viral metagenomics and targeted metabolomics from stool samples of patients during ASCT Orberg et al. uncovered a microbiome signature of Lachnospiraceae and Oscillospiraceae and their associated bacteriophages, correlating with the production of immunomodulatory metabolites (Orberg et al., [2024](#))

- Modern assessment of clinical interactions by “omic” approaches brings a new challenge to translational research as each new finding needs to be confirmed and validated in prospective studies. Large consortia dedicated to prospective clinical data and sample collection will be needed to provide adequate clinical instruments to meet these diagnostic and scientific challenges, as it was started within the biomarker “MAGIC” consortium for acute GvHD (MAGIC Manuscript, JCI Insight 2017).

Controversy As in other disease models, the major unresolved issue is the precise pathophysiological role of dysbiosis and microbiota damage in GvHD. One perspective is that dysbiosis simply acts as a factor that lowers the threshold for the initiation of inflammation. However, it is also possible that specific bacteria contribute to particular mechanisms, such as the destruction of the mucus layer or epithelial damage caused by strain-specific

epitheliolysins. Clearly, more in-depth analyses are needed to clarify these mechanisms. The same question applies to the association between microbiota shifts and relapse—are there specific mechanisms by which certain bacteria stimulate anti-tumor immune responses? Furthermore, it remains unclear whether specific immune responses involving T and B cells (such as Th17 cells, follicular T helper cells, or IgA-producing plasma cells) are involved in these observed pathophysiological associations. This topic is still under active debate and can be quite emotional, as the observations challenge long-standing dogmas in clinical practice. The question arises: should these findings lead to changes in clinical strategies? Are we ready to modify antibiotic prophylaxis and treatment protocols or to consider fecal microbiota transplantation (FMT) as a standard therapy in heavily immunosuppressed patients?

History

Allogeneic stem cell transplantation is a standard treatment for patients with high-risk leukemias; however, it carries the risk of immunological complications due to the activation of donor lymphocytes. Consequently, graft-versus-host disease (GvHD) is the primary complication and a significant cause of treatment-related mortality following this procedure. Early studies demonstrated a reduced incidence of GvHD in animals raised in germ-free conditions or those receiving maximal isolation and decontamination. This finding, along with clinical trials showing a decrease in neutropenic infections through the use of prophylactic antibiotics, has led to the widespread adoption of maximal decontamination as a standard of care for many years.

However, with the increasing understanding of the interactions between innate and adaptive immunity, as well as insights gained from modern molecular microbiota sequencing, there is a growing need to reevaluate these strategies. It appears that, in the majority of patients, dysbiosis rather than decontamination is achieved.

Highlights

Dysbiosis induced by prophylactic and therapeutic antibiotics is a new and independent risk factor of acute GvHD:

- GvHD itself potentiates dysbiosis as Paneth cells are the most sensitive targets of intestinal GvHD, resulting in diminished release of antimicrobial peptides.
- Increased systemic levels of Reg3 α , an antimicrobial Paneth cell peptide, are sensitive and predictive biomarkers of intestinal GvHD but also early indicators of intestinal mucosal damage caused by antibiotic-induced dysbiosis
- Maintaining and protecting or reinstating commensal bacteria seems to be a new option to modulate GvHD.
- Antibiotic stewardship for treatment of neutropenic fever, as well as fecal microbiota transfer, are both promising options to modulate microbiota composition in order to prevent and to treat acute GvHD

References

Ames, N. J., Sulima, P., Ngo, T., Barb, J., Munson, P. J., Paster, B. J., & Hart, T. C. (2012). A characterization of the oral microbiome in allogeneic stem cell transplant patients. *PLoS One*, 7(10), e47628. <https://doi.org/10.1371/journal.pone.0047628>. Epub 2012 Oct 29. PubMed PMID: 23144704; PubMed Central PMCID: PMC3483166.

[[PubMed](#)][[PubMedCentral](#)]

Andermann, T. M., Rezvani, A., & Bhatt, A. S. (2016). Microbiota manipulation with prebiotics and probiotics in patients undergoing stem cell transplantation. *Current Hematologic Malignancy Reports*, 11(1), 19–28.

[[PubMed](#)][[PubMedCentral](#)]

Apostolova, P., & Zeiser, R. (2016). The role of purine metabolites as DAMPs in acute graft-versus-host disease. *Frontiers in Immunology*, 7, 439. eCollection 2016.

[[PubMed](#)][[PubMedCentral](#)]

Arpaia, N., Campbell, C., Fan, X., Dikiy, S., van der Veeken, J., deRoos, P., Liu, H., Cross, J. R., Pfeffer, K., Coffey, P. J., & Rudensky, A. Y. (2013). Metabolites produced by commensal bacteria promote peripheral regulatory T-cell generation. *Nature*, 504, 451–455.

[<https://doi.org/10.1038/nature12726>][[PubMed](#)][[PubMedCentral](#)]

Battipaglia, G., Malard, F., Rubio, M. T., Ruggeri, A., Mamez, A. C., Brissot, E., Giannotti, F., Dulery, R., Joly, A. C., Baylatry, K. M. J., Tankovic, J., Beaugier, L., Sokol, H., & Mohty, M. (2019). Fecal microbiota transplantation before or after allogeneic hematopoietic transplantation in patients with hematologic malignancies carrying multidrug-resistance bacteria. *Haematologica*, 104(8), 1682–1688.

[[PubMed](#)][[PubMedCentral](#)]

Beelen, D. W., Elmaagacli, A., Müller, K. D., Hirche, H., & Schaefer, U. W. (1999). Influence of intestinal bacterial decontamination using metronidazole and ciprofloxacin or ciprofloxacin alone on the development of acute graft-versus-host disease after marrow transplantation in patients with hematologic malignancies: Final results and long-term follow-up of an open-label prospective randomized trial. *Blood*, 93(10), 3267–3275.

[[PubMed](#)]

Bevins, C. L., Stange, E. F., & Wehkamp, J. (2009). Decreased Paneth cell defensin expression in ileal Crohn's disease is independent of inflammation, but linked to the NOD2 1007fs genotype. *Gut*, 58(6), 882–883. discussion 883–4. PubMed PMID: 19433600.

Bilinski, J., Robak, K., Peric, Z., Marchel, H., Karakulska-Prystupiu, E., Halaburda, K., Rusicka, P., Swoboda-Kopiec, E., Wroblewska, M., Wiktor-Jedrzejczak, W., & Basak, G. W. (2016). Impact of gut colonization by antibiotic-resistant bacteria on the outcomes of allogeneic hematopoietic stem cell transplantation: A retrospective, single-center study. *Biology of Blood and Marrow Transplantation*, 22(6), 1087–1093. <https://doi.org/10.1016/j.bbmt.2016.02.009>. Epub 2016 Feb 18. PubMed PMID: 26900084.

[[PubMed](#)]

Bilinski, J., Grzesiowski, P., Sorensen, N., Madry, K., Muszynski, J., Robak, K., Wroblewska, M., Dzieciatkowski, T., Dulny, G., Dwilewicz-Trojaczek, J., Wiktor-Jedrzejczak, W., & Basak, G. W. (2017). Fecal microbiota transplantation in patients with blood disorders inhibits gut colonization with antibiotic-resistant bacteria: Results of a prospective, single-center study. *Clinical Infectious Diseases*, 65(3), 364–370. <https://doi.org/10.1093/cid/cix252>. PubMed PMID: 28369341.

[[PubMed](#)]

Chun, E., Lavoie, S., Fonseca-Pereira, D., Bae, S., Michaud, M., Hoveyda, H. R., Fraser, G. L., Gallini Comeau, C. A., Glickman, J. N., Fuller, M. H., Layden, B. T., & Garrett, W. S. (2019). Metabolite-sensing receptor Ffar2 regulates Colonic Group 3 innate lymphoid cells and gut immunity. *Immunity*, 51(5), 871–884.

[[PubMed](#)][[PubMedCentral](#)]

Cooke, K. R., Gerbitz, A., Crawford, J. M., Teshima, T., Hill, G. R., Tesolin, A., Rossignol, D. P., & Ferrara, J. L. (2001). LPS antagonism reduces graft-versus-host disease and preserves graft-versus-leukemia activity after experimental bone marrow transplantation. *The Journal of Clinical Investigation*, 107(12), 1581–1589.

[[PubMed](#)][[PubMedCentral](#)]

Cooke, K. R., Olkiewicz, K., Erickson, N., & Ferrara, J. L. (2002). The role of endotoxin and the innate immune response in the pathophysiology of acute graft versus host disease. *Journal of Endotoxin Research*, 8(6), 441–448.

[[PubMed](#)]

DeFilipp, Z., Hohmann, E., Jenq, R. R., & Chen, Y.-B. (2019). Fecal microbiota transplantation: Restoring the injured microbiome after allogeneic hematopoietic cell transplantation. *Biology of Blood and Marrow Transplantation*, 25(1), e17–e22.

[[PubMed](#)]

DeFilipp, Z., Damania, A. V., Kim, H. T., Chang, C. C., El-Jawahri, A., McAfee, S. L., Bottoms, A. S., Toncheva, V., Smith, M. M., Dolaher, M., Perry, L., White, M., Diana, B., Connolly, S., Dey, B. R., Frigault, M. J., Newcomb, R. A., O'Donnell, P. V., Spitzer, T. R., Mansour, M. K., Weber, D., Ajami, N. J., Hohmann, E., Jenq, R. R., & Chen, Y. B. (2024). Third-party fecal microbiota transplantation for high-risk treatment-naïve acute GVHD of the lower GI tract. *Blood Advances*, 8(9), 2074–2084.

[[PubMed](#)][[PubMedCentral](#)]

Doki, N., Suyama, M., Sasajima, S., Ota, J., Igarashi, A., Mimura, I., Morita, H., Fujioka, Y., Sugiyama, D., Nishikawa, H., Shimazu, Y., Suda, W., Takeshita, K., Atarashi, K., Hattori, M., Sato, E., Watakabe-Inamoto, K., Yoshioka, K., Najima, Y., Kobayashi, T., Kakihana, K., Takahashi, N., Sakamaki, H., Honda, K., & Ohashi, K. (2017). Clinical impact of pre-transplant gut microbial diversity on outcomes of allogeneic hematopoietic stem cell transplantation. *Annals of Hematology*, 96(9), 1517–1523. <https://doi.org/10.1007/s00277-017-3069-8>. Epub 2017 Jul 21. PubMed PMID: 28733895.

[[PubMed](#)]

Ekmekci, I., von Klitzing, E., Fiebig, U., Escher, U., Neumann, C., Bacher, P., Scheffold, A., Kühl, A. A., Bereswill, S., & Heimesaat, M. M. (2017). Immune responses to broad-spectrum antibiotic treatment and fecal microbiota transplantation in mice. *Frontiers in Immunology*, 8, 397. <https://doi.org/10.3389/fimmu.2017.00397>

[[PubMed](#)][[PubMedCentral](#)]

Eriguchi, Y., Takashima, S., Oka, H., Shimoji, S., Nakamura, K., Uryu, H., Shimoda, S., Iwasaki, H., Shimono, N., Ayabe, T., Akashi, K., & Teshima, T. (2012). Graft-versus-host disease disrupts intestinal microbial ecology by inhibiting Paneth cell production of α -defensins. *Blood*, 120(1), 223–231.

[[PubMed](#)]

Eriguchi, Y., Uryu, H., Nakamura, K., Shimoji, S., Takashima, S., Iwasaki, H., Miyamoto, T., Shimono, N., Hashimoto, D., Akashi, K., Ayabe, T., & Teshima, T. (2013). Reciprocal expression of enteric antimicrobial proteins in intestinal graft-versus-host disease. *Biology of Blood and Marrow Transplantation*, 19(10), 1525–1529.

[[PubMed](#)]

Eriguchi, Y., Nakamura, K., Hashimoto, D., Shimoda, S., Shimono, N., Akashi, K., Ayabe, T., & Teshima, T. (2015). Decreased secretion of Paneth cell α -defensins in graft-versus-host disease. *Transplant Infectious Disease*, 17(5), 702–706.

[[PubMed](#)]

Ferrara, J. L., Levine, J. E., Reddy, P., & Holler, E. (2009). Graft-versus-host disease. *Lancet*, 373(9674), 1550–1561. [https://doi.org/10.1016/S0140-6736\(09\)60237-3](https://doi.org/10.1016/S0140-6736(09)60237-3)

[[PubMed](#)][[PubMedCentral](#)]

Ferrara, J. L., Harris, A. C., Greenson, J. K., Braun, T. M., Holler, E., Teshima, T., Levine, J. E., Choi, S. W., Huber, E., Landfried, K., Akashi, K., Vander Lugt, M., Reddy, P., Chin, A., Zhang, Q., Hanash, S., & Paczesny, S. (2011). Regenerating islet-derived 3-alpha is a biomarker of gastrointestinal graft-versus-host disease. *Blood*, 118(25), 6702–6708.

[[PubMed](#)][[PubMedCentral](#)]

Gafter-Gvili, A., Fraser, A., Paul, M., van de Wetering, M., Kremer, L., & Leibovici, L. (2005). Antibiotic prophylaxis for bacterial infections in afebrile neutropenic patients following chemotherapy. *Cochrane Database Systematic Review*, (4), CD004386. Review. Update in: *Cochrane Database Syst Rev*. 2012;1:CD004386.

Gerbitz, A., Schultz, M., Wilke, A., Linde, H. J., Schölmerich, J., Andreesen, R., & Holler, E. (2004). Probiotic effects on experimental graft-versus-host disease: Let them eat yogurt. *Blood*, 103(11), 4365–4367.

[[PubMed](#)]

Ghimire, S., Matos, C., Caioni, M., Weber, D., Peter, K., Holler, E., Kreutz, M., & Renner, K. (2018). Indoxyl 3-sulfate inhibits maturation and activation of human monocyte-derived dendritic cells. *Immunobiology*, 223(2), 239–245.

[[PubMed](#)]

Ghimire, S., Ederer, K. U., Meedt, E., Weber, D., Matos, C., Hiergeist, A., Zeman, F., Wolff, D., Edinger, M., Poeck, H., Herr, W., Gessner, A., Holler, E., & Bülow, S. (2022). Low intestinal IL22 associates with increased transplant-related mortality after allogeneic stem cell transplantation. *Frontiers in Immunology*, 13, 857400.

[[PubMed](#)][[PubMedCentral](#)]

Goeser, F., Sifft, B., Stein-Thoeringer, C., Farowski, F., Strassburg, C. P., Brossart, P., Higgins, P. G., Scheid, C., Wolf, D., Holderried, T. A. W., Vehreschild, M. J. G. T., & Cruz Aguilar, M. R. (2021). Fecal microbiota transfer for refractory intestinal graft-versus-host disease - experience from two German tertiary centers. *European Journal of Haematology*, 107(2), 229–245.

[[PubMed](#)]

Golob, J. L., Pergam, S. A., Srinivasan, S., Fiedler, T. L., Liu, C., Garcia, K., Mielcarek, M., Ko, D., Aker, S., Marquis, S., Loeffelholz, T., Plantinga, A., Wu, M. C., Celustka, K., Morrison, A., Woodfield, M., & Fredricks, D. N. (2017). The stool microbiota at neutrophil recovery is predictive for severe acute graft versus host disease after hematopoietic cell transplantation. *Clinical Infectious Diseases*. <https://doi.org/10.1093/cid/cix699>. [Epub ahead of print] PubMed PMID: 29020185.

Gonzales, F., Bruno, B., Alarcón Fuentes, M., De Berranger, E., Guimber, D., Behal, H., Gandemer, V., Spiegel, A., Sirvent, A., Yakoub-Agha, I., Nelken, B., Duhamel, A., & Seguy, D. (2017). Better early outcome with enteral rather than parenteral nutrition in children undergoing MAC allo-SCT. *Clinical Nutrition*, pii: S0261-5614(17)31365-1. <https://doi.org/10.1016/j.clnu.2017.10.005>. [Epub ahead of print] PubMed PMID: 29097037.

Gooley, T. A., Chien, J. W., Pergam, S. A., Hingorani, S., Sorrow, M. L., Boeckh, M., Martin, P. J., Sandmaier, B. M., Marr, K. A., Appelbaum, F. R., Storb, R., & McDonald, G. B. (2010). Reduced mortality after allogeneic hematopoietic-cell transplantation. *The New England Journal of Medicine*, 363(22), 2091–2101.

[\[PubMed\]](#)[\[PubMedCentral\]](#)

Gorshein, E., Wei, C., Ambrosy, S., Budney, S., Vivas, J., Shenkerman, A., Manago, J., McGrath, M. K., Tyno, A., Lin, Y., Patel, V., Gharibo, M., Schaar, D., Jenq, R. R., Khiabani, H., & Strair, R. (2017). Lactobacillus rhamnosus GG probiotic enteric regimen does not appreciably alter the gut microbiome or provide protection against GVHD after allogeneic hematopoietic stem cell transplantation. *Clinical Transplantation*, 31(5). <https://doi.org/10.1111/ctr.12947>. Epub 2017 Mar 31.

Hanash, A. M., Dudakov, J. A., Hua, G., O'Connor, M. H., Young, L. F., Singer, N. V., West, M. L., Jenq, R. R., Holland, A. M., Kappel, L. W., Ghosh, A., Tsai, J. J., Rao, U. K., Yim, N. L., Smith, O. M., Velardi, E., Hawryluk, E. B., Murphy, G. F., Liu, C., Fouser, L. A., Kolesnick, R., Blazar, B. R., & van den Brink, M. R. (2012). Interleukin-22 protects intestinal stem cells from immune-mediated tissue damage and regulates sensitivity to graft versus host disease. *Immunity*, 37(2), 339–350.

[\[PubMed\]](#)[\[PubMedCentral\]](#)

Harris, B., Morjaria, S. M., Littmann, E. R., Geyer, A. I., Stover, D. E., Barker, J. N., Giralt, S. A., Taur, Y., & Pamer, E. G. (2016). Gut microbiota predict pulmonary infiltrates after allogeneic hematopoietic cell transplantation. *American Journal of Respiratory and Critical Care Medicine*, 194(4), 450–463.

[\[PubMed\]](#)[\[PubMedCentral\]](#)

Hayase, E., Hayase, T., Jamal, M. A., Miyama, T., Chang, C. C., Ortega, M. R., Ahmed, S. S., Karmouch, J. L., Sanchez, C. A., Brown, A. N., El-Himri, R. K., Flores, I. I., McDaniel, L. K., Pham, D., Halsey, T., Frenk, A. C., Chapa, V. A., Heckel, B. E., Jin, Y., Tsai, W. B., Prasad, R., Tan, L., Veillon, L., Ajami, N. J., Wargo, J. A., Galloway-Peña, J., Shelburne, S., Chemaly, R. F., Davey, L., Glowacki, R. W. P., Liu, C., Rondon, G., Alousi, A. M., Molldrem, J. J., Champlin, R. E., Shpall, E. J., Valdivia, R. H., Martens, E. C., Lorenzi, P. L., & Jenq, R. R. (2022). Mucus-degrading Bacteroides link carbapenems to aggravated graft-versus-host disease. *Cell*, 185(20), 3705–3719.

[\[PubMed\]](#)[\[PubMedCentral\]](#)

Holler, E., Kolb, H. J., Möller, A., Kempeni, J., Liesenfeld, S., Pechumer, H., Lehmacher, W., Ruckdeschel, G., Gleixner, B., Riedner, C., et al. (1990). Increased serum levels of tumor necrosis factor alpha precede major complications of bone marrow transplantation. *Blood*, 75(4), 1011–1016. PubMed PMID: 2405918.

[\[PubMed\]](#)

Holler, E., Rogler, G., Brenmoehl, J., Hahn, J., Herfarth, H., Greinix, H., Dickinson, A. M., Socié, G., Wolff, D., Fischer, G., Jackson, G., Rocha, V., Steiner, B., Eissner, G., Marienhagen, J., Schoelmerich, J., & Andreesen, R. (2006). Prognostic significance of NOD2/CARD15 variants in HLA-identical sibling hematopoietic stem cell transplantation: Effect on long-term outcome is confirmed in 2 independent cohorts and may be modulated by the type of gastrointestinal decontamination. *Blood*, 107(10), 4189–4193.

[\[PubMed\]](#)

Holler, E., Butzhammer, P., Schmid, K., Hundsruker, C., Koestler, J., Peter, K., Zhu, W., Sporrer, D., Hehlhans, T., Kreutz, M., Holler, B., Wolff, D., Edinger, M., Andreesen, R., Levine, J. E., Ferrara, J. L., Gessner, A., Spang, R., & Oefner, P. J. (2014). Metagenomic analysis of the stool microbiome in patients receiving allogeneic stem cell transplantation: Loss of diversity is associated with use of

systemic antibiotics and more pronounced in gastrointestinal graft-versus-host disease. *Biology of Blood and Marrow Transplantation*, 20(5), 640–645.

[\[PubMed\]](#)[\[PubMedCentral\]](#)

Hülsdünker, J., Ottmüller, K. J., Neeff, H. P., Koyama, M., Gao, Z., Thomas, O. S., Follo, M., Al-Ahmad, A., Prinz, G., Duquesne, S., Dierbach, H., Kirschnek, S., Lämmermann, T., Blaser, M. J., Fife, B. T., Blazar, B. R., Beilhack, A., Hill, G. R., Häcker, G., & Zeiser, R. (2018). Neutrophils provide cellular communication between ileum and mesenteric lymph nodes at graft-versus-host disease onset. *Blood*, 131(16), 1858–1869.

[\[PubMed\]](#)[\[PubMedCentral\]](#)

Hülsdünker, J., Thomas, O. S., Haring, E., Unger, S., Gonzalo, N. N., Tugues, S., Gao, Z., Duquesne, S., Cywes-Bentley, C., Oyardi, O., Kirschnek, S., Schmitt-Graeff, A., Pabst, O., Koenecke, C., Duyster, J., Apostolova, P., Blaser, M. J., Becher, B., Pier, G. B., Häcker, G., & Zeiser, R. (2019). Immunization against poly-N-acetylglucosamine reduces neutrophil activation and GVHD while sparing microbial diversity. *Proceedings of the National Academy of Sciences of the United States of America*, 116(41), 20700–20706.

[\[PubMed\]](#)[\[PubMedCentral\]](#)

Innes, A. J., Mullish, B. H., Ghani, R., Szydło, R. M., Apperley, J. F., Olavarria, E., Palanicawandar, R., Kanfer, E. J., Milojkovic, D., McDonald, J. A. K., Brannigan, E. T., Thursz, M. R., Williams, H. R. T., Davies, F. J., Marchesi, J. R., & Pavlů, J. (2021). Fecal microbiota transplant mitigates adverse outcomes seen in patients colonized with multidrug-resistant organisms undergoing allogeneic hematopoietic cell transplantation. *Frontiers in Cellular and Infection Microbiology*, 11, 684659.

[\[PubMed\]](#)[\[PubMedCentral\]](#)

Jarosch, S., Köhlen, J., Ghimire, S., Orberg, E. T., Hammel, M., Gaag, D., Evert, M., Janssen, K. P., Hiergeist, A., Gessner, A., Weber, D., Meedt, E., Poeck, H., D'Ippolito, E., Holler, E., & Busch, D. H. (2023). Multimodal immune cell phenotyping in GI biopsies reveals microbiome-related T cell modulations in human GvHD. *Cell Reports Medicine*, 4(7), 101125.

[\[PubMed\]](#)[\[PubMedCentral\]](#)

Jenq, R. R., Ubeda, C., Taur, Y., Menezes, C. C., Khanin, R., Dudakov, J. A., Liu, C., West, M. L., Singer, N. V., Equinda, M. J., Gobourne, A., Lipuma, L., Young, L. F., Smith, O. M., Ghosh, A., Hanash, A. M., Goldberg, J. D., Aoyama, K., Blazar, B. R., Pamer, E. G., & van den Brink, M. R. (2012). Regulation of intestinal inflammation by microbiota following allogeneic bone marrow transplantation. *The Journal of Experimental Medicine*, 209(5), 903–911.

[\[PubMed\]](#)[\[PubMedCentral\]](#)

Jenq, R. R., Taur, Y., Devlin, S. M., Ponce, D. M., Goldberg, J. D., Ahr, K. F., Littmann, E. R., Ling, L., Gobourne, A. C., Miller, L. C., Docampo, M. D., Peled, J. U., Arpaia, N., Cross, J. R., Peets, T. K., Lumish, M. A., Shono, Y., Dudakov, J. A., Poeck, H., Hanash, A. M., Barker, J. N., Perales, M. A., Giral, S. A., Pamer, E. G., & van den Brink, M. R. (2015). Intestinal blautia is associated with reduced death from graft-versus-host disease. *Biology of Blood and Marrow Transplantation*, 21(8), 1373–1383.

[\[PubMed\]](#)[\[PubMedCentral\]](#)

Kakihana, K., Fujioka, Y., Suda, W., Najima, Y., Kuwata, G., Sasajima, S., Mimura, I., Morita, H., Sugiyama, D., Nishikawa, H., Hattori, M., Hino, Y., Ikegawa, S., Yamamoto, K., Toya, T., Doki, N.,

Koizumi, K., Honda, K., & Ohashi, K. (2016). Fecal microbiota transplantation for patients with steroid-resistant acute graft-versus-host disease of the gut. *Blood*, 128(16), 2083–2088.

[[PubMed](#)][[PubMedCentral](#)]

Kaysen, A., Heintz-Buschart, A., Muller, E. E. L., Narayanasamy, S., Wampach, L., Laczny, C. C., Graf, N., Simon, A., Franke, K., Bittenbring, J., Wilmes, P., & Schneider, J. G. (2017). Integrated meta-omic analyses of the gastrointestinal tract microbiome in patients undergoing allogeneic hematopoietic stem cell transplantation. *Translational Research*, 186, 79–94.e1. <https://doi.org/10.1016/j.trsl.2017.06.008>. Epub 2017 Jun 20. PubMed PMID: 28686852.

[[PubMed](#)]

Koyama, M., Mukhopadhyay, P., Schuster, J. S., Henden, A. S., Hülsdünker, J., Varelias, A., Vetizou, M., Kuns, R. D., Robb, R. J., Zhang, P., Blazar, B. R., Thomas, R., Begun, J., Waddell, N., Trinchieri, G., Zeiser, R., Clouston, A. D., Degli-Esposti, M. A., & Hill, G. R. (2019). MHC class II antigen presentation by the intestinal epithelium initiates graft-versus-host disease and is influenced by the microbiota. *Immunity*, 51(5), 885–898.

[[PubMed](#)][[PubMedCentral](#)]

Koyama, M., Hippe, D. S., Srinivasan, S., Proll, S. C., Miltiadous, O., Li, N., Zhang, P., Ensbey, K. S., Hoffman, N. G., Schmidt, C. R., Yeh, A. C., Minnie, S. A., Strenk, S. M., Fiedler, T. L., Hattangady, N., Kowalsky, J., Grady, W. M., Degli-Esposti, M. A., Varelias, A., Clouston, A. D., van den Brink, M. R. M., Dey, N., Randolph, T. W., Markey, K. A., Fredricks, D. N., & Hill, G. R. (2023). Intestinal microbiota controls graft-versus-host disease independent of donor-host genetic disparity. *Immunity*, 56(8), 1876–1893.

[[PubMed](#)][[PubMedCentral](#)]

Ladas, E. J., Bhatia, M., Chen, L., Sandler, E., Petrovic, A., Berman, D. M., Hamblin, F., Gates, M., Hawks, R., Sung, L., & Nieder, M. (2016). The safety and feasibility of probiotics in children and adolescents undergoing hematopoietic cell transplantation. *Bone Marrow Transplantation*, 51(2), 262–266.

[[PubMed](#)]

Lee, Y. J., Arguello, E. S., Jenq, R. R., Littmann, E., Kim, G. J., Miller, L. C., Ling, L., Figueroa, C., Robilotti, E., Perales, M. A., Barker, J. N., Giral, S., van den Brink, M. R. M., Pamer, E. G., & Taur, Y. (2017). Protective factors in the intestinal microbiome against *Clostridium difficile* infection in recipients of allogeneic hematopoietic stem cell transplantation. *The Journal of Infectious Diseases*, 215(7), 1117–1123. <https://doi.org/10.1093/infdis/jix011>. PubMed PMID: 28498996; PubMed Central PMCID: PMC5426375.

[[PubMed](#)][[PubMedCentral](#)]

Legoff, J., Resche-Rigon, M., Bouquet, J., Robin, M., Naccache, S. N., Mercier-Delarue, S., Federman, S., Samayoa, E., Rousseau, C., Piron, P., Kapel, N., Simon, F., Socié, G., & Chiu, C. Y. (2017). The eukaryotic gut virome in hematopoietic stem cell transplantation: New clues in enteric graft-versus-host disease. *Nature Medicine*, 23(9), 1080–1085. <https://doi.org/10.1038/nm.4380>. Epub 2017 Jul 31. PubMed PMID: 28759053.

[[PubMed](#)]

Lehrnbecher, T., Fisher, B. T., Phillips, B., Alexander, S., Ammann, R. A., Beauchemin, M., Carlesse, F., Castagnola, E., Davis, B. L., Dupuis, L. L., Egan, G., Groll, A. H., Haeusler, G. M., Santolaya, M.,

Steinbach, W. J., van de Wetering, M., Wolf, J., Cabral, S., Robinson, P. D., Sung, L. (2020). Guideline for Antibacterial Prophylaxis Administration in Pediatric Cancer and Hematopoietic Stem Cell Transplantation. *Clinical Infectious Diseases*, 71(1), 226–236.

Levine, J. E., Huber, E., Hammer, S. T., Harris, A. C., Greenson, J. K., Braun, T. M., Ferrara, J. L., & Holler, E. (2013). Low Paneth cell numbers at onset of gastrointestinal graft-versus-host disease identify patients at high risk for nonrelapse mortality. *Blood*, 122(8), 1505–1509.
[PubMed][PubMedCentral]

Liu, C., Frank, D. N., Horch, M., Chau, S., Ir, D., Horch, E. A., Tretina, K., van Besien, K., Lozupone, C. A., & Nguyen, V. H. (2017). Associations between acute gastrointestinal GvHD and the baseline gut microbiota of allogeneic hematopoietic stem cell transplant recipients and donors. *Bone Marrow Transplantation*. <https://doi.org/10.1038/bmt.2017.200>. [Epub ahead of print].

Malard, F., Vekhoff, A., Lapusan, S., Isnard, F., D'incan-Corda, E., Rey, J., Saillard, C., Thomas, X., Ducastelle-Lepretre, S., Paubelle, E., Larcher, M. V., Rocher, C., Recher, C., Tavitian, S., Bertoli, S., Michallet, A. S., Gilis, L., Peterlin, P., Chevallier, P., Nguyen, S., Plantamura, E., Boucinha, L., Gasc, C., Michallet, M., Dore, J., Legrand, O., & Mohty, M. (2021). Gut microbiota diversity after autologous fecal microbiota transfer in acute myeloid leukemia patients. *Nature Communications*, 12(1), 3084.
[PubMed][PubMedCentral]

Malard, F., Loschi, M., Huynh, A., Cluzeau, T., Guenounou, S., Legrand, F., Magro, L., Orvain, C., Charbonnier, A., Panz-Klapuch, M., Desmier, D., Mear, J. B., Cornillon, J., Robin, C., Daguindau, E., Bilger, K., Vehreschild, M. J. G. T., Chevallier, P., Labussière-Wallet, H., Mediavilla, C., Couturier, M. A., Bulabois, C.-E., Camus, V., Chantepie, S., Ceballos, P., Gaugler, B., Holler, E., Doré, J., Prestat, E., Gasc, C., Plantamura, E., & Mohty, M. (2023). Pooled allogeneic faecal microbiota MaaT013 for steroid-resistant gastrointestinal acute graft-versus-host disease: A single-arm, multicentre phase 2 trial. *EClinicalMedicine*, 26(62), 102111.

Mandalia, A., Ward, A., Tauxe, W., Kraft, C. S., & Dhere, T. (2016). Fecal transplant is as effective and safe in immunocompromised as non-immunocompromised patients for *Clostridium difficile*. *International Journal of Colorectal Disease*, 31(5), 1059–1060.
[PubMed]

Manges, A. R., Steiner, T. S., & Wright, A. J. (2016). Fecal microbiota transplantation for the intestinal decolonization of extensively antimicrobial-resistant opportunistic pathogens: A review. *Infectious Diseases (London)*, 48(8), 587–592.

Margolis, E. B., Maron, G., Sun, Y., Dallas, R. H., Allison, K. J., Ferrolino, J., Ross, H. S., Davis, A. E., Jia, Q., Turner, P., Mackay, V., Morin, C. E., Triplett, B. M., Klein, E. J., Englund, J. A., Tang, L., & Hayden, R. (2023). Microbiota predict infections and acute graft-versus-host disease after pediatric allogeneic hematopoietic stem cell transplantation. *The Journal of Infectious Diseases*, 228(5), 627–636.
[PubMed][PubMedCentral]

Masetti, R., Leardini, D., Muratore, E., Fabbrini, M., D'Amico, F., Zama, D., Baccelli, F., Gottardi, F., Belotti, T., Ussowicz, M., Fraczkievicz, J., Cesaro, S., Zecca, M., Merli, P., Candela, M., Pession, A., Locatelli, F., Prete, A., Brigidi, P., & Turrone, S. (2023). Gut microbiota diversity before allogeneic

hematopoietic stem cell transplantation as a predictor of mortality in children. *Blood*, 142(16), 1387–1398.

[[PubMed](#)][[PubMedCentral](#)]

Mathewson, N. D., Jenq, R., Mathew, A. V., Koenigskecht, M., Hanash, A., Toubai, T., Oravec-Wilson, K., Wu, S. R., Sun, Y., Rossi, C., Fujiwara, H., Byun, J., Shono, Y., Lindemans, C., Calafiore, M., Schmidt, T. C., Honda, K., Young, V. B., Pennathur, S., van den Brink, M., & Reddy, P. (2016). Gut microbiome-derived metabolites modulate intestinal epithelial cell damage and mitigate graft-versus-host disease. *Nature Immunology*, 17(5), 505–513.

[[PubMed](#)][[PubMedCentral](#)]

Meedt, E., Hiergeist, A., Gessner, A., Dettmer, K., Liebisch, G., Ghimire, S., Poeck, H., Edinger, M., Wolff, D., Herr, W., & Weber, D. (2022). Prolonged suppression of butyrate-producing bacteria is associated with acute gastrointestinal graft-vs-host disease and transplantation-related mortality after allogeneic stem cell transplantation. *Clinical Infectious Diseases*, 74, 614–621.

[[PubMed](#)]

Montassier, E., Al-Ghalith, G. A., Ward, T., Corvec, S., Gastinne, T., Potel, G., Moreau, P., de la Cochetiere, M. F., Batard, E., & Knights, D. (2016). Pretreatment gut microbiome predicts chemotherapy-related bloodstream infection. *Genome Medicine*, 8(1), 49. <https://doi.org/10.1186/s13073-016-0301-4>. Erratum in: *Genome Med.* 2016;8(1):61. PubMed PMID: 27121964; PubMed Central PMCID: PMC4848771.

[[PubMed](#)][[PubMedCentral](#)]

Norona, J., Apostolova, P., Schmidt, D., Ihlemann, R., Reischmann, N., Taylor, G., Köhler, N., de Heer, J., Heeg, S., Andrieux, G., Siranosian, B. A., Schmitt-Graeff, A., Pfeifer, D., Catalano, A., Frew, I. J., Proietti, M., Grimbacher, B., Bulashevskaya, A., Bhatt, A. S., Brummer, T., Clauditz, T., Zabelina, T., Kroeger, N., Blazar, B. R., Boerries, M., Ayuk, F., & Zeiser, R. (2020). Glucagon-like peptide 2 for intestinal stem cell and Paneth cell repair during graft-versus-host disease in mice and humans. *Blood*, 136(12), 1442–1455.

[[PubMed](#)][[PubMedCentral](#)]

Orberg, E. T., Meedt, E., Hiergeist, A., Xue, J., Heinrich, P., Ru, J., Ghimire, S., Miltiadous, O., Lindner, S., Tiefgraber, M., Gödel, S., Eismann, T., Schwarz, A., Göttert, S., Jarosch, S., Steiger, K., Schulz, C. H., Gigl, M., Fischer, J. C., Janssen, K.-P., Quante, M., Heidegger, S., Herhaus, P., Verbeek, M., Ruland, J., van den Brink, M. R. M., Weber, D., Edinger, M., Wolff, D., Busch, D. H., Kleigrew, K., Herr, W., Bassermann, F., Gessner, A., Deng, L., Holler, E., & Poeck, H. (2024). Bacteria and bacteriophage consortia are associated with protective intestinal metabolites in patients receiving stem cell transplantation. *Nature Cancer*, 5(1), 187–208.

Paczesny, S., Krijanovski, O. I., Braun, T. M., Choi, S. W., Clouthier, S. G., Kuick, R., Misek, D. E., Cooke, K. R., Kitko, C. L., Weyand, A., Bickley, D., Jones, D., Whitfield, J., Reddy, P., Levine, J. E., Hanash, S. M., & Ferrara, J. L. (2009). A biomarker panel for acute graft-versus-host disease. *Blood*, 113(2), 273–278.

[[PubMed](#)]

Peled, J. U., Jenq, R. R., Holler, E., & van den Brink, M. R. (2016). Role of gut flora after bone marrow transplantation. *Nature Microbiology*, 1, 16036. <https://doi.org/10.1038/nmicrobiol.2016.36>

[[PubMed](#)][[PubMedCentral](#)]

Peled, J. U., Devlin, S. M., Staffas, A., Lumish, M., Khanin, R., Littmann, E. R., Ling, L., Kosuri, S., Maloy, M., Slingerland, J. B., Ahr, K. F., Porosnicu Rodriguez, K. A., Shono, Y., Slingerland, A. E., Docampo, M. D., Sung, A. D., Weber, D., Alousi, A. M., Gyurkocza, B., Ponce, D. M., Barker, J. N., Perales, M. A., Giralt, S. A., Taur, Y., Pamer, E. G., Jenq, R. R., & van den Brink, M. R. M. (2017). Intestinal microbiota and relapse after hematopoietic-cell transplantation. *Journal of Clinical Oncology*, 35(15), 1650–1659.

[[PubMed](#)][[PubMedCentral](#)]

Peled, J. U., Gomes, A. L. C., Devlin, S. M., Littmann, E. R., Taur, Y., Sung, A. D., Weber, D., Hashimoto, D., Slingerland, A. E., Slingerland, J. B., Maloy, M., Clurman, A. G., Stein-Thoeringer, C. K., Markey, K. A., Docampo, M. D., Burgos da Silva, M., Khan, N., Gessner, A., Messina, J. A., Romero, K., Lew, M. V., Bush, A., Bohannon, L., Brereton, D. G., Fontana, E., Amoretti, L. A., Wright, R. J., Armijo, G. K., Shono, Y., Sanchez-Escamilla, M., Castillo Flores, N., Alarcon Tomas, A., Lin, R. J., Yáñez San Segundo, L., Shah, G. L., Cho, C., Scordo, M., Politikos, I., Hayasaka, K., Hasegawa, Y., Gyurkocza, B., Ponce, D. M., Barker, J. N., Perales, M. A., Giralt, S. A., Jenq, R. R., Teshima, T., Chao, N. J., Holler, E., Xavier, J. B., Pamer, E. G., van den Brink, M. R. M. (2020). Microbiota as Predictor of Mortality in Allogeneic Hematopoietic-Cell Transplantation. *New England Journal of Medicine*, 382(9), 822–834.

Penack, O., Smith, O. M., Cunningham-Bussel, A., Liu, X., Rao, U., Yim, N., Na, I. K., Holland, A. M., Ghosh, A., Lu, S. X., Jenq, R. R., Liu, C., Murphy, G. F., Brandl, K., & van den Brink, M. R. (2009). NOD2 regulates hematopoietic cell function during graft-versus-host disease. *The Journal of Experimental Medicine*, 206(10), 2101–2110. <https://doi.org/10.1084/jem.20090623>. Epub 2009 Sep 8. PubMed PMID: 19737867; PubMed Central PMCID: PMC2757869.

[[PubMed](#)][[PubMedCentral](#)]

Pflug, N., Kluth, S., Vehreschild, J. J., Bahlo, J., Tacke, D., Biehl, L., Eichhorst, B., Fischer, K., Cramer, P., Fink, A. M., von Bergwelt-Baildon, M., Stilgenbauer, S., Hallek, M., Cornely, O. A., & Vehreschild, M. J. (2016). Efficacy of antineoplastic treatment is associated with the use of antibiotics that modulate intestinal microbiota. *Oncoimmunology*, 5(6), e1150399. <https://doi.org/10.1080/2162402X.2016.1150399>. eCollection 2016 Jun. PubMed PMID: 27471619; PubMed Central PMCID: PMC4938364.

[[PubMed](#)][[PubMedCentral](#)]

Piguet, P. F., Grau, G. E., Allet, B., & Vassalli, P. (1987). Tumor necrosis factor/cachectin is an effector of skin and gut lesions of the acute phase of graft-vs.-host disease. *The Journal of Experimental Medicine*, 166(5), 1280–1289.

[[PubMed](#)][[PubMedCentral](#)]

Rashidi, A., Peled, J. U., Ebadi, M., Rehman, T. U., Elhusseini, H., Marcello, L. T., Halaweish, H., Kaiser, T., Holtan, S. G., Khoruts, A., Weisdorf, D. J., & Staley, C. (2022). Protective effect of intestinal *Blautia* against neutropenic fever in allogeneic transplant recipients. *Clinical Infectious Diseases*, 75(11), 1912–1920.

[[PubMed](#)][[PubMedCentral](#)]

Rashidi, A., Ebadi, M., Rehman, T. U., Elhusseini, H., Kazadi, D., Halaweish, H., Khan, M. H., Hoeschen, A., Cao, Q., Luo, X., Kabage, A. J., Lopez, L., Holtan, S. G., Weisdorf, D. J., Khoruts, A., & Staley, C. (2023). Randomized double-blind phase II trial of Fecal microbiota transplantation versus placebo in allogeneic hematopoietic cell transplantation and AML. *Journal of Clinical Oncology*, 41(34), 5306–5319.

[PubMed][PubMedCentral]

Reddy, P., Sun, Y., Toubai, T., Duran-Struuck, R., Clouthier, S. G., Weisiger, E., Maeda, Y., Tawara, I., Krijanovski, O., Gatz, E., Liu, C., Malter, C., Mascagni, P., Dinarello, C. A., & Ferrara, J. L. (2008). Histone deacetylase inhibition modulates indoleamine 2,3-dioxygenase-dependent DC functions and regulates experimental graft-versus-host disease in mice. *The Journal of Clinical Investigation*, 118(7), 2562–2573. Review.

[PubMed][PubMedCentral]

Riwe, M., & Reddy, P. (2017). Microbial metabolites and graft versus host disease. *American Journal of Transplantation*. <https://doi.org/10.1111/ajt.14443>. [Epub ahead of print] Review. PubMed PMID: 28742948.

Riwe, M. M., Golob, J. L., Magenau, J., Shan, M., Dick, G., Braun, T., Schmidt, T. M., Pawarode, A., Anand, S., Ghosh, M., Maciejewski, J., King, D., Choi, S., Yanik, G., Geer, M., Hillman, E., Lyssiotis, C. A., Tewari, M., & Reddy, P. (2023). Feasibility of a dietary intervention to modify gut microbial metabolism in patients with hematopoietic stem cell transplantation. *Nature Medicine*, 29(11), 2805–2813.

[PubMed][PubMedCentral]

Routy, B., Letendre, C., Enot, D., Chénard-Poirier, M., Mehraj, V., Séguin, N. C., Guenda, K., Gagnon, K., Woerther, P. L., Ghez, D., & Lachance, S. (2016). The influence of gut-decontamination prophylactic antibiotics on acute graft-versus-host disease and survival following allogeneic hematopoietic stem cell transplantation. *Oncoimmunology*, 6(1), e1258506. <https://doi.org/10.1080/2162402X.2016.1258506>. eCollection 2017.

[PubMed][PubMedCentral]

Routy, B., Le Chatelier, E., Derosa, L., Duong, C. P. M., Alou, M. T., Daillère, R., Fluckiger, A., Messaoudene, M., Rauber, C., Roberti, M. P., Fidelle, M., Flament, C., Poirier-Colame, V., Opolon, P., Klein, C., Iribarren, K., Mondragón, L., Jacquilot, N., Qu, B., Ferrere, G., Clémenson, C., Mezquita, L., Masip, J. R., Naltet, C., Brosseau, S., Kaderbhai, C., Richard, C., Rizvi, H., Levenez, F., Galleron, N., Quinquis, B., Pons, N., Ryffel, B., Minard-Colin, V., Gonin, P., Soria, J. C., Deutsch, E., Lorient, Y., Ghiringhelli, F., Zalcman, G., Goldwasser, F., Escudier, B., Hellmann, M. D., Eggermont, A., Raoult, D., Albiges, L., Kroemer, G., & Zitvogel, L. (2017). Gut microbiome influences efficacy of PD-1-based immunotherapy against epithelial tumors. *Science*. <https://doi.org/10.1126/science.aan3706>. pii: ean3706. [Epub ahead of print] PubMed PMID: 29097494.

Routy, B., Le Chatelier, E., Derosa, L., Duong, C. P. M., Alou, M. T., Daillère, R., Fluckiger, A., Messaoudene, M., Rauber, C., Roberti, M. P., Fidelle, M., Flament, C., Poirier-Colame, V., Opolon, P., Klein, C., Iribarren, K., Mondragón, L., Jacquilot, N., Qu, B., Ferrere, G., Clémenson, C., Mezquita, L., Masip, J. R., Naltet, C., Brosseau, S., Kaderbhai, C., Richard, C., Rizvi, H., Levenez, F., Galleron, N., Quinquis, B., Pons, N., Ryffel, B., Minard-Colin, V., Gonin, P., Soria, J. C., Deutsch, E., Lorient, Y., Ghiringhelli, F., Zalcman, G., Goldwasser, F., Escudier, B., Hellmann, M. D., Eggermont, A., Raoult, D., Albiges, L., Kroemer, G., & Zitvogel, L. (2018). Gut microbiome influences efficacy of PD-1-based immunotherapy against epithelial tumors. *Science*, 359(6371), 91–97.

[PubMed]

Schmeiser, T., Kurrle, E., Arnold, R., Heit, W., Krieger, D., Kubanek, B., & Heimpel, H. (1985). The influence of the microbial flora on incidence of graft-versus-host disease in human allogeneic bone

marrow transplantation. *Progress in Clinical and Biological Research*, 181, 433–435.

[\[PubMed\]](#)

Schwab, L., Goroncy, L., Palaniyandi, S., Gautam, S., Triantafyllou, A., Mocsai, A., Reichardt, W., Karlsson, F. J., Radhakrishnan, S. V., Hanke, K., Schmitt-Graeff, A., Freudenberg, M., von Loewenich, F. D., Wolf, P., Leonhardt, F., Baxan, N., Pfeifer, D., Schmah, O., Schönle, A., Martin, S. F., Mertelsmann, R., Duyster, J., Finke, J., Prinz, M., Henneke, P., Häcker, H., Hildebrandt, G. C., Häcker, G., & Zeiser, R. (2014). Neutrophil granulocytes recruited upon translocation of intestinal bacteria enhance graft-versus-host disease via tissue damage. *Nature Medicine*, 20(6), 648–654.

[\[PubMed\]](#)

Shelburne, S. A., Ajami, N. J., Chibucos, M. C., Beird, H. C., Tarrand, J., Galloway-Peña, J., Albert, N., Chemaly, R. F., Ghantaji, S. S., Marsh, L., Pemmaraju, N., Andreeff, M., Shpall, E. J., Wargo, J. A., Rezvani, K., Alousi, A., Bruno, V. M., Futreal, P. A., Petrosino, J. F., & Kontoyiannis, D. P. (2015). Implementation of a pan-genomic approach to investigate holobiont-infecting microbe interaction: A case report of a leukemic patient with invasive mucormycosis. *PLoS One*, 10(11), e0139851.

[\[PubMed\]](#)[\[PubMedCentral\]](#)

Shono, Y., Docampo, M. D., Peled, J. U., Perobelli, S. M., Velardi, E., Tsai, J. J., Slingerland, A. E., Smith, O. M., Young, L. F., Gupta, J., Lieberman, S. R., Jay, H. V., Ahr, K. F., Porosnicu Rodriguez, K. A., Xu, K., Calarfiore, M., Poeck, H., Caballero, S., Devlin, S. M., Rapaport, F., Dudakov, J. A., Hanash, A. M., Gyurkocza, B., Murphy, G. F., Gomes, C., Liu, C., Moss, E. L., Falconer, S. B., Bhatt, A. S., Taur, Y., Pamer, E. G., van den Brink, M. R. M., & Jenq, R. R. (2016). Increased GVHD-related mortality with broad-spectrum antibiotic use after allogeneic hematopoietic stem cell transplantation in human patients and mice. *Science Translational Medicine*, 8(339), 339ra71.

[\[PubMed\]](#)[\[PubMedCentral\]](#)

Spencer, C. N., & Wargo, J. A. (2021). Dietary fiber and probiotics influence the gut microbiome and melanoma immunotherapy response. *Science*, 374(6575), 1632–1640.

[\[PubMed\]](#)[\[PubMedCentral\]](#)

Spindelboeck, W., Schulz, E., Uhl, B., Kashofer, K., Aigelsreiter, A., Zinke-Cerwenka, W., Mulabecirovic, A., Kump, P. K., Halwachs, B., Gorkiewicz, G., Sill, H., Greinix, H., Högenauer, C., & Neumeister, P. (2017). Repeated fecal microbiota transplantations attenuate diarrhea and lead to sustained changes in the fecal microbiota in acute, refractory gastrointestinal graft-versus-host-disease. *Haematologica*, 102(5), e210–e213.

[\[PubMed\]](#)[\[PubMedCentral\]](#)

Stein-Thoeringer, C. K., Nichols, K. B., Lazrak, A., Docampo, M. D., Slingerland, A. E., Slingerland, J. B., Clurman, A. G., Armijo, G., Gomes, A. L. C., Shono, Y., Staffas, A., Burgos da Silva, M., Devlin, S. M., Markey, K. A., Bajic, D., Pinedo, R., Tsakmaklis, A., Littmann, E. R., Pastore, A., Taur, Y., Monette, S., Arcila, M. E., Pickard, A. J., Maloy, M., Wright, R. J., Amoretti, L. A., Fontana, E., Pham, D., Jamal, M. A., Weber, D., Sung, A. D., Hashimoto, D., Scheid, C., Xavier, J. B., Messina, J. A., Romero, K., Lew, M., Bush, A., Bohannon, L., Hayasaka, K., Hasegawa, Y., Vehreschild, M. J. G. T., Cross, J. R., Ponce, D. M., Perales, M. A., Giralt, S. A., Jenq, R. R., Teshima, T., Holler, E., Chao, N. J., Pamer, E. G., Peled, J. U., van den Brink, M. R. M. (2019). Lactose drives Enterococcus expansion to promote graft-versus-host disease. *Science*, 366(6469), 1143–1149.

Stoma, I., Littmann, E. R., Peled, J. U., Giral, S., van den Brink, M. R. M., Pamer, E. G., & Taur, Y. (2021). Compositional flux within the intestinal microbiota and risk for bloodstream infection with gram-negative bacteria. *Clinical Infectious Diseases*, 73(11), e4627–e4635.

[\[PubMed\]](#) [\[PubMedCentral\]](#)

Storb, R., Prentice, R. L., Buckner, C. D., Clift, R. A., Appelbaum, F., Deeg, J., Doney, K., Hansen, J. A., Mason, M., Sanders, J. E., Singer, J., Sullivan, K. M., Witherspoon, R. P., & Thomas, E. D. (1983). Graft-versus-host disease and survival in patients with aplastic anemia treated by marrow grafts from HLA-identical siblings. Beneficial effect of a protective environment. *The New England Journal of Medicine*, 308(6), 302–307.

[\[PubMed\]](#)

Su, Y., Li, X., Li, D., & Sun, J. (2021). Fecal microbiota transplantation shows marked shifts in the multi-omic profiles of porcine post-weaning Diarrhea. *Frontiers in Microbiology*, 12, 619460.

[\[PubMed\]](#) [\[PubMedCentral\]](#)

Svahn, B. M., Remberger, M., Myrbäck, K. E., Holmberg, K., Eriksson, B., Hentschke, P., Aschan, J., Barkholt, L., & Ringdén, O. (2002). Home care during the pancytopenic phase after allogeneic hematopoietic stem cell transplantation is advantageous compared with hospital care. *Blood*, 100(13), 4317–4324.

[\[PubMed\]](#)

Taur, Y., Xavier, J. B., Lipuma, L., Ubeda, C., Goldberg, J., Gobourne, A., Lee, Y. J., Dubin, K. A., Socci, N. D., Viale, A., Perales, M. A., Jenq, R. R., van den Brink, M. R., & Pamer, E. G. (2012). Intestinal domination and the risk of bacteremia in patients undergoing allogeneic hematopoietic stem cell transplantation. *Clinical Infectious Diseases*, 55(7), 905–914.

[\[PubMed\]](#) [\[PubMedCentral\]](#)

Taur, Y., Jenq, R. R., Perales, M. A., Littmann, E. R., Morjaria, S., Ling, L., No, D., Gobourne, A., Viale, A., Dahi, P. B., Ponce, D. M., Barker, J. N., Giral, S., van den Brink, M., & Pamer, E. G. (2014). The effects of intestinal tract bacterial diversity on mortality following allogeneic hematopoietic stem cell transplantation. *Blood*, 124(7), 1174–1182.

[\[PubMed\]](#) [\[PubMedCentral\]](#)

Taur, Y., Coyte, K., Schluter, J., Robilotti, E., Figueroa, C., Gjonbalaj, M., Littmann, E. R., Ling, L., Miller, L., Gyaltsen, Y., Fontana, E., Morjaria, S., Gyurkocza, B., Perales, M. A., Castro-Malaspina, H., Tamari, R., Ponce, D., Koehne, G., Barker, J., Jakubowski, A., Papadopoulos, E., Dahi, P., Sauter, C., Shaffer, B., Young, J. W., Peled, J., Meagher, R. C., Jenq, R. R., van den Brink, M. R. M., Giral, S. A., Pamer, E. G., & Xavier, J. B. (2018). Reconstitution of the gut microbiota of antibiotic-treated patients by autologous fecal microbiota transplant. *Science Translational Medicine*, 10(460), eaap9489.

[\[PubMed\]](#) [\[PubMedCentral\]](#)

Tawara, I., Liu, C., Tamaki, H., Toubai, T., Sun, Y., Evers, R., Nieves, E., Mathewson, N., Nunez, G., & Reddy, P. (2013). Influence of donor microbiota on the severity of experimental graft-versus-host-disease. *Biology of Blood and Marrow Transplantation*, 19(1), 164–168.

[\[PubMed\]](#)

Thomas, A. M., Fidelle, M., Routy, B., Kroemer, G., Wargo, J. A., Segata, N., & Zitvogel, L. (2023). Gut OncoMicrobiome Signatures (GOMS) as next-generation biomarkers for cancer immunotherapy.

Nature Reviews. Clinical Oncology, 20(9), 583–603.

[[PubMed](#)][[PubMedCentral](#)]

Turner, A. M., Li, L., Monk, I. R., Lee, J. Y. H., Ingle, D. J., Portelli, S., Sherry, N. L., Isles, N., Seemann, T., Sharkey, L. K., Walsh, C. J., Reid, G. E., Nie, S., Eijkelkamp, B. A., Holmes, N. E., Collis, B., Vogrin, S., Hiergeist, A., Weber, D., Gessner, A., Holler, E., Ascher, D. B., Duchene, S., Scott, N. E., Stinear, T. P., Kwong, J. C., Gorrie, C. L., Howden, B. P., & Carter, G. P. (2024). Rifaximin prophylaxis causes resistance to the last-resort antibiotic daptomycin. *Nature*, 635(8040), 969–977.

[<https://doi.org/10.1038/s41586-024-08095-4>][[PubMed](#)][[PubMedCentral](#)]

van Bekkum, D. W., & Knaan, S. (1977). Role of bacterial microflora in development of intestinal lesions from graft-versus-host reaction. *Journal of the National Cancer Institute*, 58(3), 787–790.

[[PubMed](#)]

van Bekkum, D. W., Roodenburg, J., Heidt, P. J., & van der Waaij, D. (1974). Mitigation of secondary disease of allogeneic mouse radiation chimeras by modification of the intestinal microflora. *Journal of the National Cancer Institute*, 52(2), 401–404.

[[PubMed](#)]

van Lier, Y. F., Davids, M., Haverkate, N. J. E., de Groot, P. F., Donker, M. L., Meijer, E., Heubel-Moenen, F. C. J. I., Nur, E., Zeerleder, S. S., Nieuwdorp, M., Blom, B., & Hazenberg, M. D. (2020). Donor fecal microbiota transplantation ameliorates intestinal graft-versus-host disease in allogeneic hematopoietic cell transplant recipients. *Science Translational Medicine*, 12(556), eaaz8926.

[[PubMed](#)]

Varelias, A., Ormerod, K. L., Bunting, M. D., Koyama, M., Gartlan, K. H., Kuns, R. D., Lachner, N., Locke, K. R., Lim, C. Y., Henden, A. S., Zhang, P., Clouston, A. D., Hasnain, S. Z., McGuckin, M. A., Blazar, B. R., MacDonald, K. P., Hugenholtz, P., & Hill, G. R. (2017). Acute graft-versus-host disease is regulated by an IL-17-sensitive microbiome. *Blood*, 129(15), 2172–2185.

[[PubMed](#)][[PubMedCentral](#)]

Vossen, J. M., Guiot, H. F., Lankester, A. C., Vossen, A. C., Bredius, R. G., Wolterbeek, R., Bakker, H. D., & Heidt, P. J. (2014). Complete suppression of the gut microbiome prevents acute graft-versus-host disease following allogeneic bone marrow transplantation. *PLoS One*, 9(9), e105706. <https://doi.org/10.1371/journal.pone.0105706>. eCollection 2014. PubMed PMID: 25180821; PubMed Central PMCID: PMC4152127.

[[PubMed](#)][[PubMedCentral](#)]

Vriesendorp, H. M., Klapwijk, W. M., Visser, T. P., Zurcher, C., & van Bekkum, D. W. (1982). Alternatives to donor matching for control of graft-versus-host disease. *Immunogenetics*, 15(1), 79–94.

[<https://doi.org/10.1007/BF00375505>][[PubMed](#)]

Webb, B. J., Brunner, A., Ford, C. D., Gazdik, M. A., Petersen, F. B., & Hoda, D. (2016). Fecal microbiota transplantation for recurrent *Clostridium difficile* infection in hematopoietic stem cell transplant recipients. *Transplant Infectious Disease*, 18(4), 628–633.

[[PubMed](#)]

Weber, D., Oefner, P. J., Hiergeist, A., Koestler, J., Gessner, A., Weber, M., Hahn, J., Wolff, D., Stämmler,

F., Spang, R., Herr, W., Dettmer, K., & Holler, E. (2015). Low urinary indoxyl sulfate levels early after transplantation reflect a disrupted microbiome and are associated with poor outcome. *Blood*, 126(14), 1723–1728.

[[PubMed](#)]

Weber, D., Oefner, P. J., Dettmer, K., Hiergeist, A., Koestler, J., Gessner, A., Weber, M., Stämmeler, F., Hahn, J., Wolff, D., Herr, W., & Holler, E. (2016). Rifaximin preserves intestinal microbiota balance in patients undergoing allogeneic stem cell transplantation. *Bone Marrow Transplantation*, 51(8), 1087–1092.

[[PubMed](#)]

Weber, D., Frauenschläger, K., Ghimire, S., Peter, K., Panzer, I., Hiergeist, A., Weber, M., Kutny, D., Wolff, D., Grube, M., Huber, E., Oefner, P., Gessner, A., Hehlhans, T., Herr, W., & Holler, E. (2017a). The association between acute graft-versus-host disease and antimicrobial peptide expression in the gastrointestinal tract after allogeneic stem cell transplantation. *PLoS One*, 12(9), e0185265.

<https://doi.org/10.1371/journal.pone.0185265>. eCollection 2017.

[[PubMed](#)][[PubMedCentral](#)]

Weber, D., Jenq, R. R., Peled, J. U., Taur, Y., Hiergeist, A., Koestler, J., Dettmer, K., Weber, M., Wolff, D., Hahn, J., Pamer, E. G., Herr, W., Gessner, A., Oefner, P. J., van den Brink, M. R., & Holler, E. (2017b). Microbiota disruption induced by early use of broad-spectrum antibiotics is an independent risk factor of outcome after allogeneic stem cell transplantation. *Biology of Blood and Marrow Transplantation*, 23(5), 845–852.

[[PubMed](#)][[PubMedCentral](#)]

Weber, D., Hiergeist, A., Weber, M., Ghimire, S., Salzberger, B., Wolff, D., Poeck, H., Gessner, A., Edinger, M., Herr, W., Meedt, E., & Holler, E. (2023a). Restrictive versus permissive use of broad-spectrum antibiotics in patients receiving allogeneic stem cell transplantation and with early fever due to cytokine release syndrome: Evidence for beneficial microbiota protection without increase in infectious complications. *Clinical Infectious Diseases*, 77(10), 1432–1439.

[[PubMed](#)]

Weber, D., Weber, M., Meedt, E., Ghimire, S., Wolff, D., Edinger, M., Poeck, H., Hiergeist, A., Gessner, A., Ayuk, F., Roesler, W., Wölfl, M., Kraus, M., Zeiser, R., Bertrand, H., Bader, P., Ullrich, E., Eder, M., Gleich, S., Young, R., Herr, W., Levine, J., Ferrara, J., & Holler, E. (2023b). Reg3α concentrations at day of allogeneic stem cell transplantation predict outcome and correlate with early antibiotic use. *Blood Advances*, 7(7), 1326–1335.

[[PubMed](#)][[PubMedCentral](#)]

Whangbo, J., Ritz, J., & Bhatt, A. (2017). Antibiotic-mediated modification of the intestinal microbiome in allogeneic hematopoietic stem cell transplantation. *Bone Marrow Transplantation*, 52(2), 183–190. <https://doi.org/10.1038/bmt.2016.206>. Epub 2016 Aug 15. Review. PubMed PMID: 27526283.

[[PubMed](#)]

Yang, W., Yu, T., Huang, X., Bilotta, A. J., Xu, L., Lu, Y., Sun, J., Pan, F., Zhou, J., Zhang, W., Yao, S., Maynard, C. L., Singh, N., Dann, S. M., Liu, Z., & Cong, Y. (2020). Intestinal microbiota-derived short-chain fatty acids regulation of immune cell IL-22 production and gut immunity. *Nature Communications*, 11(1), 1–18.

Yeh, A. C., Koyama, M., Waltner, O. G., Minnie, S. A., Boiko, J. R., Shabaneh, T. B., Takahashi, S., Zhang, P., Ensbey, K. S., Schmidt, C. R., Legg, S. R. W., Sekiguchi, T., Nelson, E., Bhise, S. S., Stevens, A. R., Goodpaster, T., Chakka, S., Furlan, S. N., Markey, K. A., Bleakley, M. E., Elson, C. O., Bradley, P. H., & Hill, G. R. (2024). Microbiota dictate T cell clonal selection to augment graft-versus-host disease after stem cell transplantation. *Immunity*, 57(7), 1648–1664.

[[PubMed](#)][[PubMedCentral](#)]

Zeiser, R., Penack, O., Holler, E., & Idzko, M. (2011). Danger signals activating innate immunity in graft-versus-host disease. *Journal of Molecular Medicine (Berlin)*, 89(9), 833–845.

Zitvogel, L., Daillère, R., Roberti, M. P., Routy, B., & Kroemer, G. (2017). Anticancer effects of the microbiome and its products. *Nature Reviews Microbiology*, 15(8), 465–478. <https://doi.org/10.1038/nrmicro.2017.44>. Epub 2017 May 22. Review. PubMed PMID: 28529325.

[[PubMed](#)]

14. Microbiome and Diseases: Infection

Romana R. Gerner^{1,2}✉, Éva de Hoog-Almasi¹ and Bärbel Stecher^{1,3}✉

(1) TUM School of Life Sciences, Technical University of Munich, Freising, Germany

(2) Technical University of Munich, School of Medicine and Health, Department of Internal Medicine III, TUM University Hospital, Munich, Germany

(3) German Center of Infection Research (DZIF), Braunschweig, Germany

✉ **Romana R. Gerner (Corresponding author)**

Email: romana.gerner@tum.de

✉ **Bärbel Stecher**

Email: stecher@tum.de

Abstract

The human gut is densely populated by a diverse microbial community, in which ecological niches - shaped by nutrient availability, spatial organization, and host factors – govern microbial persistence and function. Early-life colonization drives the maturation of a stable, diverse microbiota that confers colonization resistance, a key barrier against invading pathogens. This protection relies on coordinated interactions between commensals and the host, limiting pathogen establishment through nutrient competition, antimicrobial activity, and maintenance of mucosal defenses.

Despite these barriers, pathogens have evolved strategies to overcome colonization resistance, including inflammation, toxin

production, metabolic adaptation, and biofilm formation. Host susceptibility to infection is highly context-dependent and influenced by factors such as age, diet, immune status, and, particularly, antibiotic exposure, which disrupts microbiota composition.

Thus, the intestinal niche functions both as a protective ecosystem and a potential reservoir for pathogenic organisms. Emerging microbiome-based therapies aim to restore microbial balance and reinforce host defense. A deeper understanding of microbiota-host-pathogen interactions will be key to developing targeted strategies to prevent and treat intestinal infections.

Keywords Colonization resistance – Nutrient competition – Mucosal barrier – Enteric pathogens – Infection susceptibility – Microbiota-targeted therapies

14.1 The Intestinal Niche as a Reservoir of Microbiota and Enteric Pathogens

The human gut is a densely populated microbial ecosystem, where community composition is shaped by competition for space and resources. Intestinal niche is an ecological term that includes a range of conditions, specifically physiological, nutritional, microbial, and spatial factors, which determine the role and persistence of a given microbe in the intestinal ecosystem. Following birth, the gut undergoes a dynamic process of ecological succession, transitioning from a relatively low-diversity, heterogeneous microbial landscape to a more diverse and stable adult microbiota (Yatsunenko et al., [2012](#)). The microbial makeup of an adult is shaped by both host factors and microbial interactions in early life, as newly introduced microbes tend to settle into ecological niches already defined by earlier species (=historical contingency). This dependency means that early microbial exposures can have lasting effects on the composition of the mature microbiome. Ultimately, a stable community is formed that protects against invading pathogens. Colonization resistance, a widely used term for this key

microbiome function, is a universally conserved trait found across the animal kingdom, from invertebrates to humans (van der Waaij et al., [1971](#); Woelfel et al., [2024](#)).

Colonization resistance has been demonstrated to act against a wide spectrum of human pathogens, including those transmitted through contaminated food and water, such as *Salmonella enterica* spp., enteropathogenic *Escherichia coli*, *Shigella* spp., *Campylobacter* spp., *Vibrio cholerae*, and *Listeria* spp. Colonization resistance also plays a critical role in defending against gastrointestinal pathogens spread by other routes, such as *Helicobacter* spp. and *Clostridioides difficile*. Furthermore, it helps guard against opportunistic, antibiotic-resistant microbes that can exploit microbiota disruptions, particularly in immunocompromised individuals.

To cause disease, pathogens must overcome this microbial barrier by breaching occupied niches or disrupting the existing ecological balance. Freter's foundational nutrient niche theory explains microbial colonization patterns in the gut based on each microorganism's ability to persist in this environment, depending largely on the access and utilization capacity of specific, growth-limiting nutrients (Freter et al., [1983](#)). In this framework, each microbial species occupies a unique metabolic niche defined by the nutrients it can best exploit. Consequently, for an invading species, including pathogens, opportunistic, neutral, or beneficial microbes to establish itself in an already colonized gut, it must outcompete the resident microbes for at least one limiting nutrient—an outcome that is relatively rare in a mature, stable community.

However, defining what constitutes a pathogen is not always straightforward, as microbial pathogenicity exists along a continuum. The same species may function as a harmless commensal in one context and as a disease-causing agent in another, depending on host and environmental factors. For this reason, Arturo Casadevall's concept interprets each organism to have a varying level of "pathogenic potential," acknowledging each microbe's ability to cause damage to the host depending on a matrix of variables (Casadevall, [2017](#)). This

concept introduces nuance to the problematic classification of bacteria as “good,” “bad,” or “pathobiont”—a vague and simplistic term often used for microorganisms causing disease only under specific circumstances (Jochum & Stecher, [2020](#)). With regards to benefitting from pathological outcomes, pathogens can be coined as either “professional” or “accidental” pathogens (Maier et al., [2024](#)). While accidental (sometimes synonymously used with opportunistic) pathogens typically cause disease only when host defenses are impaired, professional pathogens possess specific virulence traits that enable them to infect healthy hosts.

In the following sections, we will discuss how microbes navigate and compete within the gut’s ecological landscape, whether as commensals or pathogens, and how the gut microbiota influences infection at various stages through different mechanisms. Taking all these factors into account is essential for unraveling the principles of infection and for developing strategies to reinforce gastrointestinal health.

14.2 The Complex Interplay Among Commensals, Pathogens, and the Host

14.2.1 Microbial Barrier Against Pathogens: The Interplay of Microbiota and Host Determines Susceptibility to Infection

Although healthy individuals are frequently exposed to pathogens, infections remain relatively rare (Mair-Jenkins et al., [2017](#); Molbak et al., [2014](#); Simonsen et al., [2009](#); Torok et al., [1997](#)). This is largely because pathogens often fail to establish a sufficiently high level of intestinal colonization to trigger disease (Coombes et al., [2004](#); Stecher & Hardt, [2011](#)). Importantly, pathogenicity is a highly context-dependent trait: a microorganism that causes severe illness in one host may persist asymptomatically in another for years. For instance, approximately half of the human population carries antibodies against *Clostridioides difficile*, a common intestinal pathogen, despite never

having experienced a symptomatic infection (Kyne et al., [2000](#)). Similarly, certain strains of *Escherichia coli* are regular members of a healthy gut microbiota yet can act as uropathogenic, i.e., causing an infection of the urogenital tract in susceptible individuals (Worby et al., [2022](#)). These examples underscore the need to understand the foundational principles of colonization resistance to grasp the mechanisms underlying susceptibility to intestinal infections.

Current models describe colonization resistance as arising primarily through two general mechanisms: resource competition and interference competition (Woelfel et al., [2024](#)) (Fig. [14.1](#)). Beyond, the microbiota modulates susceptibility to infections via modulating mucosal and nutritional/microbiota-nourishing immunity (Lee et al., [2024](#)).

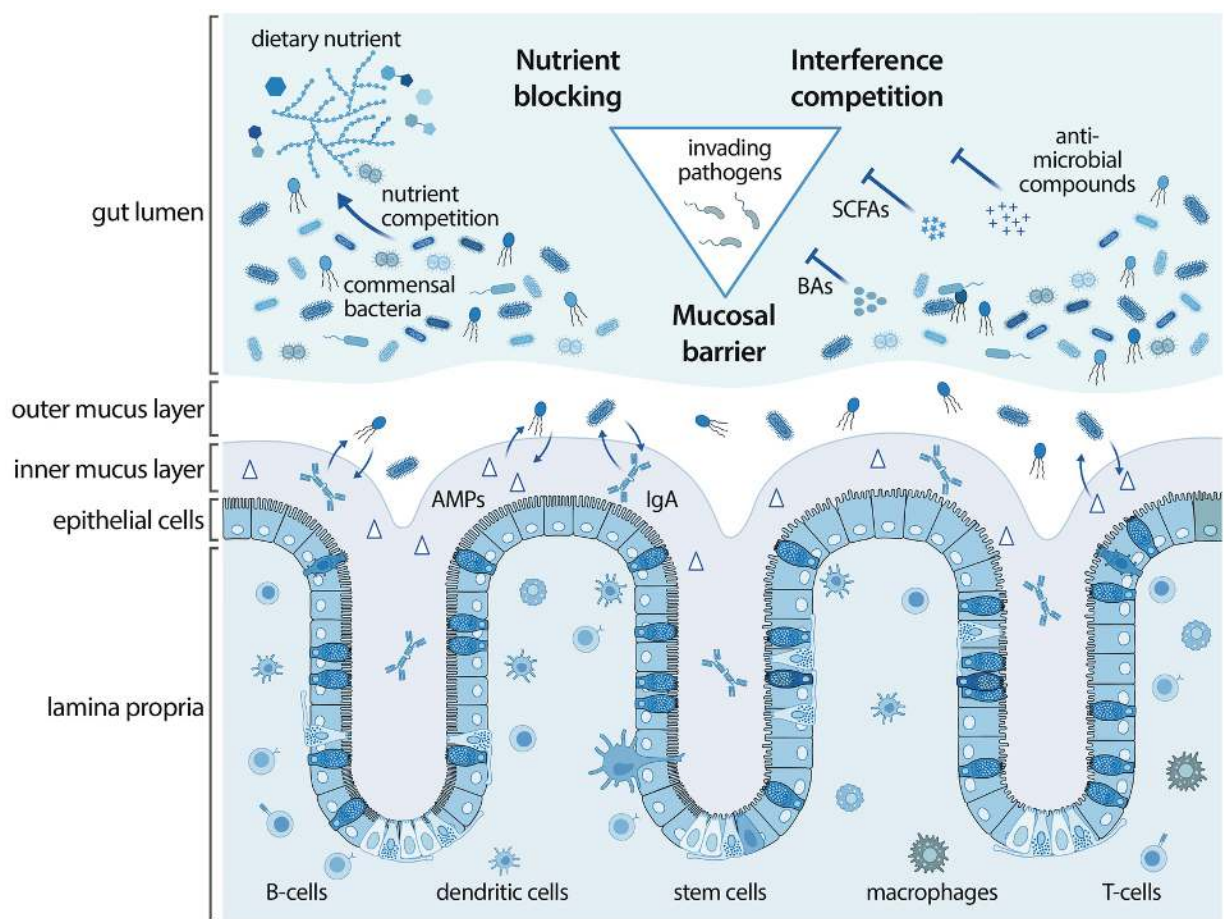


Fig. 14.1 Schematic overview of colonization resistance mechanisms in the gut environment. The gastrointestinal tract maintains a multilayered defense system that protects the host against

invading pathogens. This illustration depicts the intestinal lumen, mucus layers, and the mucosal surface, highlighting the tripartite nature of colonization resistance. One key mechanism is nutrient blocking, through which commensal microbes rapidly consume available carbon sources and micronutrients, thereby depriving potential pathogens of essential resources. A second line of defense is interference competition, where resident microbes inhibit pathogen establishment by producing inhibitory compounds such as short-chain fatty acids, converting bile acids into antimicrobial secondary forms, or secreting bacteriocins and other antagonistic molecules. The third component is the mucosal barrier, encompassing both physical and immunological host defenses that are reinforced by microbial interactions. Gut microbes contribute to the maintenance of mucus layer integrity and stimulate the production of host-derived antimicrobial peptides, immunoglobulin A (IgA), and other immune effectors. Together, these mechanisms shape a highly selective and dynamic gut ecosystem that limits pathogen colonization and supports host health

14.2.1.1 Resource Competition and Nutrient Blocking by Commensal Microbes

The gut microbiota plays a vital role in defending against pathogens, employing various strategies to prevent them from intestinal colonization. One of the fundamental mechanisms is nutrient competition, where commensal microbes outcompete pathogens for access to essential resources. This competition for limited nutrients is central to colonization resistance, as it directly impacts the ability of pathogenic microorganisms to establish themselves in the gut.

Commensals in the gut efficiently metabolize a wide range of complex diet-derived substrates, including fibers, polysaccharides, starch, and proteins, as well as simpler nutrients such as amino acids, monosaccharides, and trace elements like iron, which are essential for microbial growth and metabolic activity. This nutrient competition limits the availability of these resources to potential pathogens, a phenomenon known as nutrient blocking (Spragge et al., [2023](#)). For example, commensal bacteria such as *E. coli* and other members of the Enterobacteriaceae family are highly efficient at utilizing amino acids like serine as nutrient sources (Kitamoto et al., [2020](#)). Through preferential uptake of these nutrients, commensals effectively limit their availability to invading pathogens, thereby restricting pathogen access to essential metabolic substrates. Supporting this concept, Cabarello-Flores et al. demonstrated that the resident microbiota actively consumes dietary amino acids, compelling the murine model

pathogen *Citrobacter rodentium* to upregulate its endogenous amino acid biosynthesis pathways to sustain growth under nutrient-limiting conditions. However, dietary protein supplementation alleviates the amino acid limitation imposed by the microbiota, thereby removing the key metabolic bottleneck and enabling the expansion of *C. rodentium* (Caballero-Flores et al., [2020](#)). This finding highlights the critical role of dietary composition in shaping nutrient niches and influencing pathogen dynamics within the gut ecosystem.

Competition for carbohydrates is another key mechanism of nutrient blocking in the gut. While the intestinal lumen is rich in dietary fibers and complex carbohydrates, only select commensal microbes possess the specialized enzymes required to degrade these substrates. Through cooperative metabolic networks, commensal bacteria efficiently metabolize a broad spectrum of dietary carbohydrates—from complex polysaccharides to simple sugars—preventing pathogens from carving out their own metabolic niche and thus establishing in the gut. For instance, *E. coli* and *Klebsiella* species preferentially utilize simple sugars and oligosaccharides rather than engaging in the breakdown of complex fibers, enabling them to outcompete invading microbes. This has been exemplified by studies showing that *E. coli* restricts the growth of *Salmonella enterica* serovar Typhimurium (*S. Typhimurium*) via competitive galactitol utilization (Eberl et al., [2021](#)). Similarly, *K. oxytoca* has been shown to outcompete *K. pneumoniae* through the preferential use of β -glucoside (Osbelt et al., [2021](#)) and gluconate (Furuichi et al., [2024](#)). Such competitive exclusion mechanisms underscore how commensal-mediated carbohydrate consumption constrains the pathogens' ability to access these vital resources, thereby inhibiting their colonization potential (Sonnenburg et al., [2005](#)).

In addition to amino acids and carbohydrates, trace metals such as iron and zinc are essential for the survival and proliferation of many pathogenic organisms. Commensal microbes actively restrict access to these essential micronutrients through the production of siderophores—small, high-affinity molecules that chelate metal ions, thereby

limiting their availability to competing microbes and pathogens. Members of the Enterobacteriaceae family, including *E. coli*, are particularly proficient in siderophore biosynthesis, enabling them to sequester iron from both host-derived sources and the surrounding microbial community (Gerner et al., [2020](#)). Notably, even in the absence of endogenous siderophore production, *B. thetaiotaomicron* has been shown to scavenge siderophore-bound iron produced by *S. Typhimurium* during inflammation, illustrating the complexity of microbial competition for trace metals in the gut (Zhu et al., [2020](#)). These strategies not only provide these commensals with a competitive advantage but also deprive pathogens of the metals they need for survival and expansion (Deriu et al., [2013](#)).

The *Enterobacteriaceae* family, a prominent group of facultative anaerobic gut microbes that includes both commensals and pathogens like *Salmonella* spp., *Yersinia* and *Shigella* spp. serves as an excellent model for studying microbial resource competition. These organisms are highly adept at occupying ecological niches and competing for key nutrients, including amino acids, carbohydrates, and trace metals. Certain commensal *E. coli* strains, for example, can outcompete other microbes by producing siderophores for iron acquisition or by exploiting alternative electron acceptors, thus restricting access to these resources for competing pathogens. This competitive advantage not only contributes to the exclusion of pathogenic species but also contributes to the overall community stability of the microbiota. By outcompeting potential invaders, commensals help preserve the ecological balance in the gut, ensuring that pathogenic species do not overgrow or cause microbiome disruptions.

The relationship between the microbiota and the host immune system further enhances colonization resistance. Nutrient competition between commensals and pathogens can prevent inflammatory host immune responses, promoting a balanced immune environment. This prevents an overreaction to the commensals while ensuring that pathogens are effectively kept in check. In this way, nutrient competition not only serves as a microbial strategy for survival but also

helps maintain immune homeostasis, preventing infection and promoting a healthy gut ecosystem (Hooper et al., [2012](#)).

In conclusion, resource competition is a fundamental mechanism by which commensal microbes prevent pathogen colonization. By outcompeting pathogens for essential nutrients like amino acids, carbohydrates, and trace metals, commensals help maintain the health and stability of the gut microbiota. This competition, in turn, plays a key role in supporting the host's immune system and preventing infections, highlighting the intricate interplay between the microbiota, nutrients, and host immunity.

14.2.1.2 Interference Competition Mechanisms

To prevent the colonization of incoming microbes, resident gut bacteria engage in interference competition by producing antimicrobial compounds such as bacteriocins and bioactive metabolites.

Furthermore, the gut microbiota can convert host, diet-, and drug-derived metabolites into antimicrobial compounds, which in turn interfere with pathogens. These strategies help maintain colonization resistance and protect against pathogen overgrowth (Sorbara & Pamer, [2019](#)).

Resident microbes secrete bacteriocins, a diverse class of ribosomally (and in some cases non-ribosomally) synthesized peptides with direct antimicrobial activity (Heilbronner et al., [2021](#)). Members of the Lactobacillales order are well-characterized producers of such compounds. For example, *Lactococcus lactis* secretes nisin, a ribosomally synthesized pore-forming lantibiotic with broad-spectrum activity against Gram-positive bacteria (Asaduzzaman & Sonomoto, [2009](#)). Similarly, *Blautia producta* produces a lantibiotic capable of inhibiting vancomycin-resistant *Enterococcus faecium* in vivo (Kim et al., [2019](#)). Other bacteriocins, such as thuricin produced by *Bacillus thuringiensis*, exhibit narrower activity spectra (Rea et al., [2010](#)). Among Gram-negative bacteria, *E. coli* strains, including the probiotic *E. coli* Nissle 1917, produce colicins and microcins with potent activity against closely related *Enterobacteriaceae*, including the human

pathogen *S. Typhimurium* (Kirkup & Riley, [2004](#); Patzer et al., [2003](#); Sassone-Corsi et al., [2016](#)). Beyond *E. coli*, other commensals such as *Bacteroides fragilis* have also been reported to produce strain-specific bacteriocins with either narrow or broad-spectrum antimicrobial activity (Chatzidaki-Livanis et al., [2017](#); Hecht et al., [2016](#)). Of note, some commensals produce genotoxic compounds with antimicrobial properties. For instance, *Klebsiella oxytoca* synthesizes the genotoxin tilimycin, which can induce DNA damage and elevated mutational rates in competing bacteria (Kienesberger et al., [2022](#)). Tilimycin production is upregulated in response to high carbohydrate availability and has been shown to inhibit *S. Typhimurium* in a context-dependent manner (Osbelt et al., [2024](#)).

Furthermore, many Gram-negative bacteria encode type VI secretion systems (T6SS), which allow the direct injection of toxic effector molecules into neighboring bacteria or eukaryotic cells through cell–cell contact (Silverman et al., [2012](#)). These effectors can hydrolyze peptidoglycans or deplete essential molecules like NAD(P)⁺, leading to target cell death. Self-intoxication is prevented by the expression of cognate immunity proteins in the producer cells (Russell et al., [2014](#)).

Another physical antagonism strategy is the contact-dependent inhibition (CDI) system, which is functionally similar to T6SS but structurally distinct. CDI systems involve the delivery of polymorphic toxic proteins via cell-surface receptors, typically encoded by *E. coli*, *Burkholderia*, and *Bacteroides* species. These systems inhibit growth of susceptible strains upon cell–cell contact and have been implicated in shaping strain composition and spatial structure in the gut (Anderson et al., [2014](#); Ruhe et al., [2020](#)).

One important class of antimicrobial metabolites is secondary bile acids, which are derived from primary bile acids synthesized in the human liver and secreted into the duodenum to emulsify dietary fats. Although most primary bile acids are reabsorbed in the ileum, approximately 5% reach the colon, where they are metabolized by anaerobic bacteria into secondary bile acids (Marion et al., [2020](#)). Several secondary bile acids—such as deoxycholic acid, lithocholic acid,

ursodeoxycholic acid, and ω -muricholic acid—have been shown to strongly inhibit both sporulation and vegetative growth of *C. difficile* (Francis et al., [2013](#); Weingarden et al., [2016](#)).

Another relevant class of microbiota-derived metabolites comprises the short-chain fatty acids (SCFAs) acetate, propionate, and butyrate, which are generated through the fermentation of dietary fibers. SCFAs contribute to gut homeostasis by acidifying the intestinal lumen, exerting direct antimicrobial effects, and modulating host epithelial integrity and immune responses in ways that restrict pathogen expansion (Fukuda et al., [2011](#); Parada Venegas et al., [2019](#); Sorbara & Pamer, [2019](#)). Similarly, lactate, a strong organic acid produced by bifidobacteria and lactic acid bacteria, lowers the luminal pH and prevents colonization by pathogens (Alcon-Giner et al., [2020](#)). Notably, butyrate has been shown to enhance mucosal barrier function and reduce epithelial oxygenation, thereby limiting the expansion of facultative anaerobes such as *S. Typhimurium* (Rivera-Chavez et al., [2016](#)). Collectively, these metabolites act through both direct and indirect antimicrobial mechanisms and host-mediated pathways to suppress enteric pathogens.

Indole and its derivatives, produced from tryptophan by commensals such as *E. coli* and *Bacteroides* spp., represent another class of resistance-promoting metabolites. Indole has been shown to strengthen intestinal epithelial barriers, suppress virulence gene expression in pathogens like *EHEC* and *S. Typhimurium*, and reduce inflammation (Bansal et al., [2010](#); Lee & Lee, [2010](#)). These effects indirectly contribute to colonization resistance by limiting the ability of pathogens to establish infection niches.

Altogether, these diverse mechanisms illustrate how resident microbiota inhibit incoming microbes through multiple, often synergistic strategies. This multifaceted interference contributes to the stability and resilience of the gut microbial ecosystem and protects against pathogen invasion.

14.2.1.3 Impact of the Microbiota on Mucosal Barrier Function and Protection from Infection

The gut mucus layer is essential for maintaining intestinal health by acting as a physicochemical barrier that limits pathogen invasion and microbial access to the underlying epithelium. Produced by goblet cells, the composition and structural characteristics of mucus vary along the gastrointestinal tract and are profoundly influenced by the resident microbiota. In the small intestine, mucus forms a relatively loose and permeable diffusion barrier, enriched with antimicrobial peptides to deter microbial encroachment (McGuckin et al., [2011](#)). In contrast, the colonic mucus is organized into two distinct layers: an outer, loose layer that harbors commensal microbes, and an inner, dense layer that remains largely free of bacteria, thereby maintaining spatial segregation between microbes and the epithelial surface. (Johansson & Hansson, [2016](#); Johansson et al., [2008](#); Li et al., [2015](#)). The critical role of the microbiome in shaping the intestinal mucus barrier is underscored by observations in germ-free mice, which exhibit an underdeveloped mucus layer, most notably a markedly thinner inner layer. Similarly, antibiotic treatment results in disruption and thinning of the mucus layer, enabling the translocation of microbes and rendering mice more susceptible to *C. rodentium* (Sawaed et al., [2024](#); Wlodarska et al., [2011](#)). Beyond mere presence, the specific composition of the microbiota critically influences both the structural integrity and barrier function of the mucus layer (Jakobsson et al., [2015](#); Johansson et al., [2008](#)).

A central structural component of the mucus barrier are mucins—large, heavily glycosylated proteins that confer the characteristic viscosity and gel-like consistency to mucus. These molecules are essential for restricting microbial contact with the epithelium and for entrapping pathogens. Two major classes of mucins are distinguished in the gut: transmembrane mucins and gel-forming mucins (Johansson & Hansson, [2016](#)). Transmembrane mucins, including MUC3, MUC4, MUC12, MUC13, and MUC17, are anchored in the apical membrane of epithelial cells, forming the glycocalyx. These mucins are constitutively

expressed by enterocytes and goblet cells under homeostatic conditions, providing a continuous protective interface. In contrast, MUC1 and MUC16 are typically upregulated in response to infection, serving as inducible components of the epithelial defense system (McGuckin et al., [2011](#)).

Gel-forming mucins, predominantly MUC2, form the structural backbone of the gut mucus layer. MUC2 assembles into large, multimeric networks that endow the mucus with its barrier and lubricating properties. Under physiological conditions, goblet cells store mucins in dense core granules and secrete them at a basal rate. However, during episodes of infection or inflammation, mucus secretion is rapidly upregulated (Song et al., [2023](#)). This increase in mucin secretion serves as a critical host defense mechanism, facilitating the clearance of enteric pathogens such as *C. rodentium* and limiting the proximity of commensal microbes to the epithelial surface. By reinforcing the mucosal barrier, increased mucin secretion helps prevent microbial translocation, inflammation, and tissue damage (Bergstrom et al., [2010](#)).

A specialized subset of goblet cells, known as sentinel goblet cells, resides in the colonic crypt and plays a key role in microbial surveillance. These cells recognize bacterial ligands via Toll-like receptors (TLRs), specifically TLR2, TLR4, and TLR5. Upon activation, they initiate a signaling cascade that triggers rapid MUC2 secretion from neighboring goblet cells, thereby reinforcing the mucus barrier (Birchenough et al., [2016](#)).

In addition to mucins, the mucus layer contains a range of bioactive molecules that contribute to host defense. They include members of the trefoil factor (TFF) family, Zymogen granule protein 16 (ZG16), resistin-like molecule (RELMS) family, Ly6/plaur domain-containing protein 8 (Lypd8), and Immunoglobulin A (IgA). Each of these components plays a distinct role in reinforcing the mucus barrier and preventing microbial translocation by mediating pathogen exclusion, neutralization, or immobilization within the mucus layer (Song et al., [2023](#)).

In addition to its barrier properties, the intestinal mucus layer serves as an essential habitat for mucus-associated microbes equipped with the enzymatic machinery to metabolize host-derived glycans. Mucins, as heavily glycosylated proteins, provide a rich source of carbon and nitrogen for mucolytic commensals (Glover et al., [2022](#)). *Akkermansia muciniphila*, for example, is highly specialized in degrading mucins and utilizing them as a nutrient source (Derrien et al., [2011](#); Derrien et al., [2004](#); Shuoker et al., [2023](#)). Mucin degradation by *A. muciniphila* releases mono- and oligosaccharides and generates fermentation products, notably short-chain fatty acids (SCFA). These SCFAs, such as acetate, propionate, and butyrate, play a central role in maintaining gut homeostasis by fostering the growth of other commensals and conferring immunomodulatory and metabolic benefits on the host (Belzer & de Vos, [2012](#)). However, the degradation of mucins and subsequent release of monosaccharides can create nutrient niches that favor the growth of pathogens. For example, *C. difficile* has been shown to exploit these mucin-derived sugars, facilitating its expansion and virulence in the inflamed gut (Engevik et al., [2021](#); Ng et al., [2013](#)). Additionally, studies have demonstrated that mucus-degrading microbes, such as *A. muciniphila*, can increase host susceptibility to *Salmonella* infection by altering the mucus barrier and microbial community structure (Ganesh et al., [2013](#); Rausch et al., [2015](#)). Similarly, a diet low in dietary fiber can drive the microbiota to shift its metabolic activity toward utilizing host-derived mucus glycoproteins as an alternative energy source. The erosion of the mucus layer compromises barrier integrity and enhances vulnerability to mucosal pathogens such as *C. rodentium* (Desai et al., [2016](#)).

Other prominent mucin-utilizing taxa include *Bacteroides fragilis* and *Bacteroides thetaiotaomicron*, which encode extensive repertoires of glycoside hydrolases and sulfatases that enable them to efficiently degrade and release complex glycans (Macfarlane & Gibson, [1991](#); Sarfatis et al., [2025](#); Sonnenburg et al., [2005](#)). Moreover, *B. thetaiotaomicron* can induce distinct fucosylation patterns in the

mucus, thereby creating a specialized nutrient niche and enhancing its ability to adhere to the mucosal surface (Bry et al., [1996](#)).

Beyond serving as a nutrient-rich environment, the mucus layer provides a structural habitat for non-mucolytic bacteria within the mucosal niche. The diverse array of oligosaccharide structures decorating mucins functions as potential ligands for microbial adhesins, facilitating selective interactions between commensals and host glycoproteins. For example, *Fusobacterium nucleatum*, a common oral microbe that can translocate to the gut and has been implicated in colorectal cancer, binds specifically to mucins, particularly MUC2, via its outer membrane adhesin Fap2 (Abed et al., [2016](#)). Similarly, commensal *E. coli* preferentially colonizes the outer mucus layer, where it integrates into mixed-species biofilms in close proximity to the epithelium (Conway & Cohen, [2015](#); Poulsen et al., [1994](#)). In pathological contexts, this niche can be exploited by disease-associated strains such as adherent-invasive *E. coli* (AIEC)—a pathobiont linked to inflammatory bowel disease, which exhibits enhanced capacity to adhere to and invade epithelial cells and persist within the mucus-associated biofilm community (Conway & Cohen, [2015](#)). Several enteric pathogens have evolved strategies to exploit the mucus layer in order to access the underlying epithelium and initiate infection. *S. Typhimurium*, for example, engages in near-surface swimming to detect spatial heterogeneity within the colonic mucus, allowing it to identify regions where the barrier is thinner or discontinuous and thereby reach the epithelium. In contrast, in the cecum, dense mucus primarily fills the base of the intestinal crypts, leaving the epithelial surface between crypts more exposed and permissive to infection (Furter et al., [2019](#)).

14.2.2 Evasion Strategies of Pathogens to Overcome Microbiota-Related Defense

Although the gut microbiota presents a formidable barrier to pathogen invasion, certain microbes have evolved strategies to overcome colonization resistance and establish infection. These strategies include

directly targeting commensals, exploiting metabolic byproducts of the microbiota, manipulating host immune responses, and even leveraging inflammation to their advantage (Fig. 14.2). Below, we outline several key mechanisms employed by bacterial, fungal, and viral pathogens, along with concrete examples.

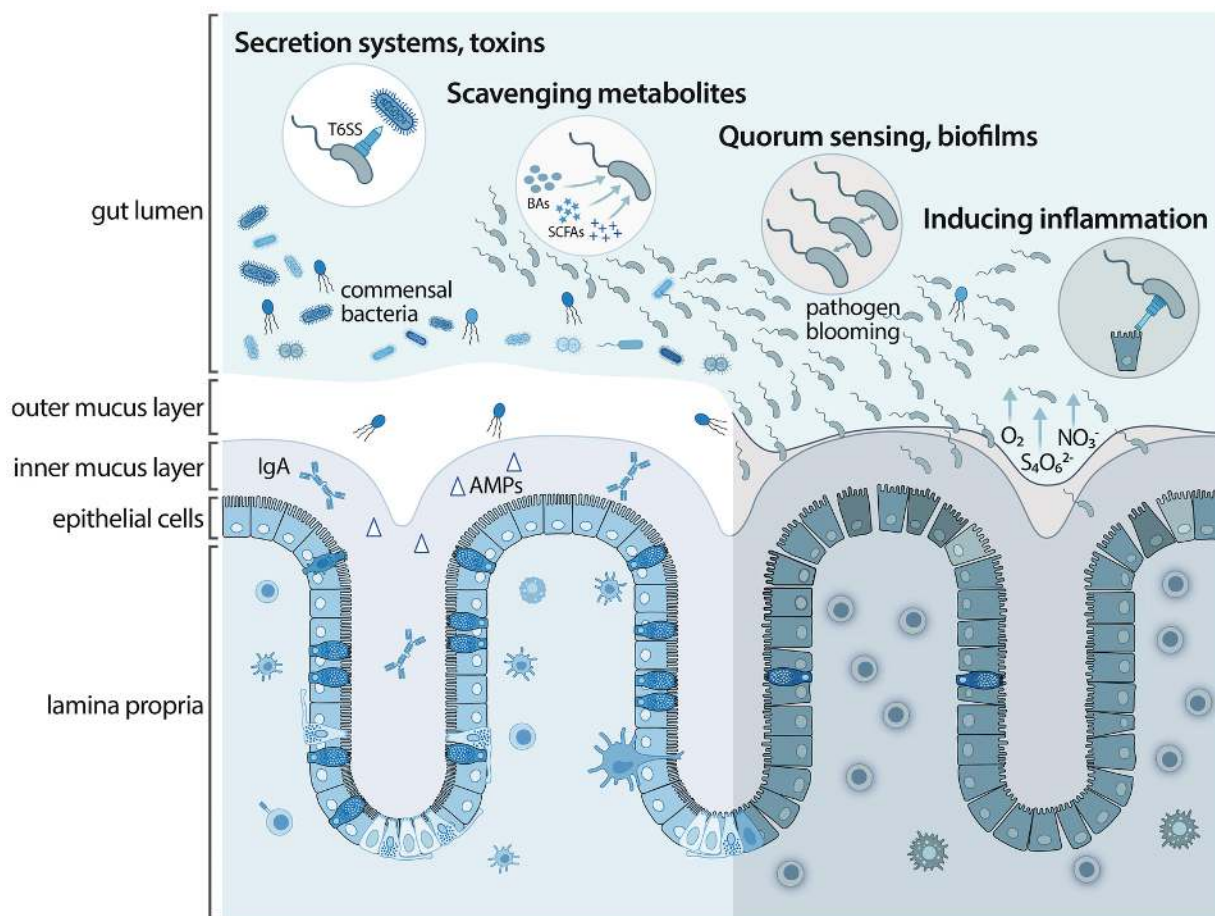


Fig. 14.2 Pathogen strategies to overcome colonization resistance in the gut. This illustration shows the gut environment under the influence of invading pathogens that actively interfere with colonization resistance to establish and expand within the host. Several microbial strategies can disrupt the protective effects of the resident microbiota and host barriers. One such strategy involves the use of secretion systems and toxins, through which pathogens directly attack and kill commensal microbes, weakening microbial competition. In addition, pathogens can exploit specific host- or microbiota-derived metabolites, such as bile acids or short-chain fatty acids, to support their own growth. Quorum sensing and biofilm formation further enhance pathogen colonization by coordinating group behaviors and establishing protective microenvironments that resist clearance. Finally, some pathogens actively induce host inflammation as a means to alter the gut landscape in their favor. Creating a niche rich in inflammation-associated nutrients such as tetrathionate, these pathogens can uniquely exploit the inflamed gut landscape. These combined

strategies contribute to the breakdown of colonization resistance and enable pathogen overgrowth

14.2.2.1 Inducing Inflammation to Reshape the Niche

One of the most effective strategies used by enteric pathogens to overcome colonization resistance is the deliberate induction of intestinal inflammation, which alters the gut environment in ways that favor pathogen expansion (Stecher et al., [2013](#); Stecher et al., [2007](#)). Inflammation disrupts the epithelial barrier, triggers immune cell infiltration, and leads to the release of reactive oxygen and nitrogen species. These changes not only suppress commensal microbes but also generate alternative nutrients and electron acceptors that certain pathogens are uniquely equipped to exploit.

A prominent example is *S. Typhimurium*, which uses its Type III Secretion System (T3SS) to actively invade intestinal epithelial cells and provoke an inflammatory response. This inflammation leads to “Enterobacteriaceae blooming” and reduction of obligate anaerobic members of the microbiota. This scenario is also observed with other enteropathogens, such as *C. rodentium*, enteropathogenic *E. coli*, and *C. difficile*. Members of the Enterobacteriaceae family, including commensal *E. coli* strains, are generally well adapted to thrive in the inflamed gut, both by exploiting nutrients that are uniquely available in this environment and resisting antimicrobial defenses. For example, inflammation leads to the production of reactive oxygen species that convert thiosulfate into tetrathionate—a compound that Enterobacteriaceae can use as a terminal electron acceptor for anaerobic respiration, giving it a growth advantage over obligate fermentative commensals (Winter et al., [2010](#)). Similarly, inflammation liberates nitrate from host cells, further enhancing the competitive fitness of facultative anaerobes like *S. Typhimurium* and certain *E. coli* strains (Lopez et al., [2012](#)). In the case of *C. difficile*, inflammation-driven dysbiosis is a key feature of its pathogenesis. Following antibiotic-induced depletion of the gut microbiota, *C. difficile* colonizes the intestine and produces toxins A and B, which damage epithelial cells and induce inflammation. This inflammatory response suppresses

the residual microbiota and increases the availability of host-derived nutrients like sialic acid and succinate—both of which can be utilized by *C. difficile* for growth (Ferreyra et al., [2014](#); Ng et al., [2013](#)). Inflammation leads to increased production of antimicrobial molecules, such as lipocalin-2, a protein that sequesters the bacterial siderophore enterobactin and thus inhibits iron acquisition. *S. Typhimurium* can modify enterobactin to salmochelin, which is not bound by lipocalin-2 and thus overcomes this host defense mechanism (Raffatellu et al., [2009](#)).

Notably, understanding how enteropathogens thrive in the inflamed gut can enable the development of novel therapeutic strategies that specifically target the metabolic pathways and virulence factors exploited under these conditions. For example, tungstate can inhibit molybdenum-cofactor-dependent microbial respiratory pathways and poise anaerobic respiration (Zhu et al., [2018](#)), while vaccination against siderophore receptors blocks bacterial iron acquisition, thereby preventing pathogen overgrowth in the inflamed gut (Gerner et al., [2022](#)).

14.2.2.2 Killing Competitors: Toxins and Secretion Systems

Pathogens often gain an upper hand by directly eliminating or suppressing microbial competitors in the gut. One approach involves the secretion of exotoxins. *C. difficile* produces TcdA and TcdB, two large glucosylating toxins encoded in a pathogenicity locus (PaLoC) that damage host epithelial cells and disrupt the resident microbiota, facilitating its own outgrowth following antibiotic treatment (Kuehne et al., [2010](#)). When internalized by epithelial cells, structural changes in the toxin lead to pore formation of the endosome and subsequent release in the cytosol (Hecht et al., [1988](#)). Epithelial cell damage then results in the symptomatic manifestation of *C. difficile* infection (CDI). Another notable example of toxin-producing pathogens is *Bacteroides fragilis*. While often a commensal, it can produce the *Bacteroides fragilis* toxin (BFT), a zinc-dependent metalloprotease that targets host cell junctions and thus damages the colonic epithelial barrier (Sears et al.,

[2014](#)). A recent study by Hill et al. revealed that BFT might facilitate niche expansion to the lamina propria, a compartment inaccessible to non-toxigenic strains (Hill et al., [2014](#)). Perhaps the most notable enteral toxins are the Shiga toxins produced by enterohemorrhagic *E. coli* (EHEC) and *Shigella dysenteriae* serotype 1 (Lee et al., [2021](#)). Shiga toxins are phage-encoded exotoxins that are the primary virulence factors associated with hemorrhagic colitis and bacterial dysentery, damaging the host through inhibiting protein synthesis and inducing cell death (Lee et al., [2016](#)).

In addition to soluble toxins, many Gram-negative pathogens use contact-dependent killing systems. The Type VI Secretion System (T6SS) is a molecular syringe that can secrete scavenging proteins to the gut lumen or directly inject toxic effectors into neighboring cells (Allsopp et al., [2020](#)). *V. cholerae*, for instance, uses its T6SS to eliminate commensal bacteria during gut colonization, thereby gaining a competitive edge (Zheng et al., [2010](#)). Beyond the killing of competitors, T6SS was also shown to be advantageous in *V. cholerae* (Borgeaud et al., [2015](#)) and *Acinetobacter baylyi* (Ringel et al., [2017](#)) via the uptake of nutrients and cellular content of the lysed prey cells, and even facilitating horizontal gene transfer. Likewise, the Type III Secretion System (T3SS), commonly found in *S. Typhimurium* and *Shigella flexneri*, injects virulence factors into host cells, which is a prerequisite of their intracellular life cycle (De Souza Santos & Orth, [2019](#)). As a result, these bacteria can invade host cells (Flint et al., [2016](#)), which enables access to nutrients and facilitates further tissue invasion (Mellouk & Enninga, [2016](#)) and the induction of inflammation.

14.2.2.3 Quorum Sensing and Biofilm Formation

Quorum sensing (QS) is a bacterial communication system that enables pathogens to measure their population density through the release and detection of small signaling molecules, such as acyl-homoserine lactones (AHLs) or autoinducing peptides (AIPs). In the gut environment, QS is a critical strategy that allows pathogens to coordinate group behaviors essential for successful colonization,

including biofilm formation, secretion of toxins, and evasion of host defenses (Miller & Bassler, [2001](#); Oliveira et al., [2023](#)).

Biofilms play a pivotal role in shielding pathogens from both host immune mechanisms and competitive microbial interactions, thereby facilitating persistence and dissemination. For example, *C. difficile* forms structured biofilms that protect it from bile acids and antimicrobial peptides in the gut, contributing to its recurrence following antibiotic treatment (Dapa & Unnikrishnan, [2013](#); Dubois et al., [2019](#)). These biofilms are regulated in part by QS-like systems, including cyclic di-GMP signaling, which modulate the transition from motile to sessile lifestyles. Similarly, *S. Typhimurium* uses the AI-2 QS system to regulate genes associated with virulence and biofilm formation in response to host-derived signals. These biofilms help the pathogen adhere to the gut mucosa and resist removal by peristalsis or microbial competition (Walawalkar et al., [2016](#)). QS also regulates the expression of the Salmonella pathogenicity islands (SPIs), which encode the Type III secretion systems critical for epithelial invasion. Another example is enterohemorrhagic *E. coli* (EHEC), which uses AI-3/epinephrine/norepinephrine sensing through the QseBC QS system to activate flagellar genes and Shiga toxin production, enhancing its capacity to colonize the gut in the presence of competing microbiota (Clarke et al., [2006](#)).

By enabling the formation of protective niches and the coordinated expression of virulence factors, QS and biofilm formation provide gut pathogens with potent tools to resist colonization resistance and establish infection in an otherwise hostile microbial ecosystem. However, it remains unclear how QS signals are modulated or disrupted by the resident microbiota. While some commensals are known to produce molecules that interfere with QS circuits, the ecological relevance of such interactions in vivo is still poorly understood. As highlighted in a recent review, the impact of commensal-derived QS interference on pathogen behavior, colonization success, or virulence expression remains an emerging and largely unexplored frontier in gut microbiome research (Oliveira et al., [2023](#)).

14.2.2.4 Exploiting Microbiota-Derived Metabolites

Pathogens often benefit from metabolites produced by the microbiota. Short-chain fatty acids (SCFAs), such as acetate and butyrate, are key microbial fermentation products that not only inhibit but can also serve as energy sources for pathogens. For instance, *S. Typhimurium* uses SCFAs to promote colonization in the inflamed gut (Bronner et al., [2018](#)). Enteropathogenic *E. coli* (EPEC), *V. cholerae*, and *Yersinia enterocolitica* have also been shown to respond to SCFAs by modulating their virulence programs (Nakanishi et al., [2009](#); Rivera-Chavez et al., [2016](#)). *Listeria monocytogenes* benefits from the presence of SCFAs, which transcriptionally enhance listeriolysin O toxin production in the anaerobic gut lumen (Rinehart et al., [2020](#)). Similarly, *S. Typhimurium* utilizes succinate as a preferred carbon source during inflammation-driven dysbiosis, using it for anaerobic respiration (Faber et al., [2017](#)).

Microbiota-derived bile acids are another important class of molecules influencing pathogenesis. *C. difficile* relies on primary bile acids as germination cues, allowing its vegetative cells to colonize the gut once the microbiota is depleted and bile acid metabolism is disrupted (Sorg & Sonenshein, [2008](#)). For example, conjugated primary bile acid taurocholic acid serves as an environmental cue for spore germination in the small intestine, since the conditions here are more favorable (Kochan et al., [2018](#)). In contrast, secondary bile acids produced by commensals typically inhibit vegetative *C. difficile* cells, illustrating the dual role of these metabolites.

14.2.2.5 Fungal and Viral Pathogens

While bacterial interactions dominate the landscape of intestinal colonization resistance, both fungal and viral communities—collectively referred to as the mycobiome and virome—are increasingly recognized as important players in gut health and disease. The most extensively studied gut fungus is *Candida albicans*, a common commensal organism that can overgrow and shift to a pathogenic state under certain conditions, such as antibiotic treatment, immunosuppression, or microbiome disruption (Perez, [2021](#)). For

instance, *C. albicans* overgrowth has been shown to impair epithelial barrier function and promote inflammation in mouse models (Mason et al., [2012](#)). In immunocompromised individuals, invasive *Candida* infections originating from the gut can lead to systemic disease, disseminating beyond the bloodstream into nearly every internal organ leading to life-threatening infections (Clancy & Nguyen, [2012](#); Zhai et al., [2020](#)). Fungal diversity in the gut is typically lower than bacterial diversity, and it is typically influenced by diet, antibiotic use, and host immunity (Iliev et al., [2012](#)). However, certain fungi, such as *Saccharomyces boulardii*, can exert beneficial effects and have been used as probiotics in gastrointestinal disorders (Czerucka et al., [2007](#); Martins et al., [2010](#)).

The gut virome, largely composed of bacterial (bacteriophages) and archaeal viruses but also eukaryotic viruses, is also an important but less well-understood component of the microbiota. Enteric viruses such as *Rotavirus*, *Norovirus*, *Astrovirus*, and *Sapovirus* are well-known, frequent causes of gastrointestinal disease (Harper et al., [2020](#)). These viral pathogens can directly damage the intestinal epithelium and modulate immune responses. For example, *Rotavirus* has been shown to inhibit the host's INF production, thus down-regulating inflammatory cytokines (Gonzalez-Ochoa et al., [2017](#)). Furthermore, *Rotavirus* toxin NSP4 directly affects mucosal tight junctions, resulting in typical diarrhea. Recent studies suggest that some probiotics, such as *Lactobacillus casei* and *Bifidobacterium adolescentis*, might offer protection against viral infections by enhancing mucosal immunity or competing for attachment sites (Gonzalez-Ochoa et al., [2017](#); Rigo-Adrover et al., [2017](#)). However, more mechanistic studies are needed to understand how microbial and viral interactions contribute to colonization resistance and susceptibility.

14.2.3 Environmental and Host Factors Compromising the Microbiota

Several factors can compromise colonization resistance, increasing susceptibility to enteric infections and the risk of pathogen overgrowth,

which may lead to systemic disease. Because colonization resistance can be weakened by diverse underlying causes, strategies for prevention and restoration must be tailored to the specific context (Fig. 14.3). The following section will explore the most common factors that affect the microbiome and colonization resistance, such as age, dietary habits, diseases, and medical interventions. Gaining a clear understanding of these conditions—and how they influence this important microbiome function—is essential for developing effective approaches to restore microbiota balance and strengthen colonization resistance against pathogenic threats.

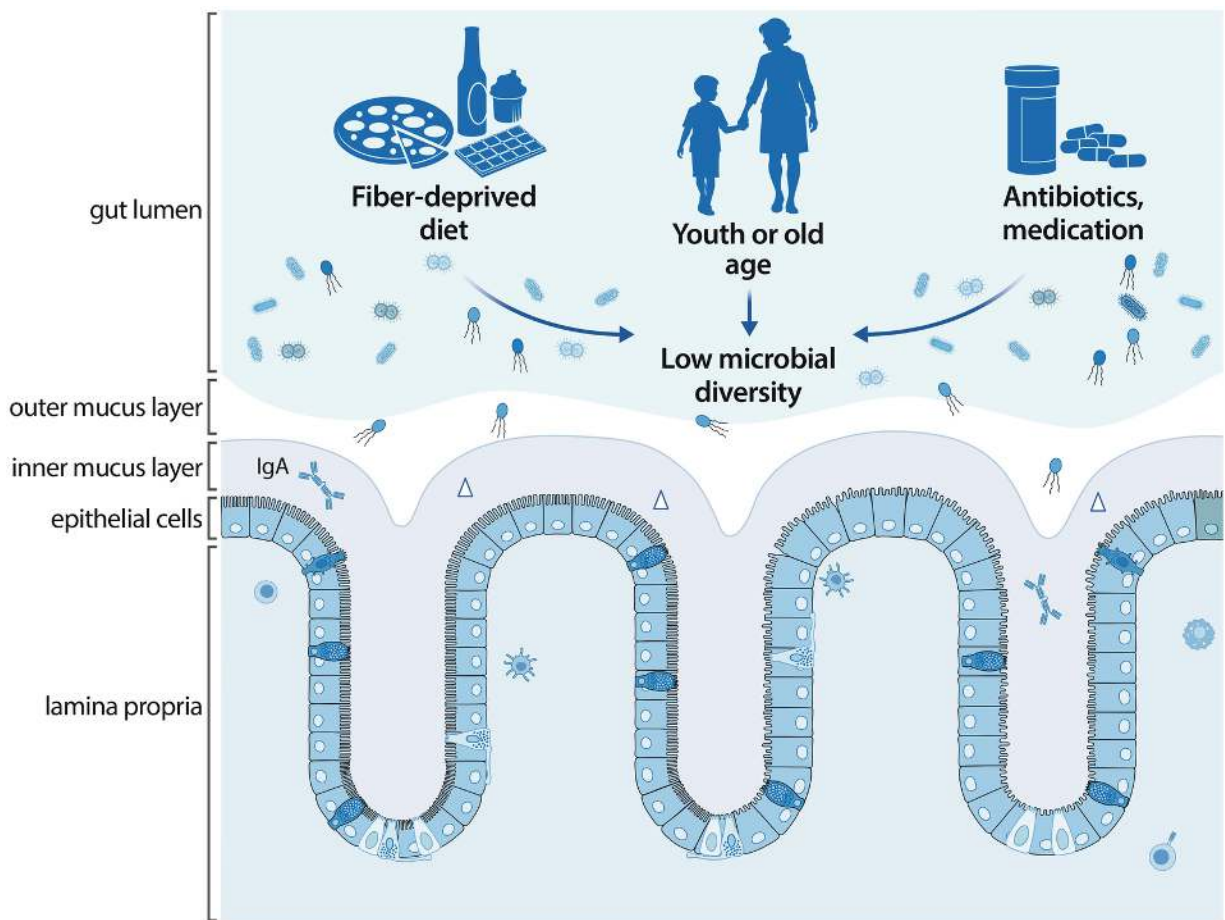


Fig. 14.3 Host- and environment-associated factors that compromise colonization resistance. This illustration depicts a gut environment characterized by a reduced microbial diversity, a thinner mucus layer, and diminished immune presence along the mucosal surface, all of which contribute to weakened colonization resistance. Several factors can contribute to such a vulnerable state. A fiber-deprived diet, lacking microbiota-accessible carbohydrates, limits the availability of diverse carbon sources necessary to sustain a functionally rich and taxonomically

diverse microbial community. This nutritional constraint reduces microbial competition and favors the expansion of opportunistic species. Similarly, the gut microbiota in very young or elderly individuals is typically lower in diversity and stability, making these age groups more susceptible to pathogen colonization. Microbial communities in early life are still developing, while in old age they may be altered by lifestyle, immune decline, or accumulated medical interventions. Another major factor compromising colonization resistance is the use of antibiotics and other medications, which can directly deplete or disturb commensal populations. Such perturbations often lead to ecological imbalances and create niches that pathogens can exploit. Together, these factors reduce the protective capacity of the gut microbiota and increase the risk of infection and dysbiosis

14.2.3.1 Age

Biological age is a key factor influencing the composition of the gut microbiota (Yatsunenko et al., [2012](#)). At birth, infants are first exposed to microbes (Perez-Munoz et al., [2017](#)), and during the initial days of life, their gut microbiome is typically of low diversity, dominated by genera such as *Bacteroides*, *Escherichia/Shigella*, and *Bifidobacteria* (Backhed et al., [2015](#)). The microbial community continues to diversify both compositionally and functionally over the first year, which contributes to the heightened susceptibility of young infants to infections during this period (Olin et al., [2018](#)). Antibiotic exposure during early life has particularly disruptive effects, significantly reducing microbial diversity—especially at the strain level—and compromising long-term microbiome stability, as shown by comparative analyses of antibiotic-treated and untreated children during the first 3 years (Yassour et al., [2016](#)). Around the age of three, inter-individual microbiome differences among children, as well as between children and adults, begin to diminish, signaling the shift toward an adult-like microbial composition (Yatsunenko et al., [2012](#)).

In healthy adults, the gut microbiome remains relatively stable over several decades (D'Argenio & Salvatore, [2015](#)). With advancing age, however, microbial diversity gradually declines. This age-related shift is less tied to chronological age and more reflective of a progressive change associated with aging (O'Toole & Jeffery, [2015](#)). In elderly individuals, the gut microbiota tends to be less diverse, with increased dominance of Bacteroidota and a notable reduction in key taxa such as *Prevotella* spp. (Claesson et al., [2011](#)), the butyrate-producing

Faecalibacterium spp., and *Eubacterium rectale*, as well as health-associated *Bifidobacterium* spp. (Claesson et al., [2012](#); Ghosh et al., [2020](#); Jeffery et al., [2016](#)). Concurrently, there is a higher prevalence of *Fusobacterium*, *Streptococcus*, *Clostridium*, and Enterobacteriaceae, which have also been associated with inflammatory gut states, the microbiome of the elderly (Ghosh et al., [2020](#); Jeffery et al., [2016](#)).

14.2.3.2 Diet

The host's diet exerts a profound influence on the composition and diversity of its gut microbiota. From early childhood, habitual dietary patterns shape the community structure, favoring, for example, *Bacteroides* or *Prevotella* dominance (Bergstrom et al., [2014](#)). Dietary fibers serve as critical energy sources for gut microbes, and their fermentation helps reduce pathogen colonization by accelerating intestinal transit (Conlon & Bird, [2014](#); Stephen & Cummings, [1980](#)).

In contrast, high-fat diets can impair barrier function and increase systemic inflammation through elevated gut permeability and microbial lipopolysaccharide translocation (Moreira et al., [2012](#)) (Munoz & Costa, [2013](#); Tan & Norhaizan, [2019](#)). The so-called Western diet, characterized by excess fats, sugars, and low fiber intake (Statovci et al., [2017](#)), is associated with obesity, metabolic dysfunction (Valdes et al., [2018](#)), and immunological disorders including IBD (Ferreira et al., [2010](#); Manichanh et al., [2006](#); Wiese et al., [2016](#)), asthma (Forsythe et al., [2007](#); MacSharry et al., [2012](#); Statovci et al., [2017](#)), psoriatic arthritis (Scher et al., [2015](#)), and coeliac disease (Schipa et al., [2010](#)), potentially through induction of dysbiotic microbiota profiles that result from poor dietary habits (Valdes et al., [2018](#)).

In the context of colonization resistance, diet plays a central role in modulating both direct and indirect interactions among the host, microbiota, and pathogens. Studies using murine models have demonstrated that a Western-style diet fosters an inflamed gut environment (Statovci et al., [2017](#)). This inflammatory state has been linked to heightened susceptibility to adherent-invasive *E. coli* (Agus et al., [2016](#)) and prolonged persistence of the Enterobacteriaceae

pathogen *C. rodentium* (An et al., [2021](#)). Mechanistically, certain dietary components can directly support pathogen expansion. For example, trehalose, a food additive, has been shown to enhance the virulence of epidemic *C. difficile* lineages (Collins et al., [2018](#)), while increased dietary amino acids can promote overgrowth of pathogenic *E. coli* strains (Caballero-Flores et al., [2020](#)).

Diet also exerts strong indirect effects by shaping the structure and metabolic output of the resident microbiota. Microbiota-accessible carbohydrates (MACs), such as pectin and resistant starches, support the growth of beneficial commensals that produce short-chain fatty acids (SCFAs). These metabolites can acidify the intestinal lumen and inhibit pathogens such as *C. difficile*. In a murine model, MAC supplementation suppressed *C. difficile* infection severity and restored colonization resistance (Hryckowian et al., [2018](#)). In contrast, high-fat diets elevate bile acid secretion, which can disrupt the protective microbiota and allow opportunistic pathogens to colonize. This mechanism was shown in mice, where fat-induced bile flux transiently reduced microbiota-mediated suppression of *Salmonella* Typhimurium, leading to pathogen overgrowth (Wotzka et al., [2019](#)).

These findings highlight that both short- and long-term dietary exposures can influence pathogen dynamics through a complex interplay of microbiota composition, metabolite production, and mucosal immunity. While diet has long been implicated in inflammatory and metabolic diseases (Hills et al., [2019](#)), its role in modulating colonization resistance against enteric pathogens is now increasingly recognized as mechanistically diverse and context-dependent.

14.2.4 Microbiome-Drug Interactions

14.2.4.1 Antibiotics, Spread of Antimicrobial Resistance, and Multidrug-Resistant Bacteria (ESKAPE Pathogens)

Antibiotics are among the most powerful factors influencing the composition of the gut microbiota. While their disruptive effects are well known, antibiotics have undeniably revolutionized medicine,

dramatically reducing mortality from infectious disease and medical procedures since their introduction in the early twentieth century (Hutchings et al., [2019](#)).

However, the rapid pace of antibiotic development, combined with their widespread and often indiscriminate use in both human and veterinary medicine, has significantly contributed to the emergence and global spread of antimicrobial resistance (AMR). This growing threat now jeopardizes many of the medical advances achieved over the past century. Beyond selecting for resistant pathogens, antibiotics inflict collateral damage on the commensal microbiota, disrupting host-microbe homeostasis and compromising colonization resistance, thereby increasing susceptibility to opportunistic infections (Keefer, [1951](#); Willing et al., [2011](#); Woelfel et al., [2024](#)). This disturbance can predispose individuals to secondary infections, most notably infections with *C. difficile* (Martin et al., [2016](#)). Another concern in clinical medicine is the global rise of multidrug-resistant infections, particularly those caused by so-called 'ESKAPE' organisms—*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Enterobacter* species, along with other members of the *Enterobacterales* group. These organisms are notorious for their ability to evade most currently available antibiotics and are among the leading causes of nosocomial infections (Miller & Arias, [2024](#)). The widespread use of broad-spectrum antibiotics not only accelerates resistance in these organisms but also disrupts the gut microbiota. For example, a recent interventional study in healthy men demonstrated that just a four-day course of antibiotics induced blooms of opportunistic taxa such as *Enterococcus faecalis* and *Fusobacterium nucleatum*, while depleting beneficial commensals like *Bifidobacterium* spp. Nonetheless, the microbiota is not entirely passive in this process; it actively contributes to ecosystem stability by conferring resilience, the ability to recover and return to its pre-disturbance state following perturbations such as antibiotic treatment. Commensal bacteria can adapt to antibiotic pressure by evolving and exchanging antibiotic resistance genes, which

facilitates microbiome recovery (Munch et al., [2023](#); Palleja et al., [2018](#)). While microbiome composition often rebounds in healthy individuals, certain shifts can persist, leaving a long-term imprint on host physiology. These durable microbial signatures have been implicated in the development of metabolic and inflammatory conditions such as diabetes and intestinal inflammation (Mikkelsen et al., [2015](#); Pan et al., [2024](#); Park et al., [2021](#)).

Given the current AMR crisis, there is an urgent need for novel antimicrobial strategies, but the discovery and clinical development of entirely new antibiotics remains a challenge (Lewis, [2020](#)). One strategy under active development involves chemical modification of existing antibiotic classes to bypass common resistance mechanisms (Miller & Arias, [2024](#)). Yet, because most of these agents still target conserved bacterial pathways such as cell wall biosynthesis or RNA/DNA metabolism, there is increasing demand for drugs with novel mechanisms and narrower spectra of activity. A particularly promising avenue exploits bacterial dependence on essential metal ions such as iron, which is critical for many bacteria, including *Enterobacteriales* (Sargun et al., [2021](#)). In parallel, microbiota-sparing alternatives are gaining traction, as they represent a critical pillar in the global response to AMR. One such approach, vaccine-enhanced competition, pairs pathogen-specific immunization with the introduction of a benign metabolic niche competitor. This dual strategy aims to pre-emptively occupy ecological niches, restoring colonization resistance and preventing pathogen expansion (Lentsch et al., [2025](#)).

14.2.4.2 Proton Pump Inhibitors

Beyond antibiotics, a wide array of non-antibiotic drugs also influences the composition and function of the gut microbiota. Conversely, the gut microbiota is capable of metabolizing and biotransforming many of these pharmaceuticals. This bidirectional interaction forms the foundation of pharmacomicrobiomics, an emerging field that investigates how microbial communities modulate drug responses and, in turn, how drugs shape the microbiome (Zimmermann et al., [2021](#)):

Recent studies suggest that non-antibiotic drugs can profoundly disrupt the gut microbiota, diminish colonization resistance, and increase susceptibility to bacterial infections (Griesshammer et al., [2025](#); Maier et al., [2018](#)).

Among the most widely prescribed medications are proton pump inhibitors (PPIs), such as esomeprazole, which are routinely used for the prevention or treatment of gastroesophageal reflux disease, as well as gastric and duodenal ulcers. Although highly effective at suppressing gastric acid secretion, PPIs have been shown to significantly reduce microbial diversity and alter the taxonomic and functional composition of the gut microbiota (Imhann et al., [2016](#)). These changes are clinically relevant, as they have been associated with an increased risk of enteric infections, including *C. difficile*, *Salmonella*, and other enteric pathogens, as well as extraintestinal infections such as pneumonia (Bouwknegt et al., [2014](#); Janarthanan et al., [2012](#); Leonard et al., [2007](#); Maret-Ouda et al., [2023](#)). This increased susceptibility is largely attributable to the loss of gastric acid as a primary defense barrier against ingested microbes. By raising gastric pH, PPIs permit the survival and downstream colonization of otherwise transient or harmful bacteria. Notably, PPI use has also been linked to an overrepresentation of oral bacteria such as *Rothia* spp. in the gut microbiome (Imhann et al., [2016](#)), likely reflecting reduced microbial elimination in the upper gastrointestinal tract. While the full clinical implications of these microbial shifts are still elusive, they highlight the need to consider microbiome-mediated effects as part of a risk-benefit consideration in long-term PPI therapy.

14.2.4.3 Non-steroidal Anti-inflammatory Drugs (NSAID)

Non-steroidal anti-inflammatory drugs (NSAIDs) exert their analgesic, antipyretic, and anti-inflammatory effects through inhibition of the cyclooxygenase enzymes COX-1 and COX-2. Despite their widespread clinical use, NSAIDs are well known to cause gastrointestinal adverse events, including peptic ulcer disease, mucosal erosions, and gastrointestinal bleeding (Bjarnason et al., [2018](#); Marlicz et al., [2014](#)).

To mitigate these complications, especially in patients at elevated risk, NSAIDs are commonly co-prescribed with PPIs, which help to protect the upper gastrointestinal mucosa. Beyond their mucosal toxicity, NSAIDs also exert significant effects on the gut microbiota. Accumulating evidence indicates that NSAID intake alters microbial composition in a drug-specific manner, which might contribute to NSAID-induced mucosal toxicity. For example, individuals taking ibuprofen exhibit increased relative abundances of *Prevotella* spp. and *Alistipes* spp., while *Bacteroides* spp. are enriched in those using naproxen (Rogers & Aronoff, [2016](#)). Notably, the concurrent use of NSAIDs and PPIs induces a distinct microbial signature compared to NSAID use alone, characterized by increased levels of *Enterococcus* spp., *Streptococcus* spp., and *Lactobacillus* spp.

These findings suggest that both NSAIDs and PPIs, individually and in combination, can disrupt the gut microbiome, potentially contributing to intestinal inflammation and compromising host-microbe homeostasis and susceptibility to enteric infections.

14.2.5 Medical Treatments and Diseases Leading to Increased Infection Susceptibility

14.2.5.1 Chemotherapy, Radiation Therapy, Checkpoint Inhibitors

Chemotherapy remains a cornerstone in the treatment of many solid and hematologic malignancies. High-intensity regimens are essential for aggressive cancers, such as certain lymphomas and acute leukemias. Likewise, both autologous and allogeneic stem cell transplantation protocols rely on high-dose chemotherapy to achieve adequate myeloablation. While the cytotoxic effects on hematopoietic cells are therapeutically desired, chemotherapy also inadvertently affects other rapidly dividing cells, most notably the epithelial cells lining the GI tract. This off-target toxicity leads to mucosal injury, disrupting the integrity of the intestinal barrier and predisposing patients to microbial translocation, systemic inflammation, and infection. The resulting condition, often referred to as gastrointestinal mucositis, represents a

major clinical complication associated with increased morbidity (Sougiannis et al., [2021](#)). It often necessitates empirical broad-spectrum antibiotic treatment, which further disrupts the gut microbiota, leads to intestinal domination of harmful bacteria, and increases the risk of secondary infections, including bloodstream infections and *C. difficile* colitis (Puerta-Alcalde et al., [2025](#); Taur et al., [2012](#)). Notably, the baseline composition of the microbiome has been associated with response to chemotherapy as well as to adverse events, and is a predictor of mortality in hematopoietic stem cell transplantation (Hakim et al., [2018](#); Hes et al., [2024](#); Peled et al., [2020](#); Terrisse et al., [2021](#)).

Moreover, neutropenic fever, a hallmark of chemotherapy-induced immunosuppression, is an early warning sign of bloodstream infection and impending sepsis. Timely and accurate identification of the causative pathogen is critical, yet conventional microbiological diagnostics are time-consuming and often detect only a limited subset of pathogens in patients with febrile neutropenia. Metagenomic next-generation sequencing has emerged as a tool to overcome these limitations by enabling unbiased, high-resolution detection of a broad spectrum of infectious agents. This approach can potentially identify the causative pathogen rapidly and with high precision, even in cases where standard culture-based methods fail (Wang et al., [2024](#)).

In parallel, patients receiving chemotherapy are frequently exposed to prolonged and broad-spectrum antibiotics, which exert strong selective pressure on the gut microbiota. This not only promotes the emergence of antibiotic-resistant organisms but also fosters colonization and subsequent infection with multidrug-resistant bacteria (Lopera et al., [2024](#)). Common culprits include extended-spectrum beta-lactamase (ESBL)- or carbapenemase-producing Enterobacterales (e.g., *E. coli*, *Klebsiella pneumoniae*), as well as other MDR pathogens such as *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Stenotrophomonas maltophilia*. Additionally, infections with vancomycin-resistant *Enterococcus* (VRE) are increasingly prevalent in this immunocompromised population (Danielsen et al.,

[2023](#); Gupta et al., [2025](#)). These infections pose significant therapeutic challenges and are associated with increased morbidity and mortality, underscoring the urgent need for microbiota-informed antimicrobial stewardship and infection prevention strategies in oncology settings.

Radiotherapy is another cornerstone of cancer treatment, employing ionizing radiation to induce genotoxic damage in tumor cells. While its direct effects on malignant tissues are well-characterized, the influence of the microbiota on radiotherapy outcomes remains less understood. However, growing evidence suggests that microbiota may modulate both therapeutic efficacy and side effects. Radiation not only targets tumor cells but also damages actively proliferating healthy tissues, including the skin, bone marrow, and epithelia lining the gastrointestinal tract. This disruption can lead to significant shifts in microbiota composition, which in turn contribute to the development and severity of radiotherapy-induced toxicities such as enteritis, dermatitis, diarrhea, and bone marrow suppression (Roy & Trinchieri, [2017](#)). Understanding these interactions could offer new opportunities to mitigate side effects and improve patient outcomes.

Beyond conventional cancer therapies, immune checkpoint inhibitors (ICI) have emerged as a transformative approach in oncology. These therapies have been successfully implemented across a range of malignancies and have significantly improved outcomes for many patients. In cancer, the host's antitumor immune response is often suppressed through inhibitory signaling pathways that prevent effective T-cell activation. ICI, such as antibodies targeting CTLA-4, PD-1, or PD-L1, block these regulatory pathways, thereby reactivating cytotoxic T-cell responses and enabling the immune system to recognize and eliminate tumor cells (Sharma et al., [2023](#)). By overcoming tumor-induced immune evasion, ICIs have redefined cancer therapy and exemplify the potential of immunotherapy. A growing body of preclinical and clinical evidence indicates that the gut microbiome plays a critical role in modulating the efficacy of ICI (Li et al., [2022](#)). Several studies have shown that specific microbial

communities can enhance antitumor immunity, whereas dysbiosis is associated with poor treatment responses (Gopalakrishnan et al., [2018](#); Routy et al., [2018](#); Sivan et al., [2015](#); Vetizou et al., [2015](#)). However, substantial interindividual variability in gut microbiota composition likely contributes to the heterogeneity in clinical outcomes observed among ICI-treated patients.

Currently, numerous clinical trials are underway to investigate whether targeted microbiome modulation—through approaches such as fecal microbiota transplantation—can enhance treatment outcomes in patients with ICI (Baruch et al., [2021](#); Davar et al., [2021](#)). Despite encouraging early findings, a major barrier to clinical transplantation remains the lack of reproducible and consistent microbiome signatures across cohorts (Lee et al., [2022](#)). To date, no single microbial species has been reliably identified as a universal predictor of treatment response, highlighting the need for integrative approaches that consider microbial function, host genetics, immune status, and environmental factors.

14.2.5.2 Immunocompromised States (HIV, Organ Transplantation)

Bacterial pathogenicity exists on a continuum that is strongly dependent on host immune status (Casadevall & Pirofski, [2003](#)). While host-microbiome interactions have been extensively studied in the context of cancer and immunotherapy, their role during impaired immunity outside oncological settings remains poorly understood.

Human immunodeficiency virus (HIV), a sexually transmitted retrovirus, selectively infects and depletes CD4⁺ T-cells, and, if left untreated, leads to acquired immunodeficiency syndrome (AIDS). The gastrointestinal tract, particularly the gut-associated lymphoid tissue (GALT), is a major site of viral replication and immunological injury. One of the earliest and most profound consequences of HIV infection is the rapid and sustained depletion of memory CD4⁺ T cells in the gut mucosa (Veazey et al., [1998](#)). Among these, a subset of CD4⁺ T cells producing IL-17 and IL-22, commonly referred to as Th17 and Th22

cells, are particularly susceptible to HIV-mediated (Favre et al., [2009](#); Kok et al., [2015](#)) depletion. These cytokines play a pivotal role in preserving mucosal barrier integrity by promoting antimicrobial peptide secretion, mucus production, and epithelial regeneration. The loss of these barrier-supporting T cell subsets disrupts mucosal immune regulation and compromises epithelial barrier integrity, resulting in intestinal inflammation and facilitating the translocation of microbes or their products from the gut lumen into systemic circulation (Deeks et al., [2013](#); Nasi et al., [2014](#)). Microbial translocation is a key driver of persistent systemic inflammation and chronic immune activation, both of which are recognized as major contributors to HIV disease progression, even in individuals receiving effective antiretroviral therapy (ART) (Ancona et al., [2021](#); Brenchley et al., [2006](#); Somsouk et al., [2015](#)). Beyond T cells, HIV-induced mucosal immune dysfunction also affects gut B cells. Recent findings show a depletion of germinal center B cells and IgA+ plasma cells in the GI tract, further compromising barrier integrity and immune homeostasis (Cossarini et al., [2024](#)).

This disruption of the mucosal barrier is believed to contribute to characteristic alterations in gut microbiome composition, independent of ART (Lozupone et al., [2013](#); Monaco et al., [2016](#)). Several microbial signatures are consistently observed in HIV patients, including an increased abundance of *Pseudomonadota*, *Erysipelotrichaceae*, *Desulfovibrionaceae*, and *Fusobacteriota*, alongside a depletion of beneficial taxa such as *Lachnospiraceae*, members of *Bacteroidota*, and *Ruminococcaceae* (Vujkovic-Cvijin et al., [2013](#); Vujkovic-Cvijin & Somsouk, [2019](#)). These compositional changes may further contribute to disease progression by modulating host immunity and metabolic functions. Notably, the loss of *Ruminococcaceae* and *Lachnospiraceae* taxa, both prominent producers of SCFA, may impair epithelial integrity and mucosal tolerance by reducing levels of beneficial metabolites such as butyrate. Concurrently, expansion of microbial taxa capable of producing immunoregulatory or proinflammatory metabolites, such as kynurenine, may exacerbate systemic inflammation and chronic

immune activation (Vujkovic-Cvijin et al., [2013](#); Vujkovic-Cvijin & Somsouk, [2019](#)).

Other states of compromised immunity include solid organ transplantation, which necessitates the use of immunosuppressive drugs to prevent graft rejection. While genetic disparities between donor and recipient are key determinants of transplant compatibility, the T cell-mediated alloresponse is also influenced by a range of environmental and host-related factors, including infections, diet, and the gut microbiota (Pirozzolo et al., [2021](#)). Among these, the microbiota has emerged as a particularly influential modulator of transplant outcomes, affecting immune reactivity, tolerance induction, and susceptibility to complications such as opportunistic infections (Pirozzolo et al., [2021](#)).

For example, in patients undergoing kidney transplantation, specific microbiome alterations have been shown to precede episodes of acute rejection (Holle et al., [2025](#)). The pre-rejection microbiome was marked by reduced microbial diversity and a notable depletion of SCFA-producing genera, including *Blautia*, *Clostridia*, and *Ruminococcus*. In contrast, potential pathobionts such as *Fusobacterium* and *Streptococcus* species were enriched. Interestingly, patients without signs of rejection exhibited progressive normalization of their gut microbiota over time, with a shift toward a composition more closely resembling that of healthy individuals.

The mechanisms by which the microbiota modulate transplant outcomes are multifaceted. Commensal microbes and their metabolites calibrate both local and systemic immune responses, influencing immune cell differentiation and function. In addition, the microbiota can alter the pharmacokinetics and efficacy of immunosuppressive drugs (Pirozzolo et al., [2021](#)). For example, *Faecalibacterium prausnitzii*, a beneficial microbe with anti-inflammatory properties (Martin et al., [2023](#)), has been shown to metabolize tacrolimus, converting it into a less potent form and potentially reducing its immunosuppressive efficacy (Guo et al., [2019](#)).

Thus, advancing our understanding of the microbiome in the context of immunosuppression and developing targeted strategies to modulate the microbial composition holds promise to improve clinical outcomes in immunosuppressed individuals.

14.2.6 Microbiome-Directed and Microbiome-Targeted Therapies (Future Perspectives)

As our understanding of the gut microbiome deepens, a new generation of microbiome-based therapies is emerging to restore colonization resistance and treat intestinal infections. These therapies aim to either modulate the microbiota to enhance beneficial functions or directly target pathogens in a precise, context-aware manner. Below, we highlight four rapidly evolving areas in microbiome research with significant therapeutic potential.

14.2.6.1 Fecal Microbiota Transplantation (FMT)

Fecal microbiota transplantation (FMT)—the transfer of minimally processed stool from a healthy donor to a recipient—remains the most established and effective microbiome-based intervention for recurrent *C. difficile* infection (rCDI). The rationale behind FMT lies in rapidly restoring a depleted or dysbiotic microbiota with a diverse, functional community capable of reestablishing colonization resistance.

Clinical trials and meta-analyses consistently show that FMT achieves cure rates exceeding 85–90% in patients with multiple *C. difficile* recurrences, often outperforming standard antibiotic therapy (Quraishi et al., [2017](#); van Nood et al., [2013](#)). The rapid resolution of symptoms following FMT correlates with the restoration of bacterial diversity and short-chain fatty acid production in the gut, which collectively inhibit *C. difficile* germination and growth. FMT has also been explored for other gastrointestinal or systemic disorders linked to dysbiosis—including inflammatory bowel disease (IBD), irritable bowel syndrome (IBS), and metabolic or neurologic diseases—but results have been variable and context-dependent. As such, regulatory authorities have been cautious, and current approvals for FMT use

remain largely restricted to rCDI. Due to concerns about variability in donor stool and the potential transmission of pathogens, the field has moved toward standardized preparations—including screened, frozen fecal capsules—and defined microbial consortia. These developments aim to maintain the efficacy of FMT while improving safety, consistency, and regulatory compliance. Indeed, FMT's success has provided the conceptual and practical foundation for the development of next-generation microbiota-based therapies. A more detailed exploration of FMT, including regulatory frameworks and applications beyond *C. difficile*, is provided in a separate chapter of this book.

14.2.6.2 Live Biotherapeutic Products (LBPs)

Live biotherapeutic products (LBPs)—defined by the US Food and Drug Administration as biological products containing live organisms applicable to disease prevention or treatment—are one of the most advanced tools in the microbiome therapeutic space. Unlike traditional probiotics, LBPs undergo stringent regulatory assessment for safety, efficacy, and manufacturing. Several LBPs are in development or clinical testing specifically to address gut infections, particularly those caused by *C. difficile*, *S. Typhimurium*, and antibiotic-resistant Enterobacteriaceae.

For example, SER-109 (Seres Therapeutics), an oral capsule composed of purified Firmicutes spores derived from healthy donors, has shown efficacy in preventing *C. difficile* recurrence and was recently approved by the FDA (Feuerstadt et al., [2022](#)). VE303, developed by Vedanta Biosciences, is a defined bacterial consortium designed to restore microbial balance after antibiotic treatment and shows promise in reducing colonization by *C. difficile* and other pathogens (Louie et al., [2023](#)). Other LBPs under investigation aim to modulate bile acid profiles, enhance mucosal barrier function, or directly inhibit pathogen growth through nutrient niche competition or bacteriocin production.

14.2.6.3 Engineered Probiotics and Biosensors

Advances in synthetic biology have enabled the engineering of probiotic strains—such as *E. coli* Nissle or *L. reuteri*—to perform programmable tasks in the gut. These include the detection of pathogenic signals, environmental cues, or host biomarkers, coupled with a defined therapeutic output. For instance, engineered strains have been developed to sense inflammation or pathogen-associated molecules and respond with the secretion of anti-inflammatory cytokines, toxins targeting pathogens, or quorum sensing inhibitors (Danino et al., [2015](#); Hwang et al., [2017](#)).

Some engineered microbes function as biosensors, able to record transient inflammatory events or exposure to specific metabolites in the gut (Kotula et al., [2014](#); Riglar et al., [2017](#)). These systems offer real-time diagnostic insight and pave the way for personalized, responsive microbiome therapeutics that adapt to the gut's dynamic environment.

Biosensors could be employed to identify individuals with a disrupted microbiome who are at increased risk of infections, and thereby identify candidates for targeted microbiota restoration. This approach is supported by the development of point-of-care biosensors capable of real-time monitoring of gut microbial composition and health biomarkers.

14.2.6.4 Phage Therapy

Another increasingly explored area of research is bacteriophage therapy, which employs viruses that selectively infect and lyse bacteria within a narrow host range (Hatfull et al., [2022](#)). Bacteriophages represent a promising tool in the fight against AMR, particularly for targeting multidrug-resistant pathogens while preserving commensal microbes. In the gut, phage therapy can selectively decolonize pathogens such as *C. difficile*, *Enterococcus faecium*, or carbapenem-resistant *K. pneumoniae*, while sparing beneficial commensals. Importantly, phages can replicate at the site of infection, increasing their local effectiveness and reducing systemic exposure.

Several preclinical and early clinical studies support the efficacy of phages in treating gut-related infections. For example, phage cocktails targeting *C. difficile* have been shown to reduce toxin production and colonization in murine models (Nale et al., [2016](#)). A personalized phage approach successfully cleared MDR *Acinetobacter baumannii* from a patient's gut microbiota after multiple unsuccessful antibiotic courses (Schooley et al., [2017](#)). Engineered phages have also been developed to deliver CRISPR-Cas payloads, enabling sequence-specific targeting of resistance genes or virulence factors (Citorik et al., [2014](#)). Moreover, phages may be combined with microbiota-restoring interventions, such as LBPs or fecal microbiota transplants (FMT), to enhance colonization resistance post-decontamination (Zuo et al., [2018](#)).

Controversy Despite decades of research, the precise mechanisms underlying microbiota-mediated colonization resistance (CR) remain incompletely understood. A key question concerns the relative importance of direct versus indirect effects of the intestinal microbiota on pathogen control. On one hand, the intestinal ecosystem is characterized by extremely low oxygen tension and redox potential (He et al., [1999](#); Savage, [1977](#)), which restricts the growth of facultative aerobes, a group that includes most enteric pathogens such as *Salmonella* and *E. coli* (Komitopoulou et al., [2004](#)). This has led to the concept that the microbiota contributes to pathogen exclusion indirectly by maintaining the anaerobic milieu through continuous metabolic activity. In this model, dysbiosis and the loss of obligate anaerobes after antibiotic treatment create a more oxidized intestinal environment that favors pathogen expansion.

Alternatively, a large body of evidence supports a direct antagonistic role of commensal microbes in suppressing pathogens. Commensals can occupy adhesion sites on the mucosal surface, compete for nutrients, or produce inhibitory compounds such as bacteriocins, short-chain fatty acids, and microcins (Chatzidaki-Livanis et al., [2017](#); Heilbronner et al., [2021](#); Sassone-Corsi et al., [2016](#)). However, identifying specific bacterial taxa or molecules

responsible for CR has proven difficult, and results vary substantially between experimental models and host backgrounds.

Adding further complexity, the intestinal microbiota also interacts intricately with the host immune system, shaping innate and adaptive immune responses that contribute to colonization resistance. Commensal microbes stimulate epithelial and mucosal immune pathways, priming the host to maintain a defense-competent state even in the absence of active infection. Yet, disentangling these immune-mediated effects from microbial competition itself remains challenging.

Thus, the current controversy centers on the relative contribution of environmental, microbial, and immunological mechanisms in maintaining colonization resistance. While these processes are likely interdependent, their quantitative importance may differ across pathogens and host conditions—a question that continues to fuel experimental and conceptual debate in the field.

History

The historical development of microbiota-mediated colonization resistance has been comprehensively reviewed by Bärbel Stecher, whose work provides a framework for understanding the key milestones in this field (Stecher, [2021](#)). The concept that the intestinal microbiota can protect the host against infections has its roots in the 1950s. At that time, Marjorie Bohnhoff and colleagues at the University of Chicago made a pivotal observation: mice treated with antibiotics became highly susceptible to *Salmonella* infection (Bohnhoff et al., [1954](#)). Similar findings were soon reported for other enteric pathogens such as *Vibrio cholerae* and *Escherichia coli* (Bartlett et al., [1977](#); Freter, [1962](#)), indicating that antibiotic-mediated disruption of the intestinal flora could compromise host resistance. In 1971, Dirk van der Waaij coined the term “colonization resistance” (CR) after demonstrating that conventional mice were naturally resistant to colonization by opportunistic bacteria like *E. coli*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*, but lost this

resistance transiently upon antibiotic treatment. Germ-free mice recolonized with microbiota from conventional mice regained CR, suggesting that microorganisms themselves were the key protective “factor” (van der Waaij et al., [1971](#)).

The development of germ-free mouse models throughout the 1960s and 1970s further advanced the understanding of microbiota-mediated protection. Germ-free mice, which exhibit exaggerated susceptibility to enteric infections and profound physiological abnormalities such as enlarged ceca (Dubos & Schaedle, [1964](#)), became essential tools to study the interplay between microbes and host physiology. Early efforts to reconstitute germ-free mice with defined bacterial consortia, such as those by Russell Schaedler and later Rolf Freter, laid the foundation for today’s concept of synthetic microbial communities. Freter’s pioneering experiments with “SynComs” composed of anaerobic isolates demonstrated that specific combinations of bacteria, particularly *Clostridium* and *Lactobacillus* species, could restore colonization resistance against enteric pathogens (Freter & Abrams, [1972](#); Itoh & Freter, [1989](#)).

In the 1980s, advances in anaerobic culturing and increasing recognition of the role of microbial diversity further solidified the idea that a complex and balanced microbiota is essential for intestinal health (Koopman et al., [1983](#); Welling et al., [1980](#)). Recent research has provided the first experimental evidence that the nutrient-blocking principle—the synergistic occupation of nutrient niches by a diverse gut microbiota—first proposed by Rolf Freter (Freter et al., [1983](#)) constitutes a central mechanism underlying colonization resistance (Spragge et al., [2023](#)). This historical trajectory—from empirical antibiotic experiments to controlled gnotobiotic studies—set the stage for modern microbiome research and therapeutic modulation of microbial communities.

A major milestone in translating microbiota research into clinical practice came decades later with the recognition of *Clostridioides difficile* as the causative agent of antibiotic-associated diarrhea (Bartlett et al., [1977](#)). The first successful fecal microbiota

transplantation (FMT) against recurrent *C. difficile* infection (van Nood et al., [2013](#)) marked a turning point, demonstrating that restoring microbial diversity could cure an otherwise intractable infection. This success story provided a proof-of-concept for microbiota-based therapies and sparked renewed interest in understanding the mechanistic basis of colonization resistance and microbial modulation in infectious diseases.

Highlights

- The intestinal microbiota forms a multilayered defense system, providing colonization resistance against a broad spectrum of bacterial, viral, and fungal pathogens.
- Nutrient competition for amino acids, carbohydrates, and trace metals represents a central mechanism through which commensals limit pathogen expansion.
- Interference competition by resident microbes, including bacteriocin production and conversion of host-derived compounds into antimicrobial metabolites, strengthens community resilience.
- Microbiota-derived metabolites such as short-chain fatty acids and secondary bile acids modulate host barrier integrity and restrict pathogen growth.
- The intestinal mucus layer, maintained by commensal–host interactions, represents a key interface regulating spatial segregation between microbes and the epithelium.
- Pathogens such as *Salmonella enterica*, *Clostridioides difficile*, and *Vibrio cholerae* have evolved strategies to overcome colonization resistance, including inflammation induction, toxin secretion, and biofilm formation.
- Host and environmental factors—including diet, age, medication, and immune status—critically shape microbiota composition and determine susceptibility to infection.

- Antibiotic use remains the strongest disruptor of colonization resistance, promoting overgrowth of opportunistic and multidrug-resistant pathogens.
- Novel microbiome-directed strategies such as fecal microbiota transplantation, live biotherapeutic products, engineered probiotics, and phage therapy are emerging to restore colonization resistance and prevent recurrent infections.
- Understanding the interplay between commensals, pathogens, and the host immune system provides the foundation for developing next-generation microbiota-based therapeutics.

References

Abed, J., Emgard, J. E., Zamir, G., Faroja, M., Almogy, G., Grenov, A., Sol, A., Naor, R., Pikarsky, E., Atlan, K. A., Mellul, A., Chaushu, S., Manson, A. L., Earl, A. M., Ou, N., Brennan, C. A., Garrett, W. S., & Bachrach, G. (2016). Fap2 mediates *Fusobacterium nucleatum* colorectal adenocarcinoma enrichment by binding to tumor-expressed Gal-GalNAc. *Cell Host & Microbe*, 20(2), 215–225. <https://doi.org/10.1016/j.chom.2016.07.006>

Agus, A., Denizot, J., Thevenot, J., Martinez-Medina, M., Massier, S., Sauvanet, P., Bernalier-Donadille, A., Denis, S., Hofman, P., Bonnet, R., Billard, E., & Barnich, N. (2016). Western diet induces a shift in microbiota composition enhancing susceptibility to Adherent-Invasive *E. coli* infection and intestinal inflammation. *Scientific Reports*, 6, 19032. <https://doi.org/10.1038/srep19032> [PubMed]

Alcon-Giner, C., Dalby, M. J., Caim, S., Ketskemety, J., Shaw, A., Sim, K., Lawson, M. A. E., Kiu, R., Leclaire, C., Chalklen, L., Kujawska, M., Mitra, S., Fardus-Reid, F., Belteki, G., McColl, K., Swann, J. R., Kroll, J. S., Clarke, P., & Hall, L. J. (2020). Microbiota supplementation with *Bifidobacterium* and *Lactobacillus* modifies the preterm infant gut microbiota and metabolome: An observational study. *Cell Reports Medicine*, 1(5), 100077. <https://doi.org/10.1016/j.xcrm.2020.100077> [PubMed]

Allsopp, L. P., Bernal, P., Nolan, L. M., & Filloux, A. (2020). Causalities of war: The connection between type VI secretion system and microbiota. *Cellular Microbiology*, 22(3), e13153. <https://doi.org/10.1111/cmi.13153> [PubMed]

An, J., Zhao, X., Wang, Y., Noriega, J., Gewirtz, A. T., & Zou, J. (2021). Western-style diet impedes colonization and clearance of *Citrobacter rodentium*. *PLoS Pathogens*, 17(4), e1009497. <https://doi.org/10.1371/journal.ppat.1009497> [PubMed]

Ancona, G., Merlini, E., Tincati, C., Barassi, A., Calcagno, A., Augello, M., Bono, V., Bai, F., Cannizzo, E. S., d'Arminio Monforte, A., & Marchetti, G. (2021). Long-term suppressive cART is not sufficient to restore intestinal permeability and gut microbiota compositional changes. *Frontiers in Immunology*, 12, 639291. <https://doi.org/10.3389/fimmu.2021.639291> [PubMed]

Anderson, M. S., Garcia, E. C., & Cotter, P. A. (2014). Kind discrimination and competitive exclusion mediated by contact-dependent growth inhibition systems shape biofilm community structure. *PLoS Pathogens*, 10(4), e1004076. <https://doi.org/10.1371/journal.ppat.1004076> [PubMed]

Asaduzzaman, S. M., & Sonomoto, K. (2009). Lantibiotics: Diverse activities and unique modes of action. *Journal of Bioscience and Bioengineering*, 107(5), 475–487. <https://doi.org/10.1016/j.jbiosc.2009.01.003> [PubMed]

Backhed, F., Roswall, J., Peng, Y., Feng, Q., Jia, H., Kovatcheva-Datchary, P., Li, Y., Xia, Y., Xie, H., Zhong, H., Khan, M. T., Zhang, J., Li, J., Xiao, L., Al-Aama, J., Zhang, D., Lee, Y. S., Kotowska, D., Colding, C., et al. (2015). Dynamics and stabilization of the human gut microbiome during the first year of life. *Cell Host & Microbe*, 17(6), 852. <https://doi.org/10.1016/j.chom.2015.05.012>

Bansal, T., Alaniz, R. C., Wood, T. K., & Jayaraman, A. (2010). The bacterial signal indole increases epithelial-cell tight-junction resistance and attenuates indicators of inflammation. *Proceedings of the National Academy of Sciences of the United States of America*, 107(1), 228–233. <https://doi.org/10.1073/pnas.0906112107> [PubMed]

Bartlett, J. G., Miao, P. V., & Gorbach, S. L. (1977). Empiric treatment with clindamycin and gentamicin of suspected sepsis due to anaerobic and aerobic bacteria. *The Journal of Infectious Diseases*, 135(Suppl), S80–S85. <https://doi.org/10.1093/infdis/135.supplement.s80> [PubMed]

Baruch, E. N., Youngster, I., Ben-Betzalel, G., Ortenberg, R., Lahat, A., Katz, L., Adler, K., Dick-Necula, D., Raskin, S., Bloch, N., Rotin, D., Anafi, L., Avivi, C., Melnichenko, J., Steinberg-Silman, Y., Mamtani, R., Harati, H., Asher, N., Shapira-Frommer, R., et al. (2021). Fecal microbiota transplant promotes response in immunotherapy-refractory melanoma patients. *Science*, 371(6529), 602–609. <https://doi.org/10.1126/science.abb5920> [PubMed]

Belzer, C., & de Vos, W. M. (2012). Microbes inside—from diversity to function: The case of Akkermansia. *The ISME Journal*, 6(8), 1449–1458. <https://doi.org/10.1038/ismej.2012.6> [PubMed]

Bergstrom, K. S., Kissoon-Singh, V., Gibson, D. L., Ma, C., Montero, M., Sham, H. P., Ryz, N., Huang, T., Velcich, A., Finlay, B. B., Chadee, K., & Vallance, B. A. (2010). Muc2 protects against lethal infectious colitis by disassociating pathogenic and commensal bacteria from the colonic mucosa. *PLoS Pathogens*, 6(5), e1000902. <https://doi.org/10.1371/journal.ppat.1000902> [PubMed]

Bergstrom, A., Skov, T. H., Bahl, M. I., Roager, H. M., Christensen, L. B., Ejlerskov, K. T., Molgaard, C., Michaelsen, K. F., & Licht, T. R. (2014). Establishment of intestinal microbiota during early life: A longitudinal, explorative study of a large cohort of Danish infants. *Applied and Environmental Microbiology*, 80(9), 2889–2900. <https://doi.org/10.1128/AEM.00342-14> [PubMed]

Birchenough, G. M., Nystrom, E. E., Johansson, M. E., & Hansson, G. C. (2016). A sentinel goblet cell guards the colonic crypt by triggering Nlrp6-dependent Muc2 secretion. *Science*, 352(6293), 1535–1542. <https://doi.org/10.1126/science.aaf7419> [PubMed]

Bjarnason, I., Scarpignato, C., Holmgren, E., Olszewski, M., Rainsford, K. D., & Lanas, A. (2018). Mechanisms of damage to the gastrointestinal tract from nonsteroidal anti-inflammatory drugs. *Gastroenterology*, 154(3), 500–514. <https://doi.org/10.1053/j.gastro.2017.10.049> [PubMed]

Bohnhoff, M., Drake, B. L., & Miller, C. P. (1954). Effect of streptomycin on susceptibility of intestinal tract to experimental Salmonella infection. *Proceedings of the Society for Experimental Biology and Medicine*, 86(1), 132–137. <https://doi.org/10.3181/00379727-86-21030> [PubMed]

Borgeaud, S., Metzger, L. C., Scrignari, T., & Blokesch, M. (2015). The type VI secretion system of vibrio cholerae fosters horizontal gene transfer. *Science*, 347(6217), 63–67. <https://doi.org/10.1126/science.1260064> [PubMed]

Bouwknegt, M., van Pelt, W., Kubbinga, M. E., Weda, M., & Havelaar, A. H. (2014). Potential association between the recent increase in campylobacteriosis incidence in the Netherlands and proton-pump inhibitor use – An ecological study. *Euro Surveillance*, 19(32). <https://doi.org/10.2807/1560-7917.es2014.19.32.20873>

Brenchley, J. M., Price, D. A., Schacker, T. W., Asher, T. E., Silvestri, G., Rao, S., Kazzaz, Z., Bornstein, E., Lambotte, O., Altmann, D., Blazar, B. R., Rodriguez, B., Teixeira-Johnson, L., Landay, A., Martin, J. N., Hecht, F. M., Picker, L. J., Lederman, M. M., Deeks, S. G., & Douek, D. C. (2006). Microbial translocation is a cause of systemic immune activation in chronic HIV infection. *Nature Medicine*, 12(12), 1365–1371. <https://doi.org/10.1038/nm1511> [PubMed]

Bronner, D. N., Faber, F., Olsan, E. E., Byndloss, M. X., Sayed, N. A., Xu, G., Yoo, W., Kim, D., Ryu, S., Lebrilla, C. B., & Baumler, A. J. (2018). Genetic ablation of butyrate utilization attenuates gastrointestinal salmonella disease. *Cell Host & Microbe*, 23(2), 266–273 e264. <https://doi.org/10.1016/j.chom.2018.01.004>

Bry, L., Falk, P. G., Midtvedt, T., & Gordon, J. I. (1996). A model of host-microbial interactions in an open mammalian ecosystem. *Science*, 273(5280), 1380–1383. <https://doi.org/10.1126/science.273.5280.1380> [PubMed]

Caballero-Flores, G., Pickard, J. M., Fukuda, S., Inohara, N., & Nunez, G. (2020). An enteric pathogen

subverts colonization resistance by evading competition for amino acids in the gut. *Cell Host & Microbe*, 28(4), 526–533 e525. <https://doi.org/10.1016/j.chom.2020.06.018>

Casadevall, A. (2017). The pathogenic potential of a microbe. *mSphere*, 2(1). <https://doi.org/10.1128/mSphere.00015-17>.

Casadevall, A., & Pirofski, L. A. (2003). The damage-response framework of microbial pathogenesis. *Nature Reviews. Microbiology*, 1(1), 17–24. <https://doi.org/10.1038/nrmicro732> [PubMed]

Chatzidaki-Livanis, M., Coyne, M. J., Roelofs, K. G., Gentyala, R. R., Caldwell, J. M., & Comstock, L. E. (2017). Gut symbiont *Bacteroides fragilis* secretes a eukaryotic-like ubiquitin protein that mediates Intraspecies antagonism. *mBio*, 8(6). <https://doi.org/10.1128/mBio.01902-17>

Citorik, R. J., Mimee, M., & Lu, T. K. (2014). Sequence-specific antimicrobials using efficiently delivered RNA-guided nucleases. *Nature Biotechnology*, 32(11), 1141–1145. <https://doi.org/10.1038/nbt.3011> [PubMed]

Claesson, M. J., Cusack, S., O'Sullivan, O., Greene-Diniz, R., de Weerd, H., Flannery, E., Marchesi, J. R., Falush, D., Dinan, T., Fitzgerald, G., Stanton, C., van Sinderen, D., O'Connor, M., Harnedy, N., O'Connor, K., Henry, C., O'Mahony, D., Fitzgerald, A. P., Shanahan, F.,...O'Toole, P. W. (2011). Composition, variability, and temporal stability of the intestinal microbiota of the elderly. *Proceedings of the National Academy of Sciences of the United States of America*, 108 Suppl 1, 4586–4591. <https://doi.org/10.1073/pnas.1000097107>. [PubMed]

Claesson, M. J., Jeffery, I. B., Conde, S., Power, S. E., O'Connor, E. M., Cusack, S., Harris, H. M., Coakley, M., Lakshminarayanan, B., O'Sullivan, O., Fitzgerald, G. F., Deane, J., O'Connor, M., Harnedy, N., O'Connor, K., O'Mahony, D., van Sinderen, D., Wallace, M., Brennan, L., et al. (2012). Gut microbiota composition correlates with diet and health in the elderly. *Nature*, 488(7410), 178–184. <https://doi.org/10.1038/nature11319> [PubMed]

Clancy, C. J., & Nguyen, M. H. (2012). The end of an era in defining the optimal treatment of invasive candidiasis. *Clinical Infectious Diseases*, 54(8), 1123–1125. <https://doi.org/10.1093/cid/cis023> [PubMed]

Clarke, M. B., Hughes, D. T., Zhu, C., Boedeker, E. C., & Sperandio, V. (2006). The QseC sensor kinase: A bacterial adrenergic receptor. *Proceedings of the National Academy of Sciences of the United States of America*, 103(27), 10420–10425. <https://doi.org/10.1073/pnas.0604343103> [PubMed]

Collins, J., Robinson, C., Danhof, H., Knetsch, C. W., van Leeuwen, H. C., Lawley, T. D., Auchtung, J. M., & Britton, R. A. (2018). Dietary trehalose enhances virulence of epidemic *Clostridium difficile*. *Nature*, 553(7688), 291–294. <https://doi.org/10.1038/nature25178> [PubMed]

Conlon, M. A., & Bird, A. R. (2014). The impact of diet and lifestyle on gut microbiota and human

health. *Nutrients*, 7(1), 17–44. <https://doi.org/10.3390/nu7010017>
[PubMed]

Conway, T., & Cohen, P. S. (2015). Commensal and pathogenic *Escherichia coli* metabolism in the gut. *Microbiology Spectrum*, 3(3). <https://doi.org/10.1128/microbiolspec.MBP-0006-2014>

Coombes, B. K., Valdez, Y., & Finlay, B. B. (2004). Evasive maneuvers by secreted bacterial proteins to avoid innate immune responses. *Current Biology*, 14(19), R856–R867. <https://doi.org/10.1016/j.cub.2004.09.043>
[PubMed]

Cossarini, F., Shang, J., Krek, A., Al-Taie, Z., Hou, R., Canales-Herrerias, P., Tokuyama, M., Tankelevich, M., Tillowitz, A., Jha, D., Livanos, A. E., Leyre, L., Uzzan, M., Martinez-Delgado, G., Taylor, M. D., Sharma, K., Bourgonje, A. R., Cruz, M., Ioannou, G., et al. (2024). Gastrointestinal germinal center B cell depletion and reduction in IgA(+) plasma cells in HIV-1 infection. *Science Immunology*, 9(100), eado0090. <https://doi.org/10.1126/sciimmunol.ado0090>
[PubMed]

Czerucka, D., Piche, T., & Rampal, P. (2007). Review article: yeast as probiotics – *Saccharomyces boulardii*. *Alimentary Pharmacology & Therapeutics*, 26(6), 767–778. <https://doi.org/10.1111/j.1365-2036.2007.03442.x>

D'Argenio, V., & Salvatore, F. (2015). The role of the gut microbiome in the healthy adult status. *Clinica Chimica Acta*, 451(Pt A), 97–102. <https://doi.org/10.1016/j.cca.2015.01.003>

Danielsen, A. S., Franconeri, L., Page, S., Myhre, A. E., Tornes, R. A., Kacelnik, O., & Bjornholt, J. V. (2023). Clinical outcomes of antimicrobial resistance in cancer patients: A systematic review of multivariable models. *BMC Infectious Diseases*, 23(1), 247. <https://doi.org/10.1186/s12879-023-08182-3>
[PubMed]

Danino, T., Prindle, A., Kwong, G. A., Skalak, M., Li, H., Allen, K., Hasty, J., & Bhatia, S. N. (2015). Programmable probiotics for detection of cancer in urine. *Science Translational Medicine*, 7(289), 289ra284. <https://doi.org/10.1126/scitranslmed.aaa3519>

Dapa, T., & Unnikrishnan, M. (2013). Biofilm formation by *Clostridium difficile*. *Gut Microbes*, 4(5), 397–402. <https://doi.org/10.4161/gmic.25862>
[PubMed]

Davar, D., Dzutsev, A. K., McCulloch, J. A., Rodrigues, R. R., Chauvin, J. M., Morrison, R. M., Deblasio, R. N., Menna, C., Ding, Q., Pagliano, O., Zidi, B., Zhang, S., Badger, J. H., Vetizou, M., Cole, A. M., Fernandes, M. R., Prescott, S., Costa, R. G. F., Balaji, A. K., et al. (2021). Fecal microbiota transplant overcomes resistance to anti-PD-1 therapy in melanoma patients. *Science*, 371(6529), 595–602. <https://doi.org/10.1126/science.abf3363>
[PubMed]

De Souza Santos, M., & Orth, K. (2019). The role of the type III secretion system in the intracellular lifestyle of enteric pathogens. *Microbiol Spectr*, 7(3). <https://doi.org/10.1128/microbiolspec.BAI-0008-2019>

Deeks, S. G., Tracy, R., & Douek, D. C. (2013). Systemic effects of inflammation on health during chronic HIV infection. *Immunity*, 39(4), 633–645. <https://doi.org/10.1016/j.immuni.2013.10.001> [PubMed]

Deriu, E., Liu, J. Z., Pezeshki, M., Edwards, R. A., Ochoa, R. J., Contreras, H., Libby, S. J., Fang, F. C., & Raffatellu, M. (2013). Probiotic bacteria reduce salmonella typhimurium intestinal colonization by competing for iron. *Cell Host & Microbe*, 14(1), 26–37. <https://doi.org/10.1016/j.chom.2013.06.007>

Derrien, M., Vaughan, E. E., Plugge, C. M., & de Vos, W. M. (2004). Akkermansia muciniphila gen. nov., sp. nov., a human intestinal mucin-degrading bacterium. *International Journal of Systematic and Evolutionary Microbiology*, 54(Pt 5), 1469–1476. <https://doi.org/10.1099/ijs.0.02873-0> [PubMed]

Derrien, M., Van Baarlen, P., Hooiveld, G., Norin, E., Muller, M., & de Vos, W. M. (2011). Modulation of mucosal immune response, tolerance, and proliferation in mice colonized by the mucin-degrader Akkermansia muciniphila. *Frontiers in Microbiology*, 2, 166. <https://doi.org/10.3389/fmicb.2011.00166> [PubMed]

Desai, M. S., Seekatz, A. M., Koropatkin, N. M., Kamada, N., Hickey, C. A., Wolter, M., Pudlo, N. A., Kitamoto, S., Terrapon, N., Muller, A., Young, V. B., Henrissat, B., Wilmes, P., Stappenbeck, T. S., Nunez, G., & Martens, E. C. (2016). A dietary fiber-deprived gut microbiota degrades the colonic mucus barrier and enhances pathogen susceptibility. *Cell*, 167(5), 1339–1353 e1321. <https://doi.org/10.1016/j.cell.2016.10.043> [PubMed]

Dubois, T., Tremblay, Y. D. N., Hamiot, A., Martin-Verstraete, I., Deschamps, J., Monot, M., Briandet, R., & Dupuy, B. (2019). A microbiota-generated bile salt induces biofilm formation in Clostridium difficile. *NPJ Biofilms Microbiomes*, 5(1), 14. <https://doi.org/10.1038/s41522-019-0087-4> [PubMed]

Dubos, R., & Schaedle, R. W. (1964). The digestive tract as an ecosystem. *The American Journal of the Medical Sciences*, 248, 267–272. <https://doi.org/10.1097/00000441-196409000-00003> [PubMed]

Eberl, C., Weiss, A. S., Jochum, L. M., Durai Raj, A. C., Ring, D., Hussain, S., Herp, S., Meng, C., Kleigrewe, K., Gigl, M., Basic, M., & Stecher, B. (2021). E. coli enhance colonization resistance against salmonella Typhimurium by competing for galactitol, a context-dependent limiting carbon source. *Cell Host & Microbe*, 29(11), 1680–1692 e1687. <https://doi.org/10.1016/j.chom.2021.09.004>

Engevik, M. A., Engevik, A. C., Engevik, K. A., Auchtung, J. M., Chang-Graham, A. L., Ruan, W., Luna, R. A., Hyser, J. M., Spinler, J. K., & Versalovic, J. (2021). Mucin-degrading microbes release monosaccharides that Chemoattract Clostridioides difficile and facilitate colonization of the human intestinal mucus layer. *ACS Infectious Diseases*, 7(5), 1126–1142. <https://doi.org/10.1021/acsinfecdis.0c00634> [PubMed]

Faber, F., Thiennimitr, P., Spiga, L., Byndloss, M. X., Litvak, Y., Lawhon, S., Andrews-Polymenis, H. L., Winter, S. E., & Baumler, A. J. (2017). Respiration of microbiota-derived 1,2-propanediol drives salmonella expansion during colitis. *PLoS Pathogens*, 13(1), e1006129. <https://doi.org/10.1371/journal.ppat.1006129>
[PubMed]

Favre, D., Lederer, S., Kanwar, B., Ma, Z. M., Proll, S., Kasakow, Z., Mold, J., Swainson, L., Barbour, J. D., Baskin, C. R., Palermo, R., Pandrea, I., Miller, C. J., Katze, M. G., & McCune, J. M. (2009). Critical loss of the balance between Th17 and T regulatory cell populations in pathogenic SIV infection. *PLoS Pathogens*, 5(2), e1000295. <https://doi.org/10.1371/journal.ppat.1000295>
[PubMed]

Ferreira, P., Cravo, M., Guerreiro, C. S., Tavares, L., Santos, P. M., & Brito, M. (2010). Fat intake interacts with polymorphisms of Caspase9, FasLigand and PPARgamma apoptotic genes in modulating Crohn's disease activity. *Clinical Nutrition*, 29(6), 819–823. <https://doi.org/10.1016/j.clnu.2010.06.008>
[PubMed]

Ferreira, J. A., Wu, K. J., Hryckowian, A. J., Bouley, D. M., Weimer, B. C., & Sonnenburg, J. L. (2014). Gut microbiota-produced succinate promotes *C. difficile* infection after antibiotic treatment or motility disturbance. *Cell Host & Microbe*, 16(6), 770–777. <https://doi.org/10.1016/j.chom.2014.11.003>

Feuerstadt, P., Louie, T. J., Lashner, B., Wang, E. E. L., Diao, L., Bryant, J. A., Sims, M., Kraft, C. S., Cohen, S. H., Berenson, C. S., Korman, L. Y., Ford, C. B., Litcfsky, K. D., Lombardo, M. J., Wortman, J. R., Wu, H., Aunins, J. G., McChalicher, C. W. J., Winkler, J. A., et al. (2022). SER-109, an Oral microbiome therapy for recurrent *Clostridioides difficile* infection. *The New England Journal of Medicine*, 386(3), 220–229. <https://doi.org/10.1056/NEJMoa2106516>
[PubMed]

Flint, A., Butcher, J., & Stintzi, A. (2016). Stress responses, adaptation, and virulence of bacterial pathogens during host gastrointestinal colonization. *Microbiol Spectr*, 4(2). <https://doi.org/10.1128/microbiolspec.VMBF-0007-2015>

Forsythe, P., Inman, M. D., & Bienenstock, J. (2007). Oral treatment with live *Lactobacillus reuteri* inhibits the allergic airway response in mice. *American Journal of Respiratory and Critical Care Medicine*, 175(6), 561–569. <https://doi.org/10.1164/rccm.200606-821OC>
[PubMed]

Francis, M. B., Allen, C. A., & Sorg, J. A. (2013). Muricholic acids inhibit *Clostridium difficile* spore germination and growth. *PLoS One*, 8(9), e73653. <https://doi.org/10.1371/journal.pone.0073653>
[PubMed]

Freter, R. (1962). In vivo and in vitro antagonism of intestinal bacteria against *Shigella flexneri*. II. The inhibitory mechanism. *The Journal of Infectious Diseases*, 110, 38–46. <https://doi.org/10.1093/infdis/110.1.38>
[PubMed]

Freter, R., & Abrams, G. D. (1972). Function of various intestinal bacteria in converting germfree

mice to the normal state. *Infection and Immunity*, 6(2), 119–126. <https://doi.org/10.1128/iai.6.2.119-126.1972>
[PubMed]

Freter, R., Brickner, H., Fekete, J., Vickerman, M. M., & Carey, K. E. (1983). Survival and implantation of *Escherichia coli* in the intestinal tract. *Infection and Immunity*, 39(2), 686–703. <https://doi.org/10.1128/iai.39.2.686-703.1983>
[PubMed]

Fukuda, S., Toh, H., Hase, K., Oshima, K., Nakanishi, Y., Yoshimura, K., Tobe, T., Clarke, J. M., Topping, D. L., Suzuki, T., Taylor, T. D., Itoh, K., Kikuchi, J., Morita, H., Hattori, M., & Ohno, H. (2011). Bifidobacteria can protect from enteropathogenic infection through production of acetate. *Nature*, 469(7331), 543–547. <https://doi.org/10.1038/nature09646>
[PubMed]

Furter, M., Sellin, M. E., Hansson, G. C., & Hardt, W. D. (2019). Mucus architecture and near-surface swimming affect distinct salmonella Typhimurium infection patterns along the murine intestinal tract. *Cell Reports*, 27(9), 2665–2678 e2663. <https://doi.org/10.1016/j.celrep.2019.04.106>
[PubMed]

Furuichi, M., Kawaguchi, T., Pust, M. M., Yasuma-Mitobe, K., Plichta, D. R., Hasegawa, N., Ohya, T., Bhattarai, S. K., Sasajima, S., Aoto, Y., Tuganbaev, T., Yaginuma, M., Ueda, M., Okahashi, N., Amafuji, K., Kiridoshi, Y., Sugita, K., Strazar, M., Avila-Pacheco, J., et al. (2024). Commensal consortia decolonize Enterobacteriaceae via ecological control. *Nature*, 633(8031), 878–886. <https://doi.org/10.1038/s41586-024-07960-6>
[PubMed]

Ganesh, B. P., Klopffleisch, R., Loh, G., & Blaut, M. (2013). Commensal *Akkermansia muciniphila* exacerbates gut inflammation in salmonella Typhimurium-infected gnotobiotic mice. *PLoS One*, 8(9), e74963. <https://doi.org/10.1371/journal.pone.0074963>
[PubMed]

Gerner, R. R., Nuccio, S. P., & Raffatellu, M. (2020). Iron at the host-microbe interface. *Molecular Aspects of Medicine*, 75, 100895. <https://doi.org/10.1016/j.mam.2020.100895>
[PubMed]

Gerner, R. R., Hossain, S., Sargun, A., Siada, K., Norton, G. J., Zheng, T., Neumann, W., Nuccio, S. P., Nolan, E. M., & Raffatellu, M. (2022). Siderophore immunization restricted colonization of adherent-invasive *Escherichia coli* and ameliorated experimental colitis. *MBio*, 13(5), e0218422. <https://doi.org/10.1128/mbio.02184-22>
[PubMed]

Ghosh, T. S., Das, M., Jeffery, I. B., & O'Toole, P. W. (2020). Adjusting for age improves identification of gut microbiome alterations in multiple diseases. *eLife*, 9. <https://doi.org/10.7554/eLife.50240>

Glover, J. S., Ticer, T. D., & Engevik, M. A. (2022). Characterizing the mucin-degrading capacity of the human gut microbiota. *Scientific Reports*, 12(1), 8456. <https://doi.org/10.1038/s41598-022-11819-z>
[PubMed]

Gonzalez-Ochoa, G., Flores-Mendoza, L. K., Icedo-Garcia, R., Gomez-Flores, R., & Tamez-Guerra, P. (2017). Modulation of rotavirus severe gastroenteritis by the combination of probiotics and prebiotics. *Archives of Microbiology*, 199(7), 953–961. <https://doi.org/10.1007/s00203-017-1400-3>

[PubMed]

Gopalakrishnan, V., Spencer, C. N., Nezi, L., Reuben, A., Andrews, M. C., Karpinets, T. V., Prieto, P. A., Vicente, D., Hoffman, K., Wei, S. C., Cogdill, A. P., Zhao, L., Hudgens, C. W., Hutchinson, D. S., Manzo, T., Petaccia de Macedo, M., Cotechini, T., Kumar, T., Chen, W. S., et al. (2018). Gut microbiome modulates response to anti-PD-1 immunotherapy in melanoma patients. *Science*, 359(6371), 97–103. <https://doi.org/10.1126/science.aan4236>

[PubMed]

Griesshammer, A., de la Cuesta-Zuluaga, J., Muller, P., Gekeler, C., Homolak, J., Chang, H., Schmitt, K., Planker, C., Schmidtchen, V., Gallage, S., Bohn, E., Nguyen, T. H., Hetzer, J., Heikenwalder, M., Huang, K. C., Zahir, T., & Maier, L. (2025). Non-antibiotics disrupt colonization resistance against enteropathogens. *Nature*. <https://doi.org/10.1038/s41586-025-09217-2>

Guo, Y., Crnkovic, C. M., Won, K. J., Yang, X., Lee, J. R., Orjala, J., Lee, H., & Jeong, H. (2019). Commensal gut bacteria convert the immunosuppressant tacrolimus to less potent metabolites. *Drug Metabolism and Disposition*, 47(3), 194–202. <https://doi.org/10.1124/dmd.118.084772>

[PubMed]

Gupta, V., Satlin, M. J., Yu, K. C., Martei, Y., Sung, L., Westblade, L. F., Howard, S., Ai, C., & Flayhart, D. C. (2025). Incidence and prevalence of antimicrobial resistance in outpatients with cancer: A multicentre, retrospective, cohort study. *The Lancet Oncology*, 26(5), 620–628. [https://doi.org/10.1016/S1470-2045\(25\)00128-7](https://doi.org/10.1016/S1470-2045(25)00128-7)

[PubMed]

Hakim, H., Dallas, R., Wolf, J., Tang, L., Schultz-Cherry, S., Darling, V., Johnson, C., Karlsson, E. A., Chang, T. C., Jeha, S., Pui, C. H., Sun, Y., Pounds, S., Hayden, R. T., Tuomanen, E., & Rosch, J. W. (2018). Gut microbiome composition predicts infection risk during chemotherapy in children with acute lymphoblastic leukemia. *Clinical Infectious Diseases*, 67(4), 541–548. <https://doi.org/10.1093/cid/ciy153>

[PubMed]

Harper, A., Vijayakumar, V., Ouwehand, A. C., Ter Haar, J., Obis, D., Espadaler, J., Binda, S., Desiraju, S., & Day, R. (2020). Viral infections, the microbiome, and probiotics. *Frontiers in Cellular and Infection Microbiology*, 10, 596166. <https://doi.org/10.3389/fcimb.2020.596166>

[PubMed]

Hatfull, G. F., Dedrick, R. M., & Schooley, R. T. (2022). Phage therapy for antibiotic-resistant bacterial infections. *Annual Review of Medicine*, 73, 197–211. <https://doi.org/10.1146/annurev-med-080219-122208>

[PubMed]

He, G., Shankar, R. A., Chzhan, M., Samouilov, A., Kuppusamy, P., & Zweier, J. L. (1999). Noninvasive measurement of anatomic structure and intraluminal oxygenation in the gastrointestinal tract of

living mice with spatial and spectral EPR imaging. *Proceedings of the National Academy of Sciences of the United States of America*, 96(8), 4586–4591. <https://doi.org/10.1073/pnas.96.8.4586>
[PubMed]

Hecht, G., Pothoulakis, C., LaMont, J. T., & Madara, J. L. (1988). Clostridium difficile toxin A perturbs cytoskeletal structure and tight junction permeability of cultured human intestinal epithelial monolayers. *The Journal of Clinical Investigation*, 82(5), 1516–1524. <https://doi.org/10.1172/JCI113760>
[PubMed]

Hecht, A. L., Casterline, B. W., Earley, Z. M., Goo, Y. A., Goodlett, D. R., & Bubeck Wardenburg, J. (2016). Strain competition restricts colonization of an enteric pathogen and prevents colitis. *EMBO Reports*, 17(9), 1281–1291. <https://doi.org/10.15252/embr.201642282>
[PubMed]

Heilbronner, S., Krismer, B., Brotz-Oesterhelt, H., & Peschel, A. (2021). The microbiome-shaping roles of bacteriocins. *Nature Reviews. Microbiology*, 19(11), 726–739. <https://doi.org/10.1038/s41579-021-00569-w>
[PubMed]

Hes, C., Desilets, A., Tonneau, M., El Ouarzadi, O., De Figueiredo Sousa, M., Bahig, H., Filion, E., Nguyen-Tan, P. F., Christopoulos, A., Benlaifaoui, M., Derosa, L., Alves Costa Silva, C., Ponce, M., Malo, J., Belkad, W., Charpentier, D., Aubin, F., Hamilou, Z., Jamal, R.,...Routy, B. (2024). Gut microbiome predicts gastrointestinal toxicity outcomes from chemoradiation therapy in patients with head and neck squamous cell carcinoma. *Oral Oncology*, 148, 106623. <https://doi.org/10.1016/j.oraloncology.2023.106623>.
[PubMed]

Hill, C., Guarner, F., Reid, G., Gibson, G. R., Merenstein, D. J., Pot, B., Morelli, L., Canani, R. B., Flint, H. J., Salminen, S., Calder, P. C., & Sanders, M. E. (2014). Expert consensus document. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nature Reviews. Gastroenterology & Hepatology*, 11(8), 506–514. <https://doi.org/10.1038/nrgastro.2014.66>

Hills, R. D., Jr., Pontefract, B. A., Mishcon, H. R., Black, C. A., Sutton, S. C., & Theberge, C. R. (2019). Gut microbiome: Profound implications for diet and disease. *Nutrients*, 11(7). <https://doi.org/10.3390/nu11071613>

Holle, J., Reitmeir, R., Behrens, F., Singh, D., Schindler, D., Potapenko, O., McParland, V., Anandakumar, H., Kanzelmeyer, N., Sommerer, C., Hartleif, S., Andrassy, J., Heemann, U., Neuenhahn, M., Forslund-Startceva, S. K., Gerhard, M., Oh, J., Wilck, N., Lober, U., & Bartolomaeus, H. (2025). Gut microbiome alterations precede graft rejection in kidney transplantation patients. *American Journal of Transplantation*. <https://doi.org/10.1016/j.ajt.2025.02.010>

Hooper, L. V., Littman, D. R., & Macpherson, A. J. (2012). Interactions between the microbiota and the immune system. *Science*, 336(6086), 1268–1273. <https://doi.org/10.1126/science.1223490>
[PubMed]

Hryckowian, A. J., Van Treuren, W., Smits, S. A., Davis, N. M., Gardner, J. O., Bouley, D. M., & Sonnenburg, J. L. (2018). Microbiota-accessible carbohydrates suppress *Clostridium difficile* infection in a murine model. *Nature Microbiology*, 3(6), 662–669. <https://doi.org/10.1038/s41564-018-0150-6>
[PubMed]

Hutchings, M. I., Truman, A. W., & Wilkinson, B. (2019). Antibiotics: Past, present and future. *Current Opinion in Microbiology*, 51, 72–80. <https://doi.org/10.1016/j.mib.2019.10.008>
[PubMed]

Hwang, I. Y., Koh, E., Wong, A., March, J. C., Bentley, W. E., Lee, Y. S., & Chang, M. W. (2017). Engineered probiotic *Escherichia coli* can eliminate and prevent *Pseudomonas aeruginosa* gut infection in animal models. *Nature Communications*, 8, 15028. <https://doi.org/10.1038/ncomms15028>
[PubMed]

Iliev, I. D., Funari, V. A., Taylor, K. D., Nguyen, Q., Reyes, C. N., Strom, S. P., Brown, J., Becker, C. A., Fleshner, P. R., Dubinsky, M., Rotter, J. I., Wang, H. L., McGovern, D. P., Brown, G. D., & Underhill, D. M. (2012). Interactions between commensal fungi and the C-type lectin receptor Dectin-1 influence colitis. *Science*, 336(6086), 1314–1317. <https://doi.org/10.1126/science.1221789>
[PubMed]

Imhann, F., Bonder, M. J., Vich Vila, A., Fu, J., Mujagic, Z., Vork, L., Tigchelaar, E. F., Jankipersadsing, S. A., Cenit, M. C., Harmsen, H. J., Dijkstra, G., Franke, L., Xavier, R. J., Jonkers, D., Wijmenga, C., Weersma, R. K., & Zhernakova, A. (2016). Proton pump inhibitors affect the gut microbiome. *Gut*, 65(5), 740–748. <https://doi.org/10.1136/gutjnl-2015-310376>
[PubMed]

Itoh, K., & Freter, R. (1989). Control of *Escherichia coli* populations by a combination of indigenous clostridia and lactobacilli in gnotobiotic mice and continuous-flow cultures. *Infection and Immunity*, 57(2), 559–565. <https://doi.org/10.1128/iai.57.2.559-565.1989>
[PubMed]

Jakobsson, H. E., Rodriguez-Pineiro, A. M., Schutte, A., Ermund, A., Boysen, P., Bemark, M., Sommer, F., Backhed, F., Hansson, G. C., & Johansson, M. E. (2015). The composition of the gut microbiota shapes the colon mucus barrier. *EMBO Reports*, 16(2), 164–177. <https://doi.org/10.15252/embr.201439263>
[PubMed]

Janarthanan, S., Ditah, I., Adler, D. G., & Ehrinpreis, M. N. (2012). *Clostridium difficile*-associated diarrhea and proton pump inhibitor therapy: A meta-analysis. *The American Journal of Gastroenterology*, 107(7), 1001–1010. <https://doi.org/10.1038/ajg.2012.179>
[PubMed]

Jeffery, I. B., Lynch, D. B., & O'Toole, P. W. (2016). Composition and temporal stability of the gut microbiota in older persons. *The ISME Journal*, 10(1), 170–182. <https://doi.org/10.1038/ismej.2015.88>
[PubMed]

Jochum, L., & Stecher, B. (2020). Label or concept – What is a pathobiont? *Trends in Microbiology*, 28(10), 789–792. <https://doi.org/10.1016/j.tim.2020.04.011>
[PubMed]

Johansson, M. E., & Hansson, G. C. (2016). Immunological aspects of intestinal mucus and mucins. *Nature Reviews. Immunology*, 16(10), 639–649. <https://doi.org/10.1038/nri.2016.88>
[PubMed]

Johansson, M. E., Phillipson, M., Petersson, J., Velcich, A., Holm, L., & Hansson, G. C. (2008). The inner of the two Muc2 mucin-dependent mucus layers in colon is devoid of bacteria. *Proceedings of the National Academy of Sciences of the United States of America*, 105(39), 15064–15069. <https://doi.org/10.1073/pnas.0803124105>
[PubMed]

Keefer, C. S. (1951). Alterations in normal bacterial flora of man and secondary infections during antibiotic therapy. *The American Journal of Medicine*, 11(6), 665–666. [https://doi.org/10.1016/0002-9343\(51\)90017-4](https://doi.org/10.1016/0002-9343(51)90017-4)
[PubMed]

Kienesberger, S., Cosic, A., Kitsera, M., Raffl, S., Hiesinger, M., Leitner, E., Halwachs, B., Gorkiewicz, G., Glabonjat, R. A., Raber, G., Lembacher-Fadum, C., Breinbauer, R., Schild, S., & Zechner, E. L. (2022). Enterotoxin tilimycin from gut-resident *Klebsiella* promotes mutational evolution and antibiotic resistance in mice. *Nature Microbiology*, 7(11), 1834–1848. <https://doi.org/10.1038/s41564-022-01260-3>
[PubMed]

Kim, S. G., Becattini, S., Moody, T. U., Shliaha, P. V., Littmann, E. R., Seok, R., Gjonbalaj, M., Eaton, V., Fontana, E., Amoretti, L., Wright, R., Caballero, S., Wang, Z. X., Jung, H. J., Morjaria, S. M., Leiner, I. M., Qin, W., Ramos, R., Cross, J. R., et al. (2019). Microbiota-derived lantibiotic restores resistance against vancomycin-resistant *Enterococcus*. *Nature*, 572(7771), 665–669. <https://doi.org/10.1038/s41586-019-1501-z>
[PubMed]

Kirkup, B. C., & Riley, M. A. (2004). Antibiotic-mediated antagonism leads to a bacterial game of rock-paper-scissors in vivo. *Nature*, 428(6981), 412–414. <https://doi.org/10.1038/nature02429>
[PubMed]

Kitamoto, S., Alteri, C. J., Rodrigues, M., Nagao-Kitamoto, H., Sugihara, K., Himpsl, S. D., Bazzi, M., Miyoshi, M., Nishioka, T., Hayashi, A., Morhardt, T. L., Kuffa, P., Grasberger, H., El-Zaatari, M., Bishu, S., Ishii, C., Hirayama, A., Eaton, K. A., Dogan, B., et al. (2020). Dietary L-serine confers a competitive fitness advantage to *Enterobacteriaceae* in the inflamed gut. *Nature Microbiology*, 5(1), 116–125. <https://doi.org/10.1038/s41564-019-0591-6>
[PubMed]

Kochan, T. J., Shoshiev, M. S., Hastie, J. L., Somers, M. J., Plotnick, Y. M., Gutierrez-Munoz, D. F., Foss, E. D., Schubert, A. M., Smith, A. D., Zimmerman, S. K., Carlson, P. E., Jr., & Hanna, P. C. (2018). Germinant synergy facilitates *Clostridium difficile* spore germination under physiological conditions. *mSphere*, 3(5). <https://doi.org/10.1128/mSphere.00335-18>

Kok, A., Hocqueloux, L., Hocini, H., Carriere, M., Lefrou, L., Guguin, A., Tisserand, P., Bonhabau, H., Avettand-Fenoel, V., Prazuck, T., Katsahian, S., Gaulard, P., Thiebaut, R., Levy, Y., & Hue, S. (2015). Early initiation of combined antiretroviral therapy preserves immune function in the gut of HIV-infected patients. *Mucosal Immunology*, 8(1), 127–140. <https://doi.org/10.1038/mi.2014.50> [PubMed]

Komitopoulou, E., Bainton, N. J., & Adams, M. R. (2004). Premature Salmonella Typhimurium growth inhibition in competition with other Gram-negative organisms is redox potential regulated via RpoS induction. *Journal of Applied Microbiology*, 97(5), 964–972. <https://doi.org/10.1111/j.1365-2672.2004.02363.x> [PubMed]

Koopman, J. P., Prins, R. A., Mullink, J. W., Welling, G. W., Kennis, H. M., & Hectors, M. P. (1983). Association of germfree mice with bacteria isolated from the intestinal tract of “normal” mice. *Zeitschrift für Versuchstierkunde*, 25(2), 57–62. <https://www.ncbi.nlm.nih.gov/pubmed/6880424> [PubMed]

Kotula, J. W., Kerns, S. J., Shaket, L. A., Siraj, L., Collins, J. J., Way, J. C., & Silver, P. A. (2014). Programmable bacteria detect and record an environmental signal in the mammalian gut. *Proceedings of the National Academy of Sciences of the United States of America*, 111(13), 4838–4843. <https://doi.org/10.1073/pnas.1321321111> [PubMed]

Kuehne, S. A., Cartman, S. T., Heap, J. T., Kelly, M. L., Cockayne, A., & Minton, N. P. (2010). The role of toxin A and toxin B in Clostridium difficile infection. *Nature*, 467(7316), 711–713. <https://doi.org/10.1038/nature09397> [PubMed]

Kyne, L., Warny, M., Qamar, A., & Kelly, C. P. (2000). Asymptomatic carriage of Clostridium difficile and serum levels of IgG antibody against toxin A. *The New England Journal of Medicine*, 342(6), 390–397. <https://doi.org/10.1056/NEJM200002103420604> [PubMed]

Lee, J. H., & Lee, J. (2010). Indole as an intercellular signal in microbial communities. *FEMS Microbiology Reviews*, 34(4), 426–444. <https://doi.org/10.1111/j.1574-6976.2009.00204.x> [PubMed]

Lee, M. S., Koo, S., Jeong, D. G., & Tesh, V. L. (2016). Shiga toxins as multi-functional proteins: Induction of host cellular stress responses, role in pathogenesis and therapeutic applications. *Toxins (Basel)*, 8(3). <https://doi.org/10.3390/toxins8030077>

Lee, K. S., Jeong, Y. J., & Lee, M. S. (2021). Escherichia coli Shiga toxins and gut microbiota interactions. *Toxins (Basel)*, 13(6). <https://doi.org/10.3390/toxins13060416>

Lee, K. A., Thomas, A. M., Bolte, L. A., Bjork, J. R., de Ruijter, L. K., Armanini, F., Asnicar, F., Blanco-Miguez, A., Board, R., Calbet-Llopart, N., Derosa, L., Dhomen, N., Brooks, K., Harland, M., Harries, M., Leeming, E. R., Lorigan, P., Manghi, P., Marais, R.,...Segata, N. (2022). Cross-cohort gut microbiome associations with immune checkpoint inhibitor response in advanced melanoma. *Nature Medicine*, 28(3), 535–544. <https://doi.org/10.1038/s41591-022-01695-5>.

[PubMed]

Lee, J. Y., Bays, D. J., Savage, H. P., & Baumler, A. J. (2024). The human gut microbiome in health and disease: Time for a new chapter? *Infection and Immunity*, 92(11), e0030224. <https://doi.org/10.1128/iai.00302-24>

[PubMed]

Lentsch, V., Woller, A., Rocker, A., Aslani, S., Moresi, C., Ruoho, N., Larsson, L., Fattinger, S. A., Wenner, N., Barazzone, E. C., Hardt, W. D., Loverdo, C., Diard, M., & Slack, E. (2025). Vaccine-enhanced competition permits rational bacterial strain replacement in the gut. *Science*, 388(6742), 74–81. <https://doi.org/10.1126/science.adp5011>

[PubMed]

Leonard, J., Marshall, J. K., & Moayyedi, P. (2007). Systematic review of the risk of enteric infection in patients taking acid suppression. *The American Journal of Gastroenterology*, 102(9), 2047–2056; quiz 2057. <https://doi.org/10.1111/j.1572-0241.2007.01275.x>

[PubMed]

Lewis, K. (2020). The science of antibiotic discovery. *Cell*, 181(1), 29–45. <https://doi.org/10.1016/j.cell.2020.02.056>

[PubMed]

Li, H., Limenitakis, J. P., Fuhrer, T., Geuking, M. B., Lawson, M. A., Wyss, M., Brugiroux, S., Keller, I., Macpherson, J. A., Rupp, S., Stolp, B., Stein, J. V., Stecher, B., Sauer, U., McCoy, K. D., & Macpherson, A. J. (2015). The outer mucus layer hosts a distinct intestinal microbial niche. *Nature Communications*, 6, 8292. <https://doi.org/10.1038/ncomms9292>

[PubMed]

Li, X., Zhang, S., Guo, G., Han, J., & Yu, J. (2022). Gut microbiome in modulating immune checkpoint inhibitors. *eBioMedicine*, 82, 104163. <https://doi.org/10.1016/j.ebiom.2022.104163>

[PubMed]

Lopera, C., Monzo, P., Aiello, T. F., Chumbita, M., Peyrony, O., Gallardo-Pizarro, A., Pitart, C., Cuervo, G., Morata, L., Bodro, M., Herrera, S., Del Rio, A., Martinez, J. A., Soriano, A., Puerta-Alcalde, P., & Garcia-Vidal, C. (2024). Prevalence and impact of multidrug-resistant bacteria in solid cancer patients with bloodstream infection: A 25-year trend analysis. *Microbiol Spectr*, 12(10), e0296123. <https://doi.org/10.1128/spectrum.02961-23>

[PubMed]

Lopez, C. A., Winter, S. E., Rivera-Chavez, F., Xavier, M. N., Poon, V., Nuccio, S. P., Tsois, R. M., & Baumler, A. J. (2012). Phage-mediated acquisition of a type III secreted effector protein boosts growth of salmonella by nitrate respiration. *mBio*, 3(3). <https://doi.org/10.1128/mBio.00143-12>

Louie, T., Golan, Y., Khanna, S., Bobilev, D., Erpelding, N., Fratazzi, C., Carini, M., Menon, R., Ruisi, M., Norman, J. M., Faith, J. J., Olle, B., Li, M., Silber, J. L., & Pardi, D. S. (2023). VE303, a defined bacterial consortium, for prevention of recurrent *Clostridioides difficile* infection: A randomized clinical trial. *The Journal of the American Medical Association*, 329(16), 1356–1366. <https://doi.org/10.1001/jama.2023.4314>

[PubMed]

Lozupone, C. A., Li, M., Campbell, T. B., Flores, S. C., Linderman, D., Gebert, M. J., Knight, R., Fontenot, A. P., & Palmer, B. E. (2013). Alterations in the gut microbiota associated with HIV-1 infection. *Cell Host & Microbe*, 14(3), 329–339. <https://doi.org/10.1016/j.chom.2013.08.006>

Macfarlane, G. T., & Gibson, G. R. (1991). Formation of glycoprotein degrading enzymes by *Bacteroides fragilis*. *FEMS Microbiology Letters*, 61(2–3), 289–293. [https://doi.org/10.1016/0378-1097\(91\)90567-t](https://doi.org/10.1016/0378-1097(91)90567-t)
[PubMed]

MacSharry, J., O'Mahony, C., Shalaby, K. H., Sheil, B., Karmouty-Quintana, H., Shanahan, F., & Martin, J. G. (2012). Immunomodulatory effects of feeding with *Bifidobacterium longum* on allergen-induced lung inflammation in the mouse. *Pulmonary Pharmacology & Therapeutics*, 25(4), 325–334. <https://doi.org/10.1016/j.pupt.2012.05.011>

Maier, L., Pruteanu, M., Kuhn, M., Zeller, G., Telzerow, A., Anderson, E. E., Brochado, A. R., Fernandez, K. C., Dose, H., Mori, H., Patil, K. R., Bork, P., & Typas, A. (2018). Extensive impact of non-antibiotic drugs on human gut bacteria. *Nature*, 555(7698), 623–628. <https://doi.org/10.1038/nature25979>
[PubMed]

Maier, L., Stein-Thoeringer, C., Ley, R. E., Brotz-Oesterhelt, H., Link, H., Ziemert, N., Wagner, S., & Peschel, A. (2024). Integrating research on bacterial pathogens and commensals to fight infections-an ecological perspective. *Lancet Microbe*, 5(8), 100843. [https://doi.org/10.1016/S2666-5247\(24\)00049-1](https://doi.org/10.1016/S2666-5247(24)00049-1)
[PubMed]

Mair-Jenkins, J., Borges-Stewart, R., harbour, C., cox-Rogers, J., Dallman, T., Ashton, P., Johnston, R., Modha, D., monk, P., & Puleston, R. (2017). Investigation using whole genome sequencing of a prolonged restaurant outbreak of *Salmonella* Typhimurium linked to the building drainage system, England, February 2015 to March 2016. *Euro Surveillance*, 22(49). <https://doi.org/10.2807/1560-7917.ES.2017.22.49.17-00037>

Manichanh, C., Rigottier-Gois, L., Bonnaud, E., Gloux, K., Pelletier, E., Frangeul, L., Nalin, R., Jarrin, C., Chardon, P., Marteau, P., Roca, J., & Dore, J. (2006). Reduced diversity of faecal microbiota in Crohn's disease revealed by a metagenomic approach. *Gut*, 55(2), 205–211. <https://doi.org/10.1136/gut.2005.073817>
[PubMed]

Maret-Ouda, J., Panula, J., Santoni, G., Xie, S., & Lagergren, J. (2023). Proton pump inhibitor use and risk of pneumonia: A self-controlled case series study. *Journal of Gastroenterology*, 58(8), 734–740. <https://doi.org/10.1007/s00535-023-02007-5>
[PubMed]

Marion, S., Desharnais, L., Studer, N., Dong, Y., Notter, M. D., Poudel, S., Menin, L., Janowczyk, A., Hettich, R. L., Hapfelmeier, S., & Bernier-Latmani, R. (2020). Biogeography of microbial bile acid transformations along the murine gut. *Journal of Lipid Research*, 61(11), 1450–1463. <https://doi.org/10.1194/jlr.RA120001021>
[PubMed]

Marlicz, W., Loniewski, I., Grimes, D. S., & Quigley, E. M. (2014). Nonsteroidal anti-inflammatory

drugs, proton pump inhibitors, and gastrointestinal injury: Contrasting interactions in the stomach and small intestine. *Mayo Clinic Proceedings*, 89(12), 1699–1709. <https://doi.org/10.1016/j.mayocp.2014.07.015>
[PubMed]

Martin, J. S., Monaghan, T. M., & Wilcox, M. H. (2016). Clostridium difficile infection: Epidemiology, diagnosis and understanding transmission. *Nature Reviews. Gastroenterology & Hepatology*, 13(4), 206–216. <https://doi.org/10.1038/nrgastro.2016.25>

Martin, R., Rios-Covian, D., Huillet, E., Auger, S., Khazaal, S., Bermudez-Humaran, L. G., Sokol, H., Chatel, J. M., & Langella, P. (2023). Faecalibacterium: A bacterial genus with promising human health applications. *FEMS Microbiology Reviews*, 47(4). <https://doi.org/10.1093/femsre/fuad039>

Martins, F. S., Dalmasso, G., Arantes, R. M., Doye, A., Lemichez, E., Lagadec, P., Imbert, V., Peyron, J. F., Rampal, P., Nicoli, J. R., & Czerucka, D. (2010). Interaction of Saccharomyces boulardii with Salmonella enterica serovar Typhimurium protects mice and modifies T84 cell response to the infection. *PLoS One*, 5(1), e8925. <https://doi.org/10.1371/journal.pone.0008925>
[PubMed]

Mason, K. L., Erb Downward, J. R., Falkowski, N. R., Young, V. B., Kao, J. Y., & Huffnagle, G. B. (2012). Interplay between the gastric bacterial microbiota and Candida albicans during postantibiotic recolonization and gastritis. *Infection and Immunity*, 80(1), 150–158. <https://doi.org/10.1128/IAI.05162-11>
[PubMed]

McGuckin, M. A., Linden, S. K., Sutton, P., & Florin, T. H. (2011). Mucin dynamics and enteric pathogens. *Nature Reviews. Microbiology*, 9(4), 265–278. <https://doi.org/10.1038/nrmicro2538>
[PubMed]

Mellouk, N., & Enninga, J. (2016). Cytosolic access of intracellular bacterial pathogens: The Shigella paradigm. *Frontiers in Cellular and Infection Microbiology*, 6, 35. <https://doi.org/10.3389/fcimb.2016.00035>
[PubMed]

Mikkelsen, K. H., Knop, F. K., Frost, M., Hallas, J., & Pottegard, A. (2015). Use of antibiotics and risk of type 2 diabetes: A population-based case-control study. *The Journal of Clinical Endocrinology and Metabolism*, 100(10), 3633–3640. <https://doi.org/10.1210/jc.2015-2696>
[PubMed]

Miller, W. R., & Arias, C. A. (2024). ESKAPE pathogens: Antimicrobial resistance, epidemiology, clinical impact and therapeutics. *Nature Reviews. Microbiology*, 22(10), 598–616. <https://doi.org/10.1038/s41579-024-01054-w>
[PubMed]

Miller, M. B., & Bassler, B. L. (2001). Quorum sensing in bacteria. *Annual Review of Microbiology*, 55, 165–199. <https://doi.org/10.1146/annurev.micro.55.1.165>
[PubMed]

Molbak, K., Simonsen, J., Jorgensen, C. S., Krogfelt, K. A., Falkenhorst, G., Ethelberg, S., Takkinen, J., &

- Emborg, H. D. (2014). Seroincidence of human infections with nontyphoid salmonella compared with data from public health surveillance and food animals in 13 European countries. *Clinical Infectious Diseases*, 59(11), 1599–1606. <https://doi.org/10.1093/cid/ciu627> [PubMed]
- Monaco, C. L., Gootenberg, D. B., Zhao, G., Handley, S. A., Ghebremichael, M. S., Lim, E. S., Lankowski, A., Baldridge, M. T., Wilen, C. B., Flagg, M., Norman, J. M., Keller, B. C., Luevano, J. M., Wang, D., Boum, Y., Martin, J. N., Hunt, P. W., Bangsberg, D. R., Siedner, M. J., et al. (2016). Altered Virome and bacterial microbiome in human immunodeficiency virus-associated acquired immunodeficiency syndrome. *Cell Host & Microbe*, 19(3), 311–322. <https://doi.org/10.1016/j.chom.2016.02.011>
- Moreira, A. P., Texeira, T. F., Ferreira, A. B., Peluzio Mdo, C., & Alfenas Rde, C. (2012). Influence of a high-fat diet on gut microbiota, intestinal permeability and metabolic endotoxaemia. *The British Journal of Nutrition*, 108(5), 801–809. <https://doi.org/10.1017/S0007114512001213> [PubMed]
- Munch, P. C., Eberl, C., Woelfel, S., Ring, D., Fritz, A., Herp, S., Lade, I., Geffers, R., Franzosa, E. A., Huttenhower, C., McHardy, A. C., & Stecher, B. (2023). Pulsed antibiotic treatments of gnotobiotic mice manifest in complex bacterial community dynamics and resistance effects. *Cell Host & Microbe*, 31(6), 1007–1020 e1004. <https://doi.org/10.1016/j.chom.2023.05.013>
- Munoz, A., & Costa, M. (2013). Nutritionally mediated oxidative stress and inflammation. *Oxidative Medicine and Cellular Longevity*, 2013, 610950. <https://doi.org/10.1155/2013/610950> [PubMed]
- Nakanishi, N., Tashiro, K., Kuhara, S., Hayashi, T., Sugimoto, N., & Tobe, T. (2009). Regulation of virulence by butyrate sensing in enterohaemorrhagic *Escherichia coli*. *Microbiology (Reading)*, 155(Pt 2), 521–530. <https://doi.org/10.1099/mic.0.023499-0> [PubMed]
- Nale, J. Y., Spencer, J., Hargreaves, K. R., Buckley, A. M., Trzepinski, P., Douce, G. R., & Clokie, M. R. (2016). Bacteriophage combinations significantly reduce *Clostridium difficile* growth in vitro and proliferation in vivo. *Antimicrobial Agents and Chemotherapy*, 60(2), 968–981. <https://doi.org/10.1128/AAC.01774-15> [PubMed]
- Nasi, M., Pinti, M., Mussini, C., & Cossarizza, A. (2014). Persistent inflammation in HIV infection: Established concepts, new perspectives. *Immunology Letters*, 161(2), 184–188. <https://doi.org/10.1016/j.imlet.2014.01.008> [PubMed]
- Ng, K. M., Ferreyra, J. A., Higginbottom, S. K., Lynch, J. B., Kashyap, P. C., Gopinath, S., Naidu, N., Choudhury, B., Weimer, B. C., Monack, D. M., & Sonnenburg, J. L. (2013). Microbiota-liberated host sugars facilitate post-antibiotic expansion of enteric pathogens. *Nature*, 502(7469), 96–99. <https://doi.org/10.1038/nature12503> [PubMed]
- O'Toole, P. W., & Jeffery, I. B. (2015). Gut microbiota and aging. *Science*, 350(6265), 1214–1215. <https://doi.org/10.1126/science.aac8469>

[[PubMed](#)]

Olin, A., Henckel, E., Chen, Y., Lakshmikanth, T., Pou, C., Mikes, J., Gustafsson, A., Bernhardsson, A. K., Zhang, C., Bohlin, K., & Brodin, P. (2018). Stereotypic immune system development in newborn children. *Cell*, 174(5), 1277–1292 e1214. <https://doi.org/10.1016/j.cell.2018.06.045>

[[PubMed](#)]

Oliveira, R. A., Cabral, V., Torcato, I., & Xavier, K. B. (2023). Deciphering the quorum-sensing lexicon of the gut microbiota. *Cell Host & Microbe*, 31(4), 500–512. <https://doi.org/10.1016/j.chom.2023.03.015>

Osbelt, L., Wende, M., Almasi, E., Derksen, E., Muthukumarasamy, U., Lesker, T. R., Galvez, E. J. C., Pils, M. C., Schalk, E., Chhatwal, P., Farber, J., Neumann-Schaal, M., Fischer, T., Schluter, D., & Strowig, T. (2021). *Klebsiella oxytoca* causes colonization resistance against multidrug-resistant *K. pneumoniae* in the gut via cooperative carbohydrate competition. *Cell Host & Microbe*, 29(11), 1663–1679 e1667. <https://doi.org/10.1016/j.chom.2021.09.003>

Osbelt, L., Almasi, E. D. H., Wende, M., Kienesberger, S., Voltz, A., Lesker, T. R., Muthukumarasamy, U., Knischewski, N., Nordmann, E., Bielecka, A. A., Giralt-Zuniga, M., Kaganovitch, E., Kuhne, C., Baier, C., Pietsch, M., Musken, M., Greweling-Pils, M. C., Breinbauer, R., Flieger, A., et al. (2024). *Klebsiella oxytoca* inhibits *Salmonella* infection through multiple microbiota-context-dependent mechanisms. *Nature Microbiology*, 9(7), 1792–1811. <https://doi.org/10.1038/s41564-024-01710-0>

[[PubMed](#)]

Palleja, A., Mikkelsen, K. H., Forslund, S. K., Kashani, A., Allin, K. H., Nielsen, T., Hansen, T. H., Liang, S., Feng, Q., Zhang, C., Pyl, P. T., Coelho, L. P., Yang, H., Wang, J., Typas, A., Nielsen, M. F., Nielsen, H. B., Bork, P., Wang, J., et al. (2018). Recovery of gut microbiota of healthy adults following antibiotic exposure. *Nature Microbiology*, 3(11), 1255–1265. <https://doi.org/10.1038/s41564-018-0257-9>

[[PubMed](#)]

Pan, R., He, Y., Yuan, J., Zhao, S., Ma, M., Chai, Z., Ji, X., Hu, X., He, C., Zhou, D., & Zhang, X. (2024). The role of antibiotic exposure as risk factor for IBD epidemic: An updated meta-analysis. *Journal of Gastroenterology and Hepatology*, 39(12), 2561–2571. <https://doi.org/10.1111/jgh.16751>

[[PubMed](#)]

Parada Venegas, D., De la Fuente, M. K., Landskron, G., Gonzalez, M. J., Quera, R., Dijkstra, G., Harmsen, H. J. M., Faber, K. N., & Hermoso, M. A. (2019). Short Chain Fatty Acids (SCFAs)-mediated gut epithelial and immune regulation and its relevance for inflammatory bowel diseases. *Frontiers in Immunology*, 10, 277. <https://doi.org/10.3389/fimmu.2019.00277>

[[PubMed](#)]

Park, S. J., Park, Y. J., Chang, J., Choi, S., Lee, G., Son, J. S., Kim, K. H., Oh, Y. H., & Park, S. M. (2021). Association between antibiotics use and diabetes incidence in a nationally representative retrospective cohort among Koreans. *Scientific Reports*, 11(1), 21681. <https://doi.org/10.1038/s41598-021-01125-5>

[[PubMed](#)]

Patzer, S. I., Baquero, M. R., Bravo, D., Moreno, F., & Hantke, K. (2003). The colicin G, H and X

determinants encode microcins M and H47, which might utilize the catecholate siderophore receptors FepA, Cir, Fiu and IroN. *Microbiology (Reading)*, 149(Pt 9), 2557–2570. <https://doi.org/10.1099/mic.0.26396-0> [PubMed]

Peled, J. U., Gomes, A. L. C., Devlin, S. M., Littmann, E. R., Taur, Y., Sung, A. D., Weber, D., Hashimoto, D., Slingerland, A. E., Slingerland, J. B., Maloy, M., Clurman, A. G., Stein-Thoeringer, C. K., Markey, K. A., Docampo, M. D., Burgos da Silva, M., Khan, N., Gessner, A., Messina, J. A., et al. (2020). Microbiota as predictor of mortality in allogeneic hematopoietic-cell transplantation. *The New England Journal of Medicine*, 382(9), 822–834. <https://doi.org/10.1056/NEJMoa1900623> [PubMed]

Perez, J. C. (2021). Fungi of the human gut microbiota: Roles and significance. *International Journal of Medical Microbiology*, 311(3), 151490. <https://doi.org/10.1016/j.ijmm.2021.151490> [PubMed]

Perez-Munoz, M. E., Arrieta, M. C., Ramer-Tait, A. E., & Walter, J. (2017). A critical assessment of the “sterile womb” and “in utero colonization” hypotheses: Implications for research on the pioneer infant microbiome. *Microbiome*, 5(1), 48. <https://doi.org/10.1186/s40168-017-0268-4> [PubMed]

Pirozzolo, I., Li, Z., Sepulveda, M., & Alegre, M. L. (2021). Influence of the microbiome on solid organ transplant survival. *The Journal of Heart and Lung Transplantation*, 40(8), 745–753. <https://doi.org/10.1016/j.healun.2021.04.004> [PubMed]

Poulsen, L. K., Lan, F., Kristensen, C. S., Hobolth, P., Molin, S., & Krogfelt, K. A. (1994). Spatial distribution of *Escherichia coli* in the mouse large intestine inferred from rRNA in situ hybridization. *Infection and Immunity*, 62(11), 5191–5194. <https://doi.org/10.1128/iai.62.11.5191-5194.1994> [PubMed]

Puerta-Alcalde, P., O’Keefe, J., Woolstencroft, R., Kaul, S., Lopez, N., Cronin, K., Lim, A., Garcia-Pouton, N., Alvarez, M., Chee, L., Espasa, M., Grafia, I., Suarez-Lledo, M., Smibert, O., Garcia-Vidal, C., Slavin, M. A., Yong, M. K., Soriano, A., & Worth, L. J. (2025). *Clostridioides difficile* infection and recurrence in cancer patients (CIRCA): A multicentre, international study. *International Journal of Infectious Diseases*, 153, 107785. <https://doi.org/10.1016/j.ijid.2025.107785> [PubMed]

Quraishi, M. N., Widlak, M., Bhala, N., Moore, D., Price, M., Sharma, N., & Iqbal, T. H. (2017). Systematic review with meta-analysis: The efficacy of faecal microbiota transplantation for the treatment of recurrent and refractory *Clostridium difficile* infection. *Alimentary Pharmacology & Therapeutics*, 46(5), 479–493. <https://doi.org/10.1111/apt.14201>

Raffatellu, M., George, M. D., Akiyama, Y., Hornsby, M. J., Nuccio, S. P., Paixao, T. A., Butler, B. P., Chu, H., Santos, R. L., Berger, T., Mak, T. W., Tsolis, R. M., Bevins, C. L., Solnick, J. V., Dandekar, S., & Baumler, A. J. (2009). Lipocalin-2 resistance confers an advantage to *Salmonella enterica* serotype Typhimurium for growth and survival in the inflamed intestine. *Cell Host & Microbe*, 5(5), 476–486. <https://doi.org/10.1016/j.chom.2009.03.011>

Rausch, P., Steck, N., Suwandi, A., Seidel, J. A., Kunzel, S., Bhullar, K., Basic, M., Bleich, A., Johnsen, J. M., Vallance, B. A., Baines, J. F., & Grassl, G. A. (2015). Expression of the blood-group-related gene B4galnt2 alters susceptibility to salmonella infection. *PLoS Pathogens*, 11(7), e1005008. <https://doi.org/10.1371/journal.ppat.1005008> [PubMed]

Rea, M. C., Sit, C. S., Clayton, E., O'Connor, P. M., Whittall, R. M., Zheng, J., Vederas, J. C., Ross, R. P., & Hill, C. (2010). Thuricin CD, a posttranslationally modified bacteriocin with a narrow spectrum of activity against *Clostridium difficile*. *Proceedings of the National Academy of Sciences of the United States of America*, 107(20), 9352–9357. <https://doi.org/10.1073/pnas.0913554107> [PubMed]

Riglar, D. T., Giessen, T. W., Baym, M., Kerns, S. J., Niederhuber, M. J., Bronson, R. T., Kotula, J. W., Gerber, G. K., Way, J. C., & Silver, P. A. (2017). Engineered bacteria can function in the mammalian gut long-term as live diagnostics of inflammation. *Nature Biotechnology*, 35(7), 653–658. <https://doi.org/10.1038/nbt.3879> [PubMed]

Rigo-Adrover, M., Perez-Berezo, T., Ramos-Romero, S., van Limpt, K., Knipping, K., Garssen, J., Knol, J., Franch, A., Castell, M., & Perez-Cano, F. J. (2017). A fermented milk concentrate and a combination of short-chain galacto-oligosaccharides/long-chain fructo-oligosaccharides/pectin-derived acidic oligosaccharides protect suckling rats from rotavirus gastroenteritis. *The British Journal of Nutrition*, 117(2), 209–217. <https://doi.org/10.1017/S0007114516004566> [PubMed]

Rinehart, E., Chapman, J., & Sun, Y. (2020). The production of Listeriolysin O and subsequent intracellular infections by *listeria monocytogenes* are regulated by exogenous short chain fatty acid mixtures. *Toxins (Basel)*, 12(4). <https://doi.org/10.3390/toxins12040218>

Ringel, P. D., Hu, D., & Basler, M. (2017). The role of type VI secretion system effectors in target cell lysis and subsequent horizontal gene transfer. *Cell Reports*, 21(13), 3927–3940. <https://doi.org/10.1016/j.celrep.2017.12.020> [PubMed]

Rivera-Chavez, F., Zhang, L. F., Faber, F., Lopez, C. A., Byndloss, M. X., Olsan, E. E., Xu, G., Velazquez, E. M., Lebrilla, C. B., Winter, S. E., & Baumlér, A. J. (2016). Depletion of butyrate-producing clostridia from the gut microbiota drives an aerobic luminal expansion of salmonella. *Cell Host & Microbe*, 19(4), 443–454. <https://doi.org/10.1016/j.chom.2016.03.004>

Rogers, M. A. M., & Aronoff, D. M. (2016). The influence of non-steroidal anti-inflammatory drugs on the gut microbiome. *Clinical Microbiology and Infection*, 22(2), 178 e171–178 e179. <https://doi.org/10.1016/j.cmi.2015.10.003>

Routy, B., Le Chatelier, E., Derosa, L., Duong, C. P. M., Alou, M. T., Daillere, R., Fluckiger, A., Messaoudene, M., Rauber, C., Roberti, M. P., Fidelle, M., Flament, C., Poirier-Colame, V., Opolon, P., Klein, C., Iribarren, K., Mondragon, L., Jacquelot, N., Qu, B.,...Zitvogel, L. (2018). Gut microbiome influences efficacy of PD-1-based immunotherapy against epithelial tumors. *Science*, 359(6371), 91–97. <https://doi.org/10.1126/science.aan3706>.

[PubMed]

Roy, S., & Trinchieri, G. (2017). Microbiota: A key orchestrator of cancer therapy. *Nature Reviews. Cancer*, 17(5), 271–285. <https://doi.org/10.1038/nrc.2017.13>

[PubMed]

Ruhe, Z. C., Low, D. A., & Hayes, C. S. (2020). Polymorphic toxins and their immunity proteins: Diversity, evolution, and mechanisms of delivery. *Annual Review of Microbiology*, 74, 497–520. <https://doi.org/10.1146/annurev-micro-020518-115638>

[PubMed]

Russell, A. B., Peterson, S. B., & Mougous, J. D. (2014). Type VI secretion system effectors: Poisons with a purpose. *Nature Reviews. Microbiology*, 12(2), 137–148. <https://doi.org/10.1038/nrmicro3185>

[PubMed]

Sarfatis, A., Wang, Y., Twumasi-Ankrah, N., & Moffitt, J. R. (2025). Highly multiplexed spatial transcriptomics in bacteria. *Science*, 387(6732), eadr0932. <https://doi.org/10.1126/science.adr0932>

[PubMed]

Sargun, A., Gerner, R. R., Raffatellu, M., & Nolan, E. M. (2021). Harnessing iron acquisition machinery to target enterobacteriaceae. *The Journal of Infectious Diseases*, 223(12 Suppl 2), S307–S313. <https://doi.org/10.1093/infdis/jiaa440>

[PubMed]

Sassone-Corsi, M., Nuccio, S. P., Liu, H., Hernandez, D., Vu, C. T., Takahashi, A. A., Edwards, R. A., & Raffatellu, M. (2016). Microcins mediate competition among Enterobacteriaceae in the inflamed gut. *Nature*, 540(7632), 280–283. <https://doi.org/10.1038/nature20557>

[PubMed]

Savage, D. C. (1977). Microbial ecology of the gastrointestinal tract. *Annual Review of Microbiology*, 31, 107–133. <https://doi.org/10.1146/annurev.mi.31.100177.000543>

[PubMed]

Sawaed, J., Zelik, L., Levin, Y., Feeney, R., Naama, M., Gordon, A., Zigdon, M., Rubin, E., Telpaz, S., Modilevsky, S., Ben-Simon, S., Awad, A., Harshuk-Shabso, S., Nuriel-Ohayon, M., Werbner, M., Schroeder, B. O., Erez, A., & Bel, S. (2024). Antibiotics damage the colonic mucus barrier in a microbiota-independent manner. *Science Advances*, 10(37), eadp4119. <https://doi.org/10.1126/sciadv.adp4119>

[PubMed]

Scher, J. U., Ubeda, C., Artacho, A., Attur, M., Isaac, S., Reddy, S. M., Marmon, S., Neimann, A., Brusca, S., Patel, T., Manasson, J., Pamer, E. G., Littman, D. R., & Abramson, S. B. (2015). Decreased bacterial diversity characterizes the altered gut microbiota in patients with psoriatic arthritis, resembling dysbiosis in inflammatory bowel disease. *Arthritis & Rheumatology*, 67(1), 128–139. <https://doi.org/10.1002/art.38892>

Schippa, S., Iebba, V., Barbato, M., Di Nardo, G., Totino, V., Checchi, M. P., Longhi, C., Maiella, G.,

Cucchiara, S., & Conte, M. P. (2010). A distinctive 'microbial signature' in celiac pediatric patients. *BMC Microbiology*, 10, 175. <https://doi.org/10.1186/1471-2180-10-175>
[PubMed]

Schooley, R. T., Biswas, B., Gill, J. J., Hernandez-Morales, A., Lancaster, J., Lessor, L., Barr, J. J., Reed, S. L., Rohwer, F., Benler, S., Segall, A. M., Taplitz, R., Smith, D. M., Kerr, K., Kumaraswamy, M., Nizet, V., Lin, L., McCauley, M. D., Strathdee, S. A., et al. (2017). Development and use of personalized bacteriophage-based therapeutic cocktails to treat a patient with a disseminated resistant *Acinetobacter baumannii* infection. *Antimicrobial Agents and Chemotherapy*, 61(10). <https://doi.org/10.1128/AAC.00954-17>

Sears, C. L., Geis, A. L., & Housseau, F. (2014). *Bacteroides fragilis* subverts mucosal biology: From symbiont to colon carcinogenesis. *The Journal of Clinical Investigation*, 124(10), 4166–4172. <https://doi.org/10.1172/JCI72334>
[PubMed]

Sharma, P., Goswami, S., Raychaudhuri, D., Siddiqui, B. A., Singh, P., Nagarajan, A., Liu, J., Subudhi, S. K., Poon, C., Gant, K. L., Herbrich, S. M., Anandhan, S., Islam, S., Amit, M., Anandappa, G., & Allison, J. P. (2023). Immune checkpoint therapy-current perspectives and future directions. *Cell*, 186(8), 1652–1669. <https://doi.org/10.1016/j.cell.2023.03.006>
[PubMed]

Shuoker, B., Pichler, M. J., Jin, C., Sakanaka, H., Wu, H., Gascuena, A. M., Liu, J., Nielsen, T. S., Holgersson, J., Nordberg Karlsson, E., Juge, N., Meier, S., Morth, J. P., Karlsson, N. G., & Abou Hachem, M. (2023). Sialidases and fucosidases of *Akkermansia muciniphila* are crucial for growth on mucin and nutrient sharing with mucus-associated gut bacteria. *Nature Communications*, 14(1), 1833. <https://doi.org/10.1038/s41467-023-37533-6>
[PubMed]

Silverman, J. M., Brunet, Y. R., Cascales, E., & Mougous, J. D. (2012). Structure and regulation of the type VI secretion system. *Annual Review of Microbiology*, 66, 453–472. <https://doi.org/10.1146/annurev-micro-121809-151619>
[PubMed]

Simonsen, J., Molbak, K., Falkenhorst, G., Krogfelt, K. A., Linneberg, A., & Teunis, P. F. (2009). Estimation of incidences of infectious diseases based on antibody measurements. *Statistics in Medicine*, 28(14), 1882–1895. <https://doi.org/10.1002/sim.3592>
[PubMed]

Sivan, A., Corrales, L., Hubert, N., Williams, J. B., Aquino-Michaels, K., Earley, Z. M., Benyamin, F. W., Lei, Y. M., Jabri, B., Alegre, M. L., Chang, E. B., & Gajewski, T. F. (2015). Commensal *Bifidobacterium* promotes antitumor immunity and facilitates anti-PD-L1 efficacy. *Science*, 350(6264), 1084–1089. <https://doi.org/10.1126/science.aac4255>
[PubMed]

Somsouk, M., Estes, J. D., Deleage, C., Dunham, R. M., Albright, R., Inadomi, J. M., Martin, J. N., Deeks, S. G., McCune, J. M., & Hunt, P. W. (2015). Gut epithelial barrier and systemic inflammation during chronic HIV infection. *AIDS*, 29(1), 43–51. <https://doi.org/10.1097/QAD.0000000000000511>
[PubMed]

Song, C., Chai, Z., Chen, S., Zhang, H., Zhang, X., & Zhou, Y. (2023). Intestinal mucus components and secretion mechanisms: What we do and do not know. *Experimental & Molecular Medicine*, 55(4), 681–691. <https://doi.org/10.1038/s12276-023-00960-y>

Sonnenburg, J. L., Xu, J., Leip, D. D., Chen, C. H., Westover, B. P., Weatherford, J., Buhler, J. D., & Gordon, J. I. (2005). Glycan foraging in vivo by an intestine-adapted bacterial symbiont. *Science*, 307(5717), 1955–1959. <https://doi.org/10.1126/science.1109051>
[PubMed]

Sorbara, M. T., & Pamer, E. G. (2019). Interbacterial mechanisms of colonization resistance and the strategies pathogens use to overcome them. *Mucosal Immunology*, 12(1), 1–9. <https://doi.org/10.1038/s41385-018-0053-0>
[PubMed]

Sorg, J. A., & Sonenshein, A. L. (2008). Bile salts and glycine as cogerminants for *Clostridium difficile* spores. *Journal of Bacteriology*, 190(7), 2505–2512. <https://doi.org/10.1128/JB.01765-07>
[PubMed]

Sougiannis, A. T., VanderVeen, B. N., Davis, J. M., Fan, D., & Murphy, E. A. (2021). Understanding chemotherapy-induced intestinal mucositis and strategies to improve gut resilience. *American Journal of Physiology. Gastrointestinal and Liver Physiology*, 320(5), G712–G719. <https://doi.org/10.1152/ajpgi.00380.2020>
[PubMed]

Spragge, F., Bakkeren, E., Jahn, M. T., E, B. N. A., Pearson, C. F., Wang, X., Pankhurst, L., Cunrath, O., & Foster, K. R. (2023). Microbiome diversity protects against pathogens by nutrient blocking. *Science*, 382(6676), eadj3502. <https://doi.org/10.1126/science.adj3502>
[PubMed]

Statovci, D., Aguilera, M., MacSharry, J., & Melgar, S. (2017). The impact of Western diet and nutrients on the microbiota and immune response at mucosal interfaces. *Frontiers in Immunology*, 8, 838. <https://doi.org/10.3389/fimmu.2017.00838>
[PubMed]

Stecher, B. (2021). Establishing causality in salmonella-microbiota-host interaction: The use of gnotobiotic mouse models and synthetic microbial communities. *International Journal of Medical Microbiology*, 311(3), 151484. <https://doi.org/10.1016/j.ijmm.2021.151484>
[PubMed]

Stecher, B., & Hardt, W. D. (2011). Mechanisms controlling pathogen colonization of the gut. *Current Opinion in Microbiology*, 14(1), 82–91. <https://doi.org/10.1016/j.mib.2010.10.003>
[PubMed]

Stecher, B., Robbiani, R., Walker, A. W., Westendorf, A. M., Barthel, M., Kremer, M., Chaffron, S., Macpherson, A. J., Buer, J., Parkhill, J., Dougan, G., von Mering, C., & Hardt, W. D. (2007). *Salmonella enterica* serovar typhimurium exploits inflammation to compete with the intestinal microbiota. *PLoS Biology*, 5(10), 2177–2189. <https://doi.org/10.1371/journal.pbio.0050244>

[PubMed]

Stecher, B., Maier, L., & Hardt, W. D. (2013). 'Blooming' in the gut: How dysbiosis might contribute to pathogen evolution. *Nature Reviews. Microbiology*, 11(4), 277–284. <https://doi.org/10.1038/nrmicro2989>

[PubMed]

Stephen, A. M., & Cummings, J. H. (1980). The microbial contribution to human faecal mass. *Journal of Medical Microbiology*, 13(1), 45–56. <https://doi.org/10.1099/00222615-13-1-45>

[PubMed]

Tan, B. L., & Norhaizan, M. E. (2019). Effect of high-fat diets on oxidative stress, cellular inflammatory response and cognitive function. *Nutrients*, 11(11). <https://doi.org/10.3390/nu11112579>

Taur, Y., Xavier, J. B., Lipuma, L., Ubeda, C., Goldberg, J., Gobourne, A., Lee, Y. J., Dubin, K. A., Socci, N. D., Viale, A., Perales, M. A., Jenq, R. R., van den Brink, M. R., & Pamer, E. G. (2012). Intestinal domination and the risk of bacteremia in patients undergoing allogeneic hematopoietic stem cell transplantation. *Clinical Infectious Diseases*, 55(7), 905–914. <https://doi.org/10.1093/cid/cis580>

[PubMed]

Terrisse, S., Derosa, L., Iebba, V., Ghiringhelli, F., Vaz-Luis, I., Kroemer, G., Fidelle, M., Christodoulidis, S., Segata, N., Thomas, A. M., Martin, A. L., Sirven, A., Everhard, S., Aprahamian, F., Nirmalathasan, N., Aarnoutse, R., Smidt, M., Ziemons, J., Caldas, C., et al. (2021). Intestinal microbiota influences clinical outcome and side effects of early breast cancer treatment. *Cell Death and Differentiation*, 28(9), 2778–2796. <https://doi.org/10.1038/s41418-021-00784-1>

[PubMed]

Torok, T. J., Tauxe, R. V., Wise, R. P., Livengood, J. R., Sokolow, R., Mauvais, S., Birkness, K. A., Skeels, M. R., Horan, J. M., & Foster, L. R. (1997). A large community outbreak of salmonellosis caused by intentional contamination of restaurant salad bars. *The Journal of the American Medical Association*, 278(5), 389–395. <https://doi.org/10.1001/jama.1997.03550050051033>

[PubMed]

Valdes, A. M., Walter, J., Segal, E., & Spector, T. D. (2018). Role of the gut microbiota in nutrition and health. *BMJ*, 361, k2179. <https://doi.org/10.1136/bmj.k2179>

[PubMed]

van der Waaij, D., Berghuis-de Vries, J. M., & Lekkerkerk, L.-V. (1971). Colonization resistance of the digestive tract in conventional and antibiotic-treated mice. *The Journal of Hygiene (London)*, 69(3), 405–411. <https://doi.org/10.1017/s0022172400021653>

van Nood, E., Vrieze, A., Nieuwdorp, M., Fuentes, S., Zoetendal, E. G., de Vos, W. M., Visser, C. E., Kuijper, E. J., Bartelsman, J. F., Tijssen, J. G., Speelman, P., Dijkgraaf, M. G., & Keller, J. J. (2013). Duodenal infusion of donor feces for recurrent *Clostridium difficile*. *The New England Journal of Medicine*, 368(5), 407–415. <https://doi.org/10.1056/NEJMoa1205037>

[PubMed]

Veazey, R. S., DeMaria, M., Chalifoux, L. V., Shvets, D. E., Pauley, D. R., Knight, H. L., Rosenzweig, M.,

Johnson, R. P., Desrosiers, R. C., & Lackner, A. A. (1998). Gastrointestinal tract as a major site of CD4+ T cell depletion and viral replication in SIV infection. *Science*, 280(5362), 427–431. <https://doi.org/10.1126/science.280.5362.427> [PubMed]

Vetizou, M., Pitt, J. M., Daillere, R., Lepage, P., Waldschmitt, N., Flament, C., Rusakiewicz, S., Routy, B., Roberti, M. P., Duong, C. P., Poirier-Colame, V., Roux, A., Becharef, S., Formenti, S., Golden, E., Cording, S., Eberl, G., Schlitzer, A., Ginhoux, F.,...Zitvogel, L. (2015). Anticancer immunotherapy by CTLA-4 blockade relies on the gut microbiota. *Science*, 350(6264), 1079–1084. <https://doi.org/10.1126/science.aad1329>. [PubMed]

Vujkovic-Cvijin, I., & Somsouk, M. (2019). HIV and the gut microbiota: Composition, consequences, and avenues for amelioration. *Current HIV/AIDS Reports*, 16(3), 204–213. <https://doi.org/10.1007/s11904-019-00441-w> [PubMed]

Vujkovic-Cvijin, I., Dunham, R. M., Iwai, S., Maher, M. C., Albright, R. G., Broadhurst, M. J., Hernandez, R. D., Lederman, M. M., Huang, Y., Somsouk, M., Deeks, S. G., Hunt, P. W., Lynch, S. V., & McCune, J. M. (2013). Dysbiosis of the gut microbiota is associated with HIV disease progression and tryptophan catabolism. *Science Translational Medicine*, 5(193), 193ra191. <https://doi.org/10.1126/scitranslmed.3006438>

Walawalkar, Y. D., Vaidya, Y., & Nayak, V. (2016). Response of salmonella Typhi to bile-generated oxidative stress: Implication of quorum sensing and persister cell populations. *Pathogens and Disease*, 74(8). <https://doi.org/10.1093/femspd/ftw090>

Wang, X., Zhang, H., Zhang, N., Zhang, S., Shuai, Y., Miao, X., Liu, Y., Qiu, L., Ren, S., Lai, S., Han, Y., Yao, H., Zhang, X., Fan, F., Sun, H., & Yi, H. (2024). Application value of metagenomic next-generation sequencing in hematological patients with high-risk febrile neutropenia. *Frontiers in Cellular and Infection Microbiology*, 14, 1366908. <https://doi.org/10.3389/fcimb.2024.1366908> [PubMed]

Weingarden, A. R., Chen, C., Zhang, N., Graiziger, C. T., Dosa, P. I., Steer, C. J., Shaughnessy, M. K., Johnson, J. R., Sadowsky, M. J., & Khoruts, A. (2016). Ursodeoxycholic acid inhibits *Clostridium difficile* spore germination and vegetative growth, and prevents the recurrence of Ileal Pouchitis associated with the infection. *Journal of Clinical Gastroenterology*, 50(8), 624–630. <https://doi.org/10.1097/MCG.0000000000000427> [PubMed]

Welling, G. W., Groen, G., Tuinte, J. H., Koopman, J. P., & Kennis, H. M. (1980). Biochemical effects on germ-free mice of association with several strains of anaerobic bacteria. *Journal of General Microbiology*, 117(1), 57–63. <https://doi.org/10.1099/00221287-117-1-57> [PubMed]

Wiese, D. M., Horst, S. N., Brown, C. T., Allaman, M. M., Hodges, M. E., Slaughter, J. C., Druce, J. P., Beaulieu, D. B., Schwartz, D. A., Wilson, K. T., & Coburn, L. A. (2016). Serum fatty acids are correlated with inflammatory cytokines in ulcerative colitis. *PLoS One*, 11(5), e0156387. <https://doi.org/10.1371/journal.pone.0156387>

[[PubMed](#)]

Willing, B. P., Russell, S. L., & Finlay, B. B. (2011). Shifting the balance: Antibiotic effects on host-microbiota mutualism. *Nature Reviews. Microbiology*, 9(4), 233–243. <https://doi.org/10.1038/nrmicro2536>

[[PubMed](#)]

Winter, S. E., Thiennimitr, P., Winter, M. G., Butler, B. P., Huseby, D. L., Crawford, R. W., Russell, J. M., Bevins, C. L., Adams, L. G., Tsois, R. M., Roth, J. R., & Baumler, A. J. (2010). Gut inflammation provides a respiratory electron acceptor for Salmonella. *Nature*, 467(7314), 426–429. <https://doi.org/10.1038/nature09415>

[[PubMed](#)]

Wlodarska, M., Willing, B., Keeney, K. M., Menendez, A., Bergstrom, K. S., Gill, N., Russell, S. L., Vallance, B. A., & Finlay, B. B. (2011). Antibiotic treatment alters the colonic mucus layer and predisposes the host to exacerbated Citrobacter rodentium-induced colitis. *Infection and Immunity*, 79(4), 1536–1545. <https://doi.org/10.1128/IAI.01104-10>

[[PubMed](#)]

Woelfel, S., Silva, M. S., & Stecher, B. (2024). Intestinal colonization resistance in the context of environmental, host, and microbial determinants. *Cell Host & Microbe*, 32(6), 820–836. <https://doi.org/10.1016/j.chom.2024.05.002>

Worby, C. J., Schreiber, H. L. t., Straub, T. J., van Dijk, L. R., Bronson, R. A., Olson, B. S., Pinkner, J. S., Obernuefemann, C. L. P., Munoz, V. L., Paharik, A. E., Azimzadeh, P. N., Walker, B. J., Desjardins, C. A., Chou, W. C., Bergeron, K., Chapman, S. B., Klim, A., Manson, A. L., Hannan, T. J., et al. (2022). Longitudinal multi-omics analyses link gut microbiome dysbiosis with recurrent urinary tract infections in women. *Nature Microbiology*, 7(5), 630–639. <https://doi.org/10.1038/s41564-022-01107-x>

[[PubMed](#)]

Wotzka, S. Y., Kreuzer, M., Maier, L., Arnoldini, M., Nguyen, B. D., Brachmann, A. O., Berthold, D. L., Zund, M., Hausmann, A., Bakkeren, E., Hoces, D., Gul, E., Beutler, M., Dolowschiak, T., Zimmermann, M., Fuhrer, T., Moor, K., Sauer, U., Typas, A., et al. (2019). Escherichia coli limits Salmonella Typhimurium infections after diet shifts and fat-mediated microbiota perturbation in mice. *Nature Microbiology*, 4(12), 2164–2174. <https://doi.org/10.1038/s41564-019-0568-5>

[[PubMed](#)]

Yassour, M., Vatanen, T., Siljander, H., Hamalainen, A. M., Harkonen, T., Ryhanen, S. J., Franzosa, E. A., Vlamakis, H., Huttenhower, C., Gevers, D., Lander, E. S., Knip, M., Group, D. S., & Xavier, R. J. (2016). Natural history of the infant gut microbiome and impact of antibiotic treatment on bacterial strain diversity and stability. *Science Translational Medicine*, 8(343), 343ra381. <https://doi.org/10.1126/scitranslmed.aad0917>

Yatsunencko, T., Rey, F. E., Manary, M. J., Trehan, I., Dominguez-Bello, M. G., Contreras, M., Magris, M., Hidalgo, G., Baldassano, R. N., Anokhin, A. P., Heath, A. C., Warner, B., Reeder, J., Kuczynski, J., Caporaso, J. G., Lozupone, C. A., Lauber, C., Clemente, J. C., Knights, D., et al. (2012). Human gut microbiome viewed across age and geography. *Nature*, 486(7402), 222–227. <https://doi.org/10.1038/nature11053>

[[PubMed](#)]

Zhai, B., Ola, M., Rolling, T., Tosini, N. L., Joshowitz, S., Littmann, E. R., Amoretti, L. A., Fontana, E., Wright, R. J., Miranda, E., Veelken, C. A., Morjaria, S. M., Peled, J. U., van den Brink, M. R. M., Babady, N. E., Butler, G., Taur, Y., & Hohl, T. M. (2020). High-resolution mycobiota analysis reveals dynamic intestinal translocation preceding invasive candidiasis. *Nature Medicine*, 26(1), 59–64. <https://doi.org/10.1038/s41591-019-0709-7>

[[PubMed](#)]

Zheng, J., Shin, O. S., Cameron, D. E., & Mekalanos, J. J. (2010). Quorum sensing and a global regulator TsrA control expression of type VI secretion and virulence in *Vibrio cholerae*. *Proceedings of the National Academy of Sciences of the United States of America*, 107(49), 21128–21133. <https://doi.org/10.1073/pnas.1014998107>

[[PubMed](#)]

Zhu, W., Winter, M. G., Byndloss, M. X., Spiga, L., Duerkop, B. A., Hughes, E. R., Buttner, L., de Lima Romao, E., Behrendt, C. L., Lopez, C. A., Sifuentes-Dominguez, L., Huff-Hardy, K., Wilson, R. P., Gillis, C. C., Tukul, C., Koh, A. Y., Burstein, E., Hooper, L. V., Baumler, A. J., & Winter, S. E. (2018). Precision editing of the gut microbiota ameliorates colitis. *Nature*, 553(7687), 208–211. <https://doi.org/10.1038/nature25172>

[[PubMed](#)]

Zhu, W., Winter, M. G., Spiga, L., Hughes, E. R., Chanin, R., Mulgaonkar, A., Pennington, J., Maas, M., Behrendt, C. L., Kim, J., Sun, X., Beiting, D. P., Hooper, L. V., & Winter, S. E. (2020). Xenosiderophore utilization promotes *Bacteroides thetaotaomicron* resilience during colitis. *Cell Host & Microbe*, 27(3), 376–388 e378. <https://doi.org/10.1016/j.chom.2020.01.010>

Zimmermann, M., Patil, K. R., Typas, A., & Maier, L. (2021). Towards a mechanistic understanding of reciprocal drug-microbiome interactions. *Molecular Systems Biology*, 17(3), e10116. <https://doi.org/10.15252/msb.202010116>

[[PubMed](#)]

Zuo, T., Wong, S. H., Lam, K., Lui, R., Cheung, K., Tang, W., Ching, J. Y. L., Chan, P. K. S., Chan, M. C. W., Wu, J. C. Y., Chan, F. K. L., Yu, J., Sung, J. J. Y., & Ng, S. C. (2018). Bacteriophage transfer during faecal microbiota transplantation in *Clostridium difficile* infection is associated with treatment outcome. *Gut*, 67(4), 634–643. <https://doi.org/10.1136/gutjnl-2017-313952>

[[PubMed](#)]

15. Microbiome and Diseases: Colorectal Cancer

Sebastian Zeissig^{1,3}✉, Florian Jokisch²✉, Kenneth Peuker¹✉ and
Klaus-Peter Janssen²✉

(1) Department of Internal Medicine A, University Medical Center
Greifswald, Greifswald, Germany

(2) School of Medicine and Health, TUM University Hospital,
Department of Surgery, Munich, Germany

(3) Universitätsklinik für Viszerale Chirurgie und Medizin, Inselspital,
Bern, Switzerland

✉ **Sebastian Zeissig**

Email: sebastian.zeissig@insel.ch

✉ **Florian Jokisch**

Email: florian.jokisch@tum.de

✉ **Kenneth Peuker**

Email: kenneth.peuker@med.uni-greifswald.de

✉ **Klaus-Peter Janssen (Corresponding author)**

Email: klaus-peter.janssen@tum.de

Abstract

Cancers of the large intestine are among the most frequent malignomas worldwide and also rank among the most frequent causes for cancer-related mortality in developed countries, with an recently increasing incidence in the younger population. Patient survival and treatment

options in the metastatic form of this disease are still relatively poor. The cell-autonomous genetic and epigenetic changes associated with carcinogenesis, and the stepwise and consecutive progression along the adenoma-carcinoma sequence in the colorectum, have been studied intensively over the last decades. However, there is a growing interest in the impact of gut microbial communities on the initiation and progression of this cancer entity. Overwhelming evidence meanwhile suggests that the microbiota, its toxins and metabolites are important and potentially causative factor for colorectal cancer (CRC), and is also involved in response to therapy. Microbial dysbiosis can lead to impairment of epithelial barrier function, imbalance in epithelial self-renewal, DNA damage, and altered immune responses, thereby fostering tumor initiation and progression.

Keywords CRC – Colorectal cancer – Tumor-infiltrating T lymphocytes (TILs) – Colibactin – Pathobionts – *Fusobacterium nucleatum* – Genotoxin

15.1 Introduction: Colorectal Cancer

Cancer is a multifactorial disease caused by genetic predispositions and environmental factors, resulting in the accumulation of genetic and epigenetic alterations leading to uncontrolled cell proliferation. The gut microbiome is increasingly recognized as causal contributor to cancer, even though only few microbial taxa were described to directly cause colorectal cancer. However, there is solid evidence that the gut microbiota and its metabolites can foster cancer formation, by acting on host epithelia and immune cells. On the other hand, specific bacterial taxa have beneficial and immune-stimulatory properties and can determine the response to cancer therapy. According to the 2025 statistics from the American Cancer Society and data from International Agency for Research on Cancer (IARC), colorectal cancer (CRC) is the third most common cancer in the USA as well as worldwide with nearly two million cases, and ranks second globally in cancer-related deaths

with a mortality rate of approximately 50% (Bray et al., [2024](#); Siegel et al., [2025](#)). CRC is known to result from a well-established sequential cascade of mutations leading to loss of function of tumor suppressor genes or activation of oncogenes, where key genetic changes are associated with various stages of cancer progression (Fearon & Vogelstein, [1990](#)). Several parallel pathways of CRC development are acknowledged, based on genetic lesions that are inherited through the germline, or, in the majority of cases, acquired somatically. The vast majority of CRCs, both hereditary and spontaneous cases, show a constitutive activation of the canonical Wnt pathway, most frequently by inactivation of the tumor suppressor gene adenomatous polyposis coli (APC), leading to an imbalance of cell proliferation over cell death. A germline mutation in the APC gene leads to a polyposis syndrome (FAP, familial adenomatous polyposis) associated with the development of CRC in all affected individuals (Powell et al., [1992](#)). Activating mutations in the proto-oncogenes Kirsten rat sarcoma viral homolog oncogene (KRAS) or NRAS occur in around 50% of cases, in the proto-oncogene BRAF (B-Raf proto-oncogene, serine/threonine kinase) in around 10% of all cases, the components of the epidermal growth factor (EGF) pathway, or the transforming growth factor- β (TGF- β) signaling cascade are also altered, somewhat less frequently (Yaeger et al., [2018](#)). Around 15% of all CRC cases show DNA microsatellite instability (MSI), caused by hereditary (HNPCC) or spontaneous deficiency in the DNA mismatch repair genes (Andre et al., [2020](#)). Incidence, prognosis and response to therapy differ between MSI-high cases and the majority of microsatellite-stable cancers (MSS). The differential association of the gut microbiome with different genetic subtypes of CRC is an important, yet still little understood area of research. Thus, CRC is a genetic disease, but the vast majority of cases occur sporadically without a known genetic predisposition. Induction, accumulation, and persistence of mutations are influenced by a variety of environmental factors, such as diet, lifestyle, alcohol consumption, obesity, and comorbidities, especially a history of inflammatory bowel disease (IBD) (Johnson et al., [2013](#); Schult et al., [2024](#)). While CRC has

typically affected older populations, cases are increasingly being diagnosed in younger adults (Sung et al., [2025](#)). In addition, the global distribution of CRC cases has changed in recent years. Populations that were previously considered low risk—such as in parts of Asia, Africa, and South America—are experiencing an increase in CRC incidence, especially in younger populations (Lui et al., [2019](#)).

It is well established that the human colon is a densely populated microbial ecosystem (Simon & Gorbach, [1984](#)). The gastrointestinal microbiota plays a crucial role in health and disease and has increasingly become the focus of CRC research. The gut microbiome can be beneficial to the host by providing protection against pathogens or by helping with digestive processes. However, the microbiota can impair the mucus barrier function or produce pro-carcinogenic compounds such as colibactin by pks + *Escherichia coli*, which will be highlighted here, together with other bacterial taxa that are meanwhile well established in the cancer context, such as *Fusobacterium nucleatum*, *Helicobacter*, or *Bacteroides fragilis*. This list is far from conclusive, and new players keep coming up. Among the exciting recent discoveries is that sulfur-metabolizing bacterial taxa like *Desulfovibrio* are associated with colorectal cancer, likely due to the fact that N-nitroso compounds and hydrogen sulfide may directly foster cancer development (Nguyen et al., [2020](#)). Short-chain fatty acids (SCFAs) like butyrate and propionate, produced by microbial fermentation of dietary fiber in the colon, have health benefits, including anti-inflammatory effects. Moreover, the gut microbiome appears to have a direct influence on lipid absorption and production. In fact, the contribution of microbiota to the host lipid metabolism has recently been reported: intestinal uptake of dietary lipids is negatively regulated by gut bacteria, whereas short-chain fatty acids derived from microbiota are used for de novo lipogenesis by the host (Kindt et al., [2018](#)). These processes are relevant for tissue regeneration, since gut microbiota are crucially required for efficient liver regeneration (Yin et al., [2023](#)), and likely also contribute to the altered lipid metabolism in colorectal cancer (Ecker et al., [2021](#); Ecker et al., [2022](#)), and by shaping

the gastrointestinal immune system. Metabolic interactions and natural compounds produced by gut bacteria are further key areas of host-microbiota interaction.

Dysbiosis, or the absence of healthy microbiota, can be a result of an active disease or induced by dietary habits, and specific microbial signatures with predictive properties have been described in stool samples from CRC (Wirbel et al., [2019](#)). However, researchers are currently still facing the conundrum: is an altered microbiome a cause or consequence of gastrointestinal cancer? An infection or nutritional imbalance leads to reduced colonization by beneficial microorganisms and an enrichment of pro-carcinogenic bacterial groups. Dysbiosis disturbs the immune system homeostasis, causing inflammation, disrupting the mucosal barrier, and increasing epithelial permeability, creating a microenvironment that further perpetuates pro-carcinogenic dysbiosis.

15.2 Role of Microbiota in Colorectal Carcinogenesis

Animal studies suggested that microorganisms play a crucial and tumor promoting role in colorectal cancer (CRC) development, using antibiotic treatment as well as germfree rodent models, to causally examine how gut microbiota affect CRC susceptibility. One of the earliest studies demonstrated a lower colonic tumor incidence in germ-free compared to conventionally housed rats after administration of the carcinogenic substances azoxymethane or dimethylhydrazine (Reddy et al., [1975](#)). Further preclinical studies, comparing the number of intestinal polyps in germfree and conventionally raised *Apc*^{Min/+} mice, a well-established genetic mouse model, suggest that the gut microbiome indeed promotes development of intestinal neoplasia. Whereas a decreased incidence of polyps in the small intestine was reported initially (Dove et al., [1997](#)), a subsequent study found a significant reduction of intestinal adenomas in both the small and large intestine of germfree mice compared with conventionally raised controls (Li et al., [2012](#)).

Antibiotic treatment of mice carrying both a truncating *APC* mutation and a defective DNA mismatch repair system significantly reduced the number of polyps in both the small and large intestine (Belcheva et al., [2014](#)). Interestingly, *APC* haplo-insufficiency of the host colonic epithelial cell alters colonic microbial interactions prior to polyposis in mouse models (Son et al., [2015](#)). Moreover, there is clear evidence that colonic tumor formation exclusively depends on the presence of gut microbiota in a transgenic mouse model driven by intestine-specific overexpression of the endoplasmic reticulum stress factor ATF6. Activated ATF6 leads to intestinal dysbiosis, which in turn promotes colorectal tumor formation (Coleman et al., [2018](#)). Taken together, it is very likely that microbiome changes contribute to the pathogenesis of sporadic colorectal cancer, indicating that the gut microbiome could present an important potential target for cancer prevention. Mouse models that develop colitis and promote neoplasia further support the involvement of microbes in tumor development. Interleukin-10 (IL-10) is an anti-inflammatory cytokine, and IL-10-deficient mice are a recognized model for human inflammatory bowel disease. *Il10*^{-/-} mice treated with the carcinogenic substance azoxymethane (AOM) develop chronic enterocolitis and colitis-associated adenocarcinomas, the severity of which is dependent on their microbiota. In contrast, germfree *Il10*^{-/-} mice do not develop colitis (Sellon et al., [1998](#)). An alternate model involves combining AOM and dextran sulfate sodium (DSS) to provide a two-step tumor model of colitis-associated cancer. DSS is an inflammatory agent that induces colonic epithelial damage and causes colitis in mice, mimicking features of human IBD. Transfer of microbiota from tumor-bearing AOM/DSS mice into germfree mice significantly increased the number and size of tumors compared to germfree mice inoculated with healthy microbiota (Zackular et al., [2013](#)). In a later study, it was observed that stool from patients with CRC could promote colorectal carcinogenesis in mice. Stool from patients with CRC increases the numbers of polyps, intestinal dysplasia, proliferation, and inflammation in germfree mice and conventional mice injected with azoxymethane compared to mice fed with stool

samples from healthy individuals (Wong et al., [2017](#)). Thus, the risk for the development of CRC can be transferred with the pro-carcinogenic microbiota. Further studies have proposed that the fecal microbiota changes as CRC progresses and can be potentially used for cancer screening and early diagnosis (Zeller et al., [2014](#)). High intake of red meat relative to fruits and vegetables was shown to be a risk factor associated with this shift in microbiota, potentially promoting carcinogenesis (Feng et al., [2015](#)). Metagenome-wide association studies (MGWAS) currently allow detailed insights into microbiome–host interactions in human disease, such as a study on stool from healthy subjects, compared to advanced adenoma and carcinoma patients (Wirbel et al., [2019](#)). This analysis identified a core set of 29 significantly enriched bacterial species in CRC, including known pathobionts like *F. nucleatum*, as well as *Slackia moorei*, *Gemella morbillorum*, *Parvimonas micra* and *Peptostreptococcus stomatis*. The authors also identified taxonomic clusters linked to specific CRC subtypes or patient groups, e.g., *Porphyromonas* was more prevalent in rectal cancer, while *Clostridiales* was significantly enriched in female CRC patients. Additionally, bacterial virulence factors such as pks from *E.coli* and fadA from *F. nucleatum* were significantly enriched in CRC (Wirbel et al., [2019](#)). These virulence factors can be considered as sophisticated microbial tools that are mainly used by the bacteria for their own benefit and survival, but can result in disruption of genetic integrity and activation of aberrant pro-carcinogenic signaling in the colonic epithelium. Furthermore, the ability of bacteria to induce specific immune responses that promote CRC development is currently under investigation, and major principles and advances are highlighted in the second part of this chapter. While CRC is associated with a characteristic microbiome signature, recent work has also demonstrated the presence of polybacterial biofilms, in particular in association with right-sided CRC, which contribute to tumor growth through IL-6-dependent epithelial STAT3 activation and polyamine production (Dejea et al., [2014](#); Johnson et al., [2015](#)). Similar observations have been made in patients with FAP (Dejea et al., [2018](#)).

Lastly, the gut microbiota has also been involved in patients' response to cancer therapy, e.g., cytotoxic chemotherapy (Roberti et al., [2020](#)).

15.3 Mechanisms by Which Bacteria Induce Aberrant Events Associated with Carcinogenesis

Microorganisms interact with their host on multiple levels, involving various physiological and pathological processes. Most relevant for carcinogenesis are: the microbial-driven induction of inflammation or immune evasion, the induction of pro-oncogenic host signaling pathways, the direct induction of DNA damage by bacterial-encoded genotoxins, and interaction with the host metabolism. Integrated tumor, immune, and microbiome analysis in human CRC currently allows elucidation of the crosstalk between gut microbiota, cancer cells, and the immune system (Roelands et al., [2023](#)).

The finding that the gastric pathogen *Helicobacter pylori* is a causative agent for gastric cancer revolutionized our thinking of the pathophysiology of gastrointestinal disorders. These bacteria are now recognized as a type I carcinogen by the WHO, and the association of *H. pylori* with gastric cancer was confirmed by many epidemiological studies (Parsonnet et al., [1991](#)), and will be dealt with in detail later in this Chapter. Briefly, studies about *H. pylori* and gastric cancer have elucidated several principles of how bacteria can manipulate epithelial cells. Meanwhile, multiple associations between colorectal and other cancer types with microbiota were made, and specific molecular pathways of how epithelial integrity can be manipulated by bacteria were described. We will summarize in this chapter some of the most recent findings about the members of the gut microbiota and gut pathogens that are linked to CRC and focus on the molecular pathways that were discovered to be linked with aberrant epithelial signaling and carcinogenesis.

15.3.1 *Salmonella enterica* Typhi/Paratyphi A

Typhoid fever is a life-threatening disease caused by the human-restricted *Salmonella enterica* serovars Typhi and Paratyphi A. Epidemiological studies have linked chronic carriage of *Salmonella enterica* Typhi/Paratyphi A with gallbladder cancer and the incidence of this adenocarcinoma is higher in countries where typhoid fever is endemic (Nagaraja & Eslick, [2014](#)). *Salmonella* resides in the gallbladders of chronic carriers intracellularly and extracellularly by forming biofilms on gallstones, which serve as a reservoir from where the bacteria are sporadically shed into the duodenum (Gonzalez-Escobedo & Gunn, [2013](#)). A key mechanism with which these human-specific *Salmonella enterica* serovars provoke genome instability is by the synthesis of the typhoid toxin that causes DNA damage (Song et al., [2013](#)). Formation of actin stress fibers and cell distension has been observed in cells exposed to typhoid toxin-producing *Salmonella* (Haghjoo & Galan, [2004](#)). The genotoxicity of the typhoid toxin is dependent on the expression of the CdtB subunit, which is functionally and structurally homologous to the mammalian DNase I (Lara-Tejero & Galan, [2000](#); Nesic et al., [2004](#)) and is translocated into the nucleus of the intoxicated cell (Guidi et al., [2013b](#)). This toxin is expressed by the bacterium only once it is internalized into the host cell and replicates within specialized vacuoles, known as *Salmonella*-containing vacuoles (Spano et al., [2008](#)). The toxin is then synthesized and released within outer membrane vesicles, where it is protected from host proteases. The toxin can be retrogradely translocated to the nucleus through the trans-Golgi network, where it causes DNA double-strand breaks (Guidi et al., [2013a](#)). It has been shown that brefeldin A, an inhibitor of the transport of proteins from the endoplasmic reticulum to the Golgi, was able to inhibit the DNase activity of the toxin (Salcedo & Holden, [2003](#)). In addition, CdtB subunit can also be directly internalized into the host cells through the endocytic pathway (Guidi et al., [2013b](#)).

Whether the described mechanisms of DNA damage induced by *Salmonella* species also occurs in the colon is still not clear, but recent studies with a study population of 140,000 cases suggest an increased presence of *Salmonella* in premalignant or malignant colonic lesions

(Mughini-Gras et al., [2018](#)). Furthermore, typhoid toxin was shown to alter intestinal inflammation and promote persistence of *Salmonella* species in the host, suggesting that a chronic exposure of epithelial cells to the toxin could occur (Del Bel Belluz et al., [2016](#)). However, another nationwide registry-based study from Denmark, with a study population of over seven million individuals, did not find a correlation between increased CRC risk and *Salmonella* infection (Duijster et al., [2021](#)). Further investigation is warranted to causally link *Salmonella* infection to increased CRC risk. One important protein that contributes to *S. enterica* infections in host cells is the 33 kDa multifunctional effector protein AvrA. The intracellular presence of AvrA was reported both in tumor-bearing mouse models and, notably, in human colorectal cancer patient samples (Lu et al., [2017](#)). After secretion, AvrA alters various host cell pathways through acetylation and ubiquitination of target proteins, such as TP53, leading to inhibition of apoptosis. Moreover, AvrA activates the JAK/STAT pathway in AOM/DSS colorectal cancer mouse models, which regulates proliferation and differentiation and can support tumorigenesis (Lu et al., [2016](#)). Taken together, *Salmonella* does appear to play a role in colon carcinogenesis, but whether it is a suitable biomarker in clinical settings remains questionable and requires further investigation.

15.3.2 *Helicobacter* and Gastrointestinal Carcinogenesis

A genus long known to be associated with cancer in the gastrointestinal region is *Helicobacter*, specifically the species *H. pylori*, but also *H. hepaticus*. The latter is a gram-negative, spiral-shaped, microaerophilic bacterium that is found predominantly in the cecum and colon of mice. The tumor-promoting effects of *H. hepaticus* have been attributed to the genotoxin cytolethal distending toxin (CDT), which is produced by multiple gram-negative bacterial species, such as *Campylobacter jejuni*. CDT is a tripartite holotoxin consisting of three subunits, CdtA, CdtB, and CdtC (Lara-Tejero & Galan, [2001](#)). CdtB is the active domain and is delivered into the target cells with the aid of the accessory proteins, CdtA and CdtC (Haghjoo & Galan, [2004](#)). CDT is essential for hepatic

and intestinal persistence of *H. hepaticus* in infected mice, leading to altered cytokine response (Ge et al., [2005](#); Pratt et al., [2006](#)). CDT plays a key role in promoting dysplastic changes and increased hepatocyte proliferation in the livers of *H. hepaticus*-infected A/JCr mice (liver disease-susceptible mice). CDT-producing *H. hepaticus* can cause overproduction of antiapoptotic proteins, upregulate the NF- κ B pathway, and cause subsequent inflammation-associated preneoplastic liver lesions (Ge et al., [2007](#)). Moreover, CDT encoded by *Campylobacter jejuni* promotes colorectal liver metastasis through JAK2-STAT3-MMP9 signaling or pulmonary metastatic mice models (He et al., [2024](#)). In contrast, genetic deletion of *cdtB* demonstrated that the genotoxin is essential for the pro-metastatic capacity of *C. jejuni* (He et al., [2024](#)). These findings demonstrate that a genotoxin produced by intratumoral bacteria may accelerate colorectal cancer metastasis. CDT is essential for modulating host immune response by downregulating anti-inflammatory cytokine IL-10 in the colon of Swiss Webster mice to cause *H. hepaticus*-induced colitis and colon carcinoma (Ge et al., [2005](#)). *H. hepaticus* has been shown to induce colon tumorigenesis both in IL10-deficient mice and in immunocompromised Rag-2-deficient mice (Erdman et al., [2003](#); Nagamine et al., [2008](#)). CdtB-deficient *H. hepaticus* mutants have been reported to cause less severe typhlocolitis in IL-10^{-/-} mice when compared to WT *H. hepaticus* (Young et al., [2004](#)). CDT-producing *H. hepaticus* causes activation of the TNF/IL-6-Stat3 signaling pathway in the ceca of Rag-2-deficient mice (Ge et al., [2017](#)). Expression of IL-10 by transferred CD4⁺ and CD25⁺ T regulatory cells in Rag-2-deficient mice inhibits the development of colitis following *H. hepaticus* infection (Erdman et al., [2003](#); Maloy et al., [2003](#)). Chronic exposure to sublethal levels of recombinant CDTs from *H. hepaticus* has been shown to induce CdtB-dependent genomic instability and anchorage-independent growth (Guidi et al., [2013a](#)). This study suggested that long-term exposure to CDT allows for the selection of cells that have overcome the tumorigenesis barrier, thus favoring tumor progression. However, the contribution of *H. hepaticus* to colorectal cancer is still under debate. Female Apc^{Min} mice infected with *H.*

hepaticus exhibited increased tumor burden compared with uninfected littermates, regarding not only the small and large intestine, but interestingly also mammary lesions, associated with an increase in myeloid-derived suppressor cells (Deng et al., [2022](#)). In contrast, *H. hepaticus* was recently not found to promote polyposis, neither in the Apc^{Min} line, nor in a combined model with mismatch-repair-deficient Apc^{Min} mice (Thakur et al., [2025](#)). In vivo, *H. pylori* has been demonstrated to persist either as free-swimming bacteria in the mucus or invade into gastric glands and attach to the apical junctions of epithelial cells (Sigal et al., [2015](#)). *H. pylori* induces an expansion of long-lived stem cells and increases proliferation (Sigal et al., [2017](#)), probably as a host cell response to the infection. The expanded stem cell compartment can be directly colonized by the bacteria, leading to changes in signaling and potentially disrupting the epithelial integrity of long-lived stem cells. Research over the last decades has uncovered several molecular mechanisms driving carcinogenesis (Amieva & El-Omar, [2008](#)), highlighting specific virulence factors linked to cancer progression. Notably, the bacterium employs a type IV secretion system to inject the CagA virulence factor into host cells. The host cells, failing to recognize CagA as foreign, phosphorylate it via c-Src and c-Abl kinases, triggering aberrant events such as altered epithelial polarity (Amieva et al., [2003](#)), increased proliferation and migration (Bagnoli et al., [2005](#)), and an elevated cancer risk associated with the presence of CagA. In addition to CagA, other virulence factors have been identified that compromise host DNA integrity by causing double-strand breaks (Koeppel et al., [2015](#)), interfering with stemness (Sigal et al., [2015](#)), affecting apoptosis, and inhibiting responses to inflammatory stimuli (Morey et al., [2018](#)). Undoubtedly, studies about *H. pylori* and gastric cancer have elucidated key principles of how bacteria can manipulate epithelial cells. Moreover, recent research has shown that *H. pylori* is not only associated with gastric ulcers and gastric cancer, but also contributes to colorectal carcinogenesis. Epidemiological studies reported that *H. pylori* infection is associated with an almost two-fold increased risk of CRC development (Boustany et al., [2023](#)). In

accordance, preclinical data show that genetic tumor mouse models Apc^{Min} and Apc^{1638N} develop twice as many tumors when infected with *H. pylori* compared to controls, despite the fact that *H. pylori* does not colonize the large intestine and is not detectable in intestinal lesions (Ralser et al., [2023](#)). Thus, the hypothesis was raised that *H. pylori* impacts CRC progression based on a complex crosstalk between the upper (stomach) and lower (colorectum) digestive tract. *H. pylori*'s virulence factors, which enable evasion of the innate immune system, also induce pro-inflammatory conditions in the colon and potentially manipulate adaptive immune responses, thereby promoting CRC (Engelsberger et al., [2024](#)). In fact, the gastric infection was found to affect intestinal homeostasis at different levels. There was an increase in pro-inflammatory cytokines such as tumor necrosis factor (TNF), interleukin-6 (IL-6), interleukin-17A (IL-17A), and interferon-gamma (IFN- γ), alongside a decrease in anti-inflammatory cytokines interleukin-10 (IL-10) and transforming growth factor-beta (TGF- β). This imbalance fostered chronic inflammation in the colon and a specific T_{H17} -type T-cell response. Of note, the *H. pylori*-positive cohort also exhibited alterations in microbiome composition, with increased abundance of mucus-degrading bacteria such as *Akkermansia muciniphila* and certain *Bacteroides* species. This “mucus-degrading microbiota signature” compromises the intestinal mucus barrier and results in greater exposure of the colonic epithelium to luminal antigens and pathogenic bacteria, promoting inflammation and potential DNA damage. These findings position *H. pylori* as a potentially modifiable risk factor for colorectal cancer. Eradication of the bacterium, restoration of a healthy gut microbiota, and strengthening of the mucus barrier are suggested as effective strategies for prevention and therapy (Engelsberger et al., [2024](#); Ralser et al., [2023](#)).

15.3.3 pks+ *Escherichia coli* and the Genotoxin Colibactin

Another bacterial “oncotoxin” or genotoxin, colibactin, was identified nearly 20 years after the discovery of CDT (Nougayrede et al., [2006](#)). Colibactin is a secondary metabolite, a polyketide/nonribosomal

peptide hybrid compound synthesized by a complex biosynthetic machinery. This toxin is encoded by a 54-kb *pks* pathogenicity island that is expressed by several *Enterobacteriaceae* members, such as *Escherichia coli* belonging to phylogenetic group B2. *E. coli* strains belonging to group B2 are less frequent in the environment but represent 30–50% of strains isolated from the feces of healthy humans in high-income countries (Escobar-Paramo et al., [2004](#)). *E. coli* is a predominant gram-negative, facultative anaerobe that colonizes the human intestine few days after birth, and B2 phylogenetic strain has been found to be especially adapted for persisting in the gut microbiota throughout the lifetime of the host (Nowrouzian et al., [2005](#); Nowrouzian & Oswald, [2012](#)). Further prominent examples of pathogenic factors and toxins produced by *E. coli* are the Shigatoxins or verotoxins, which have been used as biotechnological tools to target colorectal cancer for diagnostic or therapeutic purposes (Ehrenfeld et al., [2022](#)).

Colibactin is produced by 19 genes (*clbA* to *clbS*) present on the *pks* genomic island (also known as *clb*). Similar to CDT, infection of epithelial cells with *pks+* *E. coli* was shown to promote cell cycle arrest and a progressive enlargement of the cell body and nucleus and cause DNA double-strand breaks. The bacterial supernatant lacks toxicity, and the cytopathic effect of this toxin is contact-dependent on live bacteria with the host cell (Nougayrede et al., [2006](#)). The elucidation of the structure of colibactin and its crosslinks to DNA can be considered a breakthrough in this area of research (Xue et al., [2019](#)). Moreover, the mechanism by which colibactin is introduced into the host cell was recently unveiled, based on analysis of two different *pks+* strains: pathogenic *E. coli* 11G5 and *E. coli* Nissle 1917. Both strains harbor the *pks* island, produce similar levels of colibactin, and exhibit nearly equal fecal abundances. However, *E. coli* Nissle 1917 causes significantly less DNA damage and tumorigenesis. The study focused on two surface proteins of *E. coli*, FimH and FmlH, both of which bind to host cell glycoproteins. Sequence analysis revealed that the two bacterial strains differed in FimH, but not FmlH. A small alteration in FimH, consisting of

three amino acid substitutions, appears to determine the genotoxic potential of *pks* + *E. coli* strains (Jans et al., [2024](#)).

clbK gene of the *pks* island codes for thiazole-forming nonribosomal peptide synthases. Thiazole rings are present in pharmacophores, such as bleomycin and are known to intercalate DNA (Nougayrede et al., [2006](#); Vizcaino & Crawford, [2015](#)). The prodrug, precolibactin, is exported into the periplasm by efflux pump *clbM* and is cleaved by the periplasmic membrane-bound *clbP* peptidase to generate the active genotoxin. It was shown that precolibactin causes in vitro DNA alkylation and interstrand cross-links, demonstrating the DNA-damaging ability of this toxin (Vizcaino & Crawford, [2015](#)). It was later reported that *pks*+ *E. coli* were found in a significantly higher percentage in inflammatory bowel disease and CRC patients. Precancerous lesions exert a positive selection for the colonization and expansion of *pks*+ *E. coli* strains that were genetically diverse (Arthur et al., [2012](#); Buc et al., [2013](#); Sarshar et al., [2017](#)). These bacteria also promoted invasive carcinoma in Il10^{-/-} mice (Arthur et al., [2012](#)). Progression of inflammation and development of CRC in the Il10^{-/-} mouse model was shown to induce nine genes in the *pks* island of *E. coli* NC101 (Muehlbauer et al., [2013](#)). These studies indicate that colitis can alter microbial composition and microbial transcriptome response and cause expansion of genotoxic microorganisms that can promote colon tumorigenesis. Inflammation is essential for *E. coli* to promote CRC in AOM-treated Il10^{-/-} mice. Inflammation alters the natural barrier function of the colon epithelium, making it more readily accessible to the bacterium (Arthur et al., [2014](#)). Short exposure of intestinal epithelial cells to *pks* + *E. coli* has been shown to cause cells to divide despite incomplete DNA damage repair. Phenotypes observed include anaphase bridges, micronuclei, aneuploidy, ring chromosomes, and chromosome aberrations. Infected cells also have an increased gene mutation frequency and grow anchorage independently, demonstrating the mutagenic and transforming potential of the toxin (Cuevas-Ramos et al., [2010](#)). By detailed analysis, colibactin was found to induce specific mutational alterations in the host, with prominent single-base

substitutions and insertion and deletion (indels) type genetic lesions (Pleguezuelos-Manzano et al., [2020](#)). A consequence of this DNA damage caused by *pks + E. coli* is the senescence and senescence-associated secretory phenotype (SASP). The prooxidant and pro-inflammatory mediators secreted by senescent cells have been shown to cause DNA damage in bystander cells and promoted the growth of human colon carcinoma cells in a paracrine manner (Secher et al., [2013](#)). Further, colibactin induced SASP results in the secretion of growth factors that promote the proliferation of naïve recipient cells. Three-hour infection of tumor cells was sufficient to stimulate tumor growth of the infected cell line in a xenograft model (Cougnoux et al., [2014](#)). Further, early intestinal colonization by *pks + E. coli* in neonatal rats impairs intestinal barrier function development, making them more vulnerable to intestinal immune-mediated diseases in adulthood (Secher et al., [2015](#)). Lastly, *pks + E. coli* may contribute to the development of chemoresistance in CRC. Conditioned media from *pks + E. coli* strains, but not from Δpks controls lacking the pathogenicity island, increased resistance to the topoisomerase inhibitor irinotecan (Dalmaso et al., [2024](#)). Upon implantation in a mouse xenograft model, human CRC cells exhibited higher resistance to irinotecan upon pretreatment before engraftment with conditioned media from *pks +* strains. Taken together, SASP induced by infection with *pks + E. coli* may promote EMT, CRC progression, and the emergence of chemoresistance (Dalmaso et al., [2024](#)).

15.3.4 *Fusobacterium nucleatum*

Fusobacterium nucleatum is an anaerobic gram-negative bacterium, which is part of the normal flora of the human oral and gut mucosa. Numerous studies have shown a predominant role of *F. nucleatum* in the neoplastic process in colon cancer. Colon biopsies from adenomas showed a greater proportion of this bacterium, when compared to the adjacent normal mucosa. The same study showed that in Apc^{Min} genetic mouse model of intestinal tumorigenesis, *F. nucleatum* increased colorectal tumorigenesis by attracting tumor-infiltrating myeloid

immune cells that can promote tumor progression. Infection of $Apc^{Min/+}$ mice also resulted in the NF- κ B pro-inflammatory signature known to facilitate colorectal tumorigenesis (Kostic et al., [2013](#); Yang et al., [2017](#)). *F. nucleatum* adhesin, FadA helps the bacterium to attach and invade cancer cells. This adhesin has been shown to play an additional role in activating β -catenin and promoting tumor growth by modulating E-cadherin upon binding to it on epithelial cells. The study also showed that FadA gene levels are higher in the colon tissue from patients with adenomas compared to those of normal individuals. FadA stimulated the growth of human colon cancer cell lines that carry either an APC mutation or a β -catenin mutation and could not stimulate the growth of a noncancerous cell line, suggesting that it promotes carcinogenesis in cells with an existing tumor-initiating somatic mutation (Rubinstein et al., [2013](#)). Another surface protein of the bacterium, Fap2, has been shown to bind to a host cell factor known to be overexpressed in human colorectal adenocarcinoma, leading to fusobacterial enrichment (Abed et al., [2016](#)). Fap2 has also been reported to facilitate impairment of host antitumor immunity. Fap2 binds and activates a receptor, TIGIT, on T cells and natural killer cells. Fap2 and TIGIT interaction leads to a reduction in the cytotoxicity of infiltrating natural killer cells and lymphocytes toward tumor cells (Gur et al., [2015](#)). Fap2 has also been shown to induce apoptosis in lymphocytes (Kaplan et al., [2005](#), [2010](#)). These studies indicate that Fap2 is utilized by tumors to impair and evade the immune system. Further, some studies have shown that *F. nucleatum* high status is associated with CpG island methylator phenotype and microsatellite instability in CRCs (Tahara et al., [2014](#)), or associated with rectal cancer (Eisele et al., [2021](#)). Recent findings also propose that *Fusobacterium* colonizes distant metastases from primary CRC. The bacterium and the associated microbiome also survived mouse xenografts of human primary colorectal adenocarcinomas and were retained through sequential xenograft passages. Treatment of these xenograft-bearing mice with metronidazole antibiotic decreased *Fusobacterium* load, cancer cell proliferation, and tumor growth (Bullman et al., [2017](#)). In addition, *F.*

nucleatum was shown to promote CRC chemoresistance by modulating autophagy (Yu et al., [2017](#)). Recently, it has been proposed that colonization of the CRC tissue is a specific ability of only a small clade of *F. nucleatum* and not a general property of this species (Zepeda-Rivera et al., [2024](#)). Interestingly, *F. nucleatum* strains from primary CRC tissue, but not the oral cavity, predominantly belong to the *F. nucleatum animalis* C2 (FnaC2) clade. Nearly 200 genetic factors were found to be linked to this specific clade, enhancing its metabolic activity and ability to colonize the gastrointestinal tract. In FnaC2-infected mice, 1,296 metabolites were detected, including a significant increase in glutathione metabolism and γ -glutamyl amino acid pathways, as compared to clade FnaC1. Notably, alterations in γ -glutamyl-cysteinyl-glycine (GSH) synthesis precursors were observed. These GSH alterations, specifically shifts in oxidized-to-reduced form ratios unique to FnaC2, heighten cellular stress, drive inflammation, oxidative damage, genomic instability, and foster tumor development (Zepeda-Rivera et al., [2024](#)). In addition, new insights discovered that *F. nucleatum* positive CRC cases are more resistant to chemotherapy, attributed to an upregulation of autophagy-related proteins via the TLR4 and MyD88 pathways, enabling stress adaptation and apoptosis evasion, ultimately complicating treatment strategies for many patients (Galasso et al., [2025](#)).

15.3.5 *Bacteroides fragilis*

Bacteroides fragilis are gram-negative, obligate anaerobes and opportunistic pathogens that comprise 1–2% of the total colon microbial community (Dejea et al., [2013](#)). Based on the absence or presence of the *bft* gene, they are classified into nontoxigenic *B. fragilis* (NTBF) and enterotoxigenic *B. fragilis* (ETBF), respectively. Enterotoxigenic *B. fragilis* (ETBF) strains are implicated in diarrheal disease, inflammatory bowel disease, and colorectal cancer (Basset et al., [2004](#); Sears, [2009](#); Toprak et al., [2006](#); Wu et al., [2009](#)). Their pathogenicity is due to a 20-kDa, heat-labile, zinc-dependent metalloprotease toxin called *B. fragilis* toxin (BFT). Binding of BFT to

intestinal epithelial cells is temperature dependent and occurs only at 37 °C and is sensitive to cholesterol depletion. Metalloprotease activity of the toxin is essential for the binding, and this binding is resistant to acid washing, suggesting an irreversible interaction (Wu et al., [2006](#)). Treatment of human colonic epithelial cell lines with BFT results in a time and concentration-dependent redistribution of actin microfilaments (F-actin), as well as an increase in cell volume. This study suggested that these changes in F-actin and cell volume by ETBF infection might lead to an alteration in tight-junction integrity in the intestinal epithelium, contributing to the pathogenesis of diarrhea (Koshy et al., [1996](#)). BFT was also found to have greater biological activity on the basolateral membrane of polarized intestinal epithelial cells (Chambers et al., [1997](#); Obiso Jr. et al., [1997](#)). Later studies revealed that *B. fragilis* alters the tight junctional function of intestinal epithelial cells by cleaving tumor suppressor E-cadherin, the cellular substrate of BFT, thereby increasing mucosal permeability and activating the β -catenin signaling (Wu et al., [1998](#), [2003](#)).

Fecal carriage of ETBF is 10–20% in the healthy population, as opposed to 40% for CRC patients (Toprak et al., [2006](#)). The *bft* gene is more abundantly found in the colonic mucosa than in luminal samples of colorectal cancer (Boleij et al., [2015](#)). Increased abundance of ETBF is seen in early-stage carcinogenic lesions, underlining its potential role in the development of colorectal neoplasia (Purcell et al., [2017](#)). Of note, patients with hereditary CRC were also found to be affected with enterotoxigenic *B. fragilis* (Dejea et al., [2018](#)). Patients affected with familial adenomatous polyposis (FAP) develop benign precursor lesions at a young age and require special medical attention. Bacterial biofilms were enriched in the colon mucosa of FAP patients, composed predominantly of *Escherichia coli* and *Bacteroides fragilis*. Moreover, bacterial genes for the secreted toxins colibactin (*clbB*) and *Bacteroides fragilis* toxin (BFT), were highly enriched in colon tissue samples from FAP patients. These findings were supported by data from mouse models, showing that tumor-prone *Apc*^{Min Δ 716} mice co-colonized with colibactin-positive *E. coli* together with ETBF showed increased

production of IL-17 and DNA damage in colonic epithelium, faster tumor onset and increased mortality, compared to animals colonized with either bacterial strain alone (Dejea et al., [2018](#)).

ETBF colonization in C57BL/6 J mice results in augmentation of dextran sodium sulfate (DSS)-induced colitis (Rabizadeh et al., [2007](#)). BTF expression is essential for ETBF to induce persistent colitis in wild-type C57BL/6 mice (Rhee et al., [2009](#)). ETBF triggers a Stat3- and T_{H17}-dependent pathway of inflammation-induced cancer in ApcMin^{+/+} mice, and IL-17- and IL-23-blocking antibodies inhibited the ETBF-induced colon tumors (Wu et al., [2009](#)). ETBF-induced inflammation is sufficient to produce microadenomas within 5 days in ApcMin^{+/+} mice, but very early clearance of ETBF with antibiotics results in decreased mucosal IL-17A expression, allowing for the regression of the disease (DeStefano Shields et al., [2016](#)). BFT-stimulated IL-8 production involves tyrosine kinase-dependent activation of nuclear factor- κ B (NF- κ B) and activation of the mitogen-activated protein kinases (MAPKs) (Wu et al., [2004](#)). BFT-induced spermine oxidase (SMO) results in increased reactive oxygen species (ROS) production and consequent DNA damage in cell lines. SMO expression was upregulated in C57BL/6 mice with ETBF-induced colitis. Inhibitors of SMO significantly reduced ETBF-induced colon tumorigenesis in ApcMin^{+/+} mice (Goodwin et al., [2011](#)). Overall, research suggests that persistent ETBF infection may perturb apical junctional function and increase pro-inflammatory responses, resulting in premalignant transdifferentiation in the colon.

In a recent study on a mouse model combining mismatch-repair deficiency with an Apc-mutation, whole-exome and whole-genome sequencing were used to shed light on the ETBF pathomechanism in cancer. However, unlike *pks + E. coli*, ETBF did not seem to lead to a specific mutational signature but significantly increased tumorigenesis (Pleguezuelos-Manzano et al., [2020](#)). The researchers hypothesized that the predominantly nongenomic mechanism of ETBF is not tumor-initiating, but rather boosts tumorigenesis on the basis of a pre-existing first genetic hit, such as an APC loss of function.

A new putative mechanism of action for ETBF was recently discovered. The G protein-coupled receptor 35 (*GPR35*) is expressed throughout the entire gastrointestinal tract and is increased in different tumors. Various genome-wide association studies (GWAS) show a clear association between *GPR35* and ulcerative colitis as well as Crohn's disease. Recently, *GPR35* was identified as a regulator in early ETBF infection. *GPR35* mediates BFT sensing on the host cell surface through its downstream signaling cascade, via stimulation of G-protein-coupled receptor (GPCR) signaling via β -arrestin. Furthermore, *GPR35*-KO mice exhibited decreased colitis and reduced mortality. This association between the BFT/*GPR35* interaction and severe inflammatory bowel disease (IBD) could suggest a correlation with inflammation-associated tumorigenesis (Boleij et al., [2021](#)).

15.4 Immunological Aspects of Carcinogenesis in the Colorectum

The interactions between the immune system and solid tumors are highly complex; immune cells can have both tumor-promoting and tumor-inhibiting roles. As early as 1909, the German physician Paul Ehrlich proposed a cancer suppressive of the immune system (Strebhardt & Ullrich, [2008](#)), and deregulation of immunosurveillance is now considered a central hallmark of cancers (Hanahan & Weinberg, [2011](#)). Solid evidence indicates high intratumoral numbers of T lymphocytes, especially of CTLs and the T_{H1} -type subset, that predict good prognosis in colorectal cancer. Furthermore, there is ample preclinical and clinical evidence for tumor-enhancing effects of inflammatory processes, involving components of the immune system, as well as epithelial and mesenchymal cells. In this context, it is important to distinguish between two distinct phenomena, “inflammation-induced carcinogenesis” and “cancer-induced inflammation,” which differ mechanistically, even though they may not occur in a mutually exclusive fashion. Inflammation-induced cancers of the large intestine are well-documented, though they represent only a

minority of all clinically diagnosed cases of colorectal cancer (1–2%) (Breynaert et al., [2008](#)). Of note, patients with long-standing, active, extended inflammatory bowel diseases, such as ulcerative colitis or Crohn's disease, have an increased cancer risk (Wijnands et al., [2021](#)). However, even though the large majority of patients develop colorectal cancer in the absence of chronic inflammatory bowel disease, the immune system and inflammatory processes may still play a decisive role. The “cancer-induced inflammation” can be apparent in the formation of ectopic lymphoid structures, which are frequently associated with colorectal cancer. The structures are heterogeneous from patient to patient, as well as within given tumors, but have been described as a positive prognostic factor (Coppola et al., [2011](#); Dieu-Nosjean et al., [2014](#); Pitzalis et al., [2014](#)). Furthermore, pro-inflammatory signaling pathways are functional in many colorectal cancers, in addition to the well-established oncogenic growth factor receptor pathways (Fre et al., [2008](#)), as evidenced clinically by the demonstrated efficacy of nonsteroidal anti-inflammatory drugs in the prevention of colorectal cancer (Lasry et al., [2016](#)). Further, recent large-scale genome and transcriptome sequencing efforts have successfully defined molecular subgroups in colorectal cancer, revealing the existence of highly immunogenic tumors with good prognosis, associated with DNA mismatch repair defects, as well as mesenchymal-type tumors with signs of inflammation and bad prognosis (Dienstmann et al., [2017](#)).

A central focus of current research is to unravel the still largely unresolved mechanisms underlying T-cell infiltration and activation in colorectal tumors, as well as the functional involvement of gut microbiota in these processes. In this section, we will address the major cell types and pathways from both the adaptive and the innate branches of the immune system that are currently known to be involved in the initiation and progression of colorectal cancer and discuss their contribution to the response toward antitumoral therapies.

15.4.1 Adaptive Immunity

As stated earlier, infiltration by immune cells is strongly linked with clinical outcome in patients with colorectal cancer (Fridman et al., [2012](#); Galon et al., [2006](#)). High densities of cytotoxic effector T cells (Galon et al., [2006](#); Pages et al., [2010](#)), as well as different subsets of CD4⁺ T-helper cells, such as T-helper type 1 cells expressing IFN- γ (Tosolini et al., [2011](#)) and follicular-type T-helper (T_{FH}) cells expressing the chemokine receptor CXCR5 (Bindea et al., [2013](#)), are associated with increased postoperative survival. Tissue-resident *Lachnospiraceae*, including *Ruminococcus gnavus*, *Blautia* sp., and *Dorea*, which are enriched in normal compared to tumor tissue, can inhibit intestinal tumor growth in mice through degradation of lysoglycerophospholipids, thus supporting the activation of CD8⁺ T cells (Zhang et al., [2023](#)). In contrast, *Fusobacterium* spp. inhibit CD8⁺ T cell-dependent immunity through succinate production and inhibition of CD8⁺ T cell trafficking into the tumor microenvironment (Jiang et al., [2023](#)). Integrated tumor, immune, and microbiome analysis in human CRC supports these findings and revealed an association between a low microbiome risk score (characterized by enrichment, for example, of *Ruminococcus* species), antigen-presenting CD103⁺ dendritic cells, an immunogenic environment, and a favorable prognosis (Roelands et al., [2023](#)).

In addition to CD8⁺ T cells, CD4⁺ T_{FH} cells can contribute to anti-tumor immunity. As such, recent work demonstrated that microbiota-specific T_{FH} cells promote anti-tumor immunity in CRC models in mice through the formation of tertiary lymphoid structures (Overacre-Delgoffe et al., [2021](#)). Furthermore, ileal *B. fragilis* and *Erysipelotrichaceae* were shown to regulate T_{FH} cells and chemotherapy-induced immune responses in an IL1R1- and IL-12-dependent manner (Roberti et al., [2020](#)). In accordance with the positive role of T-helper cells, expression of HLA class II antigens was shown to be correlated with a favorable clinical outcome (Sconocchia et al., [2014](#)). However, the prognostic significance and contribution of interleukin (IL)-17-producing T-helper cells (T_{H17}) is being

controversially discussed: intratumoral infiltration of T_{H17}-type cells was associated either with worse or improved prognosis (Amicarella et al., [2017](#); Tosolini et al., [2011](#)). Recent work showed that formate derived from *Fusobacterium* spp. can support CRC progression in a T_{H17}-dependent manner (Ternes et al., [2022](#)). Somewhat surprisingly, and in contrast to observations for other solid tumor entities (Pere et al., [2012](#)), immunosuppressive Foxp3+ regulatory T cells (T_{regs}) were associated with good prognosis in colorectal cancer (Frey et al., [2010](#); Salama et al., [2009](#)). The group of J. Galon from Paris, France, has been able to establish infiltrating T-cell subsets as prognostic factors with high clinical relevance over the past decades (Galon et al., [2006](#), [2007](#); Pages et al., [2005](#), [2010](#)). Of note, increased tumor infiltration by immune cells and high expression of specific T_{H1}-type transcripts were significantly correlated with the absence of histopathological parameters of early metastatic invasion (venous, lymphatic, and perineural invasion). Importantly, the density of tumor-infiltrating T lymphocytes (TILs), which can be quantified in a standardized way by immunohistochemical staining for the cell surface marker CD3 on tissue sections, had stronger predictive power for patients' survival than the well-established clinicopathological markers, such as the tumor-node-metastasis (TNM) classification system (Galon et al., [2006](#)). The TNM system represents the current clinical standard in colorectal cancer and is accepted worldwide (UICC/AJCC), even though it has been shown to have significant limitations (Nitsche et al., [2011](#)). Upon statistical multivariate regression analysis, the TIL density was retained as an independent prognostic parameter, in contrast to the TNM stage, after adjustment to T-cell infiltration. The quantification of tumor-infiltrating CD3-positive cells further provided an impressive parameter for patient stratification. Patients with a high TIL density had a postoperative 5-year survival rate of 73%, whereas the subgroup with low T-cell density had a dismal outcome with a 5-year survival rate of only 30% (Galon et al., [2006](#)). Among the tested T-cell subsets, CD8-positive memory T cells with the specific surface marker CD45RO had

the highest prognostic impact, regarding both postoperative overall survival and disease-free survival.

Based on these impressive results, a scoring system based on histological evaluation has been proposed for colorectal as well as other solid cancers, called the “immunoscore” (Galon et al., [2014](#)). This score takes into account the relative density of TILs in the center of the tumor, as well as in the invasive margin. As the most important cell types for the score, CD45RO-/CD8-positive cells are retained. The subgroup of patients with a high density of memory CD8 T cells both in the center of the tumor and the invasive margin is associated with good prognosis, and the immunoscore is more strongly associated with disease relapse compared to the TNM system (Mlecnik et al., [2011](#)). However, despite great efforts, the quantification of TILs in colorectal cancer has still not entered routine clinical practice, since the immune infiltrate is highly heterogeneous, with great interpatient as well as intratumoral differences, and the quantification of TILs is still far from being standardized.

15.4.2 Chemokine Networks Linking Gut Microbiota and Tumor-Infiltrating Immune Cells

Colorectal carcinogenesis is a complex process influenced by genetic and environmental factors, notably inflammation, immune regulation, metabolism of dietary components, and genotoxin production—all closely linked to the gut microbiota. Chronic inflammation is a central characteristic and risk factor for colorectal cancer (CRC); patients with chronically active, extensive inflammatory bowel diseases such as ulcerative colitis and Crohn’s disease have an increased risk of developing CRC. The gut microbiota interacts with the immune system and influences inflammatory processes. For instance, transferring stool samples from CRC patients to mice led to heightened inflammation and expression of inflammatory genes (Wong et al., [2017](#)). Microorganisms can induce chemotactic factors that recruit T cells into tumors. Expression of chemokines, secreted immune mediators that chemotactically recruit T cells, was reported to correlate with high

densities of tumor-infiltrating lymphocytes (TILs) and predicted a favorable clinical outcome (Hojo et al., [2007](#); Mlecnik et al., [2010](#)). In accordance, interferon-regulated CXC chemokines are excellent predictors of survival in colorectal cancer (Kistner et al., [2017](#)). Increased intratumoral expression of CXCL9 and CXCL11 was significantly correlated with postoperative tumor-specific survival in patients with colon cancer; both parameters were retained as independent prognostic indicators upon multivariate analysis (Kistner et al., [2017](#)). Of note, the three chemokines CXCL9, CXCL10, and CXCL11 share one common receptor, CXCR3, which is expressed on T_{H1} T cells and CTLs and induces their chemotactic recruitment (Groom & Luster, [2011](#)). Further chemokines of the CXC family were found to be strongly deregulated in colorectal cancer, CXCL3 (GRO3) being associated with metastasis formation and CXCL8 (Interleukin-8) with postoperative survival (Doll et al., [2010](#)). Analysis of colorectal cancer cell lines and genetic mouse models for digestive tract cancer, as well as an ex vivo explant culture model based on resected human colon tumors, demonstrated that colorectal cancer cells produce significant amounts of CXCL11 and CXCL10 upon cytokine stimulation with IFN- γ or TNF. On the tissue level, immunohistochemical analysis revealed that tumor cells are a major source of CXCL11 (Kistner et al., [2017](#)). Moreover, high intratumoral chemokine co-expression of CXCL9, CXCL10, and CXCL11 was highly significantly correlated with the presence of CTLs and CD4+ T-helper cells within the tumor. In order to test the hypothesis that elevated CXC chemokines have a causal effect on tumorigenesis, an orthotopic mouse colorectal cancer model was established based on the isogenic murine colorectal carcinoma cells, stably expressing CXCL10. The in vivo model demonstrated that tumor growth was inhibited by intratumoral expression of CXCL10, largely mediated by the adaptive immune system (Kistner et al., [2017](#)). Thus, CXC chemokines are promising potential targets for therapeutic intervention. Moreover, their expression predicts good response to neoadjuvant radiochemotherapy in rectal cancer, as shown by analysis of three

independent patient collectives from Germany, Italy, and the USA (Agostini et al., [2015](#)).

Of note, chemokine sources and microbial-derived stimuli leading to altered chemokine production within intestinal tissue remain largely unknown. Cell-autonomous genomic changes within colorectal cancer cells have been described, which may lead to amplification or conversely loss of function of chemokine genes (Bindea et al., [2013](#)). In addition to genomic alterations, epigenetic silencing of T-helper type 1 chemokines through the polycomb repressive complex 2 has been described in colorectal cancer (Nagarsheth et al., [2016](#)). In addition to genetic and epigenetic effects, the gut microbiota play a pivotal role for chemokine secretion. There is ample evidence suggesting that gut commensal bacteria translocate across a dysfunctional epithelial barrier, stimulating the recruitment of immune cells in the lamina propria, which in turn secrete pro-inflammatory cytokines (Grivennikov et al., [2012](#)). Gut-derived microbial stimuli indeed induce the production of chemotactic factors, as well as the chemokine signaling network underlying and shaping T-cell infiltration into colorectal cancer (Cremonesi et al., [2018](#)). Previous studies showed that gut commensal bacteria translocated across the neoplastic epithelium may interact with tumor cells and induce direct protumorigenic effects or by secretion of cancer-promoting cytokines (Arthur et al., [2012](#); Grivennikov et al., [2012](#); Peucker et al., [2016](#); Rubinstein et al., [2013](#)). Of note, upon stimulation by gut commensal bacteria in vitro and in vivo, colorectal cancer cell lines showed a strongly upregulated expression of multiple chemokines, recapitulating the profiles of chemokine gene expression of primary cancer cell isolates. Interestingly, exposure of tumor cells to gut bacteria ultimately results in higher T-cell recruitment in vivo in cancer xenografts, revealing a role of gut commensal bacteria in controlling the extent of tumor infiltration by beneficial immune cells (Cremonesi et al., [2018](#)). Consistent with findings obtained in mouse models, the extent of T-cell infiltration in human colorectal cancer was significantly associated with the presence of specific bacterial families and genera. Furthermore, the

abundance of defined bacterial families was shown to be linked with the expression of specific chemokines, indicating that gut commensal bacteria induce the intratumoral production of specific immune cell-recruiting chemokines. Importantly, the composition of gut flora significantly predicted postoperative survival of patients with colorectal cancer. Even though much remains to be learned about the interactions between gut microbiota. A central unresolved question remains whether specific individual bacterial species or strains or rather complex microbial communities, contribute to altered chemokine expression patterns and immune cell infiltration in human cancer. However, recent data show that different bacterial species may promote the expression of T-cell-recruiting chemokine genes. Ex vivo analysis of human samples showed that Firmicutes and in particular Lachnospiraceae and Ruminococcaceae were most significantly associated with the expression of T-cell-attracting factors. The abundance of *Bacteroides*, *Proteobacteria*, and, in particular, *Reyranella* was associated with the expression of most T-cell-recruiting chemokines, as well as with intratumoral densities of all T-cell subsets and furthermore with a favorable disease outcome (Cremonesi et al., [2018](#)). Moreover, defined bacteria types were associated with the concomitant expression of several key chemokines, indicating their capacity to promote parallel recruitment of different T-cell populations, like CTLs and helper cells (T_{H1}-type and CXCR5-positive), observed in cases with good prognosis. Of note, *Fusobacteria* species were recently reported to be associated with reduced survival rate for colorectal cancer patients (Mima et al., [2016](#)), and were found in our study to be enriched in the subgroup of patients with low TIL density, and could evoke expression of T-cell-recruiting chemokines upon coculture with human colon cancer cell lines (Cremonesi et al., [2018](#)). It has been described that *F. nucleatum* inhibits the functions of T cells and NK cells by the inhibitory receptor TIGIT (Gur et al., [2015](#)). In contrast, a recent report demonstrated a link between the abundance of *Fusobacteria* species and increased expression of the cytokines IL-12 and TGF- β , shifting the T-cell population toward reduced Foxp3 expression, the

hallmark transcription factor of immune-inhibitory T_{regs}, and was correlated with good prognosis (Saito et al., [2016](#)). Moreover, *F. nucleatum* and *K. pneumoniae* were shown to induce the production of cytokines in cancer cells, which attract macrophages of the detrimental M2 subtype, and intratumoral enrichment of *F. nucleatum* in CRC was found to be associated with enrichment of immunosuppressive myeloid cells (Chiang et al., [2021](#); Galeano Nino et al., [2022](#)). Taken together, caution is still warranted, since different strains of a given bacterial species can be functionally different (Fig. [15.1](#)).

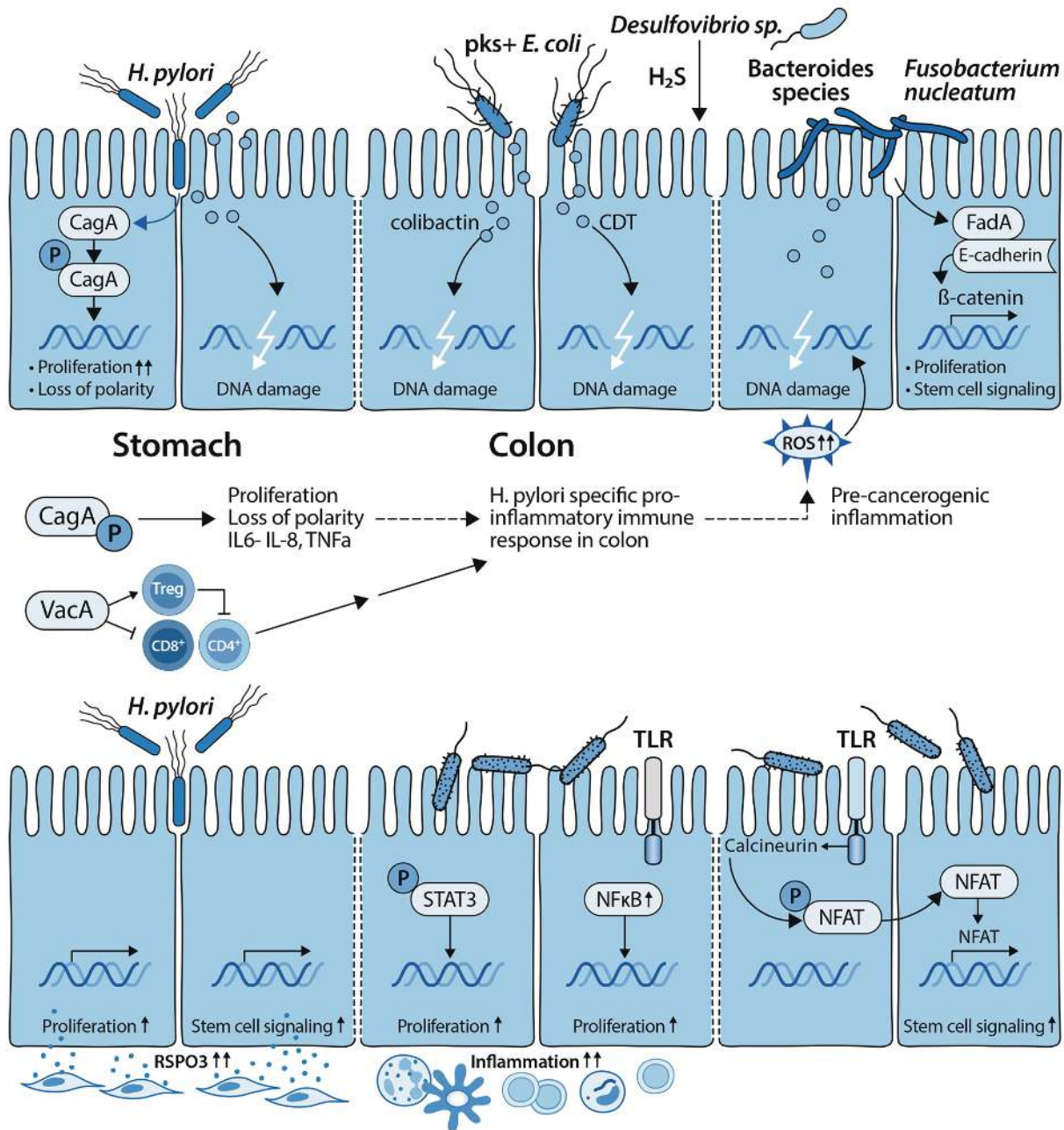


Fig. 15.1 Bacteria associated with colorectal cancer. Bacterial species associated with colorectal cancer covered in this article are visualized, and intracellular events resulting in an increased proliferation or DNA damage are highlighted

15.4.3 Innate Immunity

In addition to T cells, cell types of the innate immune system were shown to affect the prognosis and outcome of patients with colorectal cancer. Certain bacterial species might induce the recruitment of

immune cells other than T cells, such as neutrophils or natural killer cells, which can impact prognosis. In fact, infiltration of natural killer cells (NK), as well as of natural killer T cells (NKT), was associated with good prognosis in colorectal cancer (Coca et al., [1997](#); Sandel et al., [2005](#); Tachibana et al., [2005](#)). The role of macrophages in the context of cancer, however, is ambiguous (Balkwill & Mantovani, [2012](#)), and it is under debate whether tumor-associated macrophages actually constitute suitable targets for therapeutic intervention (Alahari et al., [2015](#)). Similarly, results on the prognostic role of neutrophils in CRC are controversial, with both favorable and unfavorable effects described (Droeser et al., [2013](#); Governa et al., [2017](#); Roelands et al., [2023](#); Sconocchia et al., [2011](#)). Further, an interplay between different innate cell types has been reported, since intratumoral neutrophils or natural killer cells were reported to increase the positive prognostic significance of cytotoxic T cells (Sconocchia et al., [2014](#)). Since there is even greater functional plasticity in the myeloid cell lineage, it is not surprising that multiple reciprocal interactions between macrophages and T cells have been described (Biswas & Mantovani, [2010](#)). In a simplified view, tumor-associated macrophages (TAMs) can be assigned to two subgroups, defined by surface markers, functional aspects, and their contribution to tumorigenesis: where M1 TAMs are thought to play a more tumor-suppressive role by enhancing adaptive immunity and by producing pro-inflammatory cytokines like IL-6 and TNF, whereas M2 TAMs have a tumor-promoting effect and secrete immune-inhibitory factors like IL-10 and TGF- β (Biswas & Mantovani, [2010](#)). In general, an increased density of intratumoral macrophages was found to be correlated with favorable clinical outcome (Edin et al., [2013](#)). Interestingly, recent work demonstrated that both the host and the microbiota contribute to increased urea levels in CRC, which skew macrophages toward a pro-tumoral phenotype characterized by the accumulation of polyamines (Chen et al., [2023](#)). Another innate immune cell type negatively affecting anti-tumor immunity are myeloid-derived suppressor cells (MDSCs). MDSCs can be distinguished into monocytic and granulocytic subtypes and have associated with low

levels of anti-tumor immunity in CRC and poor prognosis. (Al Zein et al., [2023](#)).

In addition to specialized innate immune cells, many inflammatory pathways have been described to be fully expressed and functional in intestinal epithelia, as well as in colorectal cancer cells (Abreu, [2010](#)). However, the molecular cross talk between colorectal cancer cells and gut microbiota remains to be elucidated. Enterocytes, as well as colonocytes, are capable of sensing gut bacteria through pattern recognition receptors, including Toll-like receptors (Abreu, [2010](#)). In addition to TLRs, the cytoplasmic NOD (nucleotide-binding and oligomerization domain) and NOD-like receptors have a critical role as pattern recognition receptors (Creagh & O'Neill, [2006](#)). Our data suggested that bacterial-induced expression of cytokines and chemokines may be initiated by Toll-like receptor-dependent signals on primary carcinoma cells from surgical explants (Kistner et al., [2017](#)). Furthermore, in vitro stimulation with TLR agonists resulted in marked induction of chemokine expression in established colorectal cancer cell lines from mice and humans. In vivo, Toll-like receptor signaling is required for both tumor initiation and progression from adenoma to carcinoma stage, as data on genetic mouse models with tissue-specific expression of the TLR-signaling adapter MyD88 have shown (Holtorf et al., [2018](#)).

Bacterial sensing not only by CRC cells but also by myeloid cells, contributes to tumor growth through cell-intrinsic activation of the calcineurin-NFAT axis. Myeloid-specific deletion of calcineurin in a genetic cancer mouse model activated protective CD8-positive T-cell responses and inhibited CRC growth (Peuker et al., [2022](#)). Hence, a microbiota-, calcineurin-, and B7H3/B7H4-dependent pathway controls anti-tumor immunity in microsatellite-stable CRC (Peuker et al., [2022](#)).

However, further studies are required to reveal which TLRs, in concert with other pattern recognition receptors, are engaged by individual bacterial species associated with colorectal cancer. In conclusion, carcinoma cells can be regarded as a major chemokine

source in colorectal cancer and likely in other solid tumor entities. Gut microbiota have a pivotal role in triggering chemokine production, which induces infiltration of T cells from blood vessels into the tumor tissue, ultimately resulting in the development of a stable antitumoral adaptive immune response and improved patient prognosis. These findings are summarized in Fig. 15.2. The recent advances in the field further our understanding of the complex interactions between gut microbiota, cancer cells, and the immune system and may enable the development of innovative treatment strategies that alter the gut flora to cause infiltration of tumor tissues by immune cell populations of favorable prognostic significance.

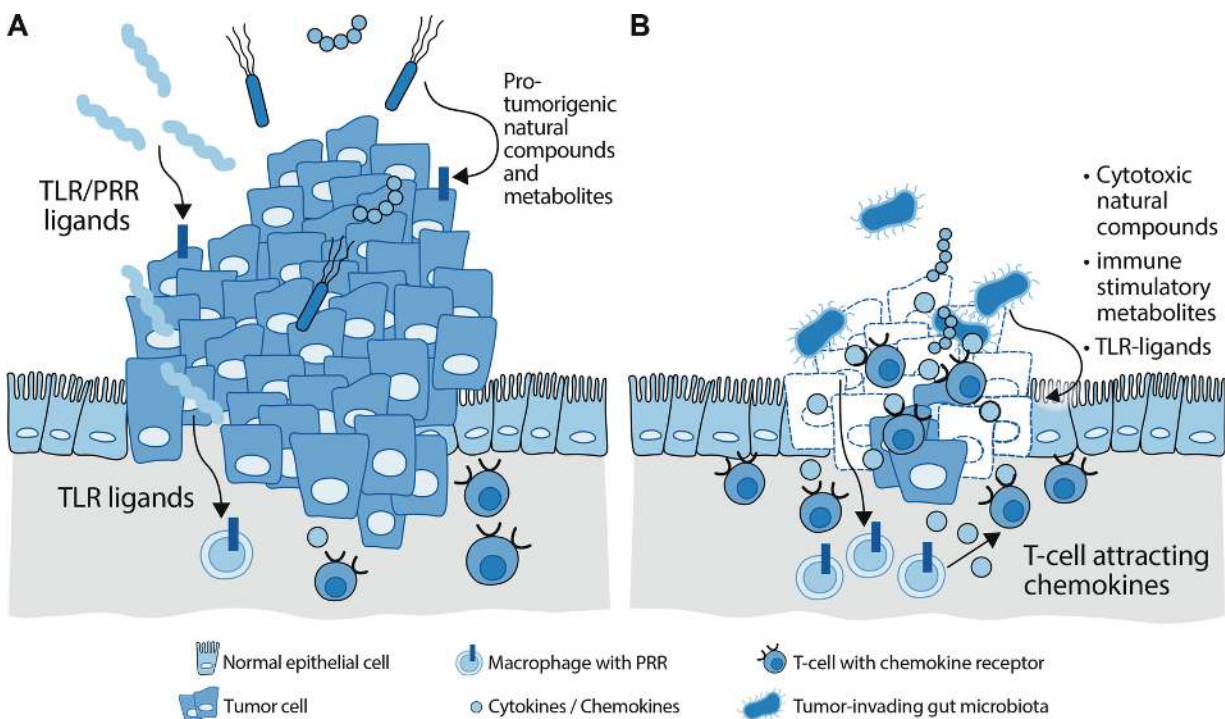


Fig. 15.2 (a) Specific gut microbiota are associated with bad prognosis and low T-cell infiltration. Microbial ligands engage TLRs and other pattern recognition receptors (PRRs) on carcinoma cells (enhancing cell survival) and immune cells, inhibiting their function. (b) In contrast, differing microbial communities are associated with favorable prognosis and high T-cell infiltration. Here, microbial signals trigger production of T-cell-attracting CXC chemokines by innate immune cells such as macrophages, leading to high densities of CTLs and T_H1 cells, with effective antitumoral immune responses

15.5 Conclusions

Several members of the gut microbiota have the potential to interfere with the epithelial cell integrity. In addition to the association between certain microbiota composition and carcinogenesis, molecular mechanisms of how bacteria induce DNA damage in epithelial cells or induce pro-carcinogenic signaling have been proposed and demonstrated. While these effects are observed in vitro, it is important to demonstrate that they also occur in vivo and indeed contribute to carcinogenesis. In addition to direct effects of bacteria on the epithelium, the microbiota is associated with differential immune states and this partly explains interindividual differences in the density of TILs, in antitumoral immune responses, and lastly in disease outcome as well as response to multimodal therapy, either chemo-radiotherapy, targeted approaches or immune therapy.

Controversy Today, there is a growing interest in the impact of gut microbial communities on the initiation and progression of CRC. CRC was originally considered to be a strictly genetic disease, but recent studies reveal that the microbiota is an important contributing factor to this malignancy. A disturbance in the microbial community and expansion of pathogenic bacteria leads to inflammation, alterations in cellular microenvironment, contributing to precancerous and ultimately malignant tumors. Microbial dysbiosis observed in cancer can be a consequence of certain microorganisms gaining a competitive advantage in the tumor microenvironment or be an active contributor to the pathology. 16S rRNA sequencing and, more recently, whole-genome shotgun sequencing have revealed the presence of lower bacterial diversity and a higher abundance of certain protumorigenic bacteria in the intestinal microbiota of CRC patients (Ahn et al., [2013](#); Flemer et al., [2017](#); Vogtmann et al., [2016](#)). Spatial aspects of the interaction of bacteria with tumor cells, as well as intratumoral heterogeneity, remain still poorly understood. A current area of controversial discussion is the actual presence (or absence) of intracellular bacteria in cancer cells, reported recently (Nejman et al., [2020](#)), but technically challenging to prove. Therefore,

cautious interpretation and appropriate controls are crucial in microbiome research, especially in the case of low-biomass sequencing approaches in cancer samples (Gihawi et al., [2023](#)). Further, epidemiological evidence strongly suggests the involvement of specific microorganisms with certain gastrointestinal malignancies. Some of these bacteria and their encoded genotoxins have been shown to have the ability to directly damage the host cell DNA or interfere with signals that control epithelial proliferation and differentiation. Interdisciplinary studies involving epidemiologists, molecular biologists, and clinicians are required to demonstrate that the processes described in animal models indeed occur in humans and drive malignant transformation. Moreover, the role of gut microbiota in the stunning failure of immune checkpoint inhibition trials in the large majority of CRC cases remains unexplained. Finally, the development of intervention strategies targeting gut microbiota for prevention or therapy of CRC is required. The effectiveness of such strategies would provide the most convincing proof of a causative role of the microbiota in CRC development.

Highlights

- Colorectal cancer (CRC) is a genetic disease, but most cases occur sporadically. Characteristic changes in the microbiota of patients with CRC suggest a causative role of the microbiota in CRC development.
- Transfer of microbiota from humans with CRC to mice increases the risk of colon tumor development compared to mice that receive microbiota from healthy individuals.
- Various bacteria that were considered commensals are now known to induce DNA damage and interfere with epithelial stem cell signaling and proliferation, indicating their potential to be pro-carcinogenic.
- Defined bacterial families are significantly associated with the intratumoral expression of specific immune cell-recruiting chemokines.

- The individual gut microbiome in patients with colorectal cancer acts in concert with genetic alterations in cancer cells to determine the extent of immune cell infiltration, with pivotal effects on disease outcome.

References

Abed, J., Emgard, J. E., Zamir, G., Faroja, M., Almogy, G., Grenov, A., et al. (2016). Fap2 mediates *Fusobacterium nucleatum* colorectal adenocarcinoma enrichment by binding to tumor-expressed Gal-GalNAc. *Cell Host and Microbe*, 20, 215–225.

[\[PubMed\]](#)

Abreu, M. T. (2010). Toll-like receptor signalling in the intestinal epithelium: How bacterial recognition shapes intestinal function. *Nature Reviews Immunology*, 10, 131–144.

[\[PubMed\]](#)

Agostini, M., Janssen, K. P., Kim, I. J., D'Angelo, E., Pizzini, S., Zangrando, A., et al. (2015). An integrative approach for the identification of prognostic and predictive biomarkers in rectal cancer. *Oncotarget*, 6, 32561–32574.

[\[PubMed\]](#)

Ahn, J., Sinha, R., Pei, Z., Dominianni, C., Wu, J., Shi, J., et al. (2013). Human gut microbiome and risk for colorectal cancer. *Journal of the National Cancer Institute*, 105, 1907–1911.

[\[PubMed\]](#)

Al Zein, M., Boukhdoud, M., Shammaa, H., Mouslem, H., El Ayoubi, L. M., Iratni, R., et al. (2023). Immunotherapy and immunoevasion of colorectal cancer. *Drug Discovery Today*, 28, 103669.

[\[PubMed\]](#)

Alahari, S. V., Dong, S., & Alahari, S. K. (2015). Are macrophages in tumors good targets for novel therapeutic approaches? *Molecules and Cells*, 38, 95–104.

[\[PubMed\]](#)

Amicarella, F., Muraro, M. G., Hirt, C., Cremonesi, E., Padovan, E., Mele, V., et al. (2017). Dual role of tumour-infiltrating T helper 17 cells in human colorectal cancer. *Gut*, 66, 692–704.

[\[PubMed\]](#)

Amieva, M. R., & El-Omar, E. M. (2008). Host-bacterial interactions in *Helicobacter pylori* infection. *Gastroenterology*, 134, 306–323.

[\[PubMed\]](#)

Amieva, M. R., Vogelmann, R., Covacci, A., Tompkins, L. S., Nelson, W. J., & Falkow, S. (2003). Disruption of the epithelial apical-junctional complex by *Helicobacter pylori* CagA. *Science*, 300, 1430–1434.

[\[PubMed\]](#)

Andre, N., Shiu, K. K., Kim, T. W., Jensen, B. V., Jensen, L. H., Punt, C., et al. (2020). Pembrolizumab in microsatellite-instability–high advanced colorectal Cancer. *The New England Journal of Medicine*, 383, 2207–2218.

[\[PubMed\]](#)

Arthur, J. C., Perez-Chanona, E., Muhlbauer, M., Tomkovich, S., Uronis, J. M., Fan, T. J., et al. (2012). Intestinal inflammation targets cancer-inducing activity of the microbiota. *Science*, 338, 120–123.

[\[PubMed\]](#)

Arthur, J. C., Gharaibeh, R. Z., Muhlbauer, M., Perez-Chanona, E., Uronis, J. M., McCafferty, J., et al. (2014). Microbial genomic analysis reveals the essential role of inflammation in bacteria-induced colorectal cancer. *Nature Communications*, 5, 4724.

[\[PubMed\]](#)

Bagnoli, F., Buti, L., Tompkins, L., Covacci, A., & Amieva, M. R. (2005). Helicobacter pylori CagA induces a transition from polarized to invasive phenotypes in MDCK cells. *Proceedings of the National Academy of Sciences of the United States of America*, 102, 16339–16344.

[\[PubMed\]](#)

Balkwill, F. R., & Mantovani, A. (2012). Cancer-related inflammation: Common themes and therapeutic opportunities. *Seminars in Cancer Biology*, 22, 33–40.

[\[PubMed\]](#)

Basset, C., Holton, J., Bazeos, A., Vaira, D., & Bloom, S. (2004). Are Helicobacter species and enterotoxigenic Bacteroides fragilis involved in inflammatory bowel disease? *Digestive Diseases and Sciences*, 49, 1425–1432.

[\[PubMed\]](#)

Belcheva, A., Irrazabal, T., Robertson, S. J., Streutker, C., Maughan, H., Rubino, S., et al. (2014). Gut microbial metabolism drives transformation of msh2-deficient colon epithelial cells. *Cell*, 158, 288–299.

[\[PubMed\]](#)

Bindea, G., Mlecnik, B., Tosolini, M., Kirilovsky, A., Waldner, M., Obenauf, A. C., et al. (2013). Spatiotemporal dynamics of intratumoral immune cells reveal the immune landscape in human cancer. *Immunity*, 39, 782–795.

[\[PubMed\]](#)

Biswas, S. K., & Mantovani, A. (2010). Macrophage plasticity and interaction with lymphocyte subsets: Cancer as a paradigm. *Nature Immunology*, 11, 889–896.

[\[PubMed\]](#)

Boleij, A., Hechenbleikner, E. M., Goodwin, A. C., Badani, R., Stein, E. M., Lazarev, M. G., et al. (2015). The Bacteroides fragilis toxin gene is prevalent in the colon mucosa of colorectal cancer patients. *Clinical Infectious Diseases*, 60, 208–215.

[\[PubMed\]](#)

Boleij, A., Fathi, P., Dalton, W., Park, B., Wu, X., Huso, D., et al. (2021). G-protein coupled receptor 35

(GPR35) regulates the colonic epithelial cell response to enterotoxigenic *Bacteroides fragilis*. *Communications Biology*, 4, 585.

[\[PubMed\]](#)

Boustany, A., Onwuzo, S., Almomani, A., & Asaad, I. (2023). Epidemiology and risk of colorectal cancer in patients with a history of *Helicobacter pylori* infection: A population-based study. *Annals of Gastroenterology*, 36, 203–207.

[\[PubMed\]](#)

Bray, F., Laversanne, M., Sung, H., Ferlay, J., Siegel, R. L., Soerjomatara, I., et al. (2024). Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: a Cancer Journal for Clinicians*, 74, 229–263.

[\[PubMed\]](#)

Breynaert, C., Vermeire, S., Rutgeerts, P., & Van Assche, G. (2008). Dysplasia and colorectal cancer in inflammatory bowel disease: A result of inflammation or an intrinsic risk? *Acta Gastroenterologica Belgica*, 71, 367–372.

Buc, E., Dubois, D., Sauvanet, P., Raisch, J., Delmas, J., Darfeuille-Michaud, A., et al. (2013). High prevalence of mucosa-associated *E. coli* producing cyclomodulin and genotoxin in colon cancer. *PLoS One*, 8, e56964.

[\[PubMed\]](#)

Bullman, S., Peadarallu, C. S., Sicinska, E., Clancy, T. E., Zhang, X., Cai, D., et al. (2017). Analysis of *Fusobacterium* persistence and antibiotic response in colorectal cancer. *Science*, 358, 1443–1448.

[\[PubMed\]](#)

Chambers, F. G., Koshy, S. S., Saidi, R. F., Clark, D. P., Moore, R. D., & Sears, C. L. (1997). *Bacteroides fragilis* toxin exhibits polar activity on monolayers of human intestinal epithelial cells (T84 cells) in vitro. *Infection and Immunity*, 65, 3561–3570.

[\[PubMed\]](#)

Chen, H., Tong, T., Lu, S. Y., Ji, L., Xuan, B., Zhao, G., et al. (2023). Urea cycle activation triggered by host-microbiota maladaptation driving colorectal tumorigenesis. *Cell Metabolism*, 35, 651–666.

[\[PubMed\]](#)

Chiang, M. K., Hsiao, P. Y., Liu, Y. Y., Tang, H. L., Chiou, C. S., Lu, M. C., et al. (2021). Two ST11 *Klebsiella pneumoniae* strains exacerbate colorectal tumorigenesis in a colitis-associated mouse model. *Gut Microbes*, 13, 1980348.

[\[PubMed\]](#)

Coca, S., Perez-Piqueras, J., Martinez, D., Colmenarejo, A., Saez, M. A., Vallejo, C., et al. (1997). The prognostic significance of intratumoral natural killer cells in patients with colorectal carcinoma. *Cancer*, 79, 2320–2328.

[\[PubMed\]](#)

Coleman, O. I., Lobner, E. M., Bierwirth, S., Sorbie, A., Waldschmitt, N., Rath, E., et al. (2018). Activated ATF6 induces intestinal dysbiosis and innate immune response to promote colorectal tumorigenesis. *Gastroenterology*, 155, 1539–1552.e12.

[\[PubMed\]](#)

Coppola, D., Nebozhyn, M., Khalil, F., Dai, H., Yeatman, T., Loboda, A., et al. (2011). Unique ectopic lymph node-like structures present in human primary colorectal carcinoma are identified by immune gene array profiling. *The American Journal of Pathology*, 179, 37–45.

[\[PubMed\]](#)

Cougnoux, A., Dalmaso, G., Martinez, R., Buc, E., Delmas, J., Gibold, L., et al. (2014). Bacterial genotoxin colibactin promotes colon tumour growth by inducing a senescence-associated secretory phenotype. *Gut*, 63, 1932–1942.

[\[PubMed\]](#)

Creagh, E. M., & O'Neill, L. A. (2006). TLRs, NLRs and RLRs: A trinity of pathogen sensors that co-operate in innate immunity. *Trends in Immunology*, 27, 352–357.

[\[PubMed\]](#)

Cremonesi, E., Governa, V., Garzon, J. F. G., Mele, V., Amicarella, F., Muraro, M. G., et al. (2018). Gut microbiota modulate T cell trafficking into human colorectal cancer. *Gut*, 67, 1984–1994.

[\[PubMed\]](#)

Cuevas-Ramos, G., Petit, C. R., Marcq, I., Boury, M., Oswald, E., & Nougayrede, J. P. (2010). *Escherichia coli* induces DNA damage in vivo and triggers genomic instability in mammalian cells. *Proceedings of the National Academy of Sciences of the United States of America*, 107, 11537–11542.

[\[PubMed\]](#)

Dalmaso, G., Cougnoux, A., Faïs, T., Bonnin, V., Mottet-Auselo, B., Nguyen, H. T., et al. (2024). Colibactin-producing *Escherichia coli* enhance resistance to chemotherapeutic drugs by promoting epithelial to mesenchymal transition and cancer stem cell emergence. *Gut Microbes*, 16, 2310215.

[\[PubMed\]](#)

Dejea, C. M., Wick, E., & Sears, C. L. (2013). Bacterial oncogenesis in the colon. *Future Microbiology*, 8, 445–460.

[\[PubMed\]](#)

Dejea, C. M., Wick, E. C., Hechenbleikner, E. M., White, J. R., Mark Welch, J. L., Rossetti, B. J., et al. (2014). Microbiota organization is a distinct feature of proximal colorectal cancers. *Proceedings of the National Academy of Sciences of the United States of America*, 111, 18321–18326.

[\[PubMed\]](#)

Dejea, C. M., Fathi, P., Craig, J. M., Boleij, A., Taddese, R., Geis, A. L., et al. (2018). Patients with familial adenomatous polyposis harbor colonic biofilms containing tumorigenic bacteria. *Science*, 359, 592–597.

[\[PubMed\]](#)

Del Bel Belluz, L., Guidi, R., Pateras, I. S., Levi, L., Mihaljevic, B., Rouf, S. F., et al. (2016). The typhoid toxin promotes host survival and the establishment of a persistent asymptomatic infection. *PLoS Pathogens*, 12, e1005528.

[\[PubMed\]](#)

Deng, H., Muthupalani, S., Erdman, S., Liu, H., Niu, Z., Wang, T. C., et al. (2022). Translocation of *Helicobacter hepaticus* synergizes with myeloid-derived suppressor cells and contributes to breast carcinogenesis. *Oncoimmunology*, 11, 2057399.

[\[PubMed\]](#)

DeStefano Shields, C. E., Van Meerbeke, S. W., Housseau, F., Wang, H., Huso, D. L., Casero, R. A., Jr., et al. (2016). Reduction of murine colon tumorigenesis driven by enterotoxigenic *Bacteroides fragilis* using cefoxitin treatment. *The Journal of Infectious Diseases*, 214, 122–129.

[\[PubMed\]](#)

Dienstmann, R., Vermeulen, L., Guinney, J., Kopetz, S., Tejpar, S., & Tabernero, J. (2017). Consensus molecular subtypes and the evolution of precision medicine in colorectal cancer. *Nature Reviews Cancer*, 17, 268.

[\[PubMed\]](#)

Dieu-Nosjean, M. C., Goc, J., Giraldo, N. A., Sautes-Fridman, C., & Fridman, W. H. (2014). Tertiary lymphoid structures in cancer and beyond. *Trends in Immunology*, 35, 571–580.

[\[PubMed\]](#)

Doll, D., Keller, L., Maak, M., Boulesteix, A. L., Siewert, J. R., Holzmann, B., et al. (2010). Differential expression of the chemokines GRO-2, GRO-3, and interleukin-8 in colon cancer and their impact on metastatic disease and survival. *International Journal of Colorectal Disease*, 25, 573–581.

[\[PubMed\]](#)

Dove, W. F., Clipson, L., Gould, K. A., Luongo, C., Marshall, D. J., Moser, A. R., et al. (1997). Intestinal neoplasia in the ApcMin mouse: Independence from the microbial and natural killer (beige locus) status. *Cancer Research*, 57, 812–814.

[\[PubMed\]](#)

Droeser, R. A., Hirt, C., Eppenberger-Castori, S., Zlobec, I., Viehl, C. T., Frey, D. M., et al. (2013). High myeloperoxidase positive cell infiltration in colorectal cancer is an independent favorable prognostic factor. *PLoS One*, 8, e64814.

[\[PubMed\]](#)

Duijster, J. W., Hansen, J. V., Franz, E., Neefjes, J. J. C., Frisch, M., Mughini-Gras, L., et al. (2021). Association between *Salmonella* infection and colon cancer: A nationwide registry-based cohort study. *Epidemiology & Infection*, 149, e56.

Ecker, J., Benedetti, E., Kindt, A. S. D., Höring, M., Perl, M., Machmüller, A. C., et al. (2021). The colorectal cancer lipidome – Identification of a robust tumor-specific lipid species signature. *Gastroenterology*, 161, 910–923.

[\[PubMed\]](#)

Ecker, J., Liebisch, G., & Janssen, K. P. (2022). Reply to: ‘The colorectal cancer lipidome: Is there any difference of lipid species between right and left colorectal cancers? *Gastroenterology*, 162, 658–659.

[\[PubMed\]](#)

Edin, S., Wikberg, M. L., Rutegard, J., Oldenborg, P. A., & Palmqvist, R. (2013). Phenotypic skewing of macrophages in vitro by secreted factors from colorectal cancer cells. *PLoS One*, 8, e74982. [\[PubMed\]](#)

Ehrenfeld, M., Schrade, A., Flisikowska, T., Perl, M., Hirsch, N. D., Sichler, A., et al. (2022). Tumor targeting with bacterial Shiga toxin B-subunit in genetic porcine models for colorectal cancer and osteosarcoma. *Molecular Cancer Therapeutics*, 21(4), 686–699. [\[PubMed\]](#)

Eisele, Y., Mallea, P. M., Gigic, B., Stephens, W. Z., Warby, C. A., Buhrke, K., et al. (2021). *Fusobacterium nucleatum* and Clinicopathologic Features of Colorectal cancer: Results from the ColoCare Study. *Clinical Colorectal Cancer*, 20(3), e165–e172. [\[PubMed\]](#)

Engelsberger, V., Gerhard, M., & Mejias-Luque, R. (2024). Effects of *Helicobacter pylori* infection on intestinal microbiota, immunity and colorectal cancer risk. *Frontiers in Cellular and Infection Microbiology*, 14, 1339750. [\[PubMed\]](#)

Erdman, S. E., Poutahidis, T., Tomczak, M., Rogers, A. B., Cormier, K., Plank, B., et al. (2003). CD4+ CD25+ regulatory T lymphocytes inhibit microbially induced colon cancer in Rag2-deficient mice. *The American Journal of Pathology*, 162, 691–702. [\[PubMed\]](#)

Escobar-Paramo, P., Grenet, K., Le Menac'h, A., Rode, L., Salgado, E., Amorin, C., et al. (2004). Large-scale population structure of human commensal *Escherichia coli* isolates. *Applied and Environmental Microbiology*, 70, 5698–5700. [\[PubMed\]](#)

Fearon, E. R., & Vogelstein, B. (1990). A genetic model for colorectal tumorigenesis. *Cell*, 61, 759–767. [\[PubMed\]](#)

Feng, Q., Liang, S., Jia, H., Stadlmayr, A., Tang, L., Lan, Z., et al. (2015). Gut microbiome development along the colorectal adenoma-carcinoma sequence. *Nature Communications*, 6, 6528. [\[PubMed\]](#)

Flemer, B., Lynch, D. B., Brown, J. M., Jeffery, I. B., Ryan, F. J., Claesson, M. J., et al. (2017). Tumour-associated and non-tumour-associated microbiota in colorectal cancer. *Gut*, 66, 633–643. [\[PubMed\]](#)

Fre, S., Vignjevic, D., Schoumacher, M., Duffy, S. L., Janssen, K.-P., Robine, S., et al. (2008). Epithelial morphogenesis and intestinal cancer: New insights in signaling mechanisms. In F. V. W. George & K. George (Eds.), *Advances in cancer research* (pp. 85–111). Academic.

Frey, D. M., Droeser, R. A., Viehl, C. T., Zlobec, I., Lugli, A., Zingg, U., et al. (2010). High frequency of tumor-infiltrating FOXP3(+) regulatory T cells predicts improved survival in mismatch repair-proficient colorectal cancer patients. *International Journal of Cancer*, 126, 2635–2643. [\[PubMed\]](#)

Fridman, W. H., Pages, F., Sautes-Fridman, C., & Galon, J. (2012). The immune contexture in human tumours: Impact on clinical outcome. *Nature Reviews Cancer*, 12, 298–306.
[\[PubMed\]](#)

Galasso, L., Termite, F., Mignini, I., Esposto, G., Borriello, R., Vitale, F., et al. (2025). Unraveling the role of *Fusobacterium nucleatum* in colorectal Cancer: molecular mechanisms and pathogenic insights. *Cancers*, 17, 368.
[\[PubMed\]](#)

Galeano Nino, J. L., Hanrui, W., LaCourse, H., Kempchinsky, K. D., Baryiames, A. G., Barber, A. B., et al. (2022). Effect of the intratumoral microbiota on spatial and cellular heterogeneity in cancer. *Nature*, 611, 810–817.
[\[https://doi.org/10.1038/s41586-022-05435-0\]](https://doi.org/10.1038/s41586-022-05435-0)[\[PubMed\]](#)

Galon, J., Costes, A., Sanchez-Cabo, F., Kirilovsky, A., Mlecnik, B., Lagorce-Pages, C., et al. (2006). Type, density, and location of immune cells within human colorectal tumors predict clinical outcome. *Science*, 313, 1960–1964.
[\[PubMed\]](#)

Galon, J., Fridman, W. H., & Pages, F. (2007). The adaptive immunologic microenvironment in colorectal cancer: A novel perspective. *Cancer Research*, 67, 1883–1886.
[\[PubMed\]](#)

Galon, J., Mlecnik, B., Bindea, G., Angell, H. K., Berger, A., Lagorce, C., et al. (2014). Towards the introduction of the ‘Immunoscore’ in the classification of malignant tumours. *The Journal of Pathology*, 232, 199–209.
[\[PubMed\]](#)

Ge, Z., Feng, Y., Whary, M. T., Nambiar, P. R., Xu, S., Ng, V., et al. (2005). Cytolethal distending toxin is essential for *Helicobacter hepaticus* colonization in outbred Swiss Webster mice. *Infection and Immunity*, 73, 3559–3567.
[\[PubMed\]](#)

Ge, Z., Rogers, A. B., Feng, Y., Lee, A., Xu, S., Taylor, N. S., et al. (2007). Bacterial cytolethal distending toxin promotes the development of dysplasia in a model of microbially induced hepatocarcinogenesis. *Cellular Microbiology*, 9, 2070–2080.
[\[PubMed\]](#)

Ge, Z., Feng, Y., Ge, L., Parry, N., Muthupalani, S., & Fox, J. G. (2017). *Helicobacter hepaticus* cytolethal distending toxin promotes intestinal carcinogenesis in 129Rag2-deficient mice. *Cellular Microbiology*, 19(7), e12728.

Gihawi, A., Cooper, C. S., & Brewer, D. S. (2023). Caution regarding the specificities of pan-cancer microbial structure. *Microbial Genomics*, 9, mgen001088.
[\[PubMed\]](#)

Gonzalez-Escobedo, G., & Gunn, J. S. (2013). Gallbladder epithelium as a niche for chronic *Salmonella* carriage. *Infection and Immunity*, 81, 2920–2930.
[\[PubMed\]](#)

Goodwin, A. C., Destefano Shields, C. E., Wu, S., Huso, D. L., Wu, X., Murray-Stewart, T. R., et al. (2011). Polyamine catabolism contributes to enterotoxigenic *Bacteroides fragilis*-induced colon tumorigenesis. *Proceedings of the National Academy of Sciences of the United States of America*, *108*, 15354–15359.

[\[PubMed\]](#)

Governa, V., Trella, E., Mele, V., Tornillo, L., Amicarella, F., Cremonesi, E., et al. (2017). The interplay between neutrophils and CD8(+) T cells improves survival in human colorectal cancer. *Clinical Cancer Research*, *23*, 3847–3858.

[\[PubMed\]](#)

Grivennikov, S. I., Wang, K., Mucida, D., Stewart, C. A., Schnabl, B., Jauch, D., et al. (2012). Adenoma-linked barrier defects and microbial products drive IL-23/IL-17-mediated tumour growth. *Nature*, *491*, 254–258.

[\[https://doi.org/10.1038/nature11465\]](https://doi.org/10.1038/nature11465) [\[PubMed\]](#)

Groom, J. R., & Luster, A. D. (2011). CXCR3 in T cell function. *Experimental Cell Research*, *317*, 620–631.

[\[PubMed\]](#)

Guidi, R., Guerra, L., Levi, L., Stenerlow, B., Fox, J. G., Josenhans, C., et al. (2013a). Chronic exposure to the cytolethal distending toxins of Gram-negative bacteria promotes genomic instability and altered DNA damage response. *Cellular Microbiology*, *15*, 98–113.

[\[PubMed\]](#)

Guidi, R., Levi, L., Rouf, S. F., Puia, S., Rhen, M., & Frisan, T. (2013b). Salmonella enterica delivers its genotoxin through outer membrane vesicles secreted from infected cells. *Cellular Microbiology*, *15*, 2034–2050.

[\[PubMed\]](#)

Gur, C., Ibrahim, Y., Isaacson, B., Yamin, R., Abed, J., Gamliel, M., et al. (2015). Binding of the Fap2 protein of *Fusobacterium nucleatum* to human inhibitory receptor TIGIT protects tumors from immune cell attack. *Immunity*, *42*, 344–355.

[\[PubMed\]](#)

Haghjoo, E., & Galan, J. E. (2004). Salmonella typhi encodes a functional cytolethal distending toxin that is delivered into host cells by a bacterial-internalization pathway. *Proceedings of the National Academy of Sciences of the United States of America*, *101*, 4614–4619.

[\[PubMed\]](#)

Hanahan, D., & Weinberg, R. A. (2011). Hallmarks of cancer: The next generation. *Cell*, *144*, 646–674.

[\[PubMed\]](#)

He, Z., Yu, J., Gong, J., Wu, J., Xuan, X., Luo, Z., et al. (2024). Campylobacter jejuni-derived cytolethal distending toxin promotes colorectal cancer metastasis. *Cell Host and Microbe*, *32*, 2080–2091.e6.

[\[PubMed\]](#)

Hojjo, S., Koizumi, K., Tsuneyama, K., Arita, Y., Cui, Z., Shinohara, K., et al. (2007). High-level

expression of chemokine CXCL16 by tumor cells correlates with a good prognosis and increased tumor-infiltrating lymphocytes in colorectal cancer. *Cancer Research*, 67, 4725–4731.

[\[PubMed\]](#)

Holtorf, A., Conrad, A., Holzmann, B., & Janssen, K. P. (2018). Cell-type specific MyD88 signaling is required for intestinal tumor initiation and progression to malignancy. *Oncoimmunology*, 7, e1466770.

[\[PubMed\]](#)

Jans, M., Kolata, M., Blancke, G., D'Hondt, A., Gräf, C., Ciers, M., et al. (2024). Colibactin-driven colon cancer requires adhesin-mediated epithelial binding. *Nature*, 635, 472–480.

[\[https://doi.org/10.1038/s41586-024-08135-z\]](https://doi.org/10.1038/s41586-024-08135-z) [\[PubMed\]](#)

Jiang, S. S., Xie, Y. L., Xiao, X. Y., Kang, Z. R., Lin, X. L., Zhang, L., et al. (2023). *Fusobacterium nucleatum*-derived succinic acid induces tumor resistance to immunotherapy in colorectal cancer. *Cell Host and Microbe*, 31, 781–797.

[\[PubMed\]](#)

Johnson, C. M., Wie, C., Ensor, J. E., Smolenski, D. J., Amos, C. I., Levin, B., et al. (2013). Meta-analyses of colorectal cancer risk factors. *Cancer Causes & Control*, 24, 1207–1222.

Johnson, C. H., Dejea, C. M., Edler, D., Hoang, L. T., Santidrian, A. F., Felding, B. H., et al. (2015). Metabolism links bacterial biofilms and colon carcinogenesis. *Cell Metabolism*, 21, 891–897.

[\[PubMed\]](#)

Kaplan, C. W., Lux, R., Huynh, T., Jewett, A., Shi, W., & Haake, S. K. (2005). *Fusobacterium nucleatum* apoptosis-inducing outer membrane protein. *Journal of Dental Research*, 84, 700–704.

[\[PubMed\]](#)

Kaplan, C. W., Ma, X., Paranjpe, A., Jewett, A., Lux, R., Kinder-Haake, S., et al. (2010). *Fusobacterium nucleatum* outer membrane proteins Fap2 and RadD induce cell death in human lymphocytes. *Infection and Immunity*, 78, 4773–4778.

[\[PubMed\]](#)

Kindt, A., Liebisch, G., Clavel, T., Haller, D., Hörmannspurger, G., Yoon, H., et al. (2018). The gut microbiota promotes hepatic fatty acid desaturation and elongation in mice. *Nature Communications*, 9, 3760.

[\[PubMed\]](#)

Kistner, L., Doll, D., Holtorf, A., Nitsche, U., & Janssen, K. P. (2017). Interferon-inducible CXC-chemokines are crucial immune modulators and survival predictors in colorectal cancer. *Oncotarget*, 8, 89998–90012.

[\[PubMed\]](#)

Koeppel, M., Garcia-Alcalde, F., Glowinski, F., Schlaermann, P., & Meyer, T. F. (2015). *Helicobacter pylori* infection causes characteristic DNA damage patterns in human cells. *Cell Reports*, 11, 1703–1713.

[\[PubMed\]](#)

Koshy, S. S., Montrose, M. H., & Sears, C. L. (1996). Human intestinal epithelial cells swell and demonstrate actin rearrangement in response to the metalloprotease toxin of *Bacteroides fragilis*. *Infection and Immunity*, 64, 5022–5028.

[\[PubMed\]](#)

Kostic, A. D., Chun, E., Robertson, L., Glickman, J. N., Gallini, C. A., Michaud, M., et al. (2013). *Fusobacterium nucleatum* potentiates intestinal tumorigenesis and modulates the tumor-immune microenvironment. *Cell Host and Microbe*, 14, 207–215.

[\[PubMed\]](#)

Lara-Tejero, M., & Galan, J. E. (2000). A bacterial toxin that controls cell cycle progression as a deoxyribonuclease I-like protein. *Science*, 290, 354–357.

[\[PubMed\]](#)

Lara-Tejero, M., & Galan, J. E. (2001). CdtA, CdtB, and CdtC form a tripartite complex that is required for cytolethal distending toxin activity. *Infection and Immunity*, 69, 4358–4365.

[\[PubMed\]](#)

Lasry, A., Zinger, A., & Ben-Neriah, Y. (2016). Inflammatory networks underlying colorectal cancer. *Nature Immunology*, 17, 230–240.

[\[PubMed\]](#)

Li, Y., Kundu, P., Seow, S. W., de Matos, C. T., Aronsson, L., Chin, K. C., et al. (2012). Gut microbiota accelerate tumor growth via c-jun and STAT3 phosphorylation in APCMin/+ mice. *Carcinogenesis*, 33, 1231–1238.

[\[PubMed\]](#)

Lu, R., Wu, S., Zhang, Y. G., Xia, Y., Zhou, Z., Kato, I., et al. (2016). Salmonella protein AvrA activates the STAT3 signaling pathway in colon cancer. *Neoplasia*, 18, 307–316.

[\[PubMed\]](#)

Lu, R., Bosland, M., Xia, Y., Zhang, Y. G., Kato, I., & Sun, J. (2017). Presence of Salmonella AvrA in colorectal tumor and its precursor lesions in mouse intestine and human specimens. *Oncotarget*, 8, 55104–55115.

[\[PubMed\]](#)

Lui, R. N., Tsoi, K. K. F., Ho, J. M. W., Lo, C. M., Chan, F. C. H., Kyaw, M. H., et al. (2019). Global increasing incidence of young-onset colorectal cancer across 5 continents: A joinpoint regression analysis of 1,922,167 cases. *Cancer Epidemiology, Biomarkers & Prevention*, 28, 1275–1282.

Maloy, K. J., Salaun, L., Cahill, R., Dougan, G., Saunders, N. J., & Powrie, F. (2003). CD4+CD25+ T(R) cells suppress innate immune pathology through cytokine-dependent mechanisms. *The Journal of Experimental Medicine*, 197, 111–119.

[\[PubMed\]](#)

Mima, K., Nishihara, R., Qian, Z. R., Cao, Y., Sukawa, Y., Nowak, J. A., et al. (2016). *Fusobacterium nucleatum* in colorectal carcinoma tissue and patient prognosis. *Gut*, 65, 1973–1980.

[\[PubMed\]](#)

Mlecnik, B., Tosolini, M., Charoentong, P., Kirilovsky, A., Bindea, G., Berger, A., et al. (2010). Biomolecular network reconstruction identifies T-cell homing factors associated with survival in colorectal cancer. *Gastroenterology*, 138, 1429–1440.

[\[PubMed\]](#)

Mlecnik, B., Tosolini, M., Kirilovsky, A., Berger, A., Bindea, G., Meatchi, T., et al. (2011). Histopathologic-based prognostic factors of colorectal cancers are associated with the state of the local immune reaction. *Journal of Clinical Oncology*, 29, 610–618.

[\[PubMed\]](#)

Morey, P., Pfannkuch, L., Pang, E., Boccellato, F., Sigal, M., Imai-Matsushima, A., Dyer, V., Koch, M., Mollenkopf, H. J., Schlaermann, P., et al. (2018). Helicobacter pylori depletes cholesterol in gastric glands to prevent interferon gamma signaling and escape the inflammatory response. *Gastroenterology*, 154, 1391–1404 e1399.

[\[PubMed\]](#)

Muehlbauer, M., Gharaibeh, R., Arthur, J. C., Fodor, A., & Jobin, C. (2013). 825 intestinal inflammation targets E. coli NC101 transcriptome response and promotes development of colorectal cancer (CRC). *Gastroenterology*, 144, S-144–S-145.

Mughini-Gras, L., Schaapveld, M., Kramers, J., Mooij, S., Neefjes-Borst, E. A., Pelt, W. V., et al. (2018). Increased colon cancer risk after severe Salmonella infection. *PLoS One*, 13, e0189721.

[\[PubMed\]](#)

Nagamine, C. M., Rogers, A. B., Fox, J. G., & Schauer, D. B. (2008). Helicobacter hepaticus promotes azoxymethane-initiated colon tumorigenesis in BALB/c-IL10-deficient mice. *International Journal of Cancer*, 122, 832–838.

[\[PubMed\]](#)

Nagaraja, V., & Eslick, G. D. (2014). Systematic review with meta-analysis: The relationship between chronic Salmonella typhi carrier status and gall-bladder cancer. *Alimentary Pharmacology & Therapeutics*, 39, 745–750.

Nagarsheth, N., Peng, D., Kryczek, I., Wu, K., Li, W., Zhao, E., et al. (2016). PRC2 epigenetically silences Th1-type chemokines to suppress effector T-cell trafficking in colon cancer. *Cancer Research*, 76, 275–282.

[\[PubMed\]](#)

Nejman, D., Livyatan, I., Fuks, G., Gavert, N., Zwang, Y., Geller, L. T., et al. (2020). The human tumor microbiome is composed of tumor type-specific intracellular bacteria. *Science*, 368, 973–980.

[\[PubMed\]](#)

Nesic, D., Hsu, Y., & Stebbins, C. E. (2004). Assembly and function of a bacterial genotoxin. *Nature*, 429, 429–433.

[\[https://doi.org/10.1038/nature02532\]](https://doi.org/10.1038/nature02532) [\[PubMed\]](#)

Nguyen, L. H., Ma, W., Wang, D. D., Cao, Y., Mallick, H., Gerbaba, T. K., et al. (2020). Association between sulfur-metabolizing bacterial communities in stool and risk of distal colorectal cancer in men. *Gastroenterology*, 158, 1313–1325.

[\[PubMed\]](#)

Nitsche, U., Maak, M., Schuster, T., Kunzli, B., Langer, R., Slotta-Huspenina, J., et al. (2011). Prediction of prognosis is not improved by the seventh and latest edition of the TNM classification for colorectal cancer in a single-center collective. *Annals of Surgery*, 254, 793–800. discussion 800–791.

[\[PubMed\]](#)

Nougayrede, J. P., Homburg, S., Taieb, F., Boury, M., Brzuszkiewicz, E., Gottschalk, G., et al. (2006). *Escherichia coli* induces DNA double-strand breaks in eukaryotic cells. *Science*, 313, 848–851.

[\[PubMed\]](#)

Nowrouzian, F. L., & Oswald, E. (2012). *Escherichia coli* strains with the capacity for long-term persistence in the bowel microbiota carry the potentially genotoxic pks island. *Microbial Pathogenesis*, 53, 180–182.

[\[PubMed\]](#)

Nowrouzian, F. L., Wold, A. E., & Adlerberth, I. (2005). *Escherichia coli* strains belonging to phylogenetic group B2 have superior capacity to persist in the intestinal microflora of infants. *The Journal of Infectious Diseases*, 191, 1078–1083.

[\[PubMed\]](#)

Obiso, R. J., Jr., Azghani, A. O., & Wilkins, T. D. (1997). The *Bacteroides fragilis* toxin fragilysin disrupts the paracellular barrier of epithelial cells. *Infection and Immunity*, 65, 1431–1439.

[\[PubMed\]](#)

Overacre-Delgoffe, A. E., Bumgarner, H. J., Cillo, A. R., Burr, A. H. P., Tometich, J. T., Bhattacharjee, A., et al. (2021). Microbiota-specific T follicular helper cells drive tertiary lymphoid structures and anti-tumor immunity against colorectal cancer. *Immunity*, 54, 2812–2824.

[\[PubMed\]](#)

Pages, F., Berger, A., Camus, M., Sanchez-Cabo, F., Costes, A., Molitor, R., et al. (2005). Effector memory T cells, early metastasis, and survival in colorectal cancer. *The New England Journal of Medicine*, 353, 2654–2666.

[\[PubMed\]](#)

Pages, F., Galon, J., Dieu-Nosjean, M. C., Tartour, E., Sautès-Fridman, C., & Fridman, W. H. (2010). Immune infiltration in human tumors: A prognostic factor that should not be ignored. *Oncogene*, 29, 1093–1102.

[\[https://doi.org/10.1038/onc.2009.416\]](https://doi.org/10.1038/onc.2009.416) [\[PubMed\]](#)

Parsonnet, J., Friedman, G. D., Vandersteen, D. P., Chang, Y., Vogelstein, J. H., Orentreich, N., et al. (1991). *Helicobacter pylori* infection and the risk of gastric carcinoma. *The New England Journal of Medicine*, 325, 1127–1131.

[\[PubMed\]](#)

Pere, H., Tanchot, C., Bayry, J., Terme, M., Taieb, J., Badoual, C., et al. (2012). Comprehensive analysis of current approaches to inhibit regulatory T cells in cancer. *Oncoimmunology*, 1, 326–333.

[\[PubMed\]](#)

Peuker, K., Muff, S., Wang, J., Künzel, S., Bosse, E., Zeissig, Y., et al. (2016). Epithelial calcineurin controls microbiota-dependent intestinal tumor development. *Nature Medicine*, 22, 506–515. [\[PubMed\]](#)

Peuker, K., Strigli, A., Tauriello, D. V. F., Hendricks, A., von Schönfels, W., Burmeister, G., et al. (2022). Microbiota-dependent activation of the myeloid calcineurin-NFAT pathway inhibits B7H3- and B7H4-dependent anti-tumor immunity in colorectal cancer. *Immunity*, 55, 701–717. [\[PubMed\]](#)

Pitzalis, C., Jones, G. W., Bombardieri, M., & Jones, S. A. (2014). Ectopic lymphoid-like structures in infection, cancer and autoimmunity. *Nature Reviews Immunology*, 14, 447–462. [\[PubMed\]](#)

Pleguezuelos-Manzano, C., Puschhof, J., Rosendahl Huber, A., van Hoeck, A., Wood, H. M., Nomburg, J., et al. (2020). Mutational signature in colorectal cancer caused by genotoxic pks+ *E. coli*. *Nature*, 580, 269–273. [\[https://doi.org/10.1038/s41586-020-2080-8\]](https://doi.org/10.1038/s41586-020-2080-8) [\[PubMed\]](#)

Powell, S. M., Zilz, N., Beazer-Barclay, Y., Bryan, T. M., Hamilton, S. R., Thibodeau, S. N., et al. (1992). APC mutations occur early during colorectal tumorigenesis. *Nature*, 359, 235–237. [\[https://doi.org/10.1038/359235a0\]](https://doi.org/10.1038/359235a0) [\[PubMed\]](#)

Pratt, J. S., Sachen, K. L., Wood, H. D., Eaton, K. A., & Young, V. B. (2006). Modulation of host immune responses by the cytolethal distending toxin of *Helicobacter hepaticus*. *Infection and Immunity*, 74, 4496–4504. [\[PubMed\]](#)

Purcell, R. V., Pearson, J., Aitchison, A., Dixon, L., Frizelle, F. A., & Keenan, J. I. (2017). Colonization with enterotoxigenic *Bacteroides fragilis* is associated with early-stage colorectal neoplasia. *PLoS One*, 12, e0171602. [\[PubMed\]](#)

Rabizadeh, S., Rhee, K. J., Wu, S., Huso, D., Gan, C. M., Golub, J. E., et al. (2007). Enterotoxigenic *bacteroides fragilis*: A potential instigator of colitis. *Inflammatory Bowel Diseases*, 13, 1475–1483. [\[PubMed\]](#)

Ralser, A., Dietl, A., Jarosch, S., Engelsberger, V., Wanisch, A., Janssen, K. P., et al. (2023). *Helicobacter pylori* promotes colorectal carcinogenesis by deregulating intestinal immunity and inducing a mucus-degrading microbiota signature. *Gut*, 72, 1258–1270. [\[PubMed\]](#)

Reddy, B. S., Narisawa, T., Wright, P., Vukusich, D., Weisburger, J. H., & Wynder, E. L. (1975). Colon carcinogenesis with azoxymethane and dimethylhydrazine in germ-free rats. *Cancer Research*, 35, 287–290. [\[PubMed\]](#)

Rhee, K. J., Wu, S., Wu, X., Huso, D. L., Karim, B., Franco, A. A., et al. (2009). Induction of persistent colitis by a human commensal, enterotoxigenic *Bacteroides fragilis*, in wild-type C57BL/6 mice. *Infection and Immunity*, 77, 1708–1718.

[\[PubMed\]](#)

Roberti, M. P., Yonekura, S., Duong, C. P. M., Picard, M., Ferrere, G., Tidjani Alou, M., et al. (2020). Chemotherapy-induced ileal crypt apoptosis and the ileal microbiome shape immunosurveillance and prognosis of proximal colon cancer. *Nature Medicine*, 26, 919–931.

[\[PubMed\]](#)

Roelands, J., Kuppen, P. J. K., Ahmed, E. I., Mall, R., Masoodi, T., Singh, P., et al. (2023). An integrated tumor, immune and microbiome atlas of colon cancer. *Nature Medicine*, 29, 1273–1286.

[\[PubMed\]](#)

Rubinstein, M. R., Wang, X., Liu, W., Hao, Y., Cai, G., & Han, Y. W. (2013). *Fusobacterium nucleatum* promotes colorectal carcinogenesis by modulating E-cadherin/beta-catenin signaling via its FadA adhesin. *Cell Host and Microbe*, 14, 195–206.

[\[PubMed\]](#)

Saito, T., Nishikawa, H., Wada, H., Nagano, Y., Sugiyama, D., Atarashi, K., et al. (2016). Two FOXP3(+)CD4(+) T cell subpopulations distinctly control the prognosis of colorectal cancers. *Nature Medicine*, 22, 679–684.

[\[PubMed\]](#)

Salama, P., Phillips, M., Grieu, F., Morris, M., Zeps, N., Joseph, D., et al. (2009). Tumor-infiltrating FOXP3+ T regulatory cells show strong prognostic significance in colorectal cancer. *Journal of Clinical Oncology*, 27, 186–192.

[\[PubMed\]](#)

Salcedo, S. P., & Holden, D. W. (2003). SseG, a virulence protein that targets Salmonella to the Golgi network. *The EMBO Journal*, 22, 5003–5014.

[\[PubMed\]](#)

Sandel, M. H., Speetjens, F. M., Menon, A. G., Albertsson, P. A., Basse, P. H., Hokland, M., et al. (2005). Natural killer cells infiltrating colorectal cancer and MHC class I expression. *Molecular Immunology*, 42, 541–546.

[\[PubMed\]](#)

Sarshar, M., Scribano, D., Marazzato, M., Ambrosi, C., Aprea, M. R., Aleandri, M., et al. (2017). Genetic diversity, phylogroup distribution and virulence gene profile of pks positive *Escherichia coli* colonizing human intestinal polyps. *Microbial Pathogenesis*, 112, 274–278.

[\[PubMed\]](#)

Schult, D., Maurer, H. C., Frolova, M., Ringelhan, M., Mayr, U., Ulrich, J., et al. (2024). Systematic evaluation of clinical, nutritional, and fecal microbial factors for their association with colorectal polyps. *Clinical and Translational Gastroenterology*, 15, e00660.

[\[PubMed\]](#)

Sconocchia, G., Zlobec, I., Lugli, A., Calabrese, D., Iezzi, G., Karamitopoulou, E., et al. (2011). Tumor infiltration by FcγRIII (CD16)+ myeloid cells is associated with improved survival in patients with colorectal carcinoma. *International Journal of Cancer*, 128, 2663–2672.

[\[PubMed\]](#)

Sconocchia, G., Eppenberger-Castori, S., Zlobec, I., Karamitopoulou, E., Arriga, R., Coppola, A., et al. (2014). HLA class II antigen expression in colorectal carcinoma tumors as a favorable prognostic marker. *Neoplasia*, 16, 31–42.

[\[PubMed\]](#)

Sears, C. L. (2009). Enterotoxigenic *bacteroides fragilis*: A rogue among symbiotes. *Clinical Microbiology Reviews*, 22, 349–369.

[\[PubMed\]](#)

Secher, T., Samba-Louaka, A., Oswald, E., & Nougayrede, J. P. (2013). Escherichia coli producing colibactin triggers premature and transmissible senescence in mammalian cells. *PLoS One*, 8, e77157.

[\[PubMed\]](#)

Secher, T., Payros, D., Brehin, C., Boury, M., Watrin, C., Gillet, M., et al. (2015). Oral tolerance failure upon neonatal gut colonization with Escherichia coli producing the genotoxin colibactin. *Infection and Immunity*, 83, 2420–2429.

[\[PubMed\]](#)

Sellon, R. K., Tonkonogy, S., Schultz, M., Dieleman, L. A., Grenther, W., Balish, E., et al. (1998). Resident enteric bacteria are necessary for development of spontaneous colitis and immune system activation in interleukin-10-deficient mice. *Infection and Immunity*, 66, 5224–5231.

[\[PubMed\]](#)

Siegel, R. L., Kratzer, T. B., Giaquinto, A. N., Sung, H., & Jemal, A. (2025). Cancer statistics, 2025. *CA: a Cancer Journal for Clinicians*, 75, 10–45.

[\[PubMed\]](#)

Sigal, M., Rothenberg, M. E., Logan, C. Y., Lee, J. Y., Honaker, R. W., Cooper, R. L., et al. (2015). Helicobacter pylori activates and expands Lgr5(+) stem cells through direct colonization of the gastric glands. *Gastroenterology*, 148, 1392–1404 e1321.

[\[PubMed\]](#)

Sigal, M., Logan, C. Y., Kapalczynska, M., Mollenkopf, H. J., Berger, H., Wiedenmann, B., et al. (2017). Stromal R-spondin orchestrates gastric epithelial stem cells and gland homeostasis. *Nature*, 548, 451–455.

[\[https://doi.org/10.1038/nature23642\]](https://doi.org/10.1038/nature23642) [\[PubMed\]](#)

Simon, G. L., & Gorbach, S. L. (1984). Intestinal flora in health and disease. *Gastroenterology*, 86, 174–193.

[\[PubMed\]](#)

Son, J. S., Khair, S., Pettet, D. W., 3rd, Ouyang, N., Tian, X., Zhang, Y., et al. (2015). Altered interactions between the gut microbiome and colonic mucosa precede polyposis in APCMin/+ mice. *PLoS One*, 10, e0127985.

[\[PubMed\]](#)

Song, J., Gao, X., & Galan, J. E. (2013). Structure and function of the Salmonella Typhi chimaeric A(2)B(5) typhoid toxin. *Nature*, 499, 350–354.

[\[https://doi.org/10.1038/nature12377\]](https://doi.org/10.1038/nature12377) [\[PubMed\]](#)

Spano, S., Ugalde, J. E., & Galan, J. E. (2008). Delivery of a Salmonella Typhi exotoxin from a host intracellular compartment. *Cell Host & Microbe*, 3, 30–38.

Strebhardt, K., & Ullrich, A. (2008). Paul Ehrlich's magic bullet concept: 100 years of progress. *Nature Reviews Cancer*, 8, 473–480.
[\[PubMed\]](#)

Sung, H., Siegel, R. L., Laversanne, M., Jiang, C., Morgan, E., Zahwe, M., et al. (2025). Colorectal cancer incidence trends in younger versus older adults: An analysis of population-based cancer registry data. *The Lancet Oncology*, 26, 51–63.
[\[PubMed\]](#)

Tachibana, T., Onodera, H., Tsuruyama, T., Mori, A., Nagayama, S., Hiai, H., et al. (2005). Increased intratumor Valpha24-positive natural killer T cells: A prognostic factor for primary colorectal carcinomas. *Clinical Cancer Research*, 11, 7322–7327.
[\[PubMed\]](#)

Tahara, T., Yamamoto, E., Suzuki, H., Maruyama, R., Chung, W., Garriga, J., et al. (2014). Fusobacterium in colonic flora and molecular features of colorectal carcinoma. *Cancer Research*, 74, 1311–1318.
[\[PubMed\]](#)

Ternes, D., Tsenkova, M., Pozdeev, V. I., Meyers, M., Koncina, E., Atatri, S., et al. (2022). The gut microbial metabolite formate exacerbates colorectal cancer progression. *Nature Metabolism*, 4, 458–475.
[\[PubMed\]](#)

Thakur, B. K., Malaise, Y., Choudhury, S. R., Neustaeter, A., Turpin, W., Streutker, C., et al. (2025). Dietary fibre counters the oncogenic potential of colibactin-producing Escherichia coli in colorectal cancer. *Nature Microbiology*. <https://doi.org/10.1038/s41564-025-01938-4>

Toprak, N. U., Yagci, A., Gulluoglu, B. M., Akin, M. L., Demirkalem, P., Celenk, T., et al. (2006). A possible role of Bacteroides fragilis enterotoxin in the aetiology of colorectal cancer. *Clinical Microbiology and Infection*, 12, 782–786.
[\[PubMed\]](#)

Tosolini, M., Kirilovsky, A., Mlecnik, B., Fredriksen, T., Mauger, S., Bindea, G., et al. (2011). Clinical impact of different classes of infiltrating T cytotoxic and helper cells (Th1, th2, treg, th17) in patients with colorectal cancer. *Cancer Research*, 71, 1263–1271.
[\[PubMed\]](#)

Vizcaino, M. I., & Crawford, J. M. (2015). The colibactin warhead crosslinks DNA. *Nature Chemistry*, 7, 411–417.
[\[PubMed\]](#)

Vogtmann, E., Hua, X., Zeller, G., Sunagawa, S., Voigt, A. Y., Hercog, R., et al. (2016). Colorectal cancer and the human gut microbiome: Reproducibility with whole-genome shotgun sequencing. *PLoS*

One, 11, e0155362.

[\[PubMed\]](#)

Wijnands, A. M., de Jong, M. E., Lutgens, M. W., Hoentjen, F., Elias, S. G., Oldenburg, B., et al. (2021). Prognostic factors for advanced colorectal neoplasia in inflammatory bowel disease: Systematic review and meta-analysis. *Gastroenterology*, 160, 1584–1598.

[\[PubMed\]](#)

Wirbel, J., Pyl, P. T., Kartal, E., Zych, K., Kashani, A., Milanese, A., et al. (2019). Meta-analysis of fecal metagenomes reveals global microbial signatures that are specific for colorectal cancer. *Nature Medicine*, 25, 679–689.

[\[PubMed\]](#)

Wong, S. H., Zhao, L., Zhang, X., Nakatsu, G., Han, J., Xu, W., et al. (2017). Gavage of fecal samples from patients with colorectal cancer promotes intestinal carcinogenesis in germ-free and conventional mice. *Gastroenterology*, 153, 1621–1633 e1626.

[\[PubMed\]](#)

Wu, S., Lim, K. C., Huang, J., Saidi, R. F., & Sears, C. L. (1998). Bacteroides fragilis enterotoxin cleaves the zonula adherens protein, E-cadherin. *Proceedings of the National Academy of Sciences of the United States of America*, 95, 14979–14984.

[\[PubMed\]](#)

Wu, S., Morin, P. J., Maouyo, D., & Sears, C. L. (2003). Bacteroides fragilis enterotoxin induces c-Myc expression and cellular proliferation. *Gastroenterology*, 124, 392–400.

[\[PubMed\]](#)

Wu, S., Powell, J., Mathioudakis, N., Kane, S., Fernandez, E., & Sears, C. L. (2004). Bacteroides fragilis enterotoxin induces intestinal epithelial cell secretion of interleukin-8 through mitogen-activated protein kinases and a tyrosine kinase-regulated nuclear factor-kappaB pathway. *Infection and Immunity*, 72, 5832–5839.

[\[PubMed\]](#)

Wu, S., Shin, J., Zhang, G., Cohen, M., Franco, A., & Sears, C. L. (2006). The Bacteroides fragilis toxin binds to a specific intestinal epithelial cell receptor. *Infection and Immunity*, 74, 5382–5390.

[\[PubMed\]](#)

Wu, S., Rhee, K. J., Albesiano, E., Rabizadeh, S., Wu, X., Yen, H. R., et al. (2009). A human colonic commensal promotes colon tumorigenesis via activation of T helper type 17 T cell responses. *Nature Medicine*, 15, 1016–1022.

[\[PubMed\]](#)

Xue, M., Kim, C. S., Healy, A. R., Wernke, K. M., Wang, Z., Frischling, M. C., et al. (2019). Structure elucidation of colibactin and its DNA cross-links. *Science*, 365, eaax2685.

[\[PubMed\]](#)

Yaeger, R., Chatila, W. K., Lipsyc, M. D., Hechtman, J. F., Cercek, A., Sanchez-Vega, F., et al. (2018). Clinical sequencing defines the genomic landscape of metastatic colorectal cancer. *Cancer Cell*, 33, 125–136.e3.

[\[PubMed\]](#)

Yang, Y., Weng, W., Peng, J., Hong, L., Yang, L., Toiyama, Y., et al. (2017). *Fusobacterium nucleatum* increases proliferation of colorectal cancer cells and tumor development in mice by activating toll-like receptor 4 signaling to nuclear factor-kappaB, and up-regulating expression of MicroRNA-21. *Gastroenterology*, 152, 851–866 e824.

[\[PubMed\]](#)

Yin, Y., Sichler, A., Ecker, J., Laschinger, M., Liebisch, G., H6ring, M., et al. (2023). Gut microbiota promote liver regeneration through hepatic membrane phospholipid biosynthesis. *Journal of Hepatology*, 78, 820–835.

[\[PubMed\]](#)

Young, V. B., Knox, K. A., Pratt, J. S., Cortez, J. S., Mansfield, L. S., Rogers, A. B., et al. (2004). In vitro and in vivo characterization of helicobacter hepaticus cytolethal distending toxin mutants. *Infection and Immunity*, 72, 2521–2527.

[\[PubMed\]](#)

Yu, T., Guo, F., Yu, Y., Sun, T., Ma, D., Han, J., et al. (2017). *Fusobacterium nucleatum* promotes chemoresistance to colorectal cancer by modulating autophagy. *Cell*, 170, 548–563 e516.

[\[PubMed\]](#)

Zackular, J. P., Baxter, N. T., Iverson, K. D., Sadler, W. D., Petrosino, J. F., Chen, G. Y., et al. (2013). The gut microbiome modulates colon tumorigenesis. *MBio*, 4, e00692–e00613.

[\[PubMed\]](#)

Zeller, G., Tap, J., Voigt, A. Y., Sunagawa, S., Kultima, J. R., Costea, P. I., et al. (2014). Potential of fecal microbiota for early-stage detection of colorectal cancer. *Molecular Systems Biology*, 10, 766.

[\[PubMed\]](#)

Zepeda-Rivera, M., Minot, S. S., Bouzek, H., Wu, H., Blanco-Míguez, A., Paolo Manghi, P., et al. (2024). A distinct *Fusobacterium nucleatum* clade dominates the colorectal cancer niche. *Nature*, 628, 424–432.

[\[https://doi.org/10.1038/s41586-024-07182-w\]](https://doi.org/10.1038/s41586-024-07182-w) [\[PubMed\]](#)

Zhang, X., Yu, D., Wu, D., Gao, X., Shao, F., Zhao, M., et al. (2023). Tissue-resident Lachnospiraceae family bacteria protect against colorectal carcinogenesis by promoting tumor immune surveillance. *Cell Host and Microbe*, 31, 418–432.

[\[PubMed\]](#)

16. Microbiome and Diseases: Metabolic Disorders

Thomas Clavel¹✉ and Josef Ecker²✉

- (1) Functional Microbiome Research Group, Institute of Medical Microbiology, University Hospital of RWTH, Aachen, Germany
- (2) Functional Lipidomics and Metabolism Research, Institute of Clinical Chemistry and Laboratory Medicine, University Hospital Regensburg, Regensburg, Germany

✉ **Thomas Clavel (Corresponding author)**
Email: tclavel@ukaachen.de

✉ **Josef Ecker**
Email: josef.ecker@ukr.de

Abstract

Following the work of pioneers at the beginning of the twentieth century and later in the 1960s, there is nowadays a renewed interest in the impact of gut microbial communities on host metabolism. Nonetheless, the contribution of the intestinal microbiota to host energy homeostasis is still unclear. The obesity pandemic is mainly due to an adverse lifestyle, including unbalanced energy intake (too high) vs. expenditure (too low), and not to the colonization of our intestine by unfavorable microbes. Nonetheless, gut microorganisms and in particular bacteria can metabolize a variety of dietary compounds, thereby releasing bioactive molecules that can influence metabolic functions of the host. Intestinal bacteria can also influence immune

responses, which are known to play a role in the development of chronic metabolic disorders. Moreover, the approach of transferring gut microbial communities via fecal transplantation to germfree animals or human subjects has demonstrated the causal role of intestinal microbes in regulating host energy homeostasis and the development of metabolic pathologies, such as Metabolic Dysfunction-associated Steatotic Liver Disease (MASLD). Experimental studies have identified single cultured bacteria that influence metabolic responses, including underlying molecular mechanisms and bioactive molecules. All these findings speak in favor of the importance of the gut microbiota for host metabolism. In the present chapter, we summarize data in a critical manner and discuss key notions pertaining to the impact of gut microbial communities on host metabolism, with emphasis on lipid metabolism, and ensuing implications for the development of metabolic disorders.

The intestine of mammals is colonized by a high diversity of microorganisms, especially bacteria, the density and diversity of which increase along the digestive tract from proximal to distal parts (see Chap. [2](#)). Gut bacteria carry out a variety of metabolic reactions and thus utilize a wide range of dietary compounds, thereby producing bioactive molecules that can regulate host physiology. This results in a mutualistic relationship that benefits both the microbial ecosystem and the host. Major changes in lifestyles throughout industrialization, including important shifts in dietary habits, improved hygiene, and access to medication (e.g., antibiotics), have affected the gut microbial ecosystem and thus microbe–host interactions. To what extent these alterations contribute to the rising occurrence of metabolic diseases in industrialized countries, in particular obesity and associated comorbidities, has been a matter of intensive research. In the following sections, we touch upon the role of the gut microbiota in metabolic health and discuss important notions in the field, from dysbiosis and causal effects to single bacterial species and molecular crosstalk.

16.1 Alterations of the Gut Microbiota linked to diet and Metabolic Disorders

Dysbiosis is a term that characterizes a disturbed ecosystem in a specific pathological condition. It refers to shifts in the diversity and composition of the gut microbiota with functional implications for a given disease. Whereas many studies have reported disease-associated changes in the gut microbiota when compared to the healthy state, it is very often unclear whether these shifts are directly relevant to the development of the given disease. Causal effects of the gut microbiota on metabolic health will be addressed below in Sect. [16.3](#). We here highlight major descriptive changes in the gut microbiota that are most often discussed in the context of metabolic disorders.

Dominant bacterial communities within the gut microbiota of individuals in industrialized countries are known to be relatively stable over time, even over decades, in the absence of major dietary and lifestyle changes. The caloric content and composition of diets are linked to the development of overweight and obesity and have also been associated with major shifts in the gut microbiota, both in laboratory animals and in human beings (Daniel et al., [2014](#); David et al., [2014](#); Fava et al., [2013](#)). However, in the short-term, the ecosystem is relatively resilient to changes induced by environmental stressors such as diet, i.e., it tends to rapidly return to original profiles upon stress cessation. In contrast, it has been observed that intake of a diet low in complex carbohydrates over four generations of mice leads to persistent alterations of the gut microbiota transmissible to the offspring (Sonnenburg et al., [2016](#)). When extrapolated to the human situation, such a phenomenon, possibly amplified by additional dietary and environmental factors, may have contributed to the loss of specific gut bacterial species over time. This is corroborated by the observation that endemic populations maintaining a traditional lifestyle seem to harbor more microbial species in their intestine compared with individuals from industrialized countries (Martinez et al., [2015](#); Yatsunenko et al., [2012](#)). It remains to be firmly proven that such

changes have really contributed to establishing a somewhat detrimental metabolic crosstalk between the gut microbiota and its host. Recently, Li et al. ([2025](#)) performed a randomized, controlled feeding trial in healthy Canadian adults using a diet that recapitulates key features of non-industrialized dietary patterns and a bacterial species rarely found in industrialized microbiomes (*Limosilactobacillus reuteri*). Despite a reduction in gut microbiota diversity, the diet increased the persistence of *L. reuteri* and restored several microbiome characteristics altered by industrialization. In addition, cardiometabolic benefits were observed independent of *L. reuteri* administration. This study provides causal evidence that microbiota features associated with a metabolically healthy state can be restored using a rationally designed diet.

Alpha-diversity, including simple enumeration of species within the ecosystem, referred to as richness, is a parameter of interest regarding the microbiota in metabolic disorders. High diversity of the gut microbiota is usually considered to be a hallmark of ecosystem stability. Different species may possess similar properties, resulting in so-called functional redundancy, guaranteeing the robustness of complex metabolic and functional networks within the ecosystem. The loss of richness of the gut microbial ecosystem has been repeatedly associated with chronic intestinal inflammation (Lee et al., [2017](#); Pascal et al., [2017](#)). In the context of metabolic disorders, a metagenomic survey of 123 non-obese vs. 169 obese Danish individuals reported that, while gene counts (a proxy for diversity) in their fecal metagenomes ranged between 200,000 and 1,000,000, most subjects were characterized by either low (ca. 450,000) or high (ca. 750,000) gene counts (Le Chatelier et al., [2013](#)). Interestingly, the group of individuals with low gene counts displayed detrimental metabolic features and responded less positively to a dietary intervention for weight loss and stabilization (Cotillard et al., [2013](#); Le Chatelier et al., [2013](#)). However, a meta-analysis of ten studies looking at the association between obesity and gut microbiota profiles as obtained by 16S rRNA gene amplicon sequencing calculated that “obese individuals averaged 7.47% lower

richness” and concluded that “obese individuals do have statistically significantly lower diversity than non-obese individuals; however, it is questionable whether the difference is biologically significant” (Sze & Schloss, [2016](#)).

Main additional features of disturbed intestinal ecosystems include an increased abundance of facultative anaerobes, mainly members of the family *Enterobacteriaceae*, and a leaky gut barrier, this latter aspect being presented in greater detail in Sect. [16.4](#) (Cani et al., [2009](#); Ott et al., [2017](#); Qin et al., [2012](#); Teixeira et al., [2012](#); Xiao et al., [2014](#)). In mouse models of diet-induced obesity, the intake of high-calorie diets is usually associated with a decrease in caecum volume and with a bloom in the family *Erysipelotrichaceae* within the phylum *Bacillota* (formerly Firmicutes) (Daniel et al., [2014](#); Fleissner et al., [2010](#); Turnbaugh et al., [2008](#)). Because of the heterogeneity of studies in terms of target pathology (e.g., obesity, diabetes, hepatic steatosis), experimental design (e.g., human study types or animal models), and methodologies (e.g., shotgun vs. targeted sequencing, different 16S rRNA gene regions amplified), it is to date still difficult to draw a uniform picture of changes in the gut microbiota associated with metabolic diseases. The case of the so-called Firmicutes-to-Bacteroidetes ratio is addressed in the “Controversy” box. Another underlying reason for inconsistent intergroup comparisons is that gut microbiota diversity and composition are, by definition, individual-specific, including functional strain-level differences (Chen-Liaw et al., [2025](#)). Hence, approaches targeting overall differences between disease and healthy states will always be limited, so that longitudinal study design and individualized strategies offer interesting perspectives (Zeevi et al., [2015](#); Mendes-Soares et al., [2019](#)).

The first population-based study of the gut microbiome using 16S rRNA gene amplicon analysis of samples from thousands of individuals reported several confounders of microbiota analysis (e.g., stool consistency) that can influence sequence-based assessment of gut dysbiosis in metabolic disorders (Falony et al., [2016](#)). This work also revealed that most data published until then likely resulted from

underpowered analysis: the authors estimated that >500 human stool samples are required to reliably assess the association between gut microbial populations and body mass index. Another study highlighted confounding effects of medication on the association between the gut microbiota and metabolic disease: failure to account for the use of metformin, a drug commonly used to treat type 2 diabetes, generated misleading results in previous studies (Forslund et al., [2015](#)). Other issues about the reproducibility of findings on the gut microbiota in metabolic health are discussed below in Sect. [16.4](#) (especially Sects. [16.4.1](#) and [16.4.4](#)) and in the “Controversy” box. Since then, direct or collateral effects of drugs on gut microbial species have been found to be very common (Maier et al., [2018](#)). Recent descriptive evidence using quantitative fecal metagenomes from 888 individuals suggests that statin therapy may act through beneficial modulation of the gut microbiome (Vieira-Silva et al., [2020](#)). Previous work in a smaller intervention cohort ($N = 40$) had reported similar findings (Wu et al., [2017](#)). The latter study demonstrated causality by observing that the positive effects of medication (e.g., reduced fat deposition) were partly conferred by metformin-induced rearrangements in gut microbial communities after transfer to germfree recipient mice.

The human metagenomic studies mentioned above and other recent findings (Fromentin et al., [2022](#)) have tested the predictive power of microbiome signatures in cardiometabolic health using an ever larger number of samples. Studies will continue to increase in size, including samples from populations outside Europe, North America, and China (Maghini et al., [2025](#)). They will also continue to explore microbiota members other than bacteria, such as the virome (Yang et al., [2021](#); Bikel et al., [2021](#)), interactions between the gut microbiome and circadian rhythm, which is disrupted in adverse metabolic conditions (Thaiss et al., [2014](#); Leone et al., [2015](#); Wang et al., [2017](#); Kuang et al., [2019](#); Reitmeier et al., [2020](#); Hoces et al., [2022](#)), and the role of specific metabolites such as biotin, bile acids, and imidazole propionate (Belda et al., [2022](#); Aydin et al., [2025](#); Molinaro et al., [2020](#)). However, a major obstacle to such studies is the fact that half of the microbiota members

and their genes are still unknown. It is therefore crucial to go beyond molecular assessment of ecosystem changes and identify key microbial species relevant to host metabolism, including the description of as-yet uncultured taxa and cryptic functions within known species. This will facilitate functional investigations of molecular mechanisms underlying microbe–host interactions in metabolic health.

16.2 From Complex Communities to Single Bacterial Species

Latest estimates of the cultured fraction of sequence-based diversity in the gut microbiota range between 40% and 60% (Almeida et al., [2021](#); Thomas & Segata, [2019](#); Hitch et al., [2021](#)). Hence, it is essential to continue efforts in isolating and characterizing gut bacteria to define their role in the development or protection of chronic disorders, including metabolic diseases. In this section, we highlight some of the few bacterial species found to be implicated in metabolic health.

Because the focus of this chapter is on commensal dwellers of the intestine, the role of probiotics is not addressed in detail. Their use for potentiating the growth of domestic animals was highlighted at the end of the 1980s (Fuller, [1989](#)). Since then, many studies on the effects of probiotics, especially lactobacilli, in the context of metabolic health have been published. Drawing clear conclusions from these studies is, however, a challenge, due to the multitude of strains and experimental approaches used (Torres et al., [2024](#); Porchia et al., [2022](#); Delzenne et al., [2011](#); Kumar et al., [2012](#); Lahtinen et al., [2012](#)). The major limitation of all studies on probiotics in metabolic diseases is the lack of rationale for selecting strains. They usually correspond to bacteria that historically have a “generally regarded as safe” status but for which there is no clearly identified mechanisms that justify their use in specific metabolic pathologies. The original concept of probiotics has evolved to include commensals isolated from the intestine, or mixtures thereof, known as live biotherapeutics (Pribyl et al., [2025](#)). However, the safety and efficacy assessment of commensal probiotics takes time

and is still rare, as demonstrated by the example of *Akkermansia muciniphila* (Cani et al., [2022](#)).

A. muciniphila, the so far uniquely described species of the phylum *Verrucomicrobia* in the human gut, is currently the most studied bacterium in the context of metabolic health. This bacterium was first described in 2004 as a novel species capable of degrading the mucin that lines up the intestinal epithelial surface (Derrien et al., [2004](#)). In 2008, the same authors showed that *A. muciniphila* is a dominant and prevalent bacterial species, which seems to colonize the human gut early in life (the earliest time point measured was 6 months of age) (Derrien et al., [2008](#)). The first report of an association between the occurrence of *A. muciniphila* and host metabolism was published in 2012, when researchers observed that an antibiotic-driven bloom of this species was linked to a lower incidence of diabetes in mice (Hansen et al., [2012](#)). One year before, two human studies had revealed conflicting results on the relation between *A. muciniphila* occurrence in feces and body weight in pregnant women and infants (Collado et al., [2010](#); Santacruz et al., [2010](#)). The first experimental proof of a causal effect of *A. muciniphila* on the improvement of high-fat diet-induced metabolic disorders was published shortly after in 2013 (Everard et al., [2013](#)), but it took another 4 years until researchers identified a purified membrane protein that appears to be at least partly responsible for positive metabolic effects (Plovier et al., [2017](#)). This molecule and the fact that pasteurized *A. muciniphila* also has beneficial effects on host metabolism qualify this species as both a probiotic and a postbiotic (Salminen et al., [2021](#)). Positive effects were later confirmed in a randomized, double-blind, placebo-controlled pilot study in 32 subjects (Depommier et al., [2019](#)), and *A. muciniphila*-based products are now commercially available. This is a breakthrough in the field, as the mechanisms underlying the beneficial effects of gut bacteria and, in particular, the molecules responsible, are very rarely described. However, it is important to note that the use of *A. muciniphila* remains context dependent (metabolic health); clinical data is needed for its use in other health conditions.

Besides *A. muciniphila*, *Christensenella minuta* is another species reported to have protective effects on host metabolism. Goodrich et al. showed that colonization of gnotobiotic mice (previously inoculated with the fecal microbiota from an obese human donor) with this species was able to reduce weight gain (Goodrich et al., [2014](#)). A recent study further showed that *C. minuta* improved metabolic disease in high-fat diet-induced obese mice in a farnesoid X receptor (FXR)-dependent manner through the production of acylated bile acids (Liu et al., [2024](#)). In addition, a newly described member of the family *Oscillospiraceae* (phylum *Bacillota*), *Dysosmobacter welbionis*, was shown to prevent diet-induced obesity in mice and proposed to affect host metabolism through the production of bioactive lipids (Le Roy et al., [2022](#); Moens de Hase et al., [2023](#)). Other gut bacterial species shown to influence host metabolic health experimentally include *Enterobacter cloacae*, *Segatella copri* (formerly *Prevotella copri*) (Hitch et al. [2022](#)), and *Thomasclavelia ramosa* (formerly *Clostridium ramosum*) (Lawson et al. [2023](#)).

In contrast to these positive effects on host metabolism, Woting et al. showed that the presence of *T. ramosa* was associated with increased body weight and fat, accompanied by higher food efficiency in gnotobiotic mice fed a high-fat diet (Woting et al., [2014](#)). In 2013, Fei and Zhao reported that a strain of *E. cloacae* isolated from the intestine of a morbidly obese patient could induce obesity and insulin resistance upon colonization of germfree mice fed with a high-fat diet (Fei & Zhao, [2013](#)). Pedersen et al. found that the occurrence of *S. copri* in the stool of 277 nondiabetic Danish individuals was linked to serum levels of branched-chain fatty acids, most likely of microbial origin, in insulin-resistant subjects (Pedersen et al., [2016](#)). The authors further demonstrated causal effects via treatment of conventional mice with the bacterium over 3 weeks, which led to aggravation of high-fat diet-induced glucose intolerance. Another study reported that the protective effects of a Mediterranean diet on cardiometabolic disease risk were stronger in subjects with reduced *S. copri* in stool (Wang et al., [2021](#)). In contrast, *S. copri* was found to be an indicator of favorable postprandial

glucose metabolism in a cohort of 1098 individuals from the UK and the USA (Asnicar et al., [2021](#)). In addition, improvement in glucose metabolism in 39 healthy subjects following 3-day consumption of barley kernel-based bread was associated with increased occurrence of fecal *Prevotella* (Kovatcheva-Datchary et al., [2015](#)). This discrepancy in results is probably related to the unclear taxonomic delineation within *Prevotellaceae* and the high intraspecies functional diversity observed for *S. copri* (Blanco-Míguez et al., [2023](#); Hitch et al., [2022](#)). Recently, Takeuchi et al. reported that the prevalent gut bacterium *Fusimonas intestini* of the family *Lachnospiraceae* facilitates diet-induced obesity in mice through the production of long-chain fatty acids such as elaidate, a trans-unsaturated fatty acid, which affected gut epithelial integrity and enhanced metabolic endotoxemia (Takeuchi et al., [2023](#)).

In summary, the plethora of reports in the literature on overall changes in the gut microbiota contrasts with the few bacterial species identified as potentially playing a role in metabolic diseases (Fig. [16.1](#)). Moreover, findings must be extrapolated with caution, because different strains of a given species can be functionally different.

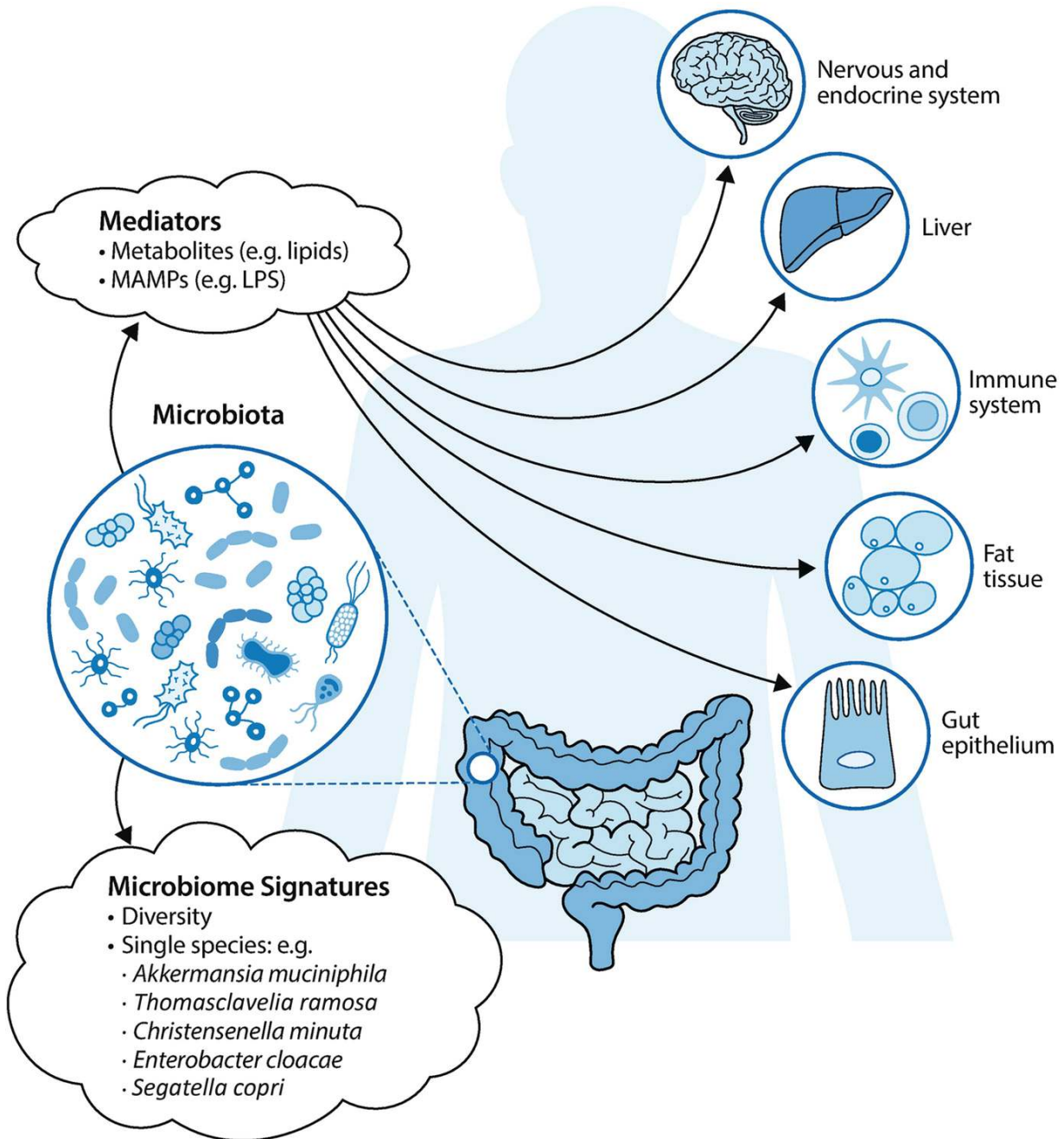


Fig. 16.1 Complex interactions between the gut microbiota and host metabolic responses. Microbe–host interactions in metabolic health result from intricate functional networks that we are just beginning to understand. The gut microbiota consists of hundreds of microbial species in the colon, approximately half of which are still unknown, which substantially limits our understanding of its functional role. Hallmarks of microbiome perturbations in metabolic diseases (microbiome signatures in the figure) are currently limited and rather vague (e.g., a decrease in diversity is also observed in chronic intestinal inflammation), partly due to the individual-specific nature of the gut microbiota (see Sect. 16.1). The number of species so far identified to regulate host metabolism is small. This is also true of the currently known mediators from these microbes, including metabolites (e.g., amino acids and lipids) or microbe-associated molecular patterns

(MAMPs), i.e., microbial molecules recognized by cells of the immune system, such as lipopolysaccharides (LPS). Species are highlighted in Sect. [16.2](#) and some of the mediators are detailed in Sects. [16.4](#) and [16.5](#). The complexity of microbe–host interactions in metabolic health also arises from the numerous host organs and systems, with which microbe-derived components can interact. The list is not exhaustive in the figure, and it is beyond the scope of this chapter to define in detail these interactions. The gut microbiota can, for instance, interact with (1) the central nervous system and thereby regulate food intake and metabolic functions (Grasset et al., [2017](#); Torres-Fuentes et al., [2017](#)), (2) the endocrine system and thereby influence growth (Schwarzer et al., [2016](#)), (3) the liver via a plethora of metabolites and cellular products drained from the intestine (Pabst et al., [2023](#)), (4) the immune system, which is known to play an important role in metabolic diseases (Kau et al., [2011](#)), (5) the brown and white adipose tissue and thereby influence energy storage and expenditure (Zietak et al., [2016](#)), (6) the gut epithelium and thereby regulate immune, endocrine, metabolic, and barrier functions (Blachier et al., [2017](#); Cherbuy et al., [1995](#); Plovier et al., [2017](#))

16.3 Evidence for the Implication of Gut Microbiota in Metabolic Pathologies

16.3.1 Microbiota Transfer Experiments

Fecal microbiota transplantation (FMT), which consists of colonizing the intestine of a recipient person (or animal) by dispensing a microbial suspension prepared using feces collected from a donor (a healthy, extensively screened donor for FMT to human recipients), is an efficient way to test causal effects of changes in microbial diversity and composition on pathophysiological parameters. Based on very high efficacy in the context of recurrent infection by the pathogen *Clostridioides difficile* (van Nood et al., [2013](#)), FMT has been applied in a variety of pathologies, including inflammatory bowel diseases (IBD) (Paramsothy et al., [2017](#)), hepatic encephalopathy (Bajaj et al., [2017](#)), and the metabolic syndrome (de Groot et al., [2017](#)). Following up on a first trial published in 2012 highlighting the improvement of insulin sensitivity after FMT in nine individuals (Vrieze et al., [2012](#)), Kootte et al. very recently demonstrated an increase in peripheral insulin sensitivity 6 weeks after allogenic (microbiota from a different donor) but not autologous (own microbiota) FMT in 38 patients with metabolic syndrome (Kootte et al., [2017a](#)). Effects were transient, as no differences were seen 18 weeks after treatment. The authors also reported that lower diversity of the recipient microbiota at baseline

was associated with positive response after treatment, stressing the need to determine fecal microbiota composition of patients at inclusion to aid in predicting the efficacy of treatment. However, the biological relevance of a relatively low (<0.25) baseline difference in the Shannon index (one parameter of ecosystem diversity) is unclear, and treatment did not lead to an increase in diversity. More studies are needed to identify recipients' microbiome signatures associated with enhanced responses to FMT. Zhang et al. (Zhang et al., [2024](#)) reported a better metabolic response (HOMA2-IR) to FMT in individuals with low fecal *Prevotella* at baseline (Sect. [16.2](#)), and a better response associated with the engraftment of butyrate-producing taxa and *Christensenellaceae* (Sect. [16.2](#)). These clinical studies clearly demonstrate causal effects of the gut microbiota in metabolic health and are very important experimental approaches to dissect microbe-host interactions. However, responses remain individual-specific, and the currently high risk-to-benefit ratio precludes routine clinical implementation. Future lines of research may include in vivo (dietary intervention) or ex vivo (continuous culture approaches) approaches to enhance fecal communities prior to FMT (Rinott et al., [2021](#)).

Besides the clinical FMT studies aforementioned, the causal role of the gut microbiota in the development of metabolic diseases has also been highlighted by the fact that diet- or obesity-driven alterations of gut microbial communities in mice can lead to detrimental metabolic responses after transfer to germfree recipients. In 2008, Turnbaugh et al. compared the effects of transferring the gut microbiota from donor mice fed with a high-calorie (Western) or standard chow diet to recipient mice kept on the standard diet. Colonization with microbiota from the diet-induced obese mice led to a significantly greater increase in body fat after 2 weeks without significant differences in feed consumption (Turnbaugh et al., [2008](#)). Because of the short-term aspect of these mouse experiments and because absolute values were not provided (the paper reported a 43 vs. 25% increase in body fat as determined by dual-energy X-ray absorptiometry), extrapolation of these experimental data is somewhat difficult. Experimental microbiota

transfer studies have recently demonstrated the causal effects of viral communities on host metabolism (Rasmussen et al., [2020](#); Borin et al., [2023](#); Mao et al., [2024](#)). Transferring the virome or bacteriophage community from lean donor mice to recipient animals fed a high-fat diet improved weight gain or blood glucose tolerance.

Regarding microbiota transfer from human to mouse, Ridaura et al. studied the impact of transferring the fecal microbiota from twin pairs discordant for obesity (i.e., one twin had a normal body mass index, while the other twin was obese) on mouse metabolism (Ridaura et al., [2013a](#)). Four twin pairs were used for colonizing mice kept on a standard diet, each sample being transferred into 3–4 mice in 1–5 independent experiments. On average, mice receiving the fecal microbiota from obese twins were characterized by a 10–15% increase in fat mass (as measured by quantitative magnetic resonance) after 4–5 weeks of colonization. Interestingly, the authors could repeat these findings by transferring cultured collections of fecal bacteria from the obese and lean donors of one twin pair instead of the whole undefined fecal communities, suggesting that it is possible to obtain single underlying species in the laboratory and reconstitute artificial communities to study molecular mechanisms. The authors also found that the increased fat mass phenotype after colonization with the obese microbiota could be reversed via cohousing with mice that had received the lean microbiota, which is interesting in terms of the permissive aspect of the obese microbiota in response to exogenous communities associated with favorable host conditions. These state-of-the-art experiments demonstrate the transmissibility of metabolic phenotypes via the gut microbiota. Nonetheless, the complexity of assessing engraftment of complex fecal ecosystems (containing a substantial fraction of yet undescribed species) from human to mice (Arrieta et al., [2016](#)), combined with the low-amplitude and short-term aspects of the fat mass changes as obtained with a limited selection of donor samples, warrants confirmation prior to generalization of the results.

Experimental evidence of the causative role of the gut microbiota exists also in the context of metabolic pathologies other than obesity

and type 2 diabetes. The liver is a central organ in host metabolism with an intricate link to the gut: the portal vein drains blood from the intestine, and the bile duct transports hepatic products into the intestinal lumen, which constitutes a flux of mediators referred to as enterohepatic circulation. Both organs form an efficient barrier to exogenous components and play key functions in nutrient absorption and metabolism, and thus in host energy homeostasis (Pabst et al., [2023](#)). The rise of obesity worldwide is associated with the development of Metabolic Dysfunction-associated Steatotic Liver Disease (MASLD) (formerly nonalcoholic fatty liver disease, NAFLD), characterized by abnormal accumulation of lipids in the liver with various complications ranging from chronic inflammation (steatohepatitis) to hepatocellular carcinoma. A study in mice previously showed that not all animals respond equally to a high caloric intake over 16 weeks: some of the mice (responders) were characterized by high levels of glycemia, systemic inflammation, and steatosis, whereas others (non-responders) did not develop any metabolic disorders despite obesity (Le Roy et al., [2013](#)). These contrasting phenotypes could be recapitulated after colonization of germfree mice with the gut microbiota from responder and non-responder donors and following the same feeding protocol. These findings suggest that gut microbiota profiling could help predict the susceptibility to develop metabolic disorders and emphasize the possibility of modulating liver pathologies via the gut microbiota. Regarding potential bioactive molecules from candidate bacterial species, recent work has proposed that microbial metabolism underlying the production of specific secondary bile acids, including 3-succinylated cholic acid, or sphingolipids, and the conversion of amino acids (in particular branched chain amino acids) affect host metabolism through the regulation of hepatic functions (Nie et al., [2024](#); Li et al., [2024](#); de Le et al., [2022](#)). Despite this data, there is so far little evidence for effective clinical applications targeting liver pathologies based on microbiome-based dietary interventions (Scorletti et al., [2020](#); Chong et al., [2020](#)).

16.3.2 Other Evidence

Colonization of the intestine of mammals following birth (Kennedy et al., [2023](#)) and throughout the first months of life is a very important physiological process for the protection against enteric infections and for the maturation of immune and metabolic responses (Chap. [3](#)). Hence, early life is an important window of opportunities where environmental factors (e.g., delivery mode, medication) or voluntary interventions (e.g., dietary supplements) may have long-lasting consequences both for the microbial ecosystem and for host health. In particular, the link between obesity and repeated use of antibiotics at an early age has been a subject of great interest (Cox & Blaser, [2015](#)). A study looking at prenatal exposure to antibiotics in the second or third trimester of pregnancy as recorded by questionnaires in a population of 436 mother–child dyads followed until 7 years of age reported a higher offspring risk of childhood obesity associated with antibiotic intake and also with caesarian section (Mueller et al., [2015](#)). In contrast, a recent randomized intervention trial including 428 infants revealed that prolonged exposure to the antibiotics trimethoprim-sulfamethoxazole did not affect weight gain throughout the first 2 years of life (Edmonson & Eickhoff, [2017](#)). These and other findings (Ajslev et al., [2011](#); Bailey et al., [2014](#); Chelimo et al., [2020](#); Leong et al., [2020](#); Kalliomaki et al., [2008](#); Saari et al., [2015](#)) show that additional work will be needed prior to drawing firm conclusions on quantifying the impact of antibiotic treatment in early life on the development of obesity. Altogether, there is enough evidence suggesting that antibiotics can influence weight development later in life, but the effects remain individual-specific because they depend on many other parameters (e.g., time, type, and duration of treatment; combination with other factors such as delivery mode and mother’s weight). With respect to the use of antibiotics later in life, a randomized, placebo-controlled, double-blind study reported that 7-day treatment with either amoxicillin or vancomycin had no clinically relevant impact on metabolic health (insulin sensitivity, postprandial hormones, systemic inflammation, gut permeability, adipocyte size) in 57 obese, prediabetic men (Reijnders et al., [2016](#)).

The implication of the host–microbiota crosstalk in metabolic diseases is also evident in the context of clinical interventions other than FMT. Bariatric surgery (also referred to as gastric bypass), which consists of reducing the size of the stomach, is one of the most efficient procedures for the treatment of morbid obesity. Gastric bypass was shown to be associated with marked over time changes in the fecal microbiota (Furet et al., [2010](#)), and later studies demonstrated the causal role of such changes by recapitulating favorable metabolic changes after colonizing germfree mice with the gut microbiota from animal (Liou et al., [2013](#)) or human (Tremaroli et al., [2015](#)) donors after surgery. Recent studies have pointed to the role of microbially produced bile acids in metabolic remodeling after bariatric surgery (Chaudhari et al., [2021](#); Chen et al., [2024](#)). With respect to diet, a clinical trial demonstrated the efficacy of a 2-week intervention with an isocaloric, carbohydrate-restricted diet on hepatic steatosis in ten obese individuals (Mardinoglu et al., [2018](#)). Rapid (as soon as 1 day after trial start) and marked reduction in liver fat and circulating triglycerides (>40% reduction on average at the end of the trial) was associated with rapid shifts in the fecal microbiota. Major changes included increased occurrence of *Streptococcus* and *Lactococcus* species linked to increased production of folate, which may have partly explained the improvement in liver fat metabolism observed in the participants. More clinical studies are needed to draw conclusions on the beneficial effects of carbohydrate-restricted or ketogenic diets on liver pathologies and the role of the gut microbiome in this process (Zhao et al., [2024](#)).

16.3.3 Microbe–Host Interactions in Malnutrition

Obesity is drawing most of the attention in the field of microbe–host interactions in metabolism due to its high incidence. However, food shortage is also a major concern worldwide. It is thus worth addressing malnutrition in this chapter on the impact of gut microbiota on metabolic health, highlighting specific underlying molecular mechanisms.

Jeffrey Gordon and his group have performed pioneering work in this research area. Via transfer of feces from infant Malawian donors to germfree mice, they showed that disturbances of the gut microbiota associated with malnutrition have causal effects on host metabolism (Blanton et al., [2016](#)). In these experiments, they identified two species, *Mediterraneibacter gnavus* (formerly *Ruminococcus gnavus*) and *Clostridium symbiosum*, which ameliorated growth and metabolic abnormalities in recipient animals when added to the microbiota from malnourished children. In another study, they found that human milk oligosaccharides that are sialylated (i.e., bound to sialic acid) were significantly less abundant in the milk collected from 6-month-postpartum mothers ($n = 88$) of severely stunted (i.e., stopped abnormally in growth) compared with control Malawian infants (Charbonneau et al., [2016](#)). In subsequent experiments in mice and pigs, the authors showed that feed supplementation with sialylated bovine milk oligosaccharides was associated with beneficial effects on growth, i.e., greater ability to utilize nutrients for anabolism. This series of studies eventually led to a new intervention for undernourished children using food specifically created to enhance microbiome responses (Chen et al., [2022](#)). Regarding the identification of specific gut bacterial players in undernutrition, Gordon and colleagues reported the occurrence of an enterotoxigenic strain of the species *Bacteroides fragilis* that was causally related to weight loss (Wagner et al., [2016](#)). In contrast, the group of François Leulier found that *Limosilactobacillus plantarum* can promote systemic growth of the host during chronic undernutrition in a strain-dependent manner and proposed a molecular mechanism that involves the somatotrophic hormone axis (Schwarzer et al., [2016](#)). Germfree mice colonized with *L. plantarum* strain WJL grew better (+25% in weight and + 6% in length) than those colonized with a control *L. plantarum* strain when fed with a diet low in proteins, fat, and vitamins from weaning on until the age of 8 weeks. These effects were associated with increased serum levels of insulin-like growth factor-1 (a protein mainly secreted by the liver after stimulation by growth hormone and involved in many cellular

processes and the growth of organs) and enhanced sensitivity to growth hormone treatment. Moreover, this work suggests mechanisms valid across phylogenetically very distant eukaryotic hosts, as strain WJL, showing effects in mice, was originally isolated from fruit flies (*Drosophila melanogaster*) and was a potent growth promoter in flies too. The same authors subsequently identified that cell wall components of *L. plantarum* are involved in the improvement of linear growth of undernourished young mice (Schwarzer et al., [2023](#)). Interestingly, diet-driven adaptation of this bacterium was shown to influence the growth of *D. melanogaster*, suggesting that co-evolutionary forces shape microbe–host interactions in metabolic health (Martino et al., [2018](#)).

In summary, there is good experimental and clinical evidence for the involvement of gut microbial communities in metabolic diseases, mostly based on interventional and microbiota transfer-based approaches. The main challenge is to define functional circuits and clinical targets.

16.4 Functional Consequences and Mechanisms

The high diversity of the gut microbiota and the multiple organs involved in host metabolism form an intricate functional network of microbe–host interactions in metabolic health. Knowledge of these functional circuits is still scarce, although some mechanisms have been repeatedly discussed in the field. They are presented below, albeit only briefly, as interactions between the gut microbiota and each of the host systems and organs, as shown in Fig. [16.1](#), are highly complex.

16.4.1 Gut Barrier

The gut mucosa is the largest area of interface between cells of our own body and the environment, larger than the skin. One of the primary functions of this interface is to separate the self (body) from nonself (environment), which is referred to as the barrier function. One

important dogma is that the gut barrier is disturbed in metabolic diseases, leading to increased translocation of pro-inflammatory molecules of microbial origin, such as lipopolysaccharides (LPS), a major component of the cell wall of Gram-negative bacteria. Experiments in genetically modified mice suffering from metabolic diseases (Brun et al., [2007](#)) and in mouse models of diet-induced obesity (Cani et al., [2007](#)) reported elevated endotoxemia, i.e., higher amounts of LPS in the blood. In 2009, Patrice Cani et al. ([2009](#)) proposed a mechanism in diet-induced obese mice by which decreased endotoxemia and improved gut barrier integrity after intake of the prebiotic inulin were linked to increased bifidobacteria numbers and elevated expression of GLP2 (glucagon-like peptide 2), a peptide secreted by enteroendocrine cells in the intestine which regulates energy absorption and mucosal morphology (Amato et al., [2016](#)).

Results pertaining to gut barrier in animal models can, however, be affected by the type of high-calorie diets used, the animal facility, and the gut region considered (Hamilton et al., [2015](#); Kless et al., [2015](#); Muller et al., [2016](#)). Moreover, the heterogeneity of LPS structures is substantial, and the immunogenic potential of various LPS can vary drastically (Steimle et al., [2016](#)). Of note, not all LPS variants can be detected by the most commonly used commercial assays, which are also sensitive to LPS contaminants from the environment (Gronbach et al., [2014](#); Gorman & Golovanov, [2022](#); Newhal et al., [2010](#); Redl et al., [1992](#)). Furthermore, it is often not clear whether LPS is measured in portal or systemic blood. This is an important parameter that allows distinguishing between levels originating directly from the gut and those potentially having systemic functions. Hence, caution must be taken when interpreting results on gut barrier as measured by LPS translocation, as they can be influenced by many technical parameters and are specific to the disease model used.

A breakthrough in the field was published by Cani and colleagues (Plovier et al., [2017](#)), who identified a bioactive molecule responsible for improvement in barrier function from *A. muciniphila* (for information on this bacterium, see Sect. [16.2](#)). Amuc_1100*, a protein of

32 kDa encoded by a specific Type IV pili gene cluster, induced a lower body weight and fat mass gain and corrected hypercholesterolemia in mice fed with a high-calorie diet. This was accompanied by restored portal plasma concentrations of LPS to values observed in control animals on a normal diet and by enhanced mRNA expression of tight junction proteins, possibly via regulation of the endocannabinoid system and TLR-2-dependent pathways (Plovier et al., [2017](#)). Moreover, there is also clinical evidence for the involvement of a leaky gut barrier in metabolic diseases. Several intervention studies reported that improvement of the metabolic health of participants was accompanied by improved barrier integrity (Damms-Machado et al., [2017](#); Karl et al., [2017](#); Krumbeck et al., [2018](#); Ott et al., [2017](#); Russo et al., [2012](#); Xiao et al., [2014](#)). Hence, it is altogether reasonable to assume that disturbances in gut physiology under conditions of metabolic stress are linked to impaired barrier function, but we are only beginning to understand how this works and what the consequences are for the host.

16.4.2 At the Crossroad Between Immune and Metabolic Responses

One functional consequence of increased fluxes of microbial molecules from the gut to the periphery via the liver is immune response activation. Adiposity is also associated with infiltration of immune cells into fat tissues (Parseus et al., [2017](#)), and it is nowadays acknowledged that the immune system plays an important role in metabolic disorders. Because interactions between the gut microbiota and immune responses are so complex, it is beyond the scope of this chapter to provide a comprehensive overview in the context of metabolic diseases, which has been reviewed by others (Kau et al., [2011](#); Brown et al., [2023](#); Zmora et al., [2017](#)). We simply highlight some interesting findings.

Interleukin-22 (IL-22) was shown to alleviate metabolic disorders and restore mucosal immunity in diabetes, as IL-22 knockout mice are prone to develop metabolic syndrome upon high-fat diet feeding, and

treatment with IL-22 restored this phenotype (Wang et al., [2014](#)). Experimental studies demonstrated that the gut microbiota regulates T-cell differentiation in the gut via mechanisms that involve IL-22, with consequences on metabolic health (Garidou et al., [2015](#); Zelante et al., [2013](#)). Dysregulation of innate immune pathways downstream of Toll-like receptor-5 or NLRP-6 and NLRP-3 inflammasomes was also shown to impact the development of metabolic diseases (Henao-Mejia et al., [2012](#); Singh et al., [2015](#); Vijay-Kumar et al., [2010](#)). An important future task is to determine the mechanisms linking local shifts in immune responses in the gut to systemic changes in metabolic responses.

With respect to changes affecting the adipose tissue, microbiota depletion by antibiotics or by using germfree mice was shown to promote browning of white adipose tissue and M2 macrophage polarization, thereby reducing obesity (Suarez-Zamorano et al., [2015](#)). The translocation of pro-inflammatory molecules from the gut to adipose tissues and to the liver is an area of intensive research (Pabst et al., [2023](#)). It is, however, unclear what the pathophysiological meaning of a so-called chronic, low-grade inflammation as proposed in overweight and obesity, really is, and what the consequences are for the development of metabolic comorbidities. Underlying mechanisms may take years prior to the establishment of phenotypes and are thus difficult to test experimentally. One human study reported that interference with the gut microbiota by a 7-day antibiotic treatment had no clinically relevant impact on metabolic health and did not induce significant changes in blood concentrations of LBP (lipopolysaccharide-binding protein), IL-6, IL-8, and TNF used as a proxy for systemic inflammation (Reijnders et al., [2016](#)).

16.4.3 Bacterial Meet Host Metabolism

Despite a renewed interest for the impact of gut microbiota on metabolic health and intensive research in the past decade, we are still not able to define precisely the caloric benefit for humans provided by the microbes in their gastrointestinal tract. The effects of microbial

colonization on intestinal tissue maturation impacting absorption capacities are also unclear.

Comparisons between germfree and conventional animals and microbiota transfer experiments have been instrumental in revealing the role of gut microorganisms in host metabolism, but they did not deliver absolute quantitative values on the contribution of the gut microbiota to energy homeostasis. In 2004, Bäckhed and colleagues demonstrated that colonization of germfree mice increased adiposity by a factor of approx. 1.4 despite lower feed intake by the germfree mice (Backhed et al., [2004](#)). However, early work in the 1960s had reported that growth differences between germfree and conventional animals depend on the reference microbiota that can vary across facilities (Pleasant, [1968](#)). Heterogeneity of models also concerns diet composition, which impacts the resistance of germfree mice to diet-induced obesity, as detailed in the “Controversy” box. In humans, following the proposition that ratios in the relative abundance of the two major phyla, *Bacillota* (formerly Firmicutes) and *Bacteroidota* (formerly Bacteroidetes), were related to obesity, Jumpertz et al. performed dietary intervention studies and estimated that a 20% increase in *Bacillota* and a corresponding decrease in *Bacteroidota* were linked to an increase of approx. 150 kcal/d in net energy retained, as estimated by measurement of energy loss in feces (Jumpertz et al., [2011](#)). However, the relevance of the Firmicutes-to-Bacteroidetes ratios is debatable, as detailed in the “Controversy” box.

A recent study measured food intake, digestion efficiency, energy expenditure, and respiratory quotient in gnotobiotic, male C57BL/6 J mice stably colonized with a community of 12 bacterial strains (Oligo mice) (Brugiroux et al., [2016](#)), enabling detailed energy balance assessment under strictly controlled conditions (Hoces et al., [2022](#)). Compared with normally colonized (SPF) and Oligo mice, germfree mice fed a standard chow diet until 12–14 weeks of age were characterized by decreased white adipose tissue mass (ca. -275 mg), increased daily food intake (ca. +0.5 g/d), and lower fecal energy density (ca. -0.15 kcal/g) but higher daily fecal dry mass (ca. +0.5 g/d).

The higher food consumption in germfree mice counterbalanced the higher energy excretion in feces, such that all mice extracted around 9 kcal/d from their food. Based on their measurements and assuming certain parameters (dry mass of a bacterium, energy stored in bacteria), the authors estimated that the fecal microbiota of colonized mice can contribute between 0.11 and 0.17 kcal/g of dry fecal mass; they concluded that, although fecal bacteria improved energy release from food, a considerable fraction of that energy remained stored in the bacteria present in feces. They further observed that the absence of microbiota did not influence daily energy expenditure in metabolically active tissues. The respiratory exchange ratio (RER) was consistently lower in germfree vs. colonized mice at all times of the day, indicative of more reliance on fat and less on glucose metabolism. Further, the RER varied between Oligo and SPF mice depending on active/resting (dark/light) phase, suggesting the existence of microbiota functions required to maintain normal host metabolism during the resting/fasting phase of the circadian cycle, which were absent in the Oligo mice.

Increased energy harvest from the diet by the gut microbiome has been proposed to underlie the development of obesity in a manner that is transmissible to offspring (Turnbaugh et al., [2006](#)). One underlying hypothesis is that high-fat diet feeding is associated with increased production of short-chain fatty acids (SCFA), primarily butyrate, used as a fuel for host cell metabolism. It has been reported that intestinal epithelial cells may derive up to 70% of their energy supply from butyrate oxidation (Roediger, [1980](#)). Moreover, studies in lambs showed that butyrate infusion in the abomasum (the fourth and final stomach compartment in ruminants) increased net nutrient flux to the portal vein and hepatic tissue (Foote & Freetly, [2016](#)). Under steady-state conditions, SCFA are produced primarily via the fermentation of complex carbohydrates by intricate bacterial trophic chains (Louis & Flint, [2017](#)). In diet-induced obesity, where diets contain low amounts of carbohydrates, butyrate may arise from the metabolism of amino acids, although it is questionable whether this can sustain production

of butyrate amounts higher than in steady-state conditions (Bui et al., [2015](#); Daniel et al., [2014](#)). Harry Flint and colleagues showed that reduced dietary intake of carbohydrates by obese subjects resulted in decreased amounts of butyrate and butyrate-producing bacteria in feces (Duncan et al., [2007](#)). Total SCFA amounts in the gut content of obese hosts may, however, increase in favor of SCFA other than butyrate. In Schwartz et al., [2010](#), Schwartz and colleagues reported that SCFA concentrations were higher in obese vs. lean subjects and that the proportion of individual SCFA changed in favor of propionate in overweight and obese subjects (Schwartz et al., [2010](#)), propionate being usable as a substrate for gluconeogenesis in the liver. Generally speaking, butyrate is a hallmark of normal fermentative processes within stable gut microbial communities, and disruption of this metabolic network has been associated with detrimental effects on host metabolism (Le Chatelier et al., [2013](#)). In mice, butyrate treatment by daily intragastric administration attenuated high-fat diet-induced steatohepatitis by improving the gastrointestinal barrier (Zhou et al., [2017](#)). In addition, a recent clinical study reported beneficial effects of oral butyrate treatment in pediatric obesity (Coppola et al., [2022](#)). In summary, whilst fermentative processes within gut microbiomes are altered following the intake of high-calorie diets and in the context of metabolic diseases, there is little evidence for a causal role of butyrate in obesity development under detrimental dietary conditions.

Another way by which the gut microbiota can regulate metabolic responses is via the production of bioactive metabolites. Although the array of molecules produced by the microbiota is vast, the majority is still unknown, and only a few bacterial metabolites are yet known to influence host metabolism. The case of branched-chain amino acids and associated bacterial species was introduced above (see Sect. [16.2](#)). Imidazole propionate, a microbially produced metabolite of histidine with elevated serum levels in prediabetes and diabetes individuals, was shown to impair insulin signaling and affect response to metformin (Koh et al., [2018](#), [2020](#); Molinaro et al., [2020](#)). Hydrogen sulfide produced by the activity of sulfate-reducing bacteria is also known to

influence metabolic capacities of the intestinal epithelium (Blachier et al., [2017](#)), although the impact on host metabolism overall is unclear. Lipids represent a major and very diverse class of compounds at the crossroad between diet, microbes, and the host. Whilst the conversion of long-chain fatty acids by microbial communities is known in environmental ecosystems (Alves et al., [2009](#)), bacterial metabolism of dietary fatty acids in the intestine is unclear. Gut bacteria are so far only known to cleave triglycerides via lipase activity and to produce conjugated fatty acids, the latter compounds having well-documented effects on host metabolism (see Sect. [16.5.5](#)) (McIntosh et al., [2009](#); Thorasin et al., [2015](#)). Gut bacteria can also metabolize bile acids produced from cholesterol in the liver. This bacterial conversion of bile acids has major consequences on their bioavailability and bioactivities (Ridlon et al., [2006](#)) and is of primary interest considering the clinical relevance of bile acids in metabolic health (Ma et al., [2017](#); Joyce et al., [2014](#); Yao et al., [2018](#); Mudaliar et al., [2013](#); Thomas et al., [2008](#)). In the following section, we provide further details on bioactive lipid molecules and the intricate interactions between the gut microbiota and host lipid metabolism.

16.5 Gut Microbiota and Host Lipid Metabolism

A wide range of lipid molecules exist in nature. Fatty acids (FAs) are key modules for various lipids, including cell membrane lipids such as glycerophospholipids or triacylglycerols (TAG), which are the major components of lipid droplets (Ecker & Liebisch, [2014](#); Wenk, [2010](#)). Excess lipids or defects in lipid storage are associated with diseases such as the metabolic syndrome, which results from unbalanced energy intake and expenditure as well as complex interactions between genetic and environmental factors, including the gut microbiota (Ussar et al., [2015](#)).

Lipids originate either from exogenous sources, i.e., diet, or from endogenous biosynthesis. They serve as precursors of important

signaling molecules such as eicosanoids with either pro- or anti-inflammatory effects (Schmitz & Ecker, [2008](#)). Numerous cellular processes, such as cell growth or differentiation, rely on de novo synthesis of FA primarily for cell membrane generation (Ecker et al., [2010a](#); van Meer et al., [2008](#)).

16.5.1 Lipid Synthesis

The transcription factors sterol regulatory element-binding proteins (SREBP), which belong to the family of basic helix-loop-helix leucine zipper transcription factors, are major regulators of cellular lipid synthesis in mammalian cells (Horton et al., [2002](#)). SREBP-2 mainly controls genes of the cholesterol pathway, and SREBP-1c preferentially regulates genes involved in FA biosynthesis. Fatty acid synthase is a well-characterized SREBP-1c target gene and a key enzyme required for endogenous FA synthesis in mammalian cells because it catalyzes the generation of palmitate (FA 16:0) from acetyl-CoA (Smith et al., [2003](#)). Palmitate is either desaturated to palmitoleate (FA 16:1 *n*-9) by stearoyl-CoA desaturase (SCD) 1 or elongated to stearate (FA 18:0) by long-chain fatty acid elongase 6 (Matsuzaka et al., [2007](#); Miyazaki & Ntambi, [2003](#)). Stearate can be desaturated to oleate (FA 18:1 *n*-9) by SCD1, and palmitoleate can be elongated to vaccinate (FA 18:1 *n*-7) by long-chain fatty acid elongase 6.

SCD1 is a critical enzyme that has been linked to various diseases, including diabetes, hypertriglyceridemia, cardiovascular disease, steatosis, bone health, and cancer (Hodson & Fielding, [2013](#); Matsuzaka et al., [2007](#)). Inhibition of SCD1 leads to severe atherosclerosis despite protecting from obesity, whereas increased SCD1 expression levels were shown to protect from endoplasmic reticulum (ER) stress and atherosclerosis (Brown et al., [2008](#); Erbay et al., [2009](#)). Dendritic cells and macrophages, which originate from common myeloid precursor cells, significantly differ in their profiles of monounsaturated fatty acids (MUFA) and phospholipids due to different SCD1 activities (Ecker et al., [2010a](#)). SCD1 activity is absolutely required for autophagy, a major pathway for degradation of cytoplasmic components (Ogasawara et al.,

[2014](#)), and in proliferating cancer cells (Snaebjornsson et al., [2020](#); Peck et al., [2016](#)). Recently, some cancer cells, such as those from hepatocellular, primary human liver and lung carcinomas, were shown to have even the capacity to desaturate palmitate to sapienate (FA 16:1 *n*-9) via fatty acid desaturase 2 (FADS2), causing independence from SCD1 and increasing cancer plasticity (Vriens et al., [2019](#)).

Linoleic acid (FA 18:2 *n*-6) and α -linolenic acid (FA 18:3 *n*-3) are precursors of polyunsaturated fatty acids (PUFA) and thus important building blocks of both pro- and anti-inflammatory mediators (Buckley et al., [2014](#); Schmitz & Ecker, [2008](#)). They are essential fatty acids because, unlike plants, mammals do not express delta-12 and delta-15 desaturases under physiological conditions, which are necessary to desaturate FA 18:1 and FA 18:2 (Pereira et al., [2003](#)). Under essential fatty acid deficiency, mead acid (FA 20:3 *n*-9) may be produced after delta-6 and delta-8 desaturation of FA 18:1 *n*-9 and accumulates in cells and tissues (Hayashi et al., [2021](#); Mead, [1956](#)). A crucial organ for lipid synthesis is the liver, which plays critical roles in many metabolic diseases, especially type 2 diabetes (Moller, [2001](#)).

16.5.2 Gut Microbiota and Host Lipid Synthesis in Metabolic Diseases

To date, a few studies have investigated the effects of the gut microbiota on host lipid synthesis. One of the first studies that linked the gut microbiota to host lipid status was published in 2004 by Bäckhed and colleagues ([2004](#)). They compared germfree and conventionalized mice and concluded that the microbiota promotes the absorption of monosaccharides from the gut, leading to an induction of hepatic FASN mRNA expression via the transcription factors carbohydrate response element-binding protein and perhaps SREBP-1. They also proposed that the presence of gut microorganisms is associated with increased lipoprotein lipase (LPL) activity through suppression of intestinal expression of fasting-induced adipocyte factor (FIAF), a circulating LPL inhibitor (Fig. [16.2](#)). LPL hydrolyzes TAG to free FA from lipoproteins and leads to deposition of FFA in adipose tissues and TAG storage. In

2007, the same research group reported that germfree animals are protected from diet-induced obesity using a Western diet rich in simple sugars and beef tallow (Backhed et al., [2007](#)) (see the “Controversy” Box). Germfree mice had higher levels of phosphorylated AMP-activated protein kinase (AMPK) in the liver and skeletal muscle, and its downstream targets were involved in β -oxidation.

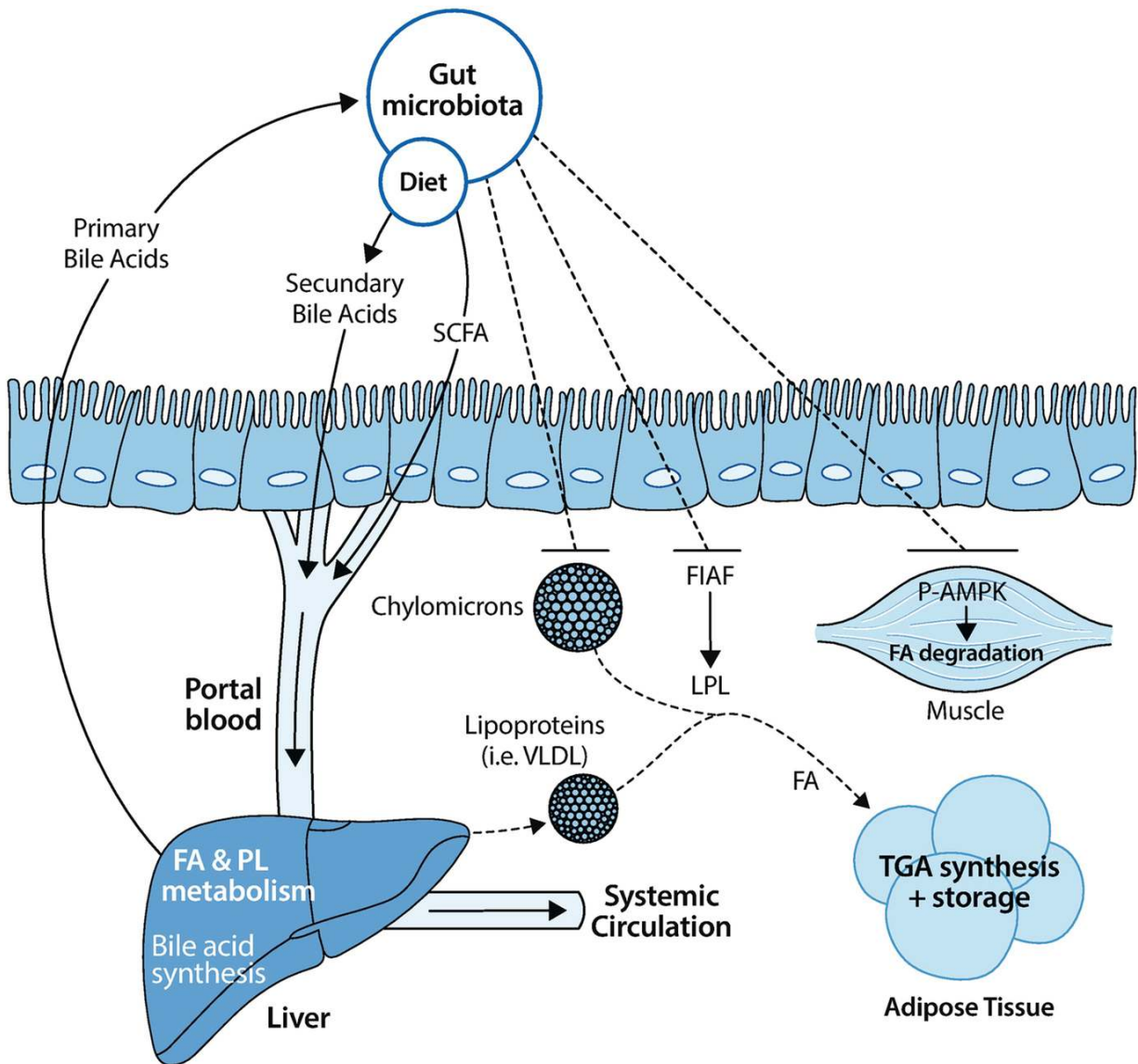


Fig. 16.2 The gut microbiota and host lipid metabolism. After fermentation of diet- and host-derived substrates (primarily carbohydrates but also proteins), the gut microbiota produces short-chain fatty acids (SCFA), such as acetate, propionate, and butyrate. Gut bacteria can also convert primary to secondary bile acids via deconjugation, dehydroxylation, and dehydrogenation. SCFA and secondary bile acids reach the liver and circulation via the portal vein and can alter host lipid

metabolism and inflammatory responses. The gut microbiota also influences fatty acid (FA) synthesis and desaturation, phospholipid acyl-chain remodeling in the liver, and beta-oxidation of FA in muscles. It is still unclear whether circulating levels of fasting-induced adipocyte factor (FIAF), a lipase inhibitor, are influenced by microbial colonization. Reduced FIAF may enhance lipoprotein lipase (LPL) activity and thus lead to degradation of lipoproteins and deposition of free fatty acids in adipose tissue, which are precursors for triacylglycerols (TAG). Future studies should also verify whether the intestinal chylomicron output can be inhibited by the gut microbiota

In 2010, Fleissner et al. ([2010](#)) reported that germfree mice fed a high-fat diet did not show major changes in circulating FIAF levels compared to conventional animals. These controversial findings show that the type of diets fed to mice, which can vary in caloric density, texture, amount, and composition of macronutrients and micronutrients, is very important (Clavel et al., [2014](#)). Unfortunately, in most studies dealing with the microbiome in models of metabolic diseases, information about the diets used is not sufficiently detailed.

A number of studies have shown that SCFA can directly inhibit hepatic FA synthesis and fat storage in white adipose tissue while enhancing β -oxidation in multiple tissues (den Besten et al., [2013](#)) (Fig. [16.2](#)). Our understanding of underlying molecular mechanisms is still incomplete. It is discussed that SCFA activate AMPK in the liver and muscles either directly or indirectly via free fatty acid receptor 2 (also known as GPCR 43), which suppresses insulin-mediated fat accumulation and promotes energy expenditure (Kimura et al., [2013](#)).

On the other hand, acetate originating from gut microbial fermentation of dietary fiber serves as a precursor for hepatic synthesis of C16 and C18 fatty acids and their related glycerophospholipid species that are also released into the circulation in mice (Fig. [16.2](#)) (Kindt et al., [2018](#)). Acetate levels in the portal vein, connecting the intestine with the liver, are ~ 0.5 mM in mice, rats, and humans quite high (Cummings et al., [1987](#); Jakobsdottir et al., [2013](#)). Antibiotic treatments eradicating major acetate producers in the murine gut lumen decreased lipid synthesis in the liver, including FASN and SCD1 expression (Kindt et al., [2018](#)). Diets containing extraordinarily high fiber contents promote, while non-degradable fiber sources blunt microbiota-driven hepatic FA and glycerophospholipid synthesis.

In the context of pathologies, a central role of this microbiome-dependent lipid metabolic pathway was shown for liver regeneration (Yin et al., [2023](#)). Partial hepatectomy (PHx) is a common clinical procedure for patients with primary liver tumors, hepatic metastasis, or parasitic cysts. Liver size and function have to be restored quickly after PHx to achieve optimal patient recovery and survival. This requires high proliferation capacity of the hepatocytes, which depends on membrane phospholipid synthesis. Antibiotic treatments decrease hepatic lipid synthesis, cell proliferation, and liver regeneration, strongly increasing mortality in mice. Germfree mice almost completely fail to regenerate their livers after PHx, but colonization with the synthetic community OMM12 can rescue hepatic phospholipid synthesis, cell proliferation, and liver regeneration (Yin et al., [2023](#)).

Toll-like receptor 5-deficient mice, which are prone to develop microbiota-dependent metabolic syndrome, have increased hepatic SCD1 expression and oleic acid (FA18:1 *n*-9) levels (Singh et al., [2015](#)). This finding might be of particular importance, since the phospholipid saturation degree contributes substantially to its flexibility. Membranes undergoing fast morphological changes contain high levels of polyunsaturated fatty acids (PUFA) (Ernst et al., [2016](#); Pinot et al., [2014](#)). Further studies have to clarify whether these gut microbiota-driven alterations of host FA and phospholipid metabolism affect biophysical membrane properties or signaling processes like eicosanoid generation and thus impact general physiology as well as inflammatory and metabolic diseases.

16.5.3 Lipid Resorption

Resorption of dietary lipids is a three-step process comprising absorption of dietary FAs from the intestinal lumen, further trafficking from the apical to the basolateral site of enterocytes, and their secretion into the circulation.

Dietary lipids are hydrolyzed in the intestinal lumen by pancreatic lipases; thereby, TAG are converted to free FA and 2-monoacylglycerols (Barret et al., [2010](#); Hussain, [2014](#)). Lipids are in general relatively

water-insoluble; therefore, a key step is their emulsification with bile acids (BA), making lipids better substrates for hydrolysis by lipases (Barret et al., [2010](#)). High levels of BA in the intestine (after contraction of the gallbladder) also induce the formation of micelles, which contain FA, cholesterol, and monoacylglycerols. Micelle formation solubilizes lipids and provides an effective mechanism for diffusion to the enterocytes. When FA concentrations are higher in the lumen than in enterocytes, the micelles move down their concentration gradient to the brush border of the mucosal cells, and the lipids may diffuse from micelles to enterocytes or are actively transported by membrane-associated proteins such as CD36, also known as FA translocase (Glatz et al., [2010](#)). In humans, the jejunum seems to be the major site of fat absorption in case of moderate food intake; the ileum becomes more important in case of a higher intake of dietary fat (Booth et al., [1961](#)).

Once FAs have entered enterocytes (from the apical site), they are transported to various organelles by FA-binding proteins and shuttled to intracellular membranes, mainly to those of the ER, where they are esterified with monoacylglycerols by monoacyltransferases to form diacylglycerols (DAGs) (Hussain, [2014](#); Hussain et al., [2013](#)). DAG can be further converted to TAG by diacylglycerol acyltransferases. For lipid secretion into the circulation, lipids are packaged into chylomicrons (CMs) in the enterocyte and secreted to the lymph. During circulation, CMs are hydrolyzed by LPL so that their products, including free fatty acids, can enter peripheral tissues. The rest of the CM forms remnant particles, the so-called CM remnants, which are removed from the circulation by the liver. FA containing less than 10–12 carbons are enough water-soluble to pass the enterocyte unmodified and are actively transported as free FA into the portal blood (Barret et al., [2010](#)).

16.5.4 Gut Microbiota and Lipid Resorption

It is hypothesized that the gut microbiota influences intestinal FA absorption, via, for instance, modulation of the luminal bile acid pool or the expression of transporters. Transit time may also influence lipid

absorption; studies in the 1960s showed that intestinal transit is faster in colonized vs. germfree mice (Abrams & Bishop, [1967](#)). While microbial colonization stimulated FA uptake and lipid droplet formation in the intestinal epithelium and liver of zebra fish, as determined using in vivo imaging and fluorescent FA analogues (Semova et al., [2012](#)), the situation in mammals is unclear. It is suggested that germfree mice have a significantly higher uptake and metabolism of dietary lipids, since elevated levels of PUFA, i.e., DHA (FA22:6 $n-3$), which are of dietary origin, were detected in the plasma, liver, and small intestinal tissue (Kindt et al., [2018](#); Liebisch et al., [2021](#)). Additionally, 40% lower CM levels were measured in the serum of conventional mice vs. GF mice (Velagapudi et al., [2010](#)). The fermentation products lactate and acetate from *Lactobacillus paracasei* and *Escherichia coli* impede CM secretion from mouse enterocytes (Araújo et al., [2020](#)). In contrast, *Clostridium bifementans* promoted TG uptake in mice fed long term with a high-fat diet (Martinez-Guryn et al., [2018](#)). A very recent study reported that small nucleolar RNA host gene 9 (Snhg9), whose expression in small intestinal epithelial cells depends on the presence of gut microbes, regulates lipid absorption and body fat accumulation (Wang et al., [2023](#)). Snhg9 interacts with cell cycle and apoptosis protein 2 (CCAR2), which inhibits sirtuin (SIRT1), a repressor of peroxisome proliferator-activated receptor γ (PPAR γ), a key transcription factor regulating host lipid metabolism. Vilin-driven Snhg9 overexpression in intestinal epithelial cells of mice limits intestinal lipid absorption and protects against diet-induced obesity.

16.5.5 Lipids as Bioactive Molecules Relevant for Microbe-Host Interactions

The intestinal epithelium is the largest and a very important interface to the external environment (Peterson & Artis, [2014](#)). Although the intestinal mucosa forms a rather tight barrier, it must also allow efficient absorption of nutrients and is in permanent contact with the luminal content, including microorganisms, their cellular components,

and metabolic products. The following lipids are considered to play an important role in microbe–host interactions:

1.

SCFA

Short-chain fatty acids are generated primarily by fermentation of complex carbohydrates by the gut microbiota, reach the circulation via the portal vein, and can then alter host metabolism and physiology substantially. Additionally to their diverse effects on the liver, muscle, and adipose tissue lipid metabolism (see Sect. [16.5.2](#)), SCFA can activate gene expression of enzymes involved in intestinal gluconeogenesis either through a cAMP-dependent mechanism (in the case of butyrate) or via a gut-brain neuronal circuit involving the free fatty acid receptor (FFAR) 3 (in the case of propionate) (De Vadder et al., [2014](#)).

2.

Bile acids

Bile acids are synthesized from cholesterol in the liver and can be metabolized to so-called secondary bile acids by bacteria in the gut (Fleishman & Kumar, [2024](#)). Besides their role in the formation of mixed micelles for lipid absorption, bile acids were shown to act as signaling molecules via membrane-bound G-protein-coupled receptors (GPCR) or via nuclear farnesoid X receptors (FXR) (Sayin et al., [2013](#)). FXR controls the expression of genes encoding enzymes involved in bile acid synthesis, metabolism, and transport of bile acids in the liver and the sinusoid (Lefebvre et al., [2009](#)).

3. *Phosphatidylcholine (PC)*

Choline derived from dietary PC can be metabolized by the gut microbiota to trimethylamine (TMA), which is further transformed by the liver to trimethylamine-N-oxide (TMAO). A metabolomics study in a large clinical cohort originally identified trimethylamine-N-oxide (TMAO) as a predictive marker for cardiovascular disease and showed that dietary supplementation of TMAO can promote atherosclerosis in mice (Wang et al., [2011](#)). Antibiotics-induced disturbances in the gut microbiota in atherosclerosis-prone mice inhibit atherosclerosis promoted by dietary choline.

...and atherosclerosis promoted by dietary cholesterol.

4.

Conjugated fatty acids (CFA)

Conjugated FA is a collective term for positional and geometric isomers of FA with conjugated double bonds. CFA can be metabolized from linoleic acid by human intestinal bacteria, including *Lactobacillus*, *Propionibacterium*, and *Bifidobacterium* species to different conjugated linoleic acid isomers such as *cis*-9, *trans*-11-CLA, *trans*-9, *trans*-11-CLA, or *trans*-10, and *cis*-12-CLA. (Devillard et al., [2007](#); McIntosh et al., [2009](#)). A major source for CLAs is also the diet, since ruminal bacteria such as *Butyrivibrio fibrisolvens* can metabolize LA to CLA (Wallace et al., [2007](#)). CLA isomers can reach the circulation and peripheral tissues (Kuhnt et al., [2006](#); Wall et al., [2009](#)). Several data from in vitro and animal studies show that CLA isomers are beneficial and inhibit the progression of several diseases, including cardiovascular and inflammatory diseases, as well as cancer (Bassaganya-Riera & Hontecillas, [2006](#); Kelley et al., [2007](#); Mitchell & McLeod, [2008](#)). CFA are metabolized in colonic cells, where they can influence cellular lipid synthesis and degradation by modulating peroxisome proliferator-activated receptor (PPAR) and liver X receptor (LXR) activity and integrate into cell membranes and neutral lipids (Degen et al., [2011](#); Ecker et al., [2009](#); [2010b](#); Moya-Camarena et al., [1999](#)).

5. *Hydroxy-fatty acids*

Whereas fatty acid saturation does not occur in mammals, intestinal lactic acid bacteria such as *Limosilactobacillus plantarum* can saturate PUFA, thereby generating hydroxy-fatty acids, which can be detected in intestinal and plasma samples of mice (Kishino et al., [2013](#)). In particular, higher levels of 10-hydroxy-fatty acids derived from linoleic and oleic acids can be detected in SPF vs. GF mice. Although in vivo functional studies have not yet been performed, it is clear that hydroxy-fatty acids act as ligands for PPAR γ , a central transcription factor for lipid metabolism and

storage involved in various metabolic diseases (Itoh et al., [2008](#)).

16.5.6 Lipidomics and the Need to Improve Data Quality

The key technology for lipid analysis is mass spectrometry. Because lipidomic approaches are nowadays able to cover almost the full lipidome (Wenk, [2010](#)), the field is currently drawing much attention as it opens new ways to study lipid biology in numerous research areas, including gut microbiome research. However, this comes along with an increasing number of studies that report poor quality lipidomics data with false identification and inaccurate/inappropriate quantification of lipid molecules (Liebisch et al., [2017](#)). Untargeted metabolomics approaches are particularly critical and rarely suitable for lipidomic investigations (Liebisch et al., [2015](#)). Adequate controls of sample handling and processing (e.g., to assure analyte stability) are often missing, as is the use of appropriate internal standards, which are essential for proper quantification and identification of lipid species and for lipid annotation. The application of stable isotope-labelled tracers combined with reliable mass spectrometric analysis is a state-of-the-art approach to gain insight into synthesis, metabolism, and fluxes of lipid species (Ecker & Liebisch, [2014](#)). Recent recommendations for scientists analyzing lipid species and reporting lipid data should enhance the lipidomic field's consistency, comparability, and repeatability (Kopczynski et al., [2024](#); Skotland et al., [2024](#)).

16.6 Conclusions

The gut microbiota is not the primary cause of obesity but contributes to interindividual differences in body weight gain and the development of comorbidities. Gut microorganisms also play an important role in malnutrition. Current needs in the field are depicted in Fig. [16.3](#). The functional importance of gut microbial communities has been demonstrated many times via microbiota transfer experiments, and we are just starting to identify individual microbial species with underlying mechanisms and bioactive molecules. It is important to remember that

gut bacteria are, per se, not good or bad, that strain-dependent functional differences exist, and that the effects of specific strains depend on the microenvironment in which they live. Hence, results must always be generalized with caution. The major task in the coming years will be to move away from descriptive work and intensify functional analysis, in order to better define the exact contribution of gut microbes to energy homeostasis and to learn how to manipulate this microbe–host crosstalk safely and with efficacy.

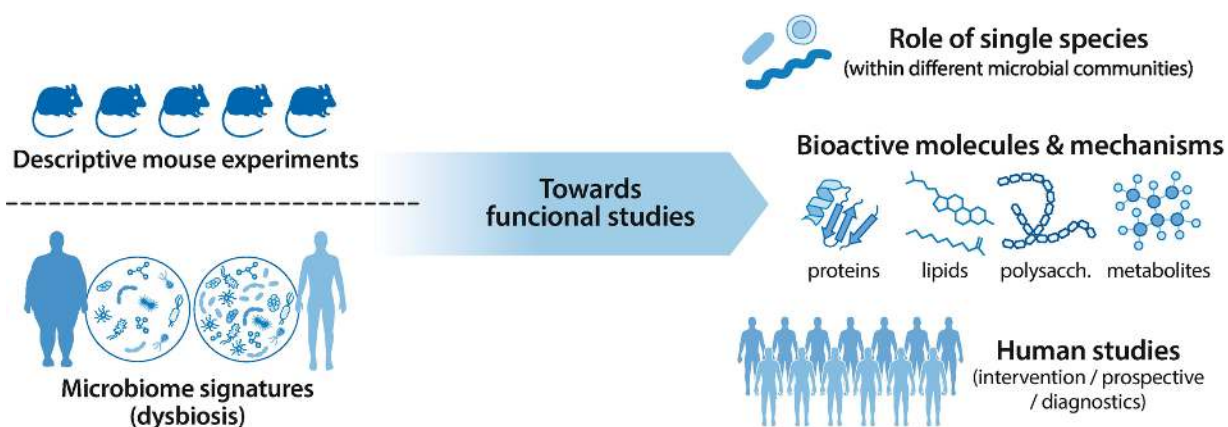


Fig. 16.3 Challenges and opportunities. Research in the field of microbe–host interactions in metabolic diseases delivered plentiful descriptive data in the past two decades. The massive investment in sequencing projects contrast to the limited number of robust microbiome signatures available to date (see Sect. 16.1). Also, efforts in finding key microbial players (single species) with a functional role in host metabolism should continue (see Sect. 16.3). Whilst the major advantage of animal experiments is the ability to test causality, perform functional studies, and characterize molecular mechanisms, many descriptive mouse studies have been published in the past. Human and molecular studies are now needed. Large, prospective studies (where individuals are followed over time) will allow us to assess the causal role of microbiome changes in the development of chronic diseases. In the clinic, it will be important to intensify work on diagnostic approaches and intervention strategies. These activities should be accompanied by the identification of additional gut microbiota-derived molecules with an impact on host metabolism, including the determination of their modes of action

Controversy Research on the gut microbiome in metabolic health is still in its infancy but has reached already the broadcast media on several occasions and is thus under the spotlight. This situation contributed to the establishment of a few misleading concepts in the field, of which we want to highlight two: (1) Firmicutes-to-Bacteroidetes ratio and (2) resistance of germfree mice to diet-

induced obesity. We cannot present these topics in the utmost detail here. We simply want to stress the fact that the research community must be careful before turning single findings into dogma that reaches a standing that goes beyond scientific evidence. As noted by others (Kennedy et al., [2023](#); Perez-Munoz et al., [2017](#)), the scientific self-correction process is nowadays slower than the transfer of information to the public, and it takes quite some efforts to verify the validity of findings that attracted the spotlight.

1. Firmicutes (current valid name *Bacillota*) and Bacteroidetes (current valid name *Bacteroidota*) are the two major phyla of bacteria in the gut of human individuals and other mammals. Ley and colleagues reported in 2005 that genetically obese (ob/ob) mice were characterized by a 50% reduction in Bacteroidetes and a proportional increase in Firmicutes (Ley et al., [2005](#)). The same authors then showed in 2006 that the Firmicutes-to-Bacteroidetes ratio was increased in feces of 12 obese human subjects compared with seven control stool samples from lean individuals (Ley et al., [2006](#)). The ratio decreased during therapy with low-calorie diets over a period of 1 year. Following this pioneering work, several other groups reported no or inverse changes in the Firmicutes-to-Bacteroidetes ratio (Duncan et al., [2008](#); Schwiertz et al., [2010](#)). A recent population-based analysis of fecal microbiota using sequencing estimated that at least >500 samples are required to reliably assess the association between gut microbial populations and body mass index. Moreover, phyla correspond to very high taxonomic levels and thus offer low resolution of analysis due to the tremendous diversity of species they contain. Low relative abundance of Bacteroidetes in obesity may make sense in view of the potential of *Prevotella* spp. to degrade fibers, but less with respect to the possible association between *Bacteroides* spp. and dietary fat (De Filippis et al., [2016](#); Kovatcheva-Datchary et al., [2015](#); Wu et al., [2011](#)). Ley and colleagues recently revisited their own view about the phylum Bacteroidetes in the context of metabolic diseases (Johnson et al.,

[2017](#)). Hence, the Firmicutes-to-Bacteroidetes ratio is by no means a reference parameter with proven validity (Walker et al., [2023](#)).

2.

In 2007, Bäckhed and colleagues published that germ-free mice fed with a Western diet did not put on weight as their colonized counterparts (Backhed et al., [2007](#)). They concluded that a hallmark of the absence of gut microbes is resistance against diet-induced obesity. In following years, Blaut and colleagues could not reproduce these findings and eventually published in 2010 that the resistance to obesity is driven by the type of diet fed to germfree mice: those fed with the Western diet as used by Bäckhed et al., characterized by higher amounts of simple sugars and fat from beef tallow, were indeed resistant to obesity, whereas those animals fed with a high-calorie diet containing wheat starch and fat from coconut oil were not (Fleissner et al., [2010](#)). Later, Klingenspor and colleagues went one step further and proposed that cholesterol is a major driver of the obesity-resistant phenotype in germfree mice (Kubeck et al., [2016](#)). By using diets that differed only by their type of fat, the authors showed that germfree mice fed with a diet rich in palm oil became obese, whereas those fed with a lard-based high-fat diet stayed lean due to increased energy expenditure, preferential carbohydrate oxidation, and increased fecal fat and energy excretion. Altogether, these findings do not refute the importance of gut microbes in energy homeostasis but draw the attention to complex diet-gut microbiome interactions and their potential influence on experimental models. It is misleading to state that germfree mice are resistant to diet-induced obesity in general, without reference to the diet used.

History

Over millions of years of evolution, our bodies have developed efficient mechanisms to harvest and store energy from food, including the interaction with gut microbes. These mechanisms have somehow

been compromised in today's era of food abundance and high-calorie diets in industrialized countries and in the thriving cities of non-industrialized countries. Diet has been a key factor in our evolution, and the microbes that colonize our gut can metabolize a wide range of nutrients and have coevolved with us, leading to the establishment of complex diet-microbe-host networks underlying energy regulation. Interactions between the diet and gut microbes and the consequences for human health were a subject of interest already at the beginning of the twentieth century (Rettger, [1915](#)). Ilya Metchnikov, the Nobel Prize Laureate for Physiology and Medicine in 1908, was very interested in the impact of lactic acid bacteria and fermented milk products on health and proposed that "aging was the result of a continuous intoxication caused by intestinal bacteria." The idea of rearing animals under sterile (germfree) conditions and observing changes in host physiology bloomed later in the 1960s when the interplay between diet and gut colonization by commensals and pathogens was studied in more detail (Dubos et al., [1969](#); Dubos & Schaedler, [1960](#); Wostmann, [1975](#)). Interestingly, it was reported that growth differences between germfree and conventional mice varied from one laboratory to another, most likely due to the instability of conventional controls (Pleasant, [1968](#)). Facility-dependent colonization of the mouse gut was used decades later to identify specific gut bacteria having distinct effects on the immune system (Ivanov et al., [2009](#)). Also in the clinics, researchers in the 1980s were already interested in studying changes in the gut microbiota after gastric bypass, with limitations of the methods available at the time (Bjornekleit et al., [1981](#)). Hence, current research activities have precedents in the literature. The renewed interest in the role of gut microbes in metabolic health, driven by new molecular methods and the use of gnotobiology, is partly explained by the ever-rising prevalence of obesity and associated metabolic disorders, and raises hopes of modulating metabolic health via the gut microbiome. There is no doubt that gut microbial communities regulate host metabolic responses, but there is an urgent need to both define exactly how the

underlying microbe–host interactions work and demonstrate the efficacy of new microbiome-based intervention strategies.

Highlights

- The gut microbiota is a very complex and dynamic ecosystem with a tremendous metabolic potential.
- Its causal role in malnutrition and the development of chronic metabolic disorders has been demonstrated via microbiota transfer experiments.
- Besides loss in ecosystem diversity and the identification of a few bacterial species with specific metabolic effects on the host, no consistent change in the gut microbiota can yet be associated with the development of metabolic disorders in a predictive way.
- Many bacterial members of the ecosystem, including those species regulating metabolic responses, and the molecules involved, remain to be identified.
- Lipids (e.g., short-chain fatty acids, bile acids, lipopolysaccharides, conjugated fatty acids) and the crosstalk between lipid metabolism and the gut microbiota are important for host metabolic health.
- More human studies, including microbiome-based personalized approaches, are required to bridge the gap between theoretical knowledge and clinical relevance.

References

Abrams, G. D., & Bishop, J. E. (1967). Effect of the normal microbial flora on gastrointestinal motility. *Proceedings of the Society for Experimental Biology and Medicine*, 126, 301–304. [\[PubMed\]](#)

Ajslev, T. A., Andersen, C. S., Gamborg, M., Sorensen, T. I., & Jess, T. (2011). Childhood overweight after establishment of the gut microbiota: The role of delivery mode, pre-pregnancy weight and early administration of antibiotics. *International Journal of Obesity*, 35, 522–529. [\[PubMed\]](#)

Almeida, A., Nayfach, S., Boland, M., Strozzi, F., Beracochea, M., Shi, Z. J., Pollard, K. S., Sakharova, E., Parks, D. H., Hugenholtz, P., Segata, N., Kyrpides, N. C., & Finn, R. D. (2021). A unified catalog of 204,938 reference genomes from the human gut microbiome. *Nature Biotechnology*, 39(1), 105–114.

[\[PubMed\]](#)

Alves, M. M., Pereira, M. A., Sousa, D. Z., Cavaleiro, A. J., Picavet, M., Smidt, H., et al. (2009). Waste lipids to energy: How to optimize methane production from long-chain fatty acids (LCFA). *Microbial Biotechnology*, 2, 538–550.

[\[PubMed\]](#)

Amato, A., Baldassano, S., & Mule, F. (2016). GLP2: An underestimated signal for improving glycaemic control and insulin sensitivity. *The Journal of Endocrinology*, 229, R57–R66.

[\[PubMed\]](#)

Arrieta, M. C., Walter, J., & Finlay, B. B. (2016). Human microbiota-associated mice: A model with challenges. *Cell Host & Microbe*, 19, 575–578.

Araújo, J. R., Tazi, A., Burlen-Defranoux, O., Vichier-Guerre, S., Nigro, G., Licandro, H., Demignot, S., & Sansonetti, P. J. (2020). Fermentation products of commensal bacteria alter enterocyte lipid metabolism. *Cell Host & Microbe*, 27(3), 358–375.e7.

Asnicar, F., Berry, S. E., Valdes, A. M., Nguyen, L. H., Piccinno, G., Drew, D. A., Leeming, E., Gibson, R., Le Roy, C., Khatib, H. A., Francis, L., Mazidi, M., Mompeo, O., Valles-Colomer, M., Tett, A., Beghini, F., Dubois, L., Bazzani, D., Thomas, A. M., et al. (2021). Microbiome connections with host metabolism and habitual diet from 1,098 deeply phenotyped individuals. *Nature Medicine*, 27(2), 321–332.

[\[PubMed\]](#)

Aydin, Ö., Wahlström, A., de Jonge, P. A., Meijnikman, A. S., Sjöland, W., Olsson, L., Henricsson, M., de Goffau, M. C., Oonk, S., Bruin, S. C., Acherman, Y. I. Z., Marschall, H.-U., Gerdes, V. E. A., Nieuwdorp, M., Bäckhed, F., & Groen, A. K. (2025). An integrated analysis of bile acid metabolism in humans with severe obesity. *Hepatology*, 81(1), 19–31.

[\[PubMed\]](#)

Azad, M. B., Bridgman, S. L., Becker, A. B., & Kozyrskyj, A. L. (2014). Infant antibiotic exposure and the development of childhood overweight and central adiposity. *International Journal of Obesity*, 38, 1290–1298.

[\[PubMed\]](#)

Backhed, F., Ding, H., Wang, T., Hooper, L. V., Koh, G. Y., Nagy, A., et al. (2004). The gut microbiota as an environmental factor that regulates fat storage. *Proceedings of the National Academy of Sciences of the United States of America*, 101, 15718–15723.

[\[PubMed\]](#)

Backhed, F., Manchester, J. K., Semenkovich, C. F., & Gordon, J. I. (2007). Mechanisms underlying the resistance to diet-induced obesity in germ-free mice. *Proceedings of the National Academy of Sciences of the United States of America*, 104, 979–984.

[\[PubMed\]](#)

Bailey, L. C., Forrest, C. B., Zhang, P., Richards, T. M., Livshits, A., DeRusso, P. A. (2014). Association of antibiotics in infancy with early childhood obesity. *JAMA Pediatrics*, 168(11), 1063–1069. <https://doi.org/10.1001/jamapediatrics.2014.1539>. PMID: 25265089.

Bajaj, J. S., Kassam, Z., Fagan, A., Gavis, E. A., Liu, E., Cox, I. J., et al. (2017). Fecal microbiota transplant from a rational stool donor improves hepatic encephalopathy: A randomized clinical trial. *Hepatology*, 66(6), 1727–1738. [\[PubMed\]](#)

Barret, K., Brooks, H., Boitano, S., & Barman, S. (2010). Chapter 27: Digestion, absorption, and nutritional principles. In *Ganong's review of medical physiology. In Lange medical book* (pp. 451–467).

Bassaganya-Riera, J., & Hontecillas, R. (2006). CLA and n-3 PUFA differentially modulate clinical activity and colonic PPAR-responsive gene expression in a pig model of experimental IBD. *Clinical Nutrition*, 25, 454–465. [\[PubMed\]](#)

Bikel, S., López-Leal, G., Cornejo-Granados, F., Gallardo-Becerra, L., García-López, R., Sánchez, F., Equihua-Medina, E., Ochoa-Romo, J. P., López-Contreras, B. E., Canizales-Quinteros, S., Hernández-Reyna, A., Mendoza-Vargas, A., & Ochoa-Leyva, A. (2021). Gut dsDNA virome shows diversity and richness alterations associated with childhood obesity and metabolic syndrome. *iScience*, 24(8), 102900. [\[PubMed\]](#)

Belda, E., Voland, L., Tremaroli, V., Falony, G., Adriouch, S., Assmann, K. E., Prifti, E., Aron-Wisniewsky, J., Debédát, J., Marquet, F., Le Roy, T., Nielsen, T., Ørbæk Andersen, M., Chakaroun, R., Gausseres, B., Chilloux, J., Adriouch, S., Dumas, M.-E., Raes, J., et al. (2022). Impairment of gut microbial biotin metabolism and host biotin status in severe obesity: Effect of biotin and prebiotic supplementation on improved metabolism. *Gut*, 71(12), 2463–2480. [\[PubMed\]](#)

Bielska, D., & Mayr, T. (2023). Gut microbiota-derived metabolites in obesity and metabolic disorders: Mechanisms and therapeutic potential. *European Journal of Pharmaceutics and Biopharmaceutics*, 182, 1–14.

Bjornekleit, A., Viddal, K. O., Midtvedt, T., & Nygaard, K. (1981). Intestinal and gastric bypass. Changes in intestinal microecology after surgical treatment of morbid obesity in man. *Scandinavian Journal of Gastroenterology*, 16, 681–687. [\[PubMed\]](#)

Blachier, F., Beaumont, M., Andriamihaja, M., Davila, A. M., Lan, A., Grauso, M., et al. (2017). Changes in the luminal environment of the colonic epithelial cells and physiopathological consequences. *The American Journal of Pathology*, 187, 476–486. [\[PubMed\]](#)

Blanco-Míguez, A., Gálvez, E. J. C., Pasolli, E., De Filippis, F., Amend, L., Huang, K. D., Manghi, P., Lesker, T.-R., Riedel, T., Cova, L., Punčochář, M., Thomas, A. M., Valles-Colomer, M., Schober, I., Hitch, T. C. A., Clavel, T., Berry, S. E., Davies, R., Wolf, J., Spector, T. D., et al. (2023). Extension of the

Segatella copri complex to 13 species with distinct large extrachromosomal elements and associations with host conditions. *Cell Host & Microbe*, 31(11), 1804–1819.e9.

Blanton, L. V., Charbonneau, M. R., Salih, T., Barratt, M. J., Venkatesh, S., Ilkaveya, O., et al. (2016). Gut bacteria that prevent growth impairments transmitted by microbiota from malnourished children. *Science*, 351, aad3311.

[\[PubMed\]](#)

Booth, C. C., Alldis, D., & Read, A. E. (1961). Studies on the site of fat absorption: 2 Fat balances after resection of varying amounts of the small intestine in man. *Gut*, 2, 168–174.

[\[PubMed\]](#)

Borin, J. M., Liu, R., Wang, Y., Wu, T.-C., Chopyk, J., Huang, L., Abril-Utrillas, N., Li, J. V., Satlin, M. J., Taur, Y., Xavier, J. B., & Allegretti, J. R. (2023). Fecal virome transplantation is sufficient to alter fecal microbiota and drive lean and obese body phenotypes in mice. *Gut Microbes*, 15(1), 2236750.

[\[PubMed\]](#)

Brown, J. M., Chung, S., Sawyer, J. K., Degirolamo, C., Alger, H. M., Nguyen, T., et al. (2008). Inhibition of stearoyl-coenzyme A desaturase 1 dissociates insulin resistance and obesity from atherosclerosis. *Circulation*, 118, 1467–1475.

[\[PubMed\]](#)

Brown, E. M., Ke, X., Hitchcock, D., Jeanfavre, S., Avila-Pacheco, J., Nakata, T., Arthur, T. D., Fornelos, N., Heim, C., Franzosa, E. A., Watson, N., Huttenhower, C., Clardy, J., & Xavier, R. J. (2023). Gut microbiome lipid metabolism and its impact on host physiology. *Cell Host & Microbe*, 31(4), 580–596.

Brun, P., Castagliuolo, I., Di Leo, V., Buda, A., Pinzani, M., Palu, G., et al. (2007). Increased intestinal permeability in obese mice: New evidence in the pathogenesis of nonalcoholic steatohepatitis. *American Journal of Physiology. Gastrointestinal and Liver Physiology*, 292, G518–G525.

[\[PubMed\]](#)

Brugiroux, S., Beutler, M., Pfann, C., Garzetti, D., Ruscheweyh, H.-J., Ring, D., Diehl, M., Herp, S., Lötscher, Y., Hussain, S., Bunk, B., Pukall, R., Huson, D. H., McHardy, A. C., McCoy, K. D., Macpherson, A. J., Loy, A., Clavel, T., Berry, D., & Stecher, B. (2016). Genome-guided design of a defined mouse microbiota that confers colonization resistance against *Salmonella enterica* serovar Typhimurium. *Nature Microbiology*, 2, 16215.

[\[PubMed\]](#)

Buckley, C. D., Gilroy, D. W., & Serhan, C. N. (2014). Proresolving lipid mediators and mechanisms in the resolution of acute inflammation. *Immunity*, 40, 315–327.

[\[PubMed\]](#)

Bui, T. P., Ritari, J., Boeren, S., de Waard, P., Plugge, C. M., & de Vos, W. M. (2015). Production of butyrate from lysine and the Amadori product fructoselysine by a human gut commensal. *Nature Communications*, 6, 10062.

[\[PubMed\]](#)

Cani, P. D., Neyrinck, A. M., Fava, F., Knauf, C., Burcelin, R. G., Tuohy, K. M., et al. (2007). Selective increases of bifidobacteria in gut microflora improve high-fat-diet-induced diabetes in mice through a mechanism associated with endotoxaemia. *Diabetologia*, 50, 2374–2383.

[<https://doi.org/10.1007/s00125-007-0791-0>][PubMed]

Cani, P. D., Possemiers, S., Van de Wiele, T., Guiot, Y., Everard, A., Rottier, O., et al. (2009). Changes in gut microbiota control inflammation in obese mice through a mechanism involving GLP-2-driven improvement of gut permeability. *Gut*, 58, 1091–1103.

[PubMed]

Cani, P. D., Depommier, C., Derrien, M., Everard, A., & de Vos, W. M. (2022). Akkermansia muciniphila: Paradigm for next-generation beneficial microorganisms. *Nature Reviews Gastroenterology & Hepatology*, 19(10), 625–637.

Charbonneau, M. R., O'Donnell, D., Blanton, L. V., Totten, S. M., Davis, J. C., Barratt, M. J., et al. (2016). Sialylated milk oligosaccharides promote microbiota-dependent growth in models of infant undernutrition. *Cell*, 164, 859–871.

[PubMed]

Chaudhari, S. N., Luo, J. N., Harris, D. A., Aliakbarian, H., Yao, L., Paik, D., Subramaniam, R., Adhikari, A. A., Vernon, A. H., Kiliç, A., Weiss, S. T., Huh, J. R., Sheu, E. G., Devlin, A. S. (2021). A microbial metabolite remodels the gut-liver axis following bariatric surgery. *Cell Host Microbe*, 29(3), 408–424.e7. <https://doi.org/10.1016/j.chom.2020.12.004>. Epub 2021 Jan 11. PMID: 33434516; PMCID: PMC7954942.

Chelimo, C., Camargo, C. A. Jr, Morton, S. M. B., Grant, C. C. (2020). Association of Repeated Antibiotic Exposure Up to Age 4 Years With Body Mass at Age 4.5 Years. *JAMA Network Open*, 3(1), e1917577. <https://doi.org/10.1001/jamanetworkopen.2019.17577>. PMID: 31968112; PMCID: PMC6991235.

Chen, R. Y., Ahmed, T., & Gordon, J. I. (2022). A microbiota-directed food intervention for undernourished children. Reply. *The New England Journal of Medicine*, 386(15), 1484.

[PubMed]

Chen, Y., Chaudhari, S. N., Harris, D. A., Roberts, C. F., Moscalu, A., Mathur, V., Zhao, L., Tavakkoli, A., Devlin, A. S., Sheu, E. G. (2024). A small intestinal bile acid modulates the gut microbiome to improve host metabolic phenotypes following bariatric surgery. *Cell Host Microbe*, 32(8), 1315–1330.e5. <https://doi.org/10.1016/j.chom.2024.06.014>. Epub 2024 Jul 22. PMID: 39043190; PMCID: PMC11332993.

Chen-Liaw, A., Aggarwala, V., Mogno, I., Haifer, C., Li, Z., Eggers, J., Helmus, D., Hart, A., Wehkamp, J., Lamousé-Smith, E. S. N., Kerby, R. L., Rey, F. E., Colombel, J. F., Kamm, M. A., Olle, B., Norman, J. M., Menon, R., Watson, A. R., Crossett, E., Terveer, E. M., Keller, J. J., Borody, T. J., Grinspan, A., Paramsothy, S., Kaakoush, N. O., Dubinsky, M. C., & Faith, J. J. (2025). Gut microbiota strain richness is species specific and affects engraftment. *Nature*, 637(8045), 422–429.

[<https://doi.org/10.1038/s41586-024-08242-x>][PubMed]

Cherbuy, C., Darcy-Vrillon, B., Morel, M. T., Pegorier, J. P., & Duee, P. H. (1995). Effect of germfree state on the capacities of isolated rat colonocytes to metabolize n-butyrate, glucose, and

glutamine. *Gastroenterology*, 109, 1890–1899.

[[PubMed](#)]

Chong, C. Y. L., Bloomfield, F. H., & O'Sullivan, J. M. (2020). Randomised double-blind placebo-controlled trial of inulin with metronidazole in non-alcoholic fatty liver disease (NAFLD).

Nutrients, 12(4), 937.

[[PubMed](#)]

Clavel, T., Desmarchelier, C., Haller, D., Gerard, P., Rohn, S., Lepage, P., et al. (2014). Intestinal microbiota in metabolic diseases: From bacterial community structure and functions to species of pathophysiological relevance. *Gut Microbes*, 5, 544–551.

[[PubMed](#)]

Collado, M. C., Isolauri, E., Laitinen, K., & Salminen, S. (2010). Effect of mother's weight on infant's microbiota acquisition, composition, and activity during early infancy: A prospective follow-up study initiated in early pregnancy. *The American Journal of Clinical Nutrition*, 92, 1023–1030.

[[PubMed](#)]

Cotillard, A., Kennedy, S. P., Kong, L. C., Prifti, E., Pons, N., Le Chatelier, E., et al. (2013). Dietary intervention impact on gut microbial gene richness. *Nature*, 500, 585–588.

[<https://doi.org/10.1038/nature12480>][[PubMed](#)]

Coppola, S., Nocerino, R., Paparo, L., Bedogni, G., Calignano, A., Di Scala, C., & de Giovanni di Santa Severina, A. F., De Filippis, F., Ercolini, D., & Berni Canani, R. (2022). Therapeutic effects of butyrate on pediatric obesity: A randomized clinical trial. *JAMA Network Open*, 5(12), e2244912.

[[PubMed](#)]

Cox, L. M., & Blaser, M. J. (2015). Antibiotics in early life and obesity. *Nature Reviews. Endocrinology*, 11, 182–190.

[[PubMed](#)]

Cummings, J. H., Pomare, E. W., Branch, W. J., Naylor, C. P., Macfarlane, G. T. (1987). Short chain fatty acids in human large intestine, portal, hepatic and venous blood. *Gut*, 28(10), 1221–1227.

[<https://doi.org/10.1136/gut.28.10.1221>]. PMID: 3678950.

Damms-Machado, A., Louis, S., Schnitzer, A., Volynets, V., Rings, A., Basrai, M., et al. (2017). Gut permeability is related to body weight, fatty liver disease, and insulin resistance in obese individuals undergoing weight reduction. *The American Journal of Clinical Nutrition*, 105, 127–135.

[[PubMed](#)]

Daniel, H., Gholami, A. M., Berry, D., Desmarchelier, C., Hahne, H., Loh, G., et al. (2014). High-fat diet alters gut microbiota physiology in mice. *The ISME Journal*, 8, 295–308.

[[PubMed](#)]

Dao, M. C., Everard, A., Aron-Wisnewsky, J., Sokolovska, N., Prifti, E., Verger, E. O., Kayser, B. D., Levenez, F., Chilloux, J., Hoyles, L., Dumas, M.-E., Rizkalla, S. W., Doré, J., Cani, P. D., & Clément, K. (2016). *Akkermansia muciniphila* and improved metabolic health during a dietary intervention in

obesity: Relationship with gut microbiome richness and ecology. *Nature Medicine*, 22(1), 107–113.

David, L. A., Maurice, C. F., Carmody, R. N., Gootenberg, D. B., Button, J. E., Wolfe, B. E., et al. (2014). Diet rapidly and reproducibly alters the human gut microbiome. *Nature*, 505, 559–563. [\[https://doi.org/10.1038/nature12820\]](https://doi.org/10.1038/nature12820) [\[PubMed\]](#)

De Filippis, F., Pellegrini, N., Laghi, L., Gobbetti, M., & Ercolini, D. (2016). Unusual sub-genus associations of faecal *Prevotella* and *Bacteroides* with specific dietary patterns. *Microbiome*, 4, 57. [\[PubMed\]](#)

de Groot, P. F., Frissen, M. N., de Clercq, N. C., & Nieuwdorp, M. (2017). Fecal microbiota transplantation in metabolic syndrome: History, present and future. *Gut Microbes*, 8, 253–267. [\[PubMed\]](#)

de Groot, P. F., Frissen, M. N., de Clercq, N. C., & Nieuwdorp, M. (2022). Fecal microbiota transplantation in metabolic syndrome: History, present and future. *The Lancet Gastroenterology & Hepatology*, 7(10), 923–935.

De Vadder, F., Kovatcheva-Datchary, P., Goncalves, D., Vinera, J., Zitoun, C., Duchampt, A., et al. (2014). Microbiota-generated metabolites promote metabolic benefits via gut-brain neural circuits. *Cell*, 156, 84–96. [\[PubMed\]](#)

Delzenne, N. M., Neyrinck, A. M., Bäckhed, F., & Cani, P. D. (2011). Targeting gut microbiota in obesity: Effects of prebiotics and probiotics. *Nature Reviews Endocrinology*, 7(11), 639–646. [\[PubMed\]](#)

Degen, C., Ecker, J., Piegholdt, S., Liebisch, G., Schmitz, G., & Jahreis, G. (2011). Metabolic and growth inhibitory effects of conjugated fatty acids in the cell line HT-29 with special regard to the conversion of t11,t13-CLA. *Biochimica et Biophysica Acta*, 1811, 1070–1080. [\[PubMed\]](#)

den Besten, G., van Eunen, K., Groen, A. K., Venema, K., Reijngoud, D. J., & Bakker, B. M. (2013). The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism. *Journal of Lipid Research*, 54, 2325–2340.

Depommier, C., Everard, A., Druart, C., Plovier, H., Van Hul, M., Vieira-Silva, S., Falony, G., Raes, J., Maiter, D., Delzenne, N. M., de Barse, M., Loumaye, A., Hermans, M. P., Thissen, J.-P., de Vos, W. M., & Cani, P. D. (2019). Supplementation with *Akkermansia muciniphila* in overweight and obese human volunteers: A proof-of-concept exploratory study. *Nature Medicine*, 25(7), 1096–1103. [\[PubMed\]](#)

Depommier, C., Everard, A., Druart, C., Plovier, H., Van Hul, M., Vieira-Silva, S., Falony, G., Raes, J., Maiter, D., Delzenne, N. M., de Barse, M., Loumaye, A., Hermans, M. P., Thissen, J.-P., de Vos, W. M., & Cani, P. D. (2023). Supplementation with *Akkermansia muciniphila* in overweight and obese human volunteers: A proof-of-concept exploratory study. *Nature Medicine*, 25(7), 1096–1103.

Derrien, M., Vaughan, E. E., Plugge, C. M., & de Vos, W. M. (2004). *Akkermansia muciniphila* gen.

nov., sp. nov., a human intestinal mucin-degrading bacterium. *International Journal of Systematic and Evolutionary Microbiology*, 54, 1469–1476.

[\[PubMed\]](#)

Derrien, M., Collado, M. C., Ben-Amor, K., Salminen, S., & de Vos, W. M. (2008). The Mucin degrader *Akkermansia muciniphila* is an abundant resident of the human intestinal tract. *Applied and Environmental Microbiology*, 74, 1646–1648.

[\[PubMed\]](#)

Devillard, E., McIntosh, F. M., Duncan, S. H., & Wallace, R. J. (2007). Metabolism of linoleic acid by human gut bacteria: Different routes for biosynthesis of conjugated linoleic acid. *Journal of Bacteriology*, 189, 2566–2570.

[\[PubMed\]](#)

Dubos, R. J., & Schaedler, R. W. (1960). The effect of the intestinal flora on the growth rate of mice, and on their susceptibility to experimental infections. *The Journal of Experimental Medicine*, 111, 407–417.

[\[PubMed\]](#)

Dubos, R., Lee, C. J., & Costello, R. (1969). Lasting biological effects of early environmental influences. V. Viability, growth, and longevity. *The Journal of Experimental Medicine*, 130, 963–977.

[\[PubMed\]](#)

Duncan, S. H., Belenguer, A., Holtrop, G., Johnstone, A. M., Flint, H. J., & Lobley, G. E. (2007). Reduced dietary intake of carbohydrates by obese subjects results in decreased concentrations of butyrate and butyrate-producing bacteria in feces. *Applied and Environmental Microbiology*, 73, 1073–1078.

[\[PubMed\]](#)

Duncan, S. H., Lobley, G. E., Holtrop, G., Ince, J., Johnstone, A. M., Louis, P., et al. (2008). Human colonic microbiota associated with diet, obesity and weight loss. *International Journal of Obesity*, 32, 1720–1724.

[\[PubMed\]](#)

Ecker, J., & Liebisch, G. (2014). Application of stable isotopes to investigate the metabolism of fatty acids, glycerophospholipid and sphingolipid species. *Progress in Lipid Research*, 54, 14–31.

[\[PubMed\]](#)

Ecker, J., Liebisch, G., Patsch, W., & Schmitz, G. (2009). The conjugated linoleic acid isomer trans-9,trans-11 is a dietary occurring agonist of liver X receptor alpha. *Biochemical and Biophysical Research Communications*, 388, 660–666.

[\[PubMed\]](#)

Ecker, J., Liebisch, G., Englmaier, M., Grandl, M., Robenek, H., & Schmitz, G. (2010a). Induction of fatty acid synthesis is a key requirement for phagocytic differentiation of human monocytes. *Proceedings of the National Academy of Sciences of the United States of America*, 107, 7817–7822.

[\[PubMed\]](#)

Ecker, J., Liebisch, G., Scherer, M., & Schmitz, G. (2010b). Differential effects of conjugated linoleic acid isomers on macrophage glycerophospholipid metabolism. *Journal of Lipid Research*, 51, 2686–2694.

[\[PubMed\]](#)

Edmonson, M. B., & Eickhoff, J. C. (2017). Weight gain and obesity in infants and young children exposed to prolonged antibiotic prophylaxis. *JAMA Pediatrics*, 171, 150–156.

[\[PubMed\]](#)

Erbay, E., Babaev, V. R., Mayers, J. R., Makowski, L., Charles, K. N., Snitow, M. E., et al. (2009). Reducing endoplasmic reticulum stress through a macrophage lipid chaperone alleviates atherosclerosis. *Nature Medicine*, 15, 1383–1391.

[\[PubMed\]](#)

Ernst, R., Ejsing, C. S., & Antonny, B. (2016). Homeoviscous adaptation and the regulation of membrane lipids. *Journal of Molecular Biology*, 428, 4776–4791.

[\[PubMed\]](#)

Everard, A., Belzer, C., Geurts, L., Ouwerkerk, J. P., Druart, C., Bindels, L. B., et al. (2013). Cross-talk between *Akkermansia muciniphila* and intestinal epithelium controls diet-induced obesity. *Proceedings of the National Academy of Sciences of the United States of America*, 110, 9066–9071.

[\[PubMed\]](#)

Falony, G., Joossens, M., Vieira-Silva, S., Wang, J., Darzi, Y., Faust, K., et al. (2016). Population-level analysis of gut microbiome variation. *Science*, 352, 560–564.

[\[PubMed\]](#)

Faubert, B., Li, K. Y., Cai, L., Hensley, C. T., Kim, J., Zacharias, L. G., Yang, C., Do, Q. N., Doucette, S., Burguete, D., Li, H., Huet, G., Yuan, Q., Wigal, T., Butt, Y., Ni, M., Torrealba, J., Oliver, D., Lenkinski, R. E., Malloy, C. R., Wachsmann, J. W., Young, J. D., Kernstine, K., DeBerardinis, R. J., & Metallo, C. M. (2017). Lactate metabolism in human lung tumors. *Cell*, 171(2), 358–371.e9.

[\[PubMed\]](#)

Fava, F., Gitau, R., Griffin, B. A., Gibson, G. R., Tuohy, K. M., & Lovegrove, J. A. (2013). The type and quantity of dietary fat and carbohydrate alter faecal microbiome and short-chain fatty acid excretion in a metabolic syndrome ‘at-risk’ population. *International Journal of Obesity*, 37, 216–223.

[\[PubMed\]](#)

Fei, N., & Zhao, L. (2013). An opportunistic pathogen isolated from the gut of an obese human causes obesity in germfree mice. *The ISME Journal*, 7, 880–884.

[\[PubMed\]](#)

Ferolla, S. M., Couto, C. A., Costa-Silva, L., Armiliato, G. N., Pereira, C. A., Martins, F. S., et al. (2016). Beneficial effect of synbiotic supplementation on hepatic steatosis and anthropometric parameters, but not on gut permeability in a population with nonalcoholic steatohepatitis. *Nutrients*, 8(7), 397.

[\[PubMed\]](#)

Fleissner, C. K., Huebel, N., Abd El-Bary, M. M., Loh, G., Klaus, S., & Blaut, M. (2010). Absence of intestinal microbiota does not protect mice from diet-induced obesity. *The British Journal of Nutrition*, 104, 919–929.

[\[PubMed\]](#)

Fleishman, J. S., & Kumar, S. (2024). Bile acid metabolism and signaling in health and disease: Molecular mechanisms and therapeutic targets. *Signal Transduction and Targeted Therapy*, 9(1), 97.

[\[PubMed\]](#)

Foote, A. P., & Freetly, H. C. (2016). Effect of abomasal butyrate infusion on net nutrient flux across the portal-drained viscera and liver of growing lambs. *Journal of Animal Science*, 94, 2962–2972.

[\[PubMed\]](#)

Forslund, K., Hildebrand, F., Nielsen, T., Falony, G., Le Chatelier, E., Sunagawa, S., et al. (2015). Disentangling type 2 diabetes and metformin treatment signatures in the human gut microbiota. *Nature*, 528, 262–266.

<https://doi.org/10.1038/nature15766> [\[PubMed\]](#)

Fromentin, S., Forslund, S. K., Chechi, K., Aron-Wisnewsky, J., Chakaroun, R., Nielsen, T., Tremaroli, V., Ji, B., Prifti, E., Myridakis, A., Chilloux, J., Andrikopoulos, P., Fan, Y., Olanipekun, M. T., Alves, R., Adamo, M., Zhao, R., Atzeni, A., Vieira-Silva, S., et al. (2022). Microbiome and metabolome features of the cardiometabolic disease spectrum. *Nature Medicine*, 28(2), 303–314.

[\[PubMed\]](#)

Fuller, R. (1989). Probiotics in man and animals. *The Journal of Applied Bacteriology*, 66, 365–378.

[\[PubMed\]](#)

Furet, J. P., Kong, L. C., Tap, J., Poitou, C., Basdevant, A., Bouillot, J. L., et al. (2010). Differential adaptation of human gut microbiota to bariatric surgery-induced weight loss: Links with metabolic and low-grade inflammation markers. *Diabetes*, 59, 3049–3057.

[\[PubMed\]](#)

Garidou, L., Pomie, C., Klopp, P., Waget, A., Charpentier, J., Aloulou, M., et al. (2015). The gut microbiota regulates intestinal CD4 T cells expressing RORgammat and controls metabolic disease. *Cell Metabolism*, 22, 100–112.

[\[PubMed\]](#)

Glatz, J. F., Luiken, J. J., & Bonen, A. (2010). Membrane fatty acid transporters as regulators of lipid metabolism: Implications for metabolic disease. *Physiological Reviews*, 90, 367–417.

[\[PubMed\]](#)

Goodrich, J. K., Waters, J. L., Poole, A. C., Sutter, J. L., Koren, O., Blekhman, R., et al. (2014). Human genetics shape the gut microbiome. *Cell*, 159, 789–799.

[\[PubMed\]](#)

Gorman, A., Golovanov, A. P. (2022). Lipopolysaccharide Structure and the Phenomenon of Low Endotoxin Recovery. *Eur J Pharm Biopharm*, 180, 289–307. <https://doi.org/10.1016/j.ejpb.2022.10.006>. Epub 2022 Oct 19. PMID: 36272656.

Grasset, E., Puel, A., Charpentier, J., Collet, X., Christensen, J. E., Terce, F., et al. (2017). A specific gut microbiota dysbiosis of type 2 diabetic mice induces GLP-1 resistance through an enteric NO-dependent and gut-brain axis mechanism. *Cell Metabolism*, 26, 278.

[[PubMed](#)]

Gronbach, K., Flade, I., Holst, O., Lindner, B., Ruscheweyh, H. J., Wittmann, A., Menz, S., Schwiertz, A., Adam, P., Stecher, B., Josenhans, C., Suerbaum, S., Gruber, A. D., Kulik, A., Huson, D., Autenrieth, I. B., Jiang, C. (2014). Endotoxicity of lipopolysaccharide as a determinant of T-cell-mediated colitis induction in mice. *Gastroenterology*, 146(3), 765–775. <https://doi.org/10.1053/j.gastro.2013.11.033>. Epub 2013 Nov 21. PMID: 24269927.

Hamilton, M. K., Boudry, G., Lemay, D. G., & Raybould, H. E. (2015). Changes in intestinal barrier function and gut microbiota in high-fat diet-fed rats are dynamic and region dependent. *American Journal of Physiology. Gastrointestinal and Liver Physiology*, 308, G840–G851.

[[PubMed](#)]

Hansen, C. H., Krych, L., Nielsen, D. S., Vogensen, F. K., Hansen, L. H., Sorensen, S. J., et al. (2012). Early life treatment with vancomycin propagates *Akkermansia muciniphila* and reduces diabetes incidence in the NOD mouse. *Diabetologia*, 55, 2285–2294.

[<https://doi.org/10.1007/s00125-012-2564-7>][[PubMed](#)]

Hayashi, Y., Lee-Okada, H. C., Nakamura, E., Tada, N., Yokomizo, T., Fujiwara, Y., Ichi, I. (2021). Ablation of fatty acid desaturase 2 (FADS2) exacerbates hepatic triacylglycerol and cholesterol accumulation in polyunsaturated fatty acid-depleted mice. *FEBS Lett*, 595(14), 1920–1932. <https://doi.org/10.1002/1873-3468.14134>. Epub 2021 Jun 1. PMID: 34008174.

Henao-Mejia, J., Elinav, E., Jin, C., Hao, L., Mehal, W. Z., Strowig, T., et al. (2012). Inflammasome-mediated dysbiosis regulates progression of NAFLD and obesity. *Nature*, 482, 179–185.

[<https://doi.org/10.1038/nature10809>][[PubMed](#)]

Hernández-Alonso, P., Camacho-Barcia, L., Bulló, M., Salas-Salvadó, J., & Ruiz-Canela, M. (2023). Gut microbiota-targeted interventions and metabolic health: Mechanisms and therapeutic opportunities. *Nature*, 621(7980), 318–329.

[<https://doi.org/10.1038/s41586-023-06390-0>]

Hill, C., Guarner, F., Reid, G., Gibson, G. R., Merenstein, D. J., Pot, B., Morelli, L., Canani, R. B., Flint, H. J., Salminen, S., Calder, P. C., & Sanders, M. E. (2014). Expert consensus document: The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nature Reviews Gastroenterology & Hepatology*, 11(8), 506–514.

Hitch, T. C. A., Afrizal, A., Riedel, T., Kioukis, A., Haller, D., Lagkouvardos, I., Overmann, J., & Clavel, T. (2021). Recent advances in culture-based gut microbiome research. *International Journal of Medical Microbiology*, 311(3), 151485.

[[PubMed](#)]

Hitch, T. C. A., Bisdorf, K., Afrizal, A., Riedel, T., Overmann, J., Strowig, T., & Clavel, T. (2022). A taxonomic note on the genus *Prevotella*: Description of four novel genera and emended

description of the genera *Hallella* and *Xylanibacter*. *Systematic and Applied Microbiology*, 45(6), 126354.

[[PubMed](#)]

Hoces, D., Lan, J., Sun, W., Geiser, T., Stäubli, M. L., Cappio Barazzzone, E., Arnoldini, M., Challa, T. D., Klug, M., Kellenberger, A., Nowok, S., Faccin, E., Macpherson, A. J., Stecher, B., Sunagawa, S., Zenobi, R., Hardt, W.-D., Wolfrum, C., & Slack, E. (2022). Metabolic reconstitution of germ-free mice by a gnotobiotic microbiota varies over the circadian cycle. *PLoS Biology*, 20(9), e3001743. <https://doi.org/10.1371/journal.pbio.3001743>. PMID: 36126044; PMCID: PMC9488797.

Hodson, L., & Fielding, B. A. (2013). Stearoyl-CoA desaturase: Rogue or innocent bystander? *Progress in Lipid Research*, 52, 15–42.

[[PubMed](#)]

Horton, J. D., Goldstein, J. L., & Brown, M. S. (2002). SREBPs: Activators of the complete program of cholesterol and fatty acid synthesis in the liver. *The Journal of Clinical Investigation*, 109, 1125–1131.

[[PubMed](#)]

Hussain, M. M. (2014). Intestinal lipid absorption and lipoprotein formation. *Current Opinion in Lipidology*, 25, 200–206.

[[PubMed](#)]

Hussain, M. M., Leung, T. M., Zhou, L., & Abu-Merhi, S. (2013). Regulating intestinal function to reduce atherogenic lipoproteins. *Clinical Lipidology*, 8, 481–490.

Itoh, T., Fairall, L., Amin, K., Inaba, Y., Szanto, A., Balint, B. L., et al. (2008). Structural basis for the activation of PPARgamma by oxidized fatty acids. *Nature Structural and Molecular Biology*, 15, 924–931.

[[PubMed](#)]

Ivanov, I. I., Atarashi, K., Manel, N., Brodie, E. L., Shima, T., Karaoz, U., et al. (2009). Induction of intestinal Th17 cells by segmented filamentous bacteria. *Cell*, 139, 485–498.

[[PubMed](#)]

Jakobsdottir, G., Jädert, C., Holm, L., Nyman, M. E. (2013). Propionic and butyric acids, formed in the caecum of rats fed highly fermentable dietary fibre, are reflected in portal and aortic serum. *Br J Nutr*, 110(9), 1565–1572. <https://doi.org/10.1017/S0007114513000809>. Epub 2013 Mar 26. PMID: 23531375.

Johnson, E. L., Heaver, S. L., Walters, W. A., & Ley, R. E. (2017). Microbiome and metabolic disease: Revisiting the bacterial phylum Bacteroidetes. *The Journal of Molecular Medicine (Berlin)*, 95, 1–8.

Joyce, S. A., MacSharry, J., Casey, P. G., Kinsella, M., Murphy, E. F., Shanahan, F., Hill, C., Gahan, C. G. (2014). Regulation of host weight gain and lipid metabolism by bacterial bile acid modification in the gut. *Proc Natl Acad Sci U S A*, 111(20), 7421–7426. <https://doi.org/10.1073/pnas.1323599111>. Epub 2014 May 5. PMID: 24799697.

Jumpertz, R., Le, D. S., Turnbaugh, P. J., Trinidad, C., Bogardus, C., Gordon, J. I., et al. (2011). Energy-

balance studies reveal associations between gut microbes, caloric load, and nutrient absorption in humans. *The American Journal of Clinical Nutrition*, 94, 58–65.

[[PubMed](#)]

Kalliomaki, M., Collado, M. C., Salminen, S., & Isolauri, E. (2008). Early differences in fecal microbiota composition in children may predict overweight. *The American Journal of Clinical Nutrition*, 87, 534–538.

[[PubMed](#)]

Karl, J. P., Margolis, L. M., Madslien, E. H., Murphy, N. E., Castellani, J. W., Gundersen, Y., et al. (2017). Changes in intestinal microbiota composition and metabolism coincide with increased intestinal permeability in young adults under prolonged physiological stress. *American Journal of Physiology. Gastrointestinal and Liver Physiology*, 312, G559–G571.

[[PubMed](#)]

Kau, A. L., Ahern, P. P., Griffin, N. W., Goodman, A. L., & Gordon, J. I. (2011). Human nutrition, the gut microbiome and the immune system. *Nature*, 474, 327–336.

[<https://doi.org/10.1038/nature10213>][[PubMed](#)]

Kelley, N. S., Hubbard, N. E., & Erickson, K. L. (2007). Conjugated linoleic acid isomers and cancer. *The Journal of Nutrition*, 137, 2599–2607.

[[PubMed](#)]

Kelsen, J. R., Wu, G. D., Duan, J., et al. (2024). Microbiota-directed complementary foods accelerate gut microbiota maturation in undernourished children. *Nature*, 629(8014), 637–645.

Kennedy, K. M., de Goffau, M. C., Perez-Muñoz, M. E., Arrieta, M. C., Bäckhed, F., Bork, P., Braun, T., Bushman, F. D., Dore, J., de Vos, W. M., Earl, A. M., Eisen, J. A., Elovitz, M. A., Ganai-Vonarburg, S. C., Gänzle, M. G., Garrett, W. S., Hall, L. J., Hornef, M. W., Huttenhower, C., Konnikova, L., Lebeer, S., Macpherson, A. J., Massey, R. C., McHardy, A. C., Koren, O., Lawley, T. D., Ley, R. E., O'Mahony, L., O'Toole, P. W., Pamer, E. G., Parkhill, J., Raes, J., Rattei, T., Salonen, A., Segal, E., Segata, N., Shanahan, F., Sloboda, D. M., Smith, G. C. S., Sokol, H., Spector, T. D., Surette, M. G., Tannock, G. W., Walker, A. W., Yassour, M., Walter, J. (2023). Questioning the fetal microbiome illustrates pitfalls of low-biomass microbial studies. *Nature*, 613(7945), 639–649. <https://doi.org/10.1038/s41586-022-05546-8>. Epub 2023 Jan 25. PMID: 36697862; PMCID: PMC11333990.

Kimura, I., Ozawa, K., Inoue, D., Imamura, T., Kimura, K., Maeda, T., et al. (2013). The gut microbiota suppresses insulin-mediated fat accumulation via the short-chain fatty acid receptor GPR43. *Nature Communications*, 4, 1829.

[[PubMed](#)]

Kindt, A., Liebisch, G., Clavel, T., Haller, D., Hörmannspurger, G., Yoon, H., Kolmeder, D., Sigrüener, A., Krautbauer, S., Seeliger, C., Ganzha, A., Schweizer, S., Morisset, R., Strowig, T., Daniel, H., Helm, D., Küster, B., Krumsiek, J., Ecker, J. (2018). The gut microbiota promotes hepatic fatty acid desaturation and elongation in mice. *Nat Commun*, 9(1), 3760. <https://doi.org/10.1038/s41467-018-05767-4>. PMID: 30218046.

Kishino, S., Takeuchi, M., Park, S. B., Hirata, A., Kitamura, N., Kunisawa, J., et al. (2013). Polyunsaturated fatty acid saturation by gut lactic acid bacteria affecting host lipid composition.

Proceedings of the National Academy of Sciences of the United States of America, 110, 17808–17813.

[[PubMed](#)]

Kless, C., Muller, V. M., Schuppel, V. L., Lichtenegger, M., Rychlik, M., Daniel, H., et al. (2015). Diet-induced obesity causes metabolic impairment independent of alterations in gut barrier integrity. *Molecular Nutrition and Food Research*, 59, 968–978.

[[PubMed](#)]

Koh, A., Molinaro, A., Ståhlman, M., Khan, M. T., Schmidt, C., Mannerås-Holm, L., Wu, H., Carreras, A., Jeong, H., Olofsson, L. E., Bergh, P. O., Gerdes, V., Hartstra, A., de Brauw, M., Perkins, R., Nieuwdorp, M., Bergström, G., Bäckhed, F. (2018). Microbially Produced Imidazole Propionate Impairs Insulin Signaling through mTORC1. *Cell*, 175(4), 947–961.e17. <https://doi.org/10.1016/j.cell.2018.09.055>. Epub 2018 Oct 25. PMID: 30401435.

Koh, A., Mannerås-Holm, L., Yunn, N. O., Nilsson, P. M., Ryu, S. H., Molinaro, A., Perkins, R., Smith, J. G., Bäckhed, F. (2020). Microbial Imidazole Propionate Affects Responses to Metformin through p38γ-Dependent Inhibitory AMPK Phosphorylation. *Cell Metab*, 32(4), 643–653.e4. <https://doi.org/10.1016/j.cmet.2020.07.012>. Epub 2020 Aug 11. PMID: 32783890.

Kootte, R. S., Levin, E., Salojärvi, J., Smits, L. P., Hartstra, A. V., Udayappan, S. D., et al. (2017a). Improvement of insulin sensitivity after lean donor feces in metabolic syndrome is driven by baseline intestinal microbiota composition. *Cell Metabolism*, 26, 611–619, e616.

[[PubMed](#)]

Kootte, R. S., Levin, E., Salojärvi, J., Smits, L. P., Hartstra, A. V., Udayappan, S. D., Hermes, G. D. A., Bouter, K. E. C., Koopen, A. M., Holst, J. J., Knop, F. K., Blaak, E. E., Zhao, J., Smidt, H., Harms, A. C., Hankemeier, T., Bergman, J. J. G. H. M., Romijn, H. A., Schaap, F. G., Olde Damink, S. W. M., Ackermans, M. T., Dallinga-Thie, G. M., Zoetendal, E. G., de Vos, W. M., Serlie, M. J., & Nieuwdorp, M. (2017b). Improvement of insulin sensitivity after lean donor feces in metabolic syndrome is driven by baseline intestinal microbiota composition. *Gastroenterology*, 153(3), 711–719.e6.

Kopczynski, D., Ejlsing, C. S., McDonald, J. G., Bamba, T., Baker, E. S., Bertrand-Michel, J., Brügger, B., Coman, C., Ellis, S. R., et al. (2024). The lipidomics reporting checklist: A framework for transparency of lipidomic experiments and repurposing resource data. *Journal of Lipid Research*, 65(9), 100621.

[[PubMed](#)]

Kovatcheva-Datchary, P., Nilsson, A., Akrami, R., Lee, Y. S., De Vadder, F., Arora, T., Hallen, A., Martens, E., Björck, I., & Bäckhed, F. (2015). Dietary fiber-induced improvement in glucose metabolism is associated with increased abundance of *Prevotella*. *Cell Metabolism*, 22(6), 971–982.

[[PubMed](#)]

Krumbeck, J. A., Rasmussen, H. E., Hutkins, R. W., Clarke, J., Shawron, K., Keshavarzian, A., & Walter, J. (2018). Probiotic *Bifidobacterium* strains and galactooligosaccharides improve intestinal barrier function in obese adults but show no synergism when used together as synbiotics. *Microbiome*, 6(1), 121.

[[PubMed](#)]

Kuang, Z., Wang, Y., Li, Y., Ye, C., Ruhn, K. A., Behrendt, C. L., Olson, E. N., & Hooper, L. V. (2019). The intestinal microbiota programs diurnal rhythms in host metabolism through histone deacetylase 3. *Science*, 365(6460), 1428–1434.

[\[PubMed\]](#)

Kubeck, R., Bonet-Ripoll, C., Hoffmann, C., Walker, A., Muller, V. M., Schuppel, V. L., et al. (2016). Dietary fat and gut microbiota interactions determine diet-induced obesity in mice. *Molecular Metabolism*, 5, 1162–1174.

[\[PubMed\]](#)

Kuhnt, K., Wagner, A., Kraft, J., Basu, S., & Jahreis, G. (2006). Dietary supplementation with 11trans- and 12trans-18:1 and oxidative stress in humans. *The American Journal of Clinical Nutrition*, 84, 981–988.

[\[PubMed\]](#)

Kumar, M., Nagpal, R., Kumar, R., Hemalatha, R., Verma, V., Kumar, A., et al. (2012). Cholesterol-lowering probiotics as potential biotherapeutics for metabolic diseases. *Experimental Diabetes Research*, 2012, 902917.

[\[PubMed\]](#)

Kurilshikov, A., Medina-Gomez, C., Bacigalupe, R., Radjabzadeh, D., Wang, J., Demirkan, A., Le Roy, C. I., Garay, J. A. R., Finnicum, C. T., Liu, X., et al. (2021). Large-scale association analyses identify host factors influencing human gut microbiome composition. *Nature Genetics*, 53(2), 156–165.

[\[PubMed\]](#)

Lagkouvardos, I., Overmann, J., & Clavel, T. (2017). Cultured microbes represent a substantial fraction of the human and mouse gut microbiota. *Gut Microbes*, 8(5), 493–503.

[\[PubMed\]](#)

Lahtinen, S. J., Davis, E., & Ouwehand, A. C. (2012). Lactobacillus species causing obesity in humans: Where is the evidence? *Beneficial Microbes*, 3, 171–174.

[\[PubMed\]](#)

Lambert, J. E., Parnell, J. A., Eksteen, B., Raman, M., Bomhof, M. R., Rioux, K. P., et al. (2015). Gut microbiota manipulation with prebiotics in patients with non-alcoholic fatty liver disease: A randomized controlled trial protocol. *BMC Gastroenterology*, 15, 169.

[\[PubMed\]](#)

Lawson, P. A., Saavedra Perez, L., & Sankaranarayanan, K. (2023). Reclassification of *Clostridium cocleatum*, *Clostridium ramosum*, *Clostridium spiroforme* and *Clostridium saccharogumia* as *Thomasclavelia cocleata* gen. nov., comb. nov., *Thomasclavelia ramosa* comb. nov., *Thomasclavelia spiroformis* comb. nov. and *Thomasclavelia saccharogumia* comb. nov. *International Journal of Systematic and Evolutionary Microbiology*, 73(1), 005694.

Le Chatelier, E., Nielsen, T., Qin, J., Prifti, E., Hildebrand, F., Falony, G., Almeida, M., Arumugam, M., Batto, J.-M., Kennedy, S., Leonard, P., Li, J., Burgdorf, K., Grarup, N., Jørgensen, T., Brandslund, I., Nielsen, H. B., Juncker, A. S., Bertalan, M., Levenez, F., Pons, N., Rasmussen, S., Sunagawa, S., Tap, J., Tims, S., Zoetendal, E. G., Brunak, S., Clément, K., Doré, J., Kleerebezem, M., Kristiansen, K., Renault,

P., Sicheritz-Pontén, T., de Vos, W. M., Zucker, J.-D., Raes, J., Hansen, T., MetaHIT Consortium, Bork, P., Wang, J., Ehrlich, S. D., & Pedersen, O. (2013). Richness of human gut microbiome correlates with metabolic markers. *Nature*, 500(7464), 541–546. [\[https://doi.org/10.1038/nature12506\]](https://doi.org/10.1038/nature12506) [\[PubMed\]](#)

Le Roy, T., Llopis, M., Lepage, P., Bruneau, A., Rabot, S., Bevilacqua, C., et al. (2013). Intestinal microbiota determines development of non-alcoholic fatty liver disease in mice. *Gut*, 62, 1787–1794. [\[PubMed\]](#)

Le, H. H., Lee, M. T., Besler, K. R., Johnson, E. L. (2022). Host hepatic metabolism is modulated by gut microbiota-derived sphingolipids. *Cell Host Microbe*, 30(6), 798–808.e7. <https://doi.org/10.1016/j.chom.2022.05.002>. Epub 2022 May 26. PMID: 35623356; PMCID: PMC9187616.

Lee, T., Clavel, T., Smirnov, K., Schmidt, A., Lagkouravdos, I., Walker, A., et al. (2017). Oral versus intravenous iron replacement therapy distinctly alters the gut microbiota and metabolome in patients with IBD. *Gut*, 66, 863–871. [\[PubMed\]](#)

Lefebvre, P., Cariou, B., Lien, F., Kuipers, F., & Staels, B. (2009). Role of bile acids and bile acid receptors in metabolic regulation. *Physiological Reviews*, 89, 147–191. [\[PubMed\]](#)

Leibel, R. L., Rosenbaum, M., & Hirsch, J. (1992). Changes in energy expenditure resulting from altered body weight. *The New England Journal of Medicine*, 332(10), 621–628.

Leone, V., Gibbons, S. M., Martinez, K., Hutchison, A. L., Huang, E. Y., Cham, C. M., Pierre, J. F., Heneghan, A. F., Nadimpalli, A., Hubert, N., Zale, E., Wang, Y., Huang, Y., Theriault, B., Dinner, A. R., Musch, M. W., Kudsk, K. A., Prendergast, B. J., Gilbert, J. A., & Chang, E. B. (2015). Effects of diurnal variation of gut microbes and high-fat feeding on host circadian clock function and metabolism. *Cell Host & Microbe*, 17(5), 681–689.

Leong, K. S. W., McLay, J., Derraik, J. G. B., Gibb, S., Shackleton, N., Taylor, R. W., Glover, M., Audas, R., Taylor, B., Milne, B. J., Cutfield, W. S. (2020). Associations of Prenatal and Childhood Antibiotic Exposure With Obesity at Age 4 Years. *JAMA Network Open*, 3(1), e1919681. <https://doi.org/10.1001/jamanetworkopen.2019.19681>. PMID: 31968118; PMCID: PMC6991276.

Ley, R. E., Backhed, F., Turnbaugh, P., Lozupone, C. A., Knight, R. D., & Gordon, J. I. (2005). Obesity alters gut microbial ecology. *Proceedings of the National Academy of Sciences of the United States of America*, 102, 11070–11075. [\[PubMed\]](#)

Ley, R. E., Turnbaugh, P. J., Klein, S., & Gordon, J. I. (2006). Microbial ecology: Human gut microbes associated with obesity. *Nature*, 444, 1022–1023. [\[https://doi.org/10.1038/4441022a\]](https://doi.org/10.1038/4441022a) [\[PubMed\]](#)

Li, T. T., Chen, X., Huo, D., Arifuzzaman, M., Qiao, S., Jin, W. B., Shi, H., Li, X. V. (2024). JRI Live Cell Bank Consortium; Iliev ID, Artis D, Guo CJ. Microbiota metabolism of intestinal amino acids impacts host nutrient homeostasis and physiology. *Cell Host Microbe*, 32(5), 661–675.e10. <https://>

doi.org/10.1016/j.chom.2024.04.004. Epub 2024 Apr 23. PMID: 38657606; PMCID: PMC11636940.

Li, F., Armet, A. M., Korpela, K., Liu, J., Margain Quevedo, R., Asnicar, F., Seethaler, B., Rusnak, T. B. S., Cole, J. L., Zhang, Z., Zhao, S., Wang, X., Gagnon, A., Deehan, E. C., Mota, J. F., Bakal, J. A., Greiner, R., Knights, D., Segata, N., Bischoff, S. C., Mereu, L., Haqq, A. M., Field, C. J., Li, L., Prado, C. M., & Walter, J. (2025). Cardiometabolic benefits of a nonindustrialized-type diet are linked to gut microbiome modulation. *Cell*, 188(5), 1226–1247.e18.

[\[PubMed\]](#)

Liebisch, G., Ejsing, C. S., & Ekroos, K. (2015). Identification and annotation of lipid species in metabolomics studies need improvement. *Clinical Chemistry*, 61, 1542–1544.

[\[PubMed\]](#)

Liebisch, G., Ekroos, K., Hermansson, M., & Ejsing, C. S. (2017). Reporting of lipidomics data should be standardized. *Biochimica et Biophysica Acta*, 1862, 747–751.

[\[PubMed\]](#)

Liebisch, G., Plagge, J., Höring, M., Seeliger, C., Ecker, J. (2021). The effect of gut microbiota on the intestinal lipidome of mice. *Int J Med Microbiol*, 311, 151488. <https://doi.org/10.1016/j.ijmm.2021.151488>. Epub 2021 Feb 25. PMID: 33662870.

Liou, A. P., Paziuk, M., Luevano, J. M., Jr, Machineni, S., Turnbaugh, P. J., & Kaplan, L. M. (2013). Conserved shifts in the gut microbiota due to gastric bypass reduce host weight and adiposity. *Science Translational Medicine*, 5, 178ra141.

Liu, C., Du, M.-X., Xie, L.-S., Wang, W.-Z., Chen, B.-S., Yun, C.-Y., Sun, X.-W., Luo, X., Jiang, Y., Wang, K., Jiang, M.-Z., Qiao, S.-S., Sun, M., Cui, B.-J., Huang, H.-J., Qu, S.-P., Li, C.-K., Wu, D., Wang, L.-S., Jiang, C., Liu, H.-W., & Liu, S.-J. (2024). Gut commensal *Christensenella minuta* modulates host metabolism via acylated secondary bile acids. *Nature Microbiology*, 9(2), 434–450.

[\[PubMed\]](#)

Louis, P., & Flint, H. J. (2017). Formation of propionate and butyrate by the human colonic microbiota. *Environmental Microbiology*, 19, 29–41.

[\[PubMed\]](#)

Luengo, A., Gui, D. Y., & Vander Heiden, M. G. (2021). Targeting metabolism for cancer therapy. *Cell*, 184(11), 2890–2906.

Ma, H., Sales, V. M., Wolf, A. R., Subramanian, S., Matthews, T. J., Chen, M., et al. (2017). Attenuated effects of bile acids on glucose metabolism and insulin sensitivity in a male mouse model of prenatal undernutrition. *Endocrinology*, 158, 2441–2452.

[\[PubMed\]](#)

Maghini, D. G., Oduaran, O. H., Wirbel, J., Olubayo, L. A. I., Smyth, N., Mathema, T., Belger, C. W., Agongo, G., Boua, P. R., Choma, S. S., Gómez-Olivé, F. X., Kisiangani, I., Mashaba, G. R., Micklesfield, L., Mohamed, S. F., Nonterah, E. A., Norris, S., Sorgho, H., Tollman, S., Wafawanaka, F., Tluway, F., Ramsay, M., Bhatt, A. S., & Hazelhurst, S. (2025). Expanding the human gut microbiome atlas of Africa. *Nature*, 638(8051), 718–728.

[\[https://doi.org/10.1038/s41586-024-08485-8\]](https://doi.org/10.1038/s41586-024-08485-8)[\[PubMed\]](#)

Moens de Hase, E., Petitfils, C., Alhouayek, M., Depommier, C., Le Faouder, P., Delzenne, N. M., Van Hul, M., Muccioli, G. G., Cenac, N., & Cani, P. D. (2023). Dysosmobacter welbionis effects on glucose, lipid, and energy metabolism are associated with specific bioactive lipids. *Journal of Lipid Research*, 64(10), 100437.

[\[PubMed\]](#)

Mao, X., Rasmussen, T. S., Kot, W., de Sordi, L., Castro-Mejía, J. L., Zuffa, S., Bikel, S., Wang, Y., Wu, T.-C., Chopyk, J., Huang, L., Zhu, Q., Swann, J. R., Hansen, L. H., Vogensen, F. K., Nielsen, D. S., Xavier, J. B., & Allegretti, J. R. (2024). Transfer of modified gut viromes improves symptoms associated with metabolic syndrome in obese male mice. *Nature Communications*, 15(1), 4704.

[\[PubMed\]](#)

Mardinoglu, A., Wu, H., Bjornson, E., Zhang, C., Hakkarainen, A., Rasanen, S. M., et al. (2018). An integrated understanding of the rapid metabolic benefits of a carbohydrate-restricted diet on hepatic steatosis in humans. *Cell Metabolism*, 27(3), 559–571.e5.

[\[PubMed\]](#)

Martinez, I., Stegen, J. C., Maldonado-Gomez, M. X., Eren, A. M., Siba, P. M., Greenhill, A. R., et al. (2015). The gut microbiota of rural papua new guineans: Composition, diversity patterns, and ecological processes. *Cell Reports*, 11, 527–538.

[\[PubMed\]](#)

Martinez-Guryn, K., Hubert, N., Frazier, K., Urlass, S., Musch, M. W., Ojeda, P., Pierre, J. F., Miyoshi, J., Sontag, T. J., Cham, C. M., Reardon, C. A., Leone, V., Chang, E. B. (2018). Small Intestine Microbiota Regulate Host Digestive and Absorptive Adaptive Responses to Dietary Lipids. *Cell Host Microbe*, 23(4), 458–469.e5. <https://doi.org/10.1016/j.chom.2018.03.011>. PMID: 29649441.

Matis, G., Kulcsar, A., Turowski, V., Febel, H., Neogrady, Z., & Huber, K. (2015). Effects of oral butyrate application on insulin signaling in various tissues of chickens. *Domestic Animal Endocrinology*, 50, 26–31.

[\[PubMed\]](#)

Matsuzaka, T., Shimano, H., Yahagi, N., Kato, T., Atsumi, A., Yamamoto, T., et al. (2007). Crucial role of a long-chain fatty acid elongase, Elovl6, in obesity-induced insulin resistance. *Nature Medicine*, 13, 1193–1202.

[\[PubMed\]](#)

McIntosh, F. M., Shingfield, K. J., Devillard, E., Russell, W. R., & Wallace, R. J. (2009). Mechanism of conjugated linoleic acid and vaccenic acid formation in human faecal suspensions and pure cultures of intestinal bacteria. *Microbiology*, 155, 285–294.

[\[PubMed\]](#)

Maier, L., Pruteanu, M., Kuhn, M., Zeller, G., Telzerow, A., Anderson, E. E., Brochado, A. R., Fernandez, K. C., Dose, H., Mori, H., Patil, K. R., Bork, P., & Typas, A. (2018). Extensive impact of non-antibiotic drugs on human gut bacteria. *Nature*, 555(7698), 623–628.

[\[https://doi.org/10.1038/nature25979\]](https://doi.org/10.1038/nature25979)[\[PubMed\]](#)

Martino, M. E., Joncour, P., Leenay, R., Gervais, H., Shah, M., Hughes, S., Gillet, B., Beisel, C., Leulier, F. (2018). Bacterial Adaptation to the Host's Diet Is a Key Evolutionary Force Shaping *Drosophila-Lactobacillus* Symbiosis. *Cell Host Microbe*, 24(1), 109–119.e6. <https://doi.org/10.1016/j.chom.2018.06.001>. Epub 2018 Jun 28. PMID: 30008290.

Mead, J. F., Slaton, W. H. Jr. (1956). Metabolism of essential fatty acids. III. Isolation of 5,8,11-eicosatrienoic acid from fat-deficient rats. *Journal of Biological Chemistry*, 219(2), 705–709. PMID: 13319291.

Mendez-Lucas, A., Lin, W., Driscoll, P. C., et al. (2021). Identifying strategies to target the metabolic flexibility of tumours. *Cell*, 184(6), 1455–1472.e19.

Mendes-Soares, H., Raveh-Sadka, T., Azulay, S., Ben-Shlomo, Y., Cohen, Y., Ofek, T., Stevens, J., Bachrach, D., Kashyap, P., Segal, L., & Nelson, H. (2019). Model of personalized postprandial glycemic response to food developed for an Israeli cohort predicts responses in Midwestern American individuals. *American Journal of Clinical Nutrition*, 110(1), 63–75. [\[PubMed\]](#)

Mitchell, P. L., & McLeod, R. S. (2008). Conjugated linoleic acid and atherosclerosis: Studies in animal models. *Biochemistry and Cell Biology*, 86, 293–301. [\[PubMed\]](#)

Miyazaki, M., & Ntambi, J. M. (2003). Role of stearoyl-coenzyme A desaturase in lipid metabolism. *Prostaglandins, Leukotrienes, and Essential Fatty Acids*, 68, 113–121. [\[PubMed\]](#)

Moller, D. E. (2001). New drug targets for type 2 diabetes and the metabolic syndrome. *Nature*, 414, 821–827. <https://doi.org/10.1038/414821a> [\[PubMed\]](#)

Molinaro, A., Bel Lassen, P., Henricsson, M., Wu, H., Adriouch, S., Belda, E., Chakaroun, R., Nielsen, T., Bergh, P. O., Rouault, C., André, S., Marquet, F., Andreelli, F., Salem, J. E., Assmann, K., Bastard, J. P., Forslund, S., Le Chatelier, E., Falony, G., Pons, N., Prifti, E., Quinquis, B., Roume, H., Vieira-Silva, S., Hansen, T. H., Pedersen, H. K., Lewinter, C., Sønderskov, N. B., MetaCardis Consortium, Køber, L., Vestergaard, H., Hansen, T., Zucker, J. D., Galan, P., Dumas, M. E., Raes, J., Oppert, J. M., Letunic, I., Nielsen, J., Bork, P., Ehrlich, S. D., Stumvoll, M., Pedersen, O., Aron-Wisnewsky, J., Clément, K., Bäckhed, F. (2020). Imidazole propionate is increased in diabetes and associated with dietary patterns and altered microbial ecology. *Nature Communications*, 11(1), 5881. <https://doi.org/10.1038/s41467-020-19589-w>. PMID: 33208748.

Moya-Camarena, S. Y., Vanden Heuvel, J. P., Blanchard, S. G., Leesnitzer, L. A., & Belury, M. A. (1999). Conjugated linoleic acid is a potent naturally occurring ligand and activator of PPARalpha. *Journal of Lipid Research*, 40, 1426–1433. [\[PubMed\]](#)

Mudaliar, S., Henry, R. R., Sanyal, A. J., Morrow, L., Marschall, H. U., Kipnes, M., et al. (2013). Efficacy and safety of the farnesoid X receptor agonist obeticholic acid in patients with type 2 diabetes and nonalcoholic fatty liver disease. *Gastroenterology*, 145, 574–582.e571. [\[PubMed\]](#)

Mueller, N. T., Whyatt, R., Hoepner, L., Oberfield, S., Dominguez-Bello, M. G., Widen, E. M., et al. (2015). Prenatal exposure to antibiotics, cesarean section and risk of childhood obesity. *International Journal of Obesity*, 39, 665–670. [\[PubMed\]](#)

Muller, V. M., Zietek, T., Rohm, F., Fiamoncini, J., Lagkouvardos, I., Haller, D., et al. (2016). Gut barrier impairment by high-fat diet in mice depends on housing conditions. *Molecular Nutrition and Food Research*, 60, 897–908. [\[PubMed\]](#)

Nagpal, R., Newman, T. M., Wang, S., et al. (2024). Fecal microbiota transplantation from lean donors improves metabolic outcomes in obesity: A randomized clinical trial. *Nature Medicine*, 30(5), 1296–1306.

Newhall, K. J., Diemer, G. S., Leshinsky, N., Kerkof, K., Chute, H. T., Russell, C. B., Rees, W., Welcher, A. A., Patterson, S. D., Means, G. D. (2010). Evidence for endotoxin contamination in plastic Na⁺-heparin blood collection tube lots. *Clin Chem*, 56(9), 1483–1491. <https://doi.org/10.1373/clinchem.2006.144618>. Epub 2010 Jul 27. PMID: 20663962.

Nie, Q., Luo, X., Wang, K., Ding, Y., Jia, S., Zhao, Q., Li, M., Zhang, J., Zhuo, Y., Lin, J., Guo, C., Zhang, Z., Liu, H., Zeng, G., You, J., Sun, L., Lu, H., Ma, M., Jia, Y., Zheng, M. H., Pang, Y., Qiao, J., Jiang, C. (2024). Gut symbionts alleviate MASH through a secondary bile acid biosynthetic pathway. *Cell*, 187(11), 2717–2734.e33. <https://doi.org/10.1016/j.cell.2024.03.034>. Epub 2024 Apr 22. PMID: 38653239.

Nieuwdorp, M., Gilijamse, P. W., Pai, N., & Kaplan, L. M. (2023). Role of the microbiome in energy regulation and metabolism. *Cell Metabolism*, 35(2), 209–223.

Ogasawara, Y., Itakura, E., Kono, N., Mizushima, N., Arai, H., Nara, A., et al. (2014). Stearoyl-CoA desaturase 1 activity is required for autophagosome formation. *The Journal of Biological Chemistry*, 289, 23938–23950. [\[PubMed\]](#)

Pabst, O., Hornef, M. W., Schaap, F. G., Cerovic, V., Clavel, T., & Bruns, T. (2023). Gut-liver axis: Barriers and functional circuits. *Nature Reviews Gastroenterology & Hepatology*, 20(7), 447–461. <https://doi.org/10.1038/s41575-023-00771-6>. Epub 2023 Apr 21. PMID: 37085614.

Peck, B., Schug, Z. T., Zhang, Q., Dankworth, B., Jones, D. T., Smethurst, E., Patel, R., Mason, S., Jiang, M., Saunders, R. E., Howell, M., Mitter, R., Spencer-Dene, B., Stamp, G., McGarry, L., James, D., Shanks, E., Aboagye, E. O., Critchlow, S. E., Leung, H. Y., Harris, A. L., Wakelam, M. J. O., Gottlieb, E., & Schulze, A. (2016). Inhibition of fatty acid desaturation is detrimental to cancer cell survival in metabolically compromised environments. *Cancer & Metabolism*, 4, 6.

Porchia, L. M., Vazquez-Marroquin, G., Ochoa-Précoma, R., Lazo-de-la-Vega-Monroy, M.-L., & Gonzalez-Mejia, M. E. (2022). Probiotics' effect on visceral and subcutaneous adipose tissue: A systematic review of randomized controlled trials. *European Journal of Clinical Nutrition*, 76(12), 1646–1656. [\[PubMed\]](#)

Ott, B., Skurk, T., Hastreiter, L., Lagkouvardos, I., Fischer, S., Buttner, J., et al. (2017). Effect of

caloric restriction on gut permeability, inflammation markers, and fecal microbiota in obese women. *Scientific Reports*, 7, 11955.

[\[PubMed\]](#)

Paramsothy, S., Kamm, M. A., Kaakoush, N. O., Walsh, A. J., van den Bogaerde, J., Samuel, D., et al. (2017). Multidonor intensive faecal microbiota transplantation for active ulcerative colitis: A randomised placebo-controlled trial. *Lancet*, 389, 1218–1228.

[\[PubMed\]](#)

Parseus, A., Sommer, N., Sommer, F., Caesar, R., Molinaro, A., Stahlman, M., et al. (2017). Microbiota-induced obesity requires farnesoid X receptor. *Gut*, 66, 429–437.

[\[PubMed\]](#)

Pascal, V., Pozuelo, M., Borruel, N., Casellas, F., Campos, D., Santiago, A., et al. (2017). A microbial signature for Crohn's disease. *Gut*, 66, 813–822.

[\[PubMed\]](#)

Pedersen, H. K., Gudmundsdottir, V., Nielsen, H. B., Hyotylainen, T., Nielsen, T., Jensen, B. A., et al. (2016). Human gut microbes impact host serum metabolome and insulin sensitivity. *Nature*, 535, 376–381.

[\[https://doi.org/10.1038/nature18646\]](https://doi.org/10.1038/nature18646) [\[PubMed\]](#)

Pereira, S. L., Leonard, A. E., & Mukerji, P. (2003). Recent advances in the study of fatty acid desaturases from animals and lower eukaryotes. *Prostaglandins, Leukotrienes, and Essential Fatty Acids*, 68, 97–106.

[\[PubMed\]](#)

Perez-Munoz, M. E., Arrieta, M. C., Ramer-Tait, A. E., & Walter, J. (2017). A critical assessment of the “sterile womb” and “in utero colonization” hypotheses: Implications for research on the pioneer infant microbiome. *Microbiome*, 5, 48.

[\[https://doi.org/10.1186/s40168-017-0268-4\]](https://doi.org/10.1186/s40168-017-0268-4) [\[PubMed\]](#)

Peterson, L. W., & Artis, D. (2014). Intestinal epithelial cells: Regulators of barrier function and immune homeostasis. *Nature Reviews. Immunology*, 14, 141–153.

[\[PubMed\]](#)

Pinot, M., Vanni, S., Pagnotta, S., Lacas-Gervais, S., Payet, L. A., Ferreira, T., et al. (2014). Lipid cell biology. Polyunsaturated phospholipids facilitate membrane deformation and fission by endocytic proteins. *Science*, 345, 693–697.

[\[PubMed\]](#)

Pleasant, J. R. (1968). Characteristics of the germ-free animal. In M. E. Coates, H. A. Gordon, & B. S. Wostmann (Eds.), *The germ-free animal in research*. Academic Press.

Plovier, H., Everard, A., Druart, C., Depommier, C., Van Hul, M., Geurts, L., et al. (2017). A purified membrane protein from *Akkermansia muciniphila* or the pasteurized bacterium improves metabolism in obese and diabetic mice. *Nature Medicine*, 23, 107–113.

[\[PubMed\]](#)

Pribyl, A. L., Hugenholtz, P., Cooper, M. A. (2025). A decade of advances in human gut microbiome-derived biotherapeutics. *Nature Microbiology*, 10(2), 301–312. <https://doi.org/10.1038/s41564-024-01896-3>. Epub 2025 Jan 8. PMID: 39779879.

Qin, J., Li, Y., Cai, Z., Li, S., Zhu, J., Zhang, F., et al. (2012). A metagenome-wide association study of gut microbiota in type 2 diabetes. *Nature*, 490, 55–60. [\[https://doi.org/10.1038/nature11450\]](https://doi.org/10.1038/nature11450) [\[PubMed\]](#)

Rasmussen, T. S., Mentzel, C. M. J., Kot, W., Castro-Mejía, J. L., Zuffa, S., Swann, J. R., Hansen, L. H., Vogensen, F. K., & Nielsen, D. S. (2020). Faecal virome transplantation decreases symptoms of type 2 diabetes and obesity in a murine model. *Gut*, 69(12), 2122–2130. [\[PubMed\]](#)

Redl, H., Bahrami, S., Leichtfried, G., Schlag, G. (1992). Special collection and storage tubes for blood endotoxin and cytokine measurements. *Clin Chem*, 38(5), 764–765. PMID: 1582032.

Reijnders, D., Goossens, G. H., Hermes, G. D., Neis, E. P., van der Beek, C. M., Most, J., et al. (2016). Effects of gut microbiota manipulation by antibiotics on host metabolism in obese humans: A randomized double-blind placebo-controlled trial. *Cell Metabolism*, 24, 63–74. [\[PubMed\]](#)

Reitmeier, S., Kiessling, S., Clavel, T., List, M., Almeida, E. L., Ghosh, T. S., Neuhaus, K., Grallert, H., Troll, M., Rathmann, W., Linkohr, B., Franke, A., Le Roy, C., Bell, J., Spector, T. D., Baumbach, J., O'Toole, P. W., Peters, A., & Haller, D. (2020). Arrhythmic gut microbiome signatures predict risk of type 2 diabetes. *Cell Host & Microbe*, 28(2), 258–272.e6.

Rettger, L. F. (1915). The influence of milk feeding on mortality and growth, and on the character of the intestinal flora. *The Journal of Experimental Medicine*, 21, 365–388. [\[PubMed\]](#)

Ridaura, V. K., Faith, J. J., Rey, F. E., Cheng, J., Duncan, A. E., Kau, A. L., et al. (2013a). Gut microbiota from twins discordant for obesity modulate metabolism in mice. *Science*, 341, 1241214. [\[PubMed\]](#)

Ridaura, V. K., Faith, J. J., Rey, F. E., Cheng, J., Duncan, A. E., Kau, A. L., Griffin, N. W., Lombard, V., Henrissat, B., Bain, J. R., Muehlbauer, M. J., Ilkayeva, O., Semenkovich, C. F., Funai, K., Hayashi, D. K., Lyle, B. J., Martini, M. C., Ursell, L. K., Clemente, J. C., Van Treuren, W., Walters, W. A., Knight, R., Newgard, C. B., Heath, A. C., & Gordon, J. I. (2013b). Gut microbiota from twins discordant for obesity modulate metabolism in mice. *Nature*, 505(7484), 559–563. [\[https://doi.org/10.1038/nature12820\]](https://doi.org/10.1038/nature12820)

Ridlon, J. M., Kang, D. J., & Hylemon, P. B. (2006). Bile salt biotransformations by human intestinal bacteria. *Journal of Lipid Research*, 47, 241–259. [\[PubMed\]](#)

Rinott, E., Youngster, I., Yaskolka Meir, A., Tsaban, G., Zelicha, H., Kaplan, A., Knights, D., Tuohy, K., Fava, F., Scholz, M. U., Ziv, O., Rubin, E., Tirosh, A., Rudich, A., Blüher, M., Stumvoll, M., Ceglarek, U., Clement, K., Koren, O., Wang, D. D., Hu, F. B., Stampfer, M. J., & Shai, I. (2021). Effects of diet-

modulated autologous fecal microbiota transplantation on weight regain. *Gastroenterology*, 160(1), 158–173.e10.

[\[PubMed\]](#)

Roediger, W. E. (1980). Role of anaerobic bacteria in the metabolic welfare of the colonic mucosa in man. *Gut*, 21, 793–798.

[\[PubMed\]](#)

Le Roy, T., Moens de Hase, E., Van Hul, M., Paquot, A., Pelicaen, R., Régnier, M., Depommier, C., Druart, C., Everard, A., Maiter, D., Delzenne, N. M., Bindels, L. B., De Barse, M., Loumave, A., Hermans, M. P., Thissen, J. P., Vieira-Silva, S., Falony, G., Raes, J., Muccioli, G. G., & Cani, P. D. (2022). *Dysosmobacter welbionis* is a newly isolated human commensal bacterium preventing diet-induced obesity and metabolic disorders in mice. *Gut*, 71(3), 534–543.

[\[PubMed\]](#)

Russo, F., Linsalata, M., Clemente, C., Chiloiro, M., Orlando, A., Marconi, E., et al. (2012). Inulin-enriched pasta improves intestinal permeability and modifies the circulating levels of zonulin and glucagon-like peptide 2 in healthy young volunteers. *Nutrition Research*, 32, 940–946.

[\[PubMed\]](#)

Saari, A., Virta, L. J., Sankilampi, U., Dunkel, L., & Saxen, H. (2015). Antibiotic exposure in infancy and risk of being overweight in the first 24 months of life. *Pediatrics*, 135, 617–626.

[\[PubMed\]](#)

Salminen, S., Collado, M. C., Endo, A., Hill, C., Lebeer, S., Quigley, E. M. M., Sanders, M. E., Shamir, R., Swann, J. R., Szajewska, H., & Vinderola, G. (2021). The International Scientific Association of Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of postbiotics. *Nature Reviews Gastroenterology & Hepatology*, 18(9), 649–667.

Sanna, S., van Zuydam, N. R., Mahajan, A., Kurilshikov, A., Vila, A. V., Vösa, U., Mujagic, Z., Masclee, A. A. M., Jonkers, D. M. A. E., Oosting, M., Joosten, L. A. B., Netea, M. G., Franke, L., Zhernakova, A., McCarthy, M. I., & Wijmenga, C. (2020). Causal relationships among the gut microbiome, short-chain fatty acids and metabolic diseases. *Nature Genetics*, 51(4), 600–605.

Santacruz, A., Collado, M. C., Garcia-Valdes, L., Segura, M. T., Martin-Lagos, J. A., Anjos, T., et al. (2010). Gut microbiota composition is associated with body weight, weight gain and biochemical parameters in pregnant women. *The British Journal of Nutrition*, 104, 83–92.

[\[PubMed\]](#)

Sayin, S. I., Wahlstrom, A., Felin, J., Jantti, S., Marschall, H. U., Bamberg, K., et al. (2013). Gut microbiota regulates bile acid metabolism by reducing the levels of tauro-beta-muricholic acid, a naturally occurring FXR antagonist. *Cell Metabolism*, 17, 225–235.

[\[PubMed\]](#)

Skotland, T., Ekroos, K., McDonald, J., Ahrends, R., Liebisch, G., & Sandvig, K. (2024). Pitfalls in lipid mass spectrometry of mammalian samples — A brief guide for biologists. *Nature Reviews Molecular Cell Biology*, 25(10), 759–760.

[\[PubMed\]](#)

Schmitz, G., & Ecker, J. (2008). The opposing effects of n-3 and n-6 fatty acids. *Progress in Lipid Research*, 47, 147–155.

[[PubMed](#)]

Schmidt, T. S. B., Raes, J., & Bork, P. (2018). The human gut microbiome: From association to modulation. *Cell Host & Microbe*, 24(1), 119–130.

Schwarzer, M., Makki, K., Storelli, G., Machuca-Gayet, I., Srutkova, D., Hermanova, P., et al. (2016). *Lactobacillus plantarum* strain maintains growth of infant mice during chronic undernutrition. *Science*, 351, 854–857.

[[PubMed](#)]

Schwarzer, M., Gautam, U. K., Makki, K., Lambert, A., Brabec, T., Joly, A., Šrůtková, D., Poinso, P., Novotná, T., Geoffroy, S., Courtin, P., Hermanová, P. P., Matos, R. C., Landry, J. J. M., Gérard, C., Bulteau, A. L., Hudcovic, T., Kozáková, H., Filipp, D., Chapot-Chartier, M. P., Šinkora, M., Peretti, N., Boneca, I. G., Chamaillard, M., Vidal, H., De Vadder, F., Leulier, F. (2023). Microbe-mediated intestinal NOD2 stimulation improves linear growth of undernourished infant mice. *Science*, 379(6634), 826–833. <https://doi.org/10.1126/science.ade9767>. Epub 2023 Feb 23. PMID: 36821686.

Schwiertz, A., Taras, D., Schafer, K., Beijer, S., Bos, N. A., Donus, C., et al. (2010). Microbiota and SCFA in lean and overweight healthy subjects. *Obesity (Silver Spring)*, 18, 190–195.

[[PubMed](#)]

Scorletti, E., Afolabi, P. R., Miles, E. A., Smith, D. E., Almeshmadi, A., Alshathry, A., Childs, C. E., Kapsokefalou, M., Lynch, M., Moore, J. B., et al. (2020). Synbiotics alter fecal microbiomes, but not liver fat or fibrosis, in a randomized trial of patients with nonalcoholic fatty liver disease. *Gastroenterology*, 158(6), 1597–1610.e7.

[[PubMed](#)]

Semova, I., Carten, J. D., Stombaugh, J., Mackey, L. C., Knight, R., Farber, S. A., et al. (2012). Microbiota regulate intestinal absorption and metabolism of fatty acids in the zebrafish. *Cell Host and Microbe*, 12, 277–288.

[[PubMed](#)]

Singh, V., Chassaing, B., Zhang, L., San Yeoh, B., Xiao, X., Kumar, M., et al. (2015). Microbiota-dependent hepatic lipogenesis mediated by stearoyl CoA desaturase 1 (SCD1) promotes metabolic syndrome in TLR5-deficient mice. *Cell Metabolism*, 22, 983–996.

[[PubMed](#)]

Smith, S., Witkowski, A., & Joshi, A. K. (2003). Structural and functional organization of the animal fatty acid synthase. *Progress in Lipid Research*, 42, 289–317.

[[PubMed](#)]

Sonnenburg, E. D., Smits, S. A., Tikhonov, M., Higginbottom, S. K., Wingreen, N. S., & Sonnenburg, J. L. (2016). Diet-induced extinctions in the gut microbiota compound over generations. *Nature*, 529, 212–215.

[<https://doi.org/10.1038/nature16504>][[PubMed](#)]

Steimle, A., Autenrieth, I. B., & Frick, J. S. (2016). Structure and function: Lipid A modifications in commensals and pathogens. *International Journal of Medical Microbiology*, 306, 290–301.

[\[PubMed\]](#)

Snaebjornsson, M. T., Janaki-Raman, S., & Schulze, A. (2020). Greasing the wheels of the cancer machine: The role of lipid metabolism in cancer. *Cell Metabolism*, 31(1), 62–76.

[\[PubMed\]](#)

Suarez-Zamorano, N., Fabbiano, S., Chevalier, C., Stojanovic, O., Colin, D. J., Stevanovic, A., et al. (2015). Microbiota depletion promotes browning of white adipose tissue and reduces obesity. *Nature Medicine*, 21, 1497–1501.

[\[PubMed\]](#)

Sullivan, M. R., Danai, L. V., Lewis, C. A., Chan, S. H., Gui, D. Y., Kunchok, T., Dennstedt, E. A., Vander Heiden, M. G., & Muir, A. (2019). Quantification of microenvironmental metabolites in murine cancers reveals determinants of tumor nutrient availability. *Cell*, 177(2), 495–507.e16.

Sze, M. A., & Schloss, P. D. (2016). Looking for a signal in the noise: Revisiting obesity and the microbiome. *MBio*, 7, e01018–e01016.

[\[PubMed\]](#)

Takeuchi, T., Kameyama, K., Miyauchi, E., Nakanishi, Y., Kanaya, T., Fujii, T., Kato, T., Sasaki, T., Tachibana, N., Negishi, H., Matsui, M., & Ohno, H. (2023). Fatty acid overproduction by gut commensal microbiota exacerbates obesity. *Cell Metabolism*, 35(2), 361–375.e9.

[\[PubMed\]](#)

Teixeira, T. F., Souza, N. C., Chiarello, P. G., Franceschini, S. C., Bressan, J., Ferreira, C. L., et al. (2012). Intestinal permeability parameters in obese patients are correlated with metabolic syndrome risk factors. *Clinical Nutrition*, 31, 735–740.

[\[PubMed\]](#)

Thaiss, C. A., Zeevi, D., Levy, M., Zilberman-Schapira, G., Suez, J., Tengeler, A. C., Abramson, L., Katz, M. N., Korem, T., Zmora, N., Kuperman, Y., Biton, I., Gilad, S., Harmelin, A., Shapiro, H., Halpern, Z., Segal, E., & Elinav, E. (2023). Transkingdom control of microbiota diurnal oscillations promotes metabolic homeostasis. *Cell Metabolism*, 35(8), 1453–1468.e8.

Thaiss, C. A., Zeevi, D., Levy, M., Zilberman-Schapira, G., Suez, J., Tengeler, A. C., Abramson, L., Katz, M. N., Korem, T., Zmora, N., Kuperman, Y., Biton, I., Gilad, S., Harmelin, A., Shapiro, H., Halpern, Z., Segal, E., & Elinav, E. (2014). Transkingdom control of microbiota diurnal oscillations promotes metabolic homeostasis. *Cell*, 159(3), 514–529.

[\[PubMed\]](#)

Thomas, C., Pellicciari, R., Pruzanski, M., Auwerx, J., & Schoonjans, K. (2008). Targeting bile-acid signalling for metabolic diseases. *Nature Reviews. Drug Discovery*, 7, 678–693.

[\[PubMed\]](#)

Thomas, A. M., & Segata, N. (2019). Multiple levels of the unknown in microbiome research. *BMC Biology*, 17(1), 48.

[\[PubMed\]](#)

Thorasin, T., Hoyles, L., & McCartney, A. L. (2015). Dynamics and diversity of the 'Atopobium cluster' in the human faecal microbiota, and phenotypic characterization of 'Atopobium cluster' isolates. *Microbiology*, 161, 565–579.

[\[PubMed\]](#)

Tisdale, M. J. (1987). Cancer cachexia: Metabolic alterations and clinical manifestations. *Science*, 235(4792), 1113–1117.

Torres-Fuentes, C., Schellekens, H., Dinan, T. G., & Cryan, J. F. (2017). The microbiota-gut-brain axis in obesity. *Lancet Gastroenterology and Hepatology*, 2, 747–756.

[\[PubMed\]](#)

Torres, B., Sánchez, M. C., Virto, L., Llama-Palacios, A., Ciudad, M. J., & Collado, L. (2024). Use of probiotics in preventing and treating excess weight and obesity: A systematic review. *Obesity Science & Practice*, 10(3), e759.

Turnbaugh, P. J., Ridaura, V. K., Faith, J. J., Rey, F. E., Knight, R., & Gordon, J. I. (2009). The effect of diet on the human gut microbiome: A metagenomic analysis in humanized gnotobiotic mice. *Science Translational Medicine*, 1(6), 6ra14.

[\[PubMed\]](#)

Tremaroli, V., Karlsson, F., Werling, M., Stahlman, M., Kovatcheva-Datchary, P., Olbers, T., et al. (2015). Roux-en-Y gastric bypass and vertical banded gastroplasty induce long-term changes on the human gut microbiome contributing to fat mass regulation. *Cell Metabolism*, 22, 228–238.

[\[PubMed\]](#)

Turnbaugh, P. J., Ley, R. E., Mahowald, M. A., Magrini, V., Mardis, E. R., & Gordon, J. I. (2006). An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature*, 444, 1027–1031.

[\[https://doi.org/10.1038/nature05414\]](https://doi.org/10.1038/nature05414) [\[PubMed\]](#)

Turnbaugh, P. J., Backhed, F., Fulton, L., & Gordon, J. I. (2008). Diet-induced obesity is linked to marked but reversible alterations in the mouse distal gut microbiome. *Cell Host and Microbe*, 3, 213–223.

[\[PubMed\]](#)

Ussar, S., Griffin, N. W., Bezy, O., Fujisaka, S., Vienberg, S., Softic, S., et al. (2015). Interactions between gut microbiota, host genetics and diet modulate the predisposition to obesity and metabolic syndrome. *Cell Metabolism*, 22, 516–530.

[\[PubMed\]](#)

van Meer, G., Voelker, D. R., & Feigenson, G. W. (2008). Membrane lipids: Where they are and how they behave. *Nature Reviews. Molecular Cell Biology*, 9, 112–124.

[\[PubMed\]](#)

van Nood, E., Vrieze, A., Nieuwdorp, M., Fuentes, S., Zoetendal, E. G., de Vos, W. M., et al. (2013). Duodenal infusion of donor feces for recurrent *Clostridium difficile*. *The New England Journal of Medicine*, 368, 407–415.

[\[PubMed\]](#)

Veiga, P., Pons, N., Agrawal, A., Oozeer, R., Guyonnet, D., Brazeilles, R., Faurie, J.-M., van Hylckama Vlieg, J. E. T., Boon, N., & Varela, E. (2019). Changes of the human gut microbiome induced by a fermented milk product. *Nature*, 569(7757), 544–549.

Vieira-Silva, S., Falony, G., Belda, E., Nielsen, T., Aron-Wisnewsky, J., Chakaroun, R., Forslund, S. K., Assmann, K., Valles-Colomer, M., Nguyen, T. T. D., Proost, S., Prifti, E., Tremaroli, V., Pons, N., Le Chatelier, E., Andreelli, F., Bastard, J.-P., Coelho, L. P., Galleron, N., et al. (2020). Statin therapy is associated with lower prevalence of gut microbiota dysbiosis. *Nature*, 581(7808), 310–315. [\[https://doi.org/10.1038/s41586-020-2269-x\]](https://doi.org/10.1038/s41586-020-2269-x) [\[PubMed\]](#)

Vrieze, A., Van Nood, E., Holleman, F., Salojarvi, J., Kootte, R. S., Bartelsman, J. F. W. M., Dallinga-Thie, G. M., Ackermans, M. T., Serlie, M. J., Oozeer, R., Derrien, M., Druesne, A., Van Hylckama Vlieg, J. E. T., Bloks, V. W., Groen, A. K., Heilig, H. G. H. J., Zoetendal, E. G., Stoes, E. S., de Vos, W. M., & Nieuwdorp, M. (2012). Transfer of intestinal microbiota from lean donors increases insulin sensitivity in individuals with metabolic syndrome. *Gastroenterology*, 143(4), 913–916.e7. [\[PubMed\]](#)

Velagapudi, V. R., Hezaveh, R., Reigstad, C. S., Gopalacharyulu, P., Yetukuri, L., Islam, S., et al. (2010). The gut microbiota modulates host energy and lipid metabolism in mice. *Journal of Lipid Research*, 51, 1101–1112. [\[PubMed\]](#)

Vijay-Kumar, M., Aitken, J. D., Carvalho, F. A., Cullender, T. C., Mwangi, S., Srinivasan, S., et al. (2010). Metabolic syndrome and altered gut microbiota in mice lacking Toll-like receptor 5. *Science*, 328, 228–231. [\[PubMed\]](#)

Vriens, K., Christen, S., Parik, S., Broekaert, D., Yoshinaga, K., Talebi, A., Dehairs, J., Escalona-Noguero, C., Schmieder, R., Cornfield, T., Charlton, C., Romero-Pérez, L., Rossi, M., Rinaldi, G., Orth, M. F., Boon, R., Kerstens, A., Kwan, S. Y., Faubert, B., Méndez-Lucas, A., Kopitz, C. C., Chen, T., Fernandez-Garcia, J., Duarte, J. A. G., Schmitz, A. A., Steigemann, P., Najimi, M., Hägebarth, A., Van Ginderachter, J. A., Sokal, E., Gotoh, N., Wong, K. K., Verfaillie, C., Derua, R., Munck, S., Yuneva, M., Beretta, L., DeBerardinis, R. J., Swinnen, J. V., Hodson, L., Cassiman, D., Verslype, C., Christian, S., Grünwald, S., Grünwald, T. G. P., Fendt, S. M. (2019). Evidence for an alternative fatty acid desaturation pathway increasing cancer plasticity. *Nature*, 566(7744), 403–406. <https://doi.org/10.1038/s41586-019-0904-1>. Epub 2019 Feb 6. PMID: 30728499.

Wagner, V. E., Dey, N., Guruge, J., Hsiao, A., Ahern, P. P., Semenkovich, N. P., et al. (2016). Effects of a gut pathobiont in a gnotobiotic mouse model of childhood undernutrition. *Science Translational Medicine*, 8, 366ra164. [\[PubMed\]](#)

Walker, A. W., Hoyle, L. (2023). Human microbiome myths and misconceptions. *Nat Microbiol*, 8(8), 1392–1396. <https://doi.org/10.1038/s41564-023-01426-7>. Epub 2023 Jul 31. PMID: 37524974.

Wall, R., Ross, R. P., Shanahan, F., O'Mahony, L., O'Mahony, C., Coakley, M., et al. (2009). Metabolic activity of the enteric microbiota influences the fatty acid composition of murine and porcine

liver and adipose tissues. *The American Journal of Clinical Nutrition*, 89, 1393–1401.

[\[PubMed\]](#)

Wallace, R. J., McKain, N., Shingfield, K. J., & Devillard, E. (2007). Isomers of conjugated linoleic acids are synthesized via different mechanisms in ruminal digesta and bacteria. *Journal of Lipid Research*, 48, 2247–2254.

[\[PubMed\]](#)

Wang, Z., Klipfell, E., Bennett, B. J., Koeth, R., Levison, B. S., Dugar, B., et al. (2011). Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease. *Nature*, 472, 57–63.

[\[https://doi.org/10.1038/nature09922\]](https://doi.org/10.1038/nature09922) [\[PubMed\]](#)

Wang, X., Ota, N., Manzanillo, P., Kates, L., Zavala-Solorio, J., Eidenschenk, C., et al. (2014). Interleukin-22 alleviates metabolic disorders and restores mucosal immunity in diabetes. *Nature*, 514, 237–241.

[\[https://doi.org/10.1038/nature13564\]](https://doi.org/10.1038/nature13564) [\[PubMed\]](#)

Wang, Y., Kuang, Z., Yu, X., Ruhn, K. A., Kubo, M., & Hooper, L. V. (2017). The intestinal microbiota regulates body composition through NFIL3 and the circadian clock. *Science*, 357(6354), 912–916.

[\[PubMed\]](#)

Wang, D. D., Nguyen, L. H., Li, Y., Yan, Y., Ma, W., Rinott, E., Ivey, K. L., Shai, I., Willett, W. C., Hu, F. B., Rimm, E. B., Stampfer, M. J., Chan, A. T., & Huttenhower, C. (2021). The gut microbiome modulates the protective association between a Mediterranean diet and cardiometabolic disease risk. *Nature Medicine*, 27(2), 333–343.

[\[PubMed\]](#)

Wang, Y., Wang, M., Chen, J., Li, Y., Kuang, Z., Dende, C., Raj, P., Quinn, G., Hu, Z., Srinivasan, T., Hassell, B., Ruhn, K. A., Behrendt, C. L., Liang, T., Dou, X., Song, Z., Hooper, L. V. (2023). The gut microbiota reprograms intestinal lipid metabolism through long noncoding RNA Snhg9. *Science*, 381(6660), 851–857. <https://doi.org/10.1126/science.ade0522>. Epub 2023 Aug 24. PMID: 37616368.

Ward, P. S., & Thompson, C. B. (2012). Metabolic reprogramming: A cancer hallmark even Warburg did not anticipate. *Nature Reviews Cancer*, 12(11), 827–837.

Warburg, O. (1956). On the origin of cancer cells. *Science*, 123(3191), 309–314.

[\[PubMed\]](#)

Wastyk, H. C., Fragiadakis, G. K., Perelman, D., Dahan, D., Merrill, B. D., Yu, F. B., Topf, M., Gonzalez, C. G., Van Treuren, W., Han, S., Robinson, J. L., Elias, J. E., Sonnenburg, E. D., & Sonnenburg, J. L. (2021). Gut-microbiota-targeted diets modulate human immune status. *Cell Host & Microbe*, 29(5), 775–788.e6.

Wenk, M. R. (2010). Lipidomics: New tools and applications. *Cell*, 143, 888–895.

[\[PubMed\]](#)

Wostmann, B. S. (1975). Nutrition and metabolism of the germfree mammal. *World Review of Nutrition and Dietetics*, 22, 40–92.

[\[PubMed\]](#)

Woting, A., Pfeiffer, N., Loh, G., Klaus, S., & Blaut, M. (2014). Clostridium ramosum promotes high-fat diet-induced obesity in gnotobiotic mouse models. *MBio*, 5, e01530–e01514.

[[PubMed](#)]

Wilson, B. C., Vatanen, T., Cutfield, W. S., & O'Sullivan, J. M. (2024). The super-donor phenomenon in fecal microbiota transplantation. *Cell Host & Microbe*, 32(7), 987–999.

Wu, G. D., Chen, J., Hoffmann, C., Bittinger, K., Chen, Y. Y., Keilbaugh, S. A., et al. (2011). Linking long-term dietary patterns with gut microbial enterotypes. *Science*, 334, 105–108.

[[PubMed](#)]

Wu, H., Esteve, E., Tremaroli, V., Khan, M. T., Caesar, R., Manneras-Holm, L., et al. (2017). Metformin alters the gut microbiome of individuals with treatment-naïve type 2 diabetes, contributing to the therapeutic effects of the drug. *Nature Medicine*, 23, 850–858.

[[PubMed](#)]

Xiao, S., Fei, N., Pang, X., Shen, J., Wang, L., Zhang, B., et al. (2014). A gut microbiota-targeted dietary intervention for amelioration of chronic inflammation underlying metabolic syndrome. *FEMS Microbiology Ecology*, 87, 357–367.

[[PubMed](#)]

Yang, K., Niu, J., Zuo, T., Sun, Y., Xu, Z., Tang, W., Liu, Q., Zhang, J., Ng, E. K. W., Wong, S. K. H., Yeoh, Y. K., Chan, P. K. S., Chan, F. K. L., Miao, Y., & Ng, S. C. (2021). Alterations in the gut virome in obesity and type 2 diabetes mellitus. *Gastroenterology*, 161(4), 1257–1269.e13.

[[PubMed](#)]

Yao, L., Seaton, S. C., Ndousse-Fetter, S., Adhikari, A. A., DiBenedetto, N., Mina, A. I., Banks, A. S., Bry, L., Devlin, A. S. (2018). A selective gut bacterial bile salt hydrolase alters host metabolism. *Elife*, 7, e37182. <https://doi.org/10.7554/eLife.37182>. PMID: 30014852.

Yao, C.-H., Fowle-Grider, R., Mahieu, N. G., Liu, G.-Y., Chen, Y.-J., Wang, R., Singh, M., Potter, G. S., Gross, R. W., Schaefer, J., Johnson, S. L., & Rabinowitz, J. D. (2019). Exogenous fatty acids are the preferred source of membrane lipids in proliferating fibroblasts. *Nature*, 566(7744), 550–554.

Yatsunencko, T., Rey, F. E., Manary, M. J., Trehan, I., Dominguez-Bello, M. G., Contreras, M., et al. (2012). Human gut microbiome viewed across age and geography. *Nature*, 486, 222–227.

[<https://doi.org/10.1038/nature11053>][[PubMed](#)]

Yin, Y., Sichler, A., Ecker, J., Laschinger, M., Liebisch, G., Höring, M., Basic, M., Bleich, A., Zhang, X. J., Kübelsbeck, L., Plagge, J., Scherer, E., Wohlleber, D., Wang, J., Wang, Y., Steffani, M., Stupakov, P., Gärtner, Y., Lohöfer, F., Mogler, C., Friess, H., Hartmann, D., Holzmann, B., Hüser, N., Janssen, K. P. (2023). Gut microbiota promote liver regeneration through hepatic membrane phospholipid biosynthesis. *J Hepatol*, 78(4), 820–835. <https://doi.org/10.1016/j.jhep.2022.12.028>. Epub 2023 Jan 18. PMID: 36681162.

Yu, E. W., Gao, L., Stastka, P., Cheney, M. C., Mahabamunuge, J., Soto, M. T., Ford, C. B., Bryant, J. A., Henn, M. R., Hohmann, E. L., & Allegretti, J. R. (2023). Fecal microbiota transplantation for the improvement of metabolism in obesity: The FMT-TRIM double-blind placebo-controlled pilot trial. *Nature Medicine*, 29(6), 1439–1449.

Zeevi, D., Korem, T., Zmora, N., Israeli, D., Rothschild, D., Weinberger, A., et al. (2015). Personalized nutrition by prediction of glycemic responses. *Cell*, 163, 1079–1094.

[\[PubMed\]](#)

Zeevi, D., Korem, T., Zmora, N., Israeli, D., Rothschild, D., Weinberger, A., Ben-Yacov, O., Lador, D., Avnit-Sagi, T., Lotan-Pompan, M., Suez, J., Mahdi, J. A., Matot, E., Malka, G., Kosower, N., Rein, M., Zilberman-Schapira, G., Dohnalová, L., Pevsner-Fischer, M., Bikovsky, R., Halpern, Z., Elinav, E., & Segal, E. (2020). Personalized nutrition by prediction of glycemic responses. *Cell Host & Microbe*, 30(1), 75–88.e5.

Zelante, T., Iannitti, R. G., Cunha, C., De Luca, A., Giovannini, G., Pieraccini, G., et al. (2013). Tryptophan catabolites from microbiota engage aryl hydrocarbon receptor and balance mucosal reactivity via interleukin-22. *Immunity*, 39, 372–385.

[\[PubMed\]](#)

Zhang, Z., Mocanu, V., Deehan, E. C., Hotte, N., Zhu, Y., Wei, S., Kao, D. H., Karmali, S., Birch, D. W., Walter, J., & Madsen, K. L. (2024). Recipient microbiome-related features predicting metabolic improvement following fecal microbiota transplantation in adults with severe obesity and metabolic syndrome: A secondary analysis of a phase 2 clinical trial. *Gut Microbes*, 16(1), 2345134.

[\[PubMed\]](#)

Zhao, L., Zhang, F., Ding, X., Wu, G., Lam, Y. Y., Wang, X., Fu, H., Xue, X., Lu, C., Ma, J., Yu, L., Xu, C., Ren, Z., Xu, Y., Shen, H., Zhu, X., Shi, Y., Shen, Q., Dong, W., Liu, R., Ling, Y., Zeng, Y., Wang, X., Zhang, Q., Wang, J., & Wang, L. (2024). Gut bacteria selectively promoted by dietary fibers alleviate type 2 diabetes. *Nature*, 632(8024), 184–192.

Zhou, D., Pan, Q., Xin, F. Z., Zhang, R. N., He, C. X., Chen, G. Y., et al. (2017). Sodium butyrate attenuates high-fat diet-induced steatohepatitis in mice by improving gut microbiota and gastrointestinal barrier. *World Journal of Gastroenterology*, 23, 60–75.

[\[PubMed\]](#)

Zietak, M., Kovatcheva-Datchary, P., Markiewicz, L. H., Stahlman, M., Kozak, L. P., & Backhed, F. (2016). Altered microbiota contributes to reduced diet-induced obesity upon cold exposure. *Cell Metabolism*, 23, 1216–1223.

[\[PubMed\]](#)

Zmora, N., Bashardes, S., Levy, M., & Elinav, E. (2017). The role of the immune system in metabolic health and disease. *Cell Metabolism*, 25, 506–521.

[\[PubMed\]](#)

17. Microbiome and Diseases: Steatotic Liver Disease

Ina Bergheim¹✉ and Kai Markus Schneider²✉

- (1) Lehrstuhl Molekulare Ernährungswissenschaft—Department für Ernährungswissenschaften, Universität Wien, Wien, Germany
- (2) Medizinische Klinik 1, Universitätsklinikum Carl Gustav Carus Dresden, Technische Universität Dresden (TUD), Dresden, Germany

✉ **Ina Bergheim (Corresponding author)**
Email: ina.bergheim@univie.ac.at

✉ **Kai Markus Schneider**
Email: markus.schneider@tu-dresden.de

Abstract

Intensive research efforts aim to understand the multifaceted molecular mechanisms underlying disease onset and progression of Steatotic Liver Disease (SLD). SLD encompasses Metabolic Dysfunction-associated Steatotic Liver Disease (MASLD), MASLD with increased alcohol intake (MetALD), and alcohol-related liver disease (ALD). Taken together, MASLD and ALD are the most common liver diseases worldwide, and up to now, there are few universally accepted therapies other than lifestyle interventions, either focusing on weight reduction and physical exercise or alcohol abstinence. During the last decade, alterations of the intestinal microbiota and gut barrier function leading to an increased translocation of bacterial products have emerged as key

pathogenic factors in both diseases. In this book chapter, current knowledge and understanding of the interplay between intestinal microbiota, barrier function, and the development of SLD are summarized, with a specific focus on MASLD and ALD.

Keywords Steatotic liver disease – Microbiota – Intestinal barrier – Pathogen-associated molecular patterns – Diet – Alcohol

17.1 Introduction

As the liver receives ~70% of its blood supply from the intestine through the portal vein, an interaction between the liver and the gut, including the intestinal microbiome, has been discussed for several decades. In the 1950s, studies were published showing that germ-free animals were protected from the development of liver necrosis (György, [1954](#); Luckey et al., [1954](#)). Furthermore, the treatment of rats with diet-induced steatohepatitis with antibiotics like aureomycin protected them from the development of fibrosis and cirrhosis (Rutenburg et al., [1957](#)). Interestingly, this study showed that nonabsorbable antibiotics were markedly more efficient in delaying the progression to advanced fibrosis when compared to absorbable ones (Rutenburg et al., [1957](#)). The first reports of a novel disease entity named non-alcoholic steatohepatitis (NASH), mainly found in overweight women with type 2 diabetes, appeared in 1980 (Ludwig et al., [1980](#)). Involvement of the intestinal microbiome in steatohepatitis was suggested by a study in 1989, when a patient suffering from small bowel diverticulosis and bacterial overgrowth in the small intestine, the latter being defined as excessive bacteria in the small intestine (Dukowicz et al., [2007](#)), developed steatohepatitis (Nazim et al., [1989](#)). Over the next 30 years, an increasing number of publications suggested that alterations of the intestinal microbiota, usually coupled with intestinal barrier functional defects, are linked to the severity of liver diseases with various etiologies, mainly driven by the advances in sequencing and metabolomics technologies.

The liver receives venous blood from the intestine being rich in nutrients but also other substances ingested with the diet and derived from the intestinal microbiota that have crossed the intestinal barrier. Recent studies have proposed that the liver forms a first vascular and metabolic firewall capturing xenobiotics and endobiotics in settings of intestinal barrier dysfunction, before they may enter the blood circulation (Balmer et al., [2014](#)).

In 2023, a new consensus nomenclature under the guidance of the European Association for the Study of the Liver (EASL), together with the American Association for the Study of Liver Diseases (AASLD) in collaboration with the Asociación Latinoamericana para el Estudio del Hígado (ALEH), was released, which introduced the umbrella term of steatotic liver disease (SLD) (Rinella et al., [2023](#)). Within this classification, NAFLD became Metabolic Dysfunction-associated Steatotic Liver Disease (MASLD), which provides an affirmative and nonstigmatising description of the condition rather than a diagnosis of exclusion. Moreover, the new classification also allowed a more nuanced characterization of alcohol consumption in patients with MASLD. The new category “MASLD with increased alcohol intake” (MetALD) will be used to diagnose patients with >20/30 g alcohol per day in females and males, respectively, which is reported to affect 2–10% of Western populations (Schneider et al., [2024a](#)).

Given the very high similarity between NAFLD and MASLD, we will use the new term MASLD in this chapter when presenting knowledge and understanding of the interplay between gut microbiota, gut barrier function, and the development of MASLD, focusing on simple steatosis and its progression to metabolic dysfunction-associated steatohepatitis (MASH). Moreover, we will focus on alcohol-related liver diseases (ALD).

17.2 Metabolic Dysfunction-Associated Steatotic Liver Disease

MASLD, frequently regarded as the hepatic manifestation of the metabolic syndrome (Kim & Younossi, [2008](#)), strongly and positively correlates with overweight/obesity and insulin resistance or overt type 2 diabetes (Townsend & Newsome, [2016](#)). With still increasing prevalence, MASLD is now regarded as the most common liver disease worldwide, affecting between 20 and 30% of individuals in many industrialized and non-industrialized countries (Younossi et al., [2016](#)). Its prevalence is highest in the Middle East, the USA, and South America, and lowest in Africa (Younossi et al., [2016](#)). Furthermore, liver-specific and overall mortality of patients with MASLD were estimated to be 1.94 (range, 1.28–2.92) and 1.05 (range, 0.70–1.56) (Younossi et al., [2016](#)). MASLD can be diagnosed in patients with hepatic steatosis that fulfils at least one of the cardiometabolic risk factors (increased BMI, diabetes, hypertension, hypertriglyceridemia, low HDL) (Fig. [17.1](#)—steatotic liver disease) (Rinella et al., [2023](#)).

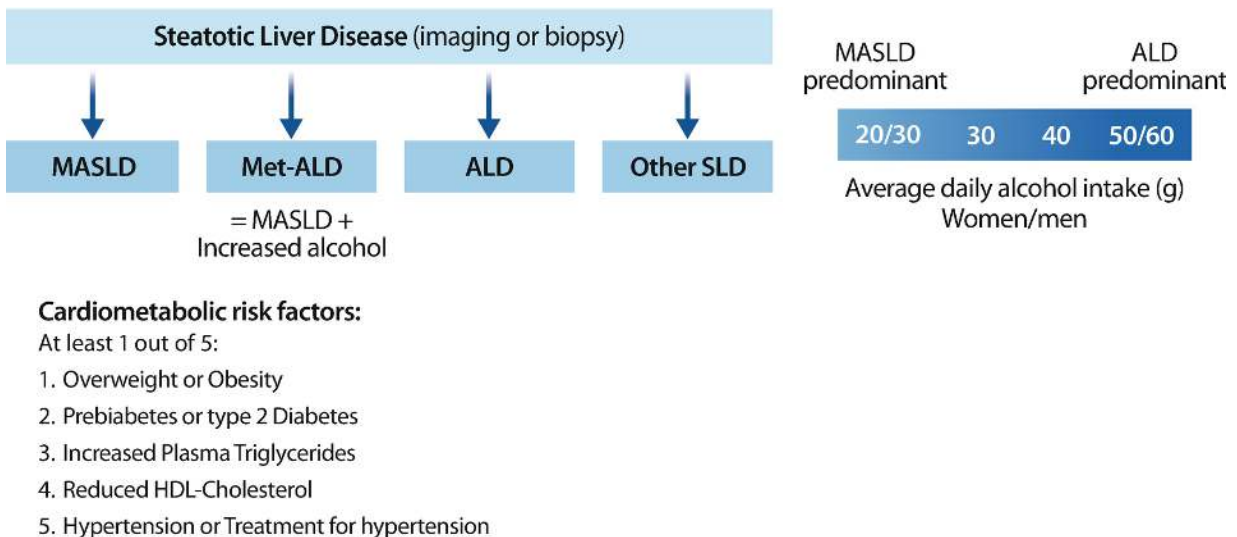


Fig. 17.1 Steatotic liver disease and cardiometabolic risk factors. *ALD* alcohol-related liver disease, *MASLD* Metabolic dysfunction-associated steatotic liver disease, *Met-ALD*, MASLD with increased alcohol intake

MASLD comprises a wide spectrum of diseases ranging from simple steatosis to steatohepatitis (metabolic dysfunction-associated steatohepatitis, MASH), regarded as the inflammatory, aggressive form of MASLD. Fibrosis and finally cirrhosis and primary hepatocellular

carcinoma (HCC) (annual progression rate for MASH ~40.76% according to Younossi et al. (2016), Neuschwander-Tetri and Caldwell (2003) (see Fig. 17.2) are considered the more progressed and non-reversible stages. Notably, once patients have developed cirrhosis, their relative risk of liver-related mortality increases more than 50-fold when compared to non-fibrotic MASLD (Dulai et al., 2017). However, the latter are rare while still serious complications of MASLD (e.g., 0.44/1000 person-years; range, 0.29–0.66) (Starley et al., 2010). In the USA (cirrhotic), MASH has become the second leading cause of liver transplantation in adults (Satapathy & Sanyal, 2015).

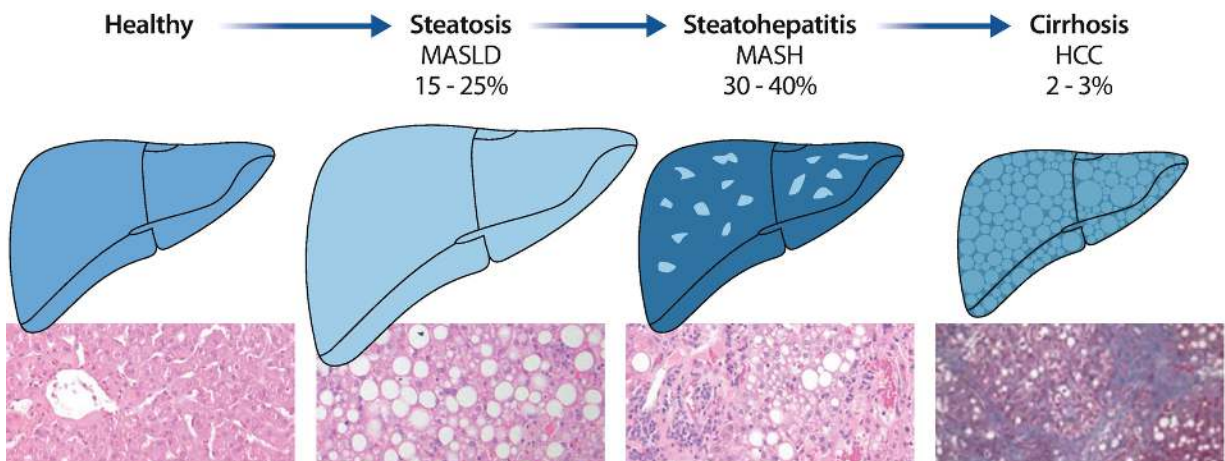


Fig. 17.2 Stages and probability of progression of steatotic liver disease. *MASLD* steatosis, *MASH* steatohepatitis, *HCC* hepatocellular carcinoma

17.2.1 Metabolic Dysfunction-Associated Steatotic Liver Disease (MASLD): Intestinal Microbiota and Gut Barrier Function

In recent years, there have been substantial efforts in understanding the molecular mechanisms involved in disease development and progression of MASLD, which have inspired several ongoing clinical trials. Within the MASLD spectrum, a subgroup is characterized by ongoing liver inflammation, identified as Metabolic-dysfunction associated steatohepatitis (MASH), which can progress to fibrosis and cirrhosis. These individuals, recognized as “at-risk MASH,” are now considered eligible for pharmacological therapies under FDA and EMA

guidelines. Currently, numerous treatment approaches assessed in clinical trials target different pathways to resolve MASH, to reverse fibrosis, or both. Several of these interventions also improve parameters beyond hepatic health, influencing blood glucose control, lipid metabolism, and body weight. Polymorphisms in genes involved in the regulation of lipid and glucose metabolism and the renin-angiotensin system have been proposed to be critical in the onset of MASLD but also in its progression to later stages (e.g., inflammation, fibrosis, and even HCC) (Miyaaki & Nakao, [2017](#); Speliotes & Schneider, [2024](#)). Indeed, numerous studies and meta-analyses have established that a genetic polymorphism of position 148 (rs738409 C/G) in the gene encoding for patatin-like phospholipase domain-containing protein (PNPLA3) is a strong genetic predisposition not only associated with hepatic fat accumulation but also the development of inflammation, fibrosis, and HCC in humans (Chen et al., [2015](#)). In recent years, several additional genetic variants have been identified. Yet, studies also suggest that the impact of PNPLA3 genetic modifications, but also those of others, on MASLD may differ among genetic groups (Chen et al., [2015](#)). Moreover, the risk of developing MASLD in carriers of genetic risk variants is strongly influenced by environmental and lifestyle factors (Speliotes & Schneider, [2024](#)). Besides genetics, physical inactivity and general overnutrition, specifically the consumption of a so-called Western-style diets, i.e., rich in saturated fatty acids and mono- and disaccharides like fructose (especially as high-fructose corn syrup) and sucrose as well as cholesterol, has been identified as a critical factor in the development and progression of MASLD (Barrea et al., [2017](#); Romero-Gomez et al., [2017](#)). The intestinal microbiota and gut barrier dysfunction have also been proposed to be critical factors in the development of MASLD (see Fig. [17.3](#)). Patients with different stages of MASLD/MASH suffer from endotoxemia associated with an increased intestinal permeability and a loss of tight junction proteins in the duodenum (Baumann et al., [2021](#); Miele et al., [2009](#); Thuy et al., [2008](#); Volynets et al., [2012](#)). These patients frequently have elevated plasma levels of the lipopolysaccharide (LPS)-binding

protein (LBP) as well as enhanced expression of the endotoxin receptor TLR (Toll-like receptor)-4 and tumor necrosis factor (TNF)- α in liver tissue (Kanuri et al., [2011](#); Ruiz et al., [2007](#); Wigg et al., [2001](#)). Furthermore, the expression of TLR4 and other TLRs, e.g., TLR1–5 (but not TLR6–10), are induced in the liver of patients with simple steatosis, to increase in MASH and MASH with beginning fibrosis (Kanuri et al., [2015](#)). It was also shown that ligands of TLR2, like peptidoglycan and lipoteichoic acids, are also higher in patients with MASH (Baumann et al., [2021](#)) and that TLR1 knockout mice are at least in part protected from the development of diet-induced MASLD. TLR1 forms a dimer with TLR2, thereby recognizing bacterial wall compounds of Gram-positive bacteria. This suggests that not only the translocation of LPS derived from Gram-negative bacteria might be increased in settings of MASLD and MASH but also that of other bacterial toxins, as well as viral and bacterial proteins, modified proteins, DNA, and RNA in humans (see Fig. [17.3](#)).

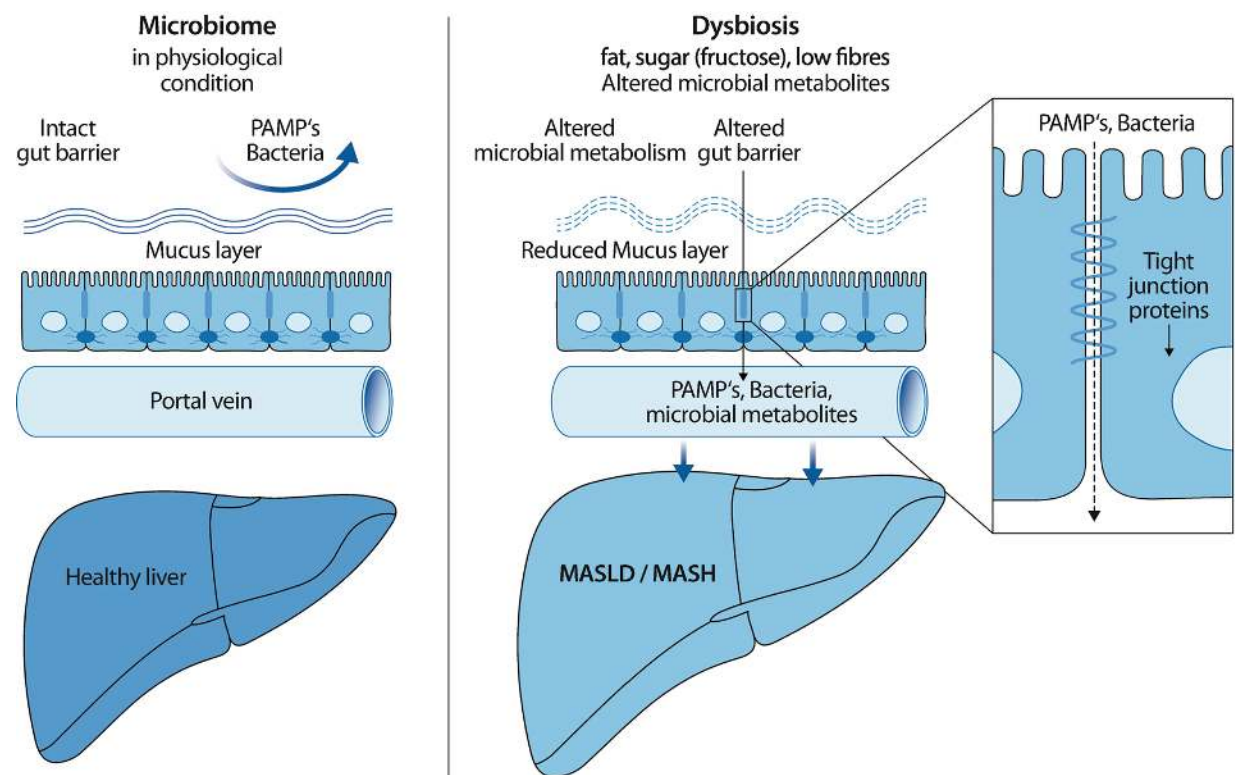


Fig. 17.3 Interaction of microbiome and host is critical in the development of MASLD. Diet can trigger dysbiosis in the small and/ or large intestine, triggering a loss of tight junction proteins and

intestinal barrier dysfunction. A subsequent translocation of bacterial products into the portal vein can induce hepatic inflammation and cause disease progression. *PAMPs*: pathogen-associated molecular patterns, *MASLD* metabolic dysfunction-associated steatotic liver disease, *MASH* steatohepatitis

Results of rodent models using mutants of leptin signaling like *ob/ob* and *db/db*, or applying various feeding schemes, such as diets rich in fructose, sucrose, and/or fat and cholesterol, and especially combinations of these macronutrients to induce MASLD, lend further support to the hypothesis that an increased translocation of bacterial endotoxins from the gut and the subsequent activation of TLR4-dependent signaling in the liver might be critical factors in the development of MASLD and especially steatohepatitis (Brun et al., [2007](#); Cani et al., [2008](#); Engstler et al., [2016](#); Engstler et al., [2017](#); Jin et al., [2016](#); Schneider et al., [2015](#); Sellmann et al., [2015](#); Spruss et al., [2011](#); Spruss & Bergheim, [2009](#); Sutter et al., [2015](#); Wagnerberger et al., [2012](#)). Thus, endotoxin reduction by treatment with non-resorbable antibiotics exerting their effect primarily in the gut rather than systemic can protect rodents from the development of diet-induced MASLD (Bergheim et al., [2008](#)), being associated with a mitigated TLR response and downstream signaling cascades in the liver (Schneider et al., [2015](#); Wagnerberger et al., [2012](#)). Similarly, in a genetic MASLD-HCC mouse model, dietary supplementation with the commensal species *Akkermansia muciniphila* was sufficient to restore intestinal barrier function and dampen hepatic inflammation.

Also, increasing orocecal transit time in MASLD patients is defined as the time from lactulose ingestion to a sustained increase of over 5 ppm above fasting levels in the end-expiratory hydrogen concentration or the interval to that of over 10 ppm (Hirakawa et al., [1988](#)). Treating them with a prokinetic drug like cisapride has been shown to ameliorate endotoxemia (Fu & Jiang, [2006](#)). Furthermore, short-term and long-term treatment of patients with steatohepatitis and with minimal hepatic encephalopathy due to liver disease in general with the non-resorbable antibiotic rifaximin decreased bacterial endotoxin levels in blood and laboratory markers of liver

disease and improved hepatic encephalopathy (Bajaj et al., [2013](#); Gangarapu et al., [2015](#)), which was linked to suppression of gut bacteria that translocate from the mouth to the intestine and cause the intestinal wall to become leaky by breaking down the protective mucus barrier (Patel et al., [2022](#)). Genetic modifications and pharmacological interventions that prevent the loss of tight junction proteins in intestinal tissue or the activation of TLR4 and downstream signaling cascades in the liver have repeatedly been shown to be associated with a marked protection against the development of MASLD and MASH (Jin et al., [2017](#); Spruss et al., [2012](#); Spruss & Bergheim, [2009](#)). In line with these findings, analyses of fecal microbiota composition revealed marked differences between patients with MASLD/MASH and controls. Bajaj et al. suggested that progressive changes in gut microbiota composition are associated with developing cirrhosis and that these changes become more severe in hepatic functional decompensation (Bajaj et al., [2014](#)). Notwithstanding, in this study, a patient population with various etiologies of cirrhosis was studied. With decreasing sequencing costs and advances in metabolomics, investigation of the functional potential of gut microbiomes via shotgun sequencing, together with released microbial metabolites, is possible on a large scale. This intensified research activities on specific microbial metabolic pathways such as bile acid- or short-chain fatty acid metabolism, which have been implicated in MASLD (Arab et al., [2017](#); Aron-Wisnewskey et al., [2020](#)). This development was paralleled by advances in experimental rodent models, which allowed functional insights into specific microbiome-dependent pathways. Boursier et al. suggested that the severity of MASLD/MASH in adults is associated with alterations of fecal microbiota composition and the microbial metabolome and that a higher relative abundance of *Bacteroides* has been independently associated with steatohepatitis, whereas *Ruminococcus* was more abundant in patients with hepatic fibrosis (Boursier et al., [2016](#); Loomba et al., [2017](#)); however, in this study, no disease-free controls were included. In line with these findings, pediatric patients with simple steatosis or steatohepatitis had lower

levels of *Oscillospira*, which were associated with a higher relative abundance of *Dorea* and *Ruminococcus* than controls (Del Chierico et al., [2017](#)). These alterations were associated with higher levels of 2-butanone and 4-methyl-2-pentanone in fecal samples of MASLD patients compared to controls (Del Chierico et al., [2017](#)). In another study, in which MASLD patients were not stratified by disease stage, it was shown that relative abundances of *Escherichia*, *Anaerobacter*, *Lactobacillus*, and *Streptococcus* were higher in patients, while those of *Ruminococcaceae* and, herein, especially the two genera *Oscillibacter* and *Flavonifractor* were higher in stool samples of healthy controls (Jiang et al., [2015](#)). In the latter study, patients with MASLD/MASH also showed lower protein levels of the tight junction proteins occludin and increased levels of proinflammatory cytokines in the duodenal mucosa, especially TNF- α and IL-6, in this part of the small intestine when compared to healthy controls. The differences between these two studies might have resulted from differences in study design (only patients with MASLD vs. a comparison with healthy controls and MASLD/MASH patients without a histologically defined stage), differences in age (e.g., pediatric patients vs. adult patients), and the lack of information on dietary intake. Indeed, one study is from China, while the other one enrolled patients in France and Italy. It has been suggested previously that mammalian gut bacterial communities are strongly dependent on dietary pattern (Muegge et al., [2011](#)) and that, for instance, the population of rumincocci can be altered through dietary intake (David et al., [2014](#)).

Another microbial metabolite that could be associated with MASLD is ethanol. Indeed, there have been several preclinical studies suggesting ethanol production by selected gut pathobionts (Li et al., [2021](#); Xue et al., [2023](#)). This phenotype had been linked to MASLD progression in rodent models (Yuan et al., [2019](#)). A recent human trial measuring portal and systemic vein concentrations with and without blockage of the liver alcohol dehydrogenase and antibiotic treatment, suggested that the gut microbiome contributes to relevant levels of ethanol production in humans as well (Meijnikman et al., [2022](#)). Such

patients may benefit from microbiome modulation, which has been successfully done using *Klebsiella*-targeting phages in mice (Gan et al., [2023](#)). Moreover, studies have also suggested that the increase of so-called endogenous alcohol is higher in patients with simple steatosis and even more so in patients with MASH, as the alcohol dehydrogenase is at least in part regulated through TNF α -dependent signaling pathways (Burger et al., [2024](#); Engstler et al., [2016](#)). In support of the latter, alcohol clearance is markedly impaired in rodents with insulin resistance and MASLD (Burger et al., [2024](#)).

17.2.2 MASLD: Interaction of Diet Pattern, Intestinal Microbiota, and the Barrier

The gut microbiome can be regarded as a signaling interface that integrates environmental influences into host physiology. Lifestyle choices such as nutrition and physical activity influence the gut microbiome (Schneider et al., [2024b](#)). In turn, the gut microbiome releases a huge number of metabolites that reach the liver via the portal circulation.

Several studies suggest that diet strongly affects human gut microbiome composition (David et al., [2014](#); Muegge et al., [2011](#); Wu et al., [2011](#)). For instance, David et al. demonstrated that microbial activity is dramatically different between herbivorous and carnivorous mammals, thereby reciprocally calibrating between carbohydrate and protein fermentation. Furthermore, it has been shown that foodborne microbes, including bacteria, fungi, and even viruses ingested with different diets colonize the gut (David et al., [2014](#)).

Results of recent rodent-based studies suggest that not only dietary content of fiber, sugars like fructose and sucrose, or saturated fats (Beilharz et al., [2016](#); Jena et al., [2016](#); Lam et al., [2015](#); Patterson et al., [2014](#)) may impact intestinal microbiota composition, but also different sources of protein, such as red meat (e.g., beef and pork), white meat (chicken and fish), and other sources of protein like casein and soy, which distinctly affect the intestinal microbiota (Zhu et al., [2015](#)). In these studies, white meat consumption was associated with a higher

relative abundance of *Lactobacillus* in the cecum, while relative abundance of *Ruminococcus* was highest in soy protein-fed animals (Zhu et al., [2017](#)). Interestingly, in rodents fed meat protein or casein, levels of circulating lipopolysaccharide-binding protein (LBP), as well as hepatic expression of CD14, both markers of innate immune activation, were significantly lower than in those animals fed soy protein (Zhu et al., [2017](#)).

Also in humans, dietary protein affects fecal microbiota composition. In a smaller study with MASLD patients ingesting a hypocaloric high-protein diet, the observed decrease in body weight and liver fat was associated with a reduced relative abundance of *Lachnospira*, whereas the relative abundance of *Blautia* and *Butyricicoccus* was increased in feces of study participants (Pataky et al., [2016](#)). The observed decrease in liver fat was negatively correlated with bacteria belonging to the phyla Firmicutes (current valid name *Bacillota*) and Bacteroidetes (current valid name *Bacteroidota*) (Pataky et al., [2016](#)). Intervention studies in healthy subjects further support the concept that nutritional imbalances, such as a diet low in choline, may induce steatosis associated with the growth of *Enterobacteriaceae* (phylum *Pseudomonadota*) that are associated with intestinal inflammation and concurrent metabolic dysfunction (Spencer et al., [2011](#)). Yet, results of this study also suggest that host factors like genetic predisposition may also affect response to different diets and that even under identical dietary conditions, the gut microbiota remains distinct between individuals over an extended time (Spencer et al., [2011](#)).

Notably, earlier and recent rodent studies clearly showed that obese animals fed a high-fat, high-carbohydrate diet harbor intestinal microbiota that more thoroughly metabolize carbohydrates and generate more caloric value from a given meal (Backhed et al., [2004](#); Ley et al., [2005](#); Sonnenburg & Backhed, [2016](#)). Transplantation of these microbiota into lean mice makes these fatter, and vice versa, colonization of obese mice with the lean microbiota leads to weight loss, subsequent to a less efficient energy production. However, data are

still controversial (also see Chap. [16](#)). Moreover, the microbiota from obese mice with steatosis has been shown to activate the intestinal and liver inflammasome, thereby promoting inflammatory steatohepatitis (Henao-Mejia et al., [2012](#)). These results could be reproduced in a small but well-performed clinical study in which obese patients with steatosis received a stool transplantation either with an obese or lean microbiota (Vrieze et al., [2012](#)), where transfer of microbiota from lean individuals was associated with higher insulin sensitivity. Finally, the beneficial effects of certain drugs like metformin on insulin resistance are in part due to the drug changing the intestinal microbiota (Wu et al., [2017](#)). Similarly, there are numerous studies that have demonstrated beneficial effects of different prebiotics on the gut microbiome. Dietary prebiotics are typically nondigestible fiber compounds that are metabolized and increase the activity of beneficial bacteria in the colon by acting as substrates for them. Dietary beta-glucan from oat has been shown to attenuate experimental steatohepatitis via its beneficial influence on the microbiome and by translocation of PAMPs (Jaeger et al., [2024](#)).

Taken together, results stemming from human studies in different regions of the world, and rodent studies including active interventions with microbiota transplantation, suggest that the intestinal microbiota composition differs markedly between patients with MASLD/MASH and healthy individuals. Studies also suggest that lifestyle and, herein, especially dietary patterns may markedly impact intestinal microbiota composition and barrier function. Indeed, recently it has been proposed that personalized microbiome-based approaches, including nutrition-based interventions or the treatment with specific pro- or prebiotics, might be an option for a preventive or supportive treatment of MASLD/MASH and the associated metabolic syndrome [for overview see (Shapiro et al., [2017](#); Wiest et al., [2017](#); Zmora et al., [2016](#))]. However, as knowledge and understanding underlying the molecular mechanisms of the interplay of intestinal microbiota and barrier and the liver is still limited in humans, as well as in rodent models of the disease, and long-term effects of altering intestinal microbiota

composition have not yet been fully understood, more research is needed before these individualized approaches to precision medicine can be taken.

17.3 Alcohol-Related Liver Disease, Intestinal Microbiota, and Intestinal Barrier Function: Current Knowledge

Despite intense public information and many public campaigns informing about the risks of high and especially chronic alcohol ingestion, alcohol consumption is still among the leading causes of liver damage in many countries worldwide (WHO, [2018](#)). For instance, in 2020, ~1.03 billion men (35.1% of the male global population aged ≥ 15 years) and ~312 million women (10.5% of female global population aged ≥ 15 years) consumed alcohol in amounts exceeding the non-drinker equivalent (NDE) (Collaborators, [2022](#)) with the latter being defined as the level of alcohol consumption at which the risk of health loss for a drinker is equivalent to that of a non-drinker. Similar to MASLD alcohol-related liver diseases (ALD) comprises a spectrum of conditions ranging from simple steatosis to alcoholic hepatitis, cirrhosis, and even hepatocellular carcinoma (Yeh & Brunt, [2014](#)). Indeed, it is estimated that ~10–15% of alcoholics will develop cirrhosis (Mann et al., [2003](#)). Yet, postmortem studies suggest even higher numbers in men exceeding a daily alcohol intake of 80 g/d (Savolainen et al., [1993](#)). Interestingly, results of a meta-analysis suggest that the same average consumption of ethanol is related to a higher risk of liver cirrhosis in women than in men (Rehm et al., [2010](#)). Furthermore, it has been suggested by several studies that (high) alcohol consumption is associated with adverse outcomes in patients with liver diseases other than MASLD [for overview see (Hagstrom, [2017](#))], which adds to the definition of a subpopulation of SLD patients, patients suffering from so-called MetALD (see Fig. [17.1](#) or Table [17.1](#)). In spite of intense research efforts, it is not clear why only a minor fraction of alcoholics develop cirrhosis, and molecular mechanisms

underlying the development of ALD are still not fully understood. Therefore, the therapies of choice for patients with ALD but also MetALD, regardless of whether suffering from alcohol use disorder (AUD) or “only” habitual too high drinking, are still primarily focused on abstinence, as AUD patients with ALD fraught with a high relapse rate (Slidrecht et al., [2019](#)).

Table 17.1 Summary of studies assessing microbiota and intestinal barrier function in patients with alcohol-related liver disease

Subjects	n	Methods	Key findings	References
AUD Controls	27 13	Jejunal fluid aspirates Anaerobic incubation	↑ Gram-negative anaerobic bacteria; coliform bacteria with AUD Correlation between gastric juice pH and NOM	Bode et al. (1984)
AUD Controls	45 60	Hydrogen breath test	Increased SIBO with AUD No difference in alcoholics with/without cirrhosis	Bode et al. (1993)
AUC Controls	66 24	Quantitative culturing of stool samples	↓ <i>Bifidobacterium</i> ; <i>Lactobacillus</i> , <i>Enterococci</i>	Kirpich et al. (2008)
AUD Controls	60 15	Fecal samples Cr-EDTA method qPCR/pyrosequencing of 16S rRNA gene	Correlation between intestinal permeability and dysbiosis with AUD ↑ <i>Lachnospiraceae</i> ↓ <i>Ruminococcaceae</i> ; <i>Bifidobacterium</i> ; <i>Lactobacillus</i> ; <i>Clostridium</i> Incomplete microbiota recovery after period of sobriety	Leclercq et al. (2014a)
AUD AH C	43 75 14	16S rRNA amplicon sequencing and PCR	Stool samples ↑ <i>Enterococcus faecalis</i>	Duan et al. (2019)
AUC AUC with hepatitis Controls	42 92 11	16S rRNA and PCR of stool samples	↑ Fungi (<i>C. albicans</i>) in AUD and AUC + ALD	Chu et al. (2020)
AH sAH C	21 15 16	Fecal samples qPCR	↓ <i>A. muciniphila</i> in AH and sAH	Grunder et al. (2018)

Subjects	n	Methods	Key findings	References
Alcohol-related liver cirrhosis (AC)				
Cirrhosis Controls	45 28	Glucose hydrogen breath test Methane breath test	Increased SIBO with cirrhosis Correlation between SIBO and the severity of liver disease	Yang et al. (1998)
Cirrhosis AC Controls	18 12 12	Fecal samples Functional gene array	Dysbiosis with cirrhosis and AC ↑ <i>Proteobacteria</i> ; <i>Fusobacteria</i> ↓ <i>Bacteroidetes</i> Correlation between enrichment of functional genes (e.g., xenobiotic metabolism, virulence) in microbiome and alcohol consumption	Chen et al. (2014)
AUD AC Controls	29 19 18	Colonic biopsy Length heterogeneity PCR Multitag pyrosequencing	Increased dysbiosis with AUD and AC ↑ <i>Proteobacteria</i> ; <i>Bacilli</i> ↓ <i>Bacteroidetes</i> ; <i>Clostridium</i> No correlation between duration of sobriety and dysbiosis	Mutlu et al. (2012)
Cirrhosis EHPVO Controls	174 28 51	Glucose hydrogen breath test	Increased SIBO with cirrhosis No correlation between SIBO and cirrhosis etiology; degree of liver dysfunction	Lakshmi et al. (2010)
Cirrhosis Controls	53 42	Lactulose hydrogen/methane breath test Multiplex PCR	Increased SIBO with cirrhosis Correlation between SIBO and bacterial translocation	Jun et al. (2010)
AC Controls	89 40	Glucose hydrogen breath test	Increased SIBO with AC Correlation between increased NOM and development of spontaneous bacterial peritonitis	Morencos (1995)
Cirrhosis AC Controls	62 16 15	Glucose hydrogen breath test	Increased SIBO with cirrhosis Correlation between SIBO and the severity of liver disease	Pande et al. (2009)

Subjects	n	Methods	Key findings	References
AUD with AC AC with no current alcohol use Controls	37 68 34	16S rRNA of duodenal, ileal, colonic mucosa, and feces	↑ Proteobacteria (Enterobacteriaceae) ↓ <i>Lachnospiraceae</i> , <i>Bacteroidaceae</i> , <i>Prevotellaceae</i>	Bajaj et al. (2017)
AC AUD	27 72	Shotgun metagenomics	Stool samples ↑ <i>Klebsiella</i> , <i>Lactococcus</i> ↑ <i>K. pneumonia</i> , <i>Lactobacillus salivarius</i> , <i>Citrobacter koseri</i> , <i>Lactococcus lactis</i> subsp. <i>cremoris</i>	Dubinkina et al. (2017)

AUD alcohol use disorder, *AH* alcohol-related hepatitis, *AC* alcohol-related cirrhosis, *EHPVO* extrahepatic portal venous obstruction, *NOM* number of microorganisms, *SIBO* small intestinal bacterial overgrowth

17.3.1 ALD: Intestinal Barrier Function, and Intestinal Microbiota

Already more than 30 years ago, the group of Bode et al. and others demonstrated that ALD is associated with elevated bacterial endotoxin levels (Bode et al., 1987; Fukui et al., 1991; Gaeta et al., 1982). Furthermore, bacterial endotoxin levels correlated with enhanced intestinal permeability in patients with ALD (Parlesak et al., 2000) as well as with disease severity (Lin et al., 1995); however, the latter study did include different etiologies of liver disease. By now, numerous animal and human studies have confirmed these findings [for an overview see (Wang et al., 2021)] but also suggest that even acute high-dose intake (a single dose of >20 g pure ethanol) is sufficient to increase bacterial endotoxin levels in peripheral blood in humans (Bala et al., 2014) (also see Fig. 17.4).

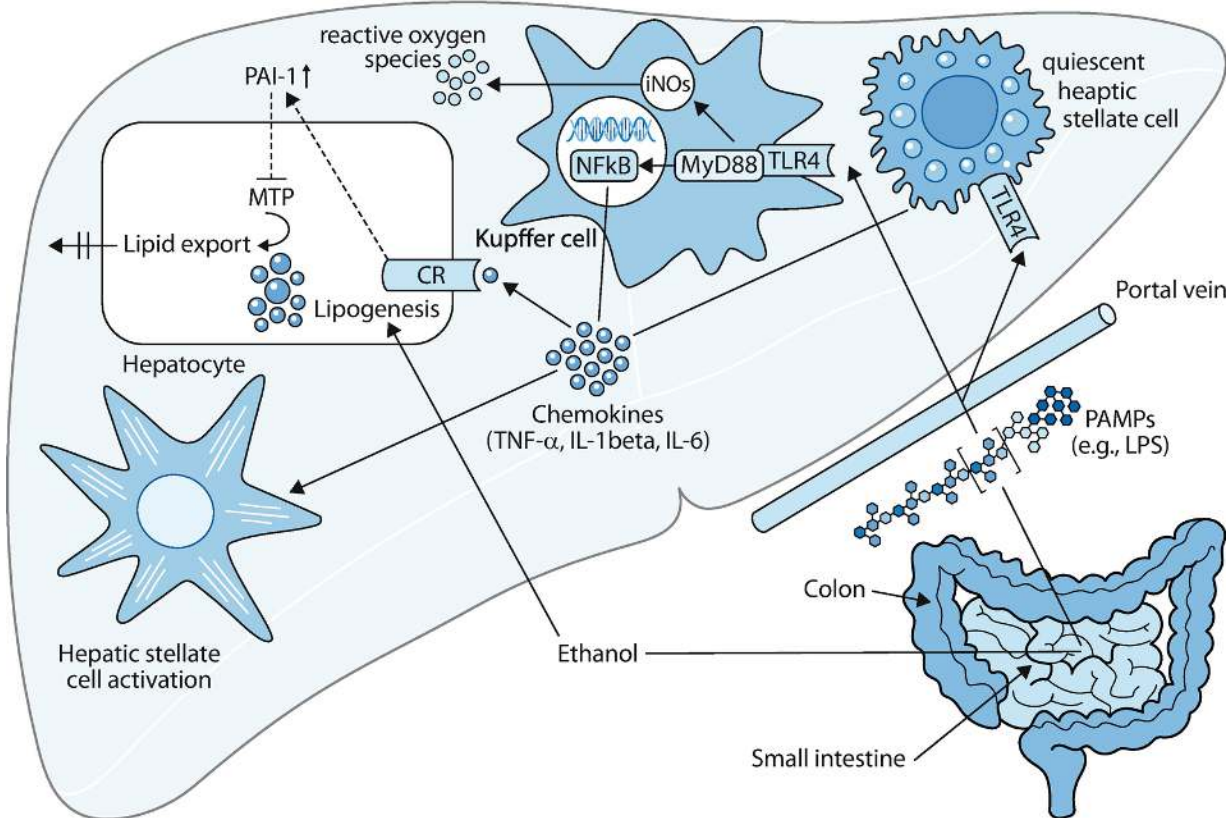


Fig. 17.4 Consequences of intestinal dysbiosis and barrier dysfunction induced by alcohol consumption on the liver. Alcohol ingestion leads to intestinal bacterial overgrowth and dysbiosis as well as barrier dysfunction. Lipopolysaccharides reach the liver through the portal vein, resulting in an induction of TLR4-dependent signaling cascades and subsequently the formation of reactive oxygen species and cytokines, which in turn alter hepatic lipid export and further add to the activation of hepatic stellate cells. CR cytokine receptors, *NFκB* nuclear factor kappa B, *TLR4* Toll-like receptor-4, *LPS* lipopolysaccharide, *PAMPs* pathogen-associated molecular patterns, *PAI-1* plasminogen activator inhibitor 1, *iNOS* inducible nitric oxide synthase, *TNF-α* tumor necrosis factor alpha, *MyD88* myeloid differentiation primary response protein 88, *MTP* microsomal triglyceride transfer protein

In line with the above reported findings, results of mouse and rat ethanol binge drinking models (5–6 g ethanol given intragastrically once) have shown that persistence of the marked hepatic fat accumulation found within 6–12 h after the acute alcohol ingestion is highly dependent upon the translocation of endotoxin (Enomoto et al., [2001](#); Wagnerberger et al., [2013](#)) Chronic and acute high-dose intake of ethanol can lead to a loss of epithelial cells from the villous tips and to hemorrhagic erosions in the lamina propria [for overview also see (Elamin et al., [2013](#))]. Furthermore, animal studies using chronic

feeding models suggest that the mucus layer and the expression of certain mucins, but also the expression of antimicrobial peptides, are altered, the latter at least in part being related to changes in the intestinal interferon (IFN) γ -STAT1 signaling cascade (Kirpich et al., [2013](#); Yue et al., [2020](#)). In the liver, gut-derived bacterial endotoxins have been suggested to add to the hypermetabolic state, e.g., increased hepatic oxygen uptake and accumulation of lipids after acute alcohol exposure (Rivera et al., [1998](#); Yuki & Thurman, [1980](#)) (for mechanisms involved also see Fig. [17.4](#)). Indeed, treatment with both non-resorbable antibiotics (that largely eliminate the microbiota) and GdCl₃ (that eliminates proinflammatory Kupffer cells) almost completely abolished these hypermetabolic alterations after acute high alcohol ingestion in rodents (Rivera et al., [1998](#)). Similar results were also reported in settings of chronic alcohol exposure in rodents (Adachi et al., [1994](#); Adachi et al., [1995](#); Koop et al., [1997](#); Uesugi et al., [2001](#)). Also, interventions, be they genetic or pharmacological, aiming to disrupt the recognition of endotoxin by liver cells in settings of chronic alcohol ingestion, are associated with a marked protection from the development of ALD in model organisms [for overview, also see (Szabo, [2015](#))].

Supporting the hypothesis that gut-derived bacterial toxins, but also exotoxins derived from *Candida albicans* (Candidalysin), and alterations of intestinal microbiota induced through the intake of alcohol may be critical in the development of ALD, some older and more recent studies suggest that ethanol consumption may alter intestinal microbiota composition (see Table [17.1](#)). Indeed, the number of anaerobic and aerobic bacteria was significantly higher in the jejunal juice of alcoholics than in controls, with a significantly higher colonization of Gram-negative and endospore-forming bacteria in patients with AUC, and that correlated closely with the pH found in gastric juice (Bode et al., [1984](#)). By using a hydrogen breath test, the same group also showed that the prevalence of small intestinal bacterial overgrowth was significantly more frequent in patients with AUC than in controls (Bode et al., [1993](#)). These results were also supported by several other groups,

all showing that small intestinal bacterial overgrowth is frequently associated with the presence of liver cirrhosis, regardless of the cause of liver damage (Casafont Morencos, [1996](#); Lakshmi et al., [2010](#); Morencos, [1995](#); Pande et al., [2009](#); Yang et al., [1998](#)). Interestingly, Jun et al. showed that the prevalence of cirrhosis was related to the presence of bacterial DNA in peripheral blood (Jun et al., [2010](#)), further supporting the hypothesis that dysbiotic alterations of intestinal microbiota are a risk factor for increased intestinal permeability. However, in this study, no details were provided regarding the etiology of cirrhosis. In line with these findings, Mutlu et al. (Mutlu et al., [2012](#)) and others (Bajaj et al., [2014](#); Dubinkina et al., [2017](#)) reported changes in mucosa-associated colonic bacterial composition in AUC patients with and without liver disease. For instance, a lower relative abundance of *Bacteroidetes* and a higher abundance of *Proteobacteria* were found in a subset of alcoholics when compared to healthy controls, and this dysbiosis also correlated with endotoxemia in the patients with AUD (Mutlu et al., [2012](#)). Furthermore, it has been shown that patients with alcohol-related liver cirrhosis may have a distinctly altered functional composition of the fecal microbiome, e.g., a depletion of functional genes involved in nutrient metabolism, including amino acids and lipid and nucleotide metabolism (Chen et al., [2014](#)).

In patients with alcoholic steatohepatitis, the abundance of fecal *A. muciniphila* is decreased when compared to controls (Grander et al., [2018](#)). The same study further showed that treating alcohol-fed mice orally with *A. muciniphila* protected animals from alcohol-induced gut leakiness and enhanced mucus thickness as well as tight junction protein expression. Similarly, Leclercq et al. ([2014a](#)) showed that patients with alcohol dependence and a high intestinal permeability, as measured by ^{51}Cr -EDTA, but without fibrosis or cirrhosis, had a lower relative abundance of *Ruminococcus*, *Faecalibacterium*, *Subdoligranulum*, *Oscillibacter*, and *Anaerofilum* than controls and patients with low intestinal permeability (Leclercq et al., [2014b](#)). After a 3-week detoxification program, the relative abundance of *Ruminococcus* and *Subdoligranulum* and the level of *Bifidobacterium* spp. and

Lactobacillus spp. were increased in subjects with a prior high intestinal permeability. Mendelian randomization analysis revealed *E. coli* as a protective factor in ALD, while *Roseburia hominis* and the members of the family *Porphyromonadaceae* were identified as risk factors for ALD (Li et al., [2024](#)). Further supporting the hypothesis that alterations of intestinal microbiota composition and an increased translocation of bacterial toxins are critical in the development of ALD it was shown that bacterial endotoxin and peptidoglycan levels and expression of TLRs in peripheral blood mononuclear cells decreased during detoxification in noncirrhotic alcohol-dependent subjects as well as patients with alcohol-related steatosis and steatohepatitis being also related with changes in markers of intestinal barrier function (Jung et al., [2021](#)). Besides alterations in bacterial composition and bacterial toxins in more recent years, it was also suggested that the changes in the abundance of fungi like *Candida albicans* and a related increase in Candidalysin may be critical with respect to patient survival (Chu et al., [2020](#)).

Notably, even a short-term 5-day treatment of patients with alcoholic psychosis and mild alcohol-induced liver injury with *Bifidobacterium bifidum* and *Lactiplantibacillus plantarum* 8PA3 in combination with the standard therapy (abstinence and vitamin supplementation) resulted in a significant increase in the number of bifidobacterial and lactobacilli and a more rapid fall of elevated transaminases in blood when compared with standard therapy alone (abstinence plus vitamins). Moreover, there are several small completed and still ongoing clinical trials assessing the effect of fecal microbiota transplantation (FMT) in alcohol-related hepatitis and cirrhosis patients [for overview see (Saha & Schnabl, [2025](#))], suggesting that FMT may be a safe measure to improve 90-day survival and reduce infections in patients with severe forms of ALD. For instance, in a study with steroid-eligible patients with severe alcohol-related hepatitis, healthy donor FMT for 7 days was related to higher 90-day survival in those receiving healthy donor FMT when compared to patients treated with prednisolone (Pande et al., [2023](#)).

Taken together, similar to the findings for MASLD, animal and human studies suggest that alterations of the intestinal microbiota composition are critical in the development of ALD (also see Figs. [17.3](#) and [17.4](#)). Studies further suggest that abstinence as the therapy of choice for ALD is associated with a rapid change of intestinal microbiota composition and intestinal barrier function; finally, probiotics but many be also fecal microbiota transplants (FMT) may aid in reconstitution of the protective mucosal barrier and alleviate endotoxin-induced liver damage. Several ongoing clinical trials target the intestinal microbiota in settings of chronic alcohol-induced liver disease to enhance recovery of patients with early stages of ALD and to assist with stabilization of cirrhosis (Saha & Schnabl, [2025](#); Wiest et al., [2017](#)). Results of these trials are expected to yield valuable information to direct future probiotic therapies for ALD and possibly also MASLD/MASH.

17.4 Conclusion

Both MASLD and ALD are multifactorial diseases associated with altered nutritional patterns, e.g., over- and malnutrition, and elevated alcohol intake, respectively. Accordingly, lifestyle interventions targeting nutritional patterns through caloric restriction and encouraging abstinence are still the primary therapeutic approaches. Nonetheless, results of animal and human studies strongly suggest that both clinical pictures are associated with alterations of both the intestinal microbiota and gut barrier function. Furthermore, results of intervention studies targeting intestinal microbiota and/or barrier may improve or even prevent disease progression, suggesting that modulating intestinal microbiota functions, e.g., microbial metabolites, might provide excellent targets for therapy and may also be the prevention of MASLD and ALD. Despite promising clinical evidence, a better characterization of the intestinal microbiota and its metabolome using larger and better characterized patient cohorts as well as unified sample collection and detection methods is needed to

develop universally acceptable treatment recommendations. Using these more generalized methodological approaches, subsets of patients suffering from ALD or MASLD may be identified that benefit from therapeutic interventions targeting the intestinal microbiome and barrier function, thereby adding to the development of a personalized microbiome-focused therapy that may serve as adjunctive treatment and that may help to prevent MASLD and ALD.

Controversy Despite being among the most common liver diseases worldwide, the molecular mechanisms underlying the development of ALD and MASLD are not fully understood. While an interaction between the intestinal microbiota, gut barrier integrity, and the development of ALD has been proposed for more than 30 years, current therapies and prevention strategies for ALD still primarily focus on lifestyle interventions, i.e., strict abstinence. However, while abstinence would be wishful in settings of chronic and excessive alcohol intake, this is often not achieved, and patients reach irreversible stages of the disease, with liver transplantation being the only remaining cure of choice. Therefore, novel (adjunctive) therapies preventing the progression of ALD are needed. Weight normalization through lifestyle interventions remains the cornerstone of both prevention and treatment for MASLD and MASH. Similar to ALD, compliance is low, and relapse rates are high. First studies targeting the intestinal microbiota and barrier bear promising results; still, as data are limited, more research is needed to unravel mechanisms underlying the interaction of host and microbiota before a personalized microbiome-focused therapy or even prevention of MASLD and ALD can be a general recommendation.

History

MASLD, MASH, and alcohol-related liver disease (ALD) are by now among the most common liver diseases worldwide. MASLD/MASH strongly correlates with the occurrence of overweight/obesity and insulin resistance, while ALD results from chronic elevated intake of

ethanol. Despite intense research efforts, molecular mechanisms involved in the development of ALD and even more so of MASLD/MASH have not yet been fully understood, as exemplified by an often unpredictable disease severity or progression, despite similar major predisposing factors. Indeed, for both diseases, lifestyle interventions, either focusing on weight reduction and increase of physical activity in the case of MASLD/MASH or the avoidance of alcohol intake in the case of ALD, are still the first lines and most effective treatments and preventive measures. However, lifestyle interventions are frequently fraught with low compliance and high relapse rates. Results of human and animal studies have shown that genetic predisposition, physical inactivity, and overnutrition as well as a diet rich in saturated fat and/or sugars, as well as excessive cholesterol, are associated with the development of MASLD/MASH. In recent years, similar to the findings in ALD, alterations of the intestinal microbiota diversity, composition, function, and gut barrier function have been proposed to be key factors for the development of MASLD and MASH. Results of intervention studies targeting intestinal microbiota and intestinal barrier function, e.g., with pre- and probiotics as well as secondary plant compounds or specific dietary patterns, are promising; however, long-term results are still missing and knowledge on molecular mechanisms and particular microbial species and metabolites is just evolving.

Highlights

- Metabolic dysfunction-associated steatotic liver disease (MASLD) is by now regarded as the most common liver disease worldwide.
- Alterations of the intestinal microbiota and increased bacterial endotoxin levels are associated with both ALD and MASLD.
- Nutrition and dietary patterns are a critical modulator of intestinal microbial communities and the development of alcohol-related liver disease, MASLD, and MASH.

- A mechanistic understanding of the interactions between the intestinal microbiota, barrier function, and the liver will help to improve the prevention and therapy of MASLD and alcohol-related liver disease.

References

Adachi, Y., Bradford, B. U., Gao, W., Bojes, H. K., & Thurman, R. G. (1994). Inactivation of Kupffer cells prevents early alcohol-induced liver injury. *Hepatology*, 20, 453–460.

[\[PubMed\]](#)

Adachi, Y., Moore, L. E., Bradford, B. U., Gao, W., & Thurman, R. G. (1995). Antibiotics prevent liver injury in rats following long-term exposure to ethanol. *Gastroenterology*, 108, 218–224.

[\[PubMed\]](#)

Arab, J. P., Karpen, S. J., Dawson, P. A., Arrese, M., & Trauner, M. (2017). Bile acids and nonalcoholic fatty liver disease: Molecular insights and therapeutic perspectives. *Hepatology*, 65, 350–362.

[\[PubMed\]](#)

Aron-Wisnewsky, J., Vigliotti, C., Witjes, J., Le, P., Holleboom, A. G., Verheij, J., Nieuwdorp, M., & Clement, K. (2020). Gut microbiota and human NAFLD: Disentangling microbial signatures from metabolic disorders. *Nature Reviews. Gastroenterology & Hepatology*, 17, 279–297.

Backhed, F., Ding, H., Wang, T., Hooper, L. V., Koh, G. Y., Nagy, A., Semenkovich, C. F., & Gordon, J. I. (2004). The gut microbiota as an environmental factor that regulates fat storage. *Proceedings of the National Academy of Sciences of the United States of America*, 101, 15718–15723.

[\[PubMed\]](#)

Bajaj, J. S., Heuman, D. M., Sanyal, A. J., Hylemon, P. B., Sterling, R. K., Stravitz, R. T., Fuchs, M., et al. (2013). Modulation of the metabiome by rifaximin in patients with cirrhosis and minimal hepatic encephalopathy. *PLoS One*, 8, e60042.

[\[PubMed\]](#)

Bajaj, J. S., Heuman, D. M., Hylemon, P. B., Sanyal, A. J., White, M. B., Monteith, P., Noble, N. A., et al. (2014). Altered profile of human gut microbiome is associated with cirrhosis and its complications. *Journal of Hepatology*, 60, 940–947.

[\[PubMed\]](#)

Bajaj, J. S., Kakiyama, G., Zhao, D., Takei, H., Fagan, A., Hylemon, P., Zhou, H., et al. (2017). Continued Alcohol misuse in human cirrhosis is associated with an impaired gut-liver axis. *Alcoholism, Clinical and Experimental Research*, 41, 1857–1865.

[\[PubMed\]](#)

Bala, S., Marcos, M., Gattu, A., Catalano, D., & Szabo, G. (2014). Acute binge drinking increases serum endotoxin and bacterial DNA levels in healthy individuals. *PLoS One*, 9, e96864.

[\[PubMed\]](#)

Balmer, M. L., Slack, E., de Gottardi, A., Lawson, M. A., Hapfelmeier, S., Miele, L., Grieco, A., et al. (2014). The liver may act as a firewall mediating mutualism between the host and its gut commensal microbiota. *Science Translational Medicine*, 6, 237ra66.

[\[PubMed\]](#)

Barrea, L., Di Somma, C., Muscogiuri, G., Tarantino, G., Tenore, G. C., Orio, F., Colao, A., & Savastano, S. (2017). Nutrition, inflammation and liver-spleen axis. *Critical Reviews in Food Science and Nutrition*, 58, 3141.

[\[PubMed\]](#)

Baumann, A., Nier, A., Hernandez-Arriaga, A., Brandt, A., Lorenzo Pisarello, M. J., Jin, C. J., Pilar, E., Camarinha-Silva, A., Schattenberg, J. M., & Bergheim, I. (2021). Toll-like receptor 1 as a possible target in non-alcoholic fatty liver disease. *Scientific Reports*, 11, 17815.

[\[PubMed\]](#)

Beilharz, J. E., Kaakoush, N. O., Maniam, J., & Morris, M. J. (2016). The effect of short-term exposure to energy-matched diets enriched in fat or sugar on memory, gut microbiota and markers of brain inflammation and plasticity. *Brain, Behavior, and Immunity*, 57, 304–313.

[\[PubMed\]](#)

Bergheim, I., Weber, S., Vos, M., Kramer, S., Volynets, V., Kaserouni, S., McClain, C. J., & Bischoff, S. C. (2008). Antibiotics protect against fructose-induced hepatic lipid accumulation in mice: Role of endotoxin. *Journal of Hepatology*, 48, 983–992.

[\[PubMed\]](#)

Bode, J. C., Bode, C., Heidelberg, R., Durr, H. K., & Martini, G. A. (1984). Jejunal microflora in patients with chronic alcohol abuse. *Hepato-Gastroenterology*, 31, 30–34.

[\[PubMed\]](#)

Bode, C., Kugler, V., & Bode, J. C. (1987). Endotoxemia in patients with alcoholic and non-alcoholic cirrhosis and in subjects with no evidence of chronic liver disease following acute alcohol excess. *Journal of Hepatology*, 4, 8–14.

[\[PubMed\]](#)

Bode, C., Koleyke, R., Schafer, K., & Bode, J. C. (1993). Breath hydrogen excretion in patients with alcoholic liver disease—evidence of small intestinal bacterial overgrowth. *Zeitschrift für Gastroenterologie*, 31, 3–7.

[\[PubMed\]](#)

Boursier, J., Mueller, O., Barret, M., Machado, M., Fizanne, L., Araujo-Perez, F., Guy, C. D., et al. (2016). The severity of nonalcoholic fatty liver disease is associated with gut dysbiosis and shift in the metabolic function of the gut microbiota. *Hepatology*, 63, 764–775.

[\[PubMed\]](#)

Brun, P., Castagliuolo, I., Di Leo, V., Buda, A., Pinzani, M., Palu, G., & Martines, D. (2007). Increased intestinal permeability in obese mice: New evidence in the pathogenesis of nonalcoholic

steatohepatitis. *American Journal of Physiology. Gastrointestinal and Liver Physiology*, 292, G518–G525.

[\[PubMed\]](#)

Burger, K., Jung, F., Staufer, K., Ladurner, R., Trauner, M., Baumann, A., Brandt, A., & Bergheim, I. (2024). MASLD is related to impaired alcohol dehydrogenase (ADH) activity and elevated blood ethanol levels: Role of TNFalpha and JNK. *Redox Biology*, 71, 103121.

[\[PubMed\]](#)

Cani, P. D., Bibiloni, R., Knauf, C., Waget, A., Neyrinck, A. M., Delzenne, N. M., & Burcelin, R. (2008). Changes in gut microbiota control metabolic endotoxemia-induced inflammation in high-fat diet-induced obesity and diabetes in mice. *Diabetes*, 57, 1470–1481.

[\[PubMed\]](#)

Casafont Morencos, F., de las Heras Castano, G., Martin Ramos, L., Lopez Arias, M. J., Ledesma, F., & Pons Romero, F. (1996). Small bowel bacterial overgrowth in patients with alcoholic cirrhosis. *Digestive Diseases and Sciences*, 41, 552–556.

[\[PubMed\]](#)

Chen, Y., Qin, N., Guo, J., Qian, G., Fang, D., Shi, D., Xu, M., et al. (2014). Functional gene arrays-based analysis of fecal microbiomes in patients with liver cirrhosis. *BMC Genomics*, 15, 753.

[\[https://doi.org/10.1186/1471-2164-15-753\]](https://doi.org/10.1186/1471-2164-15-753) [\[PubMed\]](#)

Chen, L. Z., Xin, Y. N., Geng, N., Jiang, M., Zhang, D. D., & Xuan, S. Y. (2015). PNPLA3 I148M variant in nonalcoholic fatty liver disease: Demographic and ethnic characteristics and the role of the variant in nonalcoholic fatty liver fibrosis. *World Journal of Gastroenterology*, 21, 794–802.

[\[PubMed\]](#)

Chu, H., Duan, Y., Lang, S., Jiang, L., Wang, Y., Llorente, C., Liu, J., et al. (2020). The Candida albicans exotoxin candidalysin promotes alcohol-associated liver disease. *Journal of Hepatology*, 72, 391–400.

[\[PubMed\]](#)

Collaborators, G. B. D. Alcohol. (2022). Population-level risks of alcohol consumption by amount, geography, age, sex, and year: A systematic analysis for the global burden of disease study 2020. *Lancet*, 400, 185–235.

David, L. A., Maurice, C. F., Carmody, R. N., Gootenberg, D. B., Button, J. E., Wolfe, B. E., Ling, A. V., et al. (2014). Diet rapidly and reproducibly alters the human gut microbiome. *Nature*, 505, 559–563.

[\[https://doi.org/10.1038/nature12820\]](https://doi.org/10.1038/nature12820) [\[PubMed\]](#)

Del Chierico, F., Nobili, V., Vernocchi, P., Russo, A., Stefanis, C., Gnani, D., Furlanello, C., et al. (2017). Gut microbiota profiling of pediatric nonalcoholic fatty liver disease and obese patients unveiled by an integrated meta-omics-based approach. *Hepatology*, 65, 451–464.

[\[PubMed\]](#)

Duan, Y., Llorente, C., Lang, S., Brandl, K., Chu, H., Jiang, L., White, R. C., et al. (2019). Bacteriophage targeting of gut bacterium attenuates alcoholic liver disease. *Nature*, 575, 505–511.

[\[https://doi.org/10.1038/s41586-019-1742-x\]](https://doi.org/10.1038/s41586-019-1742-x) [\[PubMed\]](#)

Dubinkina, V. B., Tyakht, A. V., Odintsova, V. Y., Yarygin, K. S., Kovarsky, B. A., Pavlenko, A. V., Ischenko, D. S., et al. (2017). Links of gut microbiota composition with alcohol dependence syndrome and alcoholic liver disease. *Microbiome*, 5, 141.
[<https://doi.org/10.1186/s40168-017-0359-2>][[PubMed](#)]

Dukowicz, A. C., Lacy, B. E., & Levine, G. M. (2007). Small intestinal bacterial overgrowth: A comprehensive review. *Gastroenterol Hepatol (New York)*, 3, 112–122.

Dulai, P. S., Singh, S., Patel, J., Soni, M., Prokop, L. J., Younossi, Z., Sebastiani, G., et al. (2017). Increased risk of mortality by fibrosis stage in nonalcoholic fatty liver disease: Systematic review and meta-analysis. *Hepatology*, 65, 1557–1565.
[[PubMed](#)]

Elamin, E. E., Masclee, A. A., Dekker, J., & Jonkers, D. M. (2013). Ethanol metabolism and its effects on the intestinal epithelial barrier. *Nutrition Reviews*, 71, 483–499.
[[PubMed](#)]

Engstler, A. J., Aumiller, T., Degen, C., Durr, M., Weiss, E., Maier, I. B., Schattenberg, J. M., Jin, C. J., Sellmann, C., & Bergheim, I. (2016). Insulin resistance alters hepatic ethanol metabolism: Studies in mice and children with non-alcoholic fatty liver disease. *Gut*, 65, 1564–1571.
[[PubMed](#)]

Engstler, A. J., Sellmann, C., Jin, C. J., Brandt, A., Herz, K., Pribs, J., & Bergheim, I. (2017). Treatment with alpha-galactosylceramide protects mice from early onset of nonalcoholic steatohepatitis: Role of intestinal barrier function. *Molecular Nutrition & Food Research*, 61(5), 1600985.

Enomoto, N., Ikejima, K., Yamashina, S., Hirose, M., Shimizu, H., Kitamura, T., Takei, Y., Sato, & Thurman, R. G. (2001). Kupffer cell sensitization by alcohol involves increased permeability to gut-derived endotoxin. *Alcoholism, Clinical and Experimental Research*, 25, 51s–54s.
[[PubMed](#)]

Fu, X. S., & Jiang, F. (2006). Cisapride decreasing orocecal transit time in patients with nonalcoholic steatohepatitis. *Hepatobiliary & Pancreatic Diseases International*, 5, 534–537.

Fukui, H., Brauner, B., Bode, J. C., & Bode, C. (1991). Plasma endotoxin concentrations in patients with alcoholic and non-alcoholic liver disease: Reevaluation with an improved chromogenic assay. *Journal of Hepatology*, 12, 162–169.
[[PubMed](#)]

Gaeta, G. B., Perna, P., Adinolfi, L. E., Utili, R., & Ruggiero, G. (1982). Endotoxemia in a series of 104 patients with chronic liver diseases: Prevalence and significance. *Digestion*, 23, 239–244.
[[PubMed](#)]

Gan, L., Feng, Y., Du, B., Fu, H., Tian, Z., Xue, G., Yan, C., et al. (2023). Bacteriophage targeting microbiota alleviates non-alcoholic fatty liver disease induced by high alcohol-producing *Klebsiella pneumoniae*. *Nature Communications*, 14, 3215.
[[PubMed](#)]

Gangarapu, V., Ince, A. T., Baysal, B., Kayar, Y., Kilic, U., Gok, O., Uysal, O., & Senturk, H. (2015).

Efficacy of rifaximin on circulating endotoxins and cytokines in patients with nonalcoholic fatty liver disease. *European Journal of Gastroenterology & Hepatology*, 27, 840–845.

Grunder, C., Adolph, T. E., Wieser, V., Lowe, P., Wrzosek, L., Gyongyosi, B., Ward, D. V., et al. (2018). Recovery of ethanol-induced Akkermansia muciniphila depletion ameliorates alcoholic liver disease. *Gut*, 67, 891–901.

[\[PubMed\]](#)

György, P. (1954). Antibiotics and liver injury. *Annals of the New York Academy of Sciences*, 57, 925–931.

[\[PubMed\]](#)

Hagstrom, H. (2017). Alcohol consumption in concomitant liver disease: How much is too much? *Current Hepatology Reports*, 16, 152–157.

[\[PubMed\]](#)

Henao-Mejia, J., Elinav, E., Jin, C., Hao, L., Mehal, W. Z., Strowig, T., Thaiss, C. A., et al. (2012). Inflammation-mediated dysbiosis regulates progression of NAFLD and obesity. *Nature*, 482, 179–185.

[\[https://doi.org/10.1038/nature10809\]](https://doi.org/10.1038/nature10809) [\[PubMed\]](#)

Hirakawa, M., Iida, M., Kohrogi, N., & Fujishima, M. (1988). Hydrogen breath test assessment of oro-cecal transit time: Comparison with barium meal study. *American Journal of Gastroenterology (Springer Nature)*, 83(12), 1361–1363.

Jaeger, J. W., Brandt, A., Gui, W., Yergaliyev, T., Hernandez-Arriaga, A., Muthu, M. M., Edlund, K., et al. (2024). Microbiota modulation by dietary oat beta-glucan prevents steatotic liver disease progression. *JHEP Reports*, 6, 100987.

[\[PubMed\]](#)

Jena, P. K., Prajapati, B., Mishra, P. K., & Seshadri, S. (2016). Influence of gut microbiota on inflammation and pathogenesis of sugar rich diet induced diabetes. *Immunome Research*, 12, 1.

Jiang, W., Wu, N., Wang, X., Chi, Y., Zhang, Y., Qiu, X., Hu, Y., Li, J., & Liu, Y. (2015). Dysbiosis gut microbiota associated with inflammation and impaired mucosal immune function in intestine of humans with non-alcoholic fatty liver disease. *Scientific Reports*, 5, 8096.

[\[PubMed\]](#)

Jin, C. J., Engstler, A. J., Sellmann, C., Ziegenhardt, D., Landmann, M., Kanuri, G., Lounis, H., Schroder, M., Vetter, W., & Bergheim, I. (2016). Sodium butyrate protects mice from the development of the early signs of non-alcoholic fatty liver disease: Role of melatonin and lipid peroxidation. *The British Journal of Nutrition*, 116(10), 1–12.

Jin, C. J., Engstler, A. J., Ziegenhardt, D., Bischoff, S. C., Trautwein, C., & Bergheim, I. (2017). Loss of lipopolysaccharide-binding protein attenuates the development of diet-induced non-alcoholic fatty liver disease in mice. *Journal of Gastroenterology and Hepatology*, 32, 708–715.

[\[PubMed\]](#)

Jun, D. W., Kim, K. T., Lee, O. Y., Chae, J. D., Son, B. K., Kim, S. H., Jo, Y. J., & Park, Y. S. (2010).

Association between small intestinal bacterial overgrowth and peripheral bacterial DNA in cirrhotic patients. *Digestive Diseases and Sciences*, 55, 1465–1471.

[\[PubMed\]](#)

Jung, F., Burger, K., Staltner, R., Brandt, A., Mueller, S., & Bergheim, I. (2021). Markers of intestinal permeability are rapidly improved by alcohol withdrawal in patients with alcohol-related liver disease. *Nutrients*, 13, 1659.

[\[PubMed\]](#)

Kanuri, G., Spruss, A., Wagnerberger, S., Bischoff, S. C., & Bergheim, I. (2011). Role of tumor necrosis factor α (TNF α) in the onset of fructose-induced nonalcoholic fatty liver disease in mice. *The Journal of Nutritional Biochemistry*, 22, 527–534.

[\[PubMed\]](#)

Kanuri, G., Ladurner, R., Skibovskaya, J., Spruss, A., Königsrainer, A., Bischoff, S. C., & Bergheim, I. (2015). Expression of toll-like receptors 1–5 but not TLR 6–10 is elevated in livers of patients with non-alcoholic fatty liver disease. *Liver International*, 35, 562–568.

[\[PubMed\]](#)

Kim, C. H., & Younossi, Z. M. (2008). Nonalcoholic fatty liver disease: A manifestation of the metabolic syndrome. *Cleveland Clinic Journal of Medicine*, 75, 721–728.

[\[PubMed\]](#)

Kirpich, I. A., Solovieva, N. V., Leikhter, S. N., Shidakova, N. A., Lebedeva, O. V., Sidorov, P. I., Bazhukova, T. A., et al. (2008). Probiotics restore bowel flora and improve liver enzymes in human alcohol-induced liver injury: A pilot study. *Alcohol*, 42, 675–682.

[\[PubMed\]](#)

Kirpich, I. A., Feng, W., Wang, Y., Liu, Y., Beier, J. I., Arteel, G. E., Falkner, K. C., Barve, S. S., & McClain, C. J. (2013). Ethanol and dietary unsaturated fat (corn oil/linoleic acid enriched) cause intestinal inflammation and impaired intestinal barrier defense in mice chronically fed alcohol. *Alcohol*, 47, 257–264.

[\[PubMed\]](#)

Koop, D. R., Klopfenstein, B., Imuro, Y., & Thurman, R. G. (1997). Gadolinium chloride blocks alcohol-dependent liver toxicity in rats treated chronically with intragastric alcohol despite the induction of CYP2E1. *Molecular Pharmacology*, 51, 944–950.

[\[PubMed\]](#)

Lakshmi, C. P., Ghoshal, U. C., Kumar, S., Goel, A., Misra, A., Mohindra, S., & Choudhuri, G. (2010). Frequency and factors associated with small intestinal bacterial overgrowth in patients with cirrhosis of the liver and extra hepatic portal venous obstruction. *Digestive Diseases and Sciences*, 55, 1142–1148.

[\[PubMed\]](#)

Lam, Y. Y., Ha, C. W. Y., Hoffmann, J., Oscarsson, J., Dinudom, A., Mather, T. J., Cook, D. I., Hunt, N. H., Caterson, I. D., & Holmes, A. J. (2015). Effects of dietary fat profile on gut permeability and microbiota and their relationships with metabolic changes in mice. *Obesity*, 23, 1429–1439.

[\[PubMed\]](#)

Leclercq, S., De Saeger, C., Delzenne, N., de Timary, P., & Stärkel, P. (2014a). Role of inflammatory pathways, blood mononuclear cells, and gut-derived bacterial products in alcohol dependence. *Biological Psychiatry*, 76, 725–733.

[\[PubMed\]](#)

Leclercq, S., Matamoros, S., Cani, P. D., Neyrinck, A. M., Jamar, F., Stärkel, P., Windey, K., et al. (2014b). Intestinal permeability, gut-bacterial dysbiosis, and behavioral markers of alcohol-dependence severity. *Proceedings of the National Academy of Sciences of the United States of America*, 111, E4485–E4E93.

[\[PubMed\]](#)

Ley, R. E., Backhed, F., Turnbaugh, P., Lozupone, C. A., Knight, R. D., & Gordon, J. I. (2005). Obesity alters gut microbial ecology. *Proceedings of the National Academy of Sciences of the United States of America*, 102, 11070–11075.

[\[PubMed\]](#)

Li, N. N., Li, W., Feng, J. X., Zhang, W. W., Zhang, R., Du, S. H., Liu, S. Y., et al. (2021). High alcohol-producing *Klebsiella pneumoniae* causes fatty liver disease through 2,3-butanediol fermentation pathway in vivo. *Gut Microbes*, 13, 1979883.

[\[PubMed\]](#)

Li, S., Zhou, C., Liu, T., Zhang, L., Liu, S., Zhao, Q., Liu, J., & Zhao, W. (2024). Causal relationships between the gut microbiota, inflammatory cytokines, and alcoholic liver disease: A Mendelian randomization analysis. *Front Endocrinol (Lausanne)*, 15, 1442603.

[\[PubMed\]](#)

Lin, R., Lee, F., Lee, S., Tsai, Y., Lin, H. C., Rei-Hwa, L., Wan-Ching, H., Cheng-Chun, H., Sun-Sang, W., & Kwang-Juei, L. (1995). Endotoxemia in patients with chronic liver diseases: Relationship to severity of liver diseases, presence of esophageal varices, and hyperdynamic circulation. *Journal of Hepatology*, 22, 165–172.

[\[PubMed\]](#)

Loomba, R., Seguritan, V., Li, W., Long, T., Klitgord, N., Bhatt, A., Dulai, P. S., et al. (2017). Gut microbiome-based metagenomic signature for non-invasive detection of advanced fibrosis in human nonalcoholic fatty liver disease. *Cell Metabolism*, 25, 1054–62.e5.

[\[PubMed\]](#)

Luckey, T. D., Reyniers, J. A., György, P., & Forbes, M. (1954). Germfree animals and liver necrosis. *Annals of the New York Academy of Sciences*, 57, 932–935.

[\[PubMed\]](#)

Ludwig, J., Viggiano, T. R., McGill, D. B., & Oh, B. J. (1980). Nonalcoholic steatohepatitis: Mayo clinic experiences with a hitherto unnamed disease. *Mayo Clinic Proceedings*, 55, 434–438.

[\[PubMed\]](#)

Mann, R. E., Smart, R. G., & Govoni, R. (2003). The epidemiology of alcoholic liver disease. *Alcohol Research & Health*, 27, 209–219.

Meijnikman, A. S., Davids, M., Herrema, H., Aydin, O., Tremaroli, V., Rios-Morales, M., Levels, H., et al.

(2022). Microbiome-derived ethanol in nonalcoholic fatty liver disease. *Nature Medicine*, 28, 2100–2106.

[\[PubMed\]](#)

Miele, L., Valenza, V., La Torre, G., Montalto, M., Cammarota, G., Ricci, R., Mascianà, R., Forgione, A., Gabrieli, M. L., & Perotti, G. (2009). Increased intestinal permeability and tight junction alterations in nonalcoholic fatty liver disease. *Hepatology*, 49, 1877–1887.

[\[PubMed\]](#)

Miyaaki, H., & Nakao, K. (2017). Significance of genetic polymorphisms in patients with nonalcoholic fatty liver disease. *Clinical Journal of Gastroenterology*, 10, 201–207.

[\[PubMed\]](#)

Morencos, F. C., de las Heras Castano, G., Martin Ramos, L., Lopez Arias, M. J., Ledesma, F., & Pons Romero, F. (1995). Small bowel bacterial overgrowth in patients with alcoholic cirrhosis. *Digestive Diseases and Sciences*, 40, 1252–1256.

[\[PubMed\]](#)

Muegge, B. D., Kuczynski, J., Knights, D., Clemente, J. C., González, A., Fontana, L., Henrissat, B., Knight, R., & Gordon, J. I. (2011). Diet drives convergence in gut microbiome functions across mammalian phylogeny and within humans. *Science (New York, N.Y.)*, 332, 970–974.

[\[PubMed\]](#)

Mutlu, E. A., Gillevet, P. M., Rangwala, H., Sikaroodi, M., Naqvi, A., Engen, P. A., Kwasny, M., Lau, C. K., & Keshavarzian, A. (2012). Colonic microbiome is altered in alcoholism. *American Journal of Physiology – Gastrointestinal and Liver Physiology*, 302, G966–GG78.

[\[PubMed\]](#)

Nazim, M., Stamp, G., & Hodgson, H. J. (1989). Non-alcoholic steatohepatitis associated with small intestinal diverticulosis and bacterial overgrowth. *Hepato-Gastroenterology*, 36, 349–351.

[\[PubMed\]](#)

Neuschwander-Tetri, B. A., & Caldwell, S. H. (2003). Nonalcoholic steatohepatitis: Summary of an AASLD single topic conference. *Hepatology*, 37, 1202–1219.

[\[PubMed\]](#)

Pande, C., Kumar, A., & Sarin, S. K. (2009). Small-intestinal bacterial overgrowth in cirrhosis is related to the severity of liver disease. *Alimentary Pharmacology & Therapeutics*, 29, 1273–1281.

Pande, A., Sharma, S., Khillan, V., Rastogi, A., Arora, V., Shasthry, S. M., Vijayaraghavan, R., Jagdish, R., Kumar, M., & Kumar, G. (2023). Fecal microbiota transplantation compared with prednisolone in severe alcoholic hepatitis patients: A randomized trial. *Hepatology International*, 17, 249–261.

[\[PubMed\]](#)

Parlesak, A., Schafer, C., Schutz, T., Bode, J. C., & Bode, C. (2000). Increased intestinal permeability to macromolecules and endotoxemia in patients with chronic alcohol abuse in different stages of alcohol-induced liver disease. *Journal of Hepatology*, 32, 742–747.

[\[PubMed\]](#)

Pataky, Z., Genton, L., Spahr, L., Lazarevic, V., Terraz, S., Gaia, N., Rubbia-Brandt, L., Golay, A., Schrenzel, J., & Pichard, C. (2016). Impact of hypocaloric hyperproteic diet on gut microbiota in overweight or obese patients with nonalcoholic fatty liver disease: A pilot study. *Digestive Diseases and Sciences*, 61, 2721–2731.

[\[PubMed\]](#)

Patel, V. C., Lee, S., McPhail, M. J. W., Da Silva, K., Guilly, S., Zamalloa, A., Witherden, E., et al. (2022). Rifaximin-alpha reduces gut-derived inflammation and mucin degradation in cirrhosis and encephalopathy: RIFSYS randomised controlled trial. *Journal of Hepatology*, 76, 332–342.

[\[PubMed\]](#)

Patterson, E., O'Doherty, R. M., Murphy, E. F., Wall, R., O'Sullivan, O., Nilaweera, K., Fitzgerald, G. F., Cotter, P. D., Ross, R. P., & Stanton, C. (2014). Impact of dietary fatty acids on metabolic activity and host intestinal microbiota composition in C57BL/6J mice. *British Journal of Nutrition*, 111, 1905–1917.

[\[PubMed\]](#)

Rehm, J., Taylor, B., Mohapatra, S., Irving, H., Baliunas, D., Patra, J., & Roerecke, M. (2010). Alcohol as a risk factor for liver cirrhosis: A systematic review and meta-analysis. *Drug and Alcohol Review*, 29, 437–445.

[\[PubMed\]](#)

Rinella, M. E., Lazarus, J. V., Ratziu, V., Francque, S. M., Sanyal, A. J., Kanwal, F., Romero, D., et al. (2023). A multisociety Delphi consensus statement on new fatty liver disease nomenclature. *Hepatology*, 78, 1966–1986.

[\[PubMed\]](#)

Rivera, C. A., Bradford, B. U., Seabra, V., & Thurman, R. G. (1998). Role of endotoxin in the hypermetabolic state after acute ethanol exposure. *The American Journal of Physiology*, 275, G1252–G1258.

[\[PubMed\]](#)

Romero-Gomez, M., Zelber-Sagi, S., & Trenell, M. (2017). Treatment of NAFLD with diet, physical activity and exercise. *Journal of Hepatology*, 67, 829.

[\[PubMed\]](#)

Ruiz, A. G., Casafont, F., Crespo, J., Cayón, A., Mayorga, M., Estebanez, A., Fernandez-Escalante, J. C., & Pons-Romero, F. (2007). Lipopolysaccharide-binding protein plasma levels and liver TNF-alpha gene expression in obese patients: Evidence for the potential role of endotoxin in the pathogenesis of non-alcoholic steatohepatitis. *Obesity Surgery*, 17, 1374.

[\[PubMed\]](#)

Rutenburg, A. M., Sonnenblick, E., Koven, I., Aprahamian, H. A., Reiner, L., & Fine, J. (1957). The role of intestinal bacteria in the development of dietary cirrhosis in rats. *The Journal of Experimental Medicine*, 106, 1–14.

[\[PubMed\]](#)

Saha, S., & Schnabl, B. (2025). Modulating the microbiome in chronic liver diseases-current evidence on the role of fecal microbiota transplantation. *Expert Review of Gastroenterology &*

Hepatology, 19, 53.

Satapathy, S. K., & Sanyal, A. J. (2015). Epidemiology and natural history of nonalcoholic fatty liver disease. *Seminars in Liver Disease*, 35, 221–235.

[\[PubMed\]](#)

Savolainen, V. T., Liesto, K., Männikkö, A., Penttilä, A., & Karhunen, P. J. (1993). Alcohol consumption and alcoholic liver disease: Evidence of a threshold level of effects of ethanol. *Alcoholism: Clinical and Experimental Research*, 17, 1112–1117.

[\[PubMed\]](#)

Schneider, K. M., Bieghs, V., Heymann, F., Hu, W., Dreymueller, D., Liao, L., Frissen, M., et al. (2015). CX3CR1 is a gatekeeper for intestinal barrier integrity in mice: Limiting steatohepatitis by maintaining intestinal homeostasis. *Hepatology*, 62, 1405–1416.

[\[PubMed\]](#)

Schneider, C. V., Schneider, K. M., Raptis, A., Huang, H., Trautwein, C., & Loomba, R. (2024a). Prevalence of at-risk MASH, MetALD and alcohol-associated steatotic liver disease in the general population. *Alimentary Pharmacology & Therapeutics*, 59, 1271–1281.

Schneider, K. M., Kummen, M., Trivedi, P. J., & Hov, J. R. (2024b). Role of microbiome in autoimmune liver diseases. *Hepatology*, 80, 965–987.

[\[PubMed\]](#)

Sellmann, C., Jin, C. J., Degen, C., De Bandt, J. P., & Bergheim, I. (2015). Oral glutamine supplementation protects female mice from nonalcoholic Steatohepatitis. *The Journal of Nutrition*, 145, 2280–2286.

[\[PubMed\]](#)

Shapiro, H., Suez, J., & Elinav, E. (2017). Personalized microbiome-based approaches to metabolic syndrome management and prevention. *Journal of Diabetes*, 9, 226–236.

[\[PubMed\]](#)

Sliedrecht, W., de Waart, R., Witkiewitz, K., & Roozen, H. G. (2019). Alcohol use disorder relapse factors: A systematic review. *Psychiatry Research*, 278, 97–115.

[\[PubMed\]](#)

Sonnenburg, J. L., & Backhed, F. (2016). Diet-microbiota interactions as moderators of human metabolism. *Nature*, 535, 56–64.

[\[https://doi.org/10.1038/nature18846\]](https://doi.org/10.1038/nature18846) [\[PubMed\]](#)

Speliotes, E. K., & Schneider, C. V. (2024). PNPLA3 I148M interacts with environmental triggers to cause human disease. *Liver International*, 45(3), e16106.

[\[PubMed\]](#)

Spencer, M. D., Hamp, T. J., Reid, R. W., Fischer, L. M., Zeisel, S. H., & Fodor, A. A. (2011). Association between composition of the human gastrointestinal microbiome and development of fatty liver with choline deficiency. *Gastroenterology*, 140, 976–986.

[\[PubMed\]](#)

Spruss, A., & Bergheim, I. (2009). Dietary fructose and intestinal barrier: Potential risk factor in the pathogenesis of nonalcoholic fatty liver disease. *The Journal of Nutritional Biochemistry*, 20, 657–662.

[\[PubMed\]](#)

Spruss, A., Kanuri, G., Uebel, K., Bischoff, S. C., & Bergheim, I. (2011). Role of the inducible nitric oxide synthase in the onset of fructose-induced steatosis in mice. *Antioxidants & Redox Signaling*, 14, 2121–2135.

Spruss, A., Kanuri, G., Stahl, C., Bischoff, S. C., & Bergheim, I. (2012). Metformin protects against the development of fructose-induced steatosis in mice: Role of the intestinal barrier function. *Laboratory Investigation*, 92, 1020–1032.

[\[PubMed\]](#)

Starley, B. Q., Calcagno, C. J., & Harrison, S. A. (2010). Nonalcoholic fatty liver disease and hepatocellular carcinoma: A weighty connection. *Hepatology*, 51, 1820–1832.

[\[PubMed\]](#)

Sutter, A. G., Palanisamy, A. P., Lench, J. H., Jessmore, A. P., & Chavin, K. D. (2015). Development of steatohepatitis in Ob/Ob mice is dependent on Toll-like receptor 4. *Annals of Hepatology*, 14, 735–743.

[\[PubMed\]](#)

Szabo, G. (2015). Gut-liver axis in alcoholic liver disease. *Gastroenterology*, 148, 30–36.

[\[PubMed\]](#)

Thuy, S., Ladurner, R., Volynets, V., Wagner, S., Strahl, S., Konigsrainer, A., Maier, K. P., Bischoff, S. C., & Bergheim, I. (2008). Nonalcoholic fatty liver disease in humans is associated with increased plasma endotoxin and plasminogen activator inhibitor 1 concentrations and with fructose intake. *The Journal of Nutrition*, 138, 1452–1455.

[\[PubMed\]](#)

Townsend, S. A., & Newsome, P. N. (2016). Non-alcoholic fatty liver disease in 2016. *British Medical Bulletin*, 119, 143–156.

[\[PubMed\]](#)

Uesugi, T., Froh, M., Arteel, G. E., Bradford, B. U., & Thurman, R. G. (2001). Toll-like receptor 4 is involved in the mechanism of early alcohol-induced liver injury in mice. *Hepatology*, 34, 101–108.

[\[PubMed\]](#)

Volynets, V., Kuper, M. A., Strahl, S., Maier, I. B., Spruss, A., Wagnerberger, S., Konigsrainer, A., Bischoff, S. C., & Bergheim, I. (2012). Nutrition, intestinal permeability, and blood ethanol levels are altered in patients with nonalcoholic fatty liver disease (NAFLD). *Digestive Diseases and Sciences*, 57, 1932–1941.

[\[PubMed\]](#)

Vrieze, A., Van Nood, E., Holleman, F., Salojarvi, J., Kootte, R. S., Bartelsman, J. F., Dallinga-Thie, G. M., et al. (2012). Transfer of intestinal microbiota from lean donors increases insulin sensitivity in individuals with metabolic syndrome. *Gastroenterology*, 143, 913–6 e7.

[\[PubMed\]](#)

Wagnerberger, S., Spruss, A., Kanuri, G., Volynets, V., Stahl, C., Bischoff, S. C., & Bergheim, I. (2012). Toll-like receptors 1-9 are elevated in livers with fructose-induced hepatic steatosis. *The British Journal of Nutrition*, 107, 1727–1738.

[\[PubMed\]](#)

Wagnerberger, S., Fiederlein, L., Kanuri, G., Stahl, C., Millonig, G., Mueller, S., Bischoff, S. C., & Bergheim, I. (2013). Sex-specific differences in the development of acute alcohol-induced liver steatosis in mice. *Alcohol and Alcoholism*, 48, 648–656.

[\[PubMed\]](#)

Wang, R., Tang, R., Li, B., Ma, X., Schnabl, B., & Tilg, H. (2021). Gut microbiome, liver immunology, and liver diseases. *Cellular & Molecular Immunology*, 18, 4–17.

Wiest, R., Albillos, A., Trauner, M., Bajaj, J. S., & Jalan, R. (2017). Targeting the gut-liver axis in liver disease. *Journal of Hepatology*, 67, 1084.

[\[PubMed\]](#)

Wigg, A. J., Roberts-Thomson, I. C., Dymock, R. B., McCarthy, P. J., Grose, R. H., & Cummins, A. G. (2001). The role of small intestinal bacterial overgrowth, intestinal permeability, endotoxaemia, and tumour necrosis factor α in the pathogenesis of non-alcoholic steatohepatitis. *Gut*, 48, 206–211.

[\[PubMed\]](#)

World Health Organization. (2018). *Global status report on alcohol and health 2018*. World Health Organization.

Wu, G. D., Chen, J., Hoffmann, C., Bittinger, K., Chen, Y. Y., Keilbaugh, S. A., Bewtra, M., et al. (2011). Linking long-term dietary patterns with gut microbial enterotypes. *Science*, 334, 105–108.

[\[PubMed\]](#)

Wu, H., Esteve, E., Tremaroli, V., Khan, M. T., Caesar, R., Manneras-Holm, L., Stahlman, M., et al. (2017). Metformin alters the gut microbiome of individuals with treatment-naïve type 2 diabetes, contributing to the therapeutic effects of the drug. *Nature Medicine*, 23, 850–858.

[\[PubMed\]](#)

Xue, G., Feng, J., Zhang, R., Du, B., Sun, Y., Liu, S., Yan, C., et al. (2023). Three *Klebsiella* species as potential pathobionts generating endogenous ethanol in a clinical cohort of patients with auto-brewery syndrome: A case control study. *eBioMedicine*, 91, 104560.

[\[PubMed\]](#)

Yang, C. Y., Chang, C. S., & Chen, G. H. (1998). Small-intestinal bacterial overgrowth in patients with liver cirrhosis, diagnosed with glucose H₂ or CH₄ breath tests. *Scandinavian Journal of Gastroenterology*, 33, 867–871.

[\[PubMed\]](#)

Yeh, M. M., & Brunt, E. M. (2014). Pathological features of fatty liver disease. *Gastroenterology*, 147, 754–764.

[\[PubMed\]](#)

Younossi, Z. M., Koenig, A. B., Abdelatif, D., Fazel, Y., Henry, L., & Wymer, M. (2016). Global epidemiology of nonalcoholic fatty liver disease-meta-analytic assessment of prevalence, incidence, and outcomes. *Hepatology*, 64, 73–84.

[\[PubMed\]](#)

Yuan, J., Chen, C., Cui, J., Lu, J., Yan, C., Wei, X., Zhao, X., et al. (2019). Fatty liver disease caused by high-Alcohol-producing *Klebsiella pneumoniae*. *Cell Metabolism*, 30, 675–688 e7.

[\[PubMed\]](#)

Yue, R., Wei, X., Zhao, J., Zhou, Z., & Zhong, W. (2020). Essential role of IFN-gamma in regulating gut antimicrobial peptides and microbiota to protect against Alcohol-induced bacterial translocation and hepatic inflammation in mice. *Frontiers in Physiology*, 11, 629141.

[\[PubMed\]](#)

Yuki, T., & Thurman, R. G. (1980). The swift increase in alcohol metabolism. Time course for the increase in hepatic oxygen uptake and the involvement of glycolysis. *Biochemical Journal*, 186, 119–126.

[\[PubMed\]](#)

Zhu, Y., Lin, X., Zhao, F., Shi, X., Li, H., Li, Y., Zhu, W., Xu, X., Li, C., & Zhou, G. (2015). Meat, dairy and plant proteins alter bacterial composition of rat gut bacteria. *Scientific Reports*, 5, 15220.

[\[PubMed\]](#)

Zhu, Y., Shi, X., Lin, X., Ye, K., Xu, X., Li, C., & Zhou, G. (2017). Beef, chicken, and soy proteins in diets induce different gut microbiota and metabolites in rats. *Frontiers in Microbiology*, 8, 1395.

[\[PubMed\]](#)

Zmora, N., Zeevi, D., Korem, T., Segal, E., & Elinav, E. (2016). Taking it personally: Personalized utilization of the human microbiome in health and disease. *Cell Host & Microbe*, 19, 12–20.

18. Microbiome and Diseases: Gut–Brain Axis and Neurological Disorders

Juliana de Castilhos^{1,2} and Christoph K. Stein-Thoeringer^{1,2} 

(¹) Department of Internal Medicine I, University Clinic Tuebingen, Tuebingen, Germany

(²) M3 Research Center for Malignome, Metabolome and Microbiome, University Clinic Tuebingen, Tuebingen, Germany

 **Christoph K. Stein-Thoeringer**

Email: christoph.stein-thoeringer@med.uni-tuebingen.de

Abstract

An expanding amount of evidence implicates the gut microbiota as a key modulator of brain development, function, and behavior. This bidirectional communication system, named the “microbiome–gut–brain axis,” relies on immune, neural, endocrine, and metabolic signaling pathways linking the gastrointestinal tract to the central nervous system. These routes involve the autonomic and enteric nervous systems, the hypothalamic–pituitary–adrenal axis, and enteroendocrine signaling, and are shaped by, e.g., metabolites as secreted microbial effectors on the host. Both preclinical and clinical studies highlight how microbial dysbiosis– alterations in gut microbial composition– may contribute to neurodevelopmental, psychiatric, and neurodegenerative conditions. This chapter dissects the mechanisms that mediate gut-to-brain communication and highlights that dysbiosis is associated with the onset and progression of neurological and psychiatric disorders, including autism spectrum disorder, major

depressive disorder, Parkinson's disease, and multiple sclerosis. By examining these disruptions, we also consider how they may inform the development of novel microbiome-targeted interventions for brain health.

18.1 Introduction

Once considered passive inhabitants of the gastrointestinal tract, microbes are now recognized as crucial regulators of host physiology—including central nervous system (CNS) development and function. This paradigm shift has been driven by growing evidence supporting the existence of the microbiota–gut–brain axis: a complex, bidirectional communication network that links the gastrointestinal microbiome with the brain through neural, immune, endocrine, and metabolic signaling (Cryan & Dinan, [2012](#); Dinan & Cryan, [2017a](#), [b](#); Mayer et al., [2014](#)).

Gnotobiotic models and high-resolution microbiome analyses have revealed that alterations in microbial composition can influence synaptic plasticity, stress reactivity, and emotional regulation. Germ-free (GF) animals show exaggerated hypothalamic–pituitary–adrenal (HPA) axis responses, altered neurotransmitter expression, and immune dysregulation—findings that parallel clinical observations in conditions such as autism spectrum disorder, major depressive disorder, and Parkinson's disease (Clarke et al., [2013](#); Hsiao et al., [2013](#); Sudo et al., [2004](#)).

In this chapter, we examine the major mechanisms underpinning microbiota–brain crosstalk, focusing on pathways such as the enteric and autonomic nervous systems, immune and endocrine signaling, enteroendocrine activity, and the HPA axis. We also explore how microbes influence neural activity and behavior. Critically, we contextualize these pathways within clinical conditions—illustrating how disruptions in microbiome composition and signaling contribute to neurodevelopmental, neuropsychiatric, and neurodegenerative disorders. By bridging basic science with translational insight, we

emphasize the growing relevance of the microbiome as both a diagnostic marker and therapeutic target in brain health.

18.2 The Microbiota–Gut–Brain Axis: Neural and Humoral Microbe-to-Brain Signaling

The gastrointestinal (GI) tract and brain maintain a dynamic, bidirectional relationship that extends beyond basic digestive processes. While several studies primarily focused on gut–brain interactions in the regulation of digestion and satiety (Clarke et al., [2024](#); Li et al., [2023](#); Moran & Thapaliya, [2021](#)), more recent investigations have shifted toward exploring how this communication influences cognitive, emotional, and behavioral outcomes (Lu et al., [2024](#); Toader et al., [2024](#); Ueda et al., [2024](#)). Disruptions in this reciprocal gut–brain network have been implicated in a variety of pathophysiological conditions, including gut inflammation (Bernstein, [2017](#); Breit et al., [2018](#)), maladaptive responses to both acute and chronic stress (Dinan & Cryan, [2017a](#); Foster et al., [2017](#); Gao et al., [2018](#); Partrick et al., [2018](#)), and alterations in mood and behavior (Abavisani et al., [2025](#); Dinan & Cryan, [2017a](#); Jaglin et al., [2018](#)). As a result, the gut–brain axis has emerged as a promising therapeutic target across a broad spectrum of neurological and psychiatric conditions, including mental health and cognitive disorders (O’Riordan et al., [2025](#); Ramadan et al., [2025](#); Ullah et al., [2023](#)). There are multiple potential routes through which the gut microbiota communicates with the brain—ranging from densely innervated, plastic neural circuits to subtle, small-molecule signaling pathways that operate both locally within the gut and remotely in the brain (Cryan et al., [2019](#)) (Fig. [18.1](#)). The following section introduces some of these communication mechanisms. Despite growing insights, the precise ways in which gut–resident microbes influence central nervous system function and behavior remain incompletely understood, highlighting the need for further mechanistic studies to unravel how signals from the gut lumen can exert such profound effects on brain activity.

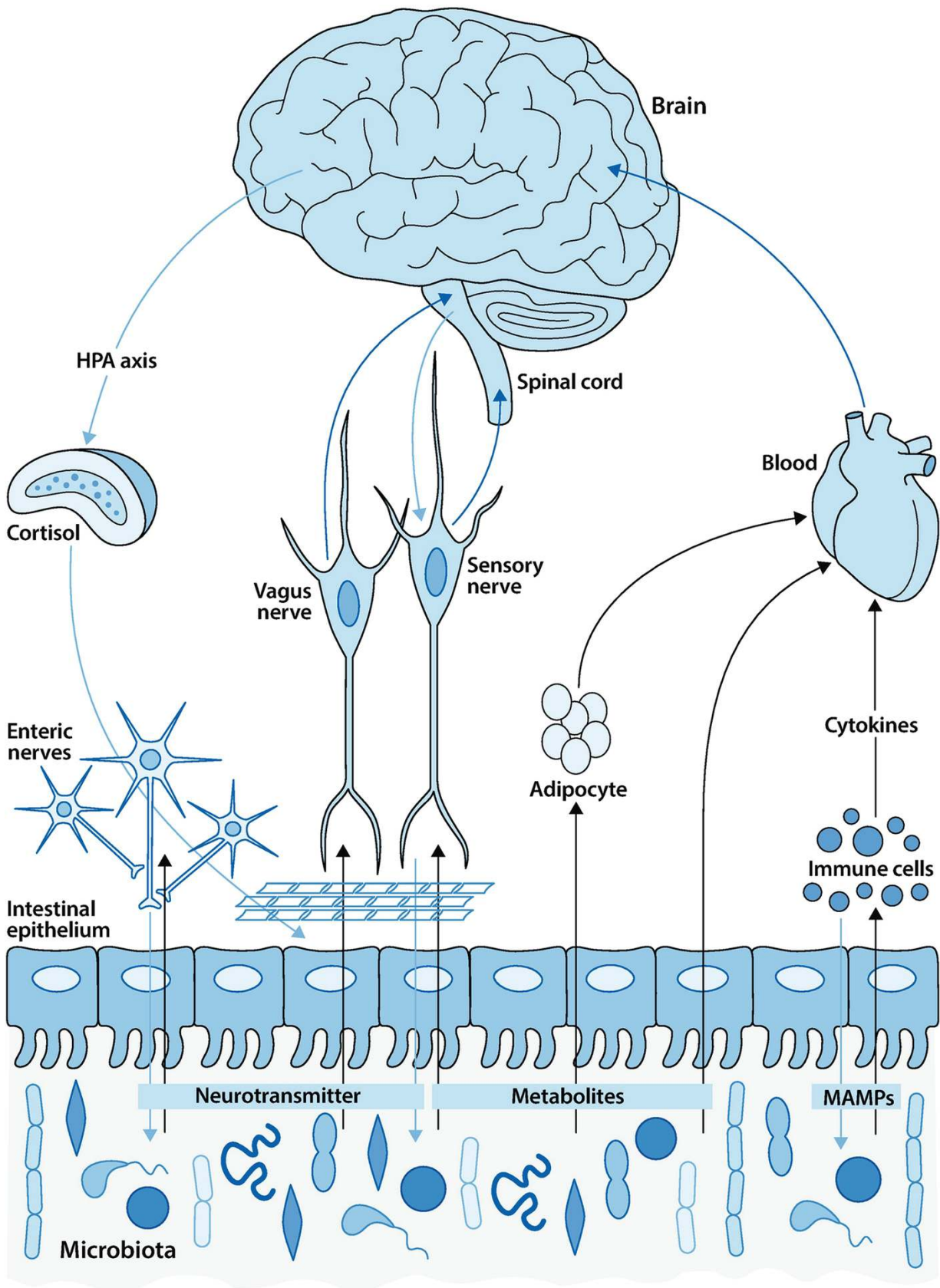


Fig. 18.1 Summary of the main interactions in the microbiota-gut-brain axis

18.2.1 Gut-Brain Signaling Pathways

18.2.1.1 The Vagus Nerve

The vagus nerve is the primary neural pathway connecting the gut and brain, composed mostly of afferent fibers (~80%) that relay visceral information to the CNS (Berthoud & Neuhuber, [2000](#); Breit et al., [2018](#)). Vagal fibers from the gut mostly reach the nucleus tractus solitarius (NTS), where glutamatergic neurons and various neuropeptides integrate and direct responses related to appetite, emotion, and memory (Altschuler et al., [1993](#); Bienkowski & Rinaman, [2013](#); Kerfoot et al., [2008](#); Rinaman, [2010](#); Roozendaal et al., [1999](#)). Through its extensive projections to the other parts of the brain, the NTS influences key neurotransmitter systems, including norepinephrine, serotonin, and dopamine, thereby acting as a central hub for gut-brain signaling (Maniscalco & Rinaman, [2018](#)). On the other hand, vagal efferent fibers, originating from the dorsal motor nucleus of the vagus (DMV) and nucleus ambiguus, constitute the motor arm of the parasympathetic nervous system and play a key role in gut regulation. Through the inflammatory reflex, afferent vagal signaling can activate these efferents, which in turn suppress intestinal inflammation and enhance epithelial barrier integrity via tight junction modulation. These effects may secondarily influence gut microbiota composition by maintaining a more stable intestinal environment (Bonaz et al., [2018](#)).

Specific microbes (e.g., *Lactobacillus rhamnosus*, *Campylobacter jejuni*, *Lactobacillus reuteri*) have been shown to modulate behavior through vagal pathways, an effect abolished by vagotomy in animal models (Goehler et al., [2005](#); Sgritta et al., [2019](#)). Evidence also shows that microbial metabolites—such as indoles, γ -aminobutyric acid (GABA), and serotonin produced by gut microbes—can activate vagal afferents, influencing emotional and cognitive behaviors (Goehler et al., [2005](#); Jaglin et al., [2018](#)). Experimental vagotomy studies underscore the critical role of intact vagal signaling in regulating mood, cognition, neurogenesis, and immune responses (O’Leary et al., [2018](#); Ronchi et

al., [2012](#)). In contrast, vagus nerve stimulation shows therapeutic promise in treating depression, epilepsy, chronic pain, and gastrointestinal inflammation by modulating neuromodulators and enhancing hippocampal plasticity (Breit et al., [2018](#); Grimonprez et al., [2015](#); Shin et al., [2019](#)).

18.2.1.2 Autonomic Nervous System (ANS)

The autonomic nervous system (ANS), comprising sympathetic and parasympathetic branches, plays a central role in gut–brain communication by regulating involuntary functions like digestion, motility, secretion, and immune responses (Wehrwein et al., [2016](#)). Through its interaction with the enteric nervous system (ENS) and modulation by the CNS and HPA axis, the ANS supports physiological homeostasis and mediates top-down and bottom-up signaling within the microbiota–gut–brain axis (Bauer et al., [2016](#); Lu et al., [2024](#)).

Microbial metabolites such as serotonin, GABA, and catecholamines influence ANS signaling, while ANS activity in turn affects gut transit, mucus secretion, and microbiota composition (Rhee et al., [2009](#)). Experimental evidence shows that microbial signals can activate vagal pathways and alter brain activity, including anxiety-like behaviors, via direct sensory input (Maniscalco & Rinaman, [2018](#); Mir et al., [2024](#)). Thus, the ANS serves as a fast, bidirectional interface through which gut microbes can rapidly impact brain function and behavior.

18.2.1.3 The Enteric Nervous System (ENS)

The enteric nervous system (ENS) is a dense and complex network of neurons embedded in the gut wall that functions semi-autonomously from the central nervous system (CNS) (Furness, [2012](#)). It is organized into two main ganglionated plexuses: the myenteric plexus, which primarily regulates gut motility, and the submucosal plexus, which controls secretion and fluid transport (Furness, [2012](#)). In addition to its local control of gastrointestinal functions, the ENS maintains continuous, bidirectional communication with the CNS. This occurs through vagal and spinal afferent pathways, as well as intestinofugal

neurons projecting to sympathetic ganglia (Furness, [2012](#); Rao & Gershon, [2016](#)). These intrinsic and extrinsic neural circuits provide a route by which signals originating in the gut lumen — including microbial metabolites—can influence both intestinal physiology and brain function (Cryan et al., [2019](#)). The ENS development unfolds in parallel with microbial colonization during early life, and studies in GF animals have demonstrated that the absence of gut microbes results in significant ENS abnormalities—ranging from impaired neurogenesis to altered neurotransmission—many of which can be reversed through microbial reconstitution (Collins et al., [2014](#); De Vadder et al., [2018](#); Hyland & Cryan, [2016](#)). This microbial influence is, in part, mediated by innate immune receptors such as Toll-like receptors (TLRs), particularly TLR2 and TLR4. These receptors enable the ENS to respond to microbial signals, and their absence leads to disrupted gut motility and physiological dysfunction, underscoring the critical role of the microbiota in ENS maturation (Brun et al., [2015](#); Caputi et al., [2017](#)). Beyond immune pathways, microbiota-derived metabolites—including serotonin, GABA, and short-chain fatty acids (SCFAs)—have been shown to modulate ENS excitability and signaling. Moreover, specific bacterial strains, such as *Lactobacillus rhamnosus* and *Bacteroides fragilis*, exert strain-dependent effects on ENS activity through epithelial interfaces and receptor-mediated mechanisms (Al-Nedawi et al., [2015](#); Kunze et al., [2009](#); Mao et al., [2013](#)). Disruption of the microbial community, for example, through antibiotic exposure, further illustrates this connection: such perturbations alter ENS architecture, reduce enteric glial cell density, and shift neurotransmitter profiles (Caputi et al., [2017](#); Kabouridis et al., [2015](#)). The ENS is also sensitive to environmental stressors. Early-life stress, particularly when coupled with dysbiosis, has been shown to modify ENS function, suggesting a complex interplay between microbiome, stress, and neurodevelopmental programming (De Palma et al., [2015](#)). Importantly, intrinsic sensory neurons of the ENS appear to serve as early sensors of microbial signals, as evidenced by GF animals

displaying reduced neuronal excitability and impaired sensory processing (McVey et al., [2013](#)).

18.2.1.4 Immune System

The gastrointestinal tract, home to the largest concentration of immune cells in the body, plays a central role in gut–brain communication through its dynamic interaction with the microbiome. At the intestinal barrier, immune cells continuously monitor microbial signals via specialized receptors such as TLRs, which detect microbial antigens and trigger the release of cytokines, chemokines, and other inflammatory mediators (Fasano & Shea-Donohue, [2005](#); Takeda & Akira, [2004](#); Vaishnavi et al., [2008](#)). These immune signals can enter systemic circulation or activate vagal and spinal afferents, thereby relaying gut-derived information to the brain (Cani et al., [2013](#); Cryan et al., [2019](#)). This immune-mediated signaling network not only protects the host from pathogens but also enables the gut microbiota to modulate brain function and behavior. Through this bidirectional dialogue, the immune system acts as a crucial transducer, translating microbial cues into neural and endocrine responses that shape central nervous system activity.

18.2.1.5 Hypothalamic–Pituitary–Adrenal (HPA) Axis

The hypothalamic–pituitary–adrenal (HPA) axis serves as a central coordinator of the stress response and represents a key non-neuronal communication route within the microbiota–gut–brain axis. In response to stress or disrupted homeostasis, the hypothalamus releases corticotropin-releasing factor (CRF), which stimulates the pituitary to secrete adrenocorticotrophic hormone (ACTH). ACTH then prompts the adrenal glands to produce glucocorticoids—such as cortisol—that mobilize the body for a “fight-or-flight” response (de Wied et al., [1993](#); Mayer, [2000](#)). Pioneering studies in GF mice demonstrated that the absence of intestinal microbiota leads to a hyperactive HPA axis, characterized by elevated corticosterone levels and altered hippocampal expression of receptors involved in CRF

regulation (Clarke et al., [2013](#); Neufeld et al., [2011](#); Sudo et al., [2004](#)). Notably, colonization with specific bacterial strains during early life normalized this stress response, indicating a critical developmental window during which the microbiota shapes neuroendocrine function. Similar patterns are observed in humans: individuals with irritable bowel syndrome (IBS) not only exhibit dysbiosis but also show exaggerated ACTH and cortisol responses to CRF infusion (Dinan et al., [2006](#); Eisenstein, [2016](#); Grenham et al., [2011](#)).

The HPA axis also engages in crosstalk with both immune and neural pathways. Psychological stress can compromise gut barrier integrity by reducing the protective mucus layer, allowing bacteria to come into direct contact with the intestinal epithelium (Allen et al., [2022](#); Jagers et al., [2022](#)). This close contact triggers proinflammatory and oxidative responses, facilitating microbial translocation and low-grade inflammation (Madison & Bailey, [2024](#)). The release of pro-inflammatory cytokines further stimulates the HPA axis, amplifying the stress response (Demaude et al., [2006](#)). In models lacking TLRs, this signaling cascade is disrupted, underscoring the importance of microbial recognition in regulating neuroendocrine activity (Gosselin & Rivest, [2008](#); O'Hara & Shanahan, [2006](#)).

Moreover, vagal afferent pathways modulate HPA axis function. Experimental vagal stimulation has been shown to increase hypothalamic CRF expression and elevate systemic levels of ACTH and corticosterone, highlighting the bidirectional interplay between neural circuits and hormonal stress pathways (Hosoi et al., [2000](#)).

18.2.2 Gut Microbiome Influences on CNS Physiology

Beyond its role in maintaining physiological homeostasis, the gut microbiome also contributes to the structural and functional organization of the brain. Microbial signals influence neurodevelopmental processes such as neurogenesis, myelination, and synaptic remodeling, while also modulating neurophysiological functions like synaptic plasticity and neurotransmitter signaling. The following sections detail how these microbial effects shape both brain

morphology and neurophysiology, offering insights into the microbiome's broader impact on CNS integrity.

18.2.2.1 Brain Morphology

Growing evidence suggests that the gut microbiome exerts a significant influence on brain morphology, shaping the structural development and maintenance of the CNS. Gut microbes have been implicated in regulating neurogenesis, myelination, dendritic architecture, microglial morphology, synaptic structure, and blood–brain barrier (BBB) integrity. Much of this knowledge derives from studies using GF animal models, which exhibit widespread morphological alterations in key brain regions (Aburto & Cryan, [2024](#); Clarke et al., [2013](#); Desbonnet et al., [2014](#); Diaz Heijtz et al., [2011](#); Erny et al., [2015](#); Gareau et al., [2011](#); Hegstrand & Hine, [1986](#); Hoban et al., [2016](#); Knox et al., [2023](#); Luczynski et al., [2016](#); Neufeld et al., [2011](#); Sudo et al., [2004](#)). GF animals display striking ultrastructural changes in neuronal dendrites and spines, particularly within the amygdala and hippocampus, along with disrupted myelination of axonal fibers in the prefrontal cortex (Ferrante et al., [2013](#); Hoban et al., [2016](#); London et al., [2013](#); Lu et al., [2018](#); Luczynski et al., [2016](#)). Additionally, abnormal morphology and activation states have been reported in other brain cell types such as microglia and astrocytes, indicating broader glial dysregulation in the absence of microbiome (Guida et al., [2018](#); Rea et al., [2016](#); Vuong et al., [2017](#)). Neurogenesis is also modulated by the gut microbiome, although there are some conflicting results. Adult GF mice can show enhanced hippocampal neurogenesis, with increased survival of newly born neurons (Ogbonnaya et al., [2015](#)), whereas other authors report that long-term antibiotic treatments or GF status can lead to decreased hippocampal neurogenesis, an effect reversible by probiotic treatment or environmental enrichment such as access to a running wheel (Möhle et al., [2016](#); Sawada et al., [2018](#)). Another key structural interface influenced by the microbiome is the blood–brain barrier. In mice with a conventional microbiome, the BBB begins to develop and restrict permeability around postnatal day 14, whereas GF mice maintain

elevated permeability and reduced expression of tight junction proteins for a longer period (Braniste et al., [2014](#)). Remarkably, colonization with SCFA-producing bacteria restores BBB integrity and selectivity (Yassin et al., [2025](#)), underscoring the microbiota's role in establishing barrier function during early development. Together, these findings indicate that the development, maturation, and renewal of multiple CNS cell types are under microbial influence. While the precise molecular mechanisms governing this host–microbe crosstalk remain to be fully elucidated, current evidence supports the gut microbiome as a key architect of brain morphology across both early-life and adult windows.

18.2.2.2 Neurophysiology

Beyond structural alterations, the gut microbiome profoundly impacts the functional dynamics of neural circuits, revealing that microbial signals can modulate fundamental processes underlying neural connectivity and function. Preclinical studies using GF animals have shown that the absence of intestinal microbes disrupts essential neurophysiological features, particularly synaptic plasticity in hippocampal circuits. One of the most well-characterized neurophysiological mechanisms influenced by the gut microbiome is synaptic plasticity, which includes long-term potentiation (LTP) and long-term depression (LTD) of synaptic strength that are crucial for learning and memory (Bliss & Cooke, [2011](#)). Initial findings demonstrated that the lack of a conventional gut microbiome impairs LTP and leads to reduced expression of brain-derived neurotrophic factor (BDNF), serotonin receptors (e.g., 5HT_{1A}), and NMDA glutamate receptor subunits in brain regions essential for emotional regulation and cognitive function, such as the hippocampus and prefrontal cortex (Bercik et al., [2011](#); Diaz Heijtz et al., [2011](#); Neufeld et al., [2011](#); Sudo et al., [2004](#)). These molecular alterations are often accompanied by increased turnover of monoamine neurotransmitters—including serotonin, dopamine, and noradrenaline—as well as impaired tryptophan metabolism, reducing the availability of this essential precursor for serotonin synthesis (Clarke et al., [2013](#)). Beyond neuronal

signaling, microbial alterations also impact glial function. For example, mice lacking S100B, a calcium-binding protein derived from astrocytes, exhibit enhanced LTP in the hippocampal CA1 region, highlighting the contribution of glial signaling to synaptic modulation and memory encoding (Nishiyama et al., [2002](#)).

The gut microbiome also regulates neurotransmitter biosynthesis and signaling pathways. While certain bacterial taxa can produce neurotransmitters such as GABA, serotonin, and dopamine, these molecules do not cross the blood–brain barrier (BBB) (Chen et al., [2021a](#)). Instead, microbiota-derived regulation occurs primarily through modulation of amino acid precursors such as tryptophan, tyrosine, and glutamate, which are absorbed from the gut, circulate in the bloodstream, and are transported across the BBB. Once in the brain, these precursors are converted into neurotransmitters by neuron- or glia-specific enzymes (Frost et al., [2014](#)). Interestingly, some gut bacteria also express enzymes capable of producing these same precursors, thus influencing the systemic pool available for central neurotransmitter synthesis (Frost et al., [2014](#)). In parallel, microbial metabolites can act on intestinal enteroendocrine cells, stimulating the local release of neurotransmitters or hormones that signal to the brain via the ENS or the vagus nerve (Kaelberer et al., [2018](#); Kaelberer et al., [2020](#); Yano et al., [2015](#)). For example, administration of *Lactobacillus rhamnosus* has been shown to alter the expression of GABA receptors in limbic and cortical brain regions—a likely result of vagal signaling rather than direct metabolite entry into the CNS (Bravo et al., [2011](#)). More broadly, several studies have linked defined microbial communities to diverse neurophysiological and behavioral phenotypes. Animal models have demonstrated that gut microbes influence motor function, circadian rhythms, stress responsiveness, anxiety-like behavior, pain perception, learning and memory, as well as feeding behavior and appetite regulation (Burokas et al., [2017](#); De Vadder et al., [2014](#); Hsiao et al., [2013](#); Kelly et al., [2017](#); Luczynski et al., [2016](#); Thaïss et al., [2014](#); Yassin et al., [2025](#)). Together, these findings highlight the critical role of the gut microbiota in shaping neurophysiological

function across multiple dimensions—from synaptic transmission and neurotrophic support to glial regulation and neurotransmitter signaling (Fig. [18.2](#)).

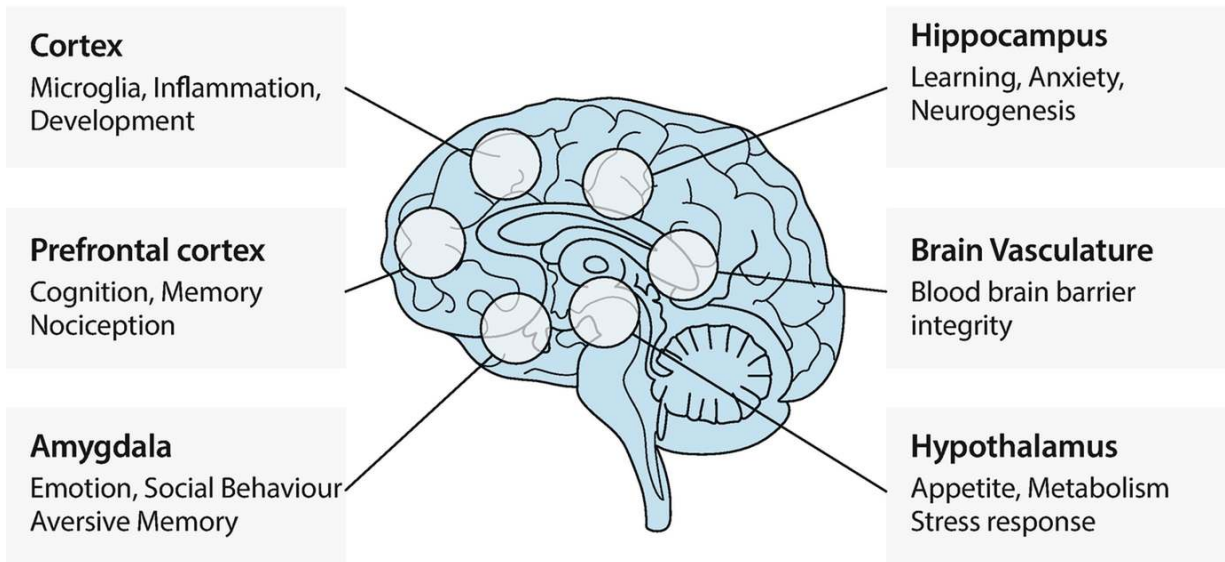


Fig. 18.2 Potential impact of the gut microbiota on several neuronal and neurobehavioral functions in distinct brain centers

18.2.3 Gut Microbiota and Brain Disorders

Having established the key roles of the gut microbiome in regulating brain structure and function, it is now evident that disruptions to this complex microbial ecosystem can contribute to the onset and progression of various neurological and psychiatric conditions (Fig. [18.3](#)). Altered microbiota composition, metabolic output, and signaling capacity have been linked to a wide range of brain disorders, from neurodevelopmental and neuropsychiatric syndromes to chronic neurodegenerative diseases. In the next sections, we examine current evidence for microbiota-related alterations in disorders such as autism spectrum disorder, attention-deficit/hyperactivity disorder, depression, anxiety, schizophrenia, Parkinson's disease, and Alzheimer's disease. By exploring both clinical findings and mechanistic insights from animal models, this chapter highlights how microbial dysbiosis may shape brain pathophysiology across the lifespan.

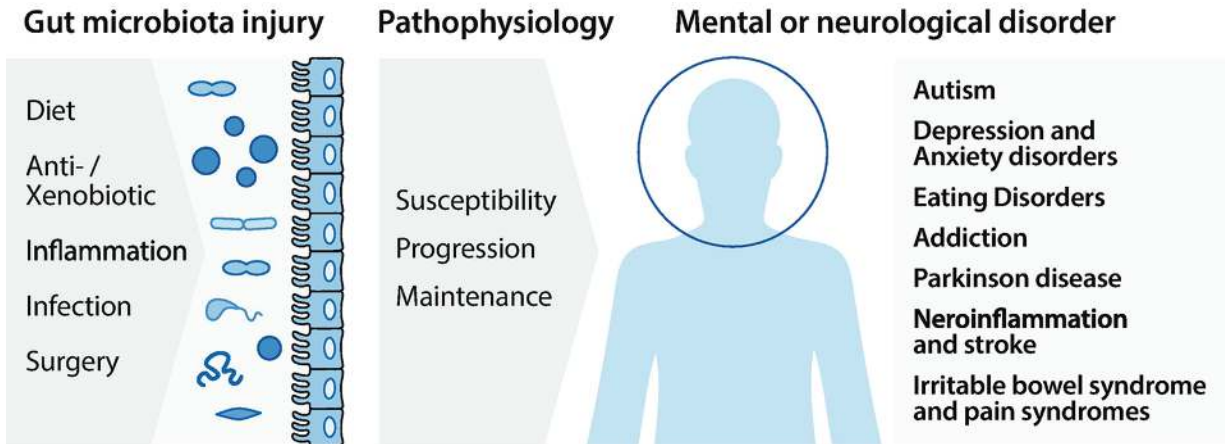


Fig. 18.3 Concept of gut microbiota disruptions influencing brain disorders

18.2.3.1 Neurological Disorders

Neurological disorders such as Alzheimer’s disease (AD), Parkinson’s disease (PD), multiple sclerosis (MS), amyotrophic lateral sclerosis (ALS), and ischemic stroke (IS) are among the leading causes of disability and mortality worldwide. These conditions involve structural, biochemical, or electrical abnormalities in the brain, spinal cord, or peripheral nerves and typically progress over time. Despite their diverse clinical manifestations—ranging from neurodegeneration and demyelination to cerebrovascular damage—growing evidence points to the gut microbiome as a shared modulator of disease risk and progression. This subchapter explores the role of the gut–brain axis in these disorders and highlights microbiome-targeted therapies as emerging avenues for intervention.

Alzheimer’s Disease (AD)

Alzheimer disease (AD) is the most common form of dementia, accounting for 60–70% of cases globally (WHO, [2025](#)). In 2020, over 55 million people were living with dementia, a number projected to exceed 150 million by 2050, with the highest growth expected in low- and middle-income countries (GBD, [2022](#)). Pathologically, AD is defined by the accumulation of extracellular amyloid- β (A β) plaques and intracellular neurofibrillary tangles composed of hyperphosphorylated tau protein (Long & Holtzman, [2019](#)). These hallmark features are

accompanied by neuroinflammation, synaptic dysfunction, and widespread metabolic alterations (Seo & Holtzman, [2024](#)). Despite decades of research, the causal mechanisms remain elusive, complicating the development of effective treatments. While most AD cases are sporadic and occur later in life, there is a clear genetic component to the disease. A small proportion of cases follow a familial inheritance pattern with early onset, and rare de novo mutations have also been reported (König & Stögmänn, [2021](#); Lanoiselée et al., [2017](#); Rocha et al., [2018](#)). Beyond genetic predisposition, age is the strongest risk factor, with incidence doubling every 5 years after age 65, as stated by the Alzheimer's Society ([2025](#)). Modifiable risk factors—including hypertension, diabetes, obesity, smoking, alcohol use, physical inactivity, depression, low education, and social isolation—contribute to approximately 40% of dementia cases, underscoring the importance of prevention and early intervention (ADI, [2025](#)).

Several studies have demonstrated that the gut microbiome composition in AD patients differs markedly from that of healthy individuals (Chaudhari et al., [2023](#); Guo et al., [2021](#); Haran et al., [2019](#); Vogt et al., [2017](#)). Notably, transferring a dysbiotic microbiome—either from humans or animal AD models—into mice can induce AD-like pathology in recipients, supporting a causal role for the microbiome in disease progression (Grabrucker et al., [2023](#); Mishra et al., [2024](#); Soriano et al., [2022](#)). Although the exact mechanisms remain unclear, current evidence suggests that microbiome dysbiosis increases gut permeability, enabling proinflammatory molecules to enter the bloodstream and trigger systemic inflammation (Mishra et al., [2024](#); Prajapati et al., [2023](#)). Initial studies showed that individuals with AD had a higher abundance of proinflammatory bacteria, such as *Escherichia/Shigella* and a lower abundance of anti-inflammatory taxa like *Eubacterium rectale*, compared to healthy controls (Cattaneo et al., [2017](#)). In parallel, fecal microbiome profiling via 16S rRNA sequencing revealed significantly reduced microbial diversity in AD patients and a compositional shift at the phylum level—specifically, decreased *Firmicutes* and increased *Bacteroidetes* (Vogt et al., [2017](#)). Subsequent

research extended these findings to individuals with mild cognitive impairment (MCI) and even preclinical AD, suggesting that microbiota alterations may precede clinical symptoms (Ferreiro et al., [2023](#); Jemimah et al., [2023](#)). However, the microbiome profiles associated with AD are not entirely coherent. For instance, while *Bacteroidetes* levels were increased in a predominantly Caucasian cohort (Vogt et al., [2017](#)), they were found to be decreased in a Chinese population (Zhuang et al., [2018](#)), indicating that geographical, dietary, and methodological factors may influence findings.

Growing evidence suggests that targeting the gut microbiota may offer a promising strategy to slow the progression of AD, given its role in modulating A β accumulation, tau pathology, neuroinflammation, and metabolic homeostasis (Seo et al., [2019](#); Seo & Holtzman, [2024](#)). Several interventions are under investigation, including probiotics, prebiotics, and fecal microbiota transplantation (FMT). Probiotics, prebiotics, and postbiotics are under investigation as less invasive tools for gut microbiome modulation. Probiotics such as *Bifidobacterium breve* have shown preliminary benefits in both animal models and early-stage human trials, including improved cognition and reduced amyloid deposition (Akhgarjand et al., [2022](#); Bicknell et al., [2023](#); Bonfili et al., [2017](#); He et al., [2022](#); Zhu et al., [2023](#)). Prebiotics also show promise, although findings are mixed. While generally beneficial, high dietary fiber intake may lead to excessive production of SCFAs, which in some animal models has been linked to increased neuroinflammation and AD pathology (Abdel-Haq et al., [2022](#); Cuervo-Zanatta et al., [2023](#); Killingsworth et al., [2020](#); Seo et al., [2023](#)). Postbiotics, such as SCFAs, are microbial metabolites with immunomodulatory and epigenetic effects, but their role in AD remains unclear. FMT has demonstrated more translational potential. In AD model mice, FMT from healthy donors reduced A β plaques, tau pathology, glial reactivity, and cognitive deficits (Kim et al., [2020](#)). In humans, case reports and small clinical studies have shown that FMT may improve cognitive symptoms, including a notable case where an

AD patient improved significantly after a single FMT administered for *Clostridioides difficile* infection (Hazan, [2020](#); Park et al., [2021](#), [2022](#)).

Parkinson's Disease (PD)

Parkinson's disease (PD) is a common, age-related neurodegenerative disorder affecting 1–2% of individuals over 65 years old, with its prevalence expected to increase as populations age (Ascherio & Schwarzschild, [2016](#); Ben-Shlomo et al., [2024](#)). It is clinically defined by motor symptoms—such as tremor, rigidity, bradykinesia, and postural instability—that emerge following significant degeneration of dopaminergic neurons in the substantia nigra pars compacta (Bloem et al., [2021](#); Gabrielli et al., [2024](#)). Beyond its motor manifestations, PD often involves widespread autonomic dysfunction, with GI symptoms, particularly constipation, frequently preceding motor onset by several years (Fasano et al., [2015](#)). The exact etiology of PD remains unresolved, but a growing body of evidence implicates α -synuclein, a neuronal protein involved in synaptic function, whose misfolding leads to the formation of Lewy bodies in both the CNS and ENS (Bloem et al., [2021](#); Fasano et al., [2015](#)). Microglial activation also contributes to PD pathogenesis; misfolded α -synuclein can overstimulate these innate immune cells, exacerbating neuroinflammation and neuronal injury (Forloni, [2023](#); Ho, [2019](#)). Increasing attention has turned to the gut–brain axis as a central component in PD, with several studies linking gut microbiota dysbiosis to enteric inflammation and neuropathy, potentially driving disease progression through inflammatory and neuroimmune mechanisms (de Vos et al., [2022](#); Gao et al., [2025](#); Perez-Pardo et al., [2018](#); Wallen et al., [2022](#); Wang et al., [2023a](#)). Notably, α -synuclein aggregates have been detected in the submucosal and myenteric plexuses of the gut prior to their appearance in the brain (Fasano et al., [2015](#)), leading Braak et al. to propose that PD may originate in the GI tract, potentially triggered by an external agent, and then spread to the brain via the vagus nerve (Braak et al., [2003](#); Hawkes et al., [2010](#)). Experimental work by Holmqvist et al. supported this model, showing that α -synuclein injected into the intestinal wall of

healthy rats traveled along the vagus nerve to the brain, mimicking a prion-like propagation (Holmqvist et al., [2014](#)).

Recent studies have consistently shown that the gut microbiome is altered in PD, with specific microbial patterns emerging across diverse cohorts. Bai et al. ([2024](#)) reported that PD patients exhibit reduced abundance of butyrate-producing taxa such as *Lachnospiraceae*, *Prevotellaceae*, and *Faecalibacterium*, alongside increases in families like *Bifidobacteriaceae*, *Lactobacillaceae*, and *Verrucomicrobiaceae* (Bai et al., [2024](#)). These shifts may disrupt SCFA production, compromise epithelial integrity, and promote gut inflammation—a scenario that may facilitate α -synuclein aggregation and neuroinflammation (Bai et al., [2024](#); Hays et al., [2024](#); Wang et al., [2024a](#)). *Bifidobacteriaceae* abundance has also been linked to higher L-DOPA metabolism, potentially influencing pharmacotherapy efficacy (Cirstea et al., [2023](#)). Notably, *Akkermansia muciniphila*, commonly elevated in PD (Gobert et al., [2016](#)), may exert dual effects: while generally associated with intestinal health, some strains have been implicated in promoting mitochondrial stress and α -synuclein aggregation under specific host conditions (Amorim Neto et al., [2022](#); Panzetta & Valdivia, [2024](#)). A metagenomic analysis by Metcalfe-Roach et al. ([2024](#)) revealed large-scale microbial disruptions in PD, with increased *Alistipes indistinctus*, *Blautia obeum*, and *Coprococcus catus*, and decreased *Blautia wexlerae* and *Roseburia intestinalis* (Metcalfe-Roach et al., [2024](#)). Functional changes in PD also included altered glutamate and purine metabolism, which may influence both brain health and disease progression (Metcalfe-Roach et al., [2024](#); Scholefield et al., [2023](#)). In a meta-analysis across five countries, Nishiwaki et al. ([2024](#)) found consistently elevated *Akkermansia muciniphila* and reduced *Roseburia intestinalis* and *Faecalibacterium prausnitzii* in PD, accompanied by reduced microbial biosynthesis of riboflavin and biotin—two vitamins involved in oxidative stress control and anti-inflammatory pathways (Kuroishi, [2015](#); Marashly & Bohlega, [2017](#)).

Various strategies aimed at restoring a balanced gut microbiome, such as probiotics, prebiotics, synbiotics, and FMT, have been explored

in studies involving patients with PD. Numerous studies have evaluated various probiotic formulations, mainly based on *Lactobacillus* and *Bifidobacterium* species, with treatment durations ranging up to 12 weeks. Despite differences in CFU dosage, sample size, and outcome measures, all studies reported significant improvements in either motor or non-motor symptoms (Cassani et al., [2011](#); Du et al., [2022](#); Georgescu et al., [2016](#); Ghalandari et al., [2023](#); Lu et al., [2021](#); Sun et al., [2022](#); Tamtaji et al., [2019](#); Tan et al., [2021](#)). Among these clinical trials, it was observed that reductions in certain taxa, such as *Eubacterium oxidoreducens*, *Eubacterium hallii*, *Odoribacter*, *Lactobacillus fermentum*, and *Klebsiella oxytoca*, while other taxa, including *Christensenella Marseille-P2437*, *Bifidobacterium animalis*, *Ruminococcaceae*, *Lachnospira*, *Faecalibacterium prausnitzii*, and *Oscillospiraceae*, increased following treatment (Du et al., [2022](#); Sun et al., [2022](#)). Some of these microbial changes were accompanied by functional enhancements, such as upregulation of species involved in SCFA, GABA, tryptophan, and secondary bile acid metabolism (Sun et al., [2022](#)). Additionally, probiotic use was linked to elevated serum acetic acid and dopamine levels, with a concurrent shift in L-tyrosine distribution, marked by decreased fecal and increased plasma concentrations, highlighting potential systemic effects relevant to dopaminergic signaling in PD (Sun et al., [2022](#)). Synbiotic interventions combining probiotics with prebiotics such as fructo-oligosaccharides (FOS) or inulin have shown beneficial effects in Parkinson's disease, particularly in alleviating gastrointestinal symptoms, though they have not consistently improved motor performance (Andreozzi et al., [2024](#); Ibrahim et al., [2020](#)). Placebo-controlled randomized clinical trials have also explored the FMT effects in individuals with mild-to-moderate PD. In a study by DuPont et al., repeated oral doses of lyophilized FMT led to temporary improvements in motor symptoms and gastrointestinal parameters, including constipation and gut motility (DuPont et al., [2023](#)). Similarly, the GUT-PARFECT trial evaluated a single nasojejunal FMT and observed notable improvements in constipation after 3–6 months and motor symptoms between 6 and 12 months, suggesting

that gastrointestinal improvements may precede neurological benefits (Bruggeman et al., [2024](#)). In contrast, a Finnish trial by Scheperjans et al. found no clinically meaningful improvements between the FMT and placebo groups (Scheperjans et al., [2024](#)).

Multiple Sclerosis (MS)

Multiple sclerosis (MS) is a chronic autoimmune disorder of the central nervous system affecting approximately 2.8 million people worldwide, with a higher incidence in young adults and females (Mohsen et al., [2025](#); Schepici et al., [2019](#); Walton et al., [2020](#)). The development of MS is shaped by a complex interplay between genetic susceptibility and environmental influence (Huang et al., [2017](#); Murayama et al., [2025](#); Nakhal et al., [2024](#); Shah et al., [2023](#)) and is characterized by the formation of inflammatory plaques—focal demyelinated areas in the brain and spinal cord—resulting from immune cell infiltration across a dysregulated blood–brain barrier, leading to myelin loss, oligodendrocyte damage, and progressive neuronal degeneration (Frohman et al., [2006](#); Rajachandrakumar & Finlayson, [2022](#)). Inflammatory processes are central to MS pathophysiology, with T helper cells driving the release of pro-inflammatory cytokines such as tumor necrosis factor alpha (TNF- α), lymphotoxin, and TGF- β , and CD8⁺ T cells contributing to direct neuronal damage (Ghasemi et al., [2017](#); Shah et al., [2023](#)).

Preclinical studies have shown that the gut microbiota plays an active role in CNS autoimmunity (Correale et al., [2022](#)). Initial clues came from transgenic mice expressing a myelin-specific T cell receptor, which developed spontaneous experimental autoimmune encephalomyelitis (EAE)—a model of multiple sclerosis—only when housed in non-sterile environments, suggesting microbial triggers were involved in disease onset (Goverman et al., [1993](#)). Further evidence emerged when antibiotic treatment, which reduces gut microbial populations, led to a decrease in mesenteric TH17 cells and subsequently reduced EAE severity, a process shown to depend on invariant natural killer T cells, highlighting the role of innate immunity

in this interaction (Yokote et al., [2008](#)). Additional experiments confirmed that oral antibiotics conferred protection against EAE by dampening pro-inflammatory responses or enhancing regulatory mechanisms (Ochoa-Repáraz et al., [2009](#)). One notable example involved *Bacteroides fragilis*, a commensal bacterium found to protect against EAE through its polysaccharide A, which activates regulatory T cells via TLR2, thereby promoting anti-inflammatory pathways and suppressing disease (Round et al., [2011](#); Wang & Kasper, [2014](#)).

Several studies have demonstrated consistent shifts in the gut microbiota composition of MS patients, especially those with relapsing-remitting multiple sclerosis (RRMS), even across diverse cohorts and treatment regimens. Increased relative abundance of taxa such as *Akkermansia*, *Faecalibacterium*, and *Coprococcus* has been observed in both treated and untreated patients (Cantarel et al., [2015](#)). Similarly, enriched levels of *Eggerthella lenta* and *Streptococcus thermophilus/salivarius*, alongside a depletion of anti-inflammatory genera including *Clostridium* spp., *Faecalibacterium prausnitzii*, and *Anaerostipes hadrus*, have been identified in RRMS patients, many of whom were receiving interferon-beta (IFN- β) or prednisolone (Miyake et al., [2015](#)). Additional shifts, such as increased abundance of *Pseudomonas*, *Pedobacter*, *Mycoplana*, and *Blautia*, were noted regardless of treatment status (Chen et al., [2016](#)). Other taxa frequently elevated in untreated patients include *Methanobrevibacter*, *Butyricimonas*, *Paraprevotella*, *Haemophilus*, and *Slackia* (Jangi et al., [2016](#); Nemati et al., [2025](#)). In mixed treatment groups, elevated levels of *Bilophila*, *Bifidobacterium*, *Desulfovibrio*, and *Christensenellaceae* were also reported (Tremlett et al., [2016](#)). Notably, *Akkermansia muciniphila* and *Acinetobacter calcoaceticus* have been recurrently associated with MS, suggesting their involvement across treatment conditions (Berer et al., [2017](#); Cekanaviciute et al., [2017](#)). Conversely, a significant reduction in *Faecalibacterium* was identified in an untreated Asian cohort (Ling et al., [2020](#)). These findings suggest that despite variability in patient demographics and therapeutic exposure—including immunomodulators like glatiramer acetate, interferon-beta,

and natalizumab—microbiome disturbances in MS follow recognizable patterns, underscoring their potential relevance to disease activity and immune regulation.

Building on these insights, several microbiome-directed strategies have recently been proposed as adjunctive therapies for MS, aiming to restore gut immune homeostasis and mitigate neuroinflammation. Among them, dietary interventions—particularly ketogenic (Brenton et al., [2022](#); Storoni & Plant, [2015](#)), anti-inflammatory (Moravejolahkami et al., [2023](#); Mousavi-Shirazi-Fard et al., [2021](#)), and modified paleo diets (Barone et al., [2019](#); Saxby et al., [2024](#))—have shown promise in improving clinical outcomes such as fatigue and quality of life, possibly through reshaping the gut microbial landscape and reducing systemic inflammation (Correale et al., [2022](#)). Probiotic supplementation has demonstrated immunomodulatory effects in preclinical models (Khailova et al., [2014](#); Klingensmith & Coopersmith, [2016](#); Krezalek et al., [2016](#)), notably enhancing regulatory T cell responses and decreasing pro-inflammatory cytokines; however, clinical evidence in MS patients remains limited and heterogeneous (Asghari et al., [2023](#); Correale et al., [2022](#); Straus Farber et al., [2024](#)). While FMT and engineered bacterial consortia are being explored, these approaches remain largely experimental and require further validation in controlled clinical trials (Correale et al., [2022](#)).

Amyotrophic Lateral Sclerosis (ALS)

Amyotrophic lateral sclerosis (ALS) is a progressive and fatal neurodegenerative disorder characterized by the degeneration of both upper and lower motor neurons, leading to muscle weakness, atrophy, paralysis, and ultimately respiratory failure—the primary cause of death (Feldman et al., [2022](#); Hardiman et al., [2017](#)). Most patients survive only 2–4 years after symptom onset, though about 10% may live longer than a decade (Kaul et al., [2024](#); Longinetti & Fang, [2019](#); Mejzini et al., [2019](#)). The disease shows significant clinical heterogeneity in both phenotype and progression, complicating diagnosis and treatment (Feldman et al., [2022](#); Marriott et al., [2023](#)).

ALS is typically classified into sporadic (90–95% of cases) and familial forms, the latter often associated with mutations in genes such as *C9orf72*, *SOD1*, *FUS*, and *TARDBP*, which disrupt RNA processing, mitochondrial function, and protein homeostasis (Alsultan et al., [2016](#); Goutman et al., [2022](#); Kaul et al., [2024](#)). Although genetic mutations account for a small subset of cases, environmental factors are believed to play a major role in ALS pathogenesis. Epidemiological studies have linked exposures such as smoking, pesticides, heavy metals, and physical exertion to increased ALS risk, and mounting evidence supports gene–environment interactions in shaping disease onset and progression (Gunnarsson & Bodin, [2018](#); Mead et al., [2023](#); Spencer et al., [2019](#); Vasta et al., [2022](#)). Beyond motor neuron loss, ALS also involves systemic disturbances, including hypermetabolism, lipid dysregulation, and immune activation, which contribute to inflammation and energy imbalance (Maksimovic et al., [2023](#); Rusina et al., [2021](#)).

The gut microbiota has recently gained attention as a potential contributor to ALS pathophysiology and clinical heterogeneity. Multiple studies have identified consistent alterations in microbial composition and metabolic function among ALS patients, with variations observed across phenotypes and disease subtypes (Boddy et al., [2021](#); Brenner et al., [2018](#); Goyal et al., [2020](#); Mazzini et al., [2018](#); Wright et al., [2018](#)). A common pattern includes reduced diversity and lower abundance of beneficial taxa such as *Firmicutes*, *Bacteroidetes*, *Oscillibacter*, *Anaerostipes*, *Lachnospiraceae*, *Megamonas*, *Roseburia intestinalis*, and *Eubacterium rectale*—all known producers of SCFAs, which play crucial roles in maintaining immune balance, mucosal integrity, and neuroprotection (Fang et al., [2016](#); Fontdevila et al., [2024](#); Gong et al., [2022](#); Kim et al., [2022](#); Liu et al., [2024](#); Rowin et al., [2017](#); Zeng et al., [2020](#)). Conversely, higher levels of potentially pro-inflammatory or opportunistic genera such as *Methanobrevibacter*, *Cyanobacteria*, *Escherichia*, and *Streptococcus* have been observed (Di Gioia et al., [2020](#); Nicholson et al., [2021](#); Zhai et al., [2019](#)). Although some studies also report increased abundance of taxa like *Lactobacillus*, *Coprococcus*,

Ruminococcaceae, and *Actinobacteria*, the functional implications of these shifts remain unclear (Liu et al., [2024](#); Zeng et al., [2020](#)). Notably, oral and gut dysbiosis appears more pronounced in patients with bulbar or cognitive symptoms compared to spinal-onset ALS, suggesting microbial signatures may correlate with disease severity (Gong et al., [2022](#); Kim et al., [2022](#)). Clinical research on microbiota-targeted interventions remains limited in ALS, with most available data coming from preclinical studies or small-scale human trials (Chen et al., [2023a](#); Cuffaro et al., [2024](#); Di Gioia et al., [2020](#); Lu et al., [2022](#); Nabakhteh et al., [2025](#); Niccolai et al., [2024](#); Yan et al., [2024](#)).

Ischemic Stroke (IS)

Ischemic stroke (IS) is a major cerebrovascular disorder caused by the sudden disruption of blood flow to the brain, leading to neurological damage. It primarily affects older adults and often results in long-term complications such as motor deficits, cognitive impairment, and depression, contributing to significant societal costs (Chen et al., [2025](#)). Without urgent intervention, the global burden of stroke—including mortality, disability, and healthcare expenses—is projected to increase by 50% by 2050 (Feigin & Owolabi, [2023](#)).

Although many studies have investigated the link between IS and the gut microbiota, detailed analyses focusing on specific microbial taxa remain limited. While it is true that poststroke microbiota alterations may partly reflect secondary factors such as reduced food intake, mobility, or medication, growing evidence indicates that stroke itself directly contributes to gut dysbiosis through neural and immunological mechanisms. Experimental models have shown that large stroke lesions can impair gastrointestinal motility and induce intestinal paralysis, leading to a dysbiotic state marked by reduced microbial diversity and overgrowth of *Bacteroidetes* (Singh et al., [2016](#)). These post-stroke microbial shifts, in turn, can worsen stroke outcomes by promoting systemic inflammation and impairing neurorecovery, suggesting a bidirectional relationship between the brain and gut after stroke (Haak et al., [2021](#)). Although studies report a reduction in

microbial diversity in both patients and animal models (Haak et al., [2021](#); Singh et al., [2016](#)), a few studies have documented unchanged or even increased diversity, likely reflecting differences in host species, stroke severity, sampling time points, and methodological approaches (Luo et al., [2024](#); Yin et al., [2015](#)). A study by Huang et al. ([2021](#)) reported that caloric restriction led to an increased abundance of *Bifidobacterium* in the gut of rats with cerebral ischemia. Moreover, oral administration of *Bifidobacterium* in stroke models significantly improved locomotor performance and reduced anxiety-like behaviors (Huang et al., [2021](#)). *Lactobacillus reuteri* has been reported to reduce neurotoxic neurotransmitters and increase neuroprotective ones, suggesting a potential role in modulating stroke-induced neurochemical imbalance (Wang et al., [2023b](#)). The SCFA-producing genus *Prevotella* is frequently depleted post-stroke, and its restoration via interventions such as FMT or melatonin has been associated with improved neurological recovery (Yin et al., [2015](#)). Findings regarding *Akkermansia muciniphila* remain inconsistent—some studies report a reduction in its abundance following stroke, while others observe an increase, potentially reflecting a compensatory mechanism to support butyrate production (Ji et al., [2017](#); Li et al., [2024a](#); Stanley et al., [2018](#); Wang et al., [2023b](#); Yao et al., [2023](#)). Conversely, a consistent increase in *Escherichia coli* and other members of the *Enterobacteriaceae* family has been documented in the post-stroke gut microbiome, raising concerns about intestinal barrier disruption and systemic inflammation (Xu et al., [2021](#); Yin et al., [2015](#)).

Probiotics and FMT are emerging as promising adjuncts in IS (Macom & Brown, [2025](#)). In a mouse model, probiotic treatment reduced infarct volume and lowered oxidative stress and TNF- α levels, although it did not improve neurological function (Akhoundzadeh et al., [2018](#)). Clinically, a meta-analysis showed that adding probiotics to enteral nutrition (EN) shortened hospital stays and reduced inflammatory cytokines (TNF- α , IL-6, IL-10), but had no effect on NIH Stroke Scale scores (NIHSS) or C-reactive protein (CRP) levels (Zhong et al., [2021](#)). Probiotic supplementation with EN also lowered infection

and dysbiosis rates in stroke patients (Chen et al., [2022](#)). FMT has similarly shown potential. Caloric restriction in donor mice increased *Bifidobacterium* and improved stroke outcomes, benefits that were transferable via FMT (Huang et al., [2021](#)). Transplants from young to aged mice enhanced gut microbiome composition and SCFA profiles (Lee et al., [2020](#)), while SCFA-enriched FMT, particularly with butyrate, also improved outcomes (Chen et al., [2019a](#)).

18.2.3.2 Psychiatric Disorders

Psychiatric disorders such as major depressive disorder (MDD), generalized anxiety disorder (GAD), bipolar disorder (BD), and schizophrenia (SZ) are complex conditions with significant personal and societal impact. Beyond well-established neurochemical and genetic factors, growing evidence highlights the gut microbiome as a potential contributor to their pathophysiology. Alterations in microbial composition and reduced diversity have been observed across these disorders, suggesting shared patterns of dysbiosis. Through the microbiota–gut–brain axis, gut microbes may influence mood, cognition, and stress responses via immune, metabolic, and neural pathways. This section explores microbiome changes in psychiatric disorders and examines emerging microbiota-based therapies.

Major Depressive Disorder (MDD)

Depression is the most prevalent psychiatric disorder globally, significantly impairing daily functioning and quality of life. According to the 2023 WHO World Report, it affects approximately 5% of the adult population worldwide (WHO, [2023](#)) and ranks among the leading contributors to global disease burden (Santomauro et al., [2021](#)). Despite advances in our understanding of its pathophysiology—including mechanisms involving monoamine imbalance, BDNF signaling, and neuroinflammation (Beurel et al., [2020](#); Colucci-D'Amato et al., [2020](#); Popova & Naumenko, [2019](#))—standard antidepressant treatments remain ineffective for up to 30% of patients with MDD (Berlim & Turecki, [2007](#)).

An expanding body of research implicates the gut–brain axis as a key player in the pathogenesis of depression. Many individuals with depression or anxiety report gastrointestinal symptoms, and the microbiota–gut–brain axis has been proposed as a framework through which gut microbes influence central nervous system function (Cao et al., [2025](#); Clerici et al., [2025](#); Khaledi et al., [2024](#); Nikdasti et al., [2025](#)). These effects are thought to occur via neuroimmune, neuroendocrine, and metabolic pathways, potentially affecting mood and cognition (Liu et al., [2023](#); Simpson et al., [2021](#)). Several studies have demonstrated that individuals diagnosed with MDD exhibit distinct alterations in gut microbiota composition at the genus level, suggesting a possible association between microbial dysbiosis and depressive symptoms (Jiang et al., [2015](#); Naseribafrouei et al., [2014](#); Zhang et al., [2021](#); Zheng et al., [2021](#); Zhong et al., [2022](#)). However, most of these studies are cross-sectional, meaning they capture microbiota profiles after the onset of MDD, and thus do not clarify whether such changes precede disease manifestation or result from it. Some preclinical studies using FMT from MDD patients into germ-free mice have shown depression-like behaviors in the recipients, pointing to a potential causal influence of gut microbiota (Kelly et al., [2016](#)). A consistent pattern across investigations reveals an increased relative abundance of potentially pro-inflammatory genera such as *Alistipes*, *Parabacteroides*, *Flavonifractor*, *Oscillibacter*, *Streptococcus*, *Eggerthella*, and *Atopobium* (Caso et al., [2021](#); Chen et al., [2018](#), [2021b](#); Chung et al., [2019](#); Jiang et al., [2015](#); Kelly et al., [2016](#); Lai et al., [2021](#); Naseribafrouei et al., [2014](#); Rong et al., [2019](#); Zhang et al., [2021](#); Zheng et al., [2021](#); Zhong et al., [2022](#)). Additionally, while some taxa like *Bifidobacterium* and *Olsenella*—generally considered beneficial—also appear enriched in certain cohorts (Chen et al., [2018](#); Chung et al., [2019](#); Dong et al., [2022](#); Lai et al., [2021](#); Rong et al., [2019](#)), their role in MDD remains to be clarified. In contrast, a depletion of SCFA-producing genera, including *Faecalibacterium*, *Prevotella*, *Dorea*, *Dialister*, *Sutterella*, *Anaerostipes*, and *Eubacterium*, has been frequently observed, while some genera, such as *Bacteroides*, *Blautia*, and *Roseburia*, have shown variable trends

across studies (Caso et al., [2021](#); Chen et al., [2021b](#); Dong et al., [2022](#); Huang et al., [2021](#); Kelly et al., [2016](#); Yang et al., [2020](#); Zhang et al., [2021](#)). These shifts may reflect impaired gut barrier integrity, immune dysregulation, or reduced neuroactive metabolite production in MDD patients.

Probiotic supplementation has been increasingly recognized for its potential role in alleviating depressive symptoms, particularly in patients with MDD (Elahinejad et al., [2025](#); Nikolova et al., [2023](#), [2025](#); Schaub et al., [2022](#)). In preclinical studies, several strains have been shown to influence cognitive and emotional processes by modulating the levels of key neurotransmitters such as GABA and serotonin (Bravo et al., [2011](#); Desbonnet et al., [2008](#)). Clinical studies suggest that probiotics may serve as a valuable adjunctive treatment, reducing the severity and prevalence of depressive symptoms in MDD patients. In a randomized, placebo-controlled study, patients with major depression who received a multi-species probiotic for 3 months exhibited significantly improved vagus nerve function, increased abundance of *Akkermansia muciniphila*, and better sleep quality compared to controls (Mörkl et al., [2025](#)). In another study, patients with MDD underwent an 8-week multi-strain probiotic intervention, which led to increased gut microbial richness and preservation of microbial diversity compared to placebo, with specific post-treatment enrichment of *Bacillaceae* correlating with reduced anxiety symptoms (Nikolova et al., [2023](#)). Clinical trials evaluating prebiotics in MDD have shown limited and inconclusive results. While some studies suggest mild benefits on metabolic outcomes or self-efficacy, prebiotic supplementation—such as inulin—has not consistently improved depressive symptoms compared to placebo (Kazemi et al., [2019](#); Tarutani et al., [2022](#); Vaghef-Mehrabani et al., [2023](#); Vaghef-Mehrabany et al., [2021](#)). These findings indicate that prebiotics alone may have minimal impact on mood in MDD, and their therapeutic value remains uncertain. Case reports and case series have indicated that FMT may serve as a promising adjunctive therapy for depression (Doll et al., [2022](#); Green et al., [2023](#)).

Generalized Anxiety Disorder (GAD)

Anxiety disorders comprise a diverse group of conditions that frequently co-occur with other psychiatric illnesses (Craske et al., [2017](#)). The most commonly diagnosed subtype of anxiety among adults in clinical practice is generalized anxiety disorder (GAD). GAD is a chronic psychiatric condition marked by excessive and uncontrollable worry that significantly impairs daily functioning. Common symptoms include fatigue, tension, poor concentration, restlessness, and sleep disturbances (APA, [2022](#)). GAD is also linked to increased risks of substance use disorders, cardiovascular disease, and suicide (Liu et al., [2019a](#); Nepon et al., [2010](#); Tyrer & Baldwin, [2006](#)). It is associated with social and economic burdens, including lower income and higher rates of singleness and divorce (Fowler, [2023](#)). Despite its prevalence, it remains underdiagnosed and under-treated, with many patients showing resistance to conventional therapies such as selective serotonin reuptake inhibitors (SSRIs) and cognitive-behavioral therapy (Penninx et al., [2021](#)).

Several studies have explored gut microbiome alterations in GAD, revealing reduced microbial richness (Chen et al., [2019b](#); Dong et al., [2021](#); Jiang et al., [2018](#)). While alpha diversity (e.g., Shannon and Simpson indices) appears unaffected, specific bacterial taxa are consistently altered. Notably, *Faecalibacterium* and *Coprococcus*—both butyrate producers linked to anti-inflammatory effects and mental health—are depleted in GAD patients (Brushett et al., [2023](#); Dong et al., [2021](#); Jiang et al., [2018](#)). One study found *Faecalibacterium* levels to be higher in remitted GAD patients compared to those in an active phase and negatively correlated with cortisol levels (Jiang et al., [2015](#)). Similarly, *Coprococcus eutactus* was reduced in GAD individuals, even after adjusting for psychotropic medication use (Brushett et al., [2023](#)). Conversely, certain taxa appear enriched in GAD, such as *Ruminococcus gnavus*, *Fusobacterium*, *Escherichia-Shigella*, and *Bacteroides* (Chen et al., [2019b](#); Jiang et al., [2018](#)). In some studies, these genera were positively correlated with symptom severity, although such findings were not replicated in larger cohorts, possibly due to methodological

differences or medication effects (Brushett et al., [2023](#); Jiang et al., [2018](#)). Interestingly, many of these microbial patterns (e.g., decreased *Faecalibacterium* and *Coprococcus*, increased *Eggerthella*) are not exclusive to GAD but are observed across various psychiatric disorders, including depression, bipolar disorder, psychosis, and schizophrenia—suggesting a transdiagnostic microbiota signature rather than disorder-specific changes (Nikolova et al., [2021](#)).

Probiotics and prebiotics have emerged as promising interventions for reducing anxiety symptoms, though most clinical trials to date have not specifically targeted individuals with a formal GAD diagnosis (Chao et al., [2020](#); Halemani et al., [2023](#); Liu et al., [2019b](#); Ribera et al., [2024](#); Zhao et al., [2023](#)). A large meta-analysis of 29 randomized controlled trials ($n = 2035$) found that probiotics and synbiotics significantly reduced anxiety symptoms, while prebiotics showed no such effect (Zhao et al., [2023](#)). Another meta-analysis focusing on healthy individuals under stress or with subclinical symptoms reported that probiotics improved subjective stress and subthreshold anxiety, although cortisol levels remained unchanged (Zhang et al., [2020](#)). One randomized controlled trial has directly examined the effects of probiotics in patients with GAD (Eskandarzadeh et al., [2021](#)). In this study, 48 medication-free patients were randomized to receive either a placebo or a multispecies probiotic (18×10^9 CFU of *Bifidobacterium longum*, *B. bifidum*, *B. lactis*, and *Lactobacillus acidophilus*) alongside 25 mg of sertraline for 8 weeks. The probiotic group showed significantly greater reductions in anxiety symptoms on the Hamilton Anxiety Rating Scale, although no differences were observed on the Beck Anxiety Inventory or State-Trait Anxiety Inventory.

Bipolar Disorder (BD)

Bipolar disorder (BD) is a severe and disabling psychiatric condition marked by recurrent episodes of mania, hypomania, and depression, typically beginning in adolescence or early adulthood (Bolton et al., [2021](#); Jain & Mitra, [2025](#)). Although its etiology remains unclear, BD is considered to arise from a complex interplay between genetic

vulnerability, environmental exposures, and epigenetic regulation (Aldinger & Schulze, [2017](#); Harrison et al., [2016](#); Harrison et al., [2018](#); Legrand et al., [2021](#); Mahgoub & Monteggia, [2013](#); Stahl et al., [2019](#)). Genetic studies suggest polygenic contributions with shared risk loci overlapping other psychiatric conditions, particularly schizophrenia (Aldinger & Schulze, [2017](#)). Neurobiologically, BD is associated with dysregulation in multiple neurotransmitter systems—especially dopamine, serotonin, norepinephrine, glutamate, and GABA—which fluctuate according to disease phase (Brady Jr. et al., [2013](#); Nikolaus et al., [2017](#); van Enkhuizen et al., [2015](#); Yüksel & Öngür, [2010](#)). These imbalances are linked to structural and functional changes in neural circuits involved in emotion and reward processing (Phillips & Swartz, [2014](#); Sepede et al., [2020](#)), as well as impairments in neurogenesis and neuroplasticity, potentially reflected in reduced BDNF levels and altered neuropeptide expression (Brockway & Crowley, [2020](#); Gandhi et al., [2020](#)).

Growing evidence indicates that individuals with BD consistently exhibit gut microbiome dysbiosis, characterized by reduced microbial diversity and altered taxonomic composition compared to healthy controls (Coello et al., [2019](#); McGuinness et al., [2022](#); Nikolova et al., [2021](#)). Among the most replicated findings is the decreased abundance of *Faecalibacterium*, a key anti-inflammatory genus linked to depressive symptoms, oxidative stress, and greater disease burden in BD (Evans et al., [2017](#); Painold et al., [2019](#); Wagner-Skacel et al., [2020](#)). Depressive episodes have also been associated with lower alpha-diversity and reduced levels of *Ruminococcaceae*, alongside increased *Coriobacteria* and *Actinobacteria* (Painold et al., [2019](#)). Despite some inconsistencies—likely due to confounding factors such as mood phase, medication use, and geography—alterations in the phyla *Firmicutes*, *Bacteroidetes*, and *Actinobacteria* are frequently reported (Nikolova et al., [2021](#)). Taxa such as *Megasphaera*, *Eggerthella*, *Klebsiella*, *Desulfovibrio*, and *Enterobacter* are often enriched in BD, although these findings require further replication (Lu et al., [2019](#); Nikolova et al., [2021](#)). Subtype-specific differences have also emerged: for example, *Collinsella* appears

elevated in BD-I compared to BD-II (McIntyre et al., [2021](#)), and higher *Morganella morganii* IgM responses have been reported in BD-I versus BD-II and MDD patients (Lucidi et al., [2021](#)). Other findings remain mixed. Some studies reported no significant differences in *Lactobacillus* or *Bifidobacterium* abundance, although both genera were associated with sleep patterns and cortisol levels (Aizawa et al., [2018](#)). Other microbial compositions may also shift with treatment: *Clostridium bartlettii* levels increased following quetiapine administration and were linked to improvements in depressive symptoms (Lai et al., [2022](#)). Although lithium remains the gold-standard therapy for BD, its clinical impact on gut microbiota remains largely unexplored (Ortega et al., [2023](#)).

Several strategies targeting the gut microbiota—such as probiotics, prebiotics, and FMT—have emerged as potential adjunctive therapies for BD. Although evidence of the use of probiotics and prebiotics in BD remains preliminary, some studies suggest promising effects. For example, supplementation with *Lactobacillus GG* and *Bifidobacterium lactis* reduced rehospitalization risk in recently discharged patients following a manic episode, particularly in those with elevated inflammatory markers (Dickerson et al., [2018](#)). In a trial involving 80 first-episode, drug-naïve BD patients, probiotics used alongside psychotropic therapy improved mania symptoms (Zeng et al., [2022](#)). Another study found that probiotic use improved attention and processing speed in euthymic BD patients over a 3-month period (Reininghaus et al., [2018](#)). Combining probiotics with prebiotic fibers may also counteract the metabolic side effects of atypical antipsychotics (Liu et al., [2021](#)). One case report described remission of both manic and depressive symptoms for 6 months following repeated FMT in a woman with BD, along with substantial weight loss (Hinton, [2020](#)).

Schizophrenia (SZ)

Schizophrenia (SZ) is a complex and disabling neuropsychiatric disorder that affects approximately 24 million people worldwide, representing a substantial global health burden (WHO, [2022](#)). Although

its pathogenesis remains incompletely understood, both genetic predisposition and environmental exposures are considered major contributors (Kong et al., [2025](#)). Increasing evidence suggests that gut microbiota alterations may play a role in SZ development and progression, though clinical investigations remain relatively limited. Early studies identified specific microbial shifts in patients with first-episode psychosis (FEP). An increased relative abundance of *Lactobacillus* spp. was observed in FEP patients compared to controls, and positively correlated with symptom severity (Schwarz et al., [2018](#)). Notably, patients with more pronounced microbiota disruptions at baseline exhibited poorer therapeutic response after 12 months of antipsychotic treatment, suggesting that gut microbial signatures may hold prognostic relevance. Complementary findings were observed in oropharyngeal microbiota, where both *Lactobacillus* and *Bifidobacterium* were enriched in SZ patients (Castro-Nallar et al., [2015](#)). In parallel, a study examining circulating microbial DNA identified increased alpha and beta diversity in SZ compared to both healthy individuals and patients with other neuropsychiatric disorders, pointing toward broader systemic microbial perturbation (Olde Loohuis et al., [2018](#)).

More recent research has further characterized the microbiota alterations across the SZ spectrum stages. In FEP cohorts, consistent findings include elevated levels of *Bacteroides* and reduced abundance of *Bifidobacterium* and *Lactobacillus* (Chen et al., [2024b](#); Ju et al., [2023](#); Yuan et al., [2022](#)). Conversely, increased *Bacteroides* abundance has been hypothesized to drive low-grade inflammation and metabolic dysfunction, which could influence SZ onset and progression (McGuinness et al., [2022](#)). At the community level, SZ is associated with a consistent reduction in alpha diversity, reflecting diminished microbial richness and phylogenetic complexity (Chen et al., [2023b](#), [2024b](#); Gao et al., [2022](#); Karpiński et al., [2023](#); Misera et al., [2025](#); Misiak et al., [2023](#); Pan et al., [2020](#); Sen et al., [2024](#); Shi et al., [2023](#); Wang et al., [2024b](#)). Additionally, beta diversity analyses reveal robust compositional differences between SZ patients—including those at

ultra-high risk, FEP, and chronic stages—and healthy controls (Chen et al., [2024b](#); Ling et al., [2024](#); Ma et al., [2020](#); Misera et al., [2025](#); Pan et al., [2020](#)). These taxonomic and diversity-based changes appear to evolve dynamically over time. Several genera—including *Lactobacillus*, *Prevotella*, and *Collinsella*—increase from the high-risk phase to chronic illness, whereas beneficial SCFA-producing taxa such as *Faecalibacterium*, *Butyricicoccus*, and *Blautia* show progressive depletion (Li et al., [2020](#)). Notably, *Bifidobacterium* and *Bacteroides* demonstrate inverse trajectories: the former increases with disease progression, while the latter declines, potentially reflecting long-term treatment exposure or adaptive host–microbiota interactions (Dias et al., [2024](#); Kelly et al., [2021](#); Ma et al., [2020](#); Vasileva et al., [2024](#)).

Considering these findings, several clinical trials have examined the therapeutic potential of microbiota-directed interventions—particularly probiotics and prebiotics—as adjuncts in schizophrenia treatment. Across multiple studies, including both randomized controlled and open-label designs, supplementation with strains of *Lactobacillus* and *Bifidobacterium* has been associated with modest improvements in psychiatric symptoms (Dickerson et al., [2014](#); Ghaderi et al., [2019](#); Kelly et al., [2021](#); Mujahid et al., [2022](#); Okubo et al., [2019](#)). Prebiotic interventions, including galacto-oligosaccharides, have also shown promise in enhancing cognitive function and modulating glutamatergic signaling, particularly in treatment-resistant populations (Kao et al., [2019](#)).

18.2.3.3 Neurodevelopmental Disorders

Neurodevelopmental disorders are conditions that emerge during early brain development and are characterized by impairments in cognitive, social, emotional, and behavioral functioning. These disorders—including autism spectrum disorder (ASD) and attention-deficit/hyperactivity disorder (ADHD)—often persist throughout life and are associated with structural, biochemical, or electrical abnormalities in the central nervous system. Increasing evidence implicates the gut microbiota in their pathophysiology, with altered

microbial profiles observed in affected individuals. These alterations may influence neurodevelopmental trajectories through immune activation, neuroinflammation, and disrupted metabolic signaling along the microbiota–gut–brain axis. This section explores the current understanding of gut microbial dysbiosis in ASD and ADHD and the emerging therapeutic potential of microbiome-targeted interventions.

Autism Spectrum Disorder (ASD)

Autism spectrum disorder (ASD) is a complex neurodevelopmental condition characterized by deficits in social interaction, communication, and the presence of restricted, repetitive behaviors (Young et al., [2020](#)). It typically manifests in early childhood and often persists into adulthood, and the prevalence of autism among 8-year-old children in the United States increased from 1.1% in 2008 to 2.3% in 2018, reaching 3.6% by 2022 (Hirota & King, [2023](#); Li & He, [2024](#)). While ASD etiology is unclear, a combination of several factors, such as genetic and environmental, immune dysregulation, inflammation, and microbiome imbalances, may play a role in the onset and development of ASD (Cheroni et al., [2020](#); Fattorusso et al., [2019](#); Han et al., [2021](#); Masini et al., [2020](#)). GI symptoms are common in ASD and include constipation, diarrhea, abdominal discomfort, and reflux (Leader et al., [2022](#)). These symptoms affect most individuals with ASD and are frequently associated with greater behavioral symptom severity, suggesting a functional interaction between GI physiology and neurodevelopmental processes (Bresnahan et al., [2015](#); Davies et al., [2021](#); Restrepo et al., [2020](#)). In parallel, several studies have reported reduced vagal tone in individuals with ASD, reflecting autonomic nervous system dysregulation (Sun et al., [2024](#); van Hoorn et al., [2019](#)). This vagal impairment may contribute to altered GI motility and affect gut–brain signaling, further supporting the involvement of the microbiota–gut–brain axis in ASD pathophysiology. Multiple studies have reported consistent shifts in gut microbial composition among individuals with ASD compared to neurotypical controls (Dan et al., [2020](#); Feng et al., [2024](#); Hazan et al., [2020](#); Ho et al., [2020](#)), though no

single taxonomic signature has been universally validated. These alterations include reduced alpha diversity and taxonomic imbalances, notably an increased abundance of *Clostridium* and *Bacteroides* (Coretti et al., [2018](#); Iglesias-Vázquez et al., [2020](#); Ma et al., [2019](#); Xu et al., [2021](#)), and a decreased presence of *Prevotella* (Dan et al., [2020](#); De Angelis et al., [2013](#); Kang et al., [2013](#); Kushak et al., [2017](#)) and *Bifidobacterium* (Andreo-Martínez et al., [2020](#); Iglesias-Vázquez et al., [2020](#)).

Microbiota-targeted interventions have emerged as promising therapeutic strategies. Among these, probiotic supplementation—particularly strains of *Lactobacillus* and *Bifidobacterium*—has demonstrated beneficial effects on both gastrointestinal and behavioral symptoms in ASD, although with variable results across studies (Hassib et al., [2025](#); Kong et al., [2021](#); Mazzone et al., [2024](#); Schmitt et al., [2023](#); Shaaban et al., [2018](#)). These genera appear to modulate gut–brain communication through neural, immune, and metabolic axes, contributing to improvements in social behavior (Engevik et al., [2021](#); O’Callaghan & van Sinderen, [2016](#)). Several clinical studies have explored the effects of probiotics, prebiotics, and FMT in children and adolescents with ASD, showing mixed but generally promising results. Probiotic supplementation in ASD has shown encouraging effects on both behavior and gut function. Kong et al. ([2021](#)) and Guidetti et al. ([2022](#)) reported improvements in social and communication symptoms, along with beneficial shifts in gut microbiota, including increases in *Roseburia*, *Veillonella*, and *Bifidobacterium longum* (Guidetti et al., [2022](#); Kong et al., [2021](#)). Shaaban et al. ([2018](#)) found reductions in autism treatment evaluation checklist (ATEC) scores and GI symptoms, with improvements in GI symptoms (constipation, abdominal pain, and flatulence) and an increase in *Bifidobacteria* and *Lactobacilli* levels after supplementation (Shaaban et al., [2018](#)). Inoue et al. ([2019](#)) reported that supplementation with partially hydrolyzed guar gum improved constipation and behavioral irritability, along with increased levels of *Blautia* and *Acidaminococcus*, and decreased *Streptococcus* and *Odoribacter* (Inoue et al., [2019](#)). Grimaldi et al.

([2018](#)) found that Bimuno galacto oligosaccharide (B-GOS) supplementation led to better bowel habits and increased social behavior, accompanied by rises in *Coprococcus* and *Dorea* species (Grimaldi et al., [2018](#)). Wang et al. ([2020a](#)) observed reductions in ATEC scores and GI symptoms after giving a synbiotic mix of four probiotics and FOS, with microbial shifts including increased *Bifidobacteriales* and decreased *Clostridium* (Wang et al., [2020b](#)). Similarly, Li et al. ([2021](#)) noted improvements in behavior and GI symptoms after administering *Bifidobacterium animalis*, with increased abundance of *Akkermansia* and *Sutterella* sp. (Li et al., [2021](#)). However, Phan et al. ([2024](#)) found no changes in core ASD symptoms after synbiotic treatment, despite microbial increases in *Lactobacillus* and *Bifidobacterium* (Phan et al., [2024](#)). In a single-case study, Aldegheri et al. ([2024](#)) reported that a combination of antibiotics, probiotics (*Bifidobacterium lactis* Bi-07 and *Lactobacillus acidophilus*), and 2'-FL human milk oligosaccharide led to improvements in aggressiveness, mood stability, and constipation in a 17-year-old male with ASD, along with a reduction in *Sutterella* abundance (Aldegheri et al., [2024](#)). More recent microbiota transfer therapy (MTT) studies in ASD have shown promising clinical and microbial outcomes. Hu et al. ([2023](#)) reported (in a single patient case study) that FMT following vancomycin pretreatment reduced ATEC, Social Responsiveness Scale (SRS), and Childhood Autism Rating Scale (CARS) scores and alleviated GI symptoms, with microbial shifts including increases in *Bacteroides* and *Ruminococcus*, and reductions in *Bifidobacterium*, *Streptococcus*, and *Faecalibacterium* (Hu et al., [2023](#)). In a larger trial, Li et al. ([2024a](#)) observed improvements in Autism Behavior Checklist (ABC), CARS, and GI symptoms after 12 weeks of oral lyophilized FMT, with follow-up confirming sustained behavioral benefit and an increase in the abundance of *Anaerostipes*, *Fusicatenibacter*, *Collinsella*, and *Dorea*, alongside a decrease in *Blautia*, *Prevotella*, and *Sellimonas* (Li et al., [2024b](#)). Chen et al. ([2024b](#)) found similar improvements using freeze-dried microbiota capsules, observing a decrease in *Collinsella* abundance (Chen et al., [2024a](#)). In a single case, Hazan et al. ([2024](#)) also

documented symptom improvements and an increase of *Bifidobacterium* and a decrease of *Lactobacillus animalis* after a single colonic MTT infusion (Hazan et al., [2024](#)).

Attention-Deficit/Hyperactivity Disorder (ADHD)

Attention-deficit/hyperactivity disorder (ADHD) is a highly prevalent neurodevelopmental condition marked by persistent inattention, hyperactivity, and impulsivity. It typically begins before age 12 and manifests in three presentations: inattentive, hyperactive-impulsive, and combined, with males more frequently affected (APA, [2022](#); Danielson et al., [2018](#); Kooij et al., [2019](#)). While ADHD is strongly heritable (70–90%), environmental factors—including prenatal exposures and gut microbiome—also contribute to its development (Faraone & Larsson, [2019](#); Nigg & Casey, [2005](#)). Clinically, ADHD is heterogeneous, involving cognitive and emotional symptoms such as executive dysfunction, emotional dysregulation, and altered reward processing (Beheshti et al., [2020](#); Posner et al., [2020](#)). Neurobiological studies implicate dopaminergic and noradrenergic dysregulation in frontostriatal circuits, along with serotonergic deficits and reduced tryptophan availability (Del Campo et al., [2011](#); Johansson et al., [2011](#); Makris et al., [2009](#)). Additionally, immune activation and oxidative stress may contribute to neuroinflammation in ADHD (Corona, [2020](#); Zhou et al., [2017](#)). Although medications are often effective, many individuals experience limited benefit or side effects, underscoring the need for complementary strategies (Childress & Sallee, [2014](#); De Crescenzo et al., [2017](#)). Emerging evidence suggests that gut dysbiosis may play a role in ADHD pathophysiology (Sandgren & Brummer, [2018](#)). Although research on the gut microbiome in ADHD is still in early stages, accumulating data suggest that dysbiosis may contribute to the inflammatory and neural alterations observed in the disorder.

Several studies have identified notable alterations in the gut microbiome composition of individuals with ADHD when compared to healthy controls. While most studies reported no significant differences in alpha and beta diversity metrics, particularly in adults, some studies

in children and adolescents found reduced diversity, suggesting possible age- or subtype-specific microbial alterations (Bundgaard-Nielsen et al., [2023](#); Jung et al., [2022](#); Li et al., [2022](#); Prehn-Kristensen et al., [2018](#); Stiernborg et al., [2023](#); Wang et al., [2020a](#)). At the taxonomic level, findings across studies were inconsistent at higher classification ranks, with contradictory results for phyla and class-level taxa such as *Clostridia* and *Clostridiales* (Aarts et al., [2017](#); Wang et al., [2022a](#)). However, more consistent differences emerged at the genus and species levels. Genera frequently increased in ADHD included *Odoribacter*, *Eggerthella*, *Agathobacter*, and *Phascolarctobacterium* (Aarts et al., [2017](#); Bundgaard-Nielsen et al., [2023](#); Lee et al., [2022](#); Wan et al., [2020](#); Wang et al., [2022a](#)), whereas *Faecalibacterium*, *Eubacterium*, and *Ruminococcus* were consistently reduced (Jiang et al., [2018](#); Jung et al., [2022](#); Lee et al., [2022](#); Li et al., [2022](#); Wan et al., [2020](#)). Specific species such as *Bacteroides uniformis* were consistently enriched in ADHD (Aarts et al., [2017](#); Wang et al., [2020a](#), [2022b](#)), while *Roseburia intestinalis*, *Ruminococcus gnavus*, and *Prevotella copri* showed conflicting results across studies (Bundgaard-Nielsen et al., [2023](#); Lee et al., [2022](#); Wan et al., [2020](#)).

Clinical studies suggest that microbiota-based interventions may improve ADHD symptoms. Probiotic treatments—particularly with *Lactobacillus rhamnosus* GG (Kumperscak et al., [2020](#); Pärtty et al., [2015](#)), *Bifidobacterium bifidum* (Wang et al., [2022b](#)) and multi-strain formulations (Sepehrmanesh et al., [2021](#))—have been associated with symptom reduction and, in some cases, improved inflammation or oxidative stress. A synbiotic blend combining *Lactobacillus* strains with prebiotics (B-glucan, inulin, pectin, starch) also showed clinical benefit (Skott et al., [2020](#)). Additionally, a FMT reported symptomatic improvement along with increased *F. prausnitzii* and reduced *B. longum* (Hooi et al., [2022](#)).

Controversy Gastrointestinal comorbidities are significantly more prevalent in patients with neurological and psychiatric disorders. Further, medication, lifestyle changes (i.e., smoking or drug use),

different diets, and other environmental factors need to be taken into account. These comorbidities and additional factors can contribute to the observed differences in the gut microbiome in these patient populations (e.g., Son et al., [2015](#)).

Preclinical studies using rodent models indicate that certain domains of psychiatric, neurologic or neurodevelopmental diseases are influenced by the gut microbiota. However, preclinical models have many limitations, especially in neuropsychiatry, and translating preclinical data into the patient population is particularly challenging for these complex disorders. Another bias in microbiota research using mouse models comes from the observation that standard laboratory mice have an altered gut microbiome with low diversity compared to the rich microbiota of wild-living mice (Rosshart et al., [2017](#)). Importantly, such a diverse natural microbiota was associated with reduced colonic inflammation, protection from influenza virus, and improved resistance against mutagen-/inflammation-induced colorectal tumorigenesis (Rosshart et al., [2017](#)).

In addition to diet and other environmental contributions, human genetic variation has emerged as another important factor that profoundly shapes the gut microbiome of an individual (Goodrich et al., [2017](#)). For instance, a strong interplay between host genetics and gut microbiota signatures has been observed in inflammatory bowel disease (Imhann et al., [2018](#)). However, this gene-microbiome intersection has not yet been studied in neuropsychiatric disorders, but appears highly needed given the intrinsic genetic component of such pathologies (Birnbaum & Weinberger, [2017](#)).

History

The idea that gut bacteria may influence brain function and mental health dates back to the nineteenth and early twentieth centuries. “Autointoxication,” “intestinal stasis,” or “intestinal intoxication” described disease processes of toxins coming from the gut and affecting the central nervous system, specifically mental health (Bested et al., [2013](#)).

Daniel R. Brower published in 1898 in JAMA on “autointoxication” and melancholia: he reported that gut bacteria produce toxin compounds like indoles and lactic acid that are “detoxified” by the liver and kidneys in healthy individuals. However, these detoxification process would fail in patients with melancholia. To “detoxify the gut and body,” physicians used colon irrigations or even surgical extractions of tonsils or teeth.

Later, probiotic formulas, mostly containing *Lactobacillus* strains, were used to “detoxify” and rebalance the gut. In this context, Ilja Metschnikow, microbiologist and Nobel laureate of 1908, wrote in 1912 (Cosmopolitan): “In effect, we fight microbe with microbe... there seems hope that we shall in time be able to transform the entire intestinal flora from a harmful to an innocuous one... the beneficent effect of this transformation must be enormous...”

With this statement, he arguably predicted the trajectory of modern microbiota-gut-brain axis research directed to understand the role of the gut microflora in neuropsychiatric disorders with anti-, pre-, and postbiotics or even FMT.

Highlights

- The intestinal microbiota is a key factor in the early development of the enteric, peripheral, and central nervous system for both neurons and glia and in neurogenesis in the adult brain.
- Microbe-to-brain communication via the gut-brain axis influences neurochemical signaling and synaptic physiology in the brain with profound impact on cognition, learning and memory, emotional behaviors, and neuroinflammatory processes.
- Animal and human studies revealed a major role of the gut commensal microbiota in the pathogenesis of neurological and neurodegenerative disorders, e.g., multiple sclerosis, ischemic stroke, or Parkinson’s disease, predominantly through microbial priming of immune responses or metabolites interfering with brain protein biochemistry.

- Increasing preclinical and clinical evidence suggests that the indigenous gut microbiota is strongly involved in the development of psychiatric and neurodevelopmental disorders such as autism spectrum disorders, schizophrenia, or major depressive episodes.
- First evidence from mouse models and early clinical trials suggests that microbiome modulation could be a beneficial adjunctive therapy in the treatment of brain disorders.

References

Aarts, E., Ederveen, T. H. A., Naaijen, J., Zwiers, M. P., Boekhorst, J., Timmerman, H. M., Smeekens, S. P., Netea, M. G., Buitelaar, J. K., Franke, B., van Hijum, S., & Arias Vasquez, A. (2017). Gut microbiome in ADHD and its relation to neural reward anticipation. *PLoS One*, 12(9), e0183509. <https://doi.org/10.1371/journal.pone.0183509> [PubMed]

Abavisani, M., Faraji, N., Ebadpour, N., Kesharwani, P., & Sahebkar, A. (2025). Beyond digestion: Exploring how the gut microbiota modulates human social behaviors. *Neuroscience*, 565, 52–62. <https://doi.org/10.1016/j.neuroscience.2024.11.068> [PubMed]

Abdel-Haq, R., Schlachetzki, J. C. M., Boktor, J. C., Cantu-Jungles, T. M., Thron, T., Zhang, M., Bostick, J. W., Khazaei, T., Chilakala, S., Morais, L. H., Humphrey, G., Keshavarzian, A., Katz, J. E., Thomson, M., Knight, R., Gradinaru, V., Hamaker, B. R., Glass, C. K., & Mazmanian, S. K. (2022). A prebiotic diet modulates microglial states and motor deficits in α -synuclein overexpressing mice. *eLife*, 11. <https://doi.org/10.7554/eLife.81453>

Aburto, M. R., & Cryan, J. F. (2024). Gastrointestinal and brain barriers: Unlocking gates of communication across the microbiota-gut-brain axis. *Nature Reviews. Gastroenterology & Hepatology*, 21(4), 222–247. <https://doi.org/10.1038/s41575-023-00890-0>

ADI. (2025). *Dementia statistics*. Retrieved June 02, from <https://www.alzint.org/about/dementia-facts-figures/dementia-statistics/>

Aizawa, E., Tsuji, H., Asahara, T., Takahashi, T., Teraishi, T., Yoshida, S., Koga, N., Hattori, K., Ota, M., & Kunugi, H. (2018). Bifidobacterium and lactobacillus counts in the gut microbiota of patients with bipolar disorder and healthy controls. *Frontiers in Psychiatry*, 9, 730. <https://doi.org/10.3389/fpsy.2018.00730> [PubMed]

Akhgarjand, C., Vahabi, Z., Shab-Bidar, S., Etesam, F., & Djafarian, K. (2022). Effects of probiotic supplements on cognition, anxiety, and physical activity in subjects with mild and moderate

Alzheimer's disease: A randomized, double-blind, and placebo-controlled study. *Frontiers in Aging Neuroscience*, 14, 1032494. <https://doi.org/10.3389/fnagi.2022.1032494>
[PubMed]

Akhoundzadeh, K., Vakili, A., Shadnoush, M., & Sadeghzadeh, J. (2018). Effects of the Oral ingestion of probiotics on brain damage in a transient model of focal cerebral ischemia in mice. *Iranian Journal of Medical Sciences*, 43(1), 32–40.
[PubMed]

Aldegheri, L., Kharrat, F., Conti, A., Monica, F., Busa, F., Campisciano, G., Zanotta, N., Cason, C., & Comar, M. (2024). Impact of human Milk oligosaccharides and probiotics on gut microbiome and mood in autism: A case report. *Microorganisms*, 12(8). <https://doi.org/10.3390/microorganisms12081625>

Aldinger, F., & Schulze, T. G. (2017). Environmental factors, life events, and trauma in the course of bipolar disorder. *Psychiatry and Clinical Neurosciences*, 71(1), 6–17. <https://doi.org/10.1111/pcn.12433>
[PubMed]

Allen, J. M., Mackos, A. R., Jagers, R. M., Brewster, P. C., Webb, M., Lin, C. H., Ladaika, C., Davies, R., White, P., Loman, B. R., & Bailey, M. T. (2022). Psychological stress disrupts intestinal epithelial cell function and mucosal integrity through microbe and host-directed processes. *Gut Microbes*, 14(1), 2035661. <https://doi.org/10.1080/19490976.2022.2035661>
[PubMed]

Al-Nedawi, K., Mian, M. F., Hossain, N., Karimi, K., Mao, Y. K., Forsythe, P., Min, K. K., Stanisz, A. M., Kunze, W. A., & Bienenstock, J. (2015). Gut commensal microvesicles reproduce parent bacterial signals to host immune and enteric nervous systems. *The FASEB Journal*, 29(2), 684–695. <https://doi.org/10.1096/fj.14-259721>
[PubMed]

Alsultan, A. A., Waller, R., Heath, P. R., & Kirby, J. (2016). The genetics of amyotrophic lateral sclerosis: Current insights. *Degenerative Neurological and Neuromuscular Disease*, 6, 49–64. <https://doi.org/10.2147/dnnd.S84956>
[PubMed]

Altschuler, S. M., Escardo, J., Lynn, R. B., & Miselis, R. R. (1993). The central organization of the vagus nerve innervating the colon of the rat. *Gastroenterology*, 104(2), 502–509. [https://doi.org/10.1016/0016-5085\(93\)90419-d](https://doi.org/10.1016/0016-5085(93)90419-d)
[PubMed]

Amorim Neto, D. P., Bosque, B. P., Pereira de Godoy, J. V., Rodrigues, P. V., Meneses, D. D., Tostes, K., Costa Tonoli, C. C., Faustino de Carvalho, H., González-Billault, C., & de Castro Fonseca, M. (2022). Akkermansia muciniphila induces mitochondrial calcium overload and α -synuclein aggregation in an enteroendocrine cell line. *iScience*, 25(3), 103908. <https://doi.org/10.1016/j.isci.2022.103908>
[PubMed]

Andreo-Martínez, P., García-Martínez, N., Sánchez-Samper, E. P., & Martínez-González, A. E. (2020). An approach to gut microbiota profile in children with autism spectrum disorder. *Environmental*

Microbiology Reports, 12(2), 115–135. <https://doi.org/10.1111/1758-2229.12810>
[PubMed]

Andreozzi, V., Cuoco, S., Balestrieri, M., Fierro, F., Ferrara, N., Erro, R., Di Filippo, M., Barbella, G., Memoli, M. C., Silvestri, A., Squillante, M., Guglielmetti, S., Barone, P., Iovino, P., & Pellecchia, M. T. (2024). Synbiotic supplementation may globally improve non-motor symptoms in patients with stable Parkinson's disease: Results from an open label single-arm study. *Scientific Reports*, 14(1), 23095. <https://doi.org/10.1038/s41598-024-74400-w>
[PubMed]

APA. (2022). *Diagnostic and statistical manual of mental disorders: DSM-5-TR* (Text Revision ed.). American Psychiatric Publishing. <https://www.psychiatry.org/psychiatrists/practice/dsm>

Ascherio, A., & Schwarzschild, M. A. (2016). The epidemiology of Parkinson's disease: Risk factors and prevention. *Lancet Neurology*, 15(12), 1257–1272. [https://doi.org/10.1016/s1474-4422\(16\)30230-7](https://doi.org/10.1016/s1474-4422(16)30230-7)
[PubMed]

Asghari, K. M., Dolatkhah, N., Ayromlou, H., Mirnasiri, F., Dadfar, T., & Hashemian, M. (2023). The effect of probiotic supplementation on the clinical and para-clinical findings of multiple sclerosis: A randomized clinical trial. *Scientific Reports*, 13(1), 18577. <https://doi.org/10.1038/s41598-023-46047-6>
[PubMed]

Bai, F., You, L., Lei, H., & Li, X. (2024). Association between increased and decreased gut microbiota abundance and Parkinson's disease: A systematic review and subgroup meta-analysis. *Experimental Gerontology*, 191, 112444. <https://doi.org/10.1016/j.exger.2024.112444>
[PubMed]

Barone, M., Turrone, S., Rampelli, S., Soverini, M., D'Amico, F., Biagi, E., Brigidi, P., Troiani, E., & Candela, M. (2019). Gut microbiome response to a modern Paleolithic diet in a Western lifestyle context. *PLoS One*, 14(8), e0220619. <https://doi.org/10.1371/journal.pone.0220619>
[PubMed]

Bauer, K. C., Huus, K. E., & Finlay, B. B. (2016). Microbes and the mind: Emerging hallmarks of the gut microbiota-brain axis. *Cellular Microbiology*, 18(5), 632–644. <https://doi.org/10.1111/cmi.12585>
[PubMed]

Beheshti, A., Chavanon, M. L., & Christiansen, H. (2020). Emotion dysregulation in adults with attention deficit hyperactivity disorder: A meta-analysis. *BMC Psychiatry*, 20(1), 120. <https://doi.org/10.1186/s12888-020-2442-7>
[PubMed]

Ben-Shlomo, Y., Darweesh, S., Llibre-Guerra, J., Marras, C., San Luciano, M., & Tanner, C. (2024). The epidemiology of Parkinson's disease. *Lancet*, 403(10423), 283–292. [https://doi.org/10.1016/s0140-6736\(23\)01419-8](https://doi.org/10.1016/s0140-6736(23)01419-8)
[PubMed]

Bercik, P., Denou, E., Collins, J., Jackson, W., Lu, J., Jury, J., Deng, Y., Blennerhassett, P., Macri, J., McCoy, K. D., Verdu, E. F., & Collins, S. M. (2011). The intestinal microbiota affect central levels of brain-derived neurotrophic factor and behavior in mice. *Gastroenterology*, 141(2), 599–609, 609.e591–593. <https://doi.org/10.1053/j.gastro.2011.04.052>
[PubMed]

Berer, K., Gerdes, L. A., Cekanaviciute, E., Jia, X., Xiao, L., Xia, Z., Liu, C., Klotz, L., Stauffer, U., Baranzini, S. E., Kumpfel, T., Hohlfeld, R., Krishnamoorthy, G., & Wekerle, H. (2017). Gut microbiota from multiple sclerosis patients enables spontaneous autoimmune encephalomyelitis in mice. *Proceedings of the National Academy of Sciences of the United States of America*, 114(40), 10719–10724. <https://doi.org/10.1073/pnas.1711233114>
[PubMed]

Berlim, M. T., & Turecki, G. (2007). What is the meaning of treatment resistant/refractory major depression (TRD)? A systematic review of current randomized trials. *European Neuropsychopharmacology*, 17(11), 696–707. <https://doi.org/10.1016/j.euroneuro.2007.03.009>
[PubMed]

Bernstein, C. N. (2017). The brain-gut Axis and stress in inflammatory bowel disease. *Gastroenterology Clinics of North America*, 46(4), 839–846. <https://doi.org/10.1016/j.gtc.2017.08.006>
[PubMed]

Berthoud, H. R., & Neuhuber, W. L. (2000). Functional and chemical anatomy of the afferent vagal system. *Autonomic Neuroscience*, 85(1–3), 1–17. [https://doi.org/10.1016/s1566-0702\(00\)00215-0](https://doi.org/10.1016/s1566-0702(00)00215-0)
[PubMed]

Bested, A. C., Logan, A. C., & Selhub, E. M. (2013). Intestinal microbiota, probiotics and mental health: from Metchnikoff to modern advances: Part I – autointoxication revisited. *Gut Pathogens*, 5(1), 5. <https://doi.org/10.1186/1757-4749-5-5>

Beurel, E., Toups, M., & Nemeroff, C. B. (2020). The bidirectional relationship of depression and inflammation: Double trouble. *Neuron*, 107(2), 234–256. <https://doi.org/10.1016/j.neuron.2020.06.002>
[PubMed]

Bicknell, B., Liebert, A., Borody, T., Herkes, G., McLachlan, C., & Kiat, H. (2023). Neurodegenerative and neurodevelopmental diseases and the gut-brain Axis: The potential of therapeutic targeting of the microbiome. *International Journal of Molecular Sciences*, 24(11). <https://doi.org/10.3390/ijms24119577>

Bienkowski, M. S., & Rinaman, L. (2013). Common and distinct neural inputs to the medial central nucleus of the amygdala and anterior ventrolateral bed nucleus of stria terminalis in rats. *Brain Structure & Function*, 218(1), 187–208. <https://doi.org/10.1007/s00429-012-0393-6>

Birnbaum, R., & Weinberger, D. R. (2017). Genetic insights into the neurodevelopmental origins of schizophrenia. *Nature Reviews Neuroscience*, 18(12), 727–740. <https://doi.org/10.1038/nrn.2017.125>

Bliss, T. V., & Cooke, S. F. (2011). Long-term potentiation and long-term depression: A clinical perspective. *Clinics (São Paulo, Brazil)*, 66(Suppl 1), 3–17. <https://doi.org/10.1590/s1807-59322011001300002>
[PubMed]

Bloem, B. R., Okun, M. S., & Klein, C. (2021). Parkinson's disease. *Lancet*, 397(10291), 2284–2303. [https://doi.org/10.1016/s0140-6736\(21\)00218-x](https://doi.org/10.1016/s0140-6736(21)00218-x)
[PubMed]

Boddy, S. L., Giovannelli, I., Sassani, M., Cooper-Knock, J., Snyder, M. P., Segal, E., Elinav, E., Barker, L. A., Shaw, P. J., & McDermott, C. J. (2021). The gut microbiome: A key player in the complexity of amyotrophic lateral sclerosis (ALS). *BMC Medicine*, 19(1), 13. <https://doi.org/10.1186/s12916-020-01885-3>
[PubMed]

Bolton, S., Warner, J., Harriss, E., Geddes, J., & Saunders, K. E. A. (2021). Bipolar disorder: Trimodal age-at-onset distribution. *Bipolar Disorders*, 23(4), 341–356. <https://doi.org/10.1111/bdi.13016>
[PubMed]

Bonaz, B., Bazin, T., & Pellissier, S. (2018). The Vagus nerve at the Interface of the microbiota-gut-brain Axis. *Frontiers in Neuroscience*, 12, 49. <https://doi.org/10.3389/fnins.2018.00049>
[PubMed]

Bonfili, L., Cekarini, V., Berardi, S., Scarpona, S., Suchodolski, J. S., Nasuti, C., Fiorini, D., Boarelli, M. C., Rossi, G., & Eleuteri, A. M. (2017). Microbiota modulation counteracts Alzheimer's disease progression influencing neuronal proteolysis and gut hormones plasma levels. *Scientific Reports*, 7(1), 2426. <https://doi.org/10.1038/s41598-017-02587-2>
[PubMed]

Braak, H., Del Tredici, K., Rüb, U., de Vos, R. A., Jansen Steur, E. N., & Braak, E. (2003). Staging of brain pathology related to sporadic Parkinson's disease. *Neurobiology of Aging*, 24(2), 197–211. [https://doi.org/10.1016/s0197-4580\(02\)00065-9](https://doi.org/10.1016/s0197-4580(02)00065-9)
[PubMed]

Brady, R. O., Jr., McCarthy, J. M., Prescott, A. P., Jensen, J. E., Cooper, A. J., Cohen, B. M., Renshaw, P. F., & Ongür, D. (2013). Brain gamma-aminobutyric acid (GABA) abnormalities in bipolar disorder. *Bipolar Disorders*, 15(4), 434–439. <https://doi.org/10.1111/bdi.12074>
[PubMed]

Braniste, V., Al-Asmakh, M., Kowal, C., Anuar, F., Abbaspour, A., Tóth, M., Korecka, A., Bakocevic, N., Ng, L. G., Kundu, P., Gulyás, B., Halldin, C., Hultenby, K., Nilsson, H., Hebert, H., Volpe, B. T., Diamond, B., & Pettersson, S. (2014). The gut microbiota influences blood-brain barrier permeability in mice. *Science Translational Medicine*, 6(263), 263ra158. <https://doi.org/10.1126/scitranslmed.3009759>
[PubMed]

Bravo, J. A., Forsythe, P., Chew, M. V., Escaravage, E., Savignac, H. M., Dinan, T. G., Bienenstock, J., & Cryan, J. F. (2011). Ingestion of Lactobacillus strain regulates emotional behavior and central GABA receptor expression in a mouse via the vagus nerve. *Proceedings of the National Academy of*

Sciences of the United States of America, 108(38), 16050–16055. <https://doi.org/10.1073/pnas.1102999108>
[PubMed]

Breit, S., Kupferberg, A., Rogler, G., & Hasler, G. (2018). Vagus nerve as modulator of the brain-gut Axis in psychiatric and inflammatory disorders. *Frontiers in Psychiatry*, 9, 44. <https://doi.org/10.3389/fpsy.2018.00044>
[PubMed]

Brenner, D., Hiergeist, A., Adis, C., Mayer, B., Gessner, A., Ludolph, A. C., & Weishaupt, J. H. (2018). The fecal microbiome of ALS patients. *Neurobiology of Aging*, 61, 132–137. <https://doi.org/10.1016/j.neurobiolaging.2017.09.023>
[PubMed]

Brenton, J. N., Lehner-Gulotta, D., Woolbright, E., Banwell, B., Bergqvist, A. G. C., Chen, S., Coleman, R., Conaway, M., & Goldman, M. D. (2022). Phase II study of ketogenic diets in relapsing multiple sclerosis: Safety, tolerability and potential clinical benefits. *Journal of Neurology, Neurosurgery, and Psychiatry*, 93(6), 637–644. <https://doi.org/10.1136/jnnp-2022-329074>
[PubMed]

Bresnahan, M., Hornig, M., Schultz, A. F., Gunnes, N., Hirtz, D., Lie, K. K., Magnus, P., Reichborn-Kjennerud, T., Roth, C., Schjølberg, S., Stoltenberg, C., Surén, P., Susser, E., & Lipkin, W. I. (2015). Association of maternal report of infant and toddler gastrointestinal symptoms with autism: Evidence from a prospective birth cohort. *JAMA Psychiatry*, 72(5), 466–474. <https://doi.org/10.1001/jamapsychiatry.2014.3034>
[PubMed]

Brockway, D. F., & Crowley, N. A. (2020). Turning the tides on neuropsychiatric diseases: The role of peptides in the prefrontal cortex. *Frontiers in Behavioral Neuroscience*, 14, 588400. <https://doi.org/10.3389/fnbeh.2020.588400>
[PubMed]

Bruggeman, A., Vandendriessche, C., Hamerlinck, H., De Looze, D., Tate, D. J., Vuylsteke, M., De Commer, L., Devolder, L., Raes, J., Verhasselt, B., Laukens, D., Vandenbroucke, R. E., & Santens, P. (2024). Safety and efficacy of faecal microbiota transplantation in patients with mild to moderate Parkinson's disease (GUT-PARFECT): A double-blind, placebo-controlled, randomised, phase 2 trial. *EclinicalMedicine*, 71, 102563. <https://doi.org/10.1016/j.eclinm.2024.102563>
[PubMed]

Brun, P., Gobbo, S., Caputi, V., Spagnol, L., Schirato, G., Pasqualin, M., Levorato, E., Palù, G., Giron, M. C., & Castagliuolo, I. (2015). Toll like receptor-2 regulates production of glial-derived neurotrophic factors in murine intestinal smooth muscle cells. *Molecular and Cellular Neurosciences*, 68, 24–35. <https://doi.org/10.1016/j.mcn.2015.03.018>
[PubMed]

Brushett, S., Gacesa, R., Vich Vila, A., Brandao Gois, M. F., Andreu-Sánchez, S., Swarte, J. C., Klaassen, M. A. Y., Collij, V., Sinha, T., Bolte, L. A., Wu, J., Swertz, M., de Kroon, M. L. A., Reijneveld, S. A., Wijmenga, C., Weersma, R. K., Fu, J., van Loo, H. M., Kurilshikov, A., & Zhernakova, A. (2023). Gut

feelings: The relations between depression, anxiety, psychotropic drugs and the gut microbiome. *Gut Microbes*, 15(2), 2281360. <https://doi.org/10.1080/19490976.2023.2281360>
[PubMed]

Bundgaard-Nielsen, C., Lauritsen, M. B., Knudsen, J. K., Rold, L. S., Larsen, M. H., Hindersson, P., Villadsen, A. B., Leutscher, P. D. C., Hagstrøm, S., Nyegaard, M., & Sørensen, S. (2023). Children and adolescents with attention deficit hyperactivity disorder and autism spectrum disorder share distinct microbiota compositions. *Gut Microbes*, 15(1), 2211923. <https://doi.org/10.1080/19490976.2023.2211923>
[PubMed]

Burokas, A., Arboleya, S., Moloney, R. D., Peterson, V. L., Murphy, K., Clarke, G., Stanton, C., Dinan, T. G., & Cryan, J. F. (2017). Targeting the microbiota-gut-brain Axis: Prebiotics have anxiolytic and antidepressant-like effects and reverse the impact of chronic stress in mice. *Biological Psychiatry*, 82(7), 472–487. <https://doi.org/10.1016/j.biopsych.2016.12.031>
[PubMed]

Cani, P. D., Everard, A., & Duparc, T. (2013). Gut microbiota, enteroendocrine functions and metabolism. *Current Opinion in Pharmacology*, 13(6), 935–940. <https://doi.org/10.1016/j.coph.2013.09.008>
[PubMed]

Cantarel, B. L., Waubant, E., Chehoud, C., Kuczynski, J., DeSantis, T. Z., Warrington, J., Venkatesan, A., Fraser, C. M., & Mowry, E. M. (2015). Gut microbiota in multiple sclerosis: Possible influence of immunomodulators. *Journal of Investigative Medicine*, 63(5), 729–734. <https://doi.org/10.1097/jim.0000000000000192>
[PubMed]

Cao, Y., Cheng, Y., Pan, W., Diao, J., Sun, L., & Meng, M. (2025). Gut microbiota variations in depression and anxiety: A systematic review. *BMC Psychiatry*, 25(1), 443. <https://doi.org/10.1186/s12888-025-06871-8>
[PubMed]

Caputi, V., Marsilio, I., Filpa, V., Cerantola, S., Orso, G., Bistoletti, M., Paccagnella, N., De Martin, S., Montopoli, M., Dall'Acqua, S., Crema, F., Di Gangi, I. M., Galuppini, F., Lante, I., Bogialli, S., Rugge, M., Debetto, P., Giaroni, C., & Giron, M. C. (2017). Antibiotic-induced dysbiosis of the microbiota impairs gut neuromuscular function in juvenile mice. *British Journal of Pharmacology*, 174(20), 3623–3639. <https://doi.org/10.1111/bph.13965>
[PubMed]

Caso, J. R., MacDowell, K. S., González-Pinto, A., García, S., de Diego-Adeliño, J., Carceller-Sindreu, M., Sarramea, F., Caballero-Villarraso, J., Gracia-García, P., De la Cámara, C., Agüera, L., Gómez-Lus, M. L., Alba, C., Rodríguez, J. M., & Leza, J. C. (2021). Gut microbiota, innate immune pathways, and inflammatory control mechanisms in patients with major depressive disorder. *Translational Psychiatry*, 11(1), 645. <https://doi.org/10.1038/s41398-021-01755-3>
[PubMed]

Cassani, E., Privitera, G., Pezzoli, G., Pusani, C., Madio, C., Iorio, L., & Barichella, M. (2011). Use of probiotics for the treatment of constipation in Parkinson's disease patients. *Minerva*

Gastroenterologica e Dietologica, 57(2), 117–121.

[[PubMed](#)]

Castro-Nallar, E., Bendall, M. L., Pérez-Losada, M., Sabuncyan, S., Severance, E. G., Dickerson, F. B., Schroeder, J. R., Yolken, R. H., & Crandall, K. A. (2015). Composition, taxonomy and functional diversity of the oropharynx microbiome in individuals with schizophrenia and controls. *PeerJ*, 3, e1140. <https://doi.org/10.7717/peerj.1140>

[[PubMed](#)]

Cattaneo, A., Cattane, N., Galluzzi, S., Provasi, S., Lopizzo, N., Festari, C., Ferrari, C., Guerra, U. P., Paghera, B., Muscio, C., Bianchetti, A., Volta, G. D., Turla, M., Cotelli, M. S., Gennuso, M., Prella, A., Zanetti, O., Lussignoli, G., Mirabile, D., et al. (2017). Association of brain amyloidosis with pro-inflammatory gut bacterial taxa and peripheral inflammation markers in cognitively impaired elderly. *Neurobiology of Aging*, 49, 60–68. <https://doi.org/10.1016/j.neurobiolaging.2016.08.019>

[[PubMed](#)]

Cekanaviciute, E., Yoo, B. B., Runia, T. F., Debelius, J. W., Singh, S., Nelson, C. A., Kanner, R., Bencosme, Y., Lee, Y. K., Hauser, S. L., Crabtree-Hartman, E., Sand, I. K., Gacias, M., Zhu, Y., Casaccia, P., Cree, B. A. C., Knight, R., Mazmanian, S. K., & Baranzini, S. E. (2017). Gut bacteria from multiple sclerosis patients modulate human T cells and exacerbate symptoms in mouse models. *Proceedings of the National Academy of Sciences of the United States of America*, 114(40), 10713–10718. <https://doi.org/10.1073/pnas.1711235114>

[[PubMed](#)]

Chao, L., Liu, C., Sutthawongwadee, S., Li, Y., Lv, W., Chen, W., Yu, L., Zhou, J., Guo, A., Li, Z., & Guo, S. (2020). Effects of probiotics on depressive or anxiety variables in healthy participants under stress conditions or with a depressive or anxiety diagnosis: A meta-analysis of randomized controlled trials. *Frontiers in Neurology*, 11, 421. <https://doi.org/10.3389/fneur.2020.00421>

[[PubMed](#)]

Chaudhari, D. S., Jain, S., Yata, V. K., Mishra, S. P., Kumar, A., Fraser, A., Kociolek, J., Dangiolo, M., Smith, A., Golden, A., Masternak, M. M., Holland, P., Agronin, M., White-Williams, C., Arikawa, A. Y., Labyak, C. A., & Yadav, H. (2023). Unique trans-kingdom microbiome structural and functional signatures predict cognitive decline in older adults. *Geroscience*, 45(5), 2819–2834. <https://doi.org/10.1007/s11357-023-00799-1>

[[PubMed](#)]

Chen, J., Chia, N., Kalari, K. R., Yao, J. Z., Novotna, M., Paz Soldan, M. M., Luckey, D. H., Marietta, E. V., Jeraldo, P. R., Chen, X., Weinshenker, B. G., Rodriguez, M., Kantarci, O. H., Nelson, H., Murray, J. A., & Mangalam, A. K. (2016). Multiple sclerosis patients have a distinct gut microbiota compared to healthy controls. *Scientific Reports*, 6, 28484. <https://doi.org/10.1038/srep28484>

[[PubMed](#)]

Chen, J. J., Zheng, P., Liu, Y. Y., Zhong, X. G., Wang, H. Y., Guo, Y. J., & Xie, P. (2018). Sex differences in gut microbiota in patients with major depressive disorder. *Neuropsychiatric Disease and Treatment*, 14, 647–655. <https://doi.org/10.2147/ndt.S159322>

[[PubMed](#)]

Chen, R., Xu, Y., Wu, P., Zhou, H., Lasanajak, Y., Fang, Y., Tang, L., Ye, L., Li, X., Cai, Z., & Zhao, J.

(2019a). Transplantation of fecal microbiota rich in short chain fatty acids and butyric acid treat cerebral ischemic stroke by regulating gut microbiota. *Pharmacological Research*, 148, 104403. <https://doi.org/10.1016/j.phrs.2019.104403>
[PubMed]

Chen, Y. H., Bai, J., Wu, D., Yu, S. F., Qiang, X. L., Bai, H., Wang, H. N., & Peng, Z. W. (2019b). Association between fecal microbiota and generalized anxiety disorder: Severity and early treatment response. *Journal of Affective Disorders*, 259, 56–66. <https://doi.org/10.1016/j.jad.2019.08.014>
[PubMed]

Chen, Y., Xu, J., & Chen, Y. (2021a). Regulation of neurotransmitters by the gut microbiota and effects on cognition in neurological disorders. *Nutrients*, 13(6). <https://doi.org/10.3390/nu13062099>

Chen, Y. H., Xue, F., Yu, S. F., Li, X. S., Liu, L., Jia, Y. Y., Yan, W. J., Tan, Q. R., Wang, H. N., & Peng, Z. W. (2021b). Gut microbiota dysbiosis in depressed women: The association of symptom severity and microbiota function. *Journal of Affective Disorders*, 282, 391–400. <https://doi.org/10.1016/j.jad.2020.12.143>
[PubMed]

Chen, X., Hu, Y., Yuan, X., Yang, J., & Ka, L. (2022). Effect of early enteral nutrition combined with probiotics in patients with stroke: A meta-analysis of randomized controlled trials. *European Journal of Clinical Nutrition*, 76(4), 592–603. <https://doi.org/10.1038/s41430-021-00986-3>
[PubMed]

Chen, P. C., Kaur, K., Ko, M. W., Huerta-Yepez, S., Jain, Y., & Jewett, A. (2023a). Regulation of cytotoxic immune effector function by AJ3 probiotic bacteria in Amyotrophic Lateral Sclerosis (ALS). *Critical Reviews in Immunology*, 43(1), 13–26. <https://doi.org/10.1615/CritRevImmunol.2023047231>
[PubMed]

Chen, Y. H., Zhou, C. H., Yu, H., Wu, W. J., Wang, Y. W., Liu, L., Hu, G. T., Li, B. J., Peng, Z. W., & Wang, H. N. (2023b). Gut microbial signatures and differences in bipolar disorder and schizophrenia of emerging adulthood. *CNS Neuroscience & Therapeutics*, 29(Suppl 1), 5–17. <https://doi.org/10.1111/cns.14044>

Chen, Q., Wu, C., Xu, J., Ye, C., Chen, X., Tian, H., Zong, N., Zhang, S., Li, L., Gao, Y., Zhao, D., Lv, X., Yang, Q., Wang, L., Cui, J., Lin, Z., Lu, J., Yang, R., Yin, F., et al. (2024a). Donor-recipient intermicrobial interactions impact transfer of subspecies and fecal microbiota transplantation outcome. *Cell Host & Microbe*, 32(3), 349–365.e344. <https://doi.org/10.1016/j.chom.2024.01.013>

Chen, Y. H., Yu, H., Xue, F., Bai, J., Guo, L., & Peng, Z. W. (2024b). 16S rRNA gene sequencing reveals altered gut microbiota in young adults with schizophrenia and prominent negative symptoms. *Brain and Behavior: A Cognitive Neuroscience Perspective*, 14(6), e3579. <https://doi.org/10.1002/brb3.3579>

Chen, L., Wang, X., Wang, S., Liu, W., Song, Z., & Liao, H. (2025). The impact of gut microbiota on the occurrence, treatment, and prognosis of ischemic stroke. *Neurobiology of Disease*, 207, 106836.

<https://doi.org/10.1016/j.nbd.2025.106836>
[PubMed]

Cheroni, C., Caporale, N., & Testa, G. (2020). Autism spectrum disorder at the crossroad between genes and environment: Contributions, convergences, and interactions in ASD developmental pathophysiology. *Molecular Autism*, 11(1), 69. <https://doi.org/10.1186/s13229-020-00370-1>
[PubMed]

Childress, A. C., & Sallee, F. R. (2014). Attention-deficit/hyperactivity disorder with inadequate response to stimulants: Approaches to management. *CNS Drugs*, 28(2), 121–129. <https://doi.org/10.1007/s40263-013-0130-6>
[PubMed]

Chung, Y. E., Chen, H. C., Chou, H. L., Chen, I. M., Lee, M. S., Chuang, L. C., Liu, Y. W., Lu, M. L., Chen, C. H., Wu, C. S., Huang, M. C., Liao, S. C., Ni, Y. H., Lai, M. S., Shih, W. L., & Kuo, P. H. (2019). Exploration of microbiota targets for major depressive disorder and mood related traits. *Journal of Psychiatric Research*, 111, 74–82. <https://doi.org/10.1016/j.jpsychires.2019.01.016>
[PubMed]

Cirstea, M. S., Creus-Cuadros, A., Lo, C., Yu, A. C., Serapio-Palacios, A., Neilson, S., Appel-Cresswell, S., & Finlay, B. B. (2023). A novel pathway of levodopa metabolism by commensal Bifidobacteria. *Scientific Reports*, 13(1), 19155. <https://doi.org/10.1038/s41598-023-45953-z>
[PubMed]

Clarke, G., Grenham, S., Scully, P., Fitzgerald, P., Moloney, R. D., Shanahan, F., Dinan, T. G., & Cryan, J. F. (2013). The microbiome-gut-brain axis during early life regulates the hippocampal serotonergic system in a sex-dependent manner. *Molecular Psychiatry*, 18(6), 666–673. <https://doi.org/10.1038/mp.2012.77>
[PubMed]

Clarke, G. S., Page, A. J., & Eldeghaidy, S. (2024). The gut-brain axis in appetite, satiety, food intake, and eating behavior: Insights from animal models and human studies. *Pharmacology Research & Perspectives*, 12(5), e70027. <https://doi.org/10.1002/prp2.70027>

Clerici, L., Bottari, D., & Bottari, B. (2025). Gut microbiome, diet and depression: Literature review of microbiological, nutritional and neuroscientific aspects. *Current Nutrition Reports*, 14(1), 30. <https://doi.org/10.1007/s13668-025-00619-2>
[PubMed]

Coello, K., Hansen, T. H., Sørensen, N., Munkholm, K., Kessing, L. V., Pedersen, O., & Vinberg, M. (2019). Gut microbiota composition in patients with newly diagnosed bipolar disorder and their unaffected first-degree relatives. *Brain, Behavior, and Immunity*, 75, 112–118. <https://doi.org/10.1016/j.bbi.2018.09.026>
[PubMed]

Collins, J., Borojevic, R., Verdu, E. F., Huizinga, J. D., & Ratcliffe, E. M. (2014). Intestinal microbiota influence the early postnatal development of the enteric nervous system. *Neurogastroenterology and Motility*, 26(1), 98–107. <https://doi.org/10.1111/nmo.12236>
[PubMed]

Colucci-D'Amato, L., Speranza, L., & Volpicelli, F. (2020). Neurotrophic factor BDNF, physiological functions and therapeutic potential in depression, neurodegeneration and brain cancer. *International Journal of Molecular Sciences*, 21(20). <https://doi.org/10.3390/ijms21207777>

Coretti, L., Paparo, L., Riccio, M. P., Amato, F., Cuomo, M., Natale, A., Borrelli, L., Corrado, G., Comegna, M., Buommino, E., Castaldo, G., Bravaccio, C., Chiariotti, L., Berni Canani, R., & Lembo, F. (2018). Gut microbiota features in Young children with autism Spectrum disorders. *Frontiers in Microbiology*, 9, 3146. <https://doi.org/10.3389/fmicb.2018.03146> [PubMed]

Corona, J. C. (2020). Role of oxidative stress and Neuroinflammation in attention-deficit/hyperactivity disorder. *Antioxidants (Basel)*, 9(11). <https://doi.org/10.3390/antiox9111039>

Correale, J., Hohlfeld, R., & Baranzini, S. E. (2022). The role of the gut microbiota in multiple sclerosis. *Nature Reviews. Neurology*, 18(9), 544–558. <https://doi.org/10.1038/s41582-022-00697-8> [PubMed]

Craske, M. G., Stein, M. B., Eley, T. C., Milad, M. R., Holmes, A., Rapee, R. M., & Wittchen, H.-U. (2017). Anxiety disorders. *Nature Reviews Disease Primers*, 3(1), 17024. <https://doi.org/10.1038/nrdp.2017.24> [PubMed]

Cryan, J. F., & Dinan, T. G. (2012). Mind-altering microorganisms: The impact of the gut microbiota on brain and behaviour. *Nature Reviews. Neuroscience*, 13(10), 701–712. <https://doi.org/10.1038/nrn3346> [PubMed]

Cryan, J. F., O'Riordan, K. J., Cowan, C. S. M., Sandhu, K. V., Bastiaanssen, T. F. S., Boehme, M., Codagnone, M. G., Cusotto, S., Fulling, C., Golubeva, A. V., Guzzetta, K. E., Jaggar, M., Long-Smith, C. M., Lyte, J. M., Martin, J. A., Molinero-Perez, A., Moloney, G., Morelli, E., Morillas, E., et al. (2019). The microbiota-gut-brain Axis. *Physiological Reviews*, 99(4), 1877–2013. <https://doi.org/10.1152/physrev.00018.2018> [PubMed]

Cuervo-Zanatta, D., Syeda, T., Sánchez-Valle, V., Irene-Fierro, M., Torres-Aguilar, P., Torres-Ramos, M. A., Shibayama-Salas, M., Silva-Olivares, A., Noriega, L. G., Torres, N., Tovar, A. R., Ruminot, I., Barros, L. F., García-Mena, J., & Perez-Cruz, C. (2023). Dietary fiber modulates the release of gut bacterial products preventing cognitive decline in an Alzheimer's mouse model. *Cellular and Molecular Neurobiology*, 43(4), 1595–1618. <https://doi.org/10.1007/s10571-022-01268-7> [PubMed]

Cuffaro, F., Lamminpää, I., Niccolai, E., & Amedei, A. (2024). Nutritional and microbiota-based approaches in amyotrophic lateral sclerosis: From prevention to treatment. *Nutrients*, 17(1). <https://doi.org/10.3390/nu17010102>

Dan, Z., Mao, X., Liu, Q., Guo, M., Zhuang, Y., Liu, Z., Chen, K., Chen, J., Xu, R., Tang, J., Qin, L., Gu, B., Liu, K., Su, C., Zhang, F., Xia, Y., Hu, Z., & Liu, X. (2020). Altered gut microbial profile is associated

with abnormal metabolism activity of Autism Spectrum Disorder. *Gut Microbes*, 11(5), 1246–1267. <https://doi.org/10.1080/19490976.2020.1747329>
[PubMed]

Danielson, M. L., Bitsko, R. H., Ghandour, R. M., Holbrook, J. R., Kogan, M. D., & Blumberg, S. J. (2018). Prevalence of parent-reported ADHD diagnosis and associated treatment among U.S. children and adolescents, 2016. *Journal of Clinical Child and Adolescent Psychology*, 47(2), 199–212. <https://doi.org/10.1080/15374416.2017.1417860>
[PubMed]

Davies, C., Mishra, D., Eshraghi, R. S., Mittal, J., Sinha, R., Bulut, E., Mittal, R., & Eshraghi, A. A. (2021). Altering the gut microbiome to potentially modulate behavioral manifestations in autism spectrum disorders: A systematic review. *Neuroscience and Biobehavioral Reviews*, 128, 549–557. <https://doi.org/10.1016/j.neubiorev.2021.07.001>
[PubMed]

De Angelis, M., Piccolo, M., Vannini, L., Siragusa, S., De Giacomo, A., Serrazzanetti, D. I., Cristofori, F., Guerzoni, M. E., Gobbetti, M., & Francavilla, R. (2013). Fecal microbiota and metabolome of children with autism and pervasive developmental disorder not otherwise specified. *PLoS One*, 8(10), e76993. <https://doi.org/10.1371/journal.pone.0076993>
[PubMed]

De Crescenzo, F., Cortese, S., Adamo, N., & Janiri, L. (2017). Pharmacological and non-pharmacological treatment of adults with ADHD: A meta-review. *Evidence-Based Mental Health*, 20(1), 4–11. <https://doi.org/10.1136/eb-2016-102415>
[PubMed]

De Palma, G., Blennerhassett, P., Lu, J., Deng, Y., Park, A. J., Green, W., Denou, E., Silva, M. A., Santacruz, A., Sanz, Y., Surette, M. G., Verdu, E. F., Collins, S. M., & Bercik, P. (2015). Microbiota and host determinants of behavioural phenotype in maternally separated mice. *Nature Communications*, 6, 7735. <https://doi.org/10.1038/ncomms8735>
[PubMed]

De Vadder, F., Kovatcheva-Datchary, P., Goncalves, D., Vinera, J., Zitoun, C., Duchampt, A., Bäckhed, F., & Mithieux, G. (2014). Microbiota-generated metabolites promote metabolic benefits via gut-brain neural circuits. *Cell*, 156(1–2), 84–96. <https://doi.org/10.1016/j.cell.2013.12.016>
[PubMed]

De Vadder, F., Grasset, E., Mannerås Holm, L., Karsenty, G., Macpherson, A. J., Olofsson, L. E., & Bäckhed, F. (2018). Gut microbiota regulates maturation of the adult enteric nervous system via enteric serotonin networks. *Proceedings of the National Academy of Sciences of the United States of America*, 115(25), 6458–6463. <https://doi.org/10.1073/pnas.1720017115>
[PubMed]

de Vos, W. M., Tilg, H., Van Hul, M., & Cani, P. D. (2022). Gut microbiome and health: Mechanistic insights. *Gut*, 71(5), 1020–1032. <https://doi.org/10.1136/gutjnl-2021-326789>
[PubMed]

de Wied, D., Diamant, M., & Fodor, M. (1993). Central nervous system effects of the

neurohypophyseal hormones and related peptides. *Frontiers in Neuroendocrinology*, 14(4), 251–302. <https://doi.org/10.1006/frne.1993.1009>
[PubMed]

Del Campo, N., Chamberlain, S. R., Sahakian, B. J., & Robbins, T. W. (2011). The roles of dopamine and noradrenaline in the pathophysiology and treatment of attention-deficit/hyperactivity disorder. *Biological Psychiatry*, 69(12), e145–e157. <https://doi.org/10.1016/j.biopsych.2011.02.036>
[PubMed]

Demaude, J., Salvador-Cartier, C., Fioramonti, J., Ferrier, L., & Bueno, L. (2006). Phenotypic changes in colonocytes following acute stress or activation of mast cells in mice: Implications for delayed epithelial barrier dysfunction. *Gut*, 55(5), 655–661. <https://doi.org/10.1136/gut.2005.078675>
[PubMed]

Desbonnet, L., Garrett, L., Clarke, G., Bienenstock, J., & Dinan, T. G. (2008). The probiotic *Bifidobacteria infantis*: An assessment of potential antidepressant properties in the rat. *Journal of Psychiatric Research*, 43(2), 164–174. <https://doi.org/10.1016/j.jpsychires.2008.03.009>
[PubMed]

Desbonnet, L., Clarke, G., Shanahan, F., Dinan, T. G., & Cryan, J. F. (2014). Microbiota is essential for social development in the mouse. *Molecular Psychiatry*, 19(2), 146–148. <https://doi.org/10.1038/mp.2013.65>
[PubMed]

Di Gioia, D., Bozzi Cionci, N., Baffoni, L., Amoruso, A., Pane, M., Mogna, L., Gaggia, F., Lucenti, M. A., Bersano, E., Cantello, R., De Marchi, F., & Mazzini, L. (2020). A prospective longitudinal study on the microbiota composition in amyotrophic lateral sclerosis. *BMC Medicine*, 18(1), 153. <https://doi.org/10.1186/s12916-020-01607-9>
[PubMed]

Dias, M. F., Nogueira, Y. J. A., Romano-Silva, M. A., & Marques de Miranda, D. (2024). Effects of antipsychotics on the gastrointestinal microbiota: A systematic review. *Psychiatry Research*, 336, 115914. <https://doi.org/10.1016/j.psychres.2024.115914>
[PubMed]

Diaz Heijtz, R., Wang, S., Anuar, F., Qian, Y., Björkholm, B., Samuelsson, A., Hibberd, M. L., Forssberg, H., & Pettersson, S. (2011). Normal gut microbiota modulates brain development and behavior. *Proceedings of the National Academy of Sciences of the United States of America*, 108(7), 3047–3052. <https://doi.org/10.1073/pnas.1010529108>
[PubMed]

Dickerson, F. B., Stallings, C., Origoni, A., Katsafanas, E., Savage, C. L., Schweinfurth, L. A., Goga, J., Khushalani, S., & Yolken, R. H. (2014). Effect of probiotic supplementation on schizophrenia symptoms and association with gastrointestinal functioning: A randomized, placebo-controlled trial. *Primary Care Companion for CNS Disorders*, 16(1). <https://doi.org/10.4088/PCC.13m01579>

Dickerson, F., Adamos, M., Katsafanas, E., Khushalani, S., Origoni, A., Savage, C., Schweinfurth, L., Stallings, C., Sweeney, K., Goga, J., & Yolken, R. H. (2018). Adjunctive probiotic microorganisms to

prevent rehospitalization in patients with acute mania: A randomized controlled trial. *Bipolar Disorders*, 20(7), 614–621. <https://doi.org/10.1111/bdi.12652>
[PubMed]

Dinan, T. G., & Cryan, J. F. (2017a). Gut-brain axis in 2016: Brain-gut-microbiota axis – mood, metabolism and behaviour. *Nature Reviews. Gastroenterology & Hepatology*, 14(2), 69–70. <https://doi.org/10.1038/nrgastro.2016.200>

Dinan, T. G., & Cryan, J. F. (2017b). The microbiome-gut-brain Axis in health and disease. *Gastroenterology Clinics of North America*, 46(1), 77–89. <https://doi.org/10.1016/j.gtc.2016.09.007>
[PubMed]

Dinan, T. G., Quigley, E. M., Ahmed, S. M., Scully, P., O'Brien, S., O'Mahony, L., O'Mahony, S., Shanahan, F., & Keeling, P. W. (2006). Hypothalamic-pituitary-gut axis dysregulation in irritable bowel syndrome: Plasma cytokines as a potential biomarker? *Gastroenterology*, 130(2), 304–311. <https://doi.org/10.1053/j.gastro.2005.11.033>
[PubMed]

Doll, J. P. K., Vázquez-Castellanos, J. F., Schaub, A. C., Schweinfurth, N., Kettelhack, C., Schneider, E., Yamanbaeva, G., Mählmann, L., Brand, S., Beglinger, C., Borgwardt, S., Raes, J., Schmidt, A., & Lang, U. E. (2022). Fecal microbiota transplantation (FMT) as an adjunctive therapy for depression-case report. *Frontiers in Psychiatry*, 13, 815422. <https://doi.org/10.3389/fpsy.2022.815422>
[PubMed]

Dong, Z., Shen, X., Hao, Y., Li, J., Li, H., Xu, H., Yin, L., & Kuang, W. (2021). Gut microbiome: A potential indicator for differential diagnosis of major depressive disorder and general anxiety disorder. *Frontiers in Psychiatry*, 12, 651536. <https://doi.org/10.3389/fpsy.2021.651536>
[PubMed]

Dong, Z., Shen, X., Hao, Y., Li, J., Xu, H., Yin, L., & Kuang, W. (2022). Gut microbiome: A potential indicator for predicting treatment outcomes in major depressive disorder. *Frontiers in Neuroscience*, 16, 813075. <https://doi.org/10.3389/fnins.2022.813075>
[PubMed]

Du, Y., Li, Y., Xu, X., Li, R., Zhang, M., Cui, Y., Zhang, L., Wei, Z., Wang, S., & Tuo, H. (2022). Probiotics for constipation and gut microbiota in Parkinson's disease. *Parkinsonism & Related Disorders*, 103, 92–97. <https://doi.org/10.1016/j.parkreldis.2022.08.022>

DuPont, H. L., Suescun, J., Jiang, Z. D., Brown, E. L., Essigmann, H. T., Alexander, A. S., DuPont, A. W., Iqbal, T., Utay, N. S., Newmark, M., & Schiess, M. C. (2023). Fecal microbiota transplantation in Parkinson's disease-A randomized repeat-dose, placebo-controlled clinical pilot study. *Frontiers in Neurology*, 14, 1104759. <https://doi.org/10.3389/fneur.2023.1104759>
[PubMed]

Eisenstein, M. (2016). Microbiome: Bacterial broadband. *Nature*, 533(7603), S104–S106. <https://doi.org/10.1038/533S104a>
[PubMed]

Elahinejad, V., Khorasanian, A. S., Tehrani-Doost, M., Khosravi-Darani, K., Mirsepasi, Z., Effatpanah, M., Askari-Rabori, R., Tajadod, S., & Jazayeri, S. (2025). Effects of probiotics as adjunctive therapy to fluoxetine on depression severity and serum brain-derived neurotrophic factor, cortisol, and adrenocorticotrophic hormone in patients with major depressive disorder: A randomized, double-blind, placebo-controlled trial. *Food Science & Nutrition*, 13(4), e4698. <https://doi.org/10.1002/fsn3.4698>

Engevik, M. A., Luck, B., Visuthranukul, C., Ihekweazu, F. D., Engevik, A. C., Shi, Z., Danhof, H. A., Chang-Graham, A. L., Hall, A., Endres, B. T., Haidacher, S. J., Horvath, T. D., Haag, A. M., Devaraj, S., Garey, K. W., Britton, R. A., Hyser, J. M., Shroyer, N. F., & Versalovic, J. (2021). Human-derived *Bifidobacterium dentium* modulates the mammalian serotonergic system and gut-brain Axis. *Cellular and Molecular Gastroenterology and Hepatology*, 11(1), 221–248. <https://doi.org/10.1016/j.jcmgh.2020.08.002> [PubMed]

Erny, D., Hrabě de Angelis, A. L., Jaitin, D., Wieghofer, P., Staszewski, O., David, E., Keren-Shaul, H., Mählakoiv, T., Jakobshagen, K., Buch, T., Schwierzeck, V., Utermöhlen, O., Chun, E., Garrett, W. S., McCoy, K. D., Diefenbach, A., Staeheli, P., Stecher, B., Amit, I., & Prinz, M. (2015). Host microbiota constantly control maturation and function of microglia in the CNS. *Nature Neuroscience*, 18(7), 965–977. <https://doi.org/10.1038/nn.4030> [PubMed]

Eskandarzadeh, S., Effatpanah, M., Khosravi-Darani, K., Askari, R., Hosseini, A. F., Reisian, M., & Jazayeri, S. (2021). Efficacy of a multispecies probiotic as adjunctive therapy in generalized anxiety disorder: A double blind, randomized, placebo-controlled trial. *Nutritional Neuroscience*, 24(2), 102–108. <https://doi.org/10.1080/1028415x.2019.1598669> [PubMed]

Evans, S. J., Bassis, C. M., Hein, R., Assari, S., Flowers, S. A., Kelly, M. B., Young, V. B., Ellingrod, V. E., & McInnis, M. G. (2017). The gut microbiome composition associates with bipolar disorder and illness severity. *Journal of Psychiatric Research*, 87, 23–29. <https://doi.org/10.1016/j.jpsychires.2016.12.007> [PubMed]

Fang, X., Wang, X., Yang, S., Meng, F., Wang, X., Wei, H., & Chen, T. (2016). Evaluation of the microbial diversity in amyotrophic lateral sclerosis using high-throughput sequencing. *Frontiers in Microbiology*, 7, 1479. <https://doi.org/10.3389/fmicb.2016.01479> [PubMed]

Faraone, S. V., & Larsson, H. (2019). Genetics of attention deficit hyperactivity disorder. *Molecular Psychiatry*, 24(4), 562–575. <https://doi.org/10.1038/s41380-018-0070-0> [PubMed]

Fasano, A., & Shea-Donohue, T. (2005). Mechanisms of disease: The role of intestinal barrier function in the pathogenesis of gastrointestinal autoimmune diseases. *Nature Clinical Practice. Gastroenterology & Hepatology*, 2(9), 416–422. <https://doi.org/10.1038/ncpgasthep0259>

Fasano, A., Visanji, N. P., Liu, L. W., Lang, A. E., & Pfeiffer, R. F. (2015). Gastrointestinal dysfunction in Parkinson's disease. *Lancet Neurology*, 14(6), 625–639. <https://doi.org/10.1016/s1474->

[4422\(15\)00007-1](#)

[\[PubMed\]](#)

Fattorusso, A., Di Genova, L., Dell'Isola, G. B., Mencaroni, E., & Esposito, S. (2019). Autism Spectrum disorders and the gut microbiota. *Nutrients*, 11(3). <https://doi.org/10.3390/nu11030521>

Feigin, V. L., & Owolabi, M. O. (2023). Pragmatic solutions to reduce the global burden of stroke: A world stroke organization-lancet neurology commission. *Lancet Neurology*, 22(12), 1160–1206.

[https://doi.org/10.1016/s1474-4422\(23\)00277-6](https://doi.org/10.1016/s1474-4422(23)00277-6)

[\[PubMed\]](#)

Feldman, E. L., Goutman, S. A., Petri, S., Mazzini, L., Savelieff, M. G., Shaw, P. J., & Sobue, G. (2022). Amyotrophic lateral sclerosis. *Lancet*, 400(10360), 1363–1380. [https://doi.org/10.1016/s0140-6736\(22\)01272-7](https://doi.org/10.1016/s0140-6736(22)01272-7)

[\[PubMed\]](#)

Feng, P., Zhang, Y., Zhao, Y., Zhao, P., & Li, E. (2024). Combined repetitive transcranial magnetic stimulation and gut microbiota modulation through the gut-brain axis for prevention and treatment of autism spectrum disorder. *Frontiers in Immunology*, 15, 1341404. <https://doi.org/10.3389/fimmu.2024.1341404>

[\[PubMed\]](#)

Ferrante, M., Migliore, M., & Ascoli, G. A. (2013). Functional impact of dendritic branch-point morphology. *The Journal of Neuroscience*, 33(5), 2156–2165. <https://doi.org/10.1523/jneurosci.3495-12.2013>

[\[PubMed\]](#)

Ferreiro, A. L., Choi, J., Ryou, J., Newcomer, E. P., Thompson, R., Bollinger, R. M., Hall-Moore, C., Ndao, I. M., Sax, L., Benzinger, T. L. S., Stark, S. L., Holtzman, D. M., Fagan, A. M., Schindler, S. E., Cruchaga, C., Butt, O. H., Morris, J. C., Tarr, P. I., Ances, B. M., & Dantas, G. (2023). Gut microbiome composition may be an indicator of preclinical Alzheimer's disease. *Science Translational Medicine*, 15(700), eabo2984. <https://doi.org/10.1126/scitranslmed.abo2984>

[\[PubMed\]](#)

Fontdevila, L., Povedano, M., Domínguez, R., Boada, J., Serrano, J. C., Pamplona, R., Ayala, V., & Portero-Otín, M. (2024). Examining the complex Interplay between gut microbiota abundance and short-chain fatty acid production in amyotrophic lateral sclerosis patients shortly after onset of disease. *Scientific Reports*, 14(1), 23497. <https://doi.org/10.1038/s41598-024-75083-z>

[\[PubMed\]](#)

Forloni, G. (2023). Alpha Synuclein: neurodegeneration and inflammation. *International Journal of Molecular Sciences*, 24(6). <https://doi.org/10.3390/ijms24065914>

Foster, J. A., Rinaman, L., & Cryan, J. F. (2017). Stress & the gut-brain axis: Regulation by the microbiome. *Neurobiology of Stress*, 7, 124–136. <https://doi.org/10.1016/j.ynstr.2017.03.001>

[\[PubMed\]](#)

Fowler, K. (2023). Generalized anxiety disorder in Canadian adults: To what extent might social support subdomains and negative social interactions predict psychological distress? *The*

Psychiatric Quarterly, 94(4), 655–673. <https://doi.org/10.1007/s11126-023-10055-6>
[PubMed]

Frohmman, E. M., Racke, M. K., & Raine, C. S. (2006). Multiple sclerosis—the plaque and its pathogenesis. *The New England Journal of Medicine*, 354(9), 942–955. <https://doi.org/10.1056/NEJMr052130>
[PubMed]

Frost, G., Sleeth, M. L., Sahuri-Arisoylu, M., Lizarbe, B., Cerdan, S., Brody, L., Anastasovska, J., Ghourab, S., Hankir, M., Zhang, S., Carling, D., Swann, J. R., Gibson, G., Viardot, A., Morrison, D., Louise Thomas, E., & Bell, J. D. (2014). The short-chain fatty acid acetate reduces appetite via a central homeostatic mechanism. *Nature Communications*, 5, 3611. <https://doi.org/10.1038/ncomms4611>
[PubMed]

Furness, J. B. (2012). The enteric nervous system and neurogastroenterology. *Nature Reviews. Gastroenterology & Hepatology*, 9(5), 286–294. <https://doi.org/10.1038/nrgastro.2012.32>

Gabrielli, M., Zileri Dal Verme, L., Zocco, M. A., Nista, E. C., Ojetti, V., & Gasbarrini, A. (2024). The role of the gastrointestinal microbiota in Parkinson's disease. *Biomolecules*, 15(1). <https://doi.org/10.3390/biom15010026>

Gandhi, A. B., Kaleem, I., Alexander, J., Hisbulla, M., Kannichamy, V., Antony, I., Mishra, V., Banerjee, A., & Khan, S. (2020). Neuroplasticity improves bipolar disorder: A review. *Cureus*, 12(10), e11241. <https://doi.org/10.7759/cureus.11241>
[PubMed]

Gao, X., Cao, Q., Cheng, Y., Zhao, D., Wang, Z., Yang, H., Wu, Q., You, L., Wang, Y., Lin, Y., Li, X., Wang, Y., Bian, J. S., Sun, D., Kong, L., Birnbaumer, L., & Yang, Y. (2018). Chronic stress promotes colitis by disturbing the gut microbiota and triggering immune system response. *Proceedings of the National Academy of Sciences of the United States of America*, 115(13), E2960–e2969. <https://doi.org/10.1073/pnas.1720696115>
[PubMed]

Gao, Y., Liu, X., Pan, M., Zeng, D., Zhou, X., Tsunoda, M., Zhang, Y., Xie, X., Wang, R., Hu, W., Li, L., Yang, H., & Song, Y. (2022). Integrated untargeted fecal metabolomics and gut microbiota strategy for screening potential biomarkers associated with schizophrenia. *Journal of Psychiatric Research*, 156, 628–638. <https://doi.org/10.1016/j.jpsychires.2022.10.072>
[PubMed]

Gao, A., Lv, J., & Su, Y. (2025). The inflammatory mechanism of Parkinson's disease: Gut microbiota metabolites affect the development of the disease through the gut-brain Axis. *Brain Sciences*, 15(2). <https://doi.org/10.3390/brainsci15020159>

Gareau, M. G., Wine, E., Rodrigues, D. M., Cho, J. H., Whary, M. T., Philpott, D. J., Macqueen, G., & Sherman, P. M. (2011). Bacterial infection causes stress-induced memory dysfunction in mice. *Gut*, 60(3), 307–317. <https://doi.org/10.1136/gut.2009.202515>
[PubMed]

GBD. (2022). Estimation of the global prevalence of dementia in 2019 and forecasted prevalence in 2050: An analysis for the global burden of disease study 2019. *The Lancet Public Health*, 7(2), e105–e125. [https://doi.org/10.1016/s2468-2667\(21\)00249-8](https://doi.org/10.1016/s2468-2667(21)00249-8)

Georgescu, D., Ancusa, O. E., Georgescu, L. A., Ionita, I., & Reisz, D. (2016). Nonmotor gastrointestinal disorders in older patients with Parkinson's disease: Is there hope? *Clinical Interventions in Aging*, 11, 1601–1608. <https://doi.org/10.2147/cia.S106284>
[PubMed]

Ghaderi, A., Banafshe, H. R., Mirhosseini, N., Moradi, M., Karimi, M. A., Mehrzad, F., Bahmani, F., & Asemi, Z. (2019). Clinical and metabolic response to vitamin D plus probiotic in schizophrenia patients. *BMC Psychiatry*, 19(1), 77. <https://doi.org/10.1186/s12888-019-2059-x>
[PubMed]

Ghalandari, N., Assarzagdegan, F., Habibi, S. A. H., Esmaily, H., & Malekpour, H. (2023). Efficacy of probiotics in improving motor function and alleviating constipation in Parkinson's disease: A randomized controlled trial. *Iranian Journal of Pharmaceutical Sciences*, 22(1), e137840. <https://doi.org/10.5812/ijpr-137840>

Ghasemi, N., Razavi, S., & Nikzad, E. (2017). Multiple sclerosis: Pathogenesis, symptoms, diagnoses and cell-based therapy. *Cell Journal*, 19(1), 1–10. <https://doi.org/10.22074/cellj.2016.4867>
[PubMed]

Gobert, A. P., Sagrestani, G., Delmas, E., Wilson, K. T., Verriere, T. G., Dapoigny, M., Del'homme, C., & Bernalier-Donadille, A. (2016). The human intestinal microbiota of constipated-predominant irritable bowel syndrome patients exhibits anti-inflammatory properties. *Scientific Reports*, 6, 39399. <https://doi.org/10.1038/srep39399>
[PubMed]

Goehler, L. E., Gaykema, R. P., Opitz, N., Reddaway, R., Badr, N., & Lyte, M. (2005). Activation in vagal afferents and central autonomic pathways: Early responses to intestinal infection with *Campylobacter jejuni*. *Brain, Behavior, and Immunity*, 19(4), 334–344. <https://doi.org/10.1016/j.bbi.2004.09.002>
[PubMed]

Gong, Z., Ba, L., Tang, J., Yang, Y., Li, Z., Liu, M., Yang, C., Ding, F., & Zhang, M. (2022). Gut microbiota links with cognitive impairment in amyotrophic lateral sclerosis: A multi-omics study. *Journal of Biomedical Research*, 37(2), 125–137. <https://doi.org/10.7555/jbr.36.20220198>
[PubMed]

Goodrich, J. K., Davenport, E. R., Clark, A. G., & Ley, R. E. (2017). The relationship between the human genome and microbiome comes into view. *Annual Review of Genetics*, 51, 413–433. <https://doi.org/10.1146/annurev-genet-110711-155532>

Gosselin, D., & Rivest, S. (2008). MyD88 signaling in brain endothelial cells is essential for the neuronal activity and glucocorticoid release during systemic inflammation. *Molecular Psychiatry*, 13(5), 480–497. <https://doi.org/10.1038/sj.mp.4002122>
[PubMed]

Goutman, S. A., Hardiman, O., Al-Chalabi, A., Chió, A., Savelieff, M. G., Kiernan, M. C., & Feldman, E. L. (2022). Recent advances in the diagnosis and prognosis of amyotrophic lateral sclerosis. *Lancet Neurology*, 21(5), 480–493. [https://doi.org/10.1016/s1474-4422\(21\)00465-8](https://doi.org/10.1016/s1474-4422(21)00465-8) [PubMed]

Goverman, J., Woods, A., Larson, L., Weiner, L. P., Hood, L., & Zaller, D. M. (1993). Transgenic mice that express a myelin basic protein-specific T cell receptor develop spontaneous autoimmunity. *Cell*, 72(4), 551–560. [https://doi.org/10.1016/0092-8674\(93\)90074-z](https://doi.org/10.1016/0092-8674(93)90074-z) [PubMed]

Goyal, N. A., Berry, J. D., Windebank, A., Staff, N. P., Maragakis, N. J., van den Berg, L. H., Genge, A., Miller, R., Baloh, R. H., Kern, R., Gothelf, Y., Lebovits, C., & Cudkowicz, M. (2020). Addressing heterogeneity in amyotrophic lateral sclerosis CLINICAL TRIALS. *Muscle & Nerve*, 62(2), 156–166. <https://doi.org/10.1002/mus.26801>

Grabrucker, S., Marizzoni, M., Silajdžić, E., Lopizzo, N., Mombelli, E., Nicolas, S., Dohm-Hansen, S., Scassellati, C., Moretti, D. V., Rosa, M., Hoffmann, K., Cryan, J. F., O’Leary, O. F., English, J. A., Lavelle, A., O’Neill, C., Thuret, S., Cattaneo, A., & Nolan, Y. M. (2023). Microbiota from Alzheimer’s patients induce deficits in cognition and hippocampal neurogenesis. *Brain*, 146(12), 4916–4934. <https://doi.org/10.1093/brain/awad303> [PubMed]

Green, J. E., McGuinness, A. J., Berk, M., Castle, D., Athan, E., Hair, C., Strandwitz, P., Loughman, A., Nierenberg, A. A., Cryan, J. F., Mohebbi, M., & Jacka, F. (2023). Safety and feasibility of faecal microbiota transplant for major depressive disorder: Study protocol for a pilot randomised controlled trial. *Pilot and Feasibility Studies*, 9(1), 5. <https://doi.org/10.1186/s40814-023-01235-z> [PubMed]

Grenham, S., Clarke, G., Cryan, J. F., & Dinan, T. G. (2011). Brain-gut-microbe communication in health and disease. *Frontiers in Physiology*, 2, 94. <https://doi.org/10.3389/fphys.2011.00094> [PubMed]

Grimaldi, R., Gibson, G. R., Vulevic, J., Giallourou, N., Castro-Mejía, J. L., Hansen, L. H., Leigh Gibson, E., Nielsen, D. S., & Costabile, A. (2018). A prebiotic intervention study in children with autism spectrum disorders (ASDs). *Microbiome*, 6(1), 133. <https://doi.org/10.1186/s40168-018-0523-3> [PubMed]

Grimonprez, A., Raedt, R., Baeken, C., Boon, P., & Vonck, K. (2015). The antidepressant mechanism of action of vagus nerve stimulation: Evidence from preclinical studies. *Neuroscience and Biobehavioral Reviews*, 56, 26–34. <https://doi.org/10.1016/j.neubiorev.2015.06.019> [PubMed]

Guida, F., Turco, F., Iannotta, M., De Gregorio, D., Palumbo, I., Sarnelli, G., Furiano, A., Napolitano, F., Boccella, S., Luongo, L., Mazzitelli, M., Usiello, A., De Filippis, F., Iannotti, F. A., Piscitelli, F., Ercolini, D., de Novellis, V., Di Marzo, V., Cuomo, R., & Maione, S. (2018). Antibiotic-induced microbiota perturbation causes gut endocannabinoidome changes, hippocampal neuroglial reorganization and depression in mice. *Brain, Behavior, and Immunity*, 67, 230–245. <https://doi.org/10.1016/j.bbi.2017.09.001>

[PubMed]

Guidetti, C., Salvini, E., Viri, M., Deidda, F., Amoruso, A., Visciglia, A., Drago, L., Calgaro, M., Vitulo, N., Pane, M., & Caucino, A. C. (2022). Randomized double-blind crossover study for evaluating a probiotic mixture on gastrointestinal and behavioral symptoms of autistic children. *Journal of Clinical Medicine*, 11(18). <https://doi.org/10.3390/jcm11185263>

Gunnarsson, L. G., & Bodin, L. (2018). Amyotrophic lateral sclerosis and occupational exposures: A systematic literature review and meta-analyses. *International Journal of Environmental Research and Public Health*, 15(11). <https://doi.org/10.3390/ijerph15112371>

Guo, M., Peng, J., Huang, X., Xiao, L., Huang, F., & Zuo, Z. (2021). Gut microbiome features of Chinese patients newly diagnosed with Alzheimer's disease or mild cognitive impairment. *Journal of Alzheimer's Disease*, 80(1), 299–310. <https://doi.org/10.3233/jad-201040>
[PubMed]

Haak, B. W., Westendorp, W. F., van Engelen, T. S. R., Brands, X., Brouwer, M. C., Vermeij, J. D., Hugenholtz, F., Verhoeven, A., Derks, R. J., Giera, M., Nederkoorn, P. J., de Vos, W. M., van de Beek, D., & Wiersinga, W. J. (2021). Disruptions of anaerobic gut bacteria are associated with stroke and post-stroke infection: A prospective case-control study. *Translational Stroke Research*, 12(4), 581–592. <https://doi.org/10.1007/s12975-020-00863-4>
[PubMed]

Halemani, K., Shetty, A. P., Thimmappa, L., Issac, A., Dhiraaj, S., Radha, K., Mishra, P., & Mathias, E. G. (2023). Impact of probiotic on anxiety and depression symptoms in pregnant and lactating women and microbiota of infants: A systematic review and meta-analysis. *Journal of Global Health*, 13, 04038. <https://doi.org/10.7189/jogh.13.04038>
[PubMed]

Han, V. X., Patel, S., Jones, H. F., & Dale, R. C. (2021). Maternal immune activation and neuroinflammation in human neurodevelopmental disorders. *Nature Reviews. Neurology*, 17(9), 564–579. <https://doi.org/10.1038/s41582-021-00530-8>
[PubMed]

Haran, J. P., Bhattarai, S. K., Foley, S. E., Dutta, P., Ward, D. V., Bucci, V., & McCormick, B. A. (2019). Alzheimer's disease microbiome is associated with dysregulation of the anti-inflammatory P-glycoprotein pathway. *mBio*, 10(3). <https://doi.org/10.1128/mBio.00632-19>

Hardiman, O., Al-Chalabi, A., Chio, A., Corr, E. M., Logroscino, G., Robberecht, W., Shaw, P. J., Simmons, Z., & van den Berg, L. H. (2017). Amyotrophic lateral sclerosis. *Nature Reviews. Disease Primers*, 3, 17071. <https://doi.org/10.1038/nrdp.2017.71>
[PubMed]

Harrison, P. J., Cipriani, A., Harmer, C. J., Nobre, A. C., Saunders, K., Goodwin, G. M., & Geddes, J. R. (2016). Innovative approaches to bipolar disorder and its treatment. *Annals of the New York Academy of Sciences*, 1366(1), 76–89. <https://doi.org/10.1111/nyas.13048>
[PubMed]

Harrison, P. J., Geddes, J. R., & Tunbridge, E. M. (2018). The emerging neurobiology of bipolar

disorder. *Trends in Neurosciences*, 41(1), 18–30. <https://doi.org/10.1016/j.tins.2017.10.006> [PubMed]

Hassib, L., Kanashiro, A., Pedrazzi, J. F. C., Vercesi, B. F., Higa, S., Arruda, Í., Soares, Y., de Jesus de Souza, A., Barichello, T., Guimarães, F. S., & Ferreira, F. R. (2025). Microbiota-based therapies as novel targets for autism spectrum disorder: A systematic review and meta-analysis. *Progress in Neuro-Psychopharmacology & Biological Psychiatry*, 139, 111385. <https://doi.org/10.1016/j.pnpbp.2025.111385>

Hawkes, C. H., Del Tredici, K., & Braak, H. (2010). A timeline for Parkinson's disease. *Parkinsonism & Related Disorders*, 16(2), 79–84. <https://doi.org/10.1016/j.parkreldis.2009.08.007>

Hays, K. E., Pfaffinger, J. M., & Ryznar, R. (2024). The interplay between gut microbiota, short-chain fatty acids, and implications for host health and disease. *Gut Microbes*, 16(1), 2393270. <https://doi.org/10.1080/19490976.2024.2393270> [PubMed]

Hazan, S. (2020). Rapid improvement in Alzheimer's disease symptoms following fecal microbiota transplantation: A case report. *The Journal of International Medical Research*, 48(6), 300060520925930. <https://doi.org/10.1177/0300060520925930> [PubMed]

Hazan, S., Spradling-Reeves, K. D., Papoutsis, A., & Walker, S. J. (2020). Shotgun metagenomic sequencing identifies Dysbiosis in triplet sibling with gastrointestinal symptoms and ASD. *Children (Basel)*, 7(12). <https://doi.org/10.3390/children7120255>

Hazan, S., Haroon, J., Jordan, S., & Walker, S. J. (2024). Improvements in gut microbiome composition and clinical symptoms following familial fecal microbiota transplantation in a nineteen-year-old adolescent with severe autism. *Journal of Medical Cases*, 15(4–5), 82–91. <https://doi.org/10.14740/jmc4209> [PubMed]

He, X., Yan, C., Zhao, S., Zhao, Y., Huang, R., & Li, Y. (2022). The preventive effects of probiotic *Akkermansia muciniphila* on D-galactose/AlCl₃ mediated Alzheimer's disease-like rats. *Experimental Gerontology*, 170, 111959. <https://doi.org/10.1016/j.exger.2022.111959> [PubMed]

Hegstrand, L. R., & Hine, R. J. (1986). Variations of brain histamine levels in germ-free and nephrectomized rats. *Neurochemical Research*, 11(2), 185–191. <https://doi.org/10.1007/bf00967967> [PubMed]

Hinton, R. (2020). A case report looking at the effects of faecal microbiota transplantation in a patient with bipolar disorder. *The Australian and New Zealand Journal of Psychiatry*, 54(6), 649–650. <https://doi.org/10.1177/0004867420912834> [PubMed]

Hirota, T., & King, B. H. (2023). Autism spectrum disorder: A review. *The Journal of the American Medical Association*, 329(2), 157–168. <https://doi.org/10.1001/jama.2022.23661>

[PubMed]

Ho, M. S. (2019). Microglia in Parkinson's disease. In A. Verkhratsky, M. S. Ho, R. Zorec, & V. Parpura (Eds.), *Neuroglia in neurodegenerative diseases* (pp. 335–353). Springer Singapore. https://doi.org/10.1007/978-981-13-9913-8_13

Ho, L. K. H., Tong, V. J. W., Syn, N., Nagarajan, N., Tham, E. H., Tay, S. K., Shorey, S., Tambyah, P. A., & Law, E. C. N. (2020). Gut microbiota changes in children with autism spectrum disorder: A systematic review. *Gut Pathogens*, 12, 6. <https://doi.org/10.1186/s13099-020-0346-1>
[PubMed]

Hoban, A. E., Stilling, R. M., Ryan, F. J., Shanahan, F., Dinan, T. G., Claesson, M. J., Clarke, G., & Cryan, J. F. (2016). Regulation of prefrontal cortex myelination by the microbiota. *Translational Psychiatry*, 6(4), e774. <https://doi.org/10.1038/tp.2016.42>
[PubMed]

Holmqvist, S., Chutna, O., Bousset, L., Aldrin-Kirk, P., Li, W., Björklund, T., Wang, Z. Y., Roybon, L., Melki, R., & Li, J. Y. (2014). Direct evidence of Parkinson pathology spread from the gastrointestinal tract to the brain in rats. *Acta Neuropathologica*, 128(6), 805–820. <https://doi.org/10.1007/s00401-014-1343-6>
[PubMed]

Hooi, S. L., Dwiyanto, J., Rasiti, H., Toh, K. Y., Wong, R. K. M., & Lee, J. W. J. (2022). A case report of improvement on ADHD symptoms after fecal microbiota transplantation with gut microbiome profiling pre- and post-procedure. *Current Medical Research and Opinion*, 38(11), 1977–1982. <https://doi.org/10.1080/03007995.2022.2129232>
[PubMed]

Hosoi, T., Okuma, Y., & Nomura, Y. (2000). Electrical stimulation of afferent vagus nerve induces IL-1 β expression in the brain and activates HPA axis. *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology*, 279(1), R141–R147. <https://doi.org/10.1152/ajpregu.2000.279.1.R141>
[PubMed]

Hsiao, E. Y., McBride, S. W., Hsien, S., Sharon, G., Hyde, E. R., McCue, T., Codelli, J. A., Chow, J., Reisman, S. E., Petrosino, J. F., Patterson, P. H., & Mazmanian, S. K. (2013). Microbiota modulate behavioral and physiological abnormalities associated with neurodevelopmental disorders. *Cell*, 155(7), 1451–1463. <https://doi.org/10.1016/j.cell.2013.11.024>
[PubMed]

Hu, C., He, T., Zou, B., Li, H., Zhao, J., Hu, C., Cui, J., Huang, Z., Shu, S., & Hao, Y. (2023). Fecal microbiota transplantation in a child with severe ASD comorbidities of gastrointestinal dysfunctions-a case report. *Frontiers in Psychiatry*, 14, 1219104. <https://doi.org/10.3389/fpsy.2023.1219104>
[PubMed]

Huang, W. J., Chen, W. W., & Zhang, X. (2017). Multiple sclerosis: Pathology, diagnosis and treatments. *Experimental and Therapeutic Medicine*, 13(6), 3163–3166. <https://doi.org/10.3892/etm.2017.4410>

[[PubMed](#)]

Huang, J. T., Mao, Y. Q., Han, B., Zhang, Z. Y., Chen, H. L., Li, Z. M., Kong, C. Y., Xu, J. Q., Cai, P. R., Zeng, Y. P., Zhao, J., Zhao, Y. P., & Wang, L. S. (2021). Calorie restriction conferred improvement effect on long-term rehabilitation of ischemic stroke via gut microbiota. *Pharmacological Research*, 170, 105726. <https://doi.org/10.1016/j.phrs.2021.105726>

[[PubMed](#)]

Hyland, N. P., & Cryan, J. F. (2016). Microbe-host interactions: Influence of the gut microbiota on the enteric nervous system. *Developmental Biology*, 417(2), 182–187. <https://doi.org/10.1016/j.ydbio.2016.06.027>

[[PubMed](#)]

Ibrahim, A., Ali, R. A. R., Manaf, M. R. A., Ahmad, N., Tajurruddin, F. W., Qin, W. Z., Desa, S. H. M., & Ibrahim, N. M. (2020). Multi-strain probiotics (Hexbio) containing MCP BCMC strains improved constipation and gut motility in Parkinson's disease: A randomised controlled trial. *PLoS One*, 15(12), e0244680. <https://doi.org/10.1371/journal.pone.0244680>

[[PubMed](#)]

Iglesias-Vázquez, L., Van Ginkel Riba, G., Arija, V., & Canals, J. (2020). Composition of gut microbiota in children with autism Spectrum disorder: A systematic review and meta-analysis. *Nutrients*, 12(3). <https://doi.org/10.3390/nu12030792>

Imhann, F., Vich Vila, A., Bonder, M. J., Fu, J., Gevers, D., Visschedijk, M. C., Spekhorst, L. M., Alberts, R., Franke, L., van Dullemen, H. M., Ter Steege, R. W. F., Huttenhower, C., Dijkstra, G., Xavier, R. J., Festen, E. A. M., Wijmenga, C., Zhernakova, A., & Weersma, R. K. (2018). Interplay of host genetics and gut microbiota underlying the onset and clinical presentation of inflammatory bowel disease. *Gut*, 67(1), 108–119. <https://doi.org/10.1136/gutjnl-2016-312135>

Inoue, R., Sakaue, Y., Kawada, Y., Tamaki, R., Yasukawa, Z., Ozeki, M., Ueba, S., Sawai, C., Nonomura, K., Tsukahara, T., & Naito, Y. (2019). Dietary supplementation with partially hydrolyzed guar gum helps improve constipation and gut dysbiosis symptoms and behavioral irritability in children with autism spectrum disorder. *Journal of Clinical Biochemistry and Nutrition*, 64(3), 217–223.

<https://doi.org/10.3164/jcbrn.18-105>

[[PubMed](#)]

Jaggers, R. M., DiSabato, D. J., Loman, B. R., Kontic, D., Spencer, K. D., Allen, J. M., Godbout, J. P., Quan, N., Gur, T. L., & Bailey, M. T. (2022). Stressor-induced reduction in cognitive behavior is associated with impaired colonic mucus layer integrity and is dependent upon the LPS-binding protein receptor CD14. *Journal of Inflammation Research*, 15, 1617–1635. <https://doi.org/10.2147/jir.S332793>

[[PubMed](#)]

Jaglin, M., Rhimi, M., Philippe, C., Pons, N., Bruneau, A., Goustard, B., Daugé, V., Maguin, E., Naudon, L., & Rabot, S. (2018). Indole, a signaling molecule produced by the gut microbiota, negatively impacts emotional behaviors in rats. *Frontiers in Neuroscience*, 12, 216. <https://doi.org/10.3389/fnins.2018.00216>

[[PubMed](#)]

Jain, A., & Mitra, P. (2025). Bipolar disorder. In *StatPearls*. StatPearls Publishing. Copyright © 2025, StatPearls Publishing LLC.

Jangi, S., Gandhi, R., Cox, L. M., Li, N., von Glehn, F., Yan, R., Patel, B., Mazzola, M. A., Liu, S., Glanz, B. L., Cook, S., Tankou, S., Stuart, F., Melo, K., Nejad, P., Smith, K., Topçuoğlu, B. D., Holden, J., Kivisäkk, P., et al. (2016). Alterations of the human gut microbiome in multiple sclerosis. *Nature Communications*, 7, 12015. <https://doi.org/10.1038/ncomms12015> [PubMed]

Jemimah, S., Chabib, C. M. M., Hadjileontiadis, L., & AlShehhi, A. (2023). Gut microbiome dysbiosis in Alzheimer's disease and mild cognitive impairment: A systematic review and meta-analysis. *PLoS One*, 18(5), e0285346. <https://doi.org/10.1371/journal.pone.0285346> [PubMed]

Ji, W., Zhu, Y., Kan, P., Cai, Y., Wang, Z., Wu, Z., & Yang, P. (2017). Analysis of intestinal microbial communities of cerebral infarction and ischemia patients based on high throughput sequencing technology and glucose and lipid metabolism. *Molecular Medicine Reports*, 16(4), 5413–5417. <https://doi.org/10.3892/mmr.2017.7227> [PubMed]

Jiang, H., Ling, Z., Zhang, Y., Mao, H., Ma, Z., Yin, Y., Wang, W., Tang, W., Tan, Z., Shi, J., Li, L., & Ruan, B. (2015). Altered fecal microbiota composition in patients with major depressive disorder. *Brain, Behavior, and Immunity*, 48, 186–194. <https://doi.org/10.1016/j.bbi.2015.03.016> [PubMed]

Jiang, H. Y., Zhang, X., Yu, Z. H., Zhang, Z., Deng, M., Zhao, J. H., & Ruan, B. (2018). Altered gut microbiota profile in patients with generalized anxiety disorder. *Journal of Psychiatric Research*, 104, 130–136. <https://doi.org/10.1016/j.jpsychires.2018.07.007> [PubMed]

Johansson, J., Landgren, M., Fernell, E., Vumma, R., Åhlin, A., Bjerkenstedt, L., & Venizelos, N. (2011). Altered tryptophan and alanine transport in fibroblasts from boys with attention-deficit/hyperactivity disorder (ADHD): An in vitro study. *Behavioral and Brain Functions*, 7, 40. <https://doi.org/10.1186/1744-9081-7-40> [PubMed]

Ju, S., Shin, Y., Han, S., Kwon, J., Choi, T. G., Kang, I., & Kim, S. S. (2023). The gut-brain Axis in schizophrenia: The implications of the gut microbiome and SCFA production. *Nutrients*, 15(20). <https://doi.org/10.3390/nu15204391>

Jung, T. H., Hwang, H. J., & Han, K. S. (2022). Correlation of attention deficit hyperactivity disorder with gut microbiota according to the dietary intake of Korean elementary school students. *PLoS One*, 17(9), e0275520. <https://doi.org/10.1371/journal.pone.0275520> [PubMed]

Kabouridis, P. S., Lasrado, R., McCallum, S., Chng, S. H., Snippert, H. J., Clevers, H., Pettersson, S., & Pachnis, V. (2015). Microbiota controls the homeostasis of glial cells in the gut lamina propria. *Neuron*, 85(2), 289–295. <https://doi.org/10.1016/j.neuron.2014.12.037> [PubMed]

Kaelberer, M. M., Buchanan, K. L., Klein, M. E., Barth, B. B., Montoya, M. M., Shen, X., & Bohórquez, D. V. (2018). A gut-brain neural circuit for nutrient sensory transduction. *Science*, 361(6408). <https://doi.org/10.1126/science.aat5236>

Kaelberer, M. M., Rupprecht, L. E., Liu, W. W., Weng, P., & Bohórquez, D. V. (2020). Neuropod cells: The emerging biology of gut-brain sensory transduction. *Annual Review of Neuroscience*, 43, 337–353. <https://doi.org/10.1146/annurev-neuro-091619-022657> [PubMed]

Kang, D. W., Park, J. G., Ilhan, Z. E., Wallstrom, G., Labaer, J., Adams, J. B., & Krajmalnik-Brown, R. (2013). Reduced incidence of *Prevotella* and other fermenters in intestinal microflora of autistic children. *PLoS One*, 8(7), e68322. <https://doi.org/10.1371/journal.pone.0068322> [PubMed]

Kao, A. C., Safarikova, J., Marquardt, T., Mullins, B., Lennox, B. R., & Burnet, P. W. J. (2019). Pro-cognitive effect of a prebiotic in psychosis: A double blind placebo controlled cross-over study. *Schizophrenia Research*, 208, 460–461. <https://doi.org/10.1016/j.schres.2019.03.003> [PubMed]

Karpiński, P., Żebrowska-Róžańska, P., Kujawa, D., Łaczmański, Ł., Samochowiec, J., Jabłoński, M., Plichta, P., Piotrowski, P., Bielawski, T., & Misiak, B. (2023). Gut microbiota alterations in schizophrenia might be related to stress exposure: Findings from the machine learning analysis. *Psychoneuroendocrinology*, 155, 106335. <https://doi.org/10.1016/j.psyneuen.2023.106335> [PubMed]

Kaul, M., Mukherjee, D., Weiner, H. L., & Cox, L. M. (2024). Gut microbiota immune cross-talk in amyotrophic lateral sclerosis. *Neurotherapeutics*, 21(6), e00469. <https://doi.org/10.1016/j.neurot.2024.e00469> [PubMed]

Kazemi, A., Noorbala, A. A., Azam, K., Eskandari, M. H., & Djafarian, K. (2019). Effect of probiotic and prebiotic vs placebo on psychological outcomes in patients with major depressive disorder: A randomized clinical trial. *Clinical Nutrition*, 38(2), 522–528. <https://doi.org/10.1016/j.clnu.2018.04.010> [PubMed]

Kelly, J. R., Borre, Y., O'Brien, C., Patterson, E., El Aidy, S., Deane, J., Kennedy, P. J., Beers, S., Scott, K., Moloney, G., Hoban, A. E., Scott, L., Fitzgerald, P., Ross, P., Stanton, C., Clarke, G., Cryan, J. F., & Dinan, T. G. (2016). Transferring the blues: Depression-associated gut microbiota induces neurobehavioural changes in the rat. *Journal of Psychiatric Research*, 82, 109–118. <https://doi.org/10.1016/j.jpsychires.2016.07.019> [PubMed]

Kelly, J. R., Minuto, C., Cryan, J. F., Clarke, G., & Dinan, T. G. (2017). Cross talk: The microbiota and neurodevelopmental disorders. *Frontiers in Neuroscience*, 11, 490. <https://doi.org/10.3389/fnins.2017.00490> [PubMed]

Kelly, J. R., Minuto, C., Cryan, J. F., Clarke, G., & Dinan, T. G. (2021). The role of the gut microbiome in

the development of schizophrenia. *Schizophrenia Research*, 234, 4–23. <https://doi.org/10.1016/j.schres.2020.02.010>
[PubMed]

Kerfoot, E. C., Chattillion, E. A., & Williams, C. L. (2008). Functional interactions between the nucleus tractus solitarius (NTS) and nucleus accumbens shell in modulating memory for arousing experiences. *Neurobiology of Learning and Memory*, 89(1), 47–60. <https://doi.org/10.1016/j.nlm.2007.09.005>
[PubMed]

Khailova, L., Petrie, B., Baird, C. H., Dominguez Rieg, J. A., & Wischmeyer, P. E. (2014). Lactobacillus rhamnosus GG and Bifidobacterium longum attenuate lung injury and inflammatory response in experimental sepsis. *PLoS One*, 9(5), e97861. <https://doi.org/10.1371/journal.pone.0097861>
[PubMed]

Khaledi, M., Sameni, F., Gholipour, A., Shahrjerdi, S., Golmohammadi, R., Gouvarchin Ghaleh, H. E., Poureslamfar, B., Hemmati, J., Mobarezpour, N., Milasi, Y. E., Rad, F., Mehboodi, M., & Owlia, P. (2024). Potential role of gut microbiota in major depressive disorder: A review. *Heliyon*, 10(12), e33157. <https://doi.org/10.1016/j.heliyon.2024.e33157>
[PubMed]

Killingsworth, J., Sawmiller, D., & Shytle, R. D. (2020). Propionate and Alzheimer's disease. *Frontiers in Aging Neuroscience*, 12, 580001. <https://doi.org/10.3389/fnagi.2020.580001>
[PubMed]

Kim, M. S., Kim, Y., Choi, H., Kim, W., Park, S., Lee, D., Kim, D. K., Kim, H. J., Choi, H., Hyun, D. W., Lee, J. Y., Choi, E. Y., Lee, D. S., Bae, J. W., & Mook-Jung, I. (2020). Transfer of a healthy microbiota reduces amyloid and tau pathology in an Alzheimer's disease animal model. *Gut*, 69(2), 283–294. <https://doi.org/10.1136/gutjnl-2018-317431>
[PubMed]

Kim, H. S., Son, J., Lee, D., Tsai, J., Wang, D., Chocron, E. S., Jeong, S., Kittrell, P., Murchison, C. F., Kennedy, R. E., Tobon, A., Jackson, C. E., & Pickering, A. M. (2022). Gut- and oral-dysbiosis differentially impact spinal- and bulbar-onset ALS, predicting ALS severity and potentially determining the location of disease onset. *BMC Neurology*, 22(1), 62. <https://doi.org/10.1186/s12883-022-02586-5>
[PubMed]

Klingensmith, N. J., & Coopersmith, C. M. (2016). The Gut as the motor of multiple organ dysfunction in critical illness. *Critical Care Clinics*, 32(2), 203–212. <https://doi.org/10.1016/j.ccc.2015.11.004>
[PubMed]

Knox, E. G., Lynch, C. M. K., Lee, Y. S., O'Driscoll, C. M., Clarke, G., Cryan, J. F., & Aburto, M. R. (2023). The gut microbiota is important for the maintenance of blood-cerebrospinal fluid barrier integrity. *The European Journal of Neuroscience*, 57(2), 233–241. <https://doi.org/10.1111/ejn.15878>
[PubMed]

Kong, X. J., Liu, J., Liu, K., Koh, M., Sherman, H., Liu, S., Tian, R., Sukijthamapan, P., Wang, J., Fong, M.,

Xu, L., Clairmont, C., Jeong, M. S., Li, A., Lopes, M., Hagan, V., Dutton, T., Chan, S. P., Lee, H., et al. (2021). Probiotic and oxytocin combination therapy in patients with autism Spectrum disorder: A randomized, double-blinded, placebo-controlled pilot trial. *Nutrients*, 13(5). <https://doi.org/10.3390/nu13051552>

Kong, L., Wang, X., Chen, G., Zhu, Y., Wang, L., Yan, M., Zeng, J., Zhou, X., Lui, S. S. Y., & Chan, R. C. K. (2025). Gut microbiome characteristics in individuals across different stages of schizophrenia spectrum disorders: A systematic review and meta-analysis. *Neuroscience and Biobehavioral Reviews*, 173, 106167. <https://doi.org/10.1016/j.neubiorev.2025.106167> [PubMed]

König, T., & Stögmänn, E. (2021). Genetics of Alzheimer's disease. *Wiener Medizinische Wochenschrift (1946)*, 171(11–12), 249–256. <https://doi.org/10.1007/s10354-021-00819-9>. (Genetik der Alzheimer-Demenz). [PubMed]

Kooij, J. J. S., Bijlenga, D., Salerno, L., Jaeschke, R., Bitter, I., Balázs, J., Thome, J., Dom, G., Kasper, S., Nunes Filipe, C., Stes, S., Mohr, P., Leppämäki, S., Casas, M., Bobes, J., McCarthy, J. M., Richarte, V., Kjemps Philipsen, A., Pehlivanidis, A., et al. (2019). Updated European consensus statement on diagnosis and treatment of adult ADHD. *European Psychiatry*, 56, 14–34. <https://doi.org/10.1016/j.eurpsy.2018.11.001> [PubMed]

Krezalek, M. A., DeFazio, J., Zaborina, O., Zaborin, A., & Alverdy, J. C. (2016). The shift of an intestinal “Microbiome” to a “Pathobiome” governs the course and outcome of sepsis following surgical injury. *Shock*, 45(5), 475–482. <https://doi.org/10.1097/shk.0000000000000534> [PubMed]

Kumperscak, H. G., Gricar, A., Ülen, I., & Micetic-Turk, D. (2020). A pilot randomized control trial with the probiotic strain lactobacillus rhamnosus GG (LGG) in ADHD: Children and adolescents report better health-related quality of life. *Frontiers in Psychiatry*, 11, 181. <https://doi.org/10.3389/fpsy.2020.00181> [PubMed]

Kunze, W. A., Mao, Y. K., Wang, B., Huizinga, J. D., Ma, X., Forsythe, P., & Bienenstock, J. (2009). Lactobacillus reuteri enhances excitability of colonic AH neurons by inhibiting calcium-dependent potassium channel opening. *Journal of Cellular and Molecular Medicine*, 13(8b), 2261–2270. <https://doi.org/10.1111/j.1582-4934.2009.00686.x> [PubMed]

Kuroishi, T. (2015). Regulation of immunological and inflammatory functions by biotin. *Canadian Journal of Physiology and Pharmacology*, 93(12), 1091–1096. <https://doi.org/10.1139/cjpp-2014-0460> [PubMed]

Kushak, R. I., Winter, H. S., Buie, T. M., Cox, S. B., Phillips, C. D., & Ward, N. L. (2017). Analysis of the duodenal microbiome in autistic individuals: Association with carbohydrate digestion. *Journal of Pediatric Gastroenterology and Nutrition*, 64(5), e110–e116. <https://doi.org/10.1097/mpg.0000000000001458>

[[PubMed](#)]

Lai, W. T., Deng, W. F., Xu, S. X., Zhao, J., Xu, D., Liu, Y. H., Guo, Y. Y., Wang, M. B., He, F. S., Ye, S. W., Yang, Q. F., Liu, T. B., Zhang, Y. L., Wang, S., Li, M. Z., Yang, Y. J., Xie, X. H., & Rong, H. (2021). Shotgun metagenomics reveals both taxonomic and tryptophan pathway differences of gut microbiota in major depressive disorder patients. *Psychological Medicine*, 51(1), 90–101. <https://doi.org/10.1017/s0033291719003027>

[[PubMed](#)]

Lai, J., Li, A., Jiang, J., Yuan, X., Zhang, P., Xi, C., Wu, L., Wang, Z., Chen, J., Lu, J., Lu, S., Mou, T., Zhou, H., Wang, D., Huang, M., Dong, F., Li, M. D., Xu, Y., Song, X., & Hu, S. (2022). Metagenomic analysis reveals gut bacterial signatures for diagnosis and treatment outcome prediction in bipolar depression. *Psychiatry Research*, 307, 114326. <https://doi.org/10.1016/j.psychres.2021.114326>

[[PubMed](#)]

Lanoiselée, H. M., Nicolas, G., Wallon, D., Rovelet-Lecrux, A., Lacour, M., Rousseau, S., Richard, A. C., Pasquier, F., Rollin-Sillaire, A., Martinaud, O., Quillard-Muraine, M., de la Sayette, V., Boutoleau-Bretonniere, C., Etcharry-Bouyx, F., Chauviré, V., Sarazin, M., le Ber, I., Epelbaum, S., Jonveaux, T., et al. (2017). APP, PSEN1, and PSEN2 mutations in early-onset Alzheimer disease: A genetic screening study of familial and sporadic cases. *PLoS Medicine*, 14(3), e1002270. <https://doi.org/10.1371/journal.pmed.1002270>

[[PubMed](#)]

Leader, G., Abberton, C., Cunningham, S., Gilmartin, K., Grudzien, M., Higgins, E., Joshi, L., Whelan, S., & Mannion, A. (2022). Gastrointestinal symptoms in autism spectrum disorder: A systematic review. *Nutrients*, 14(7). <https://doi.org/10.3390/nu14071471>

Lee, J., d'Aigle, J., Atadja, L., Quaicoe, V., Honarpisheh, P., Ganesh, B. P., Hassan, A., Graf, J., Petrosino, J., Putluri, N., Zhu, L., Durgan, D. J., Bryan, R. M., Jr., McCullough, L. D., & Venna, V. R. (2020). Gut microbiota-derived short-chain fatty acids promote Poststroke recovery in aged mice. *Circulation Research*, 127(4), 453–465. <https://doi.org/10.1161/circresaha.119.316448>

[[PubMed](#)]

Lee, M. J., Lai, H. C., Kuo, Y. L., & Chen, V. C. (2022). Association between gut microbiota and emotional-behavioral symptoms in children with attention-deficit/hyperactivity disorder. *Journal of Personalized Medicine*, 12(10). <https://doi.org/10.3390/jpm12101634>

Legrand, A., Iftimovici, A., Khayachi, A., & Chaumette, B. (2021). Epigenetics in bipolar disorder: A critical review of the literature. *Psychiatric Genetics*, 31(1), 1–12. <https://doi.org/10.1097/ypg.0000000000000267>

[[PubMed](#)]

Li, C., & He, W. Q. (2024). Prevalence and treatment of autism spectrum disorder in the United States, 2016–2022. *Autism Research*, 17(9), 1916–1927. <https://doi.org/10.1002/aur.3228>

[[PubMed](#)]

Li, S., Zhuo, M., Huang, X., Huang, Y., Zhou, J., Xiong, D., Li, J., Liu, Y., Pan, Z., Li, H., Chen, J., Li, X., Xiang, Z., Wu, F., & Wu, K. (2020). Altered gut microbiota associated with symptom severity in schizophrenia. *PeerJ*, 8, e9574. <https://doi.org/10.7717/peerj.9574>

[[PubMed](#)]

Li, Y. Q., Sun, Y. H., Liang, Y. P., Zhou, F., Yang, J., & Jin, S. L. (2021). Effect of probiotics combined with applied behavior analysis in the treatment of children with autism spectrum disorder: A prospective randomized controlled trial. *Zhongguo Dang Dai Er Ke Za Zhi*, 23(11), 1103–1110. <https://doi.org/10.7499/j.issn.1008-8830.2108085>. (益生菌联合应用行为分析法治疗儿童孤独症谱系障碍的前瞻性随机对照研究.).

[[PubMed](#)]

Li, Y., Sun, H., Huang, Y., Yin, A., Zhang, L., Han, J., Lyu, Y., Xu, X., Zhai, Y., Sun, H., Wang, P., Zhao, J., Sun, S., Dong, H., Zhu, F., Wang, Q., Augusto Rohde, L., Xie, X., Sun, X., & Xiong, L. (2022). Gut metagenomic characteristics of ADHD reveal low *Bacteroides ovatus*-associated host cognitive impairment. *Gut Microbes*, 14(1), 2125747. <https://doi.org/10.1080/19490976.2022.2125747>

[[PubMed](#)]

Li, S., Liu, M., Cao, S., Liu, B., Li, D., Wang, Z., Sun, H., Cui, Y., & Shi, Y. (2023). The mechanism of the gut-brain Axis in regulating food intake. *Nutrients*, 15(17). <https://doi.org/10.3390/nu15173728>

Li, K., Ding, W., Li, X., Gao, H., Wang, S., Li, T., Zhao, H., & Zhang, S. (2024a). Intestinal *Akkermansia muciniphila* is beneficial to functional recovery following ischemic stroke. *Journal of Neuroimmune Pharmacology*, 19(1), 43. <https://doi.org/10.1007/s11481-024-10146-6>

[[PubMed](#)]

Li, Y., Xiao, P., Cao, R., Le, J., Xu, Q., Xiao, F., Ye, L., Wang, X., Wang, Y., & Zhang, T. (2024b). Effects and microbiota changes following oral lyophilized fecal microbiota transplantation in children with autism spectrum disorder. *Frontiers in Pediatrics*, 12, 1369823. <https://doi.org/10.3389/fped.2024.1369823>

[[PubMed](#)]

Ling, Z., Cheng, Y., Yan, X., Shao, L., Liu, X., Zhou, D., Zhang, L., Yu, K., & Zhao, L. (2020). Alterations of the fecal microbiota in Chinese patients with multiple sclerosis. *Frontiers in Immunology*, 11, 590783. <https://doi.org/10.3389/fimmu.2020.590783>

[[PubMed](#)]

Ling, Z., Lan, Z., Cheng, Y., Liu, X., Li, Z., Yu, Y., Wang, Y., Shao, L., Zhu, Z., Gao, J., Lei, W., Ding, W., & Liao, R. (2024). Altered gut microbiota and systemic immunity in Chinese patients with schizophrenia comorbid with metabolic syndrome. *Journal of Translational Medicine*, 22(1), 729. <https://doi.org/10.1186/s12967-024-05533-9>

[[PubMed](#)]

Liu, H., Tian, Y., Liu, Y., Nigatu, Y. T., & Wang, J. (2019a). Relationship between major depressive disorder, generalized anxiety disorder and coronary artery disease in the US general population. *Journal of Psychosomatic Research*, 119, 8–13. <https://doi.org/10.1016/j.jpsychores.2019.01.007>

[[PubMed](#)]

Liu, R. T., Walsh, R. F. L., & Sheehan, A. E. (2019b). Prebiotics and probiotics for depression and anxiety: A systematic review and meta-analysis of controlled clinical trials. *Neuroscience and Biobehavioral Reviews*, 102, 13–23. <https://doi.org/10.1016/j.neubiorev.2019.03.023>

[[PubMed](#)]

Liu, C., Kang, D., Xiao, J., Huang, Y., Peng, X., Wang, W., Xie, P., Yang, Y., Zhao, J., & Wu, R. (2021). Dietary fiber and probiotics for the treatment of atypical antipsychotic-induced metabolic side effects: Study protocol for a randomized, double-blind, placebo-controlled trial. *Trials*, 22(1), 159. <https://doi.org/10.1186/s13063-021-05123-w>

[PubMed]

Liu, L., Wang, H., Chen, X., Zhang, Y., Zhang, H., & Xie, P. (2023). Gut microbiota and its metabolites in depression: From pathogenesis to treatment. *eBioMedicine*, 90, 104527. <https://doi.org/10.1016/j.ebiom.2023.104527>

[PubMed]

Liu, K., Guo, Q., Ding, Y., Luo, L., Huang, J., & Zhang, Q. (2024). Alterations in nasal microbiota of patients with amyotrophic lateral sclerosis. *Chinese Medical Journal*, 137(2), 162–171. <https://doi.org/10.1097/cm9.0000000000002701>

[PubMed]

London, A., Cohen, M., & Schwartz, M. (2013). Microglia and monocyte-derived macrophages: Functionally distinct populations that act in concert in CNS plasticity and repair. *Frontiers in Cellular Neuroscience*, 7, 34. <https://doi.org/10.3389/fncel.2013.00034>

[PubMed]

Long, J. M., & Holtzman, D. M. (2019). Alzheimer disease: An update on pathobiology and treatment strategies. *Cell*, 179(2), 312–339. <https://doi.org/10.1016/j.cell.2019.09.001>

[PubMed]

Longinetti, E., & Fang, F. (2019). Epidemiology of amyotrophic lateral sclerosis: An update of recent literature. *Current Opinion in Neurology*, 32(5), 771–776. <https://doi.org/10.1097/wco.0000000000000730>

[PubMed]

Lu, J., Lu, L., Yu, Y., Cluette-Brown, J., Martin, C. R., & Claud, E. C. (2018). Effects of intestinal microbiota on brain development in humanized Gnotobiotic mice. *Scientific Reports*, 8(1), 5443. <https://doi.org/10.1038/s41598-018-23692-w>

[PubMed]

Lu, Q., Lai, J., Lu, H., Ng, C., Huang, T., Zhang, H., Ding, K., Wang, Z., Jiang, J., Hu, J., Lu, J., Lu, S., Mou, T., Wang, D., Du, Y., Xi, C., Lyu, H., Chen, J., Xu, Y., et al. (2019). Gut microbiota in bipolar depression and its relationship to brain function: An advanced exploration. *Frontiers in Psychiatry*, 10, 784. <https://doi.org/10.3389/fpsy.2019.00784>

[PubMed]

Lu, C. S., Chang, H. C., Weng, Y. H., Chen, C. C., Kuo, Y. S., & Tsai, Y. C. (2021). The add-on effect of lactobacillus plantarum PS128 in patients with Parkinson's disease: A pilot study. *Frontiers in Nutrition*, 8, 650053. <https://doi.org/10.3389/fnut.2021.650053>

[PubMed]

Lu, G., Wen, Q., Cui, B., Li, Q., & Zhang, F. (2022). Washed microbiota transplantation stopped the deterioration of amyotrophic lateral sclerosis: The first case report and narrative review. *Journal of Biomedical Research*, 37(1), 69–76. <https://doi.org/10.7555/jbr.36.20220088>

[PubMed]

Lu, S., Zhao, Q., Guan, Y., Sun, Z., Li, W., Guo, S., & Zhang, A. (2024). The communication mechanism of the gut-brain axis and its effect on central nervous system diseases: A systematic review. *Biomedicine & Pharmacotherapy*, 178, 117207. <https://doi.org/10.1016/j.biopha.2024.117207>

Lucidi, L., Pettorruso, M., Vellante, F., Di Carlo, F., Ceci, F., Santovito, M. C., Di Muzio, I., Fornaro, M., Ventriglio, A., Tomasetti, C., Valchera, A., Gentile, A., Kim, Y. K., Martinotti, G., Fraticelli, S., Di Giannantonio, M., & De Berardis, D. (2021). Gut microbiota and bipolar disorder: An overview on a novel biomarker for diagnosis and treatment. *International Journal of Molecular Sciences*, 22(7). <https://doi.org/10.3390/ijms22073723>

Luczynski, P., McVey Neufeld, K. A., Oriach, C. S., Clarke, G., Dinan, T. G., & Cryan, J. F. (2016). Growing up in a bubble: Using germ-free animals to assess the influence of the gut microbiota on brain and behavior. *The International Journal of Neuropsychopharmacology*, 19(8). <https://doi.org/10.1093/ijnp/pyw020>

Luo, Y., Chang, G., Yu, G., Lin, Y., Zhang, Q., Wang, Z., & Han, J. (2024). Unveiling the negative association of *Faecalibacterium prausnitzii* with ischemic stroke severity, impaired prognosis and pro-inflammatory markers. *Heliyon*, 10(5), e26651. <https://doi.org/10.1016/j.heliyon.2024.e26651>

[PubMed]

Ma, B., Liang, J., Dai, M., Wang, J., Luo, J., Zhang, Z., & Jing, J. (2019). Altered gut microbiota in Chinese children with autism Spectrum disorders. *Frontiers in Cellular and Infection Microbiology*, 9, 40. <https://doi.org/10.3389/fcimb.2019.00040>

[PubMed]

Ma, X., Asif, H., Dai, L., He, Y., Zheng, W., Wang, D., Ren, H., Tang, J., Li, C., Jin, K., Li, Z., & Chen, X. (2020). Alteration of the gut microbiome in first-episode drug-naïve and chronic medicated schizophrenia correlate with regional brain volumes. *Journal of Psychiatric Research*, 123, 136–144. <https://doi.org/10.1016/j.jpsychires.2020.02.005>

[PubMed]

Macom, R. V., & Brown, C. M. (2025). Gastrointestinal dysfunction and Dysbiosis in ischemic stroke: Opportunities for therapeutic intervention. *Pharmaceuticals (Basel)*, 18(3). <https://doi.org/10.3390/ph18030320>

Madison, A. A., & Bailey, M. T. (2024). Stressed to the Core: Inflammation and intestinal permeability link stress-related gut microbiota shifts to mental health outcomes. *Biological Psychiatry*, 95(4), 339–347. <https://doi.org/10.1016/j.biopsych.2023.10.014>

[PubMed]

Mahgoub, M., & Monteggia, L. M. (2013). Epigenetics and psychiatry. *Neurotherapeutics*, 10(4), 734–741. <https://doi.org/10.1007/s13311-013-0213-6>

[PubMed]

Makris, N., Biederman, J., Monuteaux, M. C., & Seidman, L. J. (2009). Towards conceptualizing a neural systems-based anatomy of attention-deficit/hyperactivity disorder. *Developmental*

Neuroscience, 31(1–2), 36–49. <https://doi.org/10.1159/000207492>
[PubMed]

Maksimovic, K., Youssef, M., You, J., Sung, H. K., & Park, J. (2023). Evidence of metabolic dysfunction in Amyotrophic Lateral Sclerosis (ALS) patients and animal models. *Biomolecules*, 13(5). <https://doi.org/10.3390/biom13050863>

Maniscalco, J. W., & Rinaman, L. (2018). Vagal interoceptive modulation of motivated behavior. *Physiology (Bethesda)*, 33(2), 151–167. <https://doi.org/10.1152/physiol.00036.2017>
[PubMed]

Mao, Y. K., Kasper, D. L., Wang, B., Forsythe, P., Bienenstock, J., & Kunze, W. A. (2013). *Bacteroides fragilis* polysaccharide A is necessary and sufficient for acute activation of intestinal sensory neurons. *Nature Communications*, 4, 1465. <https://doi.org/10.1038/ncomms2478>
[PubMed]

Marashly, E. T., & Bohlega, S. A. (2017). Riboflavin has neuroprotective potential: Focus on Parkinson's disease and migraine. *Frontiers in Neurology*, 8, 333. <https://doi.org/10.3389/fneur.2017.00333>
[PubMed]

Marriott, H., Kabiljo, R., Hunt, G. P., Khleifat, A. A., Jones, A., Troakes, C., Pfaff, A. L., Quinn, J. P., Koks, S., Dobson, R. J., Schwab, P., Al-Chalabi, A., & Iacoangeli, A. (2023). Unsupervised machine learning identifies distinct ALS molecular subtypes in post-mortem motor cortex and blood expression data. *Acta Neuropathologica Communications*, 11(1), 208. <https://doi.org/10.1186/s40478-023-01686-8>
[PubMed]

Masini, E., Loi, E., Vega-Benedetti, A. F., Carta, M., Doneddu, G., Fadda, R., & Zavattari, P. (2020). An overview of the Main genetic, epigenetic and environmental factors involved in autism Spectrum disorder focusing on synaptic activity. *International Journal of Molecular Sciences*, 21(21). <https://doi.org/10.3390/ijms21218290>

Mayer, E. A. (2000). The neurobiology of stress and gastrointestinal disease. *Gut*, 47(6), 861–869. <https://doi.org/10.1136/gut.47.6.861>
[PubMed]

Mayer, E. A., Knight, R., Mazmanian, S. K., Cryan, J. F., & Tillisch, K. (2014). Gut microbes and the brain: Paradigm shift in neuroscience. *The Journal of Neuroscience*, 34(46), 15490–15496. <https://doi.org/10.1523/jneurosci.3299-14.2014>
[PubMed]

Mazzini, L., Mogna, L., De Marchi, F., Amoruso, A., Pane, M., Aloisio, I., Cionci, N. B., Gaggia, F., Lucenti, A., Bersano, E., Cantello, R., Di Gioia, D., & Mogna, G. (2018). Potential role of gut microbiota in ALS pathogenesis and possible novel therapeutic strategies. *Journal of Clinical Gastroenterology*, 52(Suppl 1). Proceedings from the 9th Probiotics, Prebiotics and New Foods, Nutraceuticals and Botanicals for Nutrition & Human and Microbiota Health Meeting, held in Rome, Italy from September 10 to 12, 2017, S68–S70. <https://doi.org/10.1097/mcg.0000000000001042>

Mazzone, L., Dooling, S. W., Volpe, E., Uljarević, M., Waters, J. L., Sabatini, A., Arturi, L., Abate, R., Riccioni, A., Siracusano, M., Pereira, M., Engstrand, L., Cristofori, F., Adduce, D., Francavilla, R., Costa-Mattioli, M., & Hardan, A. Y. (2024). Precision microbial intervention improves social behavior but not autism severity: A pilot double-blind randomized placebo-controlled trial. *Cell Host & Microbe*, 32(1), 106–116.e106. <https://doi.org/10.1016/j.chom.2023.11.021>

McGuinness, A. J., Davis, J. A., Dawson, S. L., Loughman, A., Collier, F., O'Hely, M., Simpson, C. A., Green, J., Marx, W., Hair, C., Guest, G., Mohebbi, M., Berk, M., Stupart, D., Watters, D., & Jacka, F. N. (2022). A systematic review of gut microbiota composition in observational studies of major depressive disorder, bipolar disorder and schizophrenia. *Molecular Psychiatry*, 27(4), 1920–1935. <https://doi.org/10.1038/s41380-022-01456-3>
[PubMed]

McIntyre, R. S., Subramaniapillai, M., Shekotikhina, M., Carmona, N. E., Lee, Y., Mansur, R. B., Brietzke, E., Fus, D., Coles, A. S., Iacobucci, M., Park, C., Potts, R., Amer, M., Gillard, J., James, C., Anglin, R., & Surette, M. G. (2021). Characterizing the gut microbiota in adults with bipolar disorder: A pilot study. *Nutritional Neuroscience*, 24(3), 173–180. <https://doi.org/10.1080/1028415x.2019.1612555>
[PubMed]

McVey, K. A. N., Mao, Y. K., Bienenstock, J., Foster, J. A., & Kunze, W. A. (2013). The microbiome is essential for normal gut intrinsic primary afferent neuron excitability in the mouse. *Neurogastroenterology and Motility*, 25(2), 183–e188. <https://doi.org/10.1111/nmo.12049>

Mead, R. J., Shan, N., Reiser, H. J., Marshall, F., & Shaw, P. J. (2023). Amyotrophic lateral sclerosis: A neurodegenerative disorder poised for successful therapeutic translation. *Nature Reviews. Drug Discovery*, 22(3), 185–212. <https://doi.org/10.1038/s41573-022-00612-2>
[PubMed]

Mejzini, R., Flynn, L. L., Pitout, I. L., Fletcher, S., Wilton, S. D., & Akkari, P. A. (2019). ALS genetics, mechanisms, and therapeutics: Where are we now? *Frontiers in Neuroscience*, 13, 1310. <https://doi.org/10.3389/fnins.2019.01310>
[PubMed]

Metcalf-Roach, A., Cirstea, M. S., Yu, A. C., Ramay, H. R., Coker, O., Boroomand, S., Kharazyan, F., Martino, D., Sycuro, L. K., Appel-Cresswell, S., & Finlay, B. B. (2024). Metagenomic analysis reveals large-scale disruptions of the gut microbiome in Parkinson's disease. *Movement Disorders*, 39(10), 1740–1751. <https://doi.org/10.1002/mds.29959>
[PubMed]

Mir, H. D., Yang, Q., Maximin, E., Montardy, Q., Ji, S., Cheng, Q., Shan, X., Wang, L., Naudon, L., Rabot, S., & Li, L. (2024). Indole induces anxiety-like behaviour in mice mediated by brainstem locus coeruleus activation. *Neurobiology of Disease*, 200, 106606. <https://doi.org/10.1016/j.nbd.2024.106606>
[PubMed]

Misera, A., Kaczmarczyk, M., Łoniewski, I., Liśkiewicz, P., Podsiadło, K., Misiak, B., Skonieczna-Żydecka, K., & Samochowiec, J. (2025). Comparative analysis of gut microbiota in major depressive

disorder and schizophrenia during hospitalisation – the case-control, post hoc study. *Psychoneuroendocrinology*, 171, 107208. <https://doi.org/10.1016/j.psyneuen.2024.107208> [PubMed]

Mishra, S. P., Jain, S., Wang, B., Wang, S., Miller, B. C., Lee, J. Y., Borlongan, C. V., Jiang, L., Pollak, J., Taraphder, S., Layden, B. T., Rane, S. G., & Yadav, H. (2024). Abnormalities in microbiota/butyrate/FFAR3 signaling in aging gut impair brain function. *JCI. Insight*, 9(3). <https://doi.org/10.1172/jci.insight.168443>

Misiak, B., Piotrowski, P., Cyran, A., Kowalski, K., Samochowiec, J., Jabłoński, M., Plichta, P., Łaczmański, Ł., Żebrowska, P., Kujawa, D., Łoniewski, I., & Kaczmarczyk, M. (2023). Gut microbiota alterations in stable outpatients with schizophrenia: Findings from a case-control study. *Acta Neuropsychiatrica*, 35(3), 147–155. <https://doi.org/10.1017/neu.2022.38> [PubMed]

Miyake, S., Kim, S., Suda, W., Oshima, K., Nakamura, M., Matsuoka, T., Chihara, N., Tomita, A., Sato, W., Kim, S. W., Morita, H., Hattori, M., & Yamamura, T. (2015). Dysbiosis in the gut microbiota of patients with multiple sclerosis, with a striking depletion of species belonging to clostridia XIVA and IV clusters. *PLoS One*, 10(9), e0137429. <https://doi.org/10.1371/journal.pone.0137429> [PubMed]

Möhle, L., Mattei, D., Heimesaat, M. M., Bereswill, S., Fischer, A., Alutis, M., French, T., Hambardzumyan, D., Matzinger, P., Dunay, I. R., & Wolf, S. A. (2016). Ly6C(hi) monocytes provide a link between antibiotic-induced changes in gut microbiota and adult hippocampal neurogenesis. *Cell Reports*, 15(9), 1945–1956. <https://doi.org/10.1016/j.celrep.2016.04.074> [PubMed]

Mohsen, E., Hafeez, H., Ahmed, S., Hamed, S., & El-Mahdy, T. S. (2025). Multiple sclerosis: A story of the interaction between gut microbiome and components of the immune system. *Molecular Neurobiology*, 62(6), 7762–7775. <https://doi.org/10.1007/s12035-025-04728-5> [PubMed]

Moran, G. W., & Thapaliya, G. (2021). The gut-brain axis and its role in controlling eating behavior in intestinal inflammation. *Nutrients*, 13(3). <https://doi.org/10.3390/nu13030981>

Moravejolahkami, A. R., Chitsaz, A., Hassanzadeh, A., & Paknahad, Z. (2023). Anti-inflammatory-antioxidant modifications and synbiotics improved health-related conditions in patients with progressive forms of multiple sclerosis: A single-center, randomized clinical trial. *Complementary Therapies in Clinical Practice*, 53, 101794. <https://doi.org/10.1016/j.ctcp.2023.101794> [PubMed]

Mörkl, S., Narrath, M., Schlotmann, D., Sallmutter, M. T., Putz, J., Lang, J., Brandstätter, A., Pilz, R., Karl Lackner, H., Goswami, N., Steuber, B., Tatzer, J., Lackner, S., Holasek, S., Painold, A., Jauk, E., Wenninger, J., Horvath, A., Spicher, N., et al. (2025). Multi-species probiotic supplement enhances vagal nerve function – results of a randomized controlled trial in patients with depression and healthy controls. *Gut Microbes*, 17(1), 2492377. <https://doi.org/10.1080/19490976.2025.2492377> [PubMed]

Mousavi-Shirazi-Fard, Z., Mazloom, Z., Izadi, S., & Fararouei, M. (2021). The effects of modified anti-inflammatory diet on fatigue, quality of life, and inflammatory biomarkers in relapsing-remitting multiple sclerosis patients: A randomized clinical trial. *The International Journal of Neuroscience*, 131(7), 657–665. <https://doi.org/10.1080/00207454.2020.1750398> [PubMed]

Mujahid, E. H., Limoa, E., Syamsuddin, S., Bahar, B., Renaldi, R., Aminuddin, A., & Lisal, S. T. (2022). Effect of probiotic adjuvant therapy on improvement of clinical symptoms & interleukin 6 levels in patients with schizophrenia. *Psychiatry Investigation*, 19(11), 898–908. <https://doi.org/10.30773/pi.2022.0064> [PubMed]

Murayama, R., Cai, Y., Nakamura, H., & Hashimoto, K. (2025). Demyelination in psychiatric and neurological disorders: Mechanisms, clinical impact, and novel therapeutic strategies. *Neuroscience and Biobehavioral Reviews*, 174, 106209. <https://doi.org/10.1016/j.neubiorev.2025.106209> [PubMed]

Nabakhteh, S., Lotfi, A., Afsartaha, A., Khodadadi, E. S., Abdolghaderi, S., Mohammadpour, M., Shokri, Y., Kiani, P., Ehtiati, S., Khakshournia, S., & Khatami, S. H. (2025). Nutritional interventions in amyotrophic lateral sclerosis: From ketogenic diet and neuroprotective nutrients to the microbiota-gut-brain Axis regulation. *Molecular Neurobiology*. <https://doi.org/10.1007/s12035-025-04830-8>

Nakhal, M. M., Yassin, L. K., Alyaqoubi, R., Saeed, S., Alderei, A., Alhammadi, A., Alshehhi, M., Almehairbi, A., Al Houqani, S., BaniYas, S., Qanadilo, H., Ali, B. R., Shehab, S., Statsenko, Y., Meribout, S., Sadek, B., Akour, A., & Hamad, M. I. K. (2024). The microbiota-gut-brain Axis and neurological disorders: A comprehensive review. *Life (Basel)*, 14(10). <https://doi.org/10.3390/life14101234>

Naseribafrouei, A., Hestad, K., Avershina, E., Sekelja, M., Linløkken, A., Wilson, R., & Rudi, K. (2014). Correlation between the human fecal microbiota and depression. *Neurogastroenterology and Motility*, 26(8), 1155–1162. <https://doi.org/10.1111/nmo.12378> [PubMed]

Nemati, M. H., Yazdanpanah, E., Kazemi, R., Orooji, N., Dadfar, S., Oksenysh, V., & Haghmorad, D. (2025). Microbiota-driven mechanisms in multiple sclerosis: Pathogenesis, therapeutic strategies, and biomarker potential. *Biology-Basel*, 14(4). <https://doi.org/10.3390/biology14040435>

Nepon, J., Belik, S. L., Bolton, J., & Sareen, J. (2010). The relationship between anxiety disorders and suicide attempts: Findings from the National Epidemiologic Survey on alcohol and related conditions. *Depression and Anxiety*, 27(9), 791–798. <https://doi.org/10.1002/da.20674> [PubMed]

Neufeld, K. M., Kang, N., Bienenstock, J., & Foster, J. A. (2011). Reduced anxiety-like behavior and central neurochemical change in germ-free mice. *Neurogastroenterology and Motility*, 23(3), 255–264, e119. <https://doi.org/10.1111/j.1365-2982.2010.01620.x> [PubMed]

Niccolai, E., Martinelli, I., Quaranta, G., Nannini, G., Zucchi, E., De Maio, F., Gianferrari, G., Bibbò, S.,

Cammarota, G., Mandrioli, J., Masucci, L., & Amedei, A. (2024). Fecal microbiota transplantation in amyotrophic lateral sclerosis: Clinical protocol and evaluation of microbiota immunity Axis. *Methods in Molecular Biology*, 2761, 373–396. https://doi.org/10.1007/978-1-0716-3662-6_27 [PubMed]

Nicholson, K., Bjornevik, K., Abu-Ali, G., Chan, J., Cortese, M., Dedi, B., Jeon, M., Xavier, R., Huttenhower, C., Ascherio, A., & Berry, J. D. (2021). The human gut microbiota in people with amyotrophic lateral sclerosis. *Amyotroph Lateral Scler Frontotemporal Degener*, 22(3–4), 186–194. <https://doi.org/10.1080/21678421.2020.1828475> [PubMed]

Nigg, J. T., & Casey, B. J. (2005). An integrative theory of attention-deficit/ hyperactivity disorder based on the cognitive and affective neurosciences. *Development and Psychopathology*, 17(3), 785–806. <https://doi.org/10.1017/s0954579405050376> [PubMed]

Nikdasti, A., Khodadadi, E. S., Ferdosi, F., Dadgostar, E., Yahyazadeh, S., Heidari, P., Ehtiati, S., Vakili, O., & Khatami, S. H. (2025). Nutritional strategies in major depression disorder: From ketogenic diet to modulation of the microbiota-gut-brain Axis. *Molecular Neurobiology*, 62(3), 2973–2994. <https://doi.org/10.1007/s12035-024-04446-4> [PubMed]

Nikolaus, S., Müller, H. W., & Hautzel, H. (2017). Different patterns of dopaminergic and serotonergic dysfunction in manic, depressive and euthymic phases of bipolar disorder. *Nuklearmedizin*, 56(5), 191–200. <https://doi.org/10.3413/Nukmed-0893-17-04>. (Verschiedene Muster dopaminerger und serotonerger Dysfunktion in manischen, depressiven und euthymischen Phasen der bipolaren Störungen.) [PubMed]

Nikolova, V. L., Smith, M. R. B., Hall, L. J., Cleare, A. J., Stone, J. M., & Young, A. H. (2021). Perturbations in gut microbiota composition in psychiatric disorders: A review and meta-analysis. *JAMA Psychiatry*, 78(12), 1343–1354. <https://doi.org/10.1001/jamapsychiatry.2021.2573> [PubMed]

Nikolova, V. L., Cleare, A. J., Young, A. H., & Stone, J. M. (2023). Acceptability, tolerability, and estimates of putative treatment effects of probiotics as adjunctive treatment in patients with depression: A randomized clinical trial. *JAMA Psychiatry*, 80(8), 842–847. <https://doi.org/10.1001/jamapsychiatry.2023.1817> [PubMed]

Nikolova, V. L., Cleare, A. J., Young, A. H., & Stone, J. M. (2025). Exploring the mechanisms of action of probiotics in depression: Results from a randomized controlled pilot trial. *Journal of Affective Disorders*, 376, 241–250. <https://doi.org/10.1016/j.jad.2025.01.153> [PubMed]

Nishiwaki, H., Ueyama, J., Ito, M., Hamaguchi, T., Takimoto, K., Maeda, T., Kashiwara, K., Tsuboi, Y., Mori, H., Kurokawa, K., Katsuno, M., Hirayama, M., & Ohno, K. (2024). Meta-analysis of shotgun sequencing of gut microbiota in Parkinson's disease. *npj Parkinson's Disease*, 10. <https://doi.org/10.1038/s41531-024-00724-z>

Nishiyama, H., Knopfel, T., Endo, S., & Itohara, S. (2002). Glial protein S100B modulates long-term neuronal synaptic plasticity. *Proceedings of the National Academy of Sciences of the United States of America*, 99(6), 4037–4042. <https://doi.org/10.1073/pnas.052020999>
[PubMed]

O'Callaghan, A., & van Sinderen, D. (2016). Bifidobacteria and their role as members of the human gut microbiota. *Frontiers in Microbiology*, 7, 925. <https://doi.org/10.3389/fmicb.2016.00925>
[PubMed]

Ochoa-Repáraz, J., Mielcarz, D. W., Ditrio, L. E., Burroughs, A. R., Foureau, D. M., Haque-Begum, S., & Kasper, L. H. (2009). Role of gut commensal microflora in the development of experimental autoimmune encephalomyelitis. *Journal of Immunology*, 183(10), 6041–6050. <https://doi.org/10.4049/jimmunol.0900747>

Ogbonnaya, E. S., Clarke, G., Shanahan, F., Dinan, T. G., Cryan, J. F., & O'Leary, O. F. (2015). Adult hippocampal neurogenesis is regulated by the microbiome. *Biological Psychiatry*, 78(4), e7–e9. <https://doi.org/10.1016/j.biopsych.2014.12.023>
[PubMed]

O'Hara, A. M., & Shanahan, F. (2006). The gut flora as a forgotten organ. *EMBO Reports*, 7(7), 688–693. <https://doi.org/10.1038/sj.embor.7400731>
[PubMed]

Okubo, R., Koga, M., Katsumata, N., Odamaki, T., Matsuyama, S., Oka, M., Narita, H., Hashimoto, N., Kusumi, I., Xiao, J., & Matsuoka, Y. J. (2019). Effect of bifidobacterium breve A-1 on anxiety and depressive symptoms in schizophrenia: A proof-of-concept study. *Journal of Affective Disorders*, 245, 377–385. <https://doi.org/10.1016/j.jad.2018.11.011>
[PubMed]

Olde Loohuis, L. M., Mangul, S., Ori, A. P. S., Jospin, G., Koslicki, D., Yang, H. T., Wu, T., Boks, M. P., Lomen-Hoerth, C., Wiedau-Pazos, M., Cantor, R. M., de Vos, W. M., Kahn, R. S., Eskin, E., & Ophoff, R. A. (2018). Transcriptome analysis in whole blood reveals increased microbial diversity in schizophrenia. *Translational Psychiatry*, 8(1), 96. <https://doi.org/10.1038/s41398-018-0107-9>
[PubMed]

O'Leary, O. F., Ogbonnaya, E. S., Felice, D., Levone, B. R., Conroy, L. C., Fitzgerald, P., Bravo, J. A., Forsythe, P., Bienenstock, J., Dinan, T. G., & Cryan, J. F. (2018). The vagus nerve modulates BDNF expression and neurogenesis in the hippocampus. *European Neuropsychopharmacology*, 28(2), 307–316. <https://doi.org/10.1016/j.euroneuro.2017.12.004>
[PubMed]

O'Riordan, K. J., Moloney, G. M., Keane, L., Clarke, G., & Cryan, J. F. (2025). The gut microbiota-immune-brain axis: Therapeutic implications. *Cell Reports Medicine*, 6(3), 101982. <https://doi.org/10.1016/j.xcrm.2025.101982>
[PubMed]

Ortega, M. A., Álvarez-Mon, M. A., García-Montero, C., Fraile-Martínez, Ó., Monserrat, J., Martínez-Rozas, L., Rodríguez-Jiménez, R., Álvarez-Mon, M., & Lahera, G. (2023). Microbiota-gut-brain axis

mechanisms in the complex network of bipolar disorders: Potential clinical implications and translational opportunities. *Molecular Psychiatry*, 28(7), 2645–2673. <https://doi.org/10.1038/s41380-023-01964-w>
[PubMed]

Painold, A., Mörk, S., Kashofer, K., Halwachs, B., Dalkner, N., Bengesser, S., Birner, A., Fellendorf, F., Platzer, M., Queissner, R., Schütze, G., Schwarz, M. J., Moll, N., Holzer, P., Holl, A. K., Kapfhammer, H. P., Gorkiewicz, G., & Reininghaus, E. Z. (2019). A step ahead: Exploring the gut microbiota in inpatients with bipolar disorder during a depressive episode. *Bipolar Disorders*, 21(1), 40–49. <https://doi.org/10.1111/bdi.12682>
[PubMed]

Pan, R., Zhang, X., Gao, J., Yi, W., Wei, Q., & Su, H. (2020). Analysis of the diversity of intestinal microbiome and its potential value as a biomarker in patients with schizophrenia: A cohort study. *Psychiatry Research*, 291, 113260. <https://doi.org/10.1016/j.psychres.2020.113260>
[PubMed]

Panzetta, M. E., & Valdivia, R. H. (2024). Akkermansia in the gastrointestinal tract as a modifier of human health. *Gut Microbes*, 16(1), 2406379. <https://doi.org/10.1080/19490976.2024.2406379>
[PubMed]

Park, S. H., Lee, J. H., Shin, J., Kim, J. S., Cha, B., Lee, S., Kwon, K. S., Shin, Y. W., & Choi, S. H. (2021). Cognitive function improvement after fecal microbiota transplantation in Alzheimer's dementia patient: A case report. *Current Medical Research and Opinion*, 37(10), 1739–1744. <https://doi.org/10.1080/03007995.2021.1957807>
[PubMed]

Park, S. H., Lee, J. H., Kim, J. S., Kim, T. J., Shin, J., Im, J. H., Cha, B., Lee, S., Kwon, K. S., Shin, Y. W., Ko, S. B., & Choi, S. H. (2022). Fecal microbiota transplantation can improve cognition in patients with cognitive decline and Clostridioides difficile infection. *Aging (Albany NY)*, 14(16), 6449–6466. <https://doi.org/10.18632/aging.204230>
[PubMed]

Partrick, K. A., Chassaing, B., Beach, L. Q., McCann, K. E., Gewirtz, A. T., & Huhman, K. L. (2018). Acute and repeated exposure to social stress reduces gut microbiota diversity in Syrian hamsters. *Behavioural Brain Research*, 345, 39–48. <https://doi.org/10.1016/j.bbr.2018.02.005>
[PubMed]

Pärty, A., Kalliomäki, M., Wacklin, P., Salminen, S., & Isolauri, E. (2015). A possible link between early probiotic intervention and the risk of neuropsychiatric disorders later in childhood: A randomized trial. *Pediatric Research*, 77(6), 823–828. <https://doi.org/10.1038/pr.2015.51>
[PubMed]

Penninx, B. W., Pine, D. S., Holmes, E. A., & Reif, A. (2021). Anxiety disorders. *Lancet*, 397(10277), 914–927. [https://doi.org/10.1016/s0140-6736\(21\)00359-7](https://doi.org/10.1016/s0140-6736(21)00359-7)
[PubMed]

Perez-Pardo, P., Dodiya, H. B., Broersen, L. M., Douna, H., van Wijk, N., Lopes da Silva, S., Garssen, J., Keshavarzian, A., & Kraneveld, A. D. (2018). Gut-brain and brain-gut axis in Parkinson's disease

models: Effects of a uridine and fish oil diet. *Nutritional Neuroscience*, 21(6), 391–402. <https://doi.org/10.1080/1028415x.2017.1294555>
[PubMed]

Phan, J., Calvo, D. C., Nair, D., Jain, S., Montagne, T., Dietsche, S., Blanchard, K., Treadwell, S., Adams, J., & Krajmalnik-Brown, R. (2024). Precision synbiotics increase gut microbiome diversity and improve gastrointestinal symptoms in a pilot open-label study for autism spectrum disorder. *mSystems*, 9(5), e0050324. <https://doi.org/10.1128/msystems.00503-24>
[PubMed]

Phillips, M. L., & Swartz, H. A. (2014). A critical appraisal of neuroimaging studies of bipolar disorder: Toward a new conceptualization of underlying neural circuitry and a road map for future research. *The American Journal of Psychiatry*, 171(8), 829–843. <https://doi.org/10.1176/appi.ajp.2014.13081008>
[PubMed]

Popova, N. K., & Naumenko, V. S. (2019). Neuronal and behavioral plasticity: The role of serotonin and BDNF systems tandem. *Expert Opinion on Therapeutic Targets*, 23(3), 227–239. <https://doi.org/10.1080/14728222.2019.1572747>
[PubMed]

Posner, J., Polanczyk, G. V., & Sonuga-Barke, E. (2020). Attention-deficit hyperactivity disorder. *Lancet*, 395(10222), 450–462. [https://doi.org/10.1016/s0140-6736\(19\)33004-1](https://doi.org/10.1016/s0140-6736(19)33004-1)
[PubMed]

Prajapati, S. K., Shah, R., Alford, N., Mishra, S. P., Jain, S., Hansen, B., Sanberg, P., Molina, A. J. A., & Yadav, H. (2023). The triple Alliance: Microbiome, mitochondria, and metabolites in the context of age-related cognitive decline and Alzheimer's disease. *The Journals of Gerontology. Series A, Biological Sciences and Medical Sciences*, 78(12), 2187–2202. <https://doi.org/10.1093/gerona/glad226>
[PubMed]

Prehn-Kristensen, A., Zimmermann, A., Tittmann, L., Lieb, W., Schreiber, S., Baving, L., & Fischer, A. (2018). Reduced microbiome alpha diversity in young patients with ADHD. *PLoS One*, 13(7), e0200728. <https://doi.org/10.1371/journal.pone.0200728>
[PubMed]

Rajachandrakumar, R., & Finlayson, M. (2022). Multiple sclerosis caregiving: A systematic scoping review to map current state of knowledge. *Health & Social Care in the Community*, 30(4), e874–e897. <https://doi.org/10.1111/hsc.13687>

Ramadan, Y. N., Alqifari, S. F., Alshehri, K., Alhowiti, A., Mirghani, H., Alrasheed, T., Aljohani, F., Alghamdi, A., & Hetta, H. F. (2025). Microbiome gut-brain-Axis: Impact on brain development and mental health. *Molecular Neurobiology*. <https://doi.org/10.1007/s12035-025-04846-0>

Rao, M., & Gershon, M. D. (2016). The bowel and beyond: The enteric nervous system in neurological disorders. *Nature Reviews. Gastroenterology & Hepatology*, 13(9), 517–528. <https://doi.org/10.1038/nrgastro.2016.107>

Rea, K., Dinan, T. G., & Cryan, J. F. (2016). The microbiome: A key regulator of stress and neuroinflammation. *Neurobiology of Stress*, 4, 23–33. <https://doi.org/10.1016/j.ynstr.2016.03.001>
[PubMed]

Reininghaus, E. Z., Wetzlmair, L. C., Fellendorf, F. T., Platzer, M., Queissner, R., Birner, A., Pilz, R., Hamm, C., Maget, A., Koidl, C., Riedrich, K., Klampfer, K., Ferk, K., & Dalkner, N. (2018). The impact of probiotic supplements on cognitive parameters in euthymic individuals with bipolar disorder: A pilot study. *Neuropsychobiology*, 1–8. <https://doi.org/10.1159/000492537>

Restrepo, B., Angkustsiri, K., Taylor, S. L., Rogers, S. J., Cabral, J., Heath, B., Hechtman, A., Solomon, M., Ashwood, P., Amaral, D. G., & Nordahl, C. W. (2020). Developmental-behavioral profiles in children with autism spectrum disorder and co-occurring gastrointestinal symptoms. *Autism Research*, 13(10), 1778–1789. <https://doi.org/10.1002/aur.2354>
[PubMed]

Rhee, S. H., Pothoulakis, C., & Mayer, E. A. (2009). Principles and clinical implications of the brain-gut-enteric microbiota axis. *Nature Reviews. Gastroenterology & Hepatology*, 6(5), 306–314. <https://doi.org/10.1038/nrgastro.2009.35>

Ribera, C., Sánchez-Ortí, J. V., Clarke, G., Marx, W., Mörk, S., & Balanzá-Martínez, V. (2024). Probiotic, prebiotic, synbiotic and fermented food supplementation in psychiatric disorders: A systematic review of clinical trials. *Neuroscience and Biobehavioral Reviews*, 158, 105561. <https://doi.org/10.1016/j.neubiorev.2024.105561>
[PubMed]

Rinaman, L. (2010). Ascending projections from the caudal visceral nucleus of the solitary tract to brain regions involved in food intake and energy expenditure. *Brain Research*, 1350, 18–34. <https://doi.org/10.1016/j.brainres.2010.03.059>
[PubMed]

Rocha, A. L., Costa, A., Garrett, M. C., & Meireles, J. (2018). Difficult case of a rare form of familial Alzheimer's disease with PSEN1 P117L mutation. *BML Case Reports*, 11(1). <https://doi.org/10.1136/bcr-2018-226664>

Ronchi, G., Ryu, V., Fornaro, M., & Czaja, K. (2012). Hippocampal plasticity after a vagus nerve injury in the rat. *Neural Regeneration Research*, 7(14), 1055–1063. <https://doi.org/10.3969/j.issn.1673-5374.2012.14.003>
[PubMed]

Rong, H., Xie, X. H., Zhao, J., Lai, W. T., Wang, M. B., Xu, D., Liu, Y. H., Guo, Y. Y., Xu, S. X., Deng, W. F., Yang, Q. F., Xiao, L., Zhang, Y. L., He, F. S., Wang, S., & Liu, T. B. (2019). Similarly in depression, nuances of gut microbiota: Evidences from a shotgun metagenomics sequencing study on major depressive disorder versus bipolar disorder with current major depressive episode patients. *Journal of Psychiatric Research*, 113, 90–99. <https://doi.org/10.1016/j.jpsychires.2019.03.017>
[PubMed]

Roosendaal, B., Williams, C. L., & McGaugh, J. L. (1999). Glucocorticoid receptor activation in the rat nucleus of the solitary tract facilitates memory consolidation: Involvement of the basolateral

amygdala. *The European Journal of Neuroscience*, 11(4), 1317–1323. <https://doi.org/10.1046/j.1460-9568.1999.00537.x>
[PubMed]

Rosshart, S. P., Vassallo, B. G., Angeletti, D., Hutchinson, S. D., Morgan, A. P., Takeda, K., Hickman, H. D., McCulloch, J. A., Badger, J. H., Ajami, N. J., Trinchieri, G., Pardo-Manuel de Villena, F., Yewdell, J. W., & Rehermann, B. (2017). Wild mouse gut microbiota promotes host fitness and improves disease resistance. *Cell*, 171(5), 1015–1028.e13. <https://doi.org/10.1016/j.cell.2017.09.016>

Round, J. L., Lee, S. M., Li, J., Tran, G., Jabri, B., Chatila, T. A., & Mazmanian, S. K. (2011). The Toll-like receptor 2 pathway establishes colonization by a commensal of the human microbiota. *Science*, 332(6032), 974–977. <https://doi.org/10.1126/science.1206095>
[PubMed]

Rowin, J., Xia, Y., Jung, B., & Sun, J. (2017). Gut inflammation and dysbiosis in human motor neuron disease. *Physiological Reports*, 5(18). <https://doi.org/10.14814/phy2.13443>

Rusina, R., Vandenberghe, R., & Bruffaerts, R. (2021). Cognitive and behavioral manifestations in ALS: Beyond motor system involvement. *Diagnostics (Basel)*, 11(4). <https://doi.org/10.3390/diagnostics11040624>

Sandgren, A. M., & Brummer, R. J. M. (2018). ADHD-originating in the gut? The emergence of a new explanatory model. *Medical Hypotheses*, 120, 135–145. <https://doi.org/10.1016/j.mehy.2018.08.022>
[PubMed]

Santomauro, D. F., Mantilla Herrera, A. M., Shadid, J., Zheng, P., Ashbaugh, C., Pigott, D. M., Abbafati, C., Adolph, C., Amlag, J. O., Aravkin, A. Y., Bang-Jensen, B. L., Bertolacci, G. J., Bloom, S. S., Castellano, R., Castro, E., Chakrabarti, S., Chattopadhyay, J., Cogen, R. M., Collins, J. K., et al. (2021). Global prevalence and burden of depressive and anxiety disorders in 204 countries and territories in 2020 due to the COVID-19 pandemic. *The Lancet*, 398(10312), 1700–1712. [https://doi.org/10.1016/S0140-6736\(21\)02143-7](https://doi.org/10.1016/S0140-6736(21)02143-7)

Sawada, N., Kotani, T., Konno, T., Setiawan, J., Nishigaito, Y., Saito, Y., Murata, Y., Nibu, K. I., & Matozaki, T. (2018). Regulation by commensal bacteria of neurogenesis in the subventricular zone of adult mouse brain. *Biochemical and Biophysical Research Communications*, 498(4), 824–829. <https://doi.org/10.1016/j.bbrc.2018.03.064>
[PubMed]

Saxby, S. M., Shemirani, F., Crippes, L. J., Ehlinger, M. A., Brooks, L., Bisht, B., Titcomb, T. J., Rubenstein, L. M., Eyck, P. T., Hoth, K. F., Gill, C., Kamholz, J., Snetselaar, L. G., & Wahls, T. L. (2024). Effects of a remote multimodal intervention involving diet, walking program, and breathing exercise on quality of life among newly diagnosed people with multiple sclerosis: A quasi-experimental non-inferiority pilot study. *Degenerative Neurological and Neuromuscular Disease*, 14, 1–14. <https://doi.org/10.2147/dnnd.S441738>
[PubMed]

Schaub, A. C., Schneider, E., Vazquez-Castellanos, J. F., Schweinfurth, N., Kettelhack, C., Doll, J. P. K., Yamanbaeva, G., Mählmann, L., Brand, S., Beglinger, C., Borgwardt, S., Raes, J., Schmidt, A., & Lang, U.

E. (2022). Clinical, gut microbial and neural effects of a probiotic add-on therapy in depressed patients: A randomized controlled trial. *Translational Psychiatry*, 12(1), 227. <https://doi.org/10.1038/s41398-022-01977-z>
[PubMed]

Scheperjans, F., Levo, R., Bosch, B., Lääperi, M., Pereira, P. A. B., Smolander, O. P., Aho, V. T. E., Vetkas, N., Toivio, L., Kainulainen, V., Fedorova, T. D., Lahtinen, P., Ortiz, R., Kaasinen, V., Satokari, R., & Arkkila, P. (2024). Fecal microbiota transplantation for treatment of Parkinson disease: A randomized clinical trial. *JAMA Neurology*, 81(9), 925–938. <https://doi.org/10.1001/jamaneurol.2024.2305>
[PubMed]

Schepici, G., Silvestro, S., Bramanti, P., & Mazzon, E. (2019). The gut microbiota in multiple sclerosis: An overview of clinical trials. *Cell Transplantation*, 28(12), 1507–1527. <https://doi.org/10.1177/0963689719873890>
[PubMed]

Schmitt, L. M., Smith, E. G., Pedapati, E. V., Horn, P. S., Will, M., Lamy, M., Barber, L., Trebley, J., Meyer, K., Heiman, M., West, K. H. J., Hughes, P., Ahuja, S., & Erickson, C. A. (2023). Results of a phase Ib study of SB-121, an investigational probiotic formulation, a randomized controlled trial in participants with autism spectrum disorder. *Scientific Reports*, 13(1), 5192. <https://doi.org/10.1038/s41598-023-30909-0>
[PubMed]

Scholefield, M., Church, S. J., Taylor, G., Knight, D., Unwin, R. D., & Cooper, G. J. S. (2023). Multi-regional alterations in glucose and purine metabolic pathways in the Parkinson's disease dementia brain. *npj Parkinson's Disease*, 9(1), 66. <https://doi.org/10.1038/s41531-023-00488-y>
[PubMed]

Schwarz, E., Maukonen, J., Hyytiäinen, T., Kieseppä, T., Orešič, M., Sabunciyan, S., Mantere, O., Saarela, M., Yolken, R., & Suvisaari, J. (2018). Analysis of microbiota in first episode psychosis identifies preliminary associations with symptom severity and treatment response. *Schizophrenia Research*, 192, 398–403. <https://doi.org/10.1016/j.schres.2017.04.017>
[PubMed]

Sen, P., Prandovszky, E., Honkanen, J. K., Chen, O., Yolken, R., & Suvisaari, J. (2024). Dysregulation of microbiota in patients with first-episode psychosis is associated with symptom severity and treatment response. *Biological Psychiatry*, 95(4), 370–379. <https://doi.org/10.1016/j.biopsych.2023.10.024>
[PubMed]

Seo, D. O., & Holtzman, D. M. (2024). Current understanding of the Alzheimer's disease-associated microbiome and therapeutic strategies. *Experimental & Molecular Medicine*, 56(1), 86–94. <https://doi.org/10.1038/s12276-023-01146-2>

Seo, D. O., Boros, B. D., & Holtzman, D. M. (2019). The microbiome: A target for Alzheimer disease? *Cell Research*, 29(10), 779–780. <https://doi.org/10.1038/s41422-019-0227-7>
[PubMed]

Seo, D. O., O'Donnell, D., Jain, N., Ulrich, J. D., Herz, J., Li, Y., Lemieux, M., Cheng, J., Hu, H., Serrano, J. R., Bao, X., Franke, E., Karlsson, M., Meier, M., Deng, S., Desai, C., Dodiya, H., Lelwala-Guruge, J., Handley, S. A., et al. (2023). ApoE isoform- and microbiota-dependent progression of neurodegeneration in a mouse model of tauopathy. *Science*, 379(6628), eadd1236. <https://doi.org/10.1126/science.add1236> [PubMed]

Sepede, G., Chiacchiaretta, P., Gambi, F., Di Iorio, G., De Berardis, D., Ferretti, A., Perrucci, M. G., & Di Giannantonio, M. (2020). Bipolar disorder with and without a history of psychotic features: fMRI correlates of sustained attention. *Progress in Neuro-Psychopharmacology & Biological Psychiatry*, 98, 109817. <https://doi.org/10.1016/j.pnpbp.2019.109817>

Sepehrmanesh, Z., Shahzeidi, A., Mansournia, M. A., Ghaderi, A., & Ahmadvand, A. (2021). Clinical and metabolic reaction to probiotic supplement in children suffering attention-deficit hyperactivity disorder: A randomized, double-blind, placebo-controlled experiment. *International Archives of Health Sciences*, 8(2), 90–96. <https://doi.org/10.4103/iahs.iahs.112.20>

Sgritta, M., Dooling, S. W., Buffington, S. A., Momin, E. N., Francis, M. B., Britton, R. A., & Costa-Mattioli, M. (2019). Mechanisms underlying microbial-mediated changes in social behavior in mouse models of autism Spectrum disorder. *Neuron*, 101(2), 246–259.e246. <https://doi.org/10.1016/j.neuron.2018.11.018> [PubMed]

Shaaban, S. Y., El Gendy, Y. G., Mehanna, N. S., El-Senousy, W. M., El-Feki, H. S. A., Saad, K., & El-Asheer, O. M. (2018). The role of probiotics in children with autism spectrum disorder: A prospective, open-label study. *Nutritional Neuroscience*, 21(9), 676–681. <https://doi.org/10.1080/1028415x.2017.1347746> [PubMed]

Shah, A., Panchal, V., Patel, K., Alimohamed, Z., Kaka, N., Sethi, Y., & Patel, N. (2023). Pathogenesis and management of multiple sclerosis revisited. *Disease-a-Month*, 69(9), 101497. <https://doi.org/10.1016/j.disamonth.2022.101497> [PubMed]

Shi, L., Ju, P., Meng, X., Wang, Z., Yao, L., Zheng, M., Cheng, X., Li, J., Yu, T., Xia, Q., Yan, J., Zhu, C., & Zhang, X. (2023). Intricate role of intestinal microbe and metabolite in schizophrenia. *BMC Psychiatry*, 23(1), 856. <https://doi.org/10.1186/s12888-023-05329-z> [PubMed]

Shin, H. C., Jo, B. G., Lee, C. Y., Lee, K. W., & Namgung, U. (2019). Hippocampal activation of 5-HT(1B) receptors and BDNF production by vagus nerve stimulation in rats under chronic restraint stress. *The European Journal of Neuroscience*, 50(1), 1820–1830. <https://doi.org/10.1111/ejn.14368> [PubMed]

Simpson, C. A., Diaz-Arteche, C., Eliaby, D., Schwartz, O. S., Simmons, J. G., & Cowan, C. S. M. (2021). The gut microbiota in anxiety and depression – A systematic review. *Clinical Psychology Review*, 83, 101943. <https://doi.org/10.1016/j.cpr.2020.101943> [PubMed]

Singh, V., Roth, S., Llovera, G., Sadler, R., Garzetti, D., Stecher, B., Dichgans, M., & Liesz, A. (2016). Microbiota dysbiosis controls the Neuroinflammatory response after stroke. *The Journal of Neuroscience*, 36(28), 7428–7440. <https://doi.org/10.1523/jneurosci.1114-16.2016> [PubMed]

Skott, E., Yang, L. L., Stiernborg, M., Söderström, Å., Rùegg, J., Schalling, M., Forsell, Y., Giacobini, M., & Lavebratt, C. (2020). Effects of a synbiotic on symptoms, and daily functioning in attention deficit hyperactivity disorder – A double-blind randomized controlled trial. *Brain, Behavior, and Immunity*, 89, 9–19. <https://doi.org/10.1016/j.bbi.2020.05.056> [PubMed]

Society, A. S. (2025). *Risk factors for Alzheimer's disease*. Retrieved June 02, from <https://www.alzheimers.org.uk/about-dementia/types-dementia/who-gets-alzheimers-disease>

Son, J. S., Zheng, L. J., Rowehl, L. M., Tian, X., Zhang, Y., Zhu, W., Litcher-Kelly, L., Gadow, K. D., Gathungu, G., Robertson, C. E., Ir, D., Frank, D. N., & Li, E. (2015). Comparison of fecal microbiota in children with autism spectrum disorders and neurotypical siblings in the Simons Simplex Collection. *PLOS ONE*, 10(10), e0137725. <https://doi.org/10.1371/journal.pone.0137725>

Soriano, S., Curry, K., Wang, Q., Chow, E., Treangen, T. J., & Villapol, S. (2022). Fecal microbiota transplantation derived from Alzheimer's disease mice worsens brain trauma outcomes in wild-type controls. *International Journal of Molecular Sciences*, 23(9). <https://doi.org/10.3390/ijms23094476>

Spencer, P. S., Lagrange, E., & Camu, W. (2019). ALS and environment: Clues from spatial clustering? *Revue Neurologique (Paris)*, 175(10), 652–663. <https://doi.org/10.1016/j.neurol.2019.04.007>

Stahl, E. A., Breen, G., Forstner, A. J., McQuillin, A., Ripke, S., Trubetskoy, V., Mattheisen, M., Wang, Y., Coleman, J. R. I., Gaspar, H. A., de Leeuw, C. A., Steinberg, S., Pavlides, J. M. W., Trzaskowski, M., Byrne, E. M., Pers, T. H., Holmans, P. A., Richards, A. L., Abbott, L., et al. (2019). Genome-wide association study identifies 30 loci associated with bipolar disorder. *Nature Genetics*, 51(5), 793–803. <https://doi.org/10.1038/s41588-019-0397-8> [PubMed]

Stanley, D., Moore, R. J., & Wong, C. H. Y. (2018). An insight into intestinal mucosal microbiota disruption after stroke. *Scientific Reports*, 8(1), 568. <https://doi.org/10.1038/s41598-017-18904-8> [PubMed]

Stiernborg, M., Debelius, J. W., Yang, L. L., Skott, E., Millischer, V., Giacobini, M., Melas, P. A., Boulund, F., & Lavebratt, C. (2023). Bacterial gut microbiome differences in adults with ADHD and in children with ADHD on psychostimulant medication. *Brain, Behavior, and Immunity*, 110, 310–321. <https://doi.org/10.1016/j.bbi.2023.03.012> [PubMed]

Storoni, M., & Plant, G. T. (2015). The therapeutic potential of the ketogenic diet in treating progressive multiple sclerosis. *Multiple Sclerosis International*, 2015, 681289. <https://doi.org/10.1155/2015/681289>

[PubMed]

Straus Farber, R., Walker, E. L., Diallo, F., Onomichi, K., Riley, C., Zhang, L., Zhu, W., De Jager, P. L., & Xia, Z. (2024). A randomized cross-over trial of prebiotics and probiotics in multiple sclerosis: Trial feasibility, supplement tolerability and symptom abatement. *Multiple Sclerosis and Related Disorders*, 89, 105762. <https://doi.org/10.1016/j.msard.2024.105762>

[PubMed]

Sudo, N., Chida, Y., Aiba, Y., Sonoda, J., Oyama, N., Yu, X. N., Kubo, C., & Koga, Y. (2004). Postnatal microbial colonization programs the hypothalamic-pituitary-adrenal system for stress response in mice. *The Journal of Physiology*, 558(Pt 1), 263–275. <https://doi.org/10.1113/jphysiol.2004.063388>

[PubMed]

[PubMed]

Sun, H., Zhao, F., Liu, Y., Ma, T., Jin, H., Quan, K., Leng, B., Zhao, J., Yuan, X., Li, Z., Li, F., Kwok, L. Y., Zhang, S., Sun, Z., Zhang, J., & Zhang, H. (2022). Probiotics synergized with conventional regimen in managing Parkinson's disease. *npj Parkinson's Disease*, 8(1), 62. <https://doi.org/10.1038/s41531-022-00327-6>

[PubMed]

Sun, K., Li, Y., Zhai, Z., Yin, H., Liang, S., Zhai, F., Cui, Y., & Zhang, G. (2024). Effects of transcutaneous auricular vagus nerve stimulation and exploration of brain network mechanisms in children with high-functioning autism spectrum disorder: Study protocol for a randomized controlled trial. *Frontiers in Psychiatry*, 15, 1337101. <https://doi.org/10.3389/fpsyt.2024.1337101>

[PubMed]

[PubMed]

Takeda, K., & Akira, S. (2004). Microbial recognition by Toll-like receptors. *Journal of Dermatological Science*, 34(2), 73–82. <https://doi.org/10.1016/j.jdermsci.2003.10.002>

[PubMed]

Tamtaji, O. R., Taghizadeh, M., Daneshvar Kakhaki, R., Kouchaki, E., Bahmani, F., Borzabadi, S., Oryan, S., Mafi, A., & Asemi, Z. (2019). Clinical and metabolic response to probiotic administration in people with Parkinson's disease: A randomized, double-blind, placebo-controlled trial. *Clinical Nutrition*, 38(3), 1031–1035. <https://doi.org/10.1016/j.clnu.2018.05.018>

[PubMed]

Tan, A. H., Lim, S. Y., Chong, K. K., Manap, M. A. A. A., Hor, J. W., Lim, J. L., Low, S. C., Chong, C. W., Mahadeva, S., & Lang, A. E. (2021). Probiotics for constipation in Parkinson disease: A randomized placebo-controlled study. *Neurology*, 96(5), e772–e782. <https://doi.org/10.1212/wnl.000000000010998>

[PubMed]

[PubMed]

Tarutani, S., Omori, M., Ido, Y., Yano, M., Komatsu, T., & Okamura, T. (2022). Effects of 4G-beta-D-Galactosylsucrose in patients with depression: A randomized, double-blinded, placebo-controlled, parallel-group comparative study. *Journal of Psychiatric Research*, 148, 110–120. <https://doi.org/10.1016/j.jpsychires.2022.01.059>

[PubMed]

[PubMed]

Thaiss, C. A., Zeevi, D., Levy, M., Zilberman-Schapira, G., Suez, J., Tengeler, A. C., Abramson, L., Katz,

M. N., Korem, T., Zmora, N., Kuperman, Y., Biton, I., Gilad, S., Harmelin, A., Shapiro, H., Halpern, Z., Segal, E., & Elinav, E. (2014). Transkingdom control of microbiota diurnal oscillations promotes metabolic homeostasis. *Cell*, 159(3), 514–529. <https://doi.org/10.1016/j.cell.2014.09.048> [PubMed]

Toader, C., Dobrin, N., Costea, D., Glavan, L. A., Covache-Busuioc, R. A., Dumitrascu, D. I., Bratu, B. G., Costin, H. P., & Ciurea, A. V. (2024). Mind, mood and microbiota-gut-brain Axis in psychiatric disorders. *International Journal of Molecular Sciences*, 25(6). <https://doi.org/10.3390/ijms25063340>

Tremlett, H., Fadrosch, D. W., Faruqi, A. A., Zhu, F., Hart, J., Roalstad, S., Graves, J., Lynch, S., & Waubant, E. (2016). Gut microbiota in early pediatric multiple sclerosis: A case-control study. *European Journal of Neurology*, 23(8), 1308–1321. <https://doi.org/10.1111/ene.13026> [PubMed]

Tyrer, P., & Baldwin, D. (2006). Generalised anxiety disorder. *The Lancet*, 368(9553), 2156–2166. [https://doi.org/10.1016/S0140-6736\(06\)69865-6](https://doi.org/10.1016/S0140-6736(06)69865-6)

Ueda, E., Matsunaga, M., Fujihara, H., Kajiwar, T., Takeda, A. K., Watanabe, S., Hagihara, K., & Myowa, M. (2024). Temperament in early childhood is associated with gut microbiota composition and diversity. *Developmental Psychobiology*, 66(7), e22542. <https://doi.org/10.1002/dev.22542> [PubMed]

Ullah, H., Arbab, S., Tian, Y., Liu, C. Q., Chen, Y., Qijie, L., Khan, M. I. U., Hassan, I. U., & Li, K. (2023). The gut microbiota-brain axis in neurological disorder. *Frontiers in Neuroscience*, 17, 1225875. <https://doi.org/10.3389/fnins.2023.1225875> [PubMed]

Vaghef-Mehrabani, E., Harouni, R., Behrooz, M., Ranjbar, F., Asghari-Jafarabadi, M., & Ebrahimi-Mameghani, M. (2023). Effects of inulin supplementation on inflammatory biomarkers and clinical symptoms of women with obesity and depression on a calorie-restricted diet: A randomised controlled clinical trial. *The British Journal of Nutrition*, 129(11), 1897–1907. <https://doi.org/10.1017/s000711452200232x> [PubMed]

Vaghef-Mehrabany, E., Ranjbar, F., Asghari-Jafarabadi, M., Hosseinpour-Arjmand, S., & Ebrahimi-Mameghani, M. (2021). Calorie restriction in combination with prebiotic supplementation in obese women with depression: Effects on metabolic and clinical response. *Nutritional Neuroscience*, 24(5), 339–353. <https://doi.org/10.1080/1028415x.2019.1630985> [PubMed]

Vaishnava, S., Behrendt, C. L., & Hooper, L. V. (2008). Innate immune responses to commensal bacteria in the gut epithelium. *Journal of Pediatric Gastroenterology and Nutrition*, 46(Suppl 1), E10–E11. <https://doi.org/10.1097/01.mpg.0000313823.93841.65> [PubMed]

van Enkhuizen, J., Janowsky, D. S., Olivier, B., Minassian, A., Perry, W., Young, J. W., & Geyer, M. A. (2015). The catecholaminergic-cholinergic balance hypothesis of bipolar disorder revisited.

European Journal of Pharmacology, 753, 114–126. <https://doi.org/10.1016/j.ejphar.2014.05.063>
[PubMed]

van Hoorn, A., Carpenter, T., Oak, K., Laugharne, R., Ring, H., & Shankar, R. (2019). Neuromodulation of autism spectrum disorders using vagal nerve stimulation. *Journal of Clinical Neuroscience*, 63, 8–12. <https://doi.org/10.1016/j.jocn.2019.01.042>
[PubMed]

Vasileva, S. S., Yang, Y., Baker, A., Siskind, D., Gratten, J., & Eyles, D. (2024). Associations of the gut microbiome with treatment resistance in schizophrenia. *JAMA Psychiatry*, 81(3), 292–302. <https://doi.org/10.1001/jamapsychiatry.2023.5371>
[PubMed]

Vasta, R., Chia, R., Traynor, B. J., & Chiò, A. (2022). Unraveling the complex interplay between genes, environment, and climate in ALS. *eBioMedicine*, 75, 103795. <https://doi.org/10.1016/j.ebiom.2021.103795>
[PubMed]

Vogt, N. M., Kerby, R. L., Dill-McFarland, K. A., Harding, S. J., Merluzzi, A. P., Johnson, S. C., Carlsson, C. M., Asthana, S., Zetterberg, H., Blennow, K., Bendlin, B. B., & Rey, F. E. (2017). Gut microbiome alterations in Alzheimer's disease. *Scientific Reports*, 7(1), 13537. <https://doi.org/10.1038/s41598-017-13601-y>
[PubMed]

Vuong, H. E., Yano, J. M., Fung, T. C., & Hsiao, E. Y. (2017). The microbiome and host behavior. *Annual Review of Neuroscience*, 40, 21–49. <https://doi.org/10.1146/annurev-neuro-072116-031347>
[PubMed]

Wagner-Skacel, J., Dalkner, N., Moerkl, S., Kreuzer, K., Farzi, A., Lackner, S., Painold, A., Reininghaus, E. Z., Butler, M. I., & Bengesser, S. (2020). Sleep and microbiome in psychiatric diseases. *Nutrients*, 12(8). <https://doi.org/10.3390/nu12082198>

Wallen, Z. D., Demirkan, A., Twa, G., Cohen, G., Dean, M. N., Standaert, D. G., Sampson, T. R., & Payami, H. (2022). Metagenomics of Parkinson's disease implicates the gut microbiome in multiple disease mechanisms. *Nature Communications*, 13(1), 6958. <https://doi.org/10.1038/s41467-022-34667-x>
[PubMed]

Walton, C., King, R., Rechtman, L., Kaye, W., Leray, E., Marrie, R. A., Robertson, N., La Rocca, N., Uitdehaag, B., van der Mei, I., Wallin, M., Helme, A., Angood Napier, C., Rijke, N., & Baneke, P. (2020). Rising prevalence of multiple sclerosis worldwide: Insights from the Atlas of MS, third edition. *Multiple Sclerosis*, 26(14), 1816–1821. <https://doi.org/10.1177/1352458520970841>
[PubMed]

Wan, L., Ge, W. R., Zhang, S., Sun, Y. L., Wang, B., & Yang, G. (2020). Case-control study of the effects of gut microbiota composition on neurotransmitter metabolic pathways in children with attention deficit hyperactivity disorder. *Frontiers in Neuroscience*, 14, 127. <https://doi.org/10.3389/fnins.2020.00127>

[PubMed]

Wang, Y., & Kasper, L. H. (2014). The role of microbiome in central nervous system disorders. *Brain, Behavior, and Immunity*, 38, 1–12. <https://doi.org/10.1016/j.bbi.2013.12.015>

[PubMed]

Wang, L. J., Yang, C. Y., Chou, W. J., Lee, M. J., Chou, M. C., Kuo, H. C., Yeh, Y. M., Lee, S. Y., Huang, L. H., & Li, S. C. (2020a). Gut microbiota and dietary patterns in children with attention-deficit/hyperactivity disorder. *European Child & Adolescent Psychiatry*, 29(3), 287–297. <https://doi.org/10.1007/s00787-019-01352-2>

Wang, Y., Li, N., Yang, J. J., Zhao, D. M., Chen, B., Zhang, G. Q., Chen, S., Cao, R. F., Yu, H., Zhao, C. Y., Zhao, L., Ge, Y. S., Liu, Y., Zhang, L. H., Hu, W., Zhang, L., & Gai, Z. T. (2020b). Probiotics and fructo-oligosaccharide intervention modulate the microbiota-gut brain axis to improve autism spectrum reducing also the hyper-serotonergic state and the dopamine metabolism disorder. *Pharmacological Research*, 157, 104784. <https://doi.org/10.1016/j.phrs.2020.104784>

[PubMed]

Wang, L. J., Li, S. C., Li, S. W., Kuo, H. C., Lee, S. Y., Huang, L. H., Chin, C. Y., & Yang, C. Y. (2022a). Gut microbiota and plasma cytokine levels in patients with attention-deficit/hyperactivity disorder. *Translational Psychiatry*, 12(1), 76. <https://doi.org/10.1038/s41398-022-01844-x>

[PubMed]

Wang, L. J., Yang, C. Y., Kuo, H. C., Chou, W. J., Tsai, C. S., & Lee, S. Y. (2022b). Effect of *Bifidobacterium bifidum* on clinical characteristics and gut microbiota in attention-deficit/hyperactivity disorder. *Journal of Personalized Medicine*, 12(2). <https://doi.org/10.3390/jpm12020227>

Wang, Q., Zheng, J., Pettersson, S., Reynolds, R., & Tan, E. K. (2023a). The link between neuroinflammation and the neurovascular unit in synucleinopathies. *Science Advances*, 9(7), eabq1141. <https://doi.org/10.1126/sciadv.abq1141>

[PubMed]

Wang, R., Sun, Y., Wang, M., Li, H., Liu, S., & Liu, Z. (2023b). Therapeutic effect of *Eleutherococcus senticosus* (Rupr. & Maxim.) Maxim. leaves on ischemic stroke via the microbiota-gut-brain axis. *Phytotherapy Research*, 37(10), 4801–4818. <https://doi.org/10.1002/ptr.7947>

[PubMed]

Wang, J., He, M., Yang, M., & Ai, X. (2024a). Gut microbiota as a key regulator of intestinal mucosal immunity. *Life Sciences*, 345, 122612. <https://doi.org/10.1016/j.lfs.2024.122612>

[PubMed]

Wang, N., Yin, C., Feng, R., Jia, R., Zhou, L., Wu, W., Yu, H., Ye, Y., Gong, Z., & Li, L. (2024b). Analysis of intestinal microbiota in schizophrenic patients with type 2 diabetes mellitus. *Alpha Psychiatry*, 25(3), 375–381. <https://doi.org/10.5152/alphapsychiatry.2024.231302>

[PubMed]

Wehrwein, E. A., Orer, H. S., & Barman, S. M. (2016). Overview of the anatomy, physiology, and pharmacology of the autonomic nervous system. *Compr Physiol*, 6(3), 1239–1278. <https://doi.org/10.1002/cphy.1239>

[org/10.1002/cphy.c150037](https://doi.org/10.1002/cphy.c150037)
[PubMed]

WHO. (2022). *Schizophrenia*. Retrieved 30th May, from <https://www.who.int/news-room/fact-sheets/detail/schizophrenia>

WHO. (2023). *Depressive disorder (depression)*. World Health Organization. Retrieved 27 May, from <https://www.who.int/news-room/fact-sheets/detail/depression>

WHO. (2025). *Dementia*. Retrieved 02 June, from <https://www.who.int/news-room/fact-sheets/detail/dementia>

Wright, M. L., Fournier, C., Houser, M. C., Tansey, M., Glass, J., & Hertzberg, V. S. (2018). Potential role of the gut microbiome in ALS: A systematic review. *Biological Research for Nursing*, 20(5), 513–521. <https://doi.org/10.1177/1099800418784202>
[PubMed]

Xu, K., Gao, X., Xia, G., Chen, M., Zeng, N., Wang, S., You, C., Tian, X., Di, H., Tang, W., Li, P., Wang, H., Zeng, X., Tan, C., Meng, F., Li, H., He, Y., Zhou, H., & Yin, J. (2021). Rapid gut dysbiosis induced by stroke exacerbates brain infarction in turn. *Gut*. <https://doi.org/10.1136/gutjnl-2020-323263>

Yan, J., Chen, H., Zhang, Y., Peng, L., Wang, Z., Lan, X., Yu, S., & Yang, Y. (2024). Fecal microbiota transplantation significantly improved respiratory failure of amyotrophic lateral sclerosis. *Gut Microbes*, 16(1), 2353396. <https://doi.org/10.1080/19490976.2024.2353396>
[PubMed]

Yang, J., Zheng, P., Li, Y., Wu, J., Tan, X., Zhou, J., Sun, Z., Chen, X., Zhang, G., Zhang, H., Huang, Y., Chai, T., Duan, J., Liang, W., Yin, B., Lai, J., Huang, T., Du, Y., Zhang, P., et al. (2020). Landscapes of bacterial and metabolic signatures and their interaction in major depressive disorders. *Science Advances*, 6(49). <https://doi.org/10.1126/sciadv.aba8555>

Yano, J. M., Yu, K., Donaldson, G. P., Shastri, G. G., Ann, P., Ma, L., Nagler, C. R., Ismagilov, R. F., Mazmanian, S. K., & Hsiao, E. Y. (2015). Indigenous bacteria from the gut microbiota regulate host serotonin biosynthesis. *Cell*, 161(2), 264–276. <https://doi.org/10.1016/j.cell.2015.02.047>
[PubMed]

Yao, S., Xie, H., Wang, Y., Shen, N., Chen, Q., Zhao, Y., Gu, Q., Zhang, J., Liu, J., Sun, J., & Tong, Q. (2023). Predictive microbial feature analysis in patients with depression after acute ischemic stroke. *Frontiers in Aging Neuroscience*, 15, 1116065. <https://doi.org/10.3389/fnagi.2023.1116065>
[PubMed]

Yassin, L. K., Nakhal, M. M., Alderei, A., Almehairbi, A., Mydeen, A. B., Akour, A., & Hamad, M. I. K. (2025). Exploring the microbiota-gut-brain axis: Impact on brain structure and function. *Frontiers in Neuroanatomy*, 19, 1504065. <https://doi.org/10.3389/fnana.2025.1504065>
[PubMed]

Yin, J., Liao, S. X., He, Y., Wang, S., Xia, G. H., Liu, F. T., Zhu, J. J., You, C., Chen, Q., Zhou, L., Pan, S. Y., & Zhou, H. W. (2015). Dysbiosis of gut microbiota with reduced trimethylamine-N-oxide level in

patients with large-artery atherosclerotic stroke or transient ischemic attack. *Journal of the American Heart Association*, 4(11). <https://doi.org/10.1161/jaha.115.002699>

Yokote, H., Miyake, S., Croxford, J. L., Oki, S., Mizusawa, H., & Yamamura, T. (2008). NKT cell-dependent amelioration of a mouse model of multiple sclerosis by altering gut flora. *The American Journal of Pathology*, 173(6), 1714–1723. <https://doi.org/10.2353/ajpath.2008.080622> [PubMed]

Young, S., Hollingdale, J., Absoud, M., Bolton, P., Branney, P., Colley, W., Craze, E., Dave, M., Deeley, Q., Farrag, E., Gudjonsson, G., Hill, P., Liang, H. L., Murphy, C., Mackintosh, P., Murin, M., O'Regan, F., Ougrin, D., Rios, P., et al. (2020). Guidance for identification and treatment of individuals with attention deficit/hyperactivity disorder and autism spectrum disorder based upon expert consensus. *BMC Medicine*, 18(1), 146. <https://doi.org/10.1186/s12916-020-01585-y> [PubMed]

Yuan, X., Li, X., Kang, Y., Pang, L., Hei, G., Zhang, X., Wang, S., Zhao, X., Zhang, S., Tao, Q., Zhang, L., Zhu, Q., Lv, L., Fan, X., Hu, S., & Song, X. (2022). Gut mycobiota dysbiosis in drug-naïve, first-episode schizophrenia. *Schizophrenia Research*, 250, 76–86. <https://doi.org/10.1016/j.schres.2022.10.011> [PubMed]

Yüksel, C., & Öngür, D. (2010). Magnetic resonance spectroscopy studies of glutamate-related abnormalities in mood disorders. *Biological Psychiatry*, 68(9), 785–794. <https://doi.org/10.1016/j.biopsych.2010.06.016> [PubMed]

Zeng, Q., Shen, J., Chen, K., Zhou, J., Liao, Q., Lu, K., Yuan, J., & Bi, F. (2020). The alteration of gut microbiome and metabolism in amyotrophic lateral sclerosis patients. *Scientific Reports*, 10(1), 12998. <https://doi.org/10.1038/s41598-020-69845-8> [PubMed]

Zeng, C., Qiu, Y., Li, S., Teng, Z., Xiang, H., Chen, J., Wu, X., Cao, T., Zhang, S., Chen, Q., Wu, H., & Cai, H. (2022). Effect of probiotic supplements on oxidative stress biomarkers in first-episode bipolar disorder patients: A randomized, placebo-controlled trial. *Frontiers in Pharmacology*, 13, 829815. <https://doi.org/10.3389/fphar.2022.829815> [PubMed]

Zhai, C. D., Zheng, J. J., An, B. C., Huang, H. F., & Tan, Z. C. (2019). Intestinal microbiota composition in patients with amyotrophic lateral sclerosis: Establishment of bacterial and archaeal communities analyses. *Chinese Medical Journal*, 132(15), 1815–1822. <https://doi.org/10.1097/cm9.0000000000000351> [PubMed]

Zhang, N., Zhang, Y., Li, M., Wang, W., Liu, Z., Xi, C., Huang, X., Liu, J., Huang, J., Tian, D., Mu, J., Liao, X., & Zhai, S. (2020). Efficacy of probiotics on stress in healthy volunteers: A systematic review and meta-analysis based on randomized controlled trials. *Brain and Behavior: A Cognitive Neuroscience Perspective*, 10(9), e01699. <https://doi.org/10.1002/brb3.1699>

Zhang, Q., Yun, Y., An, H., Zhao, W., Ma, T., Wang, Z., & Yang, F. (2021). Gut microbiome composition

associated with major depressive disorder and sleep quality. *Frontiers in Psychiatry*, 12, 645045. <https://doi.org/10.3389/fpsyt.2021.645045>
[PubMed]

Zhao, Z., Xiao, G., Xia, J., Guo, H., Yang, X., Jiang, Q., Wang, H., Hu, J., & Zhang, C. (2023). Effectiveness of probiotic/prebiotic/synbiotic treatments on anxiety: A systematic review and meta-analysis of randomized controlled trials. *Journal of Affective Disorders*, 343, 9–21. <https://doi.org/10.1016/j.jad.2023.09.018>
[PubMed]

Zheng, S., Zhu, Y., Wu, W., Zhang, Q., Wang, Y., Wang, Z., & Yang, F. (2021). A correlation study of intestinal microflora and first-episode depression in Chinese patients and healthy volunteers. *Brain and Behavior: A Cognitive Neuroscience Perspective*, 11(8), e02036. <https://doi.org/10.1002/brb3.2036>

Zhong, D. Y., Li, L., Ma, R. M., & Deng, Y. H. (2021). The effect of probiotics in stroke treatment. *Evidence-based Complementary and Alternative Medicine*, 2021, 4877311. <https://doi.org/10.1155/2021/4877311>
[PubMed]

Zhong, Q., Chen, J. J., Wang, Y., Shao, W. H., Zhou, C. J., & Xie, P. (2022). Differential gut microbiota compositions related with the severity of major depressive disorder. *Frontiers in Cellular and Infection Microbiology*, 12, 907239. <https://doi.org/10.3389/fcimb.2022.907239>
[PubMed]

Zhou, R. Y., Wang, J. J., Sun, J. C., You, Y., Ying, J. N., & Han, X. M. (2017). Attention deficit hyperactivity disorder may be a highly inflammation and immune-associated disease (Review). *Molecular Medicine Reports*, 16(4), 5071–5077. <https://doi.org/10.3892/mmr.2017.7228>
[PubMed]

Zhu, G., Zhao, J., Wang, G., & Chen, W. (2023). Bifidobacterium breve HNX26M4 attenuates cognitive deficits and Neuroinflammation by regulating the gut-brain Axis in APP/PS1 mice. *Journal of Agricultural and Food Chemistry*, 71(11), 4646–4655. <https://doi.org/10.1021/acs.jafc.3c00652>
[PubMed]

Zhuang, Z. Q., Shen, L. L., Li, W. W., Fu, X., Zeng, F., Gui, L., Lü, Y., Cai, M., Zhu, C., Tan, Y. L., Zheng, P., Li, H. Y., Zhu, J., Zhou, H. D., Bu, X. L., & Wang, Y. J. (2018). Gut microbiota is altered in patients with Alzheimer's disease. *Journal of Alzheimer's Disease*, 63(4), 1337–1346. <https://doi.org/10.3233/jad-180176>
[PubMed]

19. Clinical Diagnostics for Microbiome-Based Therapies

Andreas Hiergeist¹  and André Gessner¹ 

(¹) Institute of Clinical Microbiology and Hygiene, University Hospital Regensburg, Regensburg, Germany

 **Andreas Hiergeist**

Email: andreas.hiergeist@ukr.de

 **André Gessner (Corresponding author)**

Email: andre.gessner@ukr.de

Abstract

While microbiome analytics have advanced considerably, its role as a primary clinical diagnostic tool remains suitable only for selected clinical questions and must be applied with careful consideration. High-throughput sequencing (HTS) methods—such as 16S rRNA gene and shotgun metagenomic sequencing—face challenges in standardization, interpretability, and regulatory validation, preventing their routine use in diagnostics. Nevertheless, microbiome profiling via HTS offers strong value as a monitoring and decision-support tool for microbiota-targeted therapies. A well-established example is fecal microbiota transplantation (FMT), where HTS enables donor engraftment tracking, microbial diversity assessment, and early detection of relapse risk. Similar applications are emerging in inflammatory bowel disease, decolonization of multidrug-resistant organisms, metabolic disorders, and as adjuncts to cancer immunotherapy. HTS also supports

monitoring of responses to defined microbial consortia, probiotics, prebiotics, and phage therapies. Additionally, spatial and single-cell microbiome technologies now allow resolution of host-microbe interactions at cellular and tissue levels, providing new insights into microbial function and therapeutic response. Despite these promising applications, clinical integration demands harmonized protocols, rigorous validation, and clear interpretive frameworks to ensure reproducibility and clinical relevance. This chapter outlines the current landscape and evolving potential of microbiome sequencing in medicine, emphasizing its growing utility for therapy monitoring and patient stratification—while underscoring the importance of methodological standardization for clinical translation.

Keywords Microbiome analysis – Microbiome sequencing – Fecal microbiota transfer – Spatial analysis

The human microbiome, composed of trillions of microorganisms, plays a critical role in health and disease. Advances in high-throughput sequencing (HTS) technologies have enabled detailed investigation of these complex ecosystems, moving microbiome research from basic science into clinical contexts. Once primarily used in research, sequencing is now being tested in diagnostics, infectious disease management, and personalized therapies. Amplicon (e.g., 16S rRNA, ITS) and shotgun metagenomics approaches allow for culture-independent taxonomic and functional profiling, but their clinical use is still limited due to variability in workflows, interpretation, and standardization. HTS supports applications ranging from pathogen surveillance to antimicrobial resistance gene detection. Shotgun metagenomics has shown utility in complex cases like sepsis and encephalitis, while gut microbiome profiling is being used in oncology and gastroenterology. Fecal microbiota transfer (FMT) remains the best-established microbiome-based therapy, with expanding indications beyond *Clostridioides difficile* infection. Donor engraftment can now be tracked using sequencing, and targeted therapies such as

defined consortia or phage cocktails are under development. Spatial and single-cell technologies are pushing the boundaries further. Techniques such as SAMPL-seq and INVADeseq enable micron-scale mapping of microbial communities and host-microbe transcriptomic interactions, providing unprecedented resolution of microbial niche dynamics, strain heterogeneity, and microbial-host crosstalk. These tools are expected to drive precision diagnostics, inform therapeutic response monitoring, and improve stratification in microbiome-guided medicine. Despite growing enthusiasm, robust clinical implementation faces challenges. Microbiome composition is shaped by numerous confounders, including diet, medications, inflammation, and host genetics—and even within individuals, substantial variability can occur over time, complicating longitudinal interpretation. This variability undermines the reproducibility and generalizability of microbial biomarkers. Most associations from metagenome-wide studies remain correlative, and microbial shifts—such as enrichment of *Fusobacterium* in colorectal cancer or loss of butyrate producers in IBD—may not be specific. Clinical-grade implementation of microbiome sequencing thus demands standardization, validation, and integration into existing workflows. Recent original research studies (2019–2024) underscore the importance of methodological consistency, appropriate controls, and harmonized data processing. Pilot applications of microbiome testing are emerging, but clinical translation hinges on overcoming technical variability and clearly linking microbial patterns to patient outcomes.

19.1 Clinical Diagnostics for Microbiome-Based Therapies

The integration of microbiome sequencing into clinical diagnostics is a critical step toward enabling personalized, microbiota-informed therapies. Fecal microbiota transplantation (FMT) is currently the most established application, with high efficacy for recurrent *C. difficile* infections and expanding research into IBD, metabolic disorders, and

cancer therapy support. High-throughput sequencing plays a central role in monitoring FMT by assessing donor microbiota engraftment, recipient microbiome dynamics, and therapy outcomes. For newer microbiota-based interventions—such as live biotherapeutic products, engineered consortia, and phage therapies—clinical diagnostics based on HTS can facilitate patient stratification, baseline microbiota profiling, and treatment response monitoring. Precise diagnostics may also identify contraindications or help tailor interventions to individual microbial signatures. However, clinical implementation of these diagnostics requires standardized workflows, validated biomarkers, and clear interpretive guidelines. This includes harmonized protocols for sampling, sequencing, and bioinformatic analysis, as well as rigorous integration of clinical metadata. Advances in multi-omics integration and spatial microbiome profiling are enhancing the resolution of diagnostics, making it possible to track microbiota-host interactions at the molecular level.

As the field progresses, microbiome-based diagnostics will support:

- Selection and monitoring of FMT and other microbiota-directed therapies
- Prediction of therapeutic outcomes and adverse events
- Identification of microbial risk or resilience markers
- Development of companion diagnostics for personalized medicine

In parallel, traditional diagnostics such as PCR and culture-based testing remain essential tools for donor screening in fecal microbiota transplantation (FMT). These methods are critical for identifying and excluding known pathogens (e.g., enteric bacteria, viruses, parasites) and ensuring baseline microbial safety prior to HTS-based characterization. Incorporating conventional microbiological testing enhances diagnostic reliability and fulfills clinical and regulatory standards, particularly in the context of FMT protocols.

Regulatory frameworks are evolving to keep pace with the growing clinical use of microbiome-based therapies. In the United States, the Food and Drug Administration (FDA) has approved products like

Rebyota™ (2022) and Vowst™ (2023) for the prevention of recurrent *C. difficile* infection—signaling formal recognition of fecal-derived therapeutics. Rebyota is a rectally administered suspension of donor-derived microbes, while Vowst is an oral capsule containing purified bacterial spores; both aim to restore microbial diversity and prevent pathogen recurrence. The FDA also issued safety guidelines for donor screening, requiring testing for SARS-CoV-2, multidrug-resistant organisms, and other pathogens. Microbiota-based products intended to treat or prevent disease are regulated as biological products under FDA oversight. For applications beyond *C. difficile*, Investigational New Drug (IND) applications are typically required. In the European Union, similar efforts are underway to define regulatory pathways for microbiota-based interventions, led by the European Medicines Agency (EMA) and national health agencies (Rodriguez et al., [2025](#)).

19.2 Emerging Methodological Practices and Sequencing Innovations

Advances in Sequencing Technologies The past few years have seen significant improvements in how we sequence and analyze microbiomes. While 16S rRNA gene amplicon sequencing remains a workhorse for rapid community profiling, shotgun metagenomic sequencing has become increasingly accessible and is favored for clinical applications that require species- and strain-level resolution or functional gene data (Pribyl et al., [2021](#)). Shotgun metagenomics can identify bacteria, archaea, viruses, and genes (e.g., antibiotic resistance genes) in one assay, which is valuable for clinical scenarios such as infection diagnostics or assessing a patient's resistome. Newer sequencing platforms and chemistries (e.g., Illumina NovaSeq for high-depth short reads) enable sequencing hundreds of gut samples at high coverage, improving detection of low-abundance organisms. Long-read sequencing technologies (PacBio HiFi, Oxford Nanopore) have matured and are now occasionally applied to microbiomes to resolve complete microbial genomes and plasmids from complex samples. For example,

long-read assemblies can capture strain-resolved genomes and mobile elements, aiding the identification of pathogenic strains or virulence factors directly from stool. However, long-read platforms have higher per-read error rates and lower throughput, so in practice, many studies use a hybrid approach (short-read data for quantification, long-read for assembly of reference genomes) when strain resolution is needed in the clinic. These advances benefit from increasingly portable and cost-effective sequencing platforms, democratizing access to sequencing in decentralized and resource-limited settings. However, the exponential growth in metagenomic data volume and complexity demands substantial computational infrastructure for processing, storage, and secure sharing—creating significant barriers to clinical integration and routine diagnostic implementation.

Reference Genome and Database Expansion A key development facilitating clinical microbiome analysis is the expansion of reference genome catalogs for human microbiota. Thousands of new bacterial genomes have been added via culture-based and metagenomic approaches. For instance, Forster et al. ([2019](#)) cultured and sequenced hundreds of gut bacterial isolates, creating a genome and culture collection to improve metagenomic read classification. Similarly, Almeida et al. ([2021](#)) assembled >200,000 genomes from human gut metagenomes (most from previously uncultured species), dramatically increasing the reference database for taxonomic and functional profiling. These expanded references allow clinical metagenomic data to be annotated with higher accuracy—an essential step for identifying novel or unexpected microbes in patient samples. In a clinical context, this means a higher chance of correctly pinpointing a pathogen or detecting microbial shifts associated with disease. The inclusion of previously uncharacterized bacterial species (70% of species in the Unified Human Gastrointestinal Genome catalog lacked cultured representatives (Almeida et al., [2021](#))) underscores the importance of ongoing genome discovery efforts. Clinicians and microbiome researchers now have access to improved pipelines (e.g.,

Kraken2/Bracken, MetaPhlAn 4, QIIME2) that leverage these databases for faster and more accurate classification of sequencing reads, which is critical for timely clinical interpretation.

Absolute Quantification and Multi-omics Traditional microbiome sequencing yields relative abundance data, which can obscure clinically relevant changes if total microbial load varies between samples. Emerging practices emphasize *absolute* microbiome quantification—determining the number of microbial cells or gene copies per unit sample. Approaches include flow cytometry or qPCR to measure total bacteria, the use of internal spike-in standards, and computational models. A 2024 study by Nishijima et al. showed that fecal microbial load (total cells per gram) is a major driver of variation in gut profiles and a confounder in disease associations; adjusting for load diminished many purported disease-related microbiome changes (Nishijima et al., [2024](#)). To enable routine absolute profiling, synthetic DNA spike-in methods have been developed. Zaramela et al. ([2022](#)) created a set of synthetic DNA sequences (“synDNA”) added to each sample in known quantity, which, upon sequencing, allows back-calculation of absolute abundances for microbes. This practice is gaining traction in research studies and may be incorporated into clinical lab protocols to improve the interpretability of microbiome readouts (e.g., distinguishing whether a pathogen is truly enriched or if overall biomass changed). Moreover, whole-cell microbiome spike-in standards—developed, published, and patented by the authors—have been successfully applied in a wide range of microbiome studies and are routinely used in annual ring trials to evaluate interlaboratory methodological variation (Stämmler et al., [2016](#); Hiergeist et al., [2016](#)). Beyond DNA, multi-omics analyses are emerging: combining metagenomics with metatranscriptomics (microbial gene expression), metabolomics (small-molecule profiles like short-chain fatty acids), or metaproteomics. Such integrated analyses can provide functional context—for example, revealing whether a patient’s gut microbiome is actively producing certain metabolites or harboring specific enzymatic

activities relevant to disease. While multi-omics is still primarily research-oriented, it represents a methodological frontier that could enhance clinical microbiome assessments, especially in conditions where microbial function (not just presence) is key.

Bioinformatics and Machine Learning for Clinical

Insights Handling the large, complex datasets from microbiome sequencing requires sophisticated bioinformatics. Since 2019, there have been improvements in the standardization of analytic workflows. QIIME 2, for instance, has become a widely adopted platform offering reproducible, interactive microbiome data science with robust handling of metadata and provenance tracking (Bolyen et al., [2019](#)). For clinical labs, standardized workflows are crucial for reproducibility. Machine learning (ML) techniques are increasingly used to derive diagnostic or prognostic models from microbiome data. Numerous original research studies have built classifiers that predict disease states (e.g., inflammatory bowel disease (Pasolli et al., [2016](#)), colorectal cancer (Zeller et al., [2014](#)), and type 2 diabetes (Asnicar et al., [2021](#))) based on gut microbiome composition. However, recent evaluations have raised caution: models often perform well on the dataset they were trained on but lose accuracy when tested on independent cohorts (Li et al., [2023](#)). For example, Li et al. ([2023](#)) conducted a cross-cohort validation of microbiome-based ML classifiers for 20 different diseases and found that many models did not generalize, with external areas under the curve (AUCs) dropping to ~ 0.6 despite internal AUCs ~ 0.8 . This underlines that overfitting and batch effects can plague microbiome-based diagnostics. In response, best practices for microbiome ML have been proposed, such as rigorous feature selection, normalization (rank-based methods improved model transferability (Li et al., [2023](#))), and correction for confounding factors during training. The current consensus is that while microbiome sequencing combined with ML holds promise for clinical diagnostics, models must be trained on diverse, multi-center datasets and tested prospectively to ensure reliability before clinical deployment.

19.3 Confounders and Biases in Clinical Microbiome Studies

One of the greatest challenges in using microbiome sequencing for clinical purposes is dealing with confounding factors—variables that influence microbiome readouts independently of the disease or condition of interest (Fig. [19.1](#)). During the last few years, multiple large studies dissected these confounders, emphasizing the need to control for them in study design and data analysis.

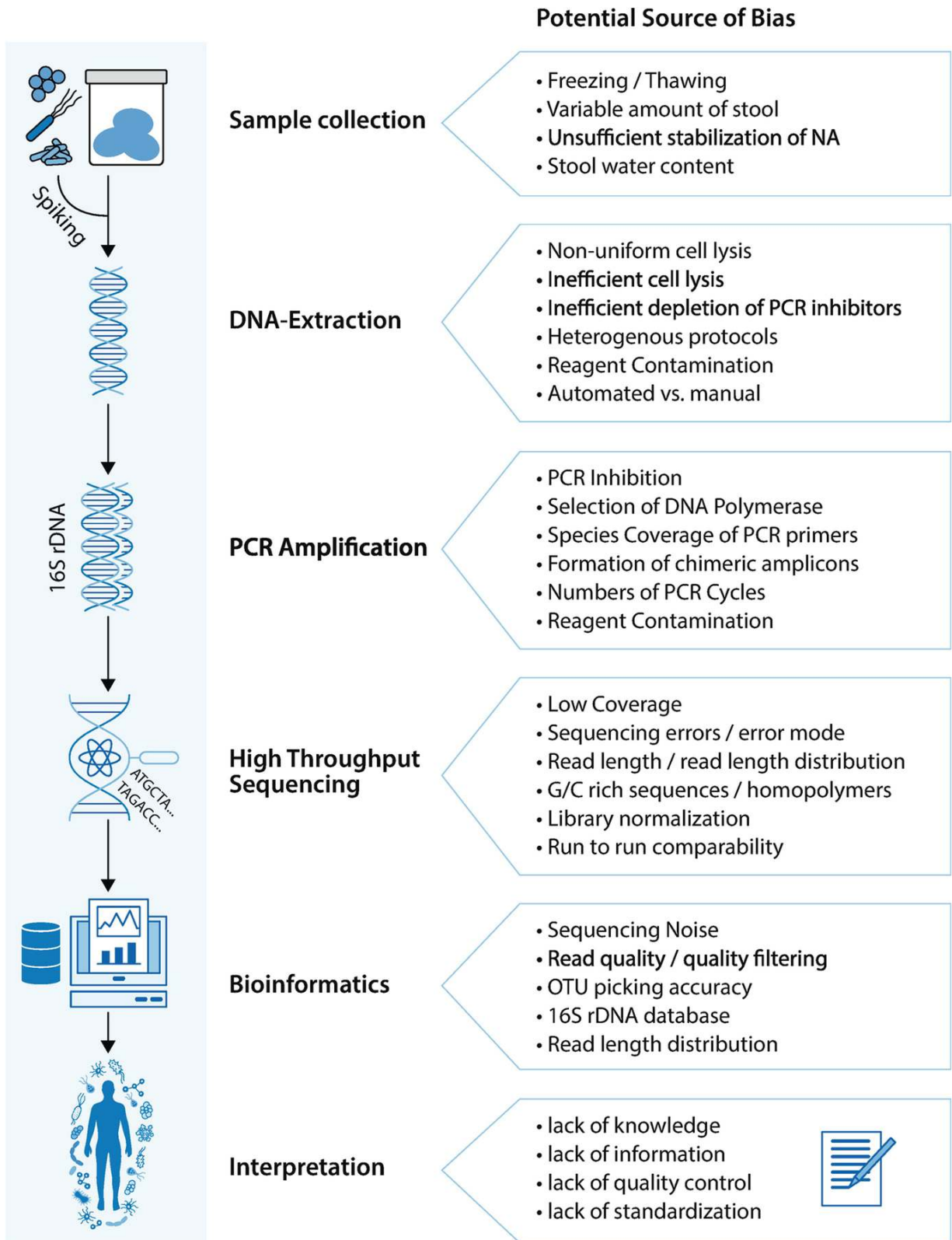


Fig. 19.1 Methodological pitfalls in marker gene-based microbiome analyses

Technical Confounders The laboratory methods used for sample collection and processing can introduce significant bias. For example, DNA extraction protocols have a known bias: bacteria with tough cell walls (Gram-positive organisms) can yield less DNA with certain kits, skewing community profiles. Ezzy et al. ([2019](#)) compared six fecal DNA extraction methods and found 60-fold differences in DNA yield and considerable shifts in detected community composition between methods—the choice of extraction kit altered which phyla dominated the profile (Ezzy et al., [2019](#)). Such variability means that if one lab uses a suboptimal extraction for certain taxa, those microbes might appear falsely low in abundance. Rauer et al. ([2025](#)) further demonstrated that extraction bias is predictable based on bacterial morphology and proposed using mock communities to computationally correct for these biases. In practice, clinical laboratories mitigate extraction bias by adopting protocols known to lyse both Gram-negative and Gram-positive bacteria effectively (e.g., bead-beating steps), and by including positive controls (defined mock communities) to monitor DNA recovery efficiency across taxa.

Another technical factor is *PCR amplification bias* in amplicon sequencing (16S/18S/ITS). Primer selection can favor certain taxa; even “universal” 16S primers have differential affinity, leading to over- or under-representation of some groups. For instance, certain Archaea or specific bacterial clades may be missed if primers do not match their rRNA gene regions well. Although shotgun sequencing circumvents PCR, it introduces other biases such as library preparation differences (e.g., GC-rich genomes might be under-represented after amplification steps). Additionally, shotgun metagenomics often captures large amounts of host (e.g., human) DNA, especially in low-biomass samples like mucosal swabs or biopsy material, reducing the sequencing depth available for microbial reads. To minimize these issues, many clinical protocols now use optimized primer sets tested for broad coverage or resort to shotgun metagenomics for comprehensive profiling despite higher cost.

Biological and Environmental Confounders Human microbiome composition is highly sensitive to diet, lifestyle, medications, and host physiology. This means that case-control studies for a disease can be confounded if these factors are not evenly distributed or accounted for. Diet is a prime example: short-term dietary intake can shift gut microbiome structure within days. Subjects consuming a high-fiber diet may naturally have different microbiomes than those on a Western diet—typically characterized by high intake of saturated fats, refined sugars, and low fiber, which could be mistaken for a disease signal if patient groups differ in diet. Personal diet logging and even controlled feeding studies are sometimes used in research to parse diet effects. In clinical practice, diet is a potential confounder for any microbiome-based diagnostic; thus, dietary habits might need consideration when interpreting results. One way to counteract confounding and move beyond correlation is to establish causality through experimental validation. For example, a study from the Elinav lab (Federici et al., [2022](#)) identified *Klebsiella pneumoniae* as a driver of microbiome-associated colitis and demonstrated that a targeted phage cocktail could reduce disease severity—highlighting a mechanistic link and a potential therapeutic strategy.

Medications are another major confounder that has come into focus with new data. Antibiotics are well-known to disrupt the gut microbiome, but non-antibiotic drugs can also have significant impacts. A large population study of 2223 individuals by Larsson et al. ([2024](#)) examined 67 common medications and found 26 drugs (including proton pump inhibitors, metformin, antidepressants, and laxatives) associated with distinct microbiome compositional changes. For example, proton pump inhibitors were linked to a higher abundance of oral bacteria in the gut (likely due to reduced gastric acidity), and metformin use was associated with an increase in *Escherichia* species and functional shifts in fermentation pathways (Larsson et al., [2024](#)). Polypharmacy (≥ 5 drugs) was correlated with enrichment of opportunistic bacteria like *Enterococcus faecalis*. These findings

reinforce earlier observations and identify new drug–microbiome interactions, underscoring that when comparing patients vs. healthy controls, differences might reflect medication effects rather than the disease itself. Clinically, this suggests that a patient’s medication history must be considered when interpreting microbiome sequencing data; it may even be beneficial to stratify or adjust analyses by drug exposures.

Host Factors Age, body mass index (BMI), bowel transit time, and inflammation are intrinsic host factors that can confound microbiome-disease associations. Tito et al. ([2024](#)) elegantly demonstrated this in a colorectal cancer (CRC) cohort. Using quantitative microbiome profiling and rigorous covariate control, they found that stool transit time (a proxy for how long stool remains in the colon, affecting microbial proliferation), fecal calprotectin level (a marker of gut inflammation), and BMI explained more variance in microbiome composition than the presence of CRC lesions (Tito et al., [2024](#)). Once they controlled for these factors, some classic CRC-associated bacteria (like *Fusobacterium nucleatum*) were no longer significantly linked to cancer, whereas other signals remained robust. This finding does not contradict the known mechanistic role of *F. nucleatum* in CRC pathogenesis, including tumor adhesion, immune evasion, and pro-inflammatory activity. Rather, it underscores that host physiology can obscure microbial biomarkers in stool-based studies, emphasizing the need to distinguish causal actors from correlative artifacts. For clinical translation, measuring and adjusting for such host factors is critical. For instance, stool form (using the Bristol stool scale) or gut transit markers can be noted alongside microbiome results to discern whether a patient’s microbial profile might simply reflect slow vs. fast transit. Low-grade inflammation can also shift the microbiome; thus, biomarkers like calprotectin help differentiate disease-specific microbiome changes from generic inflammation-related dysbiosis.

Intra-individual Variability The gut microbiome within a single person fluctuates over time (day-to-day or week-to-week variability),

which can confound single-time-point analyses. Jones et al. ([2021](#)) showed that even two stool samples collected from the same individual less than 24 h apart had detectable differences in microbiome composition and metabolite (short-chain fatty acid) levels. They also noted that sampling different spots of a single stool without homogenization led to variability in detected microbes and metabolites (Jones et al., [2021](#)). These findings highlight that a one-time sample may not perfectly represent an individual's typical microbiome. In a clinical scenario, this temporal variability means that if a microbiome-based diagnostic is borderline, a second sample might be advisable. It also supports the practice of homogenizing stool samples thoroughly before aliquoting for analysis to get a more representative snapshot of the whole specimen. Some clinical trials now incorporate multiple sampling timepoints or use an average microbiome profile to increase robustness of associations.

Cross-Cohort and Batch Effects When comparing microbiome data across different studies or sequencing runs, batch effects can act as confounders. The “Microbiome Quality Control” (MBQC) project, though initiated earlier, has continued to inform best practices through 2019–2024. Variations in wet-lab protocols and sequencing centers can produce differences sometimes larger than the biological signal of interest. For example, two labs processing an identical stool sample can report different relative abundances due to different DNA extraction, primer selection, or bioinformatics parameters. Awareness of this has led to an emphasis on standardization and the inclusion of *batch controls*. Many studies now randomize sample processing order and balance cases/controls within each sequencing run to avoid confounding disease status with batch. Also, data processing pipelines increasingly include batch correction algorithms (e.g., using tools from the *limma* package as done in Li et al., [2023](#)) to adjust for run or cohort differences. In a clinical lab setting, consistency is key: using the same validated protocol for all samples and including control materials can

help ensure that differences in a patient's sample truly reflect biology and not technical variation.

Finally, *contamination* deserves special mention, especially for clinical samples from body sites with low microbial biomass (not usually the gut, which has high biomass, but relevant when considering microbiome sequencing in sites like blood, tissues, or lungs). Several high-profile studies between 2019 and 2024 have underscored how reagent or laboratory contaminants can lead to false discovery of microbes. One striking example was the case of the “placental microbiome.” After earlier claims of a unique placenta microbiome, deeper analysis by de Goffau et al. ([2019](#)) found that nearly all bacterial DNA detected in placental samples was explained by contamination from delivery or kits, except for a few cases of *Streptococcus agalactiae* infection (de Goffau et al., [2019](#)). This taught the field a cautionary lesson: without rigorous controls, sequencing can detect DNA from bacteria that were never truly present in the specimen. In gut microbiome work, contaminants are less dominant due to the high native microbial load, but they can still affect results for low-abundance features (e.g., detection of pathogens or fungi could be masked or mimicked by contaminants). Thus, negative controls (blank DNA extraction and PCR controls) and contamination-aware analysis (removing taxa known as kit contaminants) are now standard in well-designed studies. Clinical labs performing microbiome tests will typically run reagent blanks in parallel and use decontamination algorithms to subtract background DNA, ensuring that reported organisms are genuinely from the patient sample.

In summary, recognizing and mitigating confounders is essential for clinical microbiome sequencing. Modern studies apply careful experimental design (e.g., matched controls, standardized protocols) and statistical adjustments to distinguish true disease-associated microbial signals from the noise of diet, drugs, host physiology, and technical bias. Only by addressing these challenges can we achieve the reliability required for clinical applications.

Translating microbiome sequencing into clinical practice also demands robust procedures for sample handling and stringent quality control (QC) measures. The last years have seen significant innovation in how samples are collected, preserved, and processed to maintain microbiome integrity from “bench-to-bedside.”

Stool Sample Collection and Preservation In research settings, the gold standard for fecal sample preservation has been immediate freezing at -80°C to prevent microbial growth or DNA degradation. In practice, however, especially for outpatients or multi-center studies, immediate ultra-cold freezing is often infeasible. This spurred the development and evaluation of ambient-temperature stabilization methods. Several comparative studies have examined various commercial preservation kits and simple storage conditions to identify which best maintain the microbial profile. Pribyl et al. ([2021](#)) rigorously tested five room-temperature preservation methods (including popular ones like RNAlater and OMNIgene-GUT, as well as a novel dry swab in an oxygen-free tube) against flash-freezing. They found that a dry swab with rapid desiccation (FLOQSwab in an Active Drying Tube) had the highest fidelity to frozen samples in terms of both technical reproducibility and community composition, followed closely by RNAlater and OMNIgene-GUT (Pribyl et al., [2021](#)). Methods like LifeGuard and a simple dry swab showed occasional overgrowth of *Escherichia* in some replicates, indicating that not all “stabilizers” are equal—some may inadvertently allow certain bacteria to bloom during storage, skewing results.

Other studies reinforce that the preservation method itself can introduce biases. Watson et al. ([2019](#)) observed that fecal samples stored in a DNA preservation buffer vs. immediately frozen had systematic differences: specifically, genera of Gram-negative bacteria were over-represented in the preserved samples, whereas Gram-positive genera were higher in frozen samples (Watson et al., [2019](#)). This bias likely stems from differential cell lysis or survival in the

chemical buffer. Similarly, Jones et al. ([2021](#)) noted significant compositional differences when stool was stored in OMNIgene or Norgen preservation solutions compared to freezing, which means that studies must be cautious in comparing data generated with different collection methods. The emerging consensus is that while inter-person variability usually exceeds the differences caused by preservation methods, the choice of method *does* matter, and certain kits are superior in preserving the original community structure. For clinical microbiome testing, this translates to a recommendation: use a validated preservation approach (like those that performed best in recent evaluations) and stick with it consistently. For example, Gemmell et al. ([2024](#)) found that two preservative buffers—a proprietary “PSP” buffer and RNeasy—best recapitulated the microbial diversity and metabolite profiles of fresh-frozen stool over 3 days at room temperature, outperforming other buffers (Gemmell et al., [2024](#)). Implementing such top-performing preservatives can allow patients to mail stool samples without dry ice, greatly facilitating clinical logistics, while still providing high-quality DNA for sequencing.

Stability and Storage Duration How long can a stool sample be kept under various conditions before sequencing results are affected? Earlier work suggested that short-term room temperature exposure (hours) may only minimally impact the microbiome profile if a good stabilizer is present. Recent studies extended this to longer periods and more accessible conditions. Momo Cabrera et al. ([2025](#)) reported that storing stool in a regular domestic freezer (around -20°C) for up to 6 months maintained microbial compositions, diversity, and antimicrobial resistance gene profiles comparable to fresh samples analyzed within 24 hours. This is encouraging for real-world settings, implying that even if -80°C is the ideal, a standard home freezer or short-term delay before freezing might be acceptable without compromising data (especially if whole-genome shotgun sequencing is used, which is less sensitive to minor DNA changes than amplicon sequencing)—though more validation is needed. Still, caution is

warranted: different taxa have different resilience; for instance, some fastidious anaerobes might die off quickly at higher temperatures, potentially decreasing their DNA over time. Therefore, best practice remains to minimize the time between sample passage and stabilization. Many clinical protocols instruct patients to use provided kits that either snap-freeze the sample (with cold packs) or chemically stabilize it immediately after collection.

Quality Control in Sequencing Runs Once samples reach the lab, maintaining quality through the sequencing workflow is paramount. QC measures start with DNA quality assessment—highly fragmented or impure DNA can lead to bias or sequencing failure. Labs often use standardized input amounts of DNA for library prep and include controls to ensure everything is performing as expected. It is now routine to include a *negative control* (no template) through DNA extraction and amplification. The sequencing data from these blanks help identify background contaminants. Likewise, *positive controls* are used: these can be either a “mock community” (a mixture of DNA from known organisms at known concentrations) or a previously characterized sample. Sequencing a mock community alongside patient samples tests whether the pipeline is accurately recovering known organisms in roughly the expected proportions. If the result from the mock deviates significantly, it signals a problem (e.g., a certain organism’s DNA didn’t amplify well or was biased) that must be addressed before trusting patient data. Some labs use internal standards like spike-in or contamination controls to monitor each sample’s absolute quantification and library prep efficiency (Fig. [19.2](#)).

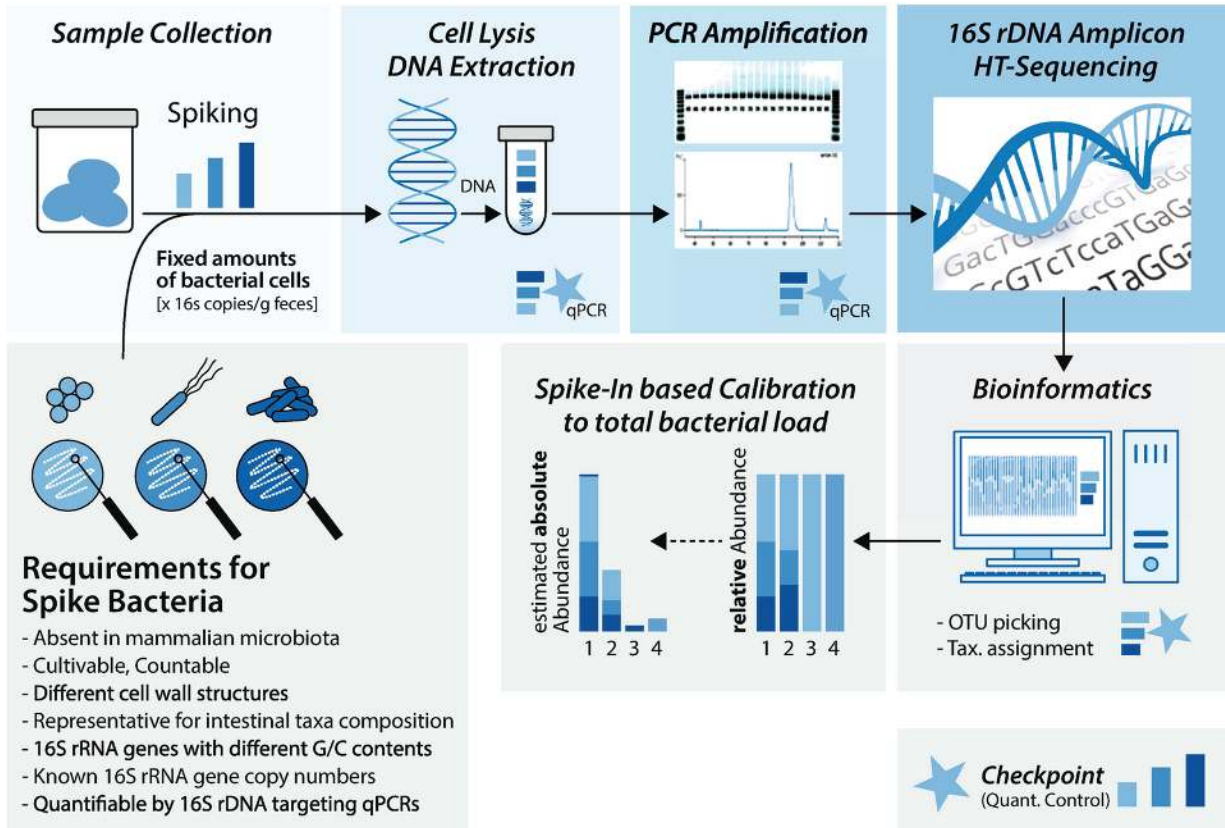


Fig. 19.2 Use of spike-in controls for 16S rRNA gene-based microbiome analyses

Another aspect of QC is *depth of sequencing*. Under-sequencing a sample (insufficient reads) can lead to missing key microbiome members, while over-sequencing wastes resources. In clinical contexts, laboratories often define a minimum number of sequencing reads per sample required for a valid result (e.g., “each stool sample must have at least 50,000 high-quality reads for 16S profiling” or much higher for metagenomics). If a sample yields fewer, it may be re-sequenced or reported as inadequate. This ensures that comparisons across patients are not confounded by unequal sequencing depth. Additionally, read quality filtering and chimera removal (for amplicon data) are applied to avoid sequencing artifacts from entering the analysis. Rauer et al. (2025) noted that higher DNA input can lead to more PCR chimeras; thus, QC protocols might adjust input DNA or cycle numbers to balance yield and artifact formation.

Data and Reporting Standards To move toward clinical-grade microbiome analysis, standardization in data processing and reporting is crucial. The microbiome research community has introduced guidelines (e.g., the STORMS reporting checklist in 2021 for human microbiome studies) that, while not a primary research article, encapsulate expert consensus on documenting methods and analyses in a reproducible way. For clinical labs, standard operating procedures (SOPs) are developed in line with regulatory requirements (like CLIA in the US or ISO standards), ensuring consistency. This includes validating the assay (analytical sensitivity/specificity, reproducibility, and repeatability tests using clinical-like samples). Proficiency testing is another component of quality assurance—pilot inter-laboratory trials have shown that when multiple labs sequence the same samples, results can vary, prompting efforts to harmonize protocols. As of 2024, organizations are beginning to offer microbiome proficiency testing schemes, where a standardized sample is sent to labs to sequence and the results are benchmarked. Such programs will likely expand as microbiome diagnostics inch closer to clinical reality.

Ensuring Clinical Utility Ultimately, any clinical test must demonstrate that it provides actionable, reliable information. For microbiome sequencing, this means robust QC not only produces accurate microbial profiles but also ensures that those profiles can be interpreted in a meaningful way for patient care. Quality control extends to the interpretation step—e.g., establishing reference ranges or “normal” microbiome variation is an ongoing effort. Large healthy cohort studies are being used to define what a typical gut microbiome looks like, against which a patient’s result might be compared. If a particular analysis yields a “dysbiosis index,” one must know the false positive/negative rates and the effect of any pre-analytical variable on that index. Continuous updates to databases (16S reference databases, genome catalogs, pathogen lists) are part of quality maintenance as well.

In summary, robust sample preservation techniques and rigorous sequencing quality control are enabling more reliable microbiome data, which is a prerequisite for clinical implementation. Innovations like ambient stable collection kits, internal spike-in controls, and cross-lab standardization have emerged between 2019 and 2024 as solutions to practical challenges. By adhering to these optimized practices and QC measures, researchers and clinicians can better ensure that a microbiome sequencing result reflects the patient's true microbial community and is reproducible and comparable across time and settings. This builds the foundation for confident clinical use, whether it be diagnosing an infection from sequence data, stratifying patients by microbiome profiles, or monitoring the success of a microbiome-targeted therapy. The coming years will likely see further refinement of these methods and increased regulatory clarity, but the advances to date have markedly improved the reliability and clinical relevance of microbiome sequencing.

19.4 Spatial and Single Cell Microbiome Analytics

Recent advances in high-throughput sequencing and spatially resolved microbiome analysis have significantly expanded the diagnostic and investigative toolkit in clinical microbiology. Three cutting-edge methods—SAMPL-seq, high-resolution spatial MGE mapping, and INVADeSeq—demonstrate how spatial and single-cell resolution technologies can enhance the understanding of microbe–host interactions and microbial community dynamics in complex human ecosystems.

Split-And-pool Metagenomic Plot-sampling sequencing (SAMPL-seq) enables micron-scale spatial profiling of the gut microbiome using split-pool barcoding and in situ 16S rRNA amplification. In a study involving over 21,000 microbial subcommunities, this method revealed conserved and temporally stable “spatial hubs” formed by bacterial taxa such as *Faecalibacterium prausnitzii*, *Phocaeicola*

vulgatus/Bacteroides dorei, and *Agathobacter rectalis* across multiple individuals. These microbial consortia demonstrated reversible spatial reorganization in response to dietary inulin supplementation, without major taxonomic shifts, underscoring the ecological importance of spatial structure as a regulatory layer in microbial community function. Given its scalability, compatibility with frozen samples, and non-invasive sample preparation, SAMPL-seq represents a robust platform for spatial microbiome analysis in clinical cohorts, especially for nutritional and therapeutic interventions targeting the gut microbiota (Grodner et al., [2024](#)).

Complementing this ecological focus, a spatial imaging framework combining single-molecule DNA-FISH and multiplexed rRNA-FISH has been developed to map mobile genetic elements (MGEs)—including antimicrobial resistance (AMR) plasmids and prophages—within complex microbiomes such as oral plaque biofilms. This method allows direct co-localization of MGEs and their host taxa at single-cell resolution. It reveals spatial clustering patterns driven by local horizontal gene transfer and clonal expansion, with taxon-specific associations: AMR plasmids with *Prevotella* spp. and *Streptococcus* spp., and prophages with *Veillonella* spp. The technology provides a foundational tool to investigate how spatial constraints influence the spread of antibiotic resistance and phage-mediated gene exchange within structured microbial communities (Galeano Niño et al., [2023](#)).

While both methods map microbial structure in extracellular environments, INVADeseq (Invasion–Adhesion–Directed Expression Sequencing) extends microbial analysis into the intracellular context. It adapts 10× Genomics’ 5′ single-cell RNA-seq protocol by including a bacterial 16S rRNA-targeting primer, enabling simultaneous capture of host and bacterial transcripts in individual cells. Applied to colorectal and oral squamous cell carcinoma tissues, INVADeseq successfully identified intracellular bacteria and quantified their transcriptional load per host cell, correlating microbial presence with distinct host gene expression profiles. The method addresses the critical limitation of standard scRNA-seq platforms, which typically miss bacterial RNA

due to poly(A) selection. INVADeseq thus provides a direct link between host cellular states and bacterial colonization at single-cell resolution, making it highly relevant for studies on infection, cancer, and immune modulation (Richardson et al., [2025](#)).

Microbe-Seq is a state-of-the-art platform that enables genomic and transcriptomic profiling of individual microbial cells without the need for cultivation. Utilizing droplet microfluidics, targeted amplification (e.g., MALBAC, MDA), and high-throughput barcoding, Microbe-seq can resolve strain-level heterogeneity, horizontal gene transfer (HGT), and rare microbial subpopulations in complex communities. This platform addresses the limitations of bulk metagenomics and 16S rRNA surveys, which average signals across populations and miss intra-species diversity and functional specialization. In clinical contexts, Microbe-seq has demonstrated utility in mapping antibiotic resistance genes at single-cell resolution, identifying immunomodulatory interactions between microbes and host immune cells, detecting microbe-associated oncogenic signatures in tumors as well as supporting microbial-based therapeutics by identifying probiotic strains with functional benefits. Nevertheless, technical challenges such as differential bacterial lysis efficiency, rRNA contamination, and limited curated single-cell microbial datasets must be addressed for broader clinical integration (Wu et al., [2024](#)).

19.5 Clinical Implementation

High-throughput sequencing is increasingly being integrated into clinical workflows to support diagnostics, therapeutic monitoring, and patient stratification. Applications include pathogen detection, profiling antimicrobial resistance, identifying microbial signatures associated with disease, and evaluating responses to microbiome-targeted therapies. While the most advanced use case remains fecal microbiota transfer, emerging strategies—such as defined microbial consortia and phage therapies—will rely on precise microbiome diagnostics. Robust clinical implementation requires standardization of

protocols, control of technical and biological confounders, and validation of bioinformatic pipelines. Integration of clinical metadata and longitudinal sampling is essential to distinguish disease-specific microbial changes from natural variability. Equally critical is the identification of reliable, reproducible biomarkers and a deeper understanding of causal disease mechanisms involving the microbiome. Many current associations between microbial features and disease lack functional validation, limiting their clinical utility. Establishing mechanistic links- such as microbial metabolites influencing host immunity or specific taxa driving inflammation—will be key to making microbiome screening a dependable and interpretable clinical tool. Spatial and single-cell microbiome technologies offer promising avenues to enhance diagnostic resolution and inform precision medicine. With careful quality control, curated databases, and reproducible workflows, microbiome sequencing can become a powerful tool for personalized clinical care. Figure [19.3](#) Clinical implementation of HTS-based microbiome analyses and their use in monitoring therapeutic responses.

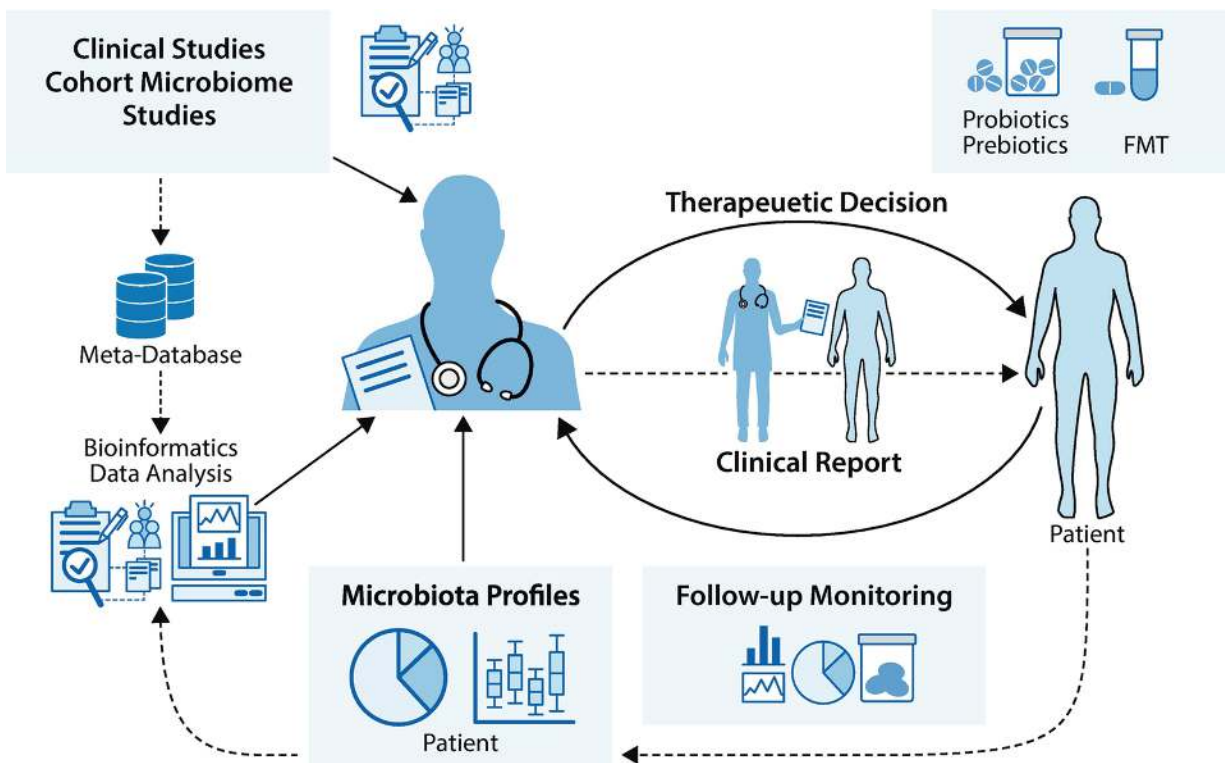


Fig. 19.3 Clinical implementation of HTS-based microbiome analyses and their use in monitoring therapeutic responses

Controversy Fecal microbiota transplantation (FMT) stands as the most validated microbiota-based therapy, particularly for recurrent *Clostridioides difficile* infections, where randomized controlled trials have demonstrated high efficacy. Its use is expanding into other areas—including inflammatory bowel disease (IBD), metabolic syndrome, and support for cancer immunotherapy—yet robust evidence is often lacking or inconsistent. The variability in donor microbiota, host response, and treatment protocols complicates reproducibility and generalizability. The long-term safety and durability of FMT effects in these broader contexts remain to be clarified.

High-throughput sequencing (HTS) plays a critical role in monitoring FMT outcomes by tracking donor engraftment and community dynamics post-intervention. It also enables early detection of microbiota shifts that may predict clinical response or relapse. However, HTS has yet to be universally adopted as a diagnostic standard due to challenges in standardization, clinical interpretation, and regulatory approval. Differences in sequencing platforms, bioinformatic pipelines, and reference databases contribute to variability, and confounding factors such as diet and medication further complicate diagnostic accuracy.

Beyond FMT, other microbiota-based interventions—such as live biotherapeutic products, engineered consortia, and phage therapies—are advancing into clinical trials. However, many of these approaches are still informed by associative rather than causative data. Without rigorous mechanistic understanding and validated microbial biomarkers, there is a risk of premature clinical application based on incomplete evidence.

To enable safe and effective integration of microbiota-based therapies, clinical HTS diagnostics must meet stringent quality and interpretability standards. This includes validated assay performance, harmonized protocols, and integration of clinical metadata. A cautious, data-driven approach will be essential to unlock the full

therapeutic and diagnostic potential of the microbiome while maintaining patient safety and scientific integrity.

History

Since Theodor Escherich's isolation of the first human gut bacterium in 1886, characterization of the gastrointestinal microbiota has evolved from culture-based techniques—revolutionized by Robert Hungate's anaerobic methods—to molecular-based approaches. The development of PCR, Sanger sequencing in the 1970s, and the use of 16S rRNA genes for bacterial phylogenetics by Carl Woese in 1990 laid the groundwork for culture-independent microbiome analysis. These techniques transformed both research and clinical microbiology by enabling precise pathogen detection and phylogenetic classification. A major breakthrough came in 2005 with the launch of next-generation sequencing (NGS) platforms by 454 Life Sciences, enabling comprehensive, culture-independent profiling of complex microbial communities. This marked the beginning of high-throughput sequencing (HTS) in microbiome research and diagnostics. HTS has since enabled the identification of microbial biomarkers, the characterization of antibiotic resistance genes, and the monitoring of microbiota-based therapies such as fecal microbiota transplantation (FMT). More recent developments include single-cell genomics, spatial transcriptomics, and nanopore-based sequencing, offering long-read capabilities and strain-level resolution. These technologies allow tracking of microbial dynamics in therapeutic contexts and hold promise for real-time diagnostics. Integrating HTS with clinical workflows enables monitoring of donor microbiota engraftment after FMT and helps assess the impact of probiotics, dietary interventions, and other microbiota-targeted therapies. As sequencing platforms become faster and more accessible, their use in microbiota-based diagnostics and personalized treatment monitoring is poised to expand.

Highlights

- High-throughput sequencing (HTS) has transformed microbiome research by enabling cultivation-independent profiling of complex microbial communities and is increasingly applied in clinical studies to explore host-microbiota associations.
- Each step of the HTS workflow—from sampling and DNA extraction to PCR, library preparation, sequencing, and data analysis—can introduce methodological bias, complicating reproducibility and comparability across studies.
- Internal quality controls, such as spike-in bacterial standards, provide essential benchmarks for evaluating protocol consistency and enabling methodological standardization.
- Clinical application of microbiome sequencing is challenged by high interindividual variability and a multitude of confounding host and environmental factors, which can obscure disease-specific microbial signals.
- Moving beyond correlative findings requires rigorous validation studies and novel tools to establish causality in microbiota-host interactions and to support the development of targeted diagnostics and therapies.

References

Almeida, A., Nayfach, S., Boland, M., et al. (2021). A unified catalog of 204,938 reference genomes from the human gut microbiome. *Nature Biotechnology*, 39(1), 105–114. <https://doi.org/10.1038/s41587-020-0603-3>

[PubMed]

Asnicar, F., Berry, S. E., Valdes, A. M., et al. (2021). Microbiome connections with host metabolism and habitual diet from 1,098 deeply phenotyped individuals. *Nature Medicine*, 27(2), 321–332.

<https://doi.org/10.1038/s41591-020-01183-8>

[PubMed]

Bolyen, E., Rideout, J. R., Dillon, M. R., Bokulich, N. A., Abnet, C. C., Al-Ghalith, G. A., Alexander, H., Alm, E. J., Arumugam, M., Asnicar, F., Bai, Y., Bisanz, J. E., Bittinger, K., Brejnrod, A., Brislawn, C. J., Brown, C. T., Callahan B. J., Caraballo-Rodríguez, A. M., Chase, J., Cope, E. K., Da Silva, R., Diener, C., Dorrestein, P. C., Douglas, G. M., Durall, D. M., Duvallet, C., Edwardson, C. F., Ernst, M., Estaki, M., Fouquier, J., Gauglitz, J. M., Gibbons, S. M., Gibson, D. L., Gonzalez, A., Gorlick, K., Guo, J., Hillmann, B., Holmes, S., Holste, H., Huttenhower, C., Huttley, G. A., Janssen, S., Jarmusch, A. K., Jiang, L.,

Kaehler, B. D., Kang, K. B., Keefe, C. R., Keim, P., Kelley, S. T., Knights, D., Koester, I., Kosciulek, T., Kreps, J., Langille, M. G. I., Lee, J., Ley, R., Liu, Y. X., Loftfield, E., Lozupone, C., Maher, M., Marotz, C., Martin, B. D., McDonald, D., McIver, L. J., Melnik, A. V., Metcalf, J. L., Morgan, S. C., Morton, J. T., Naimey, A. T., Navas-Molina, J. A., Nothias, L. F., Orchanian, S. B., Pearson, T., Peoples, S. L., Petras, D., Preuss, M. L., Pruesse, E., Rasmussen, L. B., Rivers, A., Robeson, M. S. 2nd., Rosenthal, P., Segata, N., Shaffer, M., Shiffer, A., Sinha, R., Song, S. J., Spear, J. R., Swafford, A. D., Thompson, L. R., Torres, P. J., Trinh, P., Tripathi, A., Turnbaugh, P. J., Ul-Hasan, S., van der Hooft, J. J. J., Vargas, F., Vázquez-Baeza, Y., Vogtmann, E., von Hippel, M., Walters, W., Wan, Y., Wang, M., Warren, J., Weber, K. C., Williamson, C. H. D., Willis, A. D., Xu, Z. Z., Zaneveld, J. R., Zhang, Y., Zhu, Q., Knight, R., Caporaso, J. G. (2019). Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nature Biotechnology*, 37(8), 852–857. <https://doi.org/10.1038/s41587-019-0209-9>. Erratum in: *Nat Biotechnol.* 2019 Sep;37(9):1091. <https://doi.org/10.1038/s41587-019-0252-6>. PMID: 31341288; PMCID: PMC7015180.

de Goffau, M. C., Lager, S., Salter, S. J., et al. (2019). Human placenta has no microbiome but can contain potential pathogens. *Nature*, 572(7769), 329–334. <https://doi.org/10.1038/s41586-019-1451-5> [PubMed]

Ezzy, A. C., Hagstrom, A. D., George, C., et al. (2019). Storage and handling of human faecal samples affect the gut microbiome composition: A feasibility study. *Journal of Microbiological Methods*, 164, 105668. <https://doi.org/10.1016/j.mimet.2019.105668> [PubMed]

Federici, S., Kredo-Russo, S., Valdés-Mas, R., Kviatcovsky, D., Weinstock, E., Matiuhin, Y., Silberberg, Y., Atarashi, K., Furuichi, M., Oka, A., Liu, B., Fibelman, M., Weiner, I. N., Khabra, E., Cullin, N., Ben-Yishai, N., Inbar, D., Ben-David, H., Nicenboim, J., Kowalsman, N., Lieb, W., Kario, E., Cohen, T., Geffen, Y. F., Zelcbuch, L., Cohen, A., Rappo, U., Gahali-Sass, I., Golembo, M., Lev, V., Dori-Bachash, M., Shapiro, H., Moresi, C., Cuevas-Sierra, A., Mohapatra, G., Kern, L., Zheng, D., Nobs, S. P., Suez, J., Stettner, N., Harmelin, A., Zak, N., Puttagunta, S., Bassan, M., Honda, K., Sokol, H., Bang, C., Franke, A., Schramm, C., Maharshak, N., Sartor, R. B., Sorek, R., & Elinav, E. (2022). Targeted suppression of human IBD-associated gut microbiota commensals by phage consortia for treatment of intestinal inflammation. *Cell*, 185(16), 2879–2898.e24. <https://doi.org/10.1016/j.cell.2022.07.003> [PubMed]

Forster, S. C., Kumar, N., Anonye, B. O., et al. (2019). A human gut bacterial genome and culture collection for improved metagenomic analyses. *Nature Biotechnology*, 37(2), 186–192. <https://doi.org/10.1038/s41587-018-0009-7> [PubMed]

Galeano Niño, J. L., Wu, H., LaCourse, K. D., Srinivasan, H., Fitzgibbon, M., Minot, S. S., Sather, C., Johnston, C. D., & Bullman, S. (2023). INVADEseq to identify cell-adherent or invasive bacteria and the associated host transcriptome at single-cell-level resolution. *Nature Protocols*, 18, 3355–3389. <https://doi.org/10.1038/s41596-023-00888-7> [PubMed]

Gemmell, M. R., Jayawardana, T., Koentgen, S., et al. (2024). Optimised human stool sample collection for multi-omic microbiota analysis. *Scientific Reports*, 14, 16816. <https://doi.org/10.1038/s41598-024-67499-4>

[PubMed]

Grodner, B., Shi, H., Farchione, O., Vill, A. C., Ntekas, I., Diebold, P. J., Wu, D. T., Chen, C.-Y., Kim, D. M., Zipfel, W. R., Brito, I. L., & De Vlaminc, I. (2024). Spatial mapping of mobile genetic elements and their bacterial hosts in complex microbiomes. *Nature Microbiology*, 9, 2262–2277.

<https://doi.org/10.1038/s41564-024-01735-5> [PubMed]

Hiergeist, A., Gläsner, J., Reischl, U., & Gessner, A. (2016). Analyses of intestinal microbiota: Culture versus sequencing. *ILAR Journal*, 57(2), 228–240.

<https://doi.org/10.1093/ilar/ilv017>

Jones, J., Reinke, S. N., Ali, A., et al. (2021). Fecal sample collection methods and time of day impact microbiome composition and short chain fatty acid concentrations. *Scientific Reports*, 11, 13964.

<https://doi.org/10.1038/s41598-021-93031-z>

[PubMed]

Larsson, A., Ericson, U., Jönsson, D., et al. (2024). New connections of medication use and polypharmacy with the gut microbiota composition and functional potential in a large population. *Scientific Reports*, 14, 23723. <https://doi.org/10.1038/s41598-024-71571-4>

[PubMed]

Li, M., Liu, J., Zhu, L., et al. (2023). Performance of gut microbiome as an independent diagnostic tool for 20 diseases: Cross-cohort validation of machine-learning classifiers. *Gut Microbes*, 15(1), 2155276. <https://doi.org/10.1080/19490976.2023.2155276>

Momo Cabrera, P., Bokulich, N. A., Zimmermann, P. (2025) Evaluating stool microbiome integrity after domestic freezer storage using whole-metagenome sequencing, genome assembly, and antimicrobial resistance gene analysis. *Microbiology Spectrum* 13(3), e0227824. <https://doi.org/10.1128/spectrum.02278-24>. Epub 2025 Feb 11. PMID: 39932403; PMCID: PMC11878046.

Nishijima, S., et al. (2024). Fecal microbial load is a major determinant of gut microbiome variation and a confounder for disease associations. *Cell*, 188(1), 221–236.e15. <https://doi.org/10.1016/j.cell.2024.10.022>

Pasolli, E., Truong, D. T., Malik, F., Waldron, L., & Segata, N. (2016). Machine learning meta-analysis of large metagenomic datasets: Tools and biological insights. *PLoS Computational Biology*, 12(7), e1004977. <https://doi.org/10.1371/journal.pcbi.1004977>

[PubMed]

Pribyl, A. L., Parks, D. H., Angel, N. Z., et al. (2021). Critical evaluation of faecal microbiome preservation using metagenomic analysis. *ISME Communications*, 1, 14. <https://doi.org/10.1038/s43705-021-00014-2>

[PubMed]

Rauer, L., De Tomassi, A., Müller, C. L., Hülpmusch, C., Traidl-Hoffmann, C., Reiger, M., Neumann, A. U. (2025) De-biasing microbiome sequencing data: bacterial morphology-based correction of extraction bias and correlates of chimera formation. *Microbiome* 13(1), 38. <https://doi.org/10.1186/s40168-024-01998-4>. PMID: 39905530; PMCID: PMC11792448.

Richardson, M., Zhao, S., Lin, L., Sheth, R. U., Qu, Y., Lee, J., Moody, T., Ricaurte, D., Huang, Y., Velez-Cortes, F., Urtecho, G., & Wang, H. H. (2025). SAMPL-seq reveals micron-scale spatial hubs in the human gut microbiome. *Nature Microbiology*, 10, 527–540.

[\[https://doi.org/10.1038/s41564-024-01914-4\]](https://doi.org/10.1038/s41564-024-01914-4) [\[PubMed\]](#)

Rodriguez, J., Cordaillat-Simmons, M., Pot, B., & Druart, C. (2025). The regulatory framework for microbiome-based therapies: Insights into European regulatory developments. *npj Biofilms and Microbiomes*, 11(1), 53.

[\[https://doi.org/10.1038/s41522-025-00683-0\]](https://doi.org/10.1038/s41522-025-00683-0) [\[PubMed\]](#)

Stämmli, F., Gläsner, J., Hiergeist, A., Holler, E., Weber, D., Oefner, P. J., Gessner, A., & Spang, R. (2016). Adjusting microbiome profiles for differences in microbial load by spike-in bacteria. *Microbiome*, 4, 28.

[\[https://doi.org/10.1186/s40168-016-0175-0\]](https://doi.org/10.1186/s40168-016-0175-0) [\[PubMed\]](#)

Tito, R. Y., Verbandt, S., Aguirre Vazquez, M., et al. (2024). Microbiome confounders and quantitative profiling challenge predicted microbial targets in colorectal cancer development. *Nature Medicine*, 30(7), 1339–1348. <https://doi.org/10.1038/s41591-024-02963-2>

[\[PubMed\]](#)

Watson, E. J., Giles, J., Scherer, B. L., & Blatchford, P. (2019). Human faecal collection methods demonstrate a bias in microbiome composition by cell wall structure. *Scientific Reports*, 9, 16831.

<https://doi.org/10.1038/s41598-019-53183-5>

[\[PubMed\]](#)

Wu, Y., Jing, X., Zhang, Y., & Liu, H. (2024). Advances in single-cell sequencing technology in microbiome research. *Genes & Diseases*, 11(4), 101129.

[\[https://doi.org/10.1016/j.gendis.2023.101129\]](https://doi.org/10.1016/j.gendis.2023.101129)

Zaramela, L. S., Tjuanta, M., Moyne, O., et al. (2022). synDNA – A synthetic DNA spike-in method for absolute quantification of shotgun metagenomic sequencing. *mSystems*, 7(6), e00447-22. <https://doi.org/10.1128/mSystems.00447-22>

[\[PubMed\]](#)

Zeller, G., Tap, J., Voigt, A. Y., et al. (2014). Potential of fecal microbiota for early-stage detection of colorectal cancer. *Molecular Systems Biology*, 10(11), 766. <https://doi.org/10.15252/msb.20145645>

[\[PubMed\]](#)

20. Fecal Microbiota Transplantation

Lea-Maxie Haag¹, Franziska Nowak² and Britta Siegmund¹ 

- (1) Medizinische Klinik für Gastroenterologie, Infektiologie, Rheumatologie, Charité – Universitätsmedizin Berlin, Berlin, Germany
- (2) Flow & Mass Cytometry Core Facility, Berlin Institute of Health at Charité - Universitätsmedizin Berlin, Berlin, Germany

 **Britta Siegmund**
Email: britta.siegmund@charite.de

Abstract

Fecal microbiota transplantation has emerged as a highly effective therapy for recurrent *Clostridioides difficile* infection and is now integrated into major clinical guidelines. Its success has led to growing interest in its potential for conditions linked to gut dysbiosis, such as inflammatory bowel disease, irritable bowel syndrome, metabolic disorders, and graft-versus-host disease. However, outcomes remain variable, influenced by donor selection, delivery methods, and recipient microbiome composition. Fecal microbiota transplantation exerts its effects by restoring microbial diversity, modulating immune responses, influencing bile acid metabolism, and enhancing gut barrier function. Recent advancements, including capsule-based techniques, defined microbial consortia, and synthetic microbiome therapies, aim to improve efficacy and safety. However, challenges such as pathogen transmission risks, regulatory uncertainties, and long-term effects remain key concerns. As research advances, fecal microbiota transplantation is likely to evolve toward personalized microbiome-

based therapies, leveraging metagenomic sequencing and artificial intelligence for optimized donor selection. While it has revolutionized the treatment of *Clostridioides difficile* infections, rigorous clinical trials, standardized protocols, and next-generation microbiota-based interventions will be crucial in expanding its role in precision medicine. This chapter will summarize the development of the field over the last decade and will provide an outlook on possible innovations in the foreseeable future.

Keywords Fecal microbiota transplantation – *Clostridioides difficile* infection – Recurrent CDI – Treatment efficacy – Donor screening

Transplantation has been a major focus of medical innovation for decades, traditionally associated with solid organ transplantation, with its complex challenges and breakthroughs. However, over the past 15 years, a novel and unconventional form of transplantation—fecal microbiota transplantation (FMT)—has emerged as a significant therapeutic strategy, transitioning from an experimental concept to an evidence-based intervention now included in clinical guidelines (Peery et al., [2024](#)). Unlike organ transplants, FMT does not replace a failing organ but instead modulates the gut microbiome. FMT serves distinct roles depending on the host environment. In humans, the procedure reshapes an already established intestinal microbiota, modulating microbial composition and function. In contrast, in experimental murine models, FMT is often performed in germ-free mice, where the transplanted microbiota establishes de novo colonization, allowing researchers to investigate microbial causality in health and disease (Park & Im, [2020](#)). Although reports on the beneficial effects of FMT date back decades, it remained on the fringes of mainstream medicine for many years. The surge in microbiota-related research was driven by two pivotal developments: (1) the advent of affordable, high-throughput sequencing technologies, which enabled comprehensive profiling of gut microbial communities, and (2) large-scale microbiome-

disease association studies linking gut dysbiosis to conditions ranging from metabolic disorders to neuropsychiatric diseases.

A seminal study by Turnbaugh and colleagues in 2006 demonstrated that fecal transfer from obese mice into germ-free recipients induced significant weight gain, underscoring the causal role of the gut microbiota in metabolism (Turnbaugh et al., [2006](#)). Since then, FMT has evolved from a last-resort therapy for recurrent *Clostridioides difficile* infection (rCDI) to a broader investigational tool for conditions such as inflammatory bowel disease (IBD), irritable bowel syndrome (IBS), metabolic syndrome, and even neurological disorders like Parkinson's disease and autism spectrum disorder. Moreover, FMT has been increasingly used and studied in the context of graft-versus-host disease (GVHD), particularly acute GVHD (aGVHD) following allogeneic hematopoietic stem cell transplantation (alloHCT) (Weber et al., [2024](#); Reddi et al., [2025](#)).

Alongside traditional FMT, novel approaches, including rationally designed microbial consortia and next-generation microbiome therapeutics, are being explored to refine and optimize FMT-based interventions (Camarota et al., [2017](#); Yadegar et al., [2024](#)).

This chapter will provide an overview of the historical development of FMT, its established role in treating *C. difficile* colitis, donor selection criteria, preparation methods, administration routes, and the underlying mechanisms that contribute to its clinical effects. Additionally, it will examine the expanding role of FMT in other indications, including IBD, IBS, and metabolic syndrome, while also providing an outlook on future directions in microbiome-based therapies and precision medicine approaches.

20.1 Historic View

The use of fecal matter as a therapeutic agent has a long history (Fig. [20.1](#)), with some of the earliest known applications traced back to ancient China. Records from the fourth century describe how a Chinese physician administered a suspension of human feces orally to patients

suffering from severe diarrhea. This treatment, which was believed to be life-saving, was first documented in the traditional Chinese medical text *Zhou Hou Bei Ji Fang* (Zhang et al., [2012](#)). Later, during the Ming Dynasty in the sixteenth century, the physician Li Shizhen further expanded the use of fecal-based treatments, prescribing fresh suspensions, dried feces, and even infant feces to manage gastrointestinal ailments such as diarrhea, vomiting, and constipation. His comprehensive work, *Ben Cao Gang Mu*, refers to these treatments under the term “yellow soup,” a likely attempt to improve patient acceptance (Zhang et al., [2012](#); Li, [2024](#)). In Europe, historical records suggest that similar ideas were introduced in veterinary medicine in the seventeenth century, likely as a means of restoring gut health in livestock suffering from gastrointestinal disorders (Borody et al., [2004](#); Brandt et al., [2012](#)). However, one of the more striking historical accounts comes from World War II, when German soldiers stationed in North Africa, suffering from bacterial dysentery, were reportedly treated with fresh, warm camel feces following recommendations from Bedouins. This practice, though unconventional, proved to be highly effective, suggesting an empirical understanding of the microbiome’s therapeutic potential long before the field of microbiota research formally emerged (Lewin, [2001](#)). The transition of FMT into modern medical practice began in the mid-twentieth century. In 1958, Dr. Ben Eiseman and colleagues in Denver, Colorado, published the first documented case of FMT being used as a medical treatment for pseudomembranous colitis, a severe gastrointestinal infection known to be caused by *C. difficile* (Eiseman et al., [1958](#)) (Fig. [20.2](#)). Despite its success, FMT remained an obscure treatment for decades, largely overshadowed by the development of antibiotics. It was not until the late twentieth century that the procedure gradually entered medical guidelines as a third-line recommendation in *The Sanford Guide to Antimicrobial Therapy* (Gilbert et al., [2006](#)). Over the past two decades, FMT has seen a renewed focus in clinical medicine, driven by a deeper understanding of the gut microbiome’s role in human health and the growing challenge of antibiotic-resistant *C. difficile* infections (CDI).

Landmark clinical trials in the 2010s provided robust evidence that FMT was not only effective but superior to antibiotic therapy for rCDI (van Nood et al., [2013](#); Cammarota et al., [2017](#)). These findings paved the way for the integration of FMT into major clinical guidelines, elevating it from a historical remedy to a widely recognized therapeutic approach. Within the last decade, there have been significant developments in the use of FMT for conditions beyond CDI (see Sect. [20.3](#)). In recent years, regulatory approvals for FMT products have advanced in North America and Australia. In the USA, the FDA approved *Rebyota* in 2022 and *Vowst* in 2023 for preventing rCDI. In Australia, the Therapeutic Goods Administration (TGA) introduced stricter regulations for FMT in 2021, requiring registration in the Australian Register of Therapeutic Goods. This led to the approval of *Biomictra* in 2022, the first licensed FMT product in the country. These developments highlight the increasing acceptance of FMT as a therapeutic option for gut microbiota restoration (Porcari et al., [2023](#)).

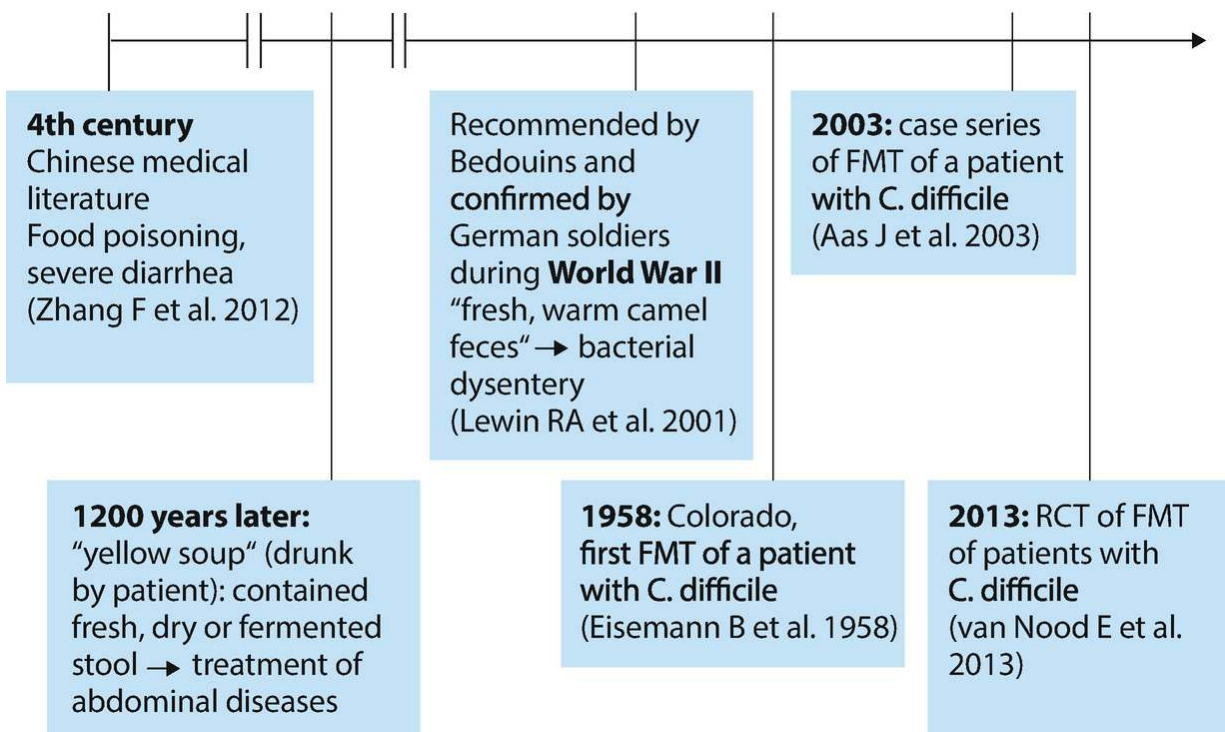


Fig. 20.1 Historic view on FMT



Fig. 20.2 Representative endoscopic images of pseudomembranous colitis. The images display the characteristic yellowish pseudomembranes associated with *Clostridioides difficile* colitis. The left image shows a milder form of the disease with fewer pseudomembranes, while the right image depicts an advanced stage of the disease with extensive, widespread pseudomembrane formation. (The endoscopic pictures were kindly provided by Prof. Dr. Christian Bojarski)

20.2 Classic Fecal Microbiota Transplantation in *Clostridioides difficile* Colitis

The emergence of CDI, formerly known as antibiotic-associated colitis, coincided with the widespread use of antibiotics. *C. difficile* colitis is also referred to as pseudomembranous colitis, a term that describes the characteristic, distinctive pathological findings in the colon during infection. The name originates from the formation of pseudomembranes—yellowish-white plaques composed of necrotic epithelial cells, fibrin, mucus, and inflammatory debris—that overlay the colonic mucosa (Fig. [20.2](#)). These lesions develop as a result of toxin-mediated epithelial damage caused by *C. difficile*, particularly toxins A and B, leading to inflammation, mucosal injury, and increased intestinal permeability.

It was not until 1978 that *C. difficile* was identified as the causative pathogen (George et al., [1978](#); Larson et al., [1978](#)). Over the past two decades, the incidence of CDI has risen sharply, partly due to the emergence of hypervirulent strains, such as *C. difficile* ribotype 027, associated with more severe disease, higher recurrence rates, and

increased mortality (Surawicz & Alexander, [2011](#); Lessa et al., [2015](#)). A retrospective study in Canada reported a fourfold increase in CDI cases between 1991 and 2000, with a tenfold rise among individuals over 65 years of age (Miller et al., [2011](#)). In the past decade, CDI incidence has shown regional variations. In Germany, cases declined by 38% between 2015 and 2019, likely due to improved infection control measures and antibiotic stewardship (Antunes et al., [2024](#)). Data from the European Centre for Disease Prevention and Control (ECDC) indicate fluctuating CDI rates across Europe between 2018 and 2020, with some countries reporting decreases while others saw continued rises (ECDC, [2024](#)). Conversely, a global analysis suggests that CDI remains a growing burden in many regions, particularly in areas with high to middle sociodemographic indices, where the age-standardized death rate increased from 0.19 in 1990 to 0.43 in 2019 (Koo et al., [2014](#); Chen et al., [2024](#)). These differences highlight the complexity of CDI epidemiology and the influence of local healthcare practices, surveillance strategies, and public health initiatives. Transmission occurs primarily via the fecal-oral route, with *C. difficile* spores persisting in healthcare environments, making hospital-acquired infections a major concern. Strict infection control measures are crucial in reducing transmission, but a detailed discussion of these strategies is beyond the scope of this chapter and is covered elsewhere (Surawicz & Alexander, [2011](#)).

By the mid-2000s, the field of intestinal microbiota research gained renewed interest, largely driven by colonization experiments in germ-free mice that demonstrated the microbiome's direct influence on host metabolism and phenotype (Turnbaugh et al., [2006](#)). Around the same time, the first case series of FMT in patients with rCDI was published, providing early clinical evidence of its therapeutic potential (Aas et al., [2003](#)). The rationale behind FMT is compelling: antibiotic therapy and certain intestinal conditions disrupt the gut microbiota, leading to an imbalance that facilitates *C. difficile* colonization. This dysbiosis results in severe diarrhea and, in some cases, life-threatening complications. Early clinical reports suggested that by performing FMT, microbial

diversity and intestinal function can be restored (Khoruts & Sadowsky, [2016](#)). Moreover, the success of the procedure appeared to be independent of the route of administration, whether via nasoduodenal tubes or endoscopically in the colon (Postigo & Kim, [2012](#)).

In 2013, the Amsterdam group presented data from the first controlled clinical trial in *The New England Journal of Medicine* (van Nood et al., [2013](#)). The study included patients suffering from rCDI and assigned them to one of the three groups:

1. An initial vancomycin regimen for 4 days, followed by bowel lavage and subsequent application of donor feces through a nasoduodenal tube.
2. A standard vancomycin regimen for 14 days.
3. A standard vancomycin regimen with bowel lavage.

The primary endpoint was the resolution of diarrhea without a relapse after 10 weeks. The study had to be stopped after the interim analysis since 81% of the group (1) had a resolution of CDI after a single infusion; two of the remaining three patients showed resolution after a second transplant. In contrast, resolution occurred only in 31% of patients in group (2) and 23% in group (3), respectively. Not surprisingly, resolution was associated with an increase in bacterial diversity comparable to healthy donors.

Since this initial landmark paper by van Nood and colleagues, several controlled trials have validated these findings and have expanded our understanding of FMT efficacy and delivery methods. Consistent success rates of nearly 90% demonstrate that in rCDI, FMT proves a reliable alternative to the antibiotics vancomycin or fidaxomicin, ultimately leading to its inclusion in major guidelines (Camarota et al., [2017](#); Quraishi et al., [2017](#); Ianiro et al., [2018](#); Hui et al., [2019](#); Hvas et al., [2019](#); Aby et al., [2022](#); Peery et al., [2024](#); Drekonja et al., [2025](#)). Moreover, the available data show significant differences in FMT for recurrent compared to refractory disease, with recurrent

diseases having a stronger evidence base and outcomes that are generally better. Therefore, more research will be needed to fully understand the efficacy and optimal protocols for FMT in refractory CDI.

The regulatory approach to FMT remains in flux globally, with ongoing discussions and evolving frameworks in many countries as they grapple with the unique challenges posed by this emerging therapeutic approach. Table [20.1](#) summarizes the regulatory approaches for FMT across countries.

Table 20.1 Regulatory approaches for FMT

Country/region	Regulatory approach	Included in guidelines
European Union	<i>Varies by member state:</i> Medicinal product: Croatia, Czech Republic, France, Germany, Ireland, Portugal, Spain, Sweden Tissue/cell legislation: Belgium, Italy Therapeutic intervention: Finland Mixed approach: Austria, Denmark	European Consensus guidelines agreed upon by experts from over 10 countries
United States	Therapeutic intervention for rCDI Drug for all other applications	FDA guidelines for rCDI since 2013
Note: The FDA has approved specific microbiome-based therapies, such as Rebyota in 2022 and Vowst in 2023, for treating rCDI.		
Canada	Biological drug	Not specified
Australia	Biological drug Class 1 or 2 biologicals (since 2020) Medicines for certain standardized products	TGA implemented regulatory requirements in 2021
Note: In November 2022, the TGA approved BiomeBank's BIOMICTRA as the world's first microbiome drug.		
United Kingdom	Unlicensed medicinal product	Regulated under the Medicines Directive

FDA Food and Drug Administration, *TGA* Therapeutic Goods Administration, *rCDI* recurrent *C. difficile* Infection

In summary, FMT’s regulatory landscape varies globally, with countries adopting different frameworks based on their healthcare systems and perspectives on FMT’s classification.

20.2.1 Donor Selection

Donor selection in FMT is subject to strict regulations due to the potential risks associated with transferring live microorganisms from one individual to another. Unlike other medical treatments, FMT involves the direct transfer of a complex microbial ecosystem, which can carry pathogens, multidrug-resistant organisms (MDRO), or even unwanted metabolic traits. To minimize these risks, rigorous screening protocols have been established to ensure donor health, prevent disease transmission, and optimize therapeutic outcomes. Over time, evolving scientific insights and emerging infectious threats have led to continuous updates in donor selection criteria, reflecting the need for both safety and efficacy in clinical applications (Cammarota et al., [2017](#); Duvallet et al., [2019](#); Haifer et al., [2020](#); Mehta et al., [2022](#)). Even though the foundational principles outlined in the 2017 consensus paper remain relevant, recent developments have refined donor selection processes to enhance safety and efficacy. Incorporating comprehensive pathogen screening, adapting to emerging infectious challenges, and employing strategic donor-recipient matching are pivotal steps in the evolution of FMT practices. While Tables [20.2](#) and [20.3](#) summarizes the noninvasive donor evaluation, including blood and stool testing, Table [20.4](#) summarizes the enhanced safety measures and optimized donor selection aspects that have entered the clinical routine in recent years.

Table 20.2 Noninvasive donor evaluation

Area of question	Specific issues
------------------	-----------------

Area of question	Specific issues
Infectious diseases	History of, exposure to: HIV, HBV, HCV, syphilis, HTLV I and II, malaria, trypanosomiasis, tuberculosis Systemic infection not controlled at the time of donation Illegal drugs, other risky behavior, recipient of transplant (ever) or blood product (<12 months), needle stick, body tattoo, piercing, earring, acupuncture (all <6 months), medical treatment in poorly hygienic conditions, risk of transmission of prions Recent parasitosis or infection with GI involvement Recent (<6 months) travel to tropical countries or countries at high risk of communicable disease or traveller's diarrhea Recent (<6 months) vaccination with a live attenuated virus Healthcare worker, individuals working with animals
GI, metabolic, and neurological disorders	History of chronic GI disorder (functional, inflammatory, malignant) or recent development of diarrhea, hematochezia High risk of GI cancer, polyposis History of neurological/neurodegenerative or psychiatric disorders Body mass index >25
Medications that can affect intestinal microbiota composition	Exposure to antibiotics, immunosuppressants, chemotherapy (<3 months) Chronic exposure to proton pump inhibitors

Adapted from Cammarota et al. ([2017](#))

GI gastrointestinal tract, *HIV* human immunodeficiency virus, *HBV* hepatitis B virus, *HCV* hepatitis C virus, *HTLV* human T lymphotropic virus

Table 20.3 Donor testing, blood, and stool

Category	Specific issues
Blood	<i>Viruses:</i> Cytomegalovirus, Epstein-Barr virus, hepatitis A/B/C/E, HIV 1/–2, (specific situation: HTLV I/II) <i>Bacteria:</i> Syphilis (<i>Treponema pallidum</i>) <i>Parasites:</i> <i>Entamoeba histolytica</i>, (specific situation: <i>Strongyloides stercoralis</i>) <i>General:</i> Complete blood cell count (+ differential), C-reactive protein, ESR, albumin, creatinine, electrolytes, aminotransferases, bilirubin, gamma-glutamyltransferase, alkaline phosphatase

Category	Specific issues
Stool	<i>Bacteria: Clostridioides difficile</i>, enteric pathogens (<i>Salmonella</i>, <i>Shigella</i>, <i>Campylobacter</i>, <i>Escherichia coli</i> 0157 H7, <i>Yersinia</i>, vancomycin-resistant enterococci, methicillin-resistant <i>Staphylococcus aureus</i>, Gram-negative multidrug-resistant bacteria), (specific situations: <i>Vibrio cholerae</i>, <i>Listeria monocytogenes</i>, <i>Helicobacter pylori</i> fecal antigen) <i>Viruses: Norovirus</i>, (specific situation: Rotavirus) <i>Parasites: Giardia lamblia</i>, <i>Cryptosporidium parvum</i>, protozoa (including <i>Blastocystis hominis</i>) and helminths, <i>Dientamoeba fragilis</i>, (specific situations: <i>Isospora</i>, <i>Microsporidia</i>) <i>General: Fecal occult blood testing, calprotectin</i>

Adapted from Cammarota et al., [2017](#)
ESR erythrocyte sedimentation rate, *HIV* human immunodeficiency virus, *HTLV* human T lymphotropic virus

Table 20.4 Enhanced safety measures

Category	Measure	Description
Enhanced safety measures	Screening for Multidrug-Resistant Organisms (MDROs)	Testing for ESBL-producing Enterobacteriaceae, VRE, CRE, and MRSA to prevent transmission of resistant pathogens
	COVID-19 Considerations	Assessing potential donors for recent exposure to SARS-CoV-2, symptoms suggestive of COVID-19, and direct virus testing when applicable
	“Super-Donor” Concept	Identifying donors with a highly diverse and rich gut microbiota who consistently achieve better clinical outcomes in FMT
Optimizing Donor Selection	Tailored Donor Selection Framework	Selecting donors based on the specific disease mechanism targeted by FMT, ensuring the microbial profile aligns with therapeutic needs
	Processing-Based Classification	Aligning donor selection criteria with the level of processing applied to the fecal material, balancing safety and efficacy considerations.

ESBL Extended spectrum beta-lactamases, *VRE* Vancomycin-resistant enterococcus, *MRSA* Multi-resistant *S. aureus*

20.2.2 Preparation of Transplant

The preparation of FMT material is the critical step to ensure safety, standardization, and optimal therapeutic efficacy. The process involves several key stages, including stool collection, homogenization, filtration, and storage, with strict adherence to hygiene and regulatory standards. Fecal material is obtained from pre-screened, healthy donors who meet stringent eligibility criteria to minimize the risk of transmitting infections or undesirable microbiota compositions (see Sect. [20.2.1](#)). The stool sample should be collected in a sterile container within a controlled environment, typically within 6 h before processing. In some protocols, stool donors are given dietary guidelines to avoid substances that could interfere with microbiome composition (Cammarota et al., [2017](#)). The fresh stool sample is mixed with a cryoprotectant solution, commonly saline, sterile water, or a glycerol-based buffer, to facilitate homogenization and preserve bacterial viability. The typical dilution ratio ranges from 1:5 to 1:10 (stool to diluent), ensuring an appropriate consistency for administration. The mixture is then thoroughly blended using a sterile blender or stomacher system to achieve a uniform suspension (Perez et al., [2016](#)). To remove undigested food particles and unwanted solid matter, the suspension undergoes filtration through gauze, mesh sieves, or specific filtration devices. This step ensures that only the microbial-rich liquid fraction is retained while eliminating unwanted materials that could obstruct delivery methods such as colonoscopy, nasojejunal tubes, or enema administration (Cammarota et al., [2017](#)). FMT preparations intended for fresh use should be administered within 6 h of processing. However, studies have demonstrated that frozen samples maintain microbial viability and therapeutic efficacy comparable to fresh preparations, allowing for standardized production and broader availability. Therefore, many clinical centers prepare frozen FMT aliquots to allow for storage and later use. In such cases, the final filtrate is divided into sterile cryovials or syringes and stored at -80°C with glycerol as a cryoprotectant (typically at 10–30% concentration). Cryopreservation helps maintain bacterial viability for extended periods while ensuring a stable, standardized microbial composition (Costello et al., [2015](#); Lee et al.,

[2016](#)). The approach of using frozen fecal microbiota preparations increases the logistical feasibility of FMT by reducing the dependency on immediate donor availability, facilitating centralized stool banking, and enhancing safety by allowing for extended screening periods before clinical use. As a result, frozen FMT samples are now widely used in clinical practice, offering a reliable and scalable solution for microbiota-based therapies. Depending on the method of delivery, FMT preparations may undergo further modifications. For capsule-based FMT, additional processing steps such as lyophilization (freeze-drying) or enteric coating may be applied to protect the microbiota from gastric acid degradation. These formulations offer a non-invasive alternative to conventional administration methods, improving patient compliance and accessibility (Cammarota et al., [2017](#)).

Research in FMT preparation is continuously evolving, with a focus on enhancing microbial viability while ensuring safety and ease of use in both clinical settings and potential home applications. Efforts to standardize protocols are ongoing, aiming to improve consistency and effectiveness across various medical conditions.

20.2.3 Preparation of Recipient and Route of Application

Proper preparation of the recipient and the selection of an appropriate route of administration are critical to the success of FMT (Fig. [20.3](#)).

Adhering to current guidelines ensures both safety and efficacy, optimizing the chances of successful microbiota engraftment and clinical improvement (Gweon et al., [2022](#)). Before undergoing FMT, recipients should undergo a comprehensive evaluation, including a detailed medical history and laboratory assessments, as described in detail in earlier sections. Bowel preparation is a key step in FMT, particularly for lower gastrointestinal (GI) administration. A polyethylene glycol-based regimen is commonly used to cleanse the colon, helping to improve mucosal contact with the transplanted microbiota. However, the need for bowel preparation and its extent can vary depending on the clinical situation and the chosen route of administration. For upper GI delivery methods, such as nasogastric or

nasoduodenal routes, patients are generally advised to fast for 8–12 hours before the procedure. This helps minimize the risk of aspiration and may also enhance the overall effectiveness of the transplant (Kim & Gluck, [2019](#); Tian et al., [2024](#)).

	Fecal microbiota transfer			
Route of administration	Orally ("yellow soup", duodenal tube, capsules)		Rectally (endoscopically, enema)	
Strategies of preparation	"Yellow soup"	Filtrate	Anaerobic preparation	"sterile" filtrate
Frequency of administration	once	Multiple times (once/week up to daily)		
Indications	<i>C. difficile</i> colitis	Ulcerative colitis	???	

Fig. 20.3 Overview of administration, preparation, and frequency of FMT

An essential component of recipient preparation is antibiotic pretreatment, particularly for patients with rCDI. The 2017 European Consensus Conference on FMT recommended completing a full course of vancomycin (or fidaxomicin) before FMT, with the final antibiotic dose administered 1 day prior to transplantation (Camarota et al., [2017](#)). This strategy aimed to suppress *C. difficile* while allowing room for microbiota engraftment. However, these guidelines lacked specific recommendations regarding antibiotic washout timing or bowel purging. In 2024, the American Gastroenterological Association (AGA) refined these recommendations, emphasizing a structured washout period and the role of bowel purging to optimize engraftment (Peery et al., [2024](#)). According to the updated guidelines, standard antibiotics for rCDI should be discontinued 1–3 days before administering FMT or investigational microbiota-based therapies. If a bowel purge is performed, FMT can be administered 1 day after stopping antibiotics. However, if no purge is used, a three-day washout period is recommended before proceeding with transplantation. For fecal microbiota spores, live-brpk (Vowst), a slightly longer antibiotic-free

period is advised, with antibiotics being stopped 2–4 days prior to FMT, followed by a split-dose bowel purge to enhance engraftment. Additionally, the AGA introduced “bridging therapy,” which involves administering a low-dose suppressive antibiotic regimen, such as vancomycin, during the interval between completing a standard antibiotic course (typically 10 days of vancomycin or fidaxomicin) and FMT. This approach aims to reduce the risk of *C. difficile* recurrence while optimizing conditions for microbiota restoration.

The selection of the administration route for FMT is influenced by factors such as the underlying condition being treated, patient preference, and potential risks associated with each method. FMT can be administered through various routes, each with distinct advantages and limitations. Colonoscopy is one of the most commonly used methods, as it allows for direct visualization of the colon and precise delivery of fecal material throughout the large intestine. This approach is particularly favored in the treatment of rCDI, where it has demonstrated high success rates. Alternatively, upper GI routes, such as nasogastric or nasoduodenal tubes, enable the delivery of fecal suspension into the upper digestive tract. While this method is less invasive than colonoscopy, it carries an inherent risk of aspiration and may be less effective for conditions primarily affecting the lower GI tract. A more recent advancement in FMT delivery is capsule ingestion, which offers a non-invasive alternative and significantly improves patient compliance. However, ongoing research is needed to optimize the viability of microbiota in capsule form and determine the appropriate dosing regimen for sustained clinical benefits.

The choice of route should be individualized, taking into account the patient’s clinical condition, anatomical considerations, and the healthcare setting’s resources. Engaging in a shared decision-making process with the patient, discussing the benefits and risks of each method, is essential to optimizing outcomes. Taken together, meticulous preparation of the recipient and thoughtful selection of the administration route are pivotal in maximizing the therapeutic potential of FMT. While the 2017 European Consensus provided

foundational recommendations, the AGA 2024 Guidelines have further refined the timing, discontinuation strategies, and the role of bowel preparation.

20.2.4 Mechanisms Behind FMT

FMT acts through multiple synergistic mechanisms. CDI arises from gut dysbiosis, depleting beneficial bacteria, particularly Firmicutes and Bacteroidetes, weakening colonization resistance. This allows *C. difficile* spores to germinate, proliferate, and produce toxins that cause colonic inflammation. FMT restores microbial diversity, repopulating the gut with commensal bacteria that reestablish metabolic balance and outcompete *C. difficile* (Khoruts & Sadowsky, [2016](#)). Through competitive exclusion, beneficial bacteria prevent *C. difficile* from accessing nutrients. Key species, such as *Bacteroides*, *Lachnospiraceae*, and *Faecalibacterium prausnitzii*, compete for sugars and amino acids while producing short-chain fatty acids (SCFAs) that create an unfavorable environment for *C. difficile*, enhance epithelial barrier integrity, and reduce gut permeability. Beneficial bacteria also regulate host gene expression by inhibiting histone deacetylases (HDACs), leading to anti-inflammatory effects (Martinez-Gili et al., [2020](#)). A key mechanism in FMT's efficacy is its impact on bile acid metabolism. Normally, gut bacteria convert primary bile acids (cholic acid, chenodeoxycholic acid) into secondary bile acids (deoxycholic acid, lithocholic acid), which inhibit *C. difficile* germination. In CDI, dysbiosis disrupts this process, increasing primary bile acids that instead promote *C. difficile* growth (Kang et al., [2019](#)). FMT restores the microbiota responsible for bile acid conversion, favoring secondary bile acids that suppress *C. difficile* and enhance colonization resistance. FMT also modulates immune responses, shifting from a pro-inflammatory to an anti-inflammatory state. CDI is linked to excessive TNF- α and IL-6, which exacerbate mucosal damage. FMT reduces these cytokines while increasing IL-10 and TGF- β , promoting mucosal healing (Alam et al., [2023](#)). Additionally, it expands regulatory T cells Tregs, suppressing inflammation and improving gut homeostasis. FMT also strengthens

intestinal barrier integrity by upregulating tight junction proteins, preventing microbial translocation (Yadegar et al., [2024](#)). Beyond microbial competition and immune modulation, FMT directly neutralizes *C. difficile* toxins. *C. difficile* produces TcdA and TcdB, which damage colonic epithelium. Certain bacteria, including *Lactobacillus*, *Bacteroides*, and *Enterococcus* spp., produce enzymes that degrade these toxins, preventing their harmful effects. Additionally, some FMT-derived bacterial strains produce bacteriocins, antimicrobial peptides that directly inhibit *C. difficile* growth (Andersen et al., [2016](#)).

20.2.5 Summarizing Thoughts for CDI

FMT has revolutionized the treatment of rCDI, offering a highly effective alternative to conventional antibiotic therapies. By restoring gut microbial diversity, FMT directly addresses the underlying dysbiosis that predisposes patients to recurrent infections. Numerous clinical trials have demonstrated its superior efficacy compared to standard antibiotic regimens, leading to its incorporation into major clinical guidelines. As a result, regulatory approvals of standardized FMT-based therapies, such as *Rebyota* and *Vowst*, mark a significant step toward integrating microbiota-based treatments into mainstream medicine. Despite its success, FMT for CDI presents challenges, particularly in donor selection, safety monitoring, and long-term outcomes. Efforts to optimize preparation methods, including the use of frozen and encapsulated formulations, have improved accessibility and feasibility. Furthermore, ongoing research aims to refine donor screening protocols and minimize potential risks, such as pathogen transmission. While FMT has transformed the management of rCDI, its role in treating refractory and severe CDI remains an area of active investigation. The development of next-generation microbiota-based therapies, such as defined bacterial consortia and synthetic microbiome formulations, may offer more controlled and standardized alternatives in the future. As research progresses, FMT continues to exemplify the therapeutic potential of microbiome restoration, providing an effective,

evidence-based solution for one of the most challenging complications of antibiotic therapy.

20.3 Other Indications

20.3.1 Ulcerative Colitis

With growing evidence linking gut dysbiosis to Ulcerative Colitis (UC) pathogenesis, FMT has been investigated as a potential strategy to restore microbial balance and modulate intestinal immunity. The first randomized controlled trials (RCTs) investigating FMT in UC, published head-to-head in 2015 by Moayyedi et al. and Rossen et al., provided early clinical evidence suggesting that FMT could induce remission in a subset of patients (Moayyedi et al., [2015](#); Rossen et al., [2015](#)). These landmark studies laid the foundation for subsequent research, and in the years that followed, multiple RCTs have further examined the efficacy, safety, and influencing factors of FMT in UC. Variability in donor selection, FMT preparation, administration routes, and patient microbiome composition has contributed to heterogeneous results, underscoring the complexity of translating FMT into standardized clinical practice. A comprehensive systematic review and meta-analysis published in 2023 aimed at evaluating the efficacy and safety of FMT in UC patients, thereby providing a robust assessment of FMT’s therapeutic potential in this context (Feng et al., [2023](#)) (Table [20.5](#)).

Table 20.5 Characteristics of included studies

Study	Year	Patient type	FMT mode	Control	Delivery route	Donor type	Evaluation time
Kedia et al.	2022	Mild to moderate UC	FMT-AID	SMT	Colonoscopy	MD	Week 8
Sarbagili et al.	2022	Active UC	FMT + UCED	UCED	Colonoscopy + enema	SD	Week 8
Haifer et al.	2022	Active UC	FMT	Placebo	Oral capsules	SD	Week 8

Study	Year	Patient type	FMT mode	Control	Delivery route	Donor type	Evaluation time
Crothers et al.	2022	Mild to moderate UC	FMT	Placebo	Colonoscopy + oral capsules	MD	Week 12
Brezina et al.	2021	Active left-sided UC	FMT	5-ASA	Enema	SD	Week 12
Pai et al.	2021	Pediatric active UC	FMT	Placebo	Enema	MD	Week 30
Fang et al.	2021	Recurrent active UC	FMT	5-ASA	Colonoscopy	SD	Week 8
Schierová et al.	2020	Active left-sided UC	FMT	5-ASA	Enema	SD	Week 12
Sood et al.	2019	Active UC	FMT + SOC	Placebo + SOC	Colonoscopy	SD	Week 48
Costello et al.	2019	Mild to moderate UC	FMT	Autologous FMT	Colonoscopy + enema	MD	Week 8
Paramsothy et al.	2017	Active UC	FMT	Placebo	Colonoscopy + enema	MD	Week 8
Rossen et al.	2015	Mild to moderate UC	FMT	Autologous FMT	Nasoduodenal tube	SD	Week 12
Moayyedi et al.	2015	Active UC	FMT	Placebo	Enema	SD	Week 7

Adapted from Feng et al. ([2023](#))

MD multiple donors, *SD* single donor, *AID* anti-inflammatory diet, *SMT* standard medical therapy, *SOC* standard of care therapy, *UCED* ulcerative colitis exclusion diet

This meta-analysis, which included 13 RCTs with a total of 580 patients (293 receiving FMT and 287 in the control group), offers robust evidence supporting the potential of FMT as a treatment for UC. The analysis revealed that FMT demonstrated significantly better clinical remission rates compared to the control group, with a risk ratio

of 1.73 (95% CI: 1.41–2.12, $P < 0.00001$). This translates to a clinical remission rate of 50.17% in the FMT group compared to 29.02% in the control group. Similarly, the FMT group showed superior results in achieving endoscopic remission, with a risk ratio of 1.74 (95% CI: 1.24–2.44, $P = 0.001$). Specifically, 26.82% of patients in the FMT group achieved endoscopic remission compared to 15.60% in the control group.

Importantly, the safety profile of FMT was comparable to control treatments, with no significant differences in the incidence of adverse reactions between the two groups. The risk ratio for adverse events was 1.00 (95% CI: 0.86–1.15, $P = 0.96$), suggesting that FMT is generally well-tolerated. The authors concluded that FMT shows promise as a treatment for inducing remission in UC patients, particularly for those who have not responded well to conventional therapies. However, they also noted that challenges remain in achieving endoscopic remission and maintaining long-term remission. The meta-analysis provides stronger evidence than previous studies due to its larger sample size and comprehensive analysis. Despite these encouraging results, the authors emphasize the need for further well-designed RCTs to standardize FMT protocols and examine long-term efficacy and safety before recommending widespread use. Ongoing and future studies are essential to refine FMT methodologies and establish standardized guidelines for a potential clinical application in UC management.

In contrast, the therapeutic role of FMT in Crohn's disease (CD), the other main form of IBD, remains uncertain based on existing clinical data. While some studies have reported modest to moderate improvements in symptoms such as abdominal pain, diarrhea, and hematochezia, with a subset of patients achieving steroid-free remission, RCTs have not demonstrated significant benefits of FMT over placebo in inducing remission in CD. For instance, a multicenter randomized trial was terminated early for futility after failing to show clinical or endoscopic remission at week 8 (Xiang et al., [2020](#); Kao et al., [2024](#)). Other prospective studies have reported only modest clinical

response rates of around 30% and have raised concerns about potential adverse events, underscoring the need for more rigorous, controlled trials to evaluate both safety and efficacy. Meta-analyses and systematic reviews further highlight the variability in outcomes and the current lack of robust evidence to support the routine use of FMT in CD (Syal et al., [2018](#); Gutin et al., [2019](#); Imdad et al., [2023](#)).

20.3.1.1 Multi-donor Approach and the Concept of a “Super-Donor”

The first study using a multi-donor FMT technique in UC was the RCT conducted by Paramsothy et al., published in 2017 in The Lancet (Paramsothy et al., [2017](#)). This multicentre, double-blind, randomized, placebo-controlled trial used an intensive-dosing, multidonor approach and involved enemas derived from between three and seven unrelated donors for each treatment. The primary endpoint at week 8 was steroid-free clinical as well as endoscopic remission. Since the primary endpoint in this study was reached, it represented the first controlled trial indicating that this strategy could indeed be successful. Most interestingly, secondary analysis revealed a donor that when included led to a better outcome (Siegmund, [2017](#)). This finding underscored the phenomenon of the existence of “super-donors,” which was first recorded in the RCT by Moayyedi et al. (Moayyedi et al., [2015](#)). In this study, of the nine patients who entered remission after FMT, seven had received fecal material from the same donor, suggesting that FMT success was donor-dependent. The super-donor effect has been further explored in subsequent research. For example, a 2019 study investigated the oral and fecal microbiota of a super-donor whose sample was used in 11 FMT recipients with CDI, all of whom experienced remission of diarrhea after a single FMT (Ortiz-Olvera et al., [2023](#)). However, it is important to note that the super-donor concept is still under investigation. Some researchers have questioned the donor-centric view, suggesting that recipient factors or procedural aspects may be equally or more important in determining FMT success (Schmidt et al., [2022](#)). Recent studies are focusing on identifying

specific microbial families associated with FMT treatment success. For instance, a recent study found that the families *Ruminococcaceae* and *Lachnospiraceae* were associated with successful clinical response in UC patients receiving FMT (Pinto et al., [2025](#)). While the super-donor phenomenon remains an intriguing area of research, more studies are needed to fully understand its role and implications in FMT for UC.

20.3.2 Irritable Bowel Syndrome and Metabolic Syndrome

Recent research has explored the potential of FMT in both IBS and metabolic syndrome. In the case of IBS, a 2023 meta-analysis of randomized controlled trials found that FMT did not result in significant global improvement of IBS symptoms compared to placebo. However, FMT administered via invasive routes showed some benefit (Wang et al., [2023](#)). A 2020 RCT demonstrated that FMT effectively improved abdominal symptoms, fatigue, and quality of life in IBS patients, emphasizing the importance of utilizing a “super-donor” for successful outcomes (El-Salhy et al., [2020](#)). Interestingly, successful modulation of microbiota composition by FMT does not necessarily correlate with symptom resolution in IBS patients (Xu et al., [2019](#)), and adverse events reported in these trials were generally minor and self-limiting, including diarrhea, abdominal pain, and constipation (Myneedu et al., [2019](#)).

In terms of metabolic syndrome, studies have indicated that FMT from lean donors can transiently improve insulin sensitivity in obese individuals (Kootte et al., [2017](#)). Animal models have shown that FMT significantly enhances glucose metabolism and insulin sensitivity in diabetic mice (Chen et al., [2023](#)). Furthermore, a prospective study found that FMT from healthy donors improved glucose metabolism and insulin sensitivity in patients with type 2 diabetes mellitus (Vrieze et al., [2012](#)). Research also suggests that FMT may alter the course of type 1 diabetes by attenuating underlying autoimmunity (de Groot et al., [2021](#)). The efficacy of FMT in metabolic syndrome appears to be influenced by the recipient’s baseline microbiota diversity and the engraftment of donor bacteria (Yu et al., [2020](#)). Collectively, these

findings highlight the potential of FMT as a therapeutic approach for both IBS and metabolic syndrome, although further research is needed to fully understand its mechanisms and optimize its efficacy.

20.4 Potential Risks and Considerations

FMT has been surprisingly well accepted by patients, often more readily than anticipated by the medical community. However, this enthusiasm also comes with risks, as some individuals assume FMT could be effective for a wide range of diseases beyond its established use and may underestimate potential complications. One of the most discussed concerns that has drawn attention is the possibility of FMT influencing metabolism and body weight. A widely cited case report described a patient who developed new-onset obesity after receiving an FMT from an overweight donor (Alang & Kelly, [2015](#)). While it is impossible to establish a direct causal link from a single case, experimental studies in germ-free mice have demonstrated that gut microbiota can shape metabolic function and body composition (Ridaura et al., [2013](#)). This raises important questions about unintended metabolic consequences. Another key issue is the risk of transmitting infections, particularly MDROs. In 2019, the US Food and Drug Administration (FDA) issued warnings after reports of severe, and in some cases fatal, infections linked to FMT from improperly screened donors. Since then, regulatory oversight has strengthened, but these incidents underscore why comprehensive donor screening and rigorous safety protocols remain critical in clinical practice. While FMT has transformed the treatment of rCDI, it is not without risks. Continued rigorous screening practices, long-term safety monitoring, and patient education remain essential to maximizing its benefits while minimizing unintended consequences.

20.5 Novel Developments, Challenges, and Future Directions

Over the past decade, FMT has evolved from an experimental therapy to an established treatment for rCDI, with expanding research into its potential applications in IBD, IBS, metabolic disorders, and even neurological conditions. Several key advancements are shaping the next generation of microbiome-based therapies and are summarized in Table [20.6](#).

Table 20.6 Novel developments

Novel developments	Explanation
Capsule-based and synthetic FMT	Traditional FMT methods via colonoscopy or enema have limitations in accessibility and patient acceptance. Encapsulated FMT formulations, such as lyophilized fecal microbiota capsules, have demonstrated efficacy comparable to traditional methods, significantly improving patient compliance. The FDA's approval of <i>Vowst</i> (oral microbiota therapy) marks an important step toward standardized, pharmaceutical-grade FMT options
Standardized and defined microbial consortia	One of the biggest challenges in FMT has been the variability in donor microbiota composition, leading to inconsistent treatment outcomes. To address this, researchers are developing <i>rationally designed microbial consortia</i> —carefully selected strains of beneficial bacteria that mimic the effects of FMT while eliminating risks associated with undefined donor stool. Companies like <i>Seres Therapeutics</i> and <i>BiomeBank</i> are at the forefront of this approach, aiming to create reproducible and safe microbiome-based therapies
Personalized microbiome medicine	Advances in metagenomic sequencing and artificial intelligence (AI) have enabled the <i>identification of patient-specific microbial profiles</i> that influence FMT outcomes. Future therapies may incorporate <i>pre-FMT microbiome analysis</i> to optimize donor-recipient matching, increasing treatment success rates for conditions like IBD and metabolic syndrome

FMT fecal microbiota transplantation

Despite its success in treating *CDI*, FMT faces several significant *scientific, clinical, and regulatory* challenges, which are displayed in Table [20.7](#).

Table 20.7 Challenges

Challenges	Explanation
------------	-------------

Challenges	Explanation
Safety and infection risk	Although FMT is generally well tolerated, concerns remain regarding the transmission of multidrug-resistant organisms (MDROs), viral infections, and opportunistic pathogens. The FDA has issued multiple safety alerts following reports of serious infections from inadequately screened donor stool. Developing robust screening protocols and alternative non-stool-based microbiome therapies is essential
Regulatory uncertainty	The classification of FMT remains a complex issue. Some regulatory agencies, such as the FDA, consider it an investigational new drug (IND), while others classify it as a biological product or tissue transplant. This has led to inconsistencies in access and approval pathways across different regions. Striking a balance between patient access and safety regulations is a pressing issue
Lack of long-term data	While short-term efficacy of FMT is well-documented, long-term effects on gut microbiota stability and host metabolism remain unclear. Concerns have been raised about potential unintended consequences, such as metabolic changes (e.g., weight gain following FMT) or immune dysregulation. More longitudinal studies are needed to fully understand the risks and benefits

FMT fecal microbiota transplantation

Looking ahead, FMT is likely to evolve into a more precise, safer, and patient-friendly intervention, driven by several promising advancements. Table [20.8](#) summarizes potential future directions

Table 20.8 Future directions

Future directions	Explanation
Synthetic and next-generation microbiome-based therapies	The transition from whole stool transplants to defined bacterial consortia and synthetic microbiome products will likely reduce variability and improve safety. Rationally designed bacterial therapies that replicate the effects of FMT without the risks of pathogen transmission are already in clinical development
Targeted microbiome engineering	Advances in CRISPR-based gene editing and bacteriophage therapy offer exciting potential for selectively modifying gut microbiota. Future treatments may involve customized microbiome editing to precisely target pathogenic bacteria while preserving beneficial microbes

Future directions	Explanation
Prebiotics and postbiotics as adjunct therapies	Instead of direct microbiota transplantation, future strategies may focus on manipulating gut microbiota through diet, prebiotics, and postbiotic compounds that promote beneficial microbial communities without the need for live donor stool
Expanding FMT applications beyond <i>C. difficile</i>	While FMT's primary success has been in treating recurrent <i>C. difficile</i> infections, ongoing research is evaluating its role in IBD, IBS, metabolic diseases (such as obesity and type 2 diabetes), and even neurodegenerative disorders (such as Parkinson's and autism spectrum disorders). Future studies will determine whether microbiota-targeted therapies can be effectively integrated into precision medicine approaches.

FMT fecal microbiota transplantation

20.6 Conclusion and Learnings

FMT has already made a profound impact on the treatment of rCDI, and ongoing advancements suggest an even broader future for microbiome-based medicine. While challenges such as safety, standardization, and regulatory oversight remain, novel approaches—including synthetic microbiome therapeutics, personalized microbiota modulation, and targeted microbial engineering—hold promise for transforming FMT into a safer, more controlled, and widely applicable therapeutic strategy. As research progresses, FMT and microbiota-based interventions are poised to become an integral part of next-generation precision medicine, shaping the future of gastrointestinal and systemic health.

Bullet Points List of Highlights

- *Fecal microbiota transplantation (FMT)* is a well-established, guideline-supported therapy for recurrent *Clostridioides difficile* infection (rCDI) and is under investigation for other microbiome-related disorders such as IBD, IBS, metabolic syndrome, and even neurological disorders like Parkinson's disease and autism spectrum disorder.

- *FMT works by restoring microbial diversity*, modulating immune responses, influencing bile acid metabolism, and strengthening gut barrier function.
 - *Donor selection and preparation* remain critical, with evolving safety measures to minimize infection risks, including rigorous pathogen screening and optimized donor-matching strategies.
 - *FMT success is influenced by multiple factors*, including the route of administration, antibiotic pre-treatment, recipient microbiome composition, and the concept of “super-donors” with highly beneficial microbiota.
 - *Safety challenges remain*, particularly regarding multidrug-resistant organism transmission, long-term metabolic effects, and standardization of protocols across global healthcare systems.
 - *Regulatory landscapes vary globally*, with the FDA approving microbiota-based therapies like Rebyota (2022) and Vowst (2023) for rCDI, while Europe, Canada, and Australia adopt differing classifications.
 - *Innovations in FMT and future directions* include capsule-based delivery, standardized microbial consortia, synthetic microbiome therapies, CRISPR-based microbiome engineering, targeted bacterial consortia, and adjunct prebiotic/postbiotic strategies—aiming to improve safety, efficacy, and personalization.
 - *FMT represents a paradigm shift* in microbiome-based medicine, with ongoing innovations likely to refine its applications in precision medicine and expand its role beyond infectious diseases.
-

References

Aas, J., Gessert, C. E., & Bakken, J. S. (2003). Recurrent *Clostridium difficile* colitis: case series involving 18 patients treated with donor stool administered via a nasogastric tube. *Clinical Infectious Diseases*, 36, 580–585. <https://doi.org/10.1086/367657> [PubMed]

Aby, E. S., Vaughn, B. P., Enns, E. A., & Rajasingham, R. (2022). Cost-effectiveness of fecal microbiota transplantation for first recurrent *Clostridioides difficile* infection. *Clinical Infectious Diseases*, 75, 1602–1609. <https://doi.org/10.1093/cid/ciac207> [PubMed]

Alam, M. Z., Maslanka, J. R., Denny, J. E., Bittinger, K., & Abt, M. C. (2023). IL10 producing regulatory T cells support fecal microbiota transplant (FMT)-mediated clearance of *Clostridioides difficile* infection. *Journal of Immunology*, 210, 61.12–61.12. <https://doi.org/10.4049/jimmunol.210.Supp.61.12>

Alang, N., & Kelly, C. R. (2015). Weight gain after fecal microbiota transplantation. *Open Forum Infectious Diseases*, 2, ofv004. <https://doi.org/10.1093/ofid/ofv004> [PubMed]

Andersen, K. K., Strokappe, N. M., Hultberg, A., Truusalu, K., Smidt, I., Mikelsaar, R.-H., Mikelsaar, M., Verrips, T., Hammarström, L., & Marcotte, H. (2016). Neutralization of *Clostridium difficile* toxin B mediated by engineered lactobacilli that produce single-domain antibodies. *Infection and Immunity*, 84, 395–406. <https://doi.org/10.1128/IAI.00870-15> [PubMed]

Antunes, A., Tricotel, A., Wilk, A., Dombrowski, S., Rinta-Kokko, H., Andersson, F. L., & Ghosh, S. (2024). Estimating excess mortality and economic burden of *Clostridioides difficile* infections and recurrences during 2015-2019: the RECUR Germany study. *BMC Infectious Diseases*, 24, 548. <https://doi.org/10.1186/s12879-024-09422-w> [PubMed]

Borody, T. J., Warren, E. F., Leis, S. M., Surace, R., Ashman, O., & Siarakas, S. (2004). Bacteriotherapy using fecal flora: toying with human motions. *Journal of Clinical Gastroenterology*, 38, 475–483. <https://doi.org/10.1097/01.mcg.0000128988.13808.dc> [PubMed]

Brandt, L. J., Aroniadis, O. C., Mellow, M., Kanatzar, A., Kelly, C., Park, T., Stollman, N., Rohlke, F., & Surawicz, C. (2012). Long-term follow-up of colonoscopic fecal microbiota transplant for recurrent *Clostridium difficile* infection. *The American Journal of Gastroenterology*, 107, 1079–1087. <https://doi.org/10.1038/ajg.2012.60> [PubMed]

Cammarota, G., Ianiro, G., Tilg, H., Rajilić-Stojanović, M., Kump, P., Satokari, R., Sokol, H., Arkkila, P., Pintus, C., Hart, A., Segal, J., Aloï, M., Masucci, L., Molinaro, A., Scaldaferri, F., Gasbarrini, G., Lopez-Sanroman, A., Link, A., De Groot, P., De Vos, W. M., Högenauer, C., Malfertheiner, P., Mattila, E., Milosavljević, T., Nieuwdorp, M., Sanguinetti, M., Simren, M., & Gasbarrini, A. (2017). European consensus conference on faecal microbiota transplantation in clinical practice. *Gut*, 66, 569–580. <https://doi.org/10.1136/gutjnl-2016-313017> [PubMed]

Chen, L., Guo, L., Feng, S., Wang, C., Cui, Z., Wang, S., Lu, Q., Chang, H., Hang, B., Snijders, A. M., Mao, J.-H., Lu, Y., & Ding, D. (2023). Fecal microbiota transplantation ameliorates type 2 diabetes via metabolic remodeling of the gut microbiota in db/db mice. *BMJ Open Diabetes Research & Care*, 11, e003282. <https://doi.org/10.1136/bmjdr-2022-003282>

Chen, Y., Xie, X., Ge, Q., He, X., Sun, Z., Li, Y., Guo, Y., Geng, C., Li, X., & Wang, C. (2024). The global burden and trend of *Clostridioides difficile* and its association with world antibiotic consumption, 1990-2019. *Journal of Global Health*, 14, 04135. <https://doi.org/10.7189/jogh.14.04135>

[PubMed]

Costello, S. P., Conlon, M. A., Vuaran, M. S., Roberts-Thomson, I. C., & Andrews, J. M. (2015). Faecal microbiota transplant for recurrent *Clostridium difficile* infection using long-term frozen stool is effective: clinical efficacy and bacterial viability data. *Alimentary Pharmacology & Therapeutics*, 42, 1011–1018. <https://doi.org/10.1111/apt.13366>

de Groot, P., Nikolic, T., Pellegrini, S., Sordi, V., Imangaliyev, S., Rampanelli, E., Hanssen, N., Attaye, I., Bakker, G., Duinkerken, G., Joosten, A., Prodan, A., Levin, E., Levels, H., Potter van Loon, B., van Bon, A., Brouwer, C., van Dam, S., Simsek, S., van Raalte, D., Stam, F., Gerdes, V., Hoogma, R., Diekman, M., Gerding, M., Rustemeijer, C., de Bakker, B., Hoekstra, J., Zwinderman, A., Bergman, J., Holleman, F., Piemonti, L., De Vos, W., Roep, B., & Nieuwdorp, M. (2021). Faecal microbiota transplantation halts progression of human new-onset type 1 diabetes in a randomised controlled trial. *Gut*, 70, 92–105. <https://doi.org/10.1136/gutjnl-2020-322630>

[PubMed]

Drekonja, D. M., Shaukat, A., Huang, Y., Zhang, J. H., Reinink, A. R., Nugent, S., Dominitz, J. A., Davis-Karim, A., Gerding, D. N., & Kyriakides, T. C. (2025). A randomized controlled trial of efficacy and safety of fecal microbiota transplant for preventing recurrent *Clostridioides difficile* infection. *Clinical Infectious Diseases*, 80, 52–60. <https://doi.org/10.1093/cid/ciae467>

[PubMed]

Duvallet, C., Zellmer, C., Panchal, P., Budree, S., Osman, M., & Alm, E. J. (2019). Framework for rational donor selection in fecal microbiota transplant clinical trials. *PLoS One*, 14, e0222881. <https://doi.org/10.1371/journal.pone.0222881>

[PubMed]

ECDC. (2024). *Clostridioides difficile* infections - Annual epidemiological report for 2018–2020. <https://www.ecdc.europa.eu/en/publications-data/clostridioides-difficile-infections-annual-epidemiological-report-2018-2020>. Accessed 27 Feb 2025.

Eiseman, B., Silen, W., Bascom, G. S., & Kauvar, A. J. (1958). Fecal enema as an adjunct in the treatment of pseudomembranous enterocolitis. *Surgery*, 44, 854–859.

[PubMed]

El-Salhy, M., Hatlebakk, J. G., Gilja, O. H., Bråthen Kristoffersen, A., & Hausken, T. (2020). Efficacy of faecal microbiota transplantation for patients with irritable bowel syndrome in a randomised, double-blind, placebo-controlled study. *Gut*, 69, 859–867. <https://doi.org/10.1136/gutjnl-2019-319630>

[PubMed]

Feng, J., Chen, Y., Liu, Y., Lin, L., Lin, X., Gong, W., Xia, R., He, J., Sheng, J., Cai, H., & Xiao, C. (2023). Efficacy and safety of fecal microbiota transplantation in the treatment of ulcerative colitis: A systematic review and meta-analysis. *Scientific Reports*, 13, 14494. <https://doi.org/10.1038/s41598-023-41182-6>

[PubMed]

George, W. L., Sutter, V. L., Goldstein, E. J., Ludwig, S. L., & Finegold, S. M. (1978). Aetiology of antimicrobial-agent-associated colitis. *The Lancet*, 1, 802–803. <https://doi.org/10.1016/s0140->

Gilbert, D. N., Ed, D. N. G., Moellering, R. C., Eliopoulos, G. M., & Sande, M. A. (2006). *The Sanford guide to antimicrobial therapy 2006*. Antimicrobial Therapy, Incorporated.

Gutin, L., Piceno, Y., Fadrosh, D., Lynch, K., Zydek, M., Kassam, Z., LaMere, B., Terdiman, J., Ma, A., Somsouk, M., Lynch, S., & El-Nachef, N. (2019). Fecal microbiota transplant for Crohn disease: A study evaluating safety, efficacy, and microbiome profile. *United European Gastroenterology Journal*, 7, 807–814. <https://doi.org/10.1177/2050640619845986>
[PubMed]

Gweon, T.-G., Lee, Y. J., Kim, K. O., Yim, S. K., Soh, J. S., Kim, S. Y., Park, J. J., Shin, S. Y., Lee, T. H., Choi, C. H., Cho, Y.-S., Yong, D., Chung, J.-W., Lee, K. J., Lee, O. Y., Choi, M.-G., Choi, M., & Gut Microbiota and Therapy Research Group Under the Korean Society of Neurogastroenterology and Motility. (2022). Clinical practice guidelines for fecal microbiota transplantation in Korea. *United European Gastroenterology Journal*, 28, 28–42. <https://doi.org/10.5056/jnm21221>

Haifer, C., Kelly, C. R., Paramsothy, S., Andresen, D., Papanicolas, L. E., McKew, G. L., Borody, T. J., Kamm, M., Costello, S. P., Andrews, J. M., Begun, J., Chan, H. T., Connor, S., Ghaly, S., Johnson, P. D., Lemberg, D. A., Paramsothy, R., Redmond, A., Sheorey, H., van der Poorten, D., & Leong, R. W. (2020). Australian consensus statements for the regulation, production and use of faecal microbiota transplantation in clinical practice. *Gut*, 69, 801–810. <https://doi.org/10.1136/gutjnl-2019-320260>
[PubMed]

Hui, W., Li, T., Liu, W., Zhou, C., & Gao, F. (2019). Fecal microbiota transplantation for treatment of recurrent *C. difficile* infection: An updated randomized controlled trial meta-analysis. *PLoS One*, 14, e0210016. <https://doi.org/10.1371/journal.pone.0210016>
[PubMed]

Hvas, C. L., Dahl Jørgensen, S. M., Jørgensen, S. P., Storgaard, M., Lemming, L., Hansen, M. M., Erikstrup, C., & Dahlerup, J. F. (2019). Fecal microbiota transplantation is superior to fidaxomicin for treatment of recurrent *Clostridium difficile* infection. *Gastroenterology*, 156, 1324–1332.e3. <https://doi.org/10.1053/j.gastro.2018.12.019>
[PubMed]

Ianiro, G., Maida, M., Burisch, J., Simonelli, C., Hold, G., Ventimiglia, M., Gasbarrini, A., & Cammarota, G. (2018). Efficacy of different faecal microbiota transplantation protocols for *Clostridium difficile* infection: A systematic review and meta-analysis. *United European Gastroenterology Journal*, 6, 1232–1244. <https://doi.org/10.1177/2050640618780762>
[PubMed]

Imdad, A., Pandit, N. G., Zaman, M., Minkoff, N. Z., Tanner-Smith, E. E., Gomez-Duarte, O. G., Acra, S., & Nicholson, M. R. (2023). Fecal transplantation for treatment of inflammatory bowel disease. *Cochrane Database of Systematic Reviews*, 4, CD012774. <https://doi.org/10.1002/14651858.CD012774.pub3>
[PubMed]

Kang, J. D., Myers, C. J., Harris, S. C., Kakiyama, G., Lee, I.-K., Yun, B.-S., Matsuzaki, K., Furukawa, M.,

Min, H.-K., Bajaj, J. S., Zhou, H., & Hylemon, P. B. (2019). Bile acid 7 α -dehydroxylating gut bacteria secrete antibiotics that inhibit clostridium difficile: role of secondary bile acids. *Cell Chemical Biology*, 26, 27–34.e4. <https://doi.org/10.1016/j.chembiol.2018.10.003> [PubMed]

Kao, D., Wong, K., Jijon, H., Moayyedi, P., Franz, R., McDougall, C., Hotte, N., Panaccione, R., Semlacher, E., Kroeker, K. I., Peerani, F., MacDonald, K. V., Xu, H., Narula, N., Turbide, C., Marshall, D. A., & Madsen, K. L. (2024). Preliminary results from a multicenter, randomized trial using fecal microbiota transplantation to induce remission in patients with mild-to-moderate Crohn's disease. *The American Journal of Gastroenterology*, 10.14309/ajg.0000000000003196.

Khoruts, A., & Sadowsky, M. J. (2016). Understanding the mechanisms of faecal microbiota transplantation. *Nature Reviews. Gastroenterology & Hepatology*, 13, 508–516. <https://doi.org/10.1038/nrgastro.2016.98>

Kim, K. O., & Gluck, M. (2019). Fecal microbiota transplantation: An update on clinical practice. *Clinical Endoscopy*, 52, 137–143. <https://doi.org/10.5946/ce.2019.009> [PubMed]

Koo, H. L., Van, J. N., Zhao, M., Ye, X., Revell, P. A., Jiang, Z.-D., Grimes, C. Z., Koo, D. C., Lasco, T., Kozinetz, C. A., Garey, K. W., & DuPont, H. L. (2014). Real-time polymerase chain reaction detection of asymptomatic Clostridium difficile colonization and rising C. difficile-associated disease rates. *Infection Control and Hospital Epidemiology*, 35, 667–673. <https://doi.org/10.1086/676433> [PubMed]

Kootte, R. S., Levin, E., Salojärvi, J., Smits, L. P., Hartstra, A. V., Udayappan, S. D., Hermes, G., Bouter, K. E., Koopen, A. M., Holst, J. J., Knop, F. K., Blaak, E. E., Zhao, J., Smidt, H., Harms, A. C., Hankemeijer, T., Bergman, J. J. G. H. M., Romijn, H. A., Schaap, F. G., Olde Damink, S. W. M., Ackermans, M. T., Dallinga-Thie, G. M., Zoetendal, E., de Vos, W. M., Serlie, M. J., Strees, E. S. G., Groen, A. K., & Nieuwdorp, M. (2017). Improvement of insulin sensitivity after lean donor feces in metabolic syndrome is driven by baseline intestinal microbiota composition. *Cell Metabolism*, 26, 611–619.e6. <https://doi.org/10.1016/j.cmet.2017.09.008> [PubMed]

Larson, H. E., Price, A. B., Honour, P., & Borriello, S. P. (1978). Clostridium difficile and the aetiology of pseudomembranous colitis. *The Lancet*, 1, 1063–1066. [https://doi.org/10.1016/s0140-6736\(78\)90912-1](https://doi.org/10.1016/s0140-6736(78)90912-1)

Lee, C. H., Steiner, T., Petrof, E. O., Smieja, M., Roscoe, D., Nematallah, A., Weese, J. S., Collins, S., Moayyedi, P., Crowther, M., Ropeleski, M. J., Jayaratne, P., Higgins, D., Li, Y., Rau, N. V., & Kim, P. T. (2016). Frozen vs fresh fecal microbiota transplantation and clinical resolution of diarrhea in patients with recurrent Clostridium difficile infection: A randomized clinical trial. *JAMA*, 315, 142–149. <https://doi.org/10.1001/jama.2015.18098> [PubMed]

Lessa, F. C., Winston, L. G., LC, M. D., & Emerging Infections Program C. difficile Surveillance Team. (2015). Burden of Clostridium difficile infection in the United States. *The New England Journal of Medicine*, 372, 2369–2370. <https://doi.org/10.1056/NEJMc1505190> [PubMed]

Lewin, R. A. (2001). More on Merde. *Perspectives in Biology and Medicine*, 44, 594–607. <https://doi.org/10.1353/pbm.2001.0067>
[PubMed]

Li, S. (2024). *Ben Cao Gang Mu, Volume I, Part A: Introduction, history, pharmacology, diseases and suitable pharmaceutical drugs I* (1st ed.). University of California Press.
<https://doi.org/10.1525/9780520395183>

Martinez-Gili, L., McDonald, J. A. K., Liu, Z., Kao, D., Allegretti, J. R., Monaghan, T. M., Barker, G. F., Miguéns Blanco, J., Williams, H. R. T., Holmes, E., Thursz, M. R., Marchesi, J. R., & Mullish, B. H. (2020). Understanding the mechanisms of efficacy of fecal microbiota transplant in treating recurrent *Clostridioides difficile* infection and beyond: the contribution of gut microbial-derived metabolites. *Gut Microbes*, 12, 1810531. <https://doi.org/10.1080/19490976.2020.1810531>
[PubMed]

Mehta, N., Wang, T., Friedman-Moraco, R. J., Carpentieri, C., Mehta, A. K., Roupshael, N., Dhere, T., Larsen, C. P., Kraft, C. S., & Woodworth, M. H. (2022). Fecal microbiota transplantation donor screening updates and research gaps for solid organ transplant recipients. *Journal of Clinical Microbiology*, 60, e00161–e00121. <https://doi.org/10.1128/JCM.00161-21>
[PubMed]

Miller, B. A., Chen, L. F., Sexton, D. J., & Anderson, D. J. (2011). Comparison of the burdens of hospital-onset, healthcare facility-associated *Clostridium difficile* Infection and of healthcare-associated infection due to methicillin-resistant *Staphylococcus aureus* in community hospitals. *Infection Control and Hospital Epidemiology*, 32, 387–390. <https://doi.org/10.1086/659156>
[PubMed]

Moayyedi, P., Surette, M. G., Kim, P. T., Libertucci, J., Wolfe, M., Onischi, C., Armstrong, D., Marshall, J. K., Kassam, Z., Reinisch, W., & Lee, C. H. (2015). Fecal microbiota transplantation induces remission in patients with active ulcerative colitis in a randomized controlled trial. *Gastroenterology*, 149, 102–109.e6. <https://doi.org/10.1053/j.gastro.2015.04.001>
[PubMed]

Myneedu, K., Deoker, A., Schmulson, M. J., & Bashashati, M. (2019). Fecal microbiota transplantation in irritable bowel syndrome: A systematic review and meta-analysis. *United European Gastroenterology Journal*, 7, 1033–1041. <https://doi.org/10.1177/2050640619866990>
[PubMed]

Ortiz-Olvera, N., Fernández-Figueroa, E. A., Argueta-Donohué, J., Miranda-Ortíz, H., & Ruiz-García, E. (2023). Case Report: Oral and fecal microbiota in a super-donor: The healthy microbiota paradigm for fecal transplantation. *Frontiers in Microbiomes*, 2, 1219960. <https://doi.org/10.3389/frmbi.2023.1219960>
[PubMed]

Paramsothy, S., Kamm, M. A., Kaakoush, N. O., Walsh, A. J., van den Bogaerde, J., Samuel, D., Leong, R. W. L., Connor, S., Ng, W., Paramsothy, R., Xuan, W., Lin, E., Mitchell, H. M., & Borody, T. J. (2017). Multidonor intensive faecal microbiota transplantation for active ulcerative colitis: A randomised

placebo-controlled trial. *The Lancet*, 389, 1218–1228. [https://doi.org/10.1016/S0140-6736\(17\)30182-4](https://doi.org/10.1016/S0140-6736(17)30182-4)

Park, J. C., & Im, S.-H. (2020). Of men in mice: The development and application of a humanized gnotobiotic mouse model for microbiome therapeutics. *Experimental & Molecular Medicine*, 52, 1383–1396. <https://doi.org/10.1038/s12276-020-0473-2>

Peery, A. F., Kelly, C. R., Kao, D., Vaughn, B. P., Lebwohl, B., Singh, S., Imdad, A., Altayar, O., & AGA Clinical Guidelines Committee. (2024). AGA clinical practice guideline on fecal microbiota-based therapies for select gastrointestinal diseases. *Gastroenterology*, 166, 409–434. <https://doi.org/10.1053/j.gastro.2024.01.008>
[PubMed]

Perez, E., Lee, C. H., & Petrof, E. O. (2016). A practical method for preparation of fecal microbiota transplantation. In A. P. Roberts & P. Mullany (Eds.), *Clostridium difficile* (pp. 259–267). Springer. https://doi.org/10.1007/978-1-4939-6361-4_19

Pinto, S., Šajbenová, D., Benincà, E., Nooij, S., Terveer, E. M., Keller, J. J., Van Der Meulen-de Jong, A. E., Bogaards, J. A., & Steyerberg, E. W. (2025). Dynamics of gut microbiota after fecal microbiota transplantation in ulcerative colitis: success linked to control of prevotellaceae. *Journal of Crohn's & Colitis*, 19, jjae137. <https://doi.org/10.1093/ecco-jcc/jjae137>

Porcari, S., Benech, N., Valles-Colomer, M., Segata, N., Gasbarrini, A., Cammarota, G., Sokol, H., & Ianaro, G. (2023). Key determinants of success in fecal microbiota transplantation: From microbiome to clinic. *Cell Host & Microbe*, 31, 712–733. <https://doi.org/10.1016/j.chom.2023.03.020>

Postigo, R., & Kim, J. H. (2012). Colonoscopic versus nasogastric fecal transplantation for the treatment of *Clostridium difficile* infection: A review and pooled analysis. *Infection*, 40, 643–648. <https://doi.org/10.1007/s15010-012-0307-9>
[PubMed]

Quraishi, M. N., Widlak, M., Bhala, N., Moore, D., Price, M., Sharma, N., & Iqbal, T. H. (2017). Systematic review with meta-analysis: The efficacy of faecal microbiota transplantation for the treatment of recurrent and refractory *Clostridium difficile* infection. *Alimentary Pharmacology & Therapeutics*, 46, 479–493. <https://doi.org/10.1111/apt.14201>

Reddi, S., Senyshyn, L., Ebadi, M., Podlesny, D., Minot, S. S., Gooley, T., Kabage, A. J., Hill, G. R., Lee, S. J., Khoruts, A., & Rashidi, A. (2025). Fecal microbiota transplantation to prevent acute graft-versus-host disease: Pre-planned interim analysis of donor effect. *Nature Communications*, 16, 1034. <https://doi.org/10.1038/s41467-025-56375-y>
[PubMed]

Ridaura, V. K., Faith, J. J., Rey, F. E., Cheng, J., Duncan, A. E., Kau, A. L., Griffin, N. W., Lombard, V., Henrissat, B., Bain, J. R., Muehlbauer, M. J., Ilkayeva, O., Semenkovich, C. F., Funai, K., Hayashi, D. K., Lyle, B. J., Martini, M. C., Ursell, L. K., Clemente, J. C., Van Treuren, W., Walters, W. A., Knight, R., Newgard, C. B., Heath, A. C., & Gordon, J. I. (2013). Gut microbiota from twins discordant for obesity modulate metabolism in mice. *Science*, 341, 1241214. <https://doi.org/10.1126/science.1241214>

[PubMed]

Rossen, N. G., Fuentes, S., van der Spek, M. J., Tijssen, J. G., Hartman, J. H. A., Duflou, A., Löwenberg, M., van den Brink, G. R., Mathus-Vliegen, E. M. H., de Vos, W. M., Zoetendal, E. G., D'Haens, G. R., & Ponsioen, C. Y. (2015). Findings from a randomized controlled trial of fecal transplantation for patients with ulcerative colitis. *Gastroenterology*, 149, 110–118.e4. <https://doi.org/10.1053/j.gastro.2015.03.045>

[PubMed]

Schmidt, T. S. B., Li, S. S., Maistrenko, O. M., Akanni, W., Coelho, L. P., Dolai, S., Fullam, A., Glazek, A. M., Hercog, R., Herrema, H., Jung, F., Kandels, S., Orakov, A., Thielemann, R., Von Stetten, M., Van Rossum, T., Benes, V., Borody, T. J., De Vos, W. M., Ponsioen, C. Y., Nieuwdorp, M., & Bork, P. (2022). Drivers and determinants of strain dynamics following fecal microbiota transplantation. *Nature Medicine*, 28, 1902–1912. <https://doi.org/10.1038/s41591-022-01913-0>

[PubMed]

Siegmund, B. (2017). Is intensity the solution for FMT in ulcerative colitis? *The Lancet*, 389, 1170–1172. [https://doi.org/10.1016/S0140-6736\(17\)30313-6](https://doi.org/10.1016/S0140-6736(17)30313-6)

Surawicz, C. M., & Alexander, J. (2011). Treatment of refractory and recurrent *Clostridium difficile* infection. *Nature Reviews. Gastroenterology & Hepatology*, 8, 330–339. <https://doi.org/10.1038/nrgastro.2011.59>

Syal, G., Kashani, A., & Shih, D. Q. (2018). Fecal microbiota transplantation in inflammatory bowel disease: A primer for internists. *The American Journal of Medicine*, 131, 1017–1024. <https://doi.org/10.1016/j.amjmed.2018.03.010>

[PubMed]

Tian, H., Wang, X., Fang, Z., Li, L., Wu, C., Bi, D., Li, N., Chen, Q., & Qin, H. (2024). Fecal microbiota transplantation in clinical practice: Present controversies and future prospects. *hLife*, 2, 269–283. <https://doi.org/10.1016/j.hlif.2024.01.006>

Turnbaugh, P. J., Ley, R. E., Mahowald, M. A., Magrini, V., Mardis, E. R., & Gordon, J. I. (2006). An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature*, 444, 1027–1031. <https://doi.org/10.1038/nature05414>

[PubMed]

van Nood, E., Vrieze, A., Nieuwdorp, M., Fuentes, S., Zoetendal, E. G., de Vos, W. M., Visser, C. E., Kuijper, E. J., Bartelsman, J. F. W. M., Tijssen, J. G. P., Speelman, P., Dijkgraaf, M. G. W., & Keller, J. J. (2013). Duodenal infusion of donor feces for recurrent *Clostridium difficile*. *The New England Journal of Medicine*, 368, 407–415. <https://doi.org/10.1056/NEJMoa1205037>

[PubMed]

Vrieze, A., Van Nood, E., Holleman, F., Salojärvi, J., Kootte, R. S., Bartelsman, J. F. W. M., Dallinga-Thie, G. M., Ackermans, M. T., Serlie, M. J., Oozeer, R., Derrien, M., Druesne, A., Van Hylckama Vlieg, J. E. T., Bloks, V. W., Groen, A. K., Heilig, H. G. H. J., Zoetendal, E. G., Stroes, E. S., de Vos, W. M., Hoekstra, J. B. L., & Nieuwdorp, M. (2012). Transfer of intestinal microbiota from lean donors increases insulin sensitivity in individuals with metabolic syndrome. *Gastroenterology*, 143, 913–916.e7. <https://doi.org/10.1053/j.gastro.2012.06.031>

[[PubMed](#)]

Wang, M., Xie, X., Zhao, S., Ma, X., Wang, Z., & Zhang, Y. (2023). Fecal microbiota transplantation for irritable bowel syndrome: A systematic review and meta-analysis of randomized controlled trials. *Frontiers in Immunology*, 14, 1136343. <https://doi.org/10.3389/fimmu.2023.1136343>

[[PubMed](#)]

Weber, D., Meedt, E., Poeck, H., Thiele-Orberg, E., Hiergeist, A., Gessner, A., & Holler, E. (2024). Fecal microbiota transfer in acute graft-versus-host disease following allogeneic stem cell transplantation. *Visceral Medicine*, 40, 210–216. <https://doi.org/10.1159/000538303>

Xiang, L., Ding, X., Li, Q., Wu, X., Dai, M., Long, C., He, Z., Cui, B., & Zhang, F. (2020). Efficacy of faecal microbiota transplantation in Crohn's disease: a new target treatment? *Microbial Biotechnology*, 13, 760–769. <https://doi.org/10.1111/1751-7915.13536>

[[PubMed](#)]

Xu, D., Chen, V. L., Steiner, C. A., Berinstein, J. A., Eswaran, S., Waljee, A. K., Higgins, P. D. R., & Owyang, C. (2019). Efficacy of fecal microbiota transplantation in irritable bowel syndrome: a systematic review and meta-analysis. *The American Journal of Gastroenterology*, 114, 1043–1050. <https://doi.org/10.14309/ajg.0000000000000198>

[[PubMed](#)]

Yadegar, A., Bar-Yoseph, H., Monaghan, T. M., Pakpour, S., Severino, A., Kuijper, E. J., Smits, W. K., Terveer, E. M., Neupane, S., Nabavi-Rad, A., Sadeghi, J., Cammarota, G., Ianiro, G., Nap-Hill, E., Leung, D., Wong, K., & Kao, D. (2024). Fecal microbiota transplantation: Current challenges and future landscapes. *Clinical Microbiology Reviews*, 37, e00060–e00022. <https://doi.org/10.1128/cmr.00060-22>

[[PubMed](#)]

Yu, E. W., Gao, L., Stastka, P., Cheney, M. C., Mahabamunuge, J., Torres Soto, M., Ford, C. B., Bryant, J. A., Henn, M. R., & Hohmann, E. L. (2020). Fecal microbiota transplantation for the improvement of metabolism in obesity: The FMT-TRIM double-blind placebo-controlled pilot trial. *PLoS Medicine*, 17, e1003051. <https://doi.org/10.1371/journal.pmed.1003051>

[[PubMed](#)]

Zhang, F., Luo, W., Shi, Y., Fan, Z., & Ji, G. (2012). Should we standardize the 1,700-year-old fecal microbiota transplantation? *The American Journal of Gastroenterology*, 107, 1755; author reply p.1755–1756. <https://doi.org/10.1038/ajg.2012.251>

[[PubMed](#)]

21. Gnotobiology

Marijana Basic¹✉ and André Bleich¹✉

(1) Institute for Laboratory Animal Science, Hannover Medical School,
Hannover, Germany

✉ **Marijana Basic**

Email: basic.marijana@mh-hannover.de

✉ **André Bleich (Corresponding author)**

Email: bleich.andre@mh-hannover.de

Abstract

In this chapter, we provide a brief overview on the historical development of gnotobiology, housing, and maintenance systems as well as procedures used today in the gnotobiotic facility/laboratory. The techniques and equipment that prompted the development of the gnotobiology field were developed more than half a century ago. However, the main principles of gnotobiotic work have remained unchanged over the years. The pioneers of gnotobiology were Nuttall and Thierfelder, who rederived the first germ-free animals. However, groundbreaking advancements in the gnotobiology field were achieved in the mid-1900s by scientists gathered around Reyniers and Trexler at the LOBUND Institute. Since its beginning, the main goals of gnotobiotic husbandry have been to provide a nucleus of pathogen-free animals for biomedical research and to elucidate the impact of microorganisms on their host health and physiology. However, to achieve these goals, the obstacles to long-term maintenance of germ-free animals needed to be overcome. The development of gnotobiotic equipment and

prerequisites for long-term maintenance accompanied with methodological progress, the creation of various mouse models and sequencing platforms, contributed to the rise of gnotobiotic research in the last two decades. Today, gnotobiology serves as a powerful platform for unraveling the mechanisms underlying the complex nature of host–microbiota interactions and enables the exploration of the roles of individual microbes in health and disease. Germ-free mice can be utilized to investigate the functionality of specific murine or human bacterial species, microbial consortia, or human fecal transplants under highly controlled conditions. Thereby, gnotobiology can reveal crucial genetic, microbial, and environmental factors underlying host–microbiota interactions.

Keywords Gnotobiology – Germ-free – Gnotobiotic – Rodents – Isolators – Gnotobiotic techniques

Gnotobiology comprises the field of studies concerned with the rearing and maintaining animals or plants that are either completely free of microorganisms (germ-free) or associated with only known microbial taxa or defined microbial communities. This field emerged as a discipline when researchers began studying the mutualistic, commensal, and parasitic relationships between microorganisms and their mammalian hosts. The term “gnotobiology” is derived from the Greek *gnotos* (well-known) and *bios* (life), reflecting the precise and defined nature of these biological systems. A gnotobiotic animal is one in which all associated life forms are fully defined, encompassing both germ-free animals and those colonized with known microorganisms. Today, gnotobiotic animal models have become a potent tool to advance our understanding of the host–microbiome interactions. These models provide an excellent platform to characterize the functional roles of individual microbial species or defined microbial communities in regulating host physiology and contributing to disease pathogenesis. In this chapter, we focus on rodent gnotobiotic models (primarily mice), as they are the most extensively used systems in biomedical research.

Despite their immense scientific value, the rederivation, maintenance, and monitoring of gnotobiotic rodents require vigilance, technical expertise, and substantial financial investment. Therefore, we would like to start this journey with the words of Thomas D. Luckey: “The essence of germ-free research is isolation. Isolation must be attained mechanically, proven scientifically, and understood philosophically” (Luckey, [1963](#)).

21.1 Biological Barriers

Today, laboratory animals, especially rodents, are housed in different biological barriers, which are designed to prevent animal contact with particular microbes. The level of barrier is defined by the exclusion list of certain microbes and by the methods used to preserve the status of the barrier. The germ-free barrier is the strictest biological barrier in animal facilities. Germ-free or *axenic* (from Greek “a” meaning without and “xenos” meaning stranger) animals are free of all foreign life forms apart from themselves, including bacteria, viruses, fungi, protozoa, and other saprophytic or parasitic life forms. However, indigenous viruses or other still uncharacterized life forms that have been integrated into the host genome and cannot be removed are considered to be an indivisible part of the animal. The specific pathogen-free (SPF) barrier is a common barrier in biomedical research institutions. The SPF animals are free from particular microbes, mostly bacteria, viruses, and parasites, that might induce disease or alter the course of the animal experiment. To exclude these pathogens, SPF animals are regularly monitored in accordance with recommendations for hygienic monitoring, such as the recommendation list of the Federation of Laboratory Animal Science Associations (FELASA) (Mähler et al., [2014](#)). However, the composition of the indigenous microbiota in these mice is unknown and varies between facilities or even within the same facility (Rausch et al., [2016](#)). The conventional housing represents the lowest barrier level, and it is outdated in modern animal facilities. The microbiological status of conventional animals is completely unknown.

These animals are colonized with an undefined microbiota and also not monitored for the presence of specified pathogens. A novel concept known as “wildlings” was introduced a few years ago. “Wildlings” refer to colonies of mice that harbor a wild mouse microbiome, including resilient natural microbiota and pathogens, while maintaining the inbred genetics of laboratory mouse strains. This model closely mimics the natural mammalian metaorganism and enhances the ability to predict complex immune responses in humans (Rosshart et al., [2019](#)).

21.2 Historical Perspective

The necessary gnotobiotic techniques and equipment were developed more than 50 years ago (Gustafsson, [1959](#); Trexler & Reynolds, [1957](#)). The development of gnotobiotic techniques was driven by two opposing hypotheses. In 1885, Louis Pasteur postulated that animal life cannot exist without symbiotic or commensal bacteria. One year later, Marcell Niencki proposed that animals without bacteria would live longer and be healthier due to the absence of repulsive microbiological by-products in the gut (Rahija, [2007](#)). Therefore, to resolve this debate, the necessary equipment needed to be established.

George H.F. Nuttall and Hans Thierfelder are considered to be the pioneers of gnotobiology and germ-free research. In 1895, they delivered the first germ-free guinea pigs by cesarean section into the sterile bell jar and kept them germ-free on sterile milk for 8 days. In a subsequent experiment, they fed the germ-free guinea pigs with sterile vegetables for up to 13 days. However, they have observed that germ-free animals gained less weight than those carrying complex microbiota, demonstrating that the gut bacteria are necessary for the digestion of the vegetable diet (Nuttall & Thierfelder, [1897](#)).

Furthermore, in 1912, E. Kuester designed the first isolator prototype, in which germ-free animals were isolated from exposure to microbes (Küster, [1915](#)). In this isolator prototype, Kuester kept goats germ-free for over a month. These results are partially opposed to Pasteur’s hypothesis, as it was shown that life without bacteria was

possible in several species for a limited time. However, it is important to note that these animals had to be fed with a diet fortified with vitamins, which are normally provided by the gut microbiota, to survive long term (Rahija, [2007](#)).

Groundbreaking work was then performed by the Laboratories of Bacteriology at the University of Notre Dame (LOBUND group), led by James A. Reyniers and Philip C. Trexler. They have established large-scale gnotobiology laboratories. The first isolators were designed as large stainless steel devices and were heavy, expensive, and difficult to handle. Additionally, these chambers were not efficient in housing large gnotobiotic colonies. One of these systems was developed by Reyniers, which was designed as an autoclave with security glass windows. The second model of this isolator type was constructed by Bengt E. Gustafsson. His isolation chamber was also designed as a steam sterilizer, but it contained a dip tank lock. Until 1957, the stainless steel isolators and steam sterilization were the backbone of the gnotobiotic technology. Subsequently, Trexler designed and introduced the first plastic polyvinyl chloride (PVC) flexible film isolator that is commonly used today (Trexler & Reynolds, [1957](#)). Furthermore, in 1960 Trexler attempted to create an entirely germ-free room. The staff entering this room was wearing full-body PVC suits. The room was entered by going through the germicidal shower, followed by immersion in a dunk tank. However, even in spite of these strict precautions, contamination with human microbiota occurred. Therefore, the idea of a germ-free room was abandoned. However, the designs and procedures developed for the first germ-free rooms served as precursors for the development of rodent barrier systems in facilities specialized for breeding and maintenance of SPF rodent colonies (Rahija, [2007](#)).

Germ-free research has developed independently at three institutions. In 1928, Gösta Glimstedt started the gnotobiotic program in Sweden at the University of Lund. In parallel, Reyniers founded the LOBUND Institute, which attracted top scientists such as Trexler and Julian R. Pleasants, and became the world's leader in germ-free research. The third program was established by Masasumi Miyakawa

15 years later in Japan at the Nagoya University (Rahija, [2007](#)). These institutes focused on the development of the equipment that would provide the physical barrier and sterile environment needed to grow germ-free animals for long-term studies. Moreover, their later studies were concentrated on the biological characterization of animals reared in a germ-free environment. During these times, many different species were rederived germ-free, including chickens, rabbits, and nonhuman primates. The standard method of germ-free rederivation was cesarean section and hand nurturing. The rearing of germ-free rodents was difficult due to their small neonatal size, which made hand rearing challenging. First-hand reared germ-free rats were reported by Gustafsson and shortly after by Reyniers (Gustafsson, [1946](#); Reyniers et al., [1946](#)). Several years later, the first germ-free mice were also successfully hand raised (Pleasant, [1959](#)). However, once the colony of germ-free mice was established, it was possible to maintain it for several generations. Hence, methods such as hysterectomy and cross-fostering of neonates by germ-free foster mothers substituted hand feeding and made the germ-free rodents maintenance easier. Reproducing colonies of germ-free rodents were available for distribution to the scientific community since the late 1950s at the LOBUND Institute and the University of Lund (Pleasant, [1959](#)). However, it is important to note that the long-term maintenance of germ-free rodent colonies was initially unsuccessful due to growth retardation and premature death caused by nutritional deficiencies. This encouraged studies to identify the vital proportion of dietary components to maintain animals under germ-free conditions. First studies have shown that in the absence of the gut bacteria, no adequate levels of vitamin K can be synthesized. Furthermore, it was shown that the sterilization process also degrades vitamins and nutrients, and therefore, the diet for germ-free rodents needs to be enriched with these components. Fortifying the rodent diet with supplements reduced the mortality in germ-free rodent colonies (Rahija, [2007](#)). Overcoming these challenges in maintaining germ-free colonies

advanced the studies on the anatomical and physiological changes associated with the germ-free environment.

A second major achievement was the application of the gnotobiotic technology for developing and maintaining SPF nucleus colonies for biomedical research, as at that time, many mice were infected with pathogens (Foster, [1959](#)).

Today, gnotobiotic animals have emerged as invaluable tools for elucidating the functions and roles of the intestinal microbiota in maintaining homeostasis, as well as in the development of pathological conditions and disorders. Interest in studying host–microbiota interactions has grown significantly, as many chronic human diseases have been associated with compositional and functional alterations of the intestinal microbiota, such as type 1 diabetes (de Goffau et al., [2014](#)), metabolic syndrome, obesity (Parekh et al., [2015](#)), inflammatory bowel disease (IBD) (Becker et al., [2015](#)), liver disease (Schwenger et al., [2019](#)), and carcinogenesis (Routy et al., [2018](#)). In recent years, it has become evident that the influence of the intestinal microbiota extends far beyond the gastrointestinal tract, with bidirectional communication pathways linking it to other organs, such as the brain and lungs (Afzaal et al., [2022](#)).

Germ-free mice and those colonized with synthetic bacterial consortia provide a platform for analyzing the impact of individual microbial species or defined communities on the host mucosal immune system. This approach has been instrumental in unraveling the mechanisms leading to physiological and/or pathological inflammation (Eun et al., [2014](#); Steck et al., [2011](#); Bolsega et al., [2019](#)). Utilizing minimal bacterial consortia allows for the reduction of microbial complexity, enabling causal analyses of a wide range of physiological and pathological processes. Furthermore, detailed investigations of microbiota–host interactions uncover important genetic, microbial, and environmental factors that contribute to the development of pathological conditions in these models and promote the discovery of new therapeutic targets. These insights are crucial for the development of novel microbiome-based therapies for patients.

21.3 Housing and Maintenance of Germ-Free or Gnotobiotic Animals

21.3.1 Isolators

For long-term maintenance in sterile conditions, gnotobiotic animals are housed in positive-pressure isolators. Isolators are designed as closed systems and thus decrease the risk of contamination. The isolator chamber creates the sterile workspace that is required to produce and maintain germ-free and gnotobiotic mice. Therefore, the isolator chamber needs to be constructed from materials that can form an impermeable physical barrier (Rahija, [2007](#); Trexler, [1983](#)).

The basic components of an isolator are an isolation chamber, air supply, air inlet filter, air outlet filter, transfer port, and arm-length gloves (Fig. [21.1a, b](#)). Isolators can be constructed as rigid, semirigid, or flexible film isolators made of plastic or stainless steel (Rahija, [2007](#)). Commonly used today are those made of plastic, e.g., flexible film isolators. These can be made of a great variety of plastic materials such as PVC, polyethylene, or nylons (Trexler, [1983](#)). However, the most used material is clear PVC. The isolator chambers can be positioned on tables that can be adjusted to the proper height to facilitate handling and provide a surface to support the isolator.

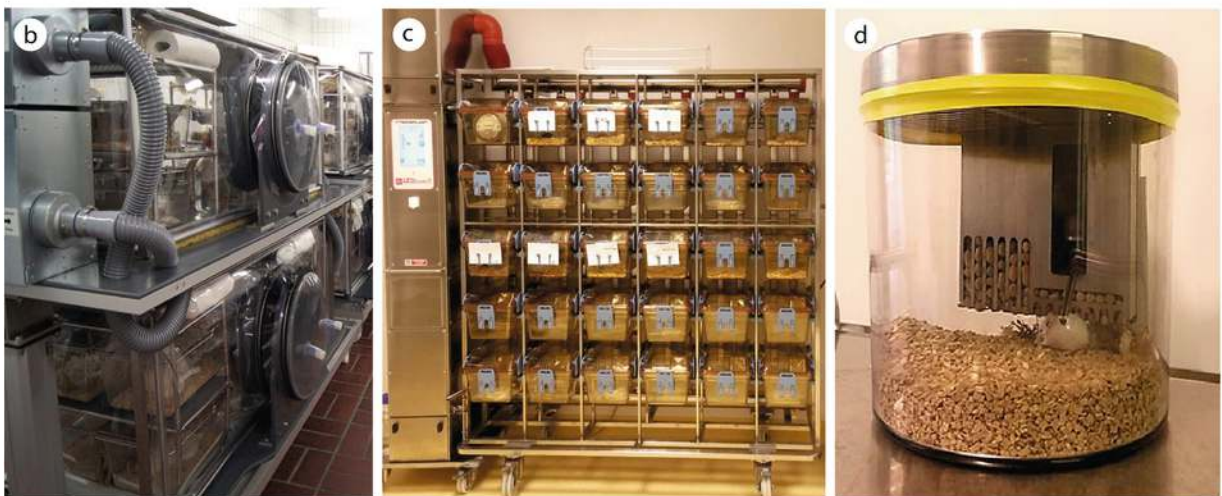
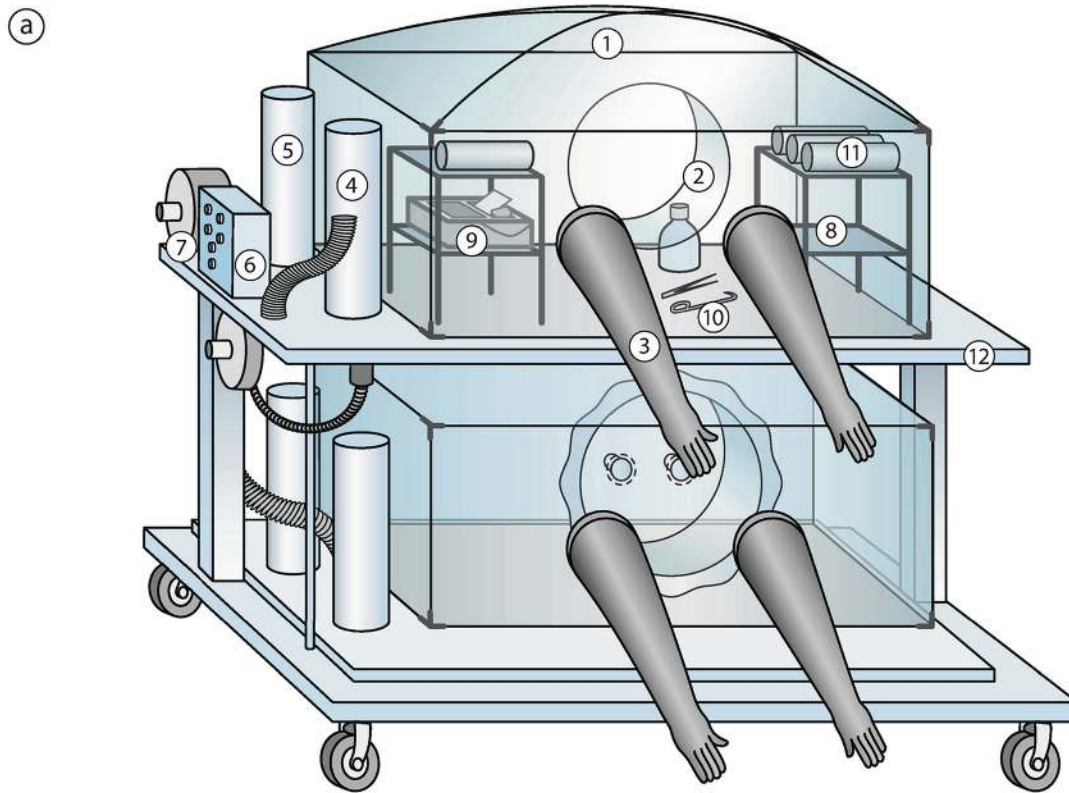


Fig. 21.1 Housing systems for germ-free and gnotobiotic rodents. (a) Scheme of the double-door port isolator (1-isolator chamber, 2-transfer port, 3-arm-length gloves, 4-air inlet filter, 5-air outlet filter, 6-console, 7-motor, 8-cage rack, 9-cage, 10-11-supplies for isolator husbandry, 12-adjustable table), (b) Isolator unit at Hannover Medical School, (c) Isocage system for short-term maintenance, (d) Gnotocage

The air inlet and outlet units contain filter medium such as a high-efficiency particulate air (HEPA) filter. Isolators are maintained under positive air pressure by central ventilation systems or decentral blower

unit. Due to positive pressure, the isolator chamber of the flexible film isolator appears as a “bubble.” Positive pressure is used to prevent entry of airborne contaminants through any puncture, and it is critical when rearing germ-free or gnotobiotic animals. Additionally, the flexible film isolators might contain a rigid frame, which supports the shape of the chamber.

Manipulation of animals and supplies in the isolator is carried out through arm-length gloves. The gloves are the most vulnerable part of the isolator and a common source of isolator contamination. It is recommended to inspect them for holes, cracks, and excessive wear before every use. The isolator gloves can be made from nitrile rubber, latex, or polyurethane and should be changed in pairs to reduce the risk of irregular wear (Rahija, [2007](#)). Additionally, to protect them from the inside, wearing cloth gloves is recommended. For occupational health and safety purposes, the glove material must be compatible with the disinfectant being used.

The transfer port is crucial, as it represents the transition area between the isolator chamber and the room environment. It is mostly made of a plastic or stainless steel cylinder, which can vary in size (Rahija, [2007](#)). The port functions as a portal for loading and sterilizing the surface of materials and supplies when entering the isolator. The other key role of the port is to maintain the physical barrier to prevent contamination of the chamber. Depending on the type, the transfer port can be fitted with a single- or double-door. The double-door port is sealed with PVC caps on both sides (Fig. [21.1a, b](#)). Sealing caps of the transfer port are equipped with small openings through which disinfectants such as peracetic acid can be introduced to decontaminate materials before they enter the isolator chamber. Single-door-type ports, which are otherwise easier to handle, require a transfer isolator for all exchange of material.

21.3.2 Short-Term Housing Systems for Gnotobiotic Animals

Each individual experiment has to be performed in one isolator, as microorganisms, once introduced into the isolator intentionally or accidentally as contaminants, rapidly spread to all cages. Therefore, each experimental group requires a separate isolator. Furthermore, these isolators must be completely disassembled and sterilized before next use (Vowles et al., [2016](#)). As these procedures are time-consuming and expensive, alternative housing options were developed for gnotobiotic short-term studies. Gnotobiotic animals can be housed shortly in microisolator cages such as static gnotocages (e.g., Han-gnotocage) or individually ventilated cage (IVC)-type isocages (Fig. [21.1c,d](#)) and handled under a class II biosafety cabinet (Nicklas et al., [2015](#)). The risk of contamination in these conditions is higher than in isolators (Hecht et al., [2014](#); Basic et al., [2021](#)). However, this is a very efficient way to perform gnotobiotic experiments, as access and handling of the animals is much easier. The gnotocages are designed as autoclavable plastic containers and sealed with a metal lid filled with HEPA filter material (Fig. [21.1d](#)). Additionally, gnotocages can also be utilized for the shipment of germ-free and gnotobiotic animals to other research institutions. Isocages such as Tecniplast Isocage P are the new cage technology developed initially for biosafety levels 3 and 4. This cage technology represents a completely closed system, in which each cage is essentially a mini isolator (Fig. [21.1c](#)). Air flows into the cage through a HEPA filter. When the cage is removed from the rack, it automatically seals and prevents any airflow into the cage. Since each cage functions as an independent hygienic unit, multiple gnotobiotic experimental groups can be conducted simultaneously without the risk of cross-contamination between the cages. Furthermore, in the event of contamination, it remains confined to the affected cage and does not compromise the entire experimental setup, unlike contamination introduced in an isolator. However, to maintain the reliability of experiments conducted in these systems, the gnotobiotic status must be regularly monitored (Basic et al., [2021](#)). Additionally, sterile handling of the cages requires extensive training and effort comparable to working with large isolators.

21.3.3 Introduction of Supply Materials into the Isolator

Each isolator contains cages with animals, as well as food, water, bedding, and all other supplies necessary for husbandry or experimental procedures. These supplies include tools such as forceps, scissors, tubes, enrichment, or hooks required to handle the mice or to transfer materials from the supply unit (cylinder or chamber) into the isolator chamber. Isolators can be loaded using a transport cylinder that is autoclaved along with all supplies intended for import into the isolator. These supply cylinders are typically used to sterilize items such as food, bedding, nesting material, or other supplies needed in the isolator. The cylinder is made out of stainless steel and wrapped with a filter medium (HEPA filter), which excludes microorganisms while allowing steam penetration during autoclaving (Rahija, [2007](#)). To ensure proper sterilization, the cylinder must be packed loosely to facilitate air and steam circulation. Once loaded with supplies, the open end of the cylinder is sealed with a polyethylene terephthalate polyester plastic film before being placed in the autoclave for sterilization (Rahija, [2007](#)). After verifying successful sterilization, the cylinder is attached to the transport port, allowing the materials to be transferred into the isolator (Fig. [21.2a-c](#)). The cylinder is attached to the transfer port with a flexible plastic transfer sleeve. The interior surfaces of the sleeve and port are disinfected/sterilized using a highly effective chemical, e.g., by spraying them with buffered peracetic acid solution to create the sterile portal between the isolator interior and the supplies inside the cylinder. During this procedure, the personnel must wear appropriate safety equipment, including masks and overalls. After disinfection, the isolator's internal door and the cylinder's external seal are opened, enabling the sterile materials to be safely introduced into the isolator.

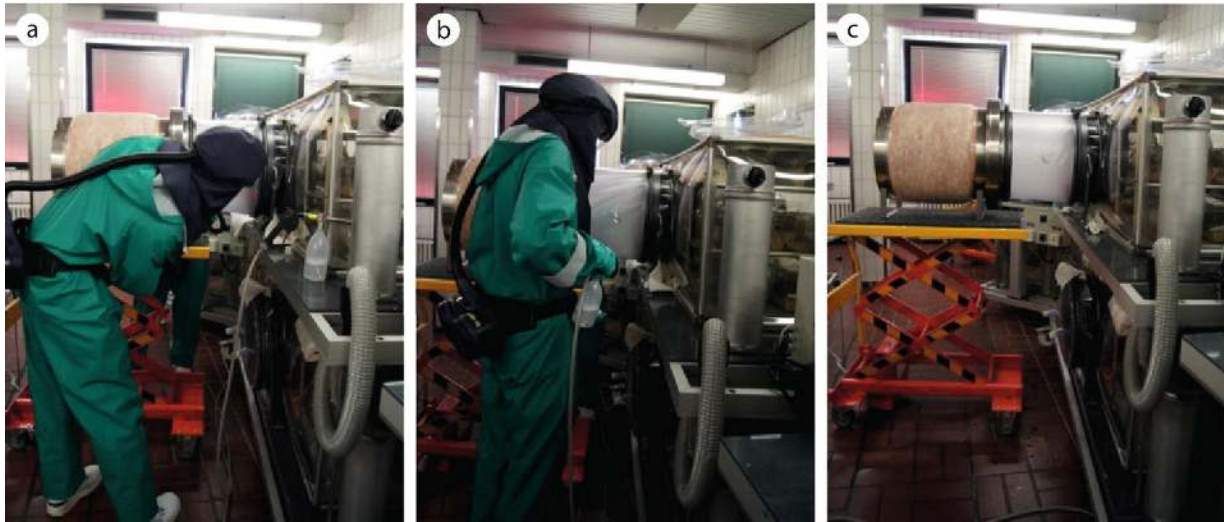


Fig. 21.2 Supply introduction into the isolator: (a) Docking of the supply cylinder onto isolator using a transfer sleeve, (b) sterilization of the transfer sleeve using peracetic acid and creating the sterile portal for supply introduction, (c) the supply cylinder is docked on the isolator through transfer sleeve and supplies are ready to be introduced into isolator

21.3.4 Sterilization Methods

Prevention of contamination is essential in all aspects of germ-free husbandry. Therefore, germ-free isolators and associated equipment must be rigorously monitored for sterility. It is essential to control and validate sterilization methods and regularly inspect equipment for damage or leaks. Several sterilization methods can be used, including autoclaving, irradiation, or gas sterilization, e.g., using ethylene oxide. Since not all supplies can be autoclaved within the transfer cylinder before they are introduced into the isolator, the surfaces of otherwise sterilized materials, as well as the interior of newly assembled isolators, must be chemically sterilized. These chemical disinfectants must be highly effective to ensure a germ-free state. Therefore, the appropriate sterilization method depends on the material type and its transfer into the isolator (Nicklas et al., [2015](#); Vowles et al., [2016](#)).

Autoclaving is the most commonly used method to sterilize materials, such as food, bedding, nesting material, water, and other supplies. Typically, autoclaving is performed at 134 °C for 30 minutes (Nicklas et al., [2015](#)). However, the specific autoclaving parameters for different materials should be validated separately by experienced staff.

To achieve optimal conditions, food and bedding should be packed into small perforated bags (Nicklas et al., [2015](#)). Autoclave cycles can be validated by monitoring the autoclave system and using bioindicators. These bioindicators are preparations of spore-forming heat-resistant organisms such as *Geobacillus stearothermophilus* (Rahija, [2007](#)). If the bioindicator shows growth, it indicates that the supply cylinder has not been sterilized and the process has failed.

The interior of all newly assembled isolators must be sterilized by using chemical sterilization agents before use. The preferred method is a 2% buffered peracetic acid solution (Nicklas et al., [2015](#); Vowles et al., [2016](#)). Alternatively, other sterilizing agents, such as chlorine dioxide or hydrogen peroxide, can be used. Peracetic acid is particularly advantageous because it can sterilize heat-sensitive materials, such as PVC. It is highly effective at low concentrations and temperatures, and works well in both liquid and vapor phases. It is also fast-acting and reliably inactivates resistant bacteria, including spores, as well as fungal spores. However, it is ineffective against parasite cysts and arthropod eggs. The decomposition products of peracetic acid are acetic acid, hydrogen peroxide, oxygen, and water. Staff working with the peracetic acid must be protected, as it is an irritant and can damage the mucosal surfaces.

Irradiation is another sterilization method, particularly used for food. For gnotobiotic husbandry, vacuum-sealed, radiation-sterilized food and bedding packages are commercially available (Rahija, [2007](#)). High doses of 50 kGy are generally sufficient to inactivate relatively resistant murine pathogens, such as parvoviruses, though this may not apply to radio-resistant bacteria (Nicklas et al., [2015](#); Reuter et al., [2011](#)). To mitigate the risk of bacterial contamination from irradiated food, all diet batches should be sampled and tested for contamination. Additionally, a dedicated isolator for verifying the sterility of the irradiated food batches should be established as an additional control measure.

21.4 Microbiological Monitoring

Laboratory animals housed in SPF barriers and colonized with endogenous microbiota are usually monitored in accordance with the FELASA recommendations. These animals are regularly tested for the presence of microorganisms that might affect animal health and modify the experimental outcome (Mähler et al., [2014](#); Nicklas et al., [2015](#)). Tests primarily focus on species-specific infectious agents, such as viruses, bacteria, and parasites, which are rarely found as contaminants in gnotobiotic animals (Nicklas et al., [2015](#); Basic et al., [2021](#)). Therefore, environmental samples have been traditionally more relevant in gnotobiotic monitoring. Contaminants that are most likely introduced into isolators include microorganisms able to stay viable under environmental conditions, such as spore-forming bacteria, micrococci, or fungi. In contrast, analyses for pathogens like mycobacteria or viruses are less critical in the hygienic monitoring of gnotobiotic colonies, as the likelihood of their introduction into an isolator is minimal (Nicklas et al., [2015](#)). However, some microbes can be transmitted during the rederivation process. Therefore, it is important to verify the germ-free status of mice after rederivation, including tests for infectious agents listed in the FELASA recommendations.

For microbiological monitoring of germ-free and gnotobiotic colonies, samples are obtained from animals, residual food, bedding, and environmental swabs from various positions. To ensure the gnotobiotic status, animals and materials are tested regularly. For germ-free animals, the goal is to confirm the absence of all foreign life forms. In contrast, monitoring of gnotobiotic animals carrying defined bacterial consortia involves verifying the presence of specific microorganisms while excluding all others. Effective microbiological monitoring of the gnotobiotic colonies requires a combination of techniques, as no single method is without limitations. Additionally, all monitoring procedures must be performed under sterile conditions to avoid contamination.

Swab samples from isolators are typically collected before animals are introduced. Once animals are present, testing of bedding, remaining food, and fresh fecal samples by culture is recommended every four weeks (Nicklas et al., [2015](#)). Full necropsy examination of animals should be conducted every 3–6 months (Nicklas et al., [2015](#)). It is important to adapt the frequency of testing based on the number of animals or supply transfers into the isolator. Diagnostic procedures include microscopic examination of gastrointestinal contents for visual assessment of bacterial morphology, microbial cultures of feces and organs using appropriate media, and conditions tailored to detect microorganisms that pose a risk in the gnotobiotic environment.

Molecular diagnostics includes culture-independent methods such as quantitative polymerase chain reaction (qPCR) or next-generation sequencing methods. This applies in particular to animals colonized with defined microbial communities, as culture-based approaches provide nonconclusive information about the status of the animals. Viral contaminations can be detected indirectly through serological assessments. It is important to note that bacterial-like particles may appear in low numbers in the feces of germ-free animals. These particles often originate from dead bacteria or plant debris in food or bedding and must be distinguished from contaminations with living bacteria (Vowles et al., [2016](#); Basic et al., [2021](#)).

Detailed guidelines for hygienic monitoring of the gnotobiotic facilities can be found in the literature (Nicklas et al., [2015](#); Vowles et al., [2016](#)).

21.5 Rederivation of Germ-Free Rodents

Rederivation refers to the process of creating germ-free animals from a colony that is not germ-free. Propagation of germ-free rodents is usually achieved by maintaining axenic colonies that are bred in germ-free isolators. However, if new germ-free strains are required, they must be rederived. Today, due to increased interest in microbiome research, the demand for new germ-free lines, including both classical

inbred and genetically manipulated mutant strains, has resulted in more frequent requests for rederivation of mice. Germ-free rodent strains can be rederived in two ways: by hysterectomy (sometimes also referred to as “cesarean section”) and fostering or by embryo transfer.

The first germ-free rodents were derived via aseptic hysterectomy (surgical removal of the uterus) performed in isolators, where the neonates were hand-reared (Pleasants, [1959](#)). Once a germ-free rodent colony was established, it became possible to derive new germ-free strains by hysterectomies directly into a germ-free isolator and fostering the offspring on a germ-free dam.

The hysterectomy method requires precise timing of pregnancy for both the donor and foster dams. Foster dams need to be mated in advance to ensure they can foster the rederived neonates. Ideally, the foster mothers give birth 1–2 days before the planned hysterectomy. Similarly, the mating of the donor dams must be carefully planned to ensure their offspring are viable and capable of surviving the stress of surgical delivery, transfer into isolators, and fostering. Namely, the pups are mature enough to survive the transfer only few hours before natural birth. However, the natural labor must not have begun, as the rupture of the amniotic sac creates an entry point for microorganisms. Another critical factor in planning the hysterectomy rederivation is the gestation period of both donor and “foster” strains. Depending on the genetic background, gestation periods can vary between 19 and 21 days. Therefore, to accurately time pregnancies, the recommended method is to pair mice overnight and check for vaginal plugs each morning.

During the hysterectomy procedure, the gravid uterus is removed and passed into the isolator through a germicidal dip tank, such as 4% iodine bath. It is important that the transfer of pups into the isolator occurs rapidly to ensure their viability and that the aseptic technique is ensured during this process. Inside the isolator, the uterus is opened, and the neonates are carefully removed, cleaned, dried with sterile cotton swabs, and stimulated to breathe. Thorough cleaning is crucial to prevent cannibalism or rejection by the surrogate mother. Cross-

fostering to germ-free dams is generally successful within the first 5–7 days post-delivery (Vowles et al., [2016](#)). However, this method has drawbacks, including a higher rate of neonatal death due to immaturity, transfer complications, or maternal neglect, as well as an increased risk of isolator contamination. For this reason, it is advisable to use a dedicated isolator for rederivation. Additionally, hysterectomy does not eliminate vertically transmitted pathogens like LCMV (lymphocytic choriomeningitis virus), LDV (lactate dehydrogenase-elevating virus), *Pasteurella pneumotropica*, and *Mycoplasma* spp. (Baker, [1998](#); Blackmore & Casillo, [1972](#)). Hence, microbiological monitoring of donor colonies should confirm the absence of these microorganisms before proceeding. If these organisms are present, the rederivation by embryo transfer may be more suitable.

The embryo transfer method, widely used today, offers advantages such as preventing vertical transmission and the mother's neglect. It is typically performed by implanting two-cell embryos into the oviduct of a pseudopregnant female. Embryos may be derived either directly from donor females or through in vitro fertilization (IVF), though direct derivation is preferred due to lower risk of microbial contamination (Janus et al., [2009](#)). Donor females are usually superovulated, mated, and sacrificed for embryo collection on day 2 of pregnancy. Two-cell embryos are collected by flushing the oviduct, then washed, and prepared for transfer (Sarvari et al., [2013](#)). Recipient germ-free females are paired with vasectomized or genetically sterile germ-free males and monitored for the presence of the vaginal plug, indicating readiness for embryo transfer. These females are removed from the isolator into a sterile cage, anesthetized, and embryos are transferred by a surgical procedure under germ-free conditions into the oviduct of the females. The procedure involves making a small incision, transferring embryos into the oviduct, and returning the females to the isolator until delivery approximately 20–21 days later.

To prevent contamination, embryo transfer must be performed using a sterile surgical platform, such as a dedicated clean bench connected to the isolator (Vowles et al., [2016](#)).

21.6 Physiology of Germ-Free Animals

Germ-free animals have adapted morphologically, anatomically, and physiologically to an environment and life without microorganisms. Numerous studies have highlighted differences in various organs between germ-free and colonized rodents. Despite these differences, germ-free animals show a healthy appearance and behavior typical of mice. Their adaptation to a germ-free environment should, thus, not be interpreted as a burden to their well-being. The most significant differences are observed in compartments that are typically colonized by microorganisms, such as the gastrointestinal tract. This section will focus on these key differences, with particular attention to the changes observed in the gastrointestinal system.

A major anatomical difference between germ-free rodents and those colonized with indigenous microbiota is an enlarged cecum (Fig. [21.3a](#)). This enlargement results from altered osmolarity in the intestinal contents due to the presence of undegraded mucopolysaccharides, which bind sodium and attract water, as well as intestinal atonia caused by the accumulation of muscle-depressant substances that are typically degraded by bacteria (Wostmann, [1981](#)). The extent of cecal enlargement is influenced by genetic factors and varies among different laboratory mouse strains (Bolsega et al., [2023](#)). In the early days of gnotobiology, the enlarged cecum often led to complications such as cecum torsion (also called cecal volvulus), which could result in the death of germ-free mice (Luckey, [1963](#)). However, with advancements in care and housing conditions, cecal torsions have become a relatively rare occurrence (Bolsega et al., [2025](#)).

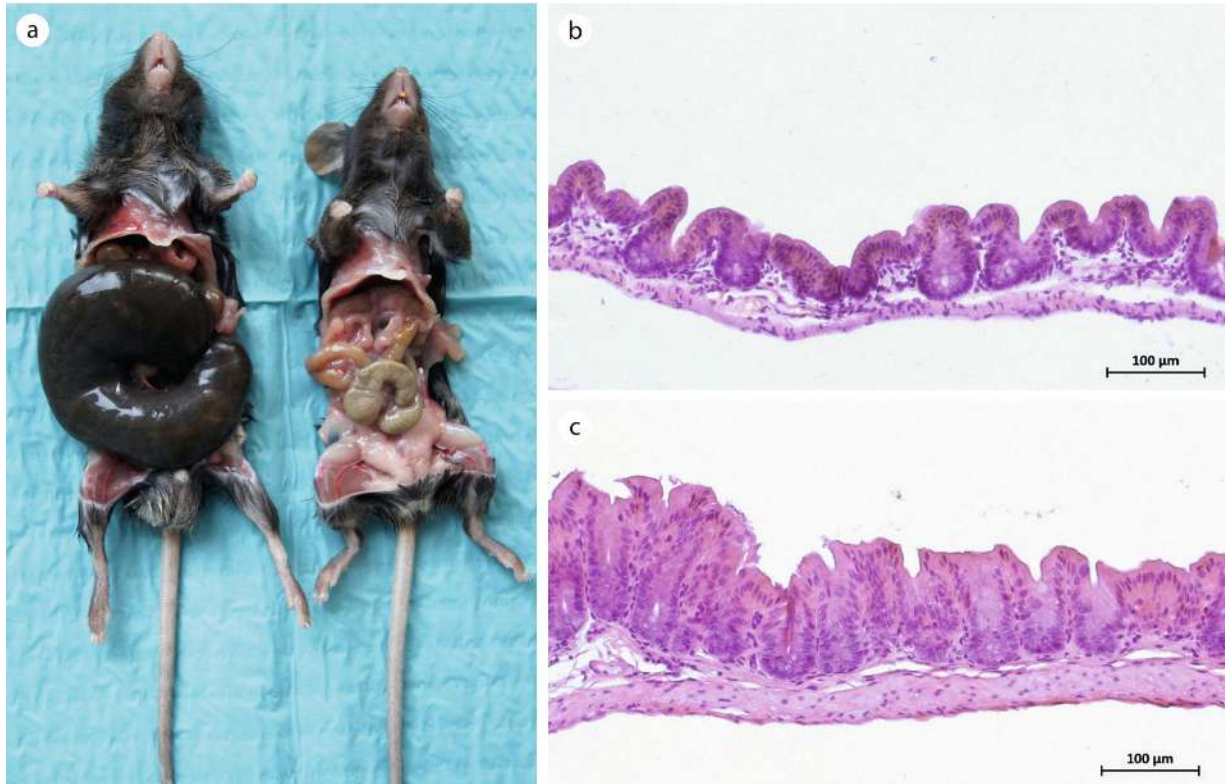


Fig. 21.3 Characteristics of germ-free mice. (a) Cecal size comparison between germ-free mouse (left) and SPF housed mice (right), (b, c) Cecal wall thickness of the mouse housed in the germ-free environment (b) and strict SPF barrier (c)

Germ-free animals also exhibit various changes in mucosal parameters compared to colonized animals. These include a reduced intestinal epithelial renewal cycle, decreased enzyme production, and increased bowel motility, and increased penetrability of the mucus layer. The intestinal wall of germ-free animals is noticeably thinner than that of mice with microbiota (Fig. [21.3b,c](#)). Additionally, in the absence of bacteria, the intestinal milieu is rather aerobic than strictly anaerobic. Fecal pellets of germ-free animals have a higher water content, making them softer compared to those of mice housed in SPF barriers. Moreover, germ-free animals show altered bile acid profiles in the intestine. Bile acids, which undergo structural and functional modifications through exposure to microbial enzymes during intestinal circulation, remain predominantly in their unmodified (conjugated and primary) forms in germ-free animals (Wostmann, [1981](#)).

The second prominent feature of germ-free animals is their underdeveloped immune system, which results from decreased antigenic stimulation due to the absence of live microbiota. Microbial colonization plays a critical role in shaping host immunity by stimulating the development of organized lymphoid tissues and enhancing immune cell functions (Round & Mazmanian, [2009](#)). Germ-free mice exhibit smaller lymph nodes and reduced levels of circulating leukocytes and serum immunoglobulins compared to mice colonized with enteric microbiota (Wostmann, [1981](#)). Additionally, gut-associated lymphoid tissue (GALT), intestinal T cells (particularly CD4+ cells), and intestinal IgA-producing cells in the intestine are markedly reduced in germ-free animals. The spleens of these free mice also exhibit fewer germinal centers and poorly formed T and B cell zones (Round & Mazmanian, [2009](#)).

Sterile organs in germ-free animals are similar to those of colonized rodents. However, some studies suggest that organs like the heart, lungs, and liver tend to be smaller in germ-free animals. Additionally, the cardiac input in germ-free animals is approximately one-third lower (Wostmann, [1981](#)). While germ-free mice grow and reproduce similarly to conventionally raised mice, their reproductive physiology is subtly affected. Female germ-free mice exhibit a prolonged diestrus phase, leading to reduced estrus frequency, which in turn lowers copulation and implantation rates (Shimizu et al., [1998](#)).

The absence of bacterial ureases in germ-free housing eliminates the typical ammonia odors associated with conventional housing environments. Ammonia is produced when bacterial ureases hydrolyze urea. Consequently, the detection of ammonia odors in germ-free housing can serve as an early indicator of bacterial contamination (Nicklas et al., [2015](#)).

21.7 Use of Germ-Free Animals for Functional Studies

Germ-free animal models serve as an invaluable experimental tool for studying microbiota-related functions and host-microbe interactions. These models enable standardized and causal analyses by colonizing germ-free animals with a single microorganism, a defined microbial community (bacterial or otherwise) bacterial, or complex microbiota such as human feces (Fig. 21.4). Many of the anatomical and physiological characteristics altered by the germ-free state can be partially or fully reversed within weeks through colonization with intestinal bacteria (Schaedler et al. 1965b). However, the timing of microbial colonization plays a pivotal role in this recovery, as certain phenotypes can only be restored if colonization occurs during the early life.

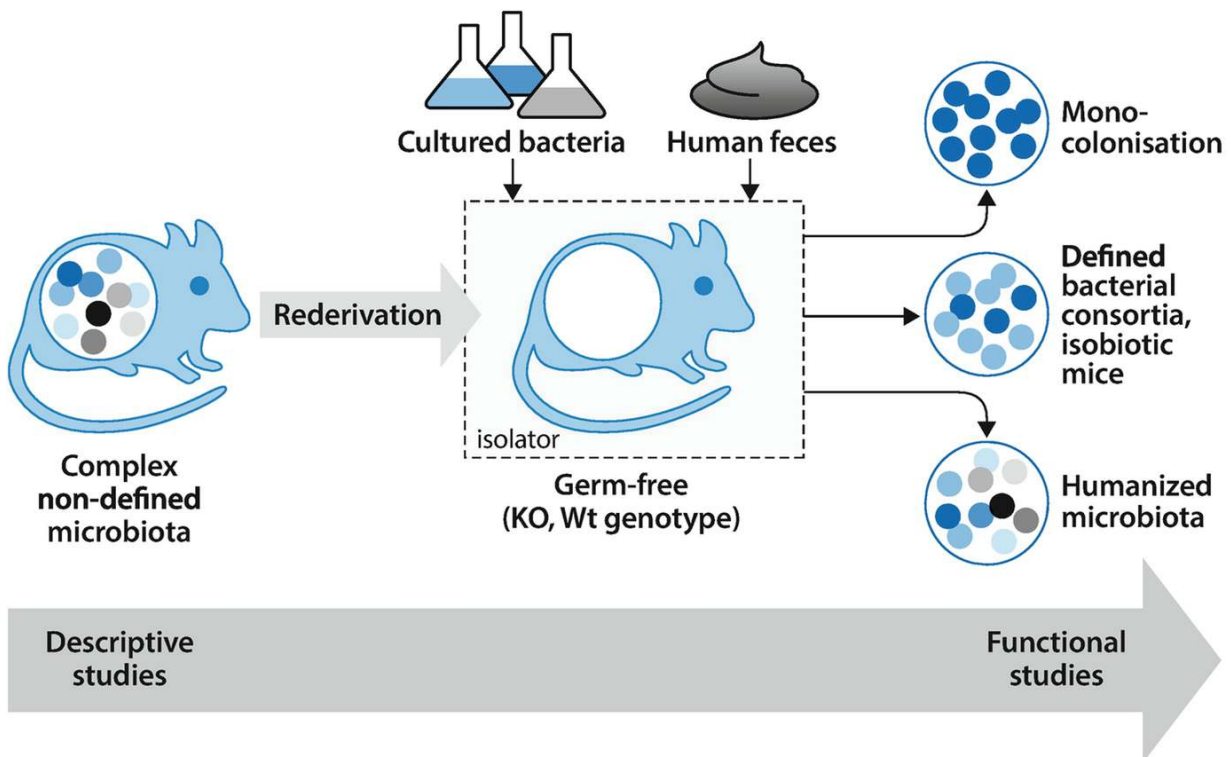


Fig. 21.4 Use of germ-free animals in functional studies. Germ-free animals are rederived from animals colonized by a complex, non-defined microbiota. The new rederived strains (specific mutant strains or wild-type strains) represent a valuable tool for functional microbiome research, as these models are fully naïve with respect to microbial colonization. Germ-free animals are free of all life forms except their own cells. Therefore, these mice can be colonized with cultured microorganisms or complex microbiota such as human feces. Colonization of germ-free animal models with a single microorganism (monocolonization) contributes to the understanding and unraveling of mechanisms of host-microbe and microbe-host interaction. Furthermore, defined

microbial consortia can be established in germ-free mice (isobiotic mice) by colonizing them with particular cultured mixtures of microorganisms. Complex microbiota can be established in germ-free mice by inoculating human or rodent feces. Colonization of germ-free mice with human feces results in establishing human microbiota in a mouse model, generating so-called “humanized gnotobiotic mice.” In summary, all listed approaches generate highly standardized models, which allow causal analysis of microbe–host interactions

Multiple studies have shown that microbial exposure and microbiota establishment during the postnatal period significantly influence long-term immune development and homeostasis. For instance, Hansen and colleagues showed that delaying microbial colonization of germ-free animals until 3 weeks of age resulted in a shift of the immune system toward a pro-inflammatory state (Hansen et al., [2012](#)). Moreover, exposing germ-free mice to microbes during the neonatal period was found to prevent the accumulation of mucosal invariant natural killer T cells, thereby reducing the risk of pathology in mouse models of IBD and asthma (Olszak et al., [2012](#)). Thus, this critical time frame is described as the “neonatal window of opportunity” (Hansen et al., [2013](#)).

Several methods are available for associating germ-free animals with microorganisms. A complex microbiota can be transferred to germ-free mice through cohousing with conventional donor mice over several weeks. Germ-free mice can also be directly inoculated with fecal material or cultured microorganisms via oral gavage. Another approach involves inoculating drinking water with pure cultures, fresh feces, or cecum content, although this method is primarily effective for oxygen-tolerant organisms.

The need for defined microbial communities has long been recognized in microbiota research. Schaedler pioneered the development of defined microbial cocktails for associating germ-free animals. These cocktails, composed of bacteria isolated from the normal mouse gut, including *Lactobacillus* spp., *Enterococcus* spp., *Enterobacter* spp., *Bacteroides* spp., and *Clostridium* spp., were instrumental in reducing mortality rates of germ-free mice when reintroduced into standard biological barriers (Schaedler et al., [1965a](#)). This work led to the creation of the Schaedler flora, the first defined

mouse-derived community. Later, Orcutt refined this consortium into the Altered Schaedler Flora (ASF), which included the major constituents of the mouse indigenous microbiota and became widely used by major commercial rodent vendors (Orcutt et al., [1987](#)). However, despite its global use, the individual ASF components remain unavailable in public strain collections, highlighting the need for accessible, standardized microbial resources.

Recent advances have introduced new synthetic microbial communities such as Oligo-Mouse-Microbiota 12 (OMM12), OMM19, and GM15. OMM12, comprising 12 well-characterized bacterial strains, has been extensively used in various research models and was initially developed to study colonization resistance against enteric bacteria (Brugiroux et al., [2016](#)). Building on this, OMM19 extends the concept by including 19 bacterial strains representing a more diverse and functionally comprehensive microbial community (Afrizal et al., [2022](#)). Similarly, GM15, another synthetic community, is tailored to better replicate the gut environment of laboratory rodents (Darnaud et al., [2021](#)). Additionally, humanized mouse models have been established using synthetic communities derived from human isolates, such as two simplified human microbial consortia (SIHUMI). One consortium includes human-derived IBD-related enteric bacteria (Wohlgemuth et al., [2011](#)), while the other comprises species selected for their numerical prevalence and fermentative abilities (Becker et al., [2011](#)). More recently, efforts have progressed toward constructing more complex, defined microbial communities that better replicate functions and diversity of the human gut microbiota. A 100-species synthetic community, MCC100, was used to supplement an “elderly” fecal microbiota in a bioreactor and was able to steer an increase in microbial diversity and abundance (Perez et al., [2021](#)). Cheng et al. assembled a defined complex human community, hCom1, composed of 104 bacterial species representing most common taxa from the human gut microbiota. This community has been challenged in gnotobiotic mice with a complex human fecal microbiota to identify open niches of the community. Subsequently, additionally identified bacterial species

have been added to the original hCom1 community to assemble a new community, hCom2, composing of 119 bacterial species. Gnotobiotic mice colonized with hCom2 exhibited colonization resistance towards *E. coli* or when challenged with complex fecal microbiota, and showed high phenotypical similarity to mice colonized with complex human fecal community (Cheng et al., [2022](#)). These simplified microbiomes enable researchers to reduce intestinal ecosystem complexity, facilitating the standardized mechanistic analysis of host-microbiota interactions. The cultivation of gut bacteria from various regions of the gastrointestinal tract and their availability in publicly accessible repositories have become essential for advancing microbiota research. These resources provide researchers with well-characterized bacterial strains for designing model-specific microbiomes and standardizing experiments. Using defined synthetic communities helps to better define phenotypes in mouse models by designing model-specific microbiomes.

In addition to defined synthetic communities, germ-free mice can also be colonized with human fecal material. This approach generates human microbiota-associated mice and allows researchers to investigate the role of the human microbiota in various diseases, such as microbiota functions related to obesity (Turnbaugh et al., [2009](#)), or resistance to cancer therapy (Routy et al., [2018](#)), and paving the way for future mechanistic studies. Moreover, fecal microbiota transplantation (FMT) is gaining interest as a novel therapeutic approach for a variety of microbiome-dependent chronic disorders. It was successfully applied in the treatment of recurrent *Clostridioides difficile* infection. Currently, the only FDA-approved microbiome-based therapies include Rebyota and Vowst, both applied for the treatment of recurrent *C. difficile* infection.

21.8 Concluding Remarks

Maintaining germ-free and gnotobiotic animals requires highly trained staff and significant financial investment, as it is both labor-intensive

and costly. Germ-free animals are anatomically and physiologically different from those raised in the regular environment. These animals have become a powerful tool for analyzing the impact of the microbiome on the host physiology as well as microbiota-driven disease mechanisms.

The association of germ-free mice with single microbes or defined synthetic communities offers a unique opportunity to investigate the protective or harmful effects of specific microorganisms, as well as to study microbial ecology within a controlled host environment. Therefore, gnotobiotic research provides tools essential for detailed analysis of host–microbe interactions under standardized conditions and enhances our ability to unravel the complexities of microbiota–host dynamics and their implications for human and animal health.

Controversy Treatment with broad-spectrum antibiotics or antibiotic cocktails, such as ampicillin, ciprofloxacin, metronidazole, streptomycin, or vancomycin, can significantly reduce the load of most bacterial species in the gut. However, this approach cannot completely eliminate the gut microbiota (Schubert et al., [2015](#)). Instead, bacterial communities are suppressed but not eradicated. This method is often used as an alternative to germ-free animals, but it comes with several limitations. Antibiotic treatment can change gut function and metabolism, promote overgrowth of antibiotic-resistant bacterial species, and contribute to the development of antimicrobial resistance (Lundberg et al., [2016](#)). Additionally, because gut microbiota play a critical role in immune system priming, exposure to microorganisms before antibiotic depletion may have lasting effects on host physiology, potentially overriding the effects of antibiotic therapy. Moreover, antibiotics do not target other components of the enteric microbiota, such as fungi, bacteriophages, and eukaryotic viruses, which can also influence intestinal homeostasis or dysbiosis (Lundberg et al., [2016](#)). These limitations make antibiotic-treated models less comprehensive, and their use is generally limited to preliminary experiments to determine whether a particular disorder

is microbiota-dependent. However, germ-free animals are also critically discussed due to their underdeveloped immune system and lack of immune system priming in early age, which is necessary for normal development of immune response (Hansen et al., [2012](#)). One way to address this issue is by establishing gnotobiotic colonies colonized with defined and stable synthetic microbial communities (Becker et al., [2011](#); Brugiroux et al., [2016](#); Schaedler et al., [1965b](#)). These colonies provide standardized animal models for studying host–microbiota interactions while ensuring that the immune system is primed and develops normally from early life. In these models, all microorganisms are well-defined, enabling precise and reproducible experiments.

Highlights

- Germ-free animals differ in their anatomical and physiological characteristics from animals carrying indigenous microbiota.
- Gnotobiotic animals are characterized by a fully defined microbiological status.
- The husbandry of gnotobiotic animals is labor-intensive, costly, and requires highly trained staff.
- Gnotobiotic models are powerful tools for investigating the effects of single microorganisms or microbial communities on host physiology and pathology under highly standardized conditions.

References

Afrizal, A., Jennings, S. A. V., Hitch, T. C. A., Riedel, T., Basic, M., Panyot, A., et al. (2022). Enhanced cultured diversity of the mouse gut microbiota enables custom-made synthetic communities. *Cell Host & Microbe*, 30, 1630–45 e25.

Afzaal, M., Saeed, F., Shah, Y. A., Hussain, M., Rabail, R., Socol, C. T., et al. (2022). Human gut microbiota in health and disease: Unveiling the relationship. *Frontiers in Microbiology*, 13, 999001. [[PubMed](#)][[PubMedCentral](#)]

Baker, D. G. (1998). Natural pathogens of laboratory mice, rats, and rabbits and their effects on research. *Clinical Microbiology Reviews*, 11, 231–266.

[\[PubMed\]](#)[\[PubMedCentral\]](#)

Basic, M., Bolsega, S., Smoczek, A., Glasner, J., Hiergeist, A., Eberl, C., et al. (2021). Monitoring and contamination incidence of gnotobiotic experiments performed in microisolator cages. *International Journal of Medical Microbiology*, 311, 151482.

Becker, N., Kunath, J., Loh, G., & Blaut, M. (2011). Human intestinal microbiota: Characterization of a simplified and stable gnotobiotic rat model. *Gut Microbes*, 2, 25–33.
[\[PubMed\]](#)

Becker, C., Neurath, M. F., & Wirtz, S. (2015). The intestinal microbiota in inflammatory bowel disease. *ILAR Journal/National Research Council, Institute of Laboratory Animal Resources*, 56, 192–204.

Blackmore, D. K., & Casillo, S. (1972). Experimental investigation of uterine infections of mice due to *Pasteurella pneumotropica*. *Journal of Comparative Pathology*, 82, 471–475.
[\[PubMed\]](#)

Bolsega, S., Basic, M., Smoczek, A., Buettner, M., Eberl, C., Ahrens, D., et al. (2019). Composition of the intestinal microbiota determines the outcome of virus-triggered colitis in mice. *Frontiers in Immunology*, 10, 1708.
[\[PubMed\]](#)[\[PubMedCentral\]](#)

Bolsega, S., Smoczek, A., Meng, C., Kleigrewe, K., Scheele, T., Meller, S., et al. (2023). The genetic background is shaping cecal enlargement in the absence of intestinal microbiota. *Nutrients*, 15.

Bolsega, S., Bleich, A., Dorsch, M., & Basic, M. (2025). Biological adaptation to a germ-free environment should not be mistaken as a burden for animals. *Laboratory Animals*.

Brugiroux, S., Beutler, M., Pfann, C., Garzetti, D., Ruscheweyh, H. J., Ring, D., et al. (2016). Genome-guided design of a defined mouse microbiota that confers colonization resistance against *Salmonella enterica* serovar Typhimurium. *Nature Microbiology*, 2, 16215.
[\[PubMed\]](#)

Cheng, A. G., Ho, P. Y., Aranda-Díaz, A., Jain, S., Yu, F. B., Meng, X., Wang, M., et al. (2022). Design, construction, and in vivo augmentation of a complex gut microbiome. *Cell*, 19, 3617–3636.

Darnaud, M., De Vadder, F., Bogeat, P., Boucinha, L., Bulteau, A. L., Bunescu, A., et al. (2021). A standardized gnotobiotic mouse model harboring a minimal 15-member mouse gut microbiota recapitulates SOPF/SPF phenotypes. *Nature Communications*, 12, 6686.
[\[PubMed\]](#)[\[PubMedCentral\]](#)

de Goffau, M. C., Fuentes, S., van den Bogert, B., Honkanen, H., de Vos, W. M., Welling, G. W., et al. (2014). Aberrant gut microbiota composition at the onset of type 1 diabetes in young children. *Diabetologia*, 8, 1569–1577.

Eun, C. S., Mishima, Y., Wohlgemuth, S., Liu, B., Bower, M., Carroll, I. M., et al. (2014). Induction of bacterial antigen-specific colitis by a simplified human microbiota consortium in gnotobiotic interleukin-10-/- mice. *Infection and Immunity*, 82, 2239–2246.

[\[PubMed\]](#)[\[PubMedCentral\]](#)

Foster, H. L. (1959). Housing of disease-free vertebrates. *Annals of the New York Academy of Sciences*, 78, 80–88.

[\[PubMed\]](#)

Gustafsson, B. (1946). Germ-free rearing of rats. *Acta Anatomica*, 2, 376–391.

[\[PubMed\]](#)

Gustafsson, B. E. (1959). Lightweight stainless steel systems for rearing germfree animals. *Annals of the New York Academy of Sciences*, 78, 17–28.

[\[PubMed\]](#)

Hansen, C. H., Nielsen, D. S., Kverka, M., Zakostelska, Z., Klimesova, K., Hudcovic, T., et al. (2012). Patterns of early gut colonization shape future immune responses of the host. *PLoS One*, 7, e34043.

[\[PubMed\]](#)[\[PubMedCentral\]](#)

Hansen, C. H., Metzdorff, S. B., & Hansen, A. K. (2013). Customizing laboratory mice by modifying gut microbiota and host immunity in an early “window of opportunity”. *Gut Microbes*, 4, 241–245.

[\[PubMed\]](#)[\[PubMedCentral\]](#)

Hecht, G., Bar-Nathan, C., Milite, G., Alon, I., Moshe, Y., Greenfeld, L., et al. (2014). A simple cage-autonomous method for the maintenance of the barrier status of germ-free mice during experimentation. *Laboratory Animals*, 48, 292–297.

[\[PubMed\]](#)

Janus, L. M., Smoczek, A., Hedrich, H. J., & Bleich, A. (2009). Risk assessment of minute virus of mice transmission during rederivation: Detection in reproductive organs, gametes, and embryos of mice after in vivo infection. *Biology of Reproduction*, 81, 1010–1015.

[\[PubMed\]](#)

Küster, E. (1915). Die keimfreie Zucht von Säugetieren. In E. Abderhalden (Ed.), *Handbuch der biochemischen Arbeitsmethoden* (pp. 311–323); 419–436.

Luckey, T. D. (1963). *Germfree life and gnotobiology*. Academic Press.

Lundberg, R., Toft, M. F., August, B., Hansen, A. K., & Hansen, C. H. (2016). Antibiotic-treated versus germ-free rodents for microbiota transplantation studies. *Gut Microbes*, 7, 68–74.

[\[PubMed\]](#)[\[PubMedCentral\]](#)

Mähler, M., Berard, M., Feinstein, R., Gallagher, A., Illgen-Wilcke, B., Pritchett-Corning, K., et al. (2014). FELASA recommendations for the health monitoring of mouse, rat, hamster, Guinea pig and rabbit colonies in breeding and experimental units. *Laboratory Animals*, 48, 178–192.

Nicklas, W., Keubler, L., & Bleich, A. (2015). Maintaining and monitoring the defined microbiota status of gnotobiotic rodents. *ILAR Journal*, 56, 241–249.

[\[PubMed\]](#)

Nuttall, G. H. F., & Thierfelder, H. (1897). Tierisches Leben ohne Bakterien im Verdauungskanal.

Zeitschrift für Physiologische Chemie, 23, 231–235.

Olszak, T., An, D., Zeissig, S., Vera, M. P., Richter, J., Franke, A., et al. (2012). Microbial exposure during early life has persistent effects on natural killer T cell function. *Science*, 336, 489–493.
[\[PubMed\]](#) [\[PubMedCentral\]](#)

Orcutt, R. P., Gianni, F. J., & Judge, R. J. (1987). Development of an “altered Schaedler flora” for NCI gnotobiotic rodents. *Microecology and Therapy*, 17, 59.

Parekh, P. J., Balart, L. A., & Johnson, D. A. (2015). The influence of the gut microbiome on obesity, metabolic syndrome and gastrointestinal disease. *Clinical and Translational Gastroenterology*, 6, e91.
[\[PubMed\]](#) [\[PubMedCentral\]](#)

Perez, M., Ntemiri, A., Tan, H., Harris, H. M. B., Roager, H. M., Ribi  re, C., et al. (2021). A synthetic consortium of 100 gut commensals modulates the composition and function in a colon model of the microbiome of elderly subjects. *Gut Microbes*, 1, 1–19.

Pleasant, J. R. (1959). Rearing germfree cesarean-born rats, mice, and rabbits through weaning. *Annals of the New York Academy of Sciences*, 78, 116–126.
[\[PubMed\]](#)

Rahija, R. J. (2007). Gnotobiotics. In J. G. Fox, M. T. Davidson, C. E. Newcomer, F. W. Quimby, & A. L. Smith (Eds.), *The mouse in biomedical research: Normative biology, husbandry, and models* (pp. 218–232). Elsevier.

Rausch, P., Basic, M., Batra, A., Bischoff, S. C., Blaut, M., Clavel, T., et al. (2016). Analysis of factors contributing to variation in the C57BL/6J fecal microbiota across German animal facilities. *International Journal of Medical Microbiology*, 5, 343–355.

Reuter, J. D., Livingston, R., & Leblanc, M. (2011). Management strategies for controlling endemic and seasonal mouse parvovirus infection in a barrier facility. *Laboratory Animal*, 40, 145–152.

Reyniers, J. A., Trexler, P. C., & Ervin, R. F. (1946). Rearing germ-free albino rats. *Lobund Reports*, 1–84.

Rosshart, S. P., Herz, J., Vassallo, B. G., Hunter, A., Wall, M. K., Badger, J. H., et al. (2019). Laboratory mice born to wild mice have natural microbiota and model human immune responses. *Science (Washington, D.C.)*, 365, 461–46+.

Round, J. L., & Mazmanian, S. K. (2009). The gut microbiota shapes intestinal immune responses during health and disease. *Nature Reviews. Immunology*, 9, 313–323.
[\[PubMed\]](#) [\[PubMedCentral\]](#)

Routy, B., Le Chatelier, E., Derosa, L., Duong, C. P. M., Alou, M. T., Daillere, R., et al. (2018). Gut microbiome influences efficacy of PD-1-based immunotherapy against epithelial tumors. *Science*, 359, 91–97.
[\[PubMed\]](#)

Sarvari, A., Naderi, M. M., Sadeghi, M. R., & Akhondi, M. M. (2013). A technique for facile and precise

transfer of mouse embryos. *Avicenna Journal Of Medical Biotechnology*, 5, 62–65.

[\[PubMed\]](#)[\[PubMedCentral\]](#)

Schaedler, R. W., Dubos, R., & Costello, R. (1965a). The development of the bacterial flora in the gastrointestinal tract of mice. *The Journal of Experimental Medicine*, 122, 59–66.

[\[PubMed\]](#)[\[PubMedCentral\]](#)

Schaedler, R. W., Dubs, R., & Costello, R. (1965b). Association of germfree mice with bacteria isolated from normal mice. *The Journal of Experimental Medicine*, 122, 77–82.

[\[PubMed\]](#)[\[PubMedCentral\]](#)

Schubert, A. M., Sinani, H., & Schloss, P. D. (2015). Antibiotic-induced alterations of the murine gut microbiota and subsequent effects on colonization resistance against *Clostridium difficile*. *MBio*, 6, e00974.

[\[PubMed\]](#)[\[PubMedCentral\]](#)

Schwenger, K. J., Clermont-Dejean, N., & Allard, J. P. (2019). The role of the gut microbiome in chronic liver disease: The clinical evidence revised. *JHEP Reports*, 1, 214–226.

[\[PubMed\]](#)[\[PubMedCentral\]](#)

Shimizu, K., Muranaka, Y., Fujimura, R., Ishida, H., Tazume, S., & Shimamura, T. (1998). Normalization of reproductive function in germfree mice following bacterial contamination. *Experimental Animals*, 47, 151–158.

[\[PubMed\]](#)

Steck, N., Hoffmann, M., Sava, I. G., Kim, S. C., Hahne, H., Tonkonogy, S. L., et al. (2011). *Enterococcus faecalis* metalloprotease compromises epithelial barrier and contributes to intestinal inflammation. *Gastroenterology*, 141, 959–971.

[\[PubMed\]](#)

Trexler, P. C. (1983). Gnotobiotics. In H. L. Forster & J. G. Fox (Eds.), *The mouse in biomedical research* (pp. 1–15). Academic Press.

Trexler, P. C., & Reynolds, L. I. (1957). Flexible film apparatus for the rearing and use of germfree animals. *Applied Microbiology*, 5, 406–412.

[\[PubMed\]](#)[\[PubMedCentral\]](#)

Turnbaugh, P. J., Ridaura, V. K., Faith, J. J., Rey, F. E., Knight, R., & Gordon, J. I. (2009). The effect of diet on the human gut microbiome: A metagenomic analysis in humanized gnotobiotic mice. *Science Translational Medicine*, 1, 6ra14.

[\[PubMed\]](#)[\[PubMedCentral\]](#)

Vowles, C. J., Anderson, N. E., & Eaton, K. A. (2016). *Gnotobiotic mouse technology an illustrated guide*. CRC Press.

Wohlgemuth, S., Bower, M., Gulati, A., & Sartor, R. B. (2011). Simplified human microbiota—A humanized gnotobiotic rodent model to study complex microbe-host interactions in ileal Crohn's disease. *Inflammatory Bowel Disease*, 17(Suppl 2), S75.

Wostmann, B. S. (1981). The germfree animal in nutritional studies. *Annual Review of Nutrition*, 1, 257–279.

[\[PubMed\]](#)

22. Functional Metabolites from the Human Gut Microbiota

Karin Kleigrewe¹, Lisa Göbner² and Tobias A. M. Gulder²✉

- (1) Bavarian Center for Biomolecular Mass Spectrometry (BayBioMS), TUM School of Life Sciences, Technical University of Munich, Munich, Germany
- (2) Department of Natural Product Biotechnology, Helmholtz Institute for Pharmaceutical Research Saarland (HIPS), Helmholtz Centre for Infection Research (HZI) and Department of Pharmacy at Saarland University, PharmaScienceHub (PSH), Saarbrücken, Germany

✉ **Tobias A. M. Gulder**
Email: tobias.gulder@helmholtz-hips.de

Abstract

The gut microbiome produces a wide array of functional metabolites through diverse mechanisms. Many of these compounds arise from microbial breakdown of dietary components, including complex carbohydrates, proteins, and secondary plant metabolites. Gut microbes can also modify host-derived compounds—such as bile acids—into bioactive metabolites. In addition, they synthesize metabolites de novo. This includes the biosynthesis of compounds associated with primary metabolism that can be found in a wide range of bacteria, but also specialized metabolites only present in single or few bacterial species that are assembled by complex biosynthetic pathways. Within this chapter, we first discuss the diversity of pathways towards relatively

simple, primary-type metabolites from gut bacteria using short-chain fatty acids as an example. Furthermore, representative examples of specialized metabolites, their biosynthesis, and proposed functions are presented.

Keywords Primary metabolites – SCFAs – Natural products – RiPPs – NRPs – PKs

22.1 Short-Chain Fatty Acids

Short-chain fatty acids (SCFAs) are volatile fatty acids containing 1 to 6 carbon atoms that can be linear or contain branched chains. They are primarily produced by the gut microbiome and serve as functional metabolites that influence the gut environment, affect colonic physiology, and provide an energy source for both intestinal microbiota and host cells. The physiological roles of SCFAs have been extensively reviewed elsewhere (Ríos-Covián et al., [2016](#)). This chapter thus focuses specifically on the biosynthetic pathways of SCFA formation. Structurally, SCFAs are classified into two main types: straight-chain fatty acids (SCFAs) and branched-chain short-chain fatty acids (BCSCFAs).

Approximately 90–95% of the SCFAs in the colon content consist of acetic, propionic, and butyric acid—typically in a ratio of 3:1:1—with a combined concentration ranging from 50 to 150 mM (Cummings et al., [1987](#)). The remaining ~5% are branched-chain SCFAs (BSCFAs), including isobutyric acid, isovaleric acid, and 2-methylbutyric acid.

22.1.1 Formic Acid Formation

Formate is the simplest short-chain fatty acid, consisting of a single carbon atom. During carbohydrate digestion, glycolytic processes convert sugars into pyruvate. Through the non-oxidative cleavage of pyruvate, formate and acetyl-CoA are generated—a reaction catalyzed by pyruvate formate lyase enzymes PflAB (Fig. [22.1](#)—dark grey, Table [22.1](#)) (Pinske & Sawers, [2016](#); Frolova et al., [2022](#); Macfarlane & Macfarlane, [2003](#)).

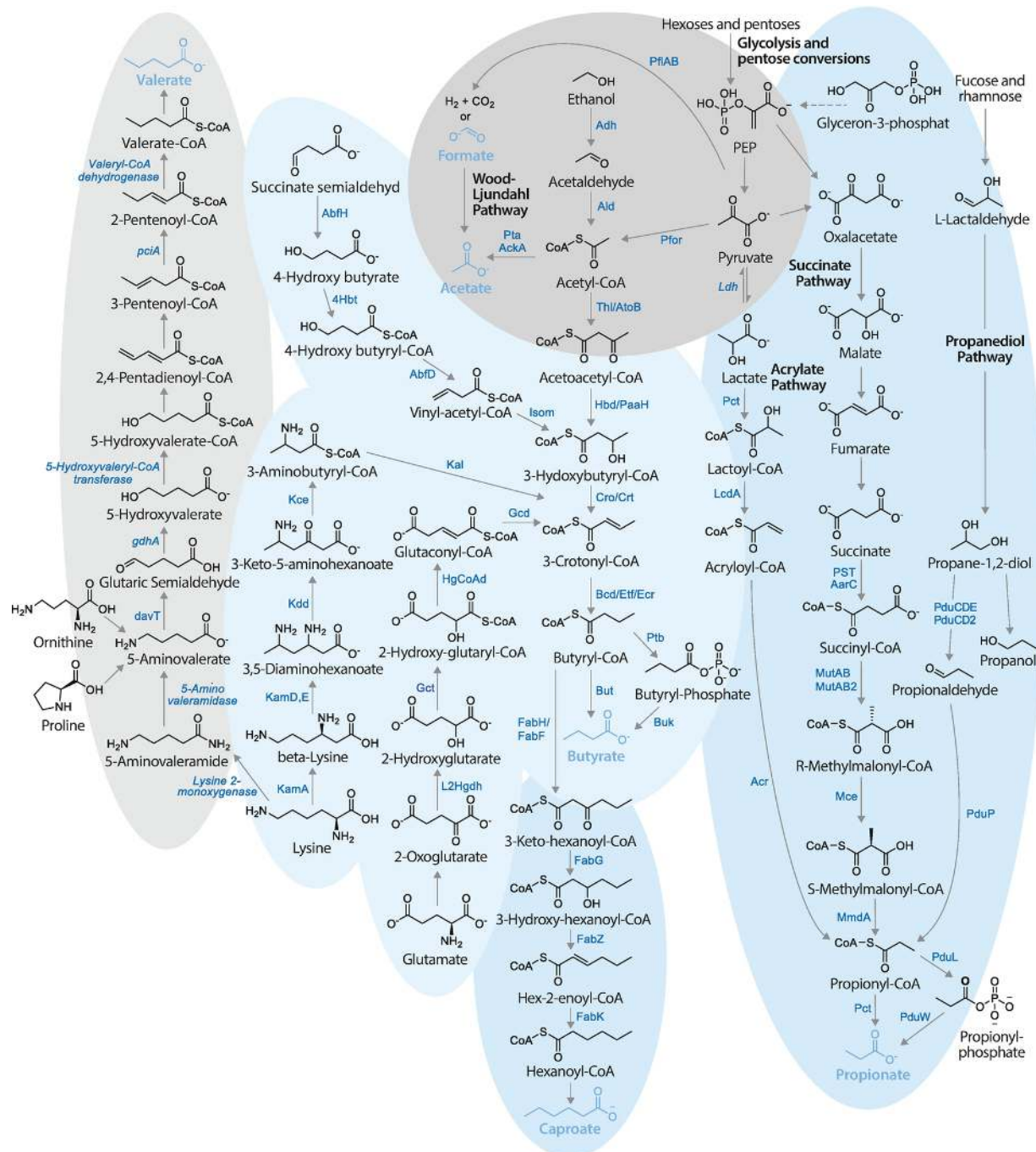


Fig. 22.1 Formation of short-chain fatty acids. (dark grey: formate and acetate formation, dark blue: propionate formation, light blue: butyrate formation, light grey: valeric acid formation, dark blue: caproate formation)

Table 22.1 Enzymes involved in the formate pathway encoded in listed bacteria (Frolova et al. 2022)

Enzyme [EC, gene]	Bacteria
-------------------	----------

Enzyme [EC, gene]	Bacteria
Formate C-acetyltransferase [2.3.1.54, <i>pflAB</i>]	Actinobacteria, Bacteroidales, Enterobacteria, Firmicutes

22.1.2 Acetic Acid Formation

The major short-chain fatty acid (SCFA), acetate, is produced either through the Wood–Ljungdahl pathway by acetogenic bacteria or via carbohydrate fermentation by most enteric bacteria (Louis et al., [2014](#)). The key reactions of the Wood–Ljungdahl pathway of CO₂ fixation consist of a methyl and carbonyl branch. In the methyl branch, one molecule of CO₂ undergoes multiple reductions to form a methyl group. In the carbonyl branch, CO₂ is reduced to carbon monoxide. CO is then condensed with a bound methyl group and coenzyme A to produce acetyl-CoA. In summary, within the Wood–Ljungdahl pathway, two one-carbon units, such as carbon dioxide or formic acid, together with hydrogen, are condensed to form acetic acid (Fig. [22.1](#)— dark grey, Table [22.2](#)) (Ragsdale & Pierce, [2008](#); Miller & Wolin, [1996](#)). Acetogenic bacteria can produce three acetate units from one molecule of glucose, whereas non-acetogenic anaerobes must dispose of reducing equivalents by forming molecules like acetate, succinate, propionate, butyrate, formate, D-lactate, L-lactate, and ethanol (Louis et al., [2014](#)). Acetate derived from acetyl-CoA is synthesized by the sequential action of phosphotransacetylase Pta and acetate kinase AckA, a process that also yields ATP (Frolova et al., [2022](#)).

Table 22.2 Enzymes involved in acetate pathways encoded in listed bacteria (Ragsdale, [2008](#); Ragsdale & Pierce, [2008](#); Frolova et al., [2022](#); Koh et al., [2016](#); Louis et al., [2014](#))

Pathway	Enzyme [gene]	Bacteria
---------	---------------	----------

Pathway	Enzyme [gene]	Bacteria
Wood-Ljungdahl pathway	Methyl branch	Acetogens: <i>Blautia hydrogenotrophica</i> <i>Clostridium</i> spp., <i>Streptococcus</i> spp.
	Formate dehydrogenase [1.2.1.43, <i>fdh</i>]	
	10-Formyl-H ₄ folate synthetase [6.3.4.3, <i>fhs</i>]	
	5,10-Methenyl-H ₄ folate cyclohydrolase [3.5.4.9, <i>folD</i>]	
	5,10-Methylene-H ₄ folate dehydrogenase [1.5.1.5, <i>folD</i>]	
	5,10-Methylene-H ₄ folate reductase [1.1.99.15, <i>metF</i>]	
	Carbonyl branch	
	Carbon monoxide dehydrogenase CODH [1.2.7.4, <i>acsA</i>]	
	Acetyl-CoA synthase ACS [2.3.1.169, <i>acsB</i>]	
	Methyltransferase MeTr [2.1.1.258, <i>acsE</i>]	
Carbohydrate fermentation	Phosphotranacetylase [2.3.1.8, <i>pta</i>]	Most enteric bacteria, e.g. <i>Akkermansia muciniphila</i> , <i>Bacteroides</i> spp., <i>Bifidobacterium</i> spp., <i>Prevotella</i> spp., <i>Ruminococcus</i> spp.
	Acetate kinase [2.7.2.1, <i>ackA</i>]	

22.1.3 Propionic Acid Formation

Propionic acid can be produced via three distinct metabolic pathways (Fig. [22.1](#)—dark blue, Table [22.3](#)) (Gonzalez-Garcia et al., [2017](#)). The succinate pathway is the most prevalent propionate-producing route in the human gut microbiota. In this pathway, succinate is first converted into succinyl-CoA by CoA-transferases such as AarC or PST, which simultaneously convert propionyl-CoA to propionate. Succinyl-CoA is

then transformed into *R*-methylmalonyl-CoA by the methylmalonyl-CoA mutase MutAB. The epimerase Mce converts this intermediate into *S*-methylmalonyl-CoA, which is subsequently decarboxylated by MmdA (methylmalonyl-CoA decarboxylase) to yield propionyl-CoA. Finally, propionate is released from propionyl-CoA (Reichardt et al., [2014](#); Frolova et al., [2022](#)).

Table 22.3 Enzymes involved in propionate pathways encoded in listed bacteria (Frolova et al., [2022](#); Reichardt et al., [2014](#); Koh et al., [2016](#))

Pathway	Enzyme [EC, gene]	Bacteria
Succinate pathway	Propanoyl-CoA:succinate CoA transferase [2.8.3.M4, <i>pst/aarC</i>]	<i>Bacteroidetes</i> , <i>Firmicutes</i> , <i>Akkermansia</i> , <i>Enterobacteria</i> , <i>Actinobacteria</i> (<i>Propionibacteria</i> / <i>Corynebacteria</i>)
	Methylmalonyl-CoA mutase [5.4.99.2, <i>mutAB/mutAB2</i>]	<i>Phascolarctobacterium succinatutens</i> , <i>Dialister</i> spp., <i>Veillonella</i> spp.
	Methylmalonyl-CoA racemase [5.1.99.1, <i>mce</i>]	
	Methylmalonyl-CoA decarboxylase [4.1.1.41, <i>mmdABCD/mmd</i>]	
	Methylmalonyl-CoA carboxytransferase [2.1.3.1, <i>mmct</i>]	
Acrylate pathway	Lactoyl-CoA dehydratase [4.2.1.54, <i>lcdABC/lcdX</i>]	Lachnospiraceae, Clostridiaceae, Peptostreptococcaceae
	Acrylyl-CoA reductase [1.3.1.95, <i>acr</i>]	<i>Megasphaera elsdenii</i> , <i>Coproccoccus catus</i>
	Propionate CoA-Transferase [2.8.3.1, <i>pct</i>]	<i>Coproccoccus catus</i> (Lachnospiraceae)
Propanediol pathway	Propanediol dehydratase [4.2.1.28, <i>pduCDE/pduCD2</i>]	Diverse Clostridia, Peptoniphilus, Veillonella, Lactobacillus, Enterococcus, Listeria, Enterobacteria, Fusobacterium
	Propionaldehyde dehydrogenase [1.2.1.87, <i>pduP</i>]	<i>Salmonella</i> spp., <i>Roseburia inulinivorans</i> , <i>Ruminococcus obeum</i>
	Phosphate propanoyltransferase [2.3.1.222, <i>pduL</i>]	
	Propionate kinase [2.7.2.15, <i>pduW</i>]	

The acrylate pathway, found in a limited number of human gut bacteria, uses lactate as a key intermediate. Lactate is first converted to lactoyl-CoA by the CoA-transferase Pct. The dehydratase complex LcdABC then converts lactoyl-CoA into acryloyl-CoA, which is reduced by the Acr reductase to form propionyl-CoA. Propionate is subsequently released from propionyl-CoA (Reichardt et al., [2014](#)).

The propanediol pathway relies on the fermentation of deoxy sugars such as fucose or rhamnose via L-lactaldehyde. These sugars are essential for activation of this route. The intermediate propane-1,2-diol is dehydrated by the enzyme complex PduCDE to form propionaldehyde. PduP, a CoA-dependent propionaldehyde dehydrogenase, converts propionaldehyde to propionyl-CoA and serves as a genetic marker for this pathway (Reichardt et al., [2014](#)). Propionyl-CoA is then transformed into propionyl-phosphate by the transferase PduL, and the final step is catalyzed by PduW kinase, which removes the phosphate group to release propionate (Frolova et al., [2022](#)).

22.1.4 Butyric Acid Formation

Butyric acid occurs naturally in certain foods, particularly in dairy products, and is also produced endogenously in the gut through microbial fermentation of indigestible carbohydrates (Saban Güler et al., [2025](#)).

(Meta)genomic analyses investigating butyrate-producing pathways and genes have identified several bacterial phyla as potential butyrate producers. These include members of Firmicutes (renamed Bacillota in 2021), as well as Actinobacteria, Bacteroidetes, Clostridia, Fusobacteria, Proteobacteria, Spirochaetes, and Thermotogae (Vital et al., [2014](#); Louis & Flint, [2009](#)). Butyrate biosynthesis occurs via four distinct metabolic pathways (Fig. [22.1](#)—light blue, Table [22.4](#)).

Table 22.4 Enzymes involved in butyrate pathways encoded in listed bacteria (Vital et al., [2014](#); Louis & Flint, [2017](#); Frolova et al., [2022](#); Koh et al., [2016](#))

Pathway	Enzyme [EC, gene]	Bacteria
---------	-------------------	----------

Pathway	Enzyme [EC, gene]	Bacteria
Acetyl-CoA pathway	Thiolase [2.3.1.16, <i>thl</i>]) β -Hydroxybutyryl-CoA dehydrogenase [1.1.1.157, <i>hbd</i>] Crotonase [4.2.1.55, <i>cro/crt</i>] Butyryl-CoA dehydrogenase [1.3.99.2, <i>bcd/etf</i>]	Ruminococcaceae, Lachnospiraceae, Clostridiaceae, Peptostreptococcaceae, Eubacteriaceae, Streptococcaceae
Lysine pathway	Lysine-2,3-aminomutase [5.4.3.2, <i>kamA</i>] β -Lysine-5,6-aminomutase [5.4.3.3, <i>kamD,E</i>] 3,5-Diaminohexanoate dehydrogenase [1.4.1.11, <i>kdd</i>] 3-Keto-5-amino hexanoate cleavage enzyme [2.3.1.247, <i>kce</i>] 3-Aminobutyryl-CoA ammonia lyase [4.2.1.14, <i>kal</i>]	<i>Faecalibacterium prausnitzii</i> , <i>Eubacterium rectale</i> , <i>Roseburia</i> spp. <i>Alistipes</i> , <i>Micromonospora</i>

Pathway	Enzyme [EC, gene]	Bacteria
Glutamate pathway	2-Hydroxyglutarate dehydrogenase [1.1.99.2, <i>L2Hgdh</i>] Glutaconate CoA transferase [2.8.3.12, <i>gctAB</i>] 2-Hydroxy-glutaryl-CoA dehydrogenase [4.2.1.167, <i>HgCoAd/hgdABC</i>] Glutaconyl-CoA decarboxylase [4.1.1.70, <i>gdcABC</i>]	Clostridiales, Acidaminococcaceae, Peptoniphilaceae, Lachnoclostridium, Fusobacterium
4-Aminobutyrate pathway	4-Hydroxybutyrate dehydrogenase [1.1.1.61, <i>abfH</i>] Butyryl-CoA:4-hydroxybutyrate CoA transferase [2.8.3.M6, <i>4hbt/cat</i>] 4-Hydroxybutyryl-CoA dehydratase [4.2.1.120, <i>abfD</i>] Vinylacetyl-CoA 3,2-isomerase [5.3.3.3, <i>isom</i>]	Lachnoclostridium, Porphyromonas, Tannerella
Butyryl-CoA:acetate CoA-transferase +route	Butyryl-CoA:acetate CoA transferase [2.8.3.8, <i>but</i>]	<i>Anaerostipes</i> spp., <i>Coprococcus catus</i> , <i>Eubacterium rectale</i> , <i>Eubacterium hallii</i> , <i>Faecalibacterium prausnitzii</i> , <i>Roseburia</i> spp.
Phosphotransbutyrylase/butyrate kinase route	Phosphate butyryltransferase [2.3.1.19, <i>ptb</i>] Buyrate kinase [2.7.2.7, <i>buk</i>]	<i>Coprococcus comes</i> , <i>Coprococcus eutactus</i>

In the acetyl-CoA pathway, two molecules of acetyl-CoA condense via the thiolase Thl to form acetoacetyl-CoA, which is sequentially reduced to 3-hydroxybutyryl-CoA by the β -hydroxybutyryl-CoA dehydrogenase Hdb, then reduced to crotonyl-CoA by the crotonase Cro/Crt (Louis & Flint, [2017](#)). Crotonyl-CoA is subsequently converted to butyryl-CoA by the electron-transferring flavoprotein butyryl-CoA dehydrogenase (Bcd/Etf) (Herrmann et al., [2008](#)).

In the glutamate pathway, butyrate is formed from glutamate degradation via 2-oxoglutarate by a transaminase reaction. Next, 2-hydroxyglutarate is formed by the 2-hydroxyglutarate dehydrogenase L2Hgdh, which is subsequently transformed to 2-hydroxyglutarate-CoA by the glutaconate CoA transferase GctAB. Next, the 2-hydroxy-glutaryl-CoA dehydrogenase HgCoAd forms glutaconyl-CoA. Again, crotonyl-CoA is synthesized by the glutaconyl-CoA decarboxylase Gdc, which is further processed as described above (Vital et al., [2014](#)).

In the lysine pathway, lysine degradation proceeds through β -lysine via the lysine-2,3-aminomutase KamA. Next, 3,5-diaminohexanoate is formed through β -lysine-5,6-aminomutase KamD and KamE. The 3,5-diaminohexanoate dehydrogenase Kdd produces 3-keto-5-amino-hexanoate, which is cleaved by the 3-keto-5-amino-hexanoate cleavage enzyme Kce to 3-aminobutyryl-CoA. This route also converges on the crotonyl-CoA intermediate by the 3-aminobutyryl-CoA ammonia lyase Kal (Vital et al., [2014](#); Louis & Flint, [2017](#)).

The 4-aminobutyrate pathway begins with the conversion of succinate semialdehyde to 4-hydroxybutyrate by the 4-hydroxybutyrate dehydrogenase AbfH, which is then transformed into 4-hydroxybutyryl-CoA by the butyryl-CoA:4-hydroxybutyrate CoA transferase 4Hbt. Next, vinyl-acetyl-CoA is formed by the 4-hydroxybutyryl-CoA dehydratase AbfD, leading again to crotonyl-CoA (Frolova et al., [2022](#)).

In all four pathways, the final step in butyrate production proceeds from butyryl-CoA via one of two enzymatic routes: Either butyryl-CoA is phosphorylated to butyryl-phosphate by the phosphate butyryl-transferase Ptb, and in the next step, butyrate kinase Buk produces butyrate (Miller & Wolin, [1996](#); Duncan et al., [2002](#); Diez-Gonzalez et

al., [1999](#)). Or the CoA group from butyryl-CoA is transferred to acetate by butyryl-CoA:acetate CoA-transferase but producing butyrate and acetyl-CoA. Genomic analyses suggest that this CoA-transferase-mediated route is more prevalent among gut bacteria than the kinase pathway (Louis & Flint, [2009](#)).

22.1.5 Valeric Acid Formation

In the human gut microbiome, *Megasphaera massiliensis* MRx0029 has been identified as a significant producer of valeric acid (Yuille et al., [2018](#)). *Megasphaera elsdenii* strains isolated from pig feces have been shown to produce valeric acid in substantial amounts (1.3–1.7 g/L) when cultured in a lactate-containing medium (3 g/L) (Yoshikawa et al., [2018](#)). Additionally, *Clostridium aminovalericum* (also referred to as *Clostridium viride* sp. nov.) forms valeric acid via the 5-aminovalerate pathway. In this pathway, 5-aminovalerate is synthesized through the reductive metabolism of proline, ornithine, or lysine (Fothergill & Guest, [1977](#); Barker et al., [1987](#)). The metabolic conversion proceeds as follows (Fig. [22.1](#)—light grey, Table [22.5](#)): 5-aminovalerate is first deaminated by the 5-aminovalerate:α-ketoglutarate transaminase and reduced by the glutamic dehydrogenase GdhA to yield ammonia and 5-hydroxyvalerate. These first reactions are utilized to produce 5-hydroxyvaleric acid as a starting material for chemical synthesis and are well studied (Kim et al., [2023](#)). Next, 5-hydroxyvalerate is activated to 5-hydroxyvaleryl-CoA by the 5-hydroxyvaleryl-CoA transferase, which undergoes oxidation and dehydration to form 2,4-pentadienoyl-CoA. This intermediate is then reduced to 3-pentenoyl-CoA, which isomerizes to 2-pentenoyl-CoA by the 3-pentenoyl-CoA:2-pentenoyl-CoA isomerase PciA. From here, 2-pentenoyl-CoA may undergo disproportionation—either oxidatively yielding acetate and propionate or reductively yielding valerate by the valeryl-CoA dehydrogenase (Buckel et al., [1994](#); Barker et al., [1987](#)).

Table 22.5 Enzymes involved in valerate pathway encoded in listed bacteria (Iino et al., [2007](#); Yuille et al., [2018](#); Yoshikawa et al., [2018](#); Staudt et al., [2025](#); Barker et al., [1987](#); Kim et al., [2023](#); Buckel, [2001](#))

Pathway	Enzyme [EC, gene]	Bacteria
5-Aminovalerate pathway	5-Aminovalerate:α.ketoglutarate transaminase [2.6.1.48, <i>davT/gabT</i>]	<i>Clostridium aminovalericum</i>
	Glutamic dehydrogenase [1.4.1.2, <i>gdhA/yqhD</i>]	<i>Oscillibacter valericigenes</i> gen. nov., sp. Nov
	5-Hydroxyvalerate dehydrogenase [1.1.1.234 <i>hvdH</i>]	<i>Magasphaera elsdenii</i>
	5-Hydroxyvaleryl-CoA transferase [unkown]	<i>Megasphaera massiliensis</i> MRx0029
	Valeryl-CoA dehydrogenase [unkown]	
	3-Pentenoyl-CoA:2-Pentenoyl-CoA Isomerase [5.3.3.6, <i>pciA</i>]	

22.1.6 Caproic Acid Formation

The formation of caproic acid (hexanoic acid) has been extensively studied, particularly in the context of sustainable biochemical production of long-chain carbon compounds (Suo et al., [2025](#)). In 1942, Barker et al. showed that *Clostridium kluyveri* produces caproic acid through the fermentation of ethanol and acetate (Barker & Taha, [1942](#)). In this metabolic pathway, ethanol acts as an electron donor and is oxidized to acetaldehyde by the alcohol dehydrogenase Adh and then further oxidized by the acetaldehyde dehydrogenase Ald to acetyl-CoA, which then enters the reverse β -oxidation cycle (Fig. [22.1](#)—dark blue, Table [22.6](#)). This cycle enables the elongation of acetate to butyrate, followed by a second elongation step leading to caproic acid (Spirito et al., [2014](#)). In detail, two units of acetyl-CoA are condensed by the acetyl-CoA transferase AtoB to acetoacetyl-CoA. Next, acetoacetyl-CoA is reduced to form 3-hydroxybutyryl-CoA by the 3-hydroxybutyryl-CoA dehydrogenase PaaH. This intermediate is dehydrated to produce 3-crotonyl-CoA by the enoyl-CoA hydratase Crt. The butyryl-CoA dehydrogenase BCD forms the next butyryl-CoA. Subsequently, butyryl-CoA is condensed with another molecule of acetyl-CoA to 3-ketohexyl-CoA by the 3-oxoacyl-ACP synthase II/III FabH/FabF. Next, the 3-oxoacyl-ACP reductase FabG forms 3-hydroxy-hexanoyl-CoA, which is then dehydrated by the 3-hydroxyacyl-ACP hydratase FabZ to hex-2-enoyl-CoA. After another reduction step by the enoyl-ACP reductase II FabK, hexanoyl-CoA is produced. The release of caproate is presumably

catalyzed through a CoA-transferase reaction (Suo et al., [2025](#); Spirito et al., [2014](#); Tao et al., [2017](#)). Instead of ethanol, some bacteria like *Ruminococcaceae* use lactate to produce caproic acid (Yuan et al., [2022](#)). In this pathway, lactate is first oxidized to pyruvate by lactate dehydrogenase Ldh. Pyruvate is then decarboxylated by pyruvate:ferredoxin oxidoreductase Pfor to form acetyl-CoA (Suo et al., [2025](#); Tao et al., [2017](#)). Furthermore, alternative carbon sources have been identified. In *Clostridium* sp. BS-1, galactitol serves as a precursor for caproic acid biosynthesis (Jeon et al., [2013](#)). In *Megasphaera elsdenii* NCIMB 702410, caproic acid production has been observed from fermentable carbohydrates such as glucose, sucrose, and starch (Choi et al., [2013](#)). Notably, strains of *Megasphaera elsdenii* have also been isolated from human gut samples, suggesting their potential relevance in the human microbiome (Sugihara et al., [1974](#)).

Table 22.6 Enzymes involved in caproate pathways encoded in listed bacteria (Barker & Taha, [1942](#); Suo et al., [2025](#); Yuan et al., [2022](#); Tao et al., [2017](#))

Pathway	Enzyme [EC, gene]	Bacteria
---------	-------------------	----------

Pathway	Enzyme [EC, gene]	Bacteria
Reverse-β-oxidaion	Acetyl-CoA acetyl-transferase [3.3.1.9, <i>atoB</i>] 3-Hydroxybutyryl-CoA dehydrogenase [1.1.1.157, <i>paaH</i>] Enoyl-CoA hydratase [4.2.1.17, <i>crt</i>] Butyryl-CoA dehydrogenase [1.3.8.1, <i>bcd</i>] Butyryl-CoA:acetate CoA-transferase [2.8.2.8, <i>bat</i>] <i>trans</i> -2-Enoyl-CoA reductase [1.3.1.44, <i>ecr</i>] Acetyl-CoA carboxylase carboxyl transferase [6.4.1.2, <i>acc</i>] ACP S-Malonyltransferase [2.3.1.39, <i>fabD</i>] 3-Oxoacyl-ACP synthase III [2.3.1.180, <i>fabH</i>] 3-Oxoacyl-ACPsynthase II [2.3.1.179, <i>fabF</i>] 3-Oxoacyl-ACP reductase [1.1.1.100, <i>fabG</i>] 3-Hydroxyacyl-ACP dehydratase [4.2.1.134, <i>fabZ</i>] Enoyl-(acyl-carrier-protein) reductase II [1.3.1.9, <i>fabK</i>]	<i>Ruminococcaceae bacterium CPB6</i>
Ethanol route	Alcohol dehydrogenase [1.1.1.1, <i>adh</i>] Acetaldehyde dehydrogenase [1.2.1.3, <i>ald</i>]	<i>Clostridium kluyveri</i>
Lactic acid route	Lactate dehydrogenase [1.1.1.27, <i>ldh</i>] Pyruvate:ferredoxin oxidoreductase [1.2.7.1, <i>pfor</i>]	<i>Ruminococcaceae, Megasphaera elsdenii,</i> <i>Caproiciproducens galactitolivorans</i> <i>Caproicibacterium lactatifermentans</i> sp. Nov., <i>Megasphaera hexanoica</i>

22.1.7 Branched-Chain Fatty Acids Formation

Branched-chain fatty acids, such as isobutyrate, 2-methylbutyrate, isovalerate, and isocaproate, are formed from branched-chain amino acids like valine, leucine, and isoleucine, and thus through proteolytic fermentation (Dai et al., [2011](#); Macfarlane et al., [1992](#); Neis et al., [2015](#);

Elsden & Hilton, [1978](#)). The initial step in this process involves the conversion of branched-chain amino acids into their corresponding α -keto acids, catalyzed by aminotransferases (Ganesan & Weimer, [2004](#)) (Fig. [22.2](#) and Table [22.7](#)). Smith and Macfarlane ([1998](#)) found that BCFA-producing bacteria constituted a variable fraction of the peptide- and amino acid-fermenting microbial community, ranging from 0.6% (for isovalerate/2-methylbutyrate producers) to 40% (for isobutyrate producers). In contrast, valerate and isocaproate were only minor components of the amino acid fermentation products in the human-derived microbiota (Smith & Macfarlane, [1998](#)).

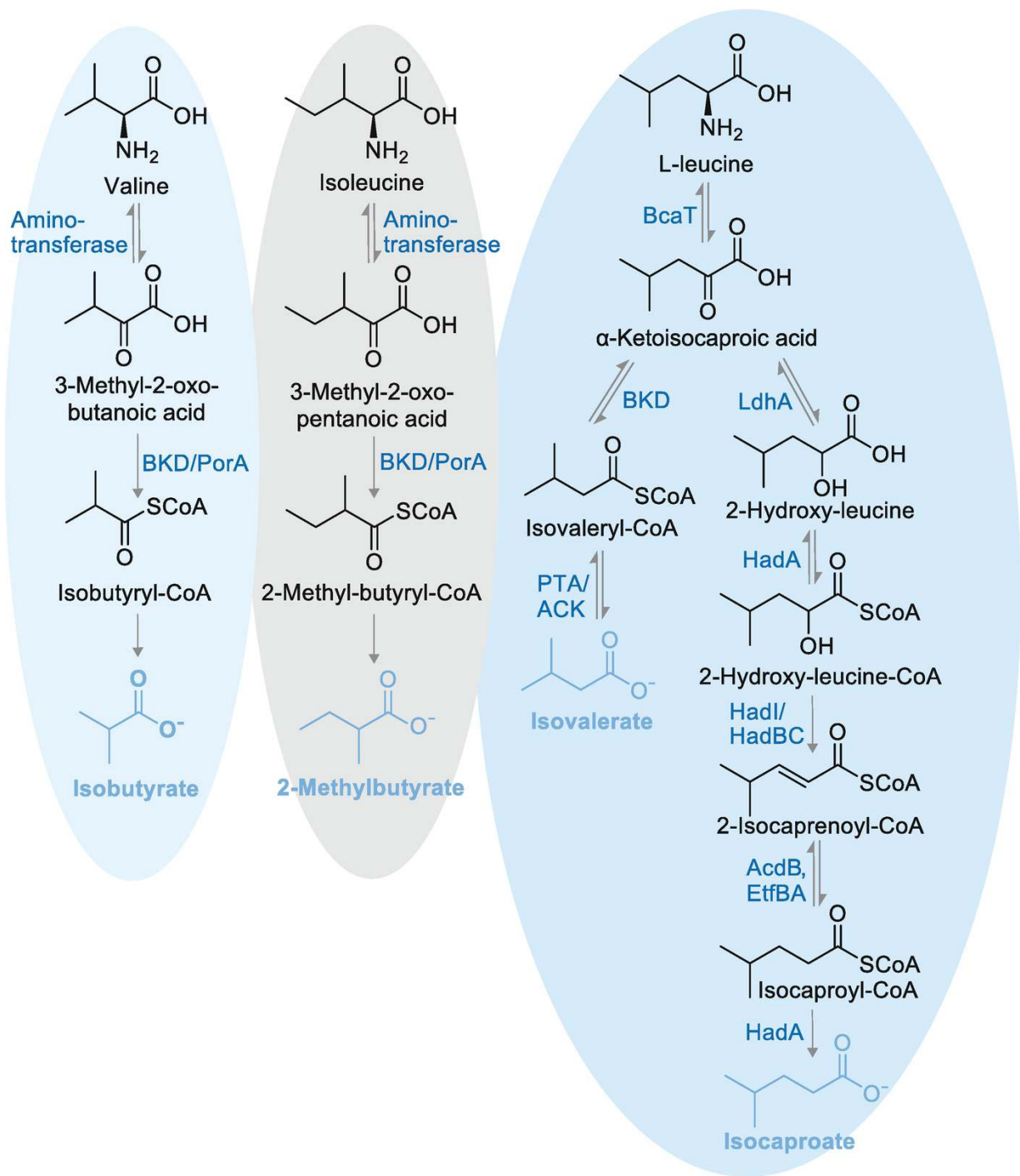


Fig. 22.2 Formation of branched-chain short-chain fatty acids. (left: isobutyrate from valine, center: 2-methylbutyrate from isoleucine, right: isovalerate and isocaproate from leucine)

Table 22.7 Enzymes involved in branched-chain fatty acid pathways encoded in listed bacteria (Guo et al., [2019](#); Dai et al., [2011](#); Kim et al., [2006](#); Díaz-Pérez et al., [2016](#))

Pathway	Enzyme [EC, gene]	Bacteria
---------	-------------------	----------

Pathway	Enzyme [EC, gene]	Bacteria
Isovalerate	Branched-chain aminotransferase [2.6.1.42, <i>bcaT</i>] Branched-chain α -ketoacid dehydrogenase complex (BKD) [1.2.4.4, <i>bkdA1A2B-lpdV</i>] Phosphotransferase [2.3.1.8, <i>pta</i>] Acyl kinase [2.7.2.1, <i>ack</i>]	<i>Peptostreptococcus</i> spp. <i>Clostridium bifermentans</i> <i>Clostridium difficile</i>
Isocaproate	Branched-chain aminotransferase [2.6.1.42, <i>bcaT</i>] <i>R</i> -2-Hydroxy-isocaproyl dehydrogenase [1.1.1.279, <i>ldhA</i>] Activator for dehydratase [EC?, <i>hadI</i>] <i>R</i> -2-Hydroxy-isocaproyl-CoA dehydratase [4.2.1.157, <i>hadBC</i>] Acyl-CoA dehydrogenase [1.3.8.7, <i>acdB</i>] Electron transferring flavoprotein [1.5.5.1, <i>etfBA</i>] <i>R</i> -2-Hydroxy-isocaproyl-CoA dehydratase [4.2.1.150, <i>hadA</i>]	<i>C. difficile</i>
Isovalerate, 2-Methylbutyrate, Isobutyrate	Pyruvate:ferredoxin oxidoreductase PFOR superfamily [1.2.7.1, <i>porA</i>]	<i>Clostridium sporogenes</i>

Amino acid degradation in the gut often involves oxidation-reduction coupling between specific amino acid pairs—a process known as the Stickland reaction, named after its discoverer (Barker, [1981](#)). Examples of such redox pairs include Arg/Ile, Ser/Pro, Ser/Arg, Val/Pro, Leu/Pro, and Ile/Pro (Liu et al., [2022](#)).

Both isovaleric acid (3-methylbutyric acid) and isocaproic acid are derived from L-leucine catabolism. Initially, L-leucine is converted into α -ketoisocaproate by the enzyme branched-chain aminotransferase (BcaT). The pathway then branches: through oxidative decarboxylation, isovaleryl-CoA is generated by the branched-chain keto acid dehydrogenase complex (BKD). Isovaleryl-CoA is subsequently converted to isovaleric acid by action of phosphotransacylase Pta and acyl kinase Ack (Díaz-Pérez et al., [2016](#)).

In *Clostridium difficile*, a reductive pathway for L-leucine fermentation leads to isocaproic acid production. Here, α -ketoisocaproate is reduced to (R)-2-hydroxyisocaproate by LdhA, a specific dehydrogenase. The enzyme HadA (a 2-hydroxyisocaproate-CoA transferase) then forms (R)-2-hydroxyisocaproyl-CoA. This intermediate is further dehydrated by HadI/HadBC (2-hydroxyisocaproyl-CoA dehydratase) to 2-isocaprenoyl-CoA, which is reduced to caprenoyl-CoA by AcdB (an acyl-CoA dehydrogenase) in conjunction with EtfBA (an electron-transferring flavoprotein complex). Finally, HadA releases isocaproic acid from the CoA-bound intermediate (Díaz-Pérez et al., [2016](#)).

The formation of isobutyrate and 2-methylbutyrate from the initial amino acids valine and isoleucine has not been studied in great detail, so the exact enzymes involved remain unknown. Interestingly, deletion of the branched-chain α -ketoacid dehydrogenase complex in *Clostridium sporogenes* did not eliminate BCFA production. However, deletion of PorA, a member of the pyruvate:ferredoxin oxidoreductase (PFOR) superfamily, abolished the formation of isovalerate, 2-methylbutyrate, and isobutyrate. Furthermore, an additional deletion of hadB completely abolished isocaproate production (Guo et al., [2019](#)). PorA is involved in the oxidative metabolism of phenylalanine and tyrosine, as shown by another study (Dodd et al., [2017](#)).

22.1.8 Bacterial Cross-Feeding

SCFA production is impacted by bacterial cross-feeding. For example, end products produced by a given microorganism can be used by another strain to produce SCFAs. For example, lactate or succinate can be utilized by different human gut bacteria to serve as substrates for butyrate and propionate producers (Ríos-Covián et al., [2016](#)). Furthermore, some strains are able to break down complex carbohydrates. An example is that some *Bifidobacterium* species cannot use inulin-type fructans but can grow on the mono- and oligosaccharides released by inulin degraders (Ríos-Covián et al., [2016](#)). This bacterial cross-feeding influences bacterial communities,

as, for example, *Bifidobacterium adolescentis* supports the growth of butyrate-producing strains of *Anerostipes*, *Roseburia*, and *Eubacterium* species with an increase in butyrate formation (Belenguer et al., [2006](#)). Due to the possibility of cross-feeding, a single strain does not need all the necessary genetic machinery to produce SCFA but might benefit from substrates of other strains. In summary, the cross-feeding of precursors adds extra complexity to the prediction of short-chain fatty acid production in gut microbiota.

22.2 De Novo Biosynthesis of Specialized Metabolites

In addition to metabolic processes that lead to the biosynthesis of typical primary metabolites, as exemplarily described for SCFAs in Sect. [22.1](#) above, the human gut microbiota contains tremendous biosynthetic potential for de novo production of specialized metabolites (Donia et al., [2014](#)), also termed natural products (NPs). In bacteria, the genes required for biosynthesis of a given NP—including those encoding proteins involved in NP precursor and core structure biosynthesis, structural diversification, regulation, transport, and frequently genes for self-resistance—are usually arranged in close spatial proximity in so-called biosynthetic gene clusters (BGCs) (Medema et al., [2015](#)). Recent advances in genome sequencing and particularly in bioinformatic tools for BGC detection, comparison, and in-depth analysis (Ren et al., [2020](#); Zhu et al., [2025](#); Navarro-Muñoz et al., [2020](#)) have revealed a plethora of such BGCs in the human microbiota, including the gut (Donia et al., [2014](#)). Importantly, NPs from different sources, traditionally mostly from plants, fungi, and environmental isolates of microorganisms, have a long history of application as drugs in human health, as they very often possess highly potent biological functions, e.g., antimicrobial, anti-inflammatory, and cytotoxic effects (Newman & Cragg, [2020](#); Atanasov et al., [2021](#)). While research into NPs originating from the human microbiome impressively accelerated in recent years (Donia & Fischbach, [2015](#); Milshteyn et al.,

[2018](#); Seyedsayamdost & Clardy, [2023](#); Dai et al., [2023](#); Wilson et al., [2017](#); Shine & Crawford, [2021](#); Wang et al., [2019](#); Butkovich et al., [2025](#)), our understanding of their structural and functional diversity is still in its infancy. This is both surprising and concerning, as the encoded NPs have the potential to significantly affect the gut ecosystem and host health. This includes potential functions in microbe–microbe interactions, e.g., caused by NPs with antimicrobial effects that might facilitate community protection, differentiation, or invasion, and microbe–host interplay, e.g., causing disturbance in host metabolism or cell integrity that might lead to the development of severe diseases. Recent studies have shown that certain diseases indeed correlate to changes in the composition of the human microbiome, in some cases even allowing correlations to individual BGCs (Schmartz et al., [2024](#); Rinninella et al., [2019](#)). Uncovering the metabolism and resulting health effects of BGCs and their encoded NPs of the human microbiota thus has the potential to help solve long-standing questions to understand, prevent, and treat gastrointestinal diseases. In this subchapter, we summarized selected examples of NPs from the gut microbiome.

22.2.1 Ribosomally Synthesized and Post-Translationally Modified Peptides (RiPPs)

A large class of NPs are *Ribosomally synthesized and Post-translationally modified Peptides* (RiPPs) (Arnison et al., [2013](#); Montalbán-López et al., [2021](#)). RiPPs are directly genetically encoded in small genes, which are translated into the respective peptide sequences with typically 20–110 amino acid residues by the ribosome (Fig. [22.3](#)). The resulting peptides are commonly composed of a so-called leader peptide followed by the core peptide. In addition, BGCs for RiPP biosynthesis usually contain genes encoding processing enzymes responsible for peptide maturation (maturases), proteolysis, and transport. The leader peptide serves as a recognition sequence for the processing enzymes, which selectively catalyze structural modifications at the core peptide important to shape its overall structure, function,

and metabolic stability. Typical modifications catalyzed by maturases include transformation at individual amino acid residues (e.g., oxygenation, alkylation, epimerization), formation of heterocyclic elements (e.g., oxazoles, thiazoles), and backbone cross-coupling reactions installing macrocyclic structural features. Final proteolytic cleavage and export of the modified core peptide conclude RiPP biosynthesis. The released mature RiPPs often possess well-defined antibiotic activity profiles and are thus thought to be key elements in competitive or symbiotic interactions of microbes in complex communities (Arnison et al., [2013](#)).

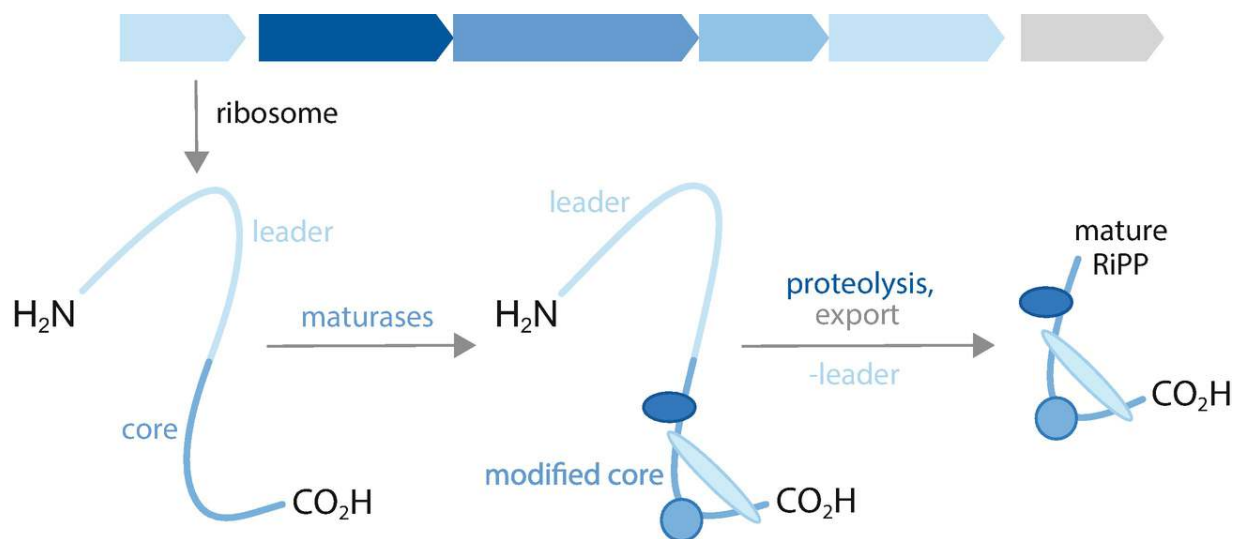


Fig. 22.3 Schematic representation of RiPP (ribosomally synthesized and post-translationally modified peptide) biosynthesis. Colored arrows on top of the graphical representation represent genes encoding the precursor peptide (light blue), maturases (dark blue), transporter (light blue), and protease (light grey), organized in a biosynthetic gene cluster (BGC). The ribosome generates the precursor peptide which is further modified by pathway-specific maturases introducing structural changes (indicated by blue oval shapes) to give a functionalized core peptide, followed by proteolytic cleavage of the leader and export of the mature RiPP

BGCs encoding RiPPs are widespread in the human gut microbiota. They can be found in both commensals and pathogens. Their systematic discovery and functional evaluation thus have the potential to lead to a better understanding of mechanisms of niche colonialization, infection, and ultimately intervention strategies (Heilbronner et al., [2021](#)). RiPPs can be divided into several subclasses

according to defining structural features (Arnison et al., [2013](#)). Major classes of RiPPs discovered from the human gut microbiome include microcins, thiazole/oxazole-modified microcins (TOMMs), lantipeptides, and sactipeptides.

Many RiPPs currently known from the human gut microbiome belong to the microcins and thiazole/oxazole-modified microcins (TOMMs) (Duquesne et al., [2007a](#)). Microcins (Mcc) commonly derive from *Escherichia coli*. They generally possess potent antibiotic activity with diverse molecular mechanisms and predominantly act against Gram-negative bacteria, particularly Enterobacteria (Duquesne et al., [2007a, b](#); Baquero et al., [2019](#); Marković et al., [2022](#); Sassone-Corsi et al., [2016](#)). These RiPPs can be further subdivided into class I (molecular mass below 5 kDa, often heavily post-translationally modified) and class IIa/b microcins (5–10 kDa).

Microcins belonging to class I that also nicely showcase the structural and functional diversity of these compounds include MccB17, MccC (also termed MccC7/C51), and MccJ25 (Duquesne et al., [2007a](#)). MccB17 is a heavily modified peptide resulting from post-translational installation of four oxazole and thiazole units by backbone cyclization utilizing four serine and cysteine residues, respectively (Fig. [22.4](#)) (Yorgey et al., [1994](#)). MccB17 is thus a representative member of the TOMMs (also classified as member of the linear azol(in)e-containing peptides or LAPs) (Arnison et al., [2013](#)). The heterocyclic units are formed by a heterotrimeric synthase complex composed of a dehydrogenase and cyclodehydratase(s) (Li et al., [1996](#)). This complex facilitates ATP-dependent cyclodehydration to the intermediate azolines followed by dehydrogenation, delivering the final azole units. MccB17 was shown to efficiently inhibit a broad diversity of Gram-negative bacteria, such as *Escherichia*, *Citrobacter*, *Klebsiella*, *Salmonella*, *Shigella*, and *Pseudomonas* (Asensio & Pérez-Díaz, [1976](#)). The antibacterial effect of MccB17 stems from inhibition of DNA gyrase (Vizán et al., [1991](#)). Further interesting examples of TOMMs include clostridiolysin S (CLS) from *Clostridium* sp. and listereolysin S (LLS) from *Listeria monocytogenes*. CLS was shown to possess hemolytic

activity and is thus thought to be involved in host tissue damage contributing to clostridial virulence (Gonzalez et al., [2010](#)), similar to the hemolytic toxin streptolysin S from *Streptococcus* sp. (Molloy et al., [2011](#)). LLS contributes to infections by epidemic *Listeria* sp. by its antibacterial effects on Gram-positive bacteria caused by contact-dependent membrane depolarization and permeabilization (Quereda et al., [2016](#); Meza-Torres et al., [2021](#)).

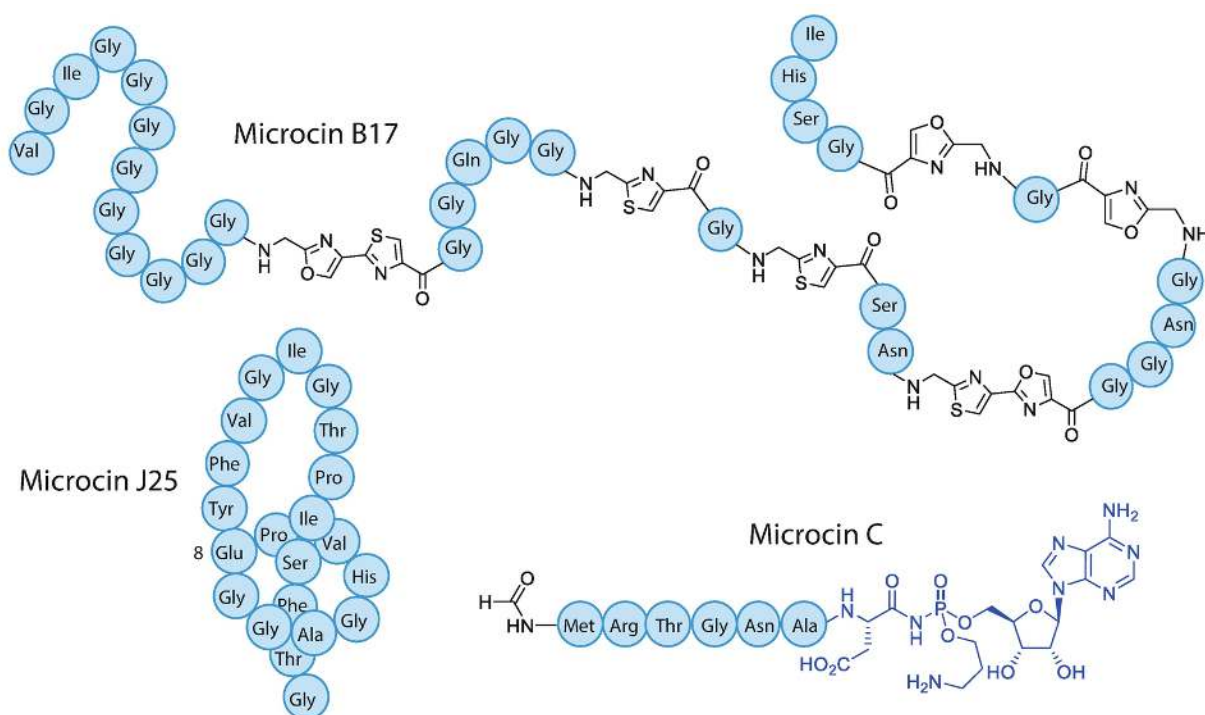


Fig. 22.4 Structures of representative microcins/TOMMs produced by members of the human gut microbiota: MccB17, MccJ25, and MccC (the proteolytically released aspartyl adenylate responsible for the antibacterial activity of MccC is shown in blue)

The short backbone of MccC, only consisting of seven amino acids, remains largely unmodified, with structural alterations solely occurring at the peptide *N*- and *C*-termini. The *N*-terminal methionine unit gets *N*-formylated, while the *C*-terminal aspartate is modified by attachment of adenosine monophosphate via an *N*-acyl phosphoramidate (Fig. [22.4](#)) (Guijarro et al., [1995](#)). The phosphate group is further linked to 1,3-propanolamine by esterification. Importantly, this *C*-terminal structural modification is responsible for the antibacterial potential of MccC.

Cleavage of the peptide bond between the alanine and aspartate moieties in MccC catalyzed by aminopeptidases of the target organisms leads to the release of an aspartyl adenylate (Fig. [22.4](#), labelled in dark blue) analog. This adenylate serves as a translational inhibitor of protein biosynthesis by targeting aspartyl tRNA synthetase (Metlitskaya et al., [2006](#); Kazakov et al., [2008](#)). MccC thereby inhibits a broad range of enterobacteria.

MccJ25 is structurally characterized by intramolecular cyclization of the *N*-terminal amino function to the acid side-chain of Glu at position 8 (Fig. [22.4](#)) (Rosengren et al., [2003](#); Wilson et al., [2003](#); Salomón & Farías, [1992](#); Ji et al., [2023](#)). The *C*-terminal peptide chain is funneled through the resulting macrocycle and this arrangement is stably maintained by steric interactions, leading to the prototypical lasso-type structure. MccJ25 inhibits protein transcription by targeting RNA polymerase and was shown to be active against *Escherichia* and *Salmonella* in the low nM range (Semenova et al., [2005](#)).

Class IIa microcins are antibiotic peptides that lack post-translational modification beyond proteolytic removal of the RiPP leader peptide. Examples include MccL, MccV (initially termed colicin V), and MccN (also named Mcc 24 or colicin 23) (Duquesne et al., [2007a](#); Arnison et al., [2013](#)). MccL, for instance, was shown to inhibit *Escherichia*, *Pseudomonas*, *Salmonella*, and *Shigella* sp. (Pons et al., [2004](#)), likely by targeting the cytoplasmic membrane (Morin et al., [2011](#)). Class IIb microcins, including MccE492, MccM, MccH47, and MccI47, are characterized by a serine-rich *C*-terminal tail. The tail can be further functionalized by linkage of its terminal serine to a *C*-glycosylated siderophore (intact, linearized, or further modified, e.g., derived from enterobactin) via an ester bond to the sugar moiety (Duquesne et al., [2007a](#); Arnison et al., [2013](#)). For example, MccH47 has potent activity against *Enterobacteriaceae*, such as *Escherichia*, *Salmonella*, and *Shigella* sp., including multi-drug resistant strains (Palmer et al., [2020](#); Laviña et al., [1990](#)).

Lantipeptides are a large family of RiPPs that are defined by the presence of intramolecular thioether bonds at lanthionine (Lan) and

methyllanthionine (MeLan) that lead to cyclic structural features (Arnison et al., [2013](#)). They can be subdivided into different classes according to the enzymatic machinery employed during thioether bond installation. The prototypical member of class I lantipeptides is nisin A (Fig. [22.5](#)), a peptide produced by lactic streptococci that has been structurally, biosynthetically, and functionally investigated for approx. one century and has, i.e., been applied as a food preservative due to its strong antibacterial activity, e.g., against *Bacillus cereus*, *Listeria monocytogenes*, enterococci, staphylococci, and streptococci (Des Field et al., [2023](#)). During peptide maturation, the precursor peptide of nisin A, NisA, is initially processed by a membrane-associated dehydratase NisB that transforms most serine and threonine residues into dehydroalanine (Dha) and dehydrobutyrine (Dhb), respectively (Fig. [22.5](#)) (Garg et al., [2013](#)). This installs electrophilic reactivity at these building blocks that is subsequently utilized by the cyclase NisC, catalyzing nucleophilic addition of the terminal thiols of the cysteine residues in the peptide backbone (Koponen et al., [2002](#); Li & van der Donk, [2007](#)). This leads to the formation of five macrocycles with Lan (addition of cysteine to Dha) and MeLan (cysteine to Dhb) units. Final transport by NisT (Kuipers et al., [2004](#)) and proteolytic cleavage of the leader sequence by NisP ultimately give nisin A (Lagedroste et al., [2017](#); Montalbán-López et al., [2018](#)). In the context of the gut microbiome, a novel nisin-type lantipeptide, nisin O (Fig. [22.5](#)), was identified from the dominant human gut bacterium *Blautia obeum* A2-162 and shown to possess antibiotic activity against *C. perfringens*, *C. difficile*, and *Lactobacillus lactis* (Hatzioanou et al., [2017](#)). Interestingly, the BGC encoding nisin O does not contain a protease for leader-peptide removal. A recent study showed that nisin-O-like BGCs can not only be found in *Blautia* sp., but also in *Dorea* and *Ruminococcus* sp. (Scadden et al., [2025](#)). All BGCs were shown to contain two bacterial two-component response regulator-histidine kinase systems and genes involved in transposition, leading to the conclusion of their distribution across species by genetic transfer. Interestingly, the leader peptide was shown to be readily cleaved by trypsin, suggesting the loss of a cognate

protease by negative selection as a consequence of the high trypsin concentrations in the gut. In addition, nisin H (Fig. 22.5) was recently identified from the porcine-gut-derived strain *Streptococcus hyointestinalis* DPC6484 and shown to possess a broad antibiotic activity profile against Gram-positive bacteria (O'Connor et al., 2015; Reiners et al., 2020).

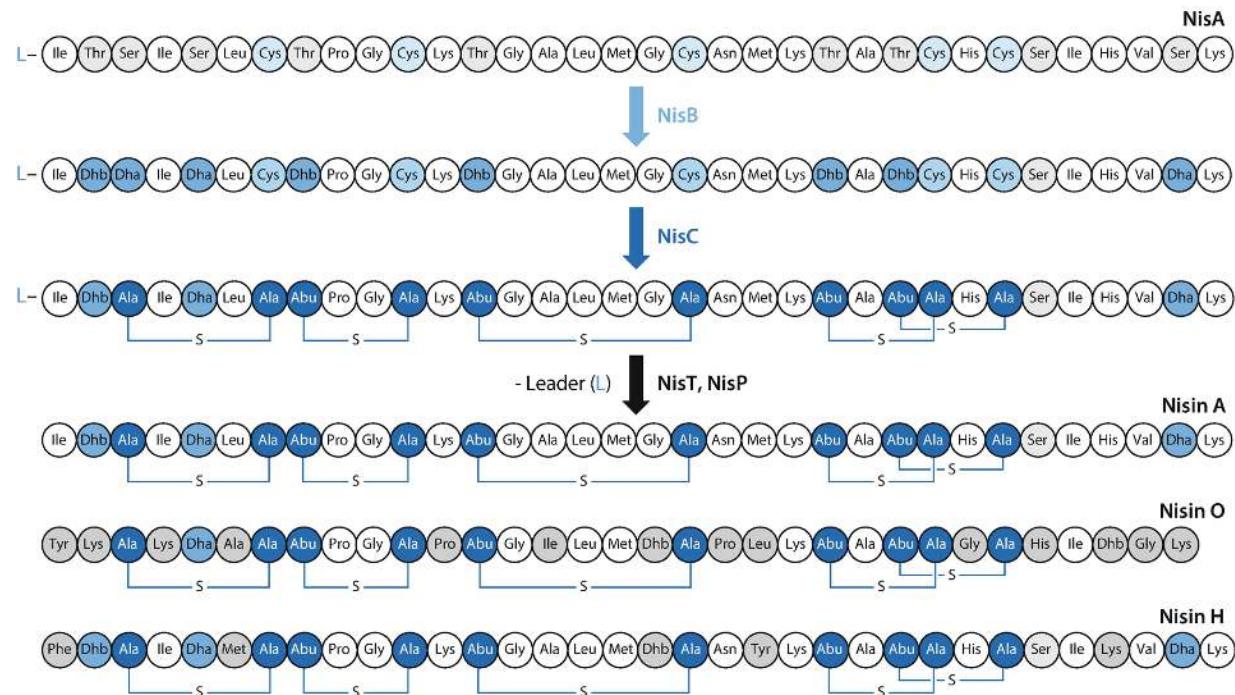


Fig. 22.5 Peptide maturation in nisin biosynthesis is exemplarily shown for nisin A: the precursor peptide NisA is processed by the dehydratase NisB, and the cyclase NisC, followed by export (NisT) and proteolytic cleavage of the leader peptide sequence L (NisP) to give nisin A. Putative structures of nisin O and nisin H are shown below. Ser/Thr residues are labelled in light grey, Cys residues in light blue. Structural changes introduced by NisB and thioether cross-links installed by NisC are highlighted in blue and dark blue, respectively. Amino acid changes in nisin O and H compared to nisin A are labelled in dark grey

Sactipeptides are a relatively new and growing family of RiPPs that is characterized by the presence of cross-links between the thiol function of cysteines and the α -carbon atoms of other amino acids present in the peptide backbone (Chen et al., 2021). The cross-link is installed by action of radical *S*-adenosyl methionine (rSAM) enzymes (Benjdia et al., 2016; Hudson et al., 2019). The first member of this class to be discovered was subtilisin A (Fig. 22.6) from *Bacillus subtilis*

(Babasaki et al., [1985](#); Zheng et al., [1999](#); Kawulka et al., [2003](#)), with many other congeners identified from *Bacillus* sp. (e.g., sporulation killing factor SKF, thuricins, huazacins) (Chen et al., [2021](#)).

Ruminococcus gnavus E1, isolated from human feces, produces the two RiPPs ruminococcins A (RumA) and C1 (RumC1). As RumA was structurally characterized as a lantipeptide (Dabard et al., [2001](#)), RumC1 was initially assumed to also belong to this class (Crosthwaite et al., [2011](#); Pujol et al., [2011](#)). However, later research unambiguously revealed RumC1 to belong to the sactipeptide class containing four α -C, S-cross links (Fig. [22.6](#)) (Balty et al., [2019](#); Chiumento et al., [2019](#); Kawulka et al., [2003](#)). Overall, five isoforms, RumC1-C5, are currently known to be encoded by producing strains (Pujol et al., [2011](#)), all of which possess potent and structure-dependent antibiotic activity, predominantly against a range of Gram-positive bacteria, with limited activity of some isoforms against Gram-negative bacteria (*Vibrio alginolyticus*, *E. coli*) (Shamseddine et al., [2023](#)). Further, structurally unique sactipeptides from other human commensals and pathogens have since been identified, including streptosactin from *Streptococcus thermophilus* (Bushin et al., [2020](#)) and suisactin from *Streptococcus suis* (Caruso & Seyedsayamdost, [2021](#)). Related to the sactipeptides are so-called SCIFF (six cysteine in 45 residues) peptides, initially discovered in silico in *Clostridia* (Haft & Basu, [2011](#)). Cross-links in these peptides can be established between cysteine and β - or γ -C atoms of backbone amino acids, with currently identified examples being freyrasin (β -C,S) from *Paenibacillus polymyxa* (Precord et al., [2019](#)) and thermocellin from *Clostridium thermocellum* (γ -C,S) (Grove et al., [2017](#)). Related SCIFF-encoding BGCs are also widespread in gut microbes, with the encoded RiPPs awaiting structural and functional characterization (Wylensek et al., [2020](#)).

not restricted to (proteinogenic) amino acids, paving the way for the direct incorporation of hundreds of different building blocks (Walsh et al., [2013](#)). The tremendous structural diversity thus addressable is even further expanded. During peptide assembly, optional catalytic domains within the NRPS module structure can be present, allowing, e.g., epimerization (E domain), methylation (MT domain), or cyclization (Cy domain) reactions. NPR biosynthesis is usually terminated by the action of a thioesterase domain (TE), which can catalyze simple hydrolysis, macro lactone- or lactamization reactions. Upon completion of NRP biosynthesis, so-called tailoring enzymes can introduce further changes, either on the still NRPS-bound or on the already released nascent peptide. Typical tailoring reactions include oxygenation and halogenation, glycosylation and prenylation, and peptide backbone cross-linking reactions. All these features combined lead to infinite structural diversity biosynthetically accessible by NRPS biosynthetic logic (Süssmuth & Mainz, [2017](#)).

Many siderophores are assembled by NPRSs (Crosa & Walsh, [2002](#)). The resulting NRPs commonly contain multiple structural features that enable efficient chelating of iron and/or other metal ions, including phenols, catechols, hydroxamates as well as carboxylic and heterocyclic moieties (Schalk, [2025](#)). Efficient iron interception provides survival advantages in competitive microbial environments, and siderophores are thus highly important factors for successful niche-colonization and/or pathogenicity (Miethke & Marahiel, [2007](#); Caza & Kronstad, [2013](#); Kramer et al., [2020](#)). Representative examples with relevance to the gut microbiome include enterobactin from *E. coli* (Raymond et al., [2003](#)), pyochelin from *Pseudomonas aeruginosa* (Cox et al., [1981](#)), and yersiniabactin from *Yersinia pestis* (Fig. [22.7](#)) (Perry & Fetherston, [2011](#)).

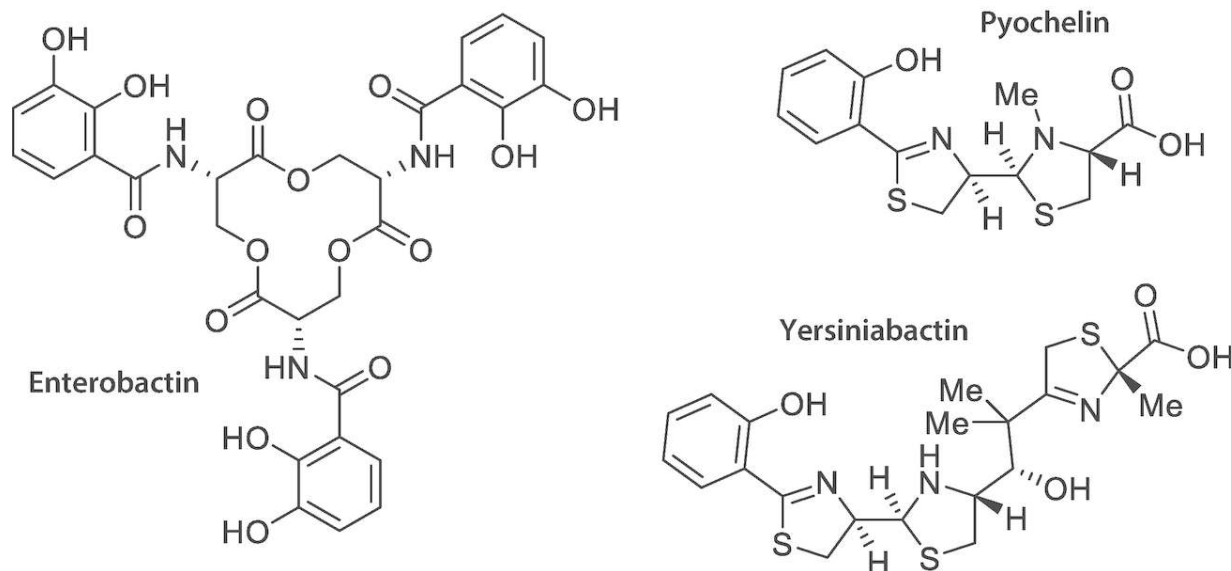


Fig. 22.7 Structures of representative examples of NRPS-derived siderophores: enterobactin, pyochelin, and yersiniabactin

While the number of known compounds assembled by NRPS machinery from the gut microbiome beyond siderophores is limited, a few representative examples nicely showcase their structural and functional breadth. For instance, broadly distributed dimodular NRPS pathways present in approx. 90% of the National Institutes of Health human microbiome stool samples encode a set of interesting small peptide products (Fig. [22.8a](#)) (Guo et al., [2017](#)). The encoded chemistry was primarily uncovered by functional expression of several such BGCs in *E. coli* as the recombinant host. The two A domains in the dimodular NRPS activate and load Val and Phe, respectively, onto two T domains. Peptide-bond formation is catalyzed by the central C domain (light blue circle and arrows), followed by reductive release by action of an R domain (blue circle and arrows) to give an intermediate dipeptide aldehyde. Spontaneous cyclization and oxygenation by molecular oxygen ultimately deliver phevalin. Depending on the NRPS system investigated, the amino-acid composition of these compounds is variable, including the depicted Val-Phe- as well as Met-Phe-, Leu-Phe-, and Phe-Phe-derived dipeptide aldehydes. These intermediates were shown to possess potent and selective inhibitory activity against several cathepsin proteases of the human cell proteome. Importantly,

the respective gut NRPS BGCs are transcribed under conditions of host colonization (Guo et al., [2017](#)). Similar NRPS BGCs producing pyrazinones related to phevalin had previously been characterized from *Staphylococcus* sp. (Zimmermann & Fischbach, [2010](#); Wyatt et al., [2010](#)), suggesting a broader role of these and potentially the intermediate dipeptide aldehydes in microbe-microbe and microbe-host interactions.

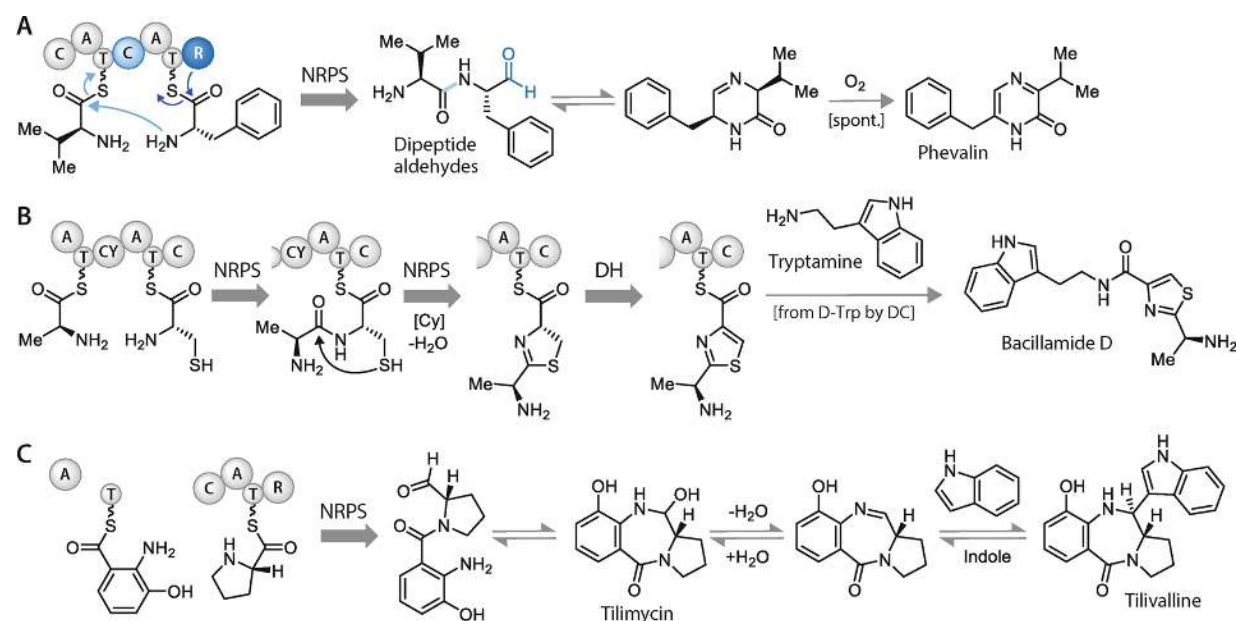


Fig. 22.8 Structures and biosyntheses of NRPS-derived NPs isolated from bacteria from the gut. NRPS domain architecture is shown using filled circles with adenylation domain (A), thiolation domain (T), condensation domain (C), reductive domain (R), and cyclization domain (Cy). (a) Biosynthesis of dipeptide aldehydes and phevalin. Peptide bond formation is catalyzed by the central C domain (light blue circle and arrows), followed by reductive release by the R domain (blue circle and arrows) (b) Biosynthesis of bacillamide D. The heterocyclic structural element is formed by action of the Cy domain followed by dehydratase (DH) mediated oxidation and tryptamine-induced offloading. (c) Biosynthesis of tilimycin and its non-enzymatic conversion into tilivalline

Bacillamide D was recently identified in a *Bacillus cereus* gut isolate by heterologous expression of a cryptic NRPS BGC (Hohmann et al., [2024](#)). Bacillamide D is likewise assembled by a dimodular NRPS (Fig. [22.8b](#)). The two A domains attach Ala and Cys to the T domains (Zhang et al., [2018](#)), with subsequent peptide-bond formation and heterocyclization catalyzed by the central Cy domain (Zhang et al.,

[2019](#)). This delivers an intermediate thiazoline, which is further oxidized by a dehydratase enzyme (DH) likewise encoded in the bacillamide BGC. The resulting thiazole intermediate is released from the NRPS by nucleophilic attack of the free amino group of tryptamine, thereby delivering bacillamide D. Tryptamine derives of decarboxylation of Trp by a pathway-specific decarboxylase (DC). Evaluation of the bioactivity profile of bacillamide D revealed potent cytotoxicity and inhibition of eukaryotic cell migration, establishing the compound as a selective cytotoxin affecting mammalian cells (Hohmann et al., [2024](#)). Interestingly, the food-poisoning pathogen *Bacillus cereus* also produces the NRPS-derived dodecadepsipeptide toxin cereulide (Ehling-Schulz et al., [2006](#)), which is responsible for the emetic effects caused by this bacterium (Agata et al., [1995](#)).

Klebsiella oxytoca is present in the gut of up to 10% of healthy human individuals and is thought to be able to cause antibiotic-associated hemorrhagic colitis (AAHC) upon antibiotic therapy (Högenauer et al., [2006](#)). This effect was attributed to the production of an enterotoxin NP, the pyrrolobenzodiazepine tilivalline (Schneditz et al., [2014](#)). Tilivalline is indeed the non-enzymatic product of the NRPS-derived tilimycin (Dornisch et al., [2017](#)). During biosynthesis, the two A domains attach Pro and 3-hydroxyanthranilic acid to the two T domains, with the C domain catalyzing amide bond formation followed by reductive release mediated by the R domain. The resulting aldehyde can undergo spontaneous non-enzymatic cyclization to tilimycin. Elimination of water from the latter delivers an intermediate imine, which can be attacked by indole to yield tilivalline (Fig. [22.8c](#)).

22.2.3 NPs Involving Polyketide Synthase (PKS)

Biosynthetic Logic

The modular nature of NRPSs also allows integration of type I polyketide synthase (PKS) biosynthetic elements. These PKS systems are likewise organized in a modular, assembly-line-type manner. Rather than amino acid building blocks, PKSs employ malonate units for the elongation of the nascent NP structure (Hertweck, [2009](#); Soohoo et al.,

[2024](#); Staunton & Weissman, [2001](#)). The malonate building blocks are also covalently attached to T domains, with building block activation and transfer being catalyzed by acyl transferase domains (AT). In contrast to C-domain catalyzed peptide bond formation, ketosynthase domains (KS) in PKS systems catalyze C,C-bond forming Claisen condensation reactions enabled by decarboxylation of the malonate units. This leads to the incorporation of acetyl units in each elongation step, reminiscent of fatty acid biosynthesis (Beld et al., [2015](#)). Diverging from typical fatty acid biosynthesis, the keto function of the incorporated acetyl units is not necessarily fully reduced after each elongation step. Depending on the presence or absence of reducing domains (ketoreductase KR, dehydratase DH, enoyl-reductase ER) in the PKS module, the oxidative state in each building block can be controlled during each elongation, thus contributing to the structural diversity of PKS products.

The combination of PKS and NRPS biosynthetic logic gives rise to tremendous structural and functional NP diversity. In the context of the human gut, mixed PKS/NRPS biosynthetic machinery is responsible for the formation of the colibactins. Since the first discovery of *E. coli*-induced DNA double-strand breaks in eukaryotic cells caused by strains harboring the so-called *pks* island in 2006 (Nougayrède et al., [2006](#)), the colibactins have been extensively researched, leading to countless exciting findings concerning the structure and biosynthesis of colibactins as well as their diverse biological functions and mechanisms. While the resulting impressive body of primary research goes well beyond the scope of this chapter, there are several excellent reviews that extensively cover this topic from different angles (Tang et al., [2022](#); Arthur, [2020](#); Addington et al., [2024](#); Azour et al., [2024](#); Zhang & Sun, [2024](#); Dougherty & Jobin, [2021](#); Bode, [2015](#); Mousa, [2022](#); Shine & Crawford, [2021](#); Balskus, [2015](#); Hirayama et al., [2022](#); Chagneau et al., [2022](#)). The most important congener of this compound family, colibactin, is involved in DNA alkylation, leading to DNA (interstrand) cross-linking (Wilson et al., [2019](#); Bossuet-Greif et al., [2018](#); Xue et al., [2019](#)). The biosynthetic assembly of colibactin is highly complex and

still not entirely elucidated (Tang et al., [2022](#); Shine & Crawford, [2021](#)). It involves 3 NRPS, 3 PKS, and 2 PKS/NRPS hybrid core biosynthetic enzymes, along with 11 accessory and tailoring enzymes. The interplay of these enzymes initially leads to the formation of the heterodimeric precolibactin 1491, which, upon transport into the periplasm, is proteolytically processed, leading to the removal of two *N*-myristoyl-D-asparagine units and triggering a heterocyclization cascade to aminoketone colibactin. The latter can be non-enzymatically oxidized and hydrolyzed to deliver colibactin. The two cyclopropyl substructures in colibactins serve as electrophiles that allow addition of biogenic nucleophiles and ring-opening, enabling DNA addition and cross linking (Wilson et al., [2019](#); Bossuet-Greif et al., [2018](#); Xue et al., [2019](#)) (Fig. [22.9](#)).

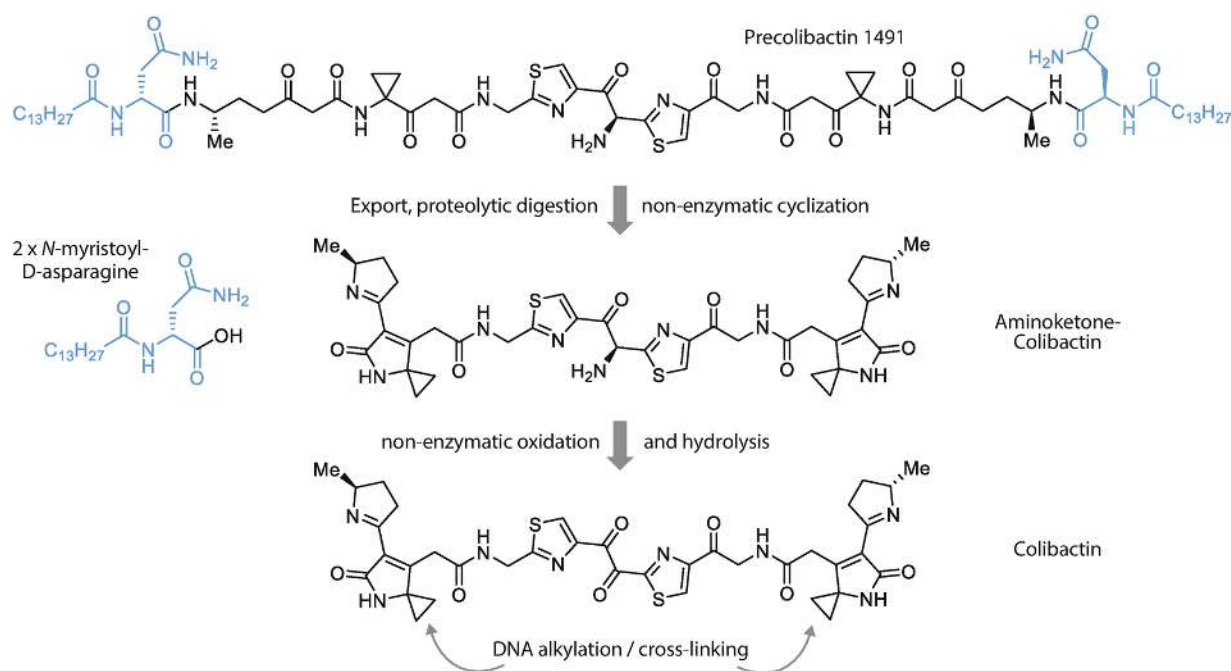


Fig. 22.9 Biosynthetic processing of precolibactin 1491 to colibactin

Apart from colibactins that are biosynthetically assembled by the interplay of PKS and NRPS biosynthetic systems, the first metabolites from bacterial type II PKSs (Wang et al., [2020](#)) have been identified by metagenomics mining and functional BGC expression (Sugimoto et al., [2019](#)). Examples include wexrubicin encoded in the genome of *Blautia*

wexlerae DSM 19850 and the metamyxins (the BGC encoding the latter identified from oral metagenomic data). Wexrubicin belongs to the class of anthracyclic polyketides, structurally related to doxorubicin or daunorubicin, clinically applied anticancer drugs. However, no cytotoxic effects were found for wexrubicin (Sugimoto et al., [2019](#)).

22.3 Conclusions

The discovery and characterization of functional metabolites from the human gut microbiota is an evolving field with significant implications for human health. Despite the progress made, several challenges remain. One major challenge is the complexity of the gut microbiome and its dynamic nature, which makes it difficult to fully understand microbe–microbe and microbe–host interactions at the organism and metabolic level and the impact of this interplay on gut microbial composition, human health, disease emergence, and progression. As a consequence, novel functional roles are continued to be discovered even for long-known and well-studied metabolites such as the SCFAs. For the limited number of specialized metabolites currently known from the gut, virtually nothing is known about their impact on the gut microbiome and, consequently, on the host, aside from very few exceptions such as the colibactins. And even for this heavily researched metabolite family, new insights are constantly emerging: a very recent study directly linked the rising incidence of colorectal cancer in young individuals under the age of 50 to early exposure to colibactin (Díaz-Gay et al. [2025](#)), clearly underpinning the importance of understanding gut microbiome specialized metabolism in general. It is therefore of utmost importance to increase our efforts towards the discovery and in-depth functional elucidation of gut-derived functional metabolites.

Publication Bibliography

Addington, E., Sandalli, S., & Roe, A. J. (2024). Current understandings of colibactin regulation. *Microbiology (Reading, England)*, 170(2). <https://doi.org/10.1099/mic.0.001427>

Agata, N., Ohta, M., Mori, M., & Isobe, M. (1995). A novel dodecadepsipeptide, cereulide, is an emetic toxin of *Bacillus cereus*. *FEMS Microbiology Letters*, 129(1), 17–20. [https://doi.org/10.1016/0378-1097\(95\)00119-P](https://doi.org/10.1016/0378-1097(95)00119-P) [PubMed]

Arnison, P. G., Bibb, M. J., Bierbaum, G., Bowers, A. A., Bugni, T. S., Bulaj, G., et al. (2013). Ribosomally synthesized and post-translationally modified peptide natural products: Overview and recommendations for a universal nomenclature. *Natural Product Reports*, 30(1), 108–160. <https://doi.org/10.1039/c2np20085f> [PubMed]

Arthur, J. C. (2020). Microbiota and colorectal cancer: Colibactin makes its mark. *Nature Reviews. Gastroenterology & Hepatology*, 17(6), 317–318. <https://doi.org/10.1038/s41575-020-0303-y>

Asensio, C., & Pérez-Díaz, J. C. (1976). A new family of low molecular weight antibiotics from enterobacteria. *Biochemical and Biophysical Research Communications*, 69(1), 7–14. [https://doi.org/10.1016/S0006-291X\(76\)80264-1](https://doi.org/10.1016/S0006-291X(76)80264-1) [PubMed]

Atanasov, A. G., Zotchev, S. B., Dirsch, V. M., & Supuran, C. T. (2021). Natural products in drug discovery: Advances and opportunities. *Nature Reviews. Drug Discovery*, 20(3), 200–216. <https://doi.org/10.1038/s41573-020-00114-z> [PubMed]

Azour, A., Al-Bayssari, C., Rolain, J.-M. (2024). Colibactin: A comprehensive review of its mechanisms of action, impact on gut homeostasis, and association with cancer.

Babasaki, K., Takao, T., Shimonishi, Y., & Kurahashi, K. (1985). Subtilosin A, a new antibiotic peptide produced by *Bacillus subtilis* 168: Isolation, structural analysis, and biogenesis. *Journal of Biochemistry*, 98(3), 585–603. <https://doi.org/10.1093/oxfordjournals.jbchem.a135315> [PubMed]

Balskus, E. P. (2015). Colibactin: Understanding an elusive gut bacterial genotoxin. *Natural Product Reports*, 32(11), 1534–1540. <https://doi.org/10.1039/c5np00091b> [PubMed]

Balty, C., Guillot, A., Fradale, L., Brewée, C., Boulay, M., Kubiak, X., et al. (2019). Ruminococcin C, an anti-clostridial sactipeptide produced by a prominent member of the human microbiota *Ruminococcus gnavus*. *The Journal of Biological Chemistry*, 294(40), 14512–14525. <https://doi.org/10.1074/jbc.RA119.009416> [PubMed]

Baquero, F., Lanza, V. F., Baquero, M.-R., Del Campo, R., & Bravo-Vázquez, D. A. (2019). Microcins in enterobacteriaceae: Peptide antimicrobials in the eco-active intestinal chemosphere. *Frontiers in Microbiology*, 10, 2261. <https://doi.org/10.3389/fmicb.2019.02261> [PubMed]

Barker, H. A. (1981). Amino acid degradation by anaerobic bacteria. *Annual Review of Biochemistry*, 50, 23–40. <https://doi.org/10.1146/annurev.bi.50.070181.000323>

[[PubMed](#)]

Barker, H. A., & Taha, S. M. (1942). Clostridium kluyverii, an organism concerned in the formation of caproic acid from ethyl alcohol. *Journal of Bacteriology*, 43(3), 347–363. <https://doi.org/10.1128/jb.43.3.347-363.1942>

[[PubMed](#)]

Barker, H. A., D'Ari, L., & Kahn, J. (1987). Enzymatic reactions in the degradation of 5-aminovaleate by Clostridium aminovaleicum. *The Journal of Biological Chemistry*, 262(19), 8994–9003.

[[https://doi.org/10.1016/S0021-9258\(18\)48036-2](https://doi.org/10.1016/S0021-9258(18)48036-2)][[PubMed](#)]

Beld, J., Lee, D. J., & Burkart, M. D. (2015). Fatty acid biosynthesis revisited: Structure elucidation and metabolic engineering. *Molecular BioSystems*, 11(1), 38–59. <https://doi.org/10.1039/c4mb00443d>

[[PubMed](#)]

Belenguer, A., Duncan, S. H., Calder, A. G., Holtrop, G., Louis, P., Lobley, G. E., & Flint, H. J. (2006). Two routes of metabolic cross-feeding between Bifidobacterium adolescentis and butyrate-producing anaerobes from the human gut. *Applied and Environmental Microbiology*, 72(5), 3593–3599. <https://doi.org/10.1128/AEM.72.5.3593-3599.2006>

[[PubMed](#)]

Benjdia, A., Guillot, A., Lefranc, B., Vaudry, H., Leprince, J., & Berteau, O. (2016). Thioether bond formation by SPASM domain radical SAM enzymes: C α H-atom abstraction in subtilisin A biosynthesis. *Chemical Communications (Cambridge, England)*, 52(37), 6249–6252. <https://doi.org/10.1039/c6cc01317a>

[[PubMed](#)]

Bode, H. B. (2015). The microbes inside us and the race for Colibactin. *Angewandte Chemie (International ed. in English)*, 54(36), 10408–10411. <https://doi.org/10.1002/anie.201505341>

[[PubMed](#)]

Bossuet-Greif, N., Vignard, J., Taieb, F., Mirey, G., Dubois, D., Petit, C., et al. (2018). The colibactin genotoxin generates DNA interstrand cross-links in infected cells. *mBio*, 9(2). <https://doi.org/10.1128/mbio.02393-17>

Buckel, W. (2001). Unusual enzymes involved in five pathways of glutamate fermentation. *Applied Microbiology and Biotechnology*, 57(3), 263–273.

[<https://doi.org/10.1007/s002530100773>][[PubMed](#)]

Buckel, W., Janssen, P. H., Schuhmann, A., Eikmanns, U., Messner, P., Sleytr, U., & Liesack, W. (1994). Clostridium viride sp. nov., a strictly anaerobic bacterium using 5-aminovaleate as growth substrate, previously assigned to Clostridium aminovaleicum. *Archives of Microbiology*, 162(6), 387–394. <https://doi.org/10.1007/BF00282102>

Bushin, L. B., Covington, B. C., Rued, B. E., Federle, M. J., & Seyedsayamdost, M. R. (2020). Discovery and biosynthesis of streptosactin, a sactipeptide with an alternative topology encoded by

commensal bacteria in the human microbiome. *Journal of the American Chemical Society*, 142(38), 16265–16275. <https://doi.org/10.1021/jacs.0c05546>
[PubMed]

Butkovich, L. V., Vining, O. B., & O'Malley, M. A. (2025). New approaches to secondary metabolite discovery from anaerobic gut microbes. *Applied Microbiology and Biotechnology*, 109(1), 12. <https://doi.org/10.1007/s00253-024-13393-y>
[PubMed]

Caruso, A., & Seyedsayamdost, M. R. (2021). Radical SAM enzyme QmpB installs two 9-membered ring sactonine macrocycles during biogenesis of a ribosomal peptide natural product. *The Journal of Organic Chemistry*, 86(16), 11284–11289. <https://doi.org/10.1021/acs.joc.1c01507>
[PubMed]

Caza, M., & Kronstad, J. W. (2013). Shared and distinct mechanisms of iron acquisition by bacterial and fungal pathogens of humans. *Frontiers in Cellular and Infection Microbiology*, 3, 80. <https://doi.org/10.3389/fcimb.2013.00080>
[PubMed]

Chagneau, C. V., Payros, D., Tang-Fichaux, M., Auvray, F., Nougayrède, J.-P., & Oswald, E. (2022). The pks Island: A bacterial Swiss army knife? Colibactin: Beyond DNA damage and cancer. *Trends in Microbiology*, 30(12), 1146–1159. <https://doi.org/10.1016/j.tim.2022.05.010>
[PubMed]

Chen, Y., Wang, J., Li, G., Yang, Y., & Ding, W. (2021). Current advancements in sactipeptide natural products. *Frontiers in Chemistry*, 9, 595991. <https://doi.org/10.3389/fchem.2021.595991>
[PubMed]

Chiumento, S., Roblin, C., Kieffer-Jaquinod, S., Tachon, S., Leprêtre, C., Basset, C., et al. (2019). Ruminococcin C, a promising antibiotic produced by a human gut symbiont. *Science Advances*, 5(9), eaaw9969. <https://doi.org/10.1126/sciadv.aaw9969>
[PubMed]

Choi, K., Jeon, B. S., Kim, B.-C., Oh, M.-K., Um, Y., & Sang, B.-I. (2013). In situ biphasic extractive fermentation for hexanoic acid production from sucrose by *Megasphaera elsdenii* NCIMB 702410. *Applied Biochemistry and Biotechnology*, 171(5), 1094–1107. <https://doi.org/10.1007/s12010-013-0310-3>
[PubMed]

Cox, C. D., Rinehart, K. L., Moore, M. L., & Cook, J. C. (1981). Pyochelin: Novel structure of an iron-chelating growth promoter for *Pseudomonas aeruginosa*. *Proceedings of the National Academy of Sciences of the United States of America*, 78(7), 4256–4260. <https://doi.org/10.1073/pnas.78.7.4256>
[PubMed]

Crosa, J. H., & Walsh, C. T. (2002). Genetics and assembly line enzymology of siderophore biosynthesis in bacteria. *Microbiology and Molecular Biology Reviews: MMBR*, 66(2), 223–249. <https://doi.org/10.1128/MMBR.66.2.223-249.2002>
[PubMed]

- Crost, E. H., Ajandouz, E. H., Villard, C., Geraert, P. A., Puigserver, A., & Fons, M. (2011). Ruminococcin C, a new anti-Clostridium perfringens bacteriocin produced in the gut by the commensal bacterium Ruminococcus gnavus E1. *Biochimie*, 93(9), 1487–1494. <https://doi.org/10.1016/j.biochi.2011.05.001> [PubMed]
- Cummings, J. H., Pomare, E. W., Branch, W. J., Naylor, C. P., & Macfarlane, G. T. (1987). Short chain fatty acids in human large intestine, portal, hepatic and venous blood. *Gut*, 28(10), 1221–1227. <https://doi.org/10.1136/gut.28.10.1221> [PubMed]
- Dabard, J., Bridonneau, C., Phillipe, C., Anglade, P., Molle, D., Nardi, M., et al. (2001). Ruminococcin A, a new lantibiotic produced by a Ruminococcus gnavus strain isolated from human feces. *Applied and Environmental Microbiology*, 67(9), 4111–4118. <https://doi.org/10.1128/AEM.67.9.4111-4118.2001> [PubMed]
- Dai, Z.-L., Wu, G., & Zhu, W.-Y. (2011). Amino acid metabolism in intestinal bacteria: Links between gut ecology and host health. *Frontiers in Bioscience (Landmark edition)*, 16(5), 1768–1786. <https://doi.org/10.2741/3820> [PubMed]
- Dai, H., Han, J., Wang, T., Yin, W.-B., Chen, Y., & Liu, H. (2023). Recent advances in gut microbiota-associated natural products: Structures, bioactivities, and mechanisms. *Natural Product Reports*, 40(6), 1078–1093. <https://doi.org/10.1039/d2np00075j> [PubMed]
- Díaz-Gay, M., dos Santos, W., Moody, S. et al. (2025). Geographic and age variations in mutational processes in colorectal cancer. *Nature*, 643, 230–240. <https://doi.org/10.1038/s41586-025-09025-8>
- Díaz-Pérez, A. L., Díaz-Pérez, C., & Campos-García, J. (2016). Bacterial l-leucine catabolism as a source of secondary metabolites. *Reviews in Environmental Science and Biotechnology*, 15(1), 1–29. <https://doi.org/10.1007/s11157-015-9385-3>
- Diez-Gonzalez, F., Bond, D. R., Jennings, E., & Russell, J. B. (1999). Alternative schemes of butyrate production in *Butyrivibrio fibrisolvens* and their relationship to acetate utilization, lactate production, and phylogeny. *Archives of Microbiology*, 171(5), 324–330. <https://doi.org/10.1007/s002030050717> [PubMed]
- Dodd, D., Spitzer, M. H., Van Treuren, W., Merrill, B. D., Hryckowian, A. J., Higginbottom, S. K., Le, A., et al. (2017). A gut bacterial pathway metabolizes aromatic amino acids into nine circulating metabolites. *Nature*, 551(7682), 648–652. <https://doi.org/10.1038/nature24661> [PubMed]
- Donia, M. S., & Fischbach, M. A. (2015). Human microbiota. Small molecules from the human microbiota. *Science*, 349(6246), 1254766. <https://doi.org/10.1126/science.1254766> [PubMed]

Donia, M. S., Cimerancic, P., Schulze, C. J., Wieland Brown, L. C., Martin, J., Mitreva, M., et al. (2014). A systematic analysis of biosynthetic gene clusters in the human microbiome reveals a common family of antibiotics. *Cell*, 158(6), 1402–1414. <https://doi.org/10.1016/j.cell.2014.08.032>
[PubMed]

Dornisch, E., Pletz, J., Glabonjat, R. A., Martin, F., Lembacher-Fadum, C., Neger, M., et al. (2017). Biosynthesis of the enterotoxic pyrrolobenzodiazepine natural product tilivalline. *Angewandte Chemie (International ed. in English)*, 56(46), 14753–14757. <https://doi.org/10.1002/anie.201707737>
[PubMed]

Dougherty, M. W., & Jobin, C. (2021). Shining a light on colibactin biology. *Toxins*, 13(5). <https://doi.org/10.3390/toxins13050346>

Duncan, S. H., Barcenilla, A., Stewart, C. S., Pryde, S. E., & Flint, H. J. (2002). Acetate utilization and butyryl coenzyme A (CoA): Acetate-CoA transferase in butyrate-producing bacteria from the human large intestine. *Applied and Environmental Microbiology*, 68(10), 5186–5190. <https://doi.org/10.1128/AEM.68.10.5186-5190.2002>
[PubMed]

Duquesne, S., Destoumieux-Garzón, D., Peduzzi, J., & Rebuffat, S. (2007a). Microcins, gene-encoded antibacterial peptides from enterobacteria. *Natural Product Reports*, 24(4), 708–734. <https://doi.org/10.1039/b516237h>
[PubMed]

Duquesne, S., Petit, V., Peduzzi, J., & Rebuffat, S. (2007b). Structural and functional diversity of microcins, gene-encoded antibacterial peptides from enterobacteria. *Journal of Molecular Microbiology and Biotechnology*, 13(4), 200–209. <https://doi.org/10.1159/000104748>
[PubMed]

Ehling-Schulz, M., Fricker, M., Grallert, H., Rieck, P., Wagner, M., & Scherer, S. (2006). Cereulide synthetase gene cluster from emetic *Bacillus cereus*: Structure and location on a mega virulence plasmid related to *Bacillus anthracis* toxin plasmid pXO1. *BMC Microbiology*, 6, 20. <https://doi.org/10.1186/1471-2180-6-20>
[PubMed]

Elsden, S. R., & Hilton, M. G. (1978). Volatile acid production from threonine, valine, leucine and isoleucine by clostridia. *Archives of Microbiology*, 117(2), 165–172. <https://doi.org/10.1007/BF00402304>
[PubMed]

Field, D., de Ullivarri, F., Miguel, Ross, R. P., & Hill, C. (2023). After a century of nisin research—where are we now? *FEMS Microbiology Reviews*, 47(3). <https://doi.org/10.1093/femsre/fuad023>

Fothergill, J. C., & Guest, J. R. (1977). Catabolism of L-lysine by *Pseudomonas aeruginosa*. *Journal of General Microbiology*, 99(1), 139–155. <https://doi.org/10.1099/00221287-99-1-139>
[PubMed]

- Frolova, M. S., Suvorova, I. A., Iablokov, S. N., Petrov, S. N., & Rodionov, D. A. (2022). Genomic reconstruction of short-chain fatty acid production by the human gut microbiota. *Frontiers in Molecular Biosciences*, 9, 949563. <https://doi.org/10.3389/fmolb.2022.949563> [PubMed]
- Ganesan, B., & Weimer, B. C. (2004). Role of aminotransferase IlvE in production of branched-chain fatty acids by *Lactococcus lactis* subsp. *lactis*. *Applied and Environmental Microbiology*, 70(1), 638–641. <https://doi.org/10.1128/AEM.70.1.638-641.2004> [PubMed]
- Garg, N., Salazar-Ocampo, L. M. A., & van der Donk, W. A. (2013). In vitro activity of the nisin dehydratase NisB. *Proceedings of the National Academy of Sciences of the United States of America*, 110(18), 7258–7263. <https://doi.org/10.1073/pnas.1222488110> [PubMed]
- Gonzalez, D. J., Lee, S. W., Hensler, M. E., Markley, A. L., Dahesh, S., Mitchell, D. A., et al. (2010). Clostridiolysin S, a post-translationally modified biotoxin from *Clostridium botulinum*. *The Journal of Biological Chemistry*, 285(36), 28220–28228. <https://doi.org/10.1074/jbc.M110.118554> [PubMed]
- Gonzalez-Garcia, R., McCubbin, T., Navone, L., Stowers, C., Nielsen, L., & Marcellin, E. (2017). Microbial propionic acid production. *Fermentation*, 3(2), 21. <https://doi.org/10.3390/fermentation3020021>
- Grove, T. L., Himes, P. M., Hwang, S., Yumerefendi, H., Bonanno, J. B., Kuhlman, B., et al. (2017). Structural insights into thioether bond formation in the biosynthesis of sactipeptides. *Journal of the American Chemical Society*, 139(34), 11734–11744. <https://doi.org/10.1021/jacs.7b01283> [PubMed]
- Guijarro, J. I., González-Pastor, J. E., Baleux, F., San Millán, J. L., Castilla, M. A., Rico, M., et al. (1995). Chemical structure and translation inhibition studies of the antibiotic microcin C7. *The Journal of Biological Chemistry*, 270(40), 23520–23532. <https://doi.org/10.1074/jbc.270.40.23520> [PubMed]
- Guo, C.-J., Chang, F.-Y., Wyche, T. P., Backus, K. M., Acker, T. M., Funabashi, M., et al. (2017). Discovery of reactive microbiota-derived metabolites that inhibit host proteases. *Cell*, 168(3), 517–526.e18. <https://doi.org/10.1016/j.cell.2016.12.021> [PubMed]
- Guo, C.-J., Allen, B. M., Hiam, K. J., Dodd, D., van Treuren, W., Higginbottom, S., et al. (2019). Depletion of microbiome-derived molecules in the host using *Clostridium* genetics. *Science (New York, N.Y.)*, 366(6471). <https://doi.org/10.1126/science.aav1282>
- Haft, D. H., & Basu, M. K. (2011). Biological systems discovery in silico: Radical S-adenosylmethionine protein families and their target peptides for posttranslational modification. *Journal of Bacteriology*, 193(11), 2745–2755. <https://doi.org/10.1128/JB.00040-11> [PubMed]
- Hatzioanou, D., Gherghisan-Filip, C., Saalbach, G., Horn, N., Wegmann, U., Duncan, S. H., et al.

(2017). Discovery of a novel lantibiotic nisin O from *Blautia obeum* A2-162, isolated from the human gastrointestinal tract. *Microbiology (Reading, England)*, 163(9), 1292–1305. <https://doi.org/10.1099/mic.0.000515>
[PubMed]

Heilbronner, S., Krismer, B., Brötz-Oesterhelt, H., & Peschel, A. (2021). The microbiome-shaping roles of bacteriocins. *Nature Reviews. Microbiology*, 19(11), 726–739. <https://doi.org/10.1038/s41579-021-00569-w>
[PubMed]

Herrmann, G., Jayamani, E., Mai, G., & Buckel, W. (2008). Energy conservation via electron-transferring flavoprotein in anaerobic bacteria. *Journal of Bacteriology*, 190(3), 784–791. <https://doi.org/10.1128/JB.01422-07>
[PubMed]

Hertweck, C. (2009). The biosynthetic logic of polyketide diversity. *Angewandte Chemie (International ed. in English)*, 48(26), 4688–4716. <https://doi.org/10.1002/anie.200806121>
[PubMed]

Hirayama, Y., Sato, M., & Watanabe, K. (2022). Advancing the biosynthetic and chemical understanding of the carcinogenic risk factor colibactin and its producers. *Biochemistry*, 61(24), 2782–2790. <https://doi.org/10.1021/acs.biochem.2c00229>
[PubMed]

Högenauer, C., Langner, C., Beubler, E., Lippe, I. T., Schicho, R., Gorkiewicz, G., et al. (2006). *Klebsiella oxytoca* as a causative organism of antibiotic-associated hemorrhagic colitis. *The New England Journal of Medicine*, 355(23), 2418–2426. <https://doi.org/10.1056/NEJMoa054765>
[PubMed]

Hohmann, M., Brunner, V., Johannes, W., Schum, D., Carroll, L. M., Liu, T., et al. (2024). Bacillamide D produced by *Bacillus cereus* from the mouse intestinal bacterial collection (miBC) is a potent cytotoxin in vitro. *Communications Biology*, 7(1), 655. <https://doi.org/10.1038/s42003-024-06208-3>
[PubMed]

Hudson, G. A., Burkhart, B. J., DiCaprio, A. J., Schwalen, C. J., Kille, B., Pogorelov, T. V., & Mitchell, D. A. (2019). Bioinformatic mapping of radical S-adenosylmethionine-dependent ribosomally synthesized and post-translationally modified peptides identifies new α , β , and γ -linked Thioether-containing peptides. *Journal of the American Chemical Society*, 141(20), 8228–8238. <https://doi.org/10.1021/jacs.9b01519>
[PubMed]

Iino, T., Mori, K., Tanaka, K., Suzuki, K.-I., & Harayama, S. (2007). *Oscillibacter valericigenes* gen. nov., sp. nov., a valerate-producing anaerobic bacterium isolated from the alimentary canal of a Japanese corbicula clam. *International Journal of Systematic and Evolutionary Microbiology*, 57(Pt 8), 1840–1845. <https://doi.org/10.1099/ijs.0.64717-0>
[PubMed]

Jeon, B. S., Moon, C., Kim, B.-C., Kim, H., Um, Y., & Sang, B.-I. (2013). In situ extractive fermentation

for the production of hexanoic acid from galactitol by *Clostridium* sp. BS-1. *Enzyme and Microbial Technology*, 53(3), 143–151. <https://doi.org/10.1016/j.enzmictec.2013.02.008> [PubMed]

Ji, Q., Zhou, B., Shen, T., Jiang, T., Cheng, C., & He, B. (2023). The lasso structure, biosynthesis, bioactivities and potential applications of Microcin J25: A novel antibacterial agent with unique mechanisms. *Engineering Microbiology*, 3(3), 100096. <https://doi.org/10.1016/j.engmic.2023.100096> [PubMed]

Kawulka, K., Sprules, T., McKay, R. T., Mercier, P., Diaper, C. M., Zuber, P., & Vederas, J. C. (2003). Structure of subtilisin A, an antimicrobial peptide from *Bacillus subtilis* with unusual posttranslational modifications linking cysteine sulfurs to alpha-carbons of phenylalanine and threonine. *Journal of the American Chemical Society*, 125(16), 4726–4727. <https://doi.org/10.1021/ja029654t> [PubMed]

Kazakov, T., Vondenhoff, G. H., Datsenko, K. A., Novikova, M., Metlitskaya, A., Wanner, B. L., & Severinov, K. (2008). *Escherichia coli* peptidase A, B, or N can process translation inhibitor microcin C. *Journal of Bacteriology*, 190(7), 2607–2610. <https://doi.org/10.1128/JB.01956-07> [PubMed]

Kim, J., Darley, D., Selmer, T., & Buckel, W. (2006). Characterization of (R)-2-hydroxyisocaproate dehydrogenase and a family III coenzyme A transferase involved in reduction of L-leucine to isocaproate by *Clostridium difficile*. *Applied and Environmental Microbiology*, 72(9), 6062–6069. <https://doi.org/10.1128/AEM.00772-06> [PubMed]

Kim, B., Oh, S. J., Hwang, J. H., Kim, H. J., Shin, N., Joo, J. C., Choi, K.-Y., et al. (2023). Complementation of reducing power for 5-hydroxyvaleric acid and 1,5-pentanediol production via glucose dehydrogenase in *Escherichia coli* whole-cell system. *Enzyme and Microbial Technology*, 170, 110305. <https://doi.org/10.1016/j.enzmictec.2023.110305> [PubMed]

Koh, A., de Vadder, F., Kovatcheva-Datchary, P., & Bäckhed, F. (2016). From dietary fiber to host physiology: Short-chain fatty acids as key bacterial metabolites. *Cell*, 165(6), 1332–1345. <https://doi.org/10.1016/j.cell.2016.05.041> [PubMed]

Koponen, O., Tolonen, M., Qiao, M., Wahlström, G., Helin, J., & Saris, P. E. J. (2002). NisB is required for the dehydration and NisC for the lanthionine formation in the post-translational modification of nisin. *Microbiology (Reading, England)*, 148(Pt 11), 3561–3568. <https://doi.org/10.1099/00221287-148-11-3561> [PubMed]

Kramer, J., Özkaya, Ö., & Kümmerli, R. (2020). Bacterial siderophores in community and host interactions. *Nature Reviews. Microbiology*, 18(3), 152–163. <https://doi.org/10.1038/s41579-019-0284-4> [PubMed]

Kuipers, A., de Boef, E., Rink, R., Fekken, S., Kluskens, L. D., Driessen, A. J. M., et al. (2004). NisT, the transporter of the lantibiotic nisin, can transport fully modified, dehydrated, and unmodified prenisin and fusions of the leader peptide with non-lantibiotic peptides. *The Journal of Biological Chemistry*, 279(21), 22176–22182. <https://doi.org/10.1074/jbc.M312789200> [PubMed]

Lagedroste, M., Smits, S. H. J., & Schmitt, L. (2017). Substrate specificity of the secreted Nisin leader peptidase NisP. *Biochemistry*, 56(30), 4005–4014. <https://doi.org/10.1021/acs.biochem.7b00524> [PubMed]

Laviña, M., Gaggero, C., & Moreno, F. (1990). Microcin H47, a chromosome-encoded microcin antibiotic of *Escherichia coli*. *Journal of Bacteriology*, 172(11), 6585–6588. <https://doi.org/10.1128/jb.172.11.6585-6588.1990> [PubMed]

Li, B., & van der Donk, W. A. (2007). Identification of essential catalytic residues of the cyclase NisC involved in the biosynthesis of nisin. *The Journal of Biological Chemistry*, 282(29), 21169–21175. <https://doi.org/10.1074/jbc.M701802200> [PubMed]

Li, Y. M., Milne, J. C., Madison, L. L., Kolter, R., & Walsh, C. T. (1996). From peptide precursors to oxazole and thiazole-containing peptide antibiotics: Microcin B17 synthase. *Science (New York, N.Y.)*, 274(5290), 1188–1193. <https://doi.org/10.1126/science.274.5290.1188> [PubMed]

Liu, L., Tao, Y., Li, Y., Deng, X., Liu, G., Yao, Y., et al. (2022). Isolation and characterization of bacteria that produce quorum sensing molecules during the fermentation and deterioration of pickles. *International Journal of Food Microbiology*, 379, 109869. <https://doi.org/10.1016/j.ijfoodmicro.2022.109869> [PubMed]

Louis, P., & Flint, H. J. (2009). Diversity, metabolism and microbial ecology of butyrate-producing bacteria from the human large intestine. *FEMS Microbiology Letters*, 294(1), 1–8. <https://doi.org/10.1111/j.1574-6968.2009.01514.x> [PubMed]

Louis, P., & Flint, H. J. (2017). Formation of propionate and butyrate by the human colonic microbiota. *Environmental Microbiology*, 19(1), 29–41. <https://doi.org/10.1111/1462-2920.13589> [PubMed]

Louis, P., Hold, G. L., & Flint, H. J. (2014). The gut microbiota, bacterial metabolites and colorectal cancer. *Nature Reviews. Microbiology*, 12(10), 661–672. <https://doi.org/10.1038/nrmicro3344> [PubMed]

Macfarlane, S., & Macfarlane, G. T. (2003). Regulation of short-chain fatty acid production. *The Proceedings of the Nutrition Society*, 62(1), 67–72. <https://doi.org/10.1079/PNS2002207> [PubMed]

Macfarlane, G. T., Gibson, G. R., Beatty, E., & Cummings, J. H. (1992). Estimation of short-chain fatty acid production from protein by human intestinal bacteria based on branched-chain fatty acid measurements. *FEMS Microbiology Letters*, 101(2), 81–88. <https://doi.org/10.1111/j.1574-6968.1992.tb05764.x>

Marković, K. G., Grujović, M. Ž., Koraćević, M. G., Nikodijević, D. D., Milutinović, M. G., Semedo-Lemsaddek, T., & Djilas, M. D. (2022). Colicins and microcins produced by Enterobacteriaceae: Characterization, mode of action, and putative applications. *International Journal of Environmental Research and Public Health*, 19(18). <https://doi.org/10.3390/ijerph191811825>

Medema, M. H., Kottmann, R., Yilmaz, P., Cummings, M., Biggins, J. B., Blin, K., et al. (2015). Minimum information about a biosynthetic gene cluster. *Nature Chemical Biology*, 11(9), 625–631. <https://doi.org/10.1038/nchembio.1890> [PubMed]

Metlitskaya, A., Kazakov, T., Kommer, A., Pavlova, O., Praetorius-Ibba, M., Ibba, M., et al. (2006). Aspartyl-tRNA synthetase is the target of peptide nucleotide antibiotic Microcin C. *The Journal of Biological Chemistry*, 281(26), 18033–18042. <https://doi.org/10.1074/jbc.M513174200> [PubMed]

Meza-Torres, J., Lelek, M., Quereda, J. J., Sachse, M., Manina, G., Ershov, D., et al. (2021). Listeriolysin S: A bacteriocin from *Listeria monocytogenes* that induces membrane permeabilization in a contact-dependent manner. *Proceedings of the National Academy of Sciences of the United States of America*, 118(40). <https://doi.org/10.1073/pnas.2108155118>

Miethke, M., & Marahiel, M. A. (2007). Siderophore-based iron acquisition and pathogen control. *Microbiology and Molecular Biology Reviews: MMBR*, 71(3), 413–451. <https://doi.org/10.1128/MMBR.00012-07> [PubMed]

Miller, T. L., & Wolin, M. J. (1996). Pathways of acetate, propionate, and butyrate formation by the human fecal microbial flora. *Applied and Environmental Microbiology*, 62(5), 1589–1592. <https://doi.org/10.1128/aem.62.5.1589-1592.1996> [PubMed]

Milshteyn, A., Colosimo, D. A., & Brady, S. F. (2018). Accessing bioactive natural products from the human microbiome. *Cell Host & Microbe*, 23(6), 725–736. <https://doi.org/10.1016/j.chom.2018.05.013>

Molloy, E. M., Cotter, P. D., Hill, C., Mitchell, D. A., & Ross, R. P. (2011). Streptolysin S-like virulence factors: The continuing saga. *Nature Reviews. Microbiology*, 9(9), 670–681. <https://doi.org/10.1038/nrmicro2624> [PubMed]

Montalbán-López, M., Deng, J., van Heel, A. J., & Kuipers, O. P. (2018). Specificity and application of the lantibiotic protease NisP. *Frontiers in Microbiology*, 9, 160. <https://doi.org/10.3389/fmicb.2018.00160> [PubMed]

Montalbán-López, M., Scott, T. A., Ramesh, S., Rahman, I. R., van Heel, A. J., Viel, J. H., et al. (2021). New developments in RiPP discovery, enzymology and engineering. *Natural Product Reports*, 38(1), 130–239. <https://doi.org/10.1039/d0np00027b>
[PubMed]

Morin, N., Lanneluc, I., Connil, N., Cottenceau, M., Pons, A. M., & Sablé, S. (2011). Mechanism of bactericidal activity of microcin L in *Escherichia coli* and *Salmonella enterica*. *Antimicrobial Agents and Chemotherapy*, 55(3), 997–1007. <https://doi.org/10.1128/AAC.01217-10>
[PubMed]

Mousa, W. K. (2022). The microbiome-product colibactin hits unique cellular targets mediating host-microbe interaction. *Frontiers in Pharmacology*, 13, 958012. <https://doi.org/10.3389/fphar.2022.958012>
[PubMed]

Navarro-Muñoz, J. C., Selem-Mojica, N., Mullowney, M. W., Kautsar, S. A., Tryon, J. H., Parkinson, E. I., et al. (2020). A computational framework to explore large-scale biosynthetic diversity. *Nature Chemical Biology*, 16(1), 60–68. <https://doi.org/10.1038/s41589-019-0400-9>
[PubMed]

Neis, E. P. J. G., Dejong, C. H. C., & Rensen, S. S. (2015). The role of microbial amino acid metabolism in host metabolism. *Nutrients*, 7(4), 2930–2946. <https://doi.org/10.3390/nu7042930>
[PubMed]

Newman, D. J., & Cragg, G. M. (2020). Natural products as sources of new drugs over the nearly four decades from 01/1981 to 09/2019. *Journal of Natural Products*, 83(3), 770–803. <https://doi.org/10.1021/acs.jnatprod.9b01285>
[PubMed]

Nougayrède, J.-P., Homburg, S., Taieb, F., Boury, M., Brzuszkiewicz, E., Gottschalk, G., et al. (2006). *Escherichia coli* induces DNA double-strand breaks in eukaryotic cells. *Science (New York, N.Y.)*, 313(5788), 848–851. <https://doi.org/10.1126/science.1127059>
[PubMed]

O'Connor, P. M., O'Shea, E. F., Guinane, C. M., O'Sullivan, O., Cotter, P. D., Ross, R. P., & Hill, C. (2015). Nisin H is a new Nisin variant produced by the gut-derived strain *Streptococcus hyointestinalis* DPC6484. *Applied and Environmental Microbiology*, 81(12), 3953–3960. <https://doi.org/10.1128/AEM.00212-15>
[PubMed]

Palmer, J. D., Mortzfeld, B. M., Piattelli, E., Silby, M. W., McCormick, B. A., & Bucci, V. (2020). Microcin H47: A class IIb microcin with potent activity against multidrug resistant Enterobacteriaceae. *ACS Infectious Diseases*, 6(4), 672–679. <https://doi.org/10.1021/acsinfecdis.9b00302>
[PubMed]

Perry, R. D., & Fetherston, J. D. (2011). Yersiniabactin iron uptake: Mechanisms and role in *Yersinia pestis* pathogenesis. *Microbes and Infection*, 13(10), 808–817. <https://doi.org/10.1016/j.micinf.2011.04.008>

[PubMed]

Pinske, C., & Sawers, R. G. (2016). Anaerobic formate and hydrogen metabolism. *EcoSal Plus*, 7(1). <https://doi.org/10.1128/ecosalplus.ESP-0011-2016>

Pons, A.-M., Delalande, F., Duarte, M., Benoit, S., Lanneluc, I., Sablé, S., et al. (2004). Genetic analysis and complete primary structure of microcin L. *Antimicrobial Agents and Chemotherapy*, 48(2), 505–513. <https://doi.org/10.1128/AAC.48.2.505-513.2004>
[PubMed]

Precord, T. W., Mahanta, N., & Mitchell, D. A. (2019). Reconstitution and substrate specificity of the thioether-forming radical S-adenosylmethionine enzyme in Freyrasin biosynthesis. *ACS Chemical Biology*, 14(9), 1981–1989. <https://doi.org/10.1021/acscchembio.9b00457>
[PubMed]

Pujol, A., Crost, E. H., Simon, G., Barbe, V., Vallenet, D., Gomez, A., & Fons, M. (2011). Characterization and distribution of the gene cluster encoding RumC, an anti-Clostridium perfringens bacteriocin produced in the gut. *FEMS Microbiology Ecology*, 78(2), 405–415. <https://doi.org/10.1111/j.1574-6941.2011.01176.x>
[PubMed]

Quereda, J. J., Dussurget, O., Nahori, M.-A., Ghoulane, A., Volant, S., Dillies, M.-A., et al. (2016). Bacteriocin from epidemic *Listeria* strains alters the host intestinal microbiota to favor infection. *Proceedings of the National Academy of Sciences of the United States of America*, 113(20), 5706–5711. <https://doi.org/10.1073/pnas.1523899113>
[PubMed]

Ragsdale, S. W. (2008). Enzymology of the wood-Ljungdahl pathway of acetogenesis. *Annals of the New York Academy of Sciences*, 1125, 129–136. <https://doi.org/10.1196/annals.1419.015>
[PubMed]

Ragsdale, S. W., & Pierce, E. (2008). Acetogenesis and the Wood-Ljungdahl pathway of CO₂ fixation. *Biochimica et Biophysica Acta*, 1784(12), 1873–1898. <https://doi.org/10.1016/j.bbapap.2008.08.012>
[PubMed]

Raymond, K. N., Dertz, E. A., & Kim, S. S. (2003). Enterobactin: An archetype for microbial iron transport. *Proceedings of the National Academy of Sciences of the United States of America*, 100(7), 3584–3588. <https://doi.org/10.1073/pnas.0630018100>
[PubMed]

Reichardt, N., Duncan, S. H., Young, P., Belenguer, A., McWilliam Leitch, C., Scott, K. P., et al. (2014). Phylogenetic distribution of three pathways for propionate production within the human gut microbiota. *The ISME Journal*, 8(6), 1323–1335. <https://doi.org/10.1038/ismej.2014.14>
[PubMed]

Reiners, J., Lagedroste, M., Gottstein, J., Adeniyi, E. T., Kalscheuer, R., Poschmann, G., et al. (2020). Insights in the antimicrobial potential of the natural Nisin variant Nisin H. *Frontiers in Microbiology*, 11, 573614. <https://doi.org/10.3389/fmicb.2020.573614>

[PubMed]

Ren, H., Shi, C., & Zhao, H. (2020). Computational tools for discovering and engineering natural product biosynthetic pathways. *iScience*, 23(1), 100795. <https://doi.org/10.1016/j.isci.2019.100795>

[PubMed]

Rinninella, E., Raoul, P., Cintoni, M., Franceschi, F., Miggiano, G. A. D., Gasbarrini, A., & Mele, M. C. (2019). What is the healthy gut microbiota composition? A changing ecosystem across age, environment, diet, and diseases. *Microorganisms*, 7(1). <https://doi.org/10.3390/microorganisms7010014>

Ríos-Covián, D., Ruas-Madiedo, P., Margolles, A., Gueimonde, M., de Los Reyes-Gavilán, C. G., & Salazar, N. (2016). Intestinal short chain fatty acids and their link with diet and human health. *Frontiers in Microbiology*, 7, 185. <https://doi.org/10.3389/fmicb.2016.00185>

[PubMed]

Rosengren, K. J., Clark, R. J., Daly, N. L., Göransson, U., Jones, A., & Craik, D. J. (2003). Microcin J25 has a threaded sidechain-to-backbone ring structure and not a head-to-tail cyclized backbone. *Journal of the American Chemical Society*, 125(41), 12464–12474. <https://doi.org/10.1021/ja0367703>

[PubMed]

Saban Güler, M., Arslan, S., Ağagündüz, D., Cerqua, I., Pagano, E., Berni Canani, R., & Capasso, R. (2025). Butyrate: A potential mediator of obesity and microbiome via different mechanisms of actions. *Food Research International (Ottawa, Ont.)*, *Food Research International*, 199, 115420. <https://doi.org/10.1016/j.foodres.2024.115420>

Salomón, R. A., & Fariás, R. N. (1992). Microcin 25, a novel antimicrobial peptide produced by *Escherichia coli*. *Journal of Bacteriology*, 174(22), 7428–7435. <https://doi.org/10.1128/jb.174.22.7428-7435.1992>

[PubMed]

Sassone-Corsi, M., Nuccio, S.-P., Liu, H., Hernandez, D., Vu, C. T., Takahashi, A. A., et al. (2016). Microcins mediate competition among Enterobacteriaceae in the inflamed gut. *Nature*, 540(7632), 280–283. <https://doi.org/10.1038/nature20557>

[PubMed]

Scadden, J., Ansorge, R., Romano, S., Telatin, A., Baker, D. J., Evans, R., et al. (2025). The nisin O cluster: species dissemination, candidate leader peptide proteases and the role of regulatory systems. *Microbiology (Reading, England)*, 171(2). <https://doi.org/10.1099/mic.0.001531>

Schalk, I. J. (2025). Bacterial siderophores: Diversity, uptake pathways and applications. *Nature Reviews. Microbiology*, 23(1), 24–40. <https://doi.org/10.1038/s41579-024-01090-6>

[PubMed]

Schmartz, G. P., Rehner, J., Gund, M. P., Keller, V., Molano, L.-A. G., Rupf, S., et al. (2024). Decoding the diagnostic and therapeutic potential of microbiota using pan-body pan-disease microbiomics. *Nature Communications*, 15(1), 8261. <https://doi.org/10.1038/s41467-024-52598-7>

[PubMed]

Schneditz, G., Rentner, J., Roier, S., Pletz, J., Herzog, K. A. T., Bückner, R., et al. (2014). Enterotoxicity of a nonribosomal peptide causes antibiotic-associated colitis. *Proceedings of the National Academy of Sciences of the United States of America*, 111(36), 13181–13186. <https://doi.org/10.1073/pnas.1403274111>

[PubMed]

Semenova, E., Yuzenkova, Y., Peduzzi, J., Rebuffat, S., & Severinov, K. (2005). Structure-activity analysis of microcinJ25: Distinct parts of the threaded lasso molecule are responsible for interaction with bacterial RNA polymerase. *Journal of Bacteriology*, 187(11), 3859–3863. <https://doi.org/10.1128/JB.187.11.3859-3863.2005>

[PubMed]

Seyedsayamdost, M. R., & Clardy, J. (2023). Discovering functional small molecules in the gut microbiome. *Current Opinion in Chemical Biology*, 75, 102309. <https://doi.org/10.1016/j.cbpa.2023.102309>

[PubMed]

Shamseddine, L., Roblin, C., Veyrier, I., Basset, C., de Macedo, L., Boyeldieu, A., et al. (2023). Mechanistic and functional aspects of the Ruminococcin C sactipeptide isoforms. *iScience*, 26(9), 107563. <https://doi.org/10.1016/j.isci.2023.107563>

[PubMed]

Shine, E. E., & Crawford, J. M. (2021). Molecules from the microbiome. *Annual Review of Biochemistry*, 90, 789–815. <https://doi.org/10.1146/annurev-biochem-080320-115307>

[PubMed]

Smith, E. A., & Macfarlane, G. T. (1998). Enumeration of amino acid fermenting bacteria in the human large intestine: Effects of pH and starch on peptide metabolism and dissimilation of amino acids. *FEMS Microbiology Ecology*, 25(4), 355–368. <https://doi.org/10.1111/j.1574-6941.1998.tb00487.x>

Soohoo, A. M., Cogan, D. P., Brodsky, K. L., & Khosla, C. (2024). Structure and mechanisms of assembly-line polyketide synthases. *Annual Review of Biochemistry*, 93(1), 471–498. <https://doi.org/10.1146/annurev-biochem-080923-043654>

[PubMed]

Spirito, C. M., Richter, H., Rabaey, K., Stams, A. J. M., & Angenent, L. T. (2014). Chain elongation in anaerobic reactor microbiomes to recover resources from waste. *Current Opinion in Biotechnology*, 27, 115–122. <https://doi.org/10.1016/j.copbio.2014.01.003>

[PubMed]

Staudt, S., Nikolka, F., Perl, M., Franz, J., Leblay, N., Yuan, X.-K., et al. (2025). Metabolization of microbial postbiotic pentanoate drives anti-cancer CAR T cells. *bioRxiv: The Preprint Server for Biology*. <https://doi.org/10.1101/2024.08.19.608538>

Staunton, J., & Weissman, K. J. (2001). Polyketide biosynthesis: A millennium review. *Natural Product Reports*, 18(4), 380–416. <https://doi.org/10.1039/a909079g>

[PubMed]

Sugihara, P. T., Sutter, V. L., Attebery, H. R., Bricknell, K. S., & Finegold, S. M. (1974). Isolation of *Acidaminococcus fermentans* and *Megasphaera elsdenii* from normal human feces. *Applied Microbiology*, 27(1), 274–275. <https://doi.org/10.1128/am.27.1.274-275.1974>

[PubMed]

Sugimoto, Y., Camacho, F. R., Wang, S., Chankhamjon, P., Odabas, A., Biswas, A., et al. (2019). A metagenomic strategy for harnessing the chemical repertoire of the human microbiome. *Science (New York, N.Y.)*, 366(6471). <https://doi.org/10.1126/science.aax9176>

Suo, M., Liu, L., Fan, H., Li, N., Pan, H., Hrynsphan, D., et al. (2025). Advancements in chain elongation technology: Transforming lactic acid into caproic acid for sustainable biochemical production. *Bioresource Technology*, 425, 132312. <https://doi.org/10.1016/j.biortech.2025.132312>

[PubMed]

Süssmuth, R. D., & Mainz, A. (2017). Nonribosomal peptide synthesis-principles and prospects. *Angewandte Chemie (International ed. in English)*, 56(14), 3770–3821. <https://doi.org/10.1002/anie.201609079>

[PubMed]

Tang, J.-W., Liu, X., Ye, W., Li, Z.-R., & Qian, P.-Y. (2022). Biosynthesis and bioactivities of microbial genotoxin colibactins. *Natural Product Reports*, 39(5), 991–1014. <https://doi.org/10.1039/d1np00050k>

[PubMed]

Tao, Y., Zhu, X., Wang, H., Wang, Y., Li, X., Jin, H., & Rui, J. (2017). Complete genome sequence of Ruminococcaceae bacterium CPB6: A newly isolated culture for efficient n-caproic acid production from lactate. *Journal of Biotechnology*, 259, 91–94. <https://doi.org/10.1016/j.jbiotec.2017.07.036>

[PubMed]

Vital, M., Howe, A. C., & Tiedje, J. M. (2014). Revealing the bacterial butyrate synthesis pathways by analyzing (meta)genomic data. *mBio*, 5(2), e00889. <https://doi.org/10.1128/mBio.00889-14>

[PubMed]

Vizán, J. L., Hernández-Chico, C., Del Castillo, I., & Moreno, F. (1991). The peptide antibiotic microcin B17 induces double-strand cleavage of DNA mediated by *E. coli* DNA gyrase. *The EMBO Journal*, 10(2), 467–476. <https://doi.org/10.1002/j.1460-2075.1991.tb07969.x>

[PubMed]

Walsh, C. T., O'Brien, R. V., & Khosla, C. (2013). Nonproteinogenic amino acid building blocks for nonribosomal peptide and hybrid polyketide scaffolds. *Angewandte Chemie International Edition*, 52(28), 7098–7124. <https://doi.org/10.1002/anie.201208344>

[PubMed]

Wang, L., Ravichandran, V., Yin, Y., Yin, J., & Zhang, Y. (2019). Natural products from mammalian gut microbiota. *Trends in Biotechnology*, 37(5), 492–504. <https://doi.org/10.1016/j.tibtech.2018.>

[10.003](#)

[\[PubMed\]](#)

Wang, J., Zhang, R., Chen, X., Sun, X., Yan, Y., Shen, X., & Yuan, Q. (2020). Biosynthesis of aromatic polyketides in microorganisms using type II polyketide synthases. *Microbial Cell Factories*, 19(1), 110. <https://doi.org/10.1186/s12934-020-01367-4>

[\[PubMed\]](#)

Wenski, S. L., Thiengmag, S., & Helfrich, E. J. N. (2022). Complex peptide natural products: Biosynthetic principles, challenges and opportunities for pathway engineering. *Synthetic and Systems Biotechnology*, 7(1), 631–647. <https://doi.org/10.1016/j.synbio.2022.01.007>

[\[PubMed\]](#)

Wilson, K.-A., Kalkum, M., Ottesen, J., Yuzenkova, J., Chait, B. T., Landick, R., et al. (2003). Structure of microcin J25, a peptide inhibitor of bacterial RNA polymerase, is a lassoed tail. *Journal of the American Chemical Society*, 125(41), 12475–12483. <https://doi.org/10.1021/ja036756q>

[\[PubMed\]](#)

Wilson, M. R., Zha, L., & Balskus, E. P. (2017). Natural product discovery from the human microbiome. *The Journal of Biological Chemistry*, 292(21), 8546–8552. <https://doi.org/10.1074/jbc.R116.762906>

[\[PubMed\]](#)

Wilson, M. R., Jiang, Y., Villalta, P. W., Stornetta, A., Boudreau, P. D., Carrá, A., et al. (2019). The human gut bacterial genotoxin colibactin alkylates DNA. *Science (New York, N.Y.)*, 363(6428). <https://doi.org/10.1126/science.aar7785>

Wyatt, M. A., Wang, W., Roux, C. M., Beasley, F. C., Heinrichs, D. E., Dunman, P. M., & Magarvey, N. A. (2010). Staphylococcus aureus nonribosomal peptide secondary metabolites regulate virulence. *Science (New York, N.Y.)*, 329(5989), 294–296. <https://doi.org/10.1126/science.1188888>

[\[PubMed\]](#)

Wylensek, D., Hitch, T. C. A., Riedel, T., Afrizal, A., Kumar, N., Wortmann, E., et al. (2020). A collection of bacterial isolates from the pig intestine reveals functional and taxonomic diversity. *Nature Communications*, 11(1), 6389. <https://doi.org/10.1038/s41467-020-19929-w>

[\[PubMed\]](#)

Xue, M., Kim, C. S., Healy, A. R., Wernke, K. M., Wang, Z., Frischling, M. C., et al. (2019). Structure elucidation of colibactin and its DNA cross-links. *Science (New York, N.Y.)*, 365(6457). <https://doi.org/10.1126/science.aax2685>

Yorgey, P., Lee, J., Kördel, J., Vivas, E., Warner, P., Jebaratnam, D., & Kolter, R. (1994). Posttranslational modifications in microcin B17 define an additional class of DNA gyrase inhibitor. *Proceedings of the National Academy of Sciences of the United States of America*, 91(10), 4519–4523. <https://doi.org/10.1073/pnas.91.10.4519>

[\[PubMed\]](#)

Yoshikawa, S., Araoka, R., Kajihara, Y., Ito, T., Miyamoto, H., & Kodama, H. (2018). Valerate production by Megasphaera elsdenii isolated from pig feces. *Journal of Bioscience and*

Bioengineering, 125(5), 519–524. <https://doi.org/10.1016/j.jbiosc.2017.12.016>
[PubMed]

Yuan, S., Jin, Z., Ali, A., Wang, C., & Liu, J. (2022). Caproic acid-producing bacteria in Chinese Baijiu brewing. *Frontiers in Microbiology*, 13, 883142. <https://doi.org/10.3389/fmicb.2022.883142>
[PubMed]

Yuille, S., Reichardt, N., Panda, S., Dunbar, H., & Mulder, I. E. (2018). Human gut bacteria as potent class I histone deacetylase inhibitors in vitro through production of butyric acid and valeric acid. *PLoS One*, 13(7), e0201073. <https://doi.org/10.1371/journal.pone.0201073>
[PubMed]

Zhang, G., & Sun, D. (2024). The synthesis of the novel *Escherichia coli* toxin-colibactin and its mechanisms of tumorigenesis of colorectal cancer. *Frontiers in Microbiology*, 15, 1501973. <https://doi.org/10.3389/fmicb.2024.1501973>
[PubMed]

Zhang, F., Wang, Y., Jiang, Q., Chen, Q., Karthik, L., Zhao, Y.-L., & Li, Z. (2018). Substrate selection of adenylation domains for nonribosomal peptide synthetase (NRPS) in bacillamide C biosynthesis by marine *Bacillus atrophaeus* C89. *Journal of Industrial Microbiology & Biotechnology*, 45(5), 335–344. <https://doi.org/10.1007/s10295-018-2028-2>

Zhang, F., Mulati, N., Wang, Y., Li, Y., Gong, S., Karthik, L., et al. (2019). Biosynthesis in vitro of bacillamide intermediate-heterocyclic AlaCysthiazole by heterologous expression of nonribosomal peptide synthetase (NRPS). *Journal of Biotechnology*, 292, 5–11. <https://doi.org/10.1016/j.jbiotec.2018.11.024>
[PubMed]

Zheng, G., Yan, L. Z., Vederas, J. C., & Zuber, P. (1999). Genes of the *sbo*-*alb* locus of *Bacillus subtilis* are required for production of the antilisterial bacteriocin subtilisin. *Journal of Bacteriology*, 181(23), 7346–7355. <https://doi.org/10.1128/jb.181.23.7346-7355.1999>
[PubMed]

Zhu, S., Xu, H., Liu, Y., Hong, Y., Yang, H., Zhou, C., & Tao, L. (2025). Computational advances in biosynthetic gene cluster discovery and prediction. *Biotechnology Advances*, 79, 108532. <https://doi.org/10.1016/j.biotechadv.2025.108532>
[PubMed]

Zimmermann, M., & Fischbach, M. A. (2010). A family of pyrazinone natural products from a conserved nonribosomal peptide synthetase in *Staphylococcus aureus*. *Chemistry & Biology*, 17(9), 925–930. <https://doi.org/10.1016/j.chembiol.2010.08.006>

23. Microbiome-Based Therapies: Phage Intervention

Joshua Lemuel Hadi^{[1](#)[2](#)[3](#)[4](#)}, Adrian Thaqi^{[1](#)[2](#)[3](#)[4](#)}, Julia Ammann^{[1](#)[2](#)[3](#)[4](#)},
Mohammadali Khan Mirzaei^{[1](#)[2](#)[3](#)[4](#)} and Li Deng^{[1](#)[2](#)[3](#)[4](#)}✉

- ([1](#)) Technical University of Munich, TUM School of Life Sciences, Freising, Germany
- ([2](#)) TUM Professor for Prevention of Microbial Diseases, Freising, Germany
- ([3](#)) TUM Central Institute of Infection Prevention (ZIP), Freising, Germany
- ([4](#)) Institute of Virology, Helmholtz Centre Munich - German Research Centre for Environmental Health, Neuherberg, Germany

✉ **Li Deng**
Email: li.deng@tum.de

Abstract

The human microbiome is linked to various health benefits and disorders. As part of the human microbial community, bacteriophages (phages) play a crucial role in regulating the microbial communities within the human gut, concomitantly giving rise to a novel research field: the human virome. Importantly, as these phages are viruses that target bacteria in a host-specific manner, various phage-based therapeutics could be developed for treating gut disorders. In this chapter, different aspects of phage therapy as a tool for modification of gut microbial composition and functionality are discussed within the context of various intestinal diseases. The discussions include the use

of fecal virome transplant as a delivery method, the application of phage-derived enzymes as antibacterial agents and the available in vitro and in vivo models for testing phage therapy. Challenges related to phage therapy are also assessed, ranging from the development of phage-resistant bacteria, non-optimal phage cocktails, and inactivation of phage particles in the human body.

Keywords Bacteriophages – Phage therapy – Human virome – Phage–host interactions – Precision microbiome modulation – Gut microbiota engineering

Highlights

- Phages, which are part of the human virome, exert control over the composition and functionality of gut microbial community, thus a key driver of gut health. This paves way for a repertoire of technologies for the manipulation of the human microbiome.
- As phages are host-specific, they could be used for the targeted elimination of bacteria in the gut. This includes the elimination of pathogenic or antibiotic-resistant bacteria or bacteria causally related to specific gut disorders.
- Challenges to be overcome include phage-resistant bacteria, the antagonistic relationship between phages in a cocktail, and the low availability of therapeutic phages in the human gut.

23.1 The Virome as a Driving Force in Human Gut Health

The human gastrointestinal tract harbors a diverse community of bacteria, archaea, viruses, fungi and other microbial eukaryotes, collectively known as the gut microbiome (Parfrey et al., [2011](#); Huttenhower et al., [2012](#); Hoffmann et al., [2013](#)). The human gut microbiome has been associated with health benefits, including immune modulation, detoxification of xenobiotics, improved

metabolism of macronutrients (lipid, protein and carbohydrate), synthesis of vitamins and protection against pathogenic bacteria—among others. In particular, the metabolites produced by the gut microbes—primarily by bacteria—play a prominent role in regulating the homeostasis in the human body (Nicholson et al., [2012](#)). Disruptions of the gut microbiome, known as dysbiosis in severe cases, have been associated with diseases, such as inflammatory bowel diseases (IBD) (Schaubeck et al., [2016](#)) and colorectal cancer (CRC) (Wong & Yu, [2023](#)), highlighting the intricate relationship of gut microbiome and human health.

The functionality of the gut microbiome is commonly defined by the interactions of different gut microbes, particularly between bacteria and bacteriophages (phages; viruses that predate on bacteria) (Mirzaei & Maurice, [2017](#)). These phages predominate the community of viruses residing within the gastrointestinal tract (i.e., gut virome)—the other members of the gut virome are eukaryotic and archaeal viruses (Tiamani et al., [2022](#)). Individual bacterial species may host multiple phages, forming the basis of a complex phage-bacteria network in the gut (Džunková et al., [2019](#)). Indeed, longitudinal studies have shown that phages replicate alongside their prokaryotic hosts in neonates, influenced by factors like diet, delivery method, environments, and direct maternal transmission (Walters et al., [2023](#); Garmaeva et al., [2024](#)). Intriguingly, these gut phages are stable over time (1–3 years) and specific to individuals, regardless of genetic relatedness (for example, between monozygotic twins or mothers and their offspring) (Reyes et al., [2010](#); Shkoporov et al., [2019](#); Lou et al., [2024](#)). However, an age-specific virome diversity has been identified (Gregory et al., [2020](#)) and a set of core and common phages that constitutes a healthy gut microbiome has also been proposed (Manrique et al., [2016](#)), indicating that the human gut virome has specific patterns that could be further explored for their potential health effects.

The human gut virome predominantly consists of the crAss-like phages, predicted to primarily infect bacteria of the phylum Bacteroidota (Dutilh et al., [2014](#); Ahlgren et al., [2017](#)), along with other

members of the *Caudoviricetes* (tailed phages; previously classified as *Caudovirales*) and members of the *Microviridae* family (icosahedral, non-tailed phages) (Liang & Bushman, [2021](#); Pargin et al., [2023](#)). These phages could be categorized into two major groups based on their lifestyles: virulent or temperate. Virulent phages, colloquially known as lytic phages, pursue a single developmental program aimed at virion production and bacterial lysis, i.e., these phages could only undergo the lytic cycle and regulate the microbial community through modulating the composition of the microbiome. Temperate phages may undergo both a lytic and a lysogenic cycle. When undergoing the lysogenic cycle, lytic genes are inactive, and the phage genome is established as a prophage, commonly integrated within the bacterial host chromosome—in certain cases, extrachromosomal replication may also occur. As prophages, temperate phages could increase bacterial fitness through horizontal gene transfer (HGT), i.e., modification of functionality. Some intestinal phages belonging to the family *Inoviridae* (filamentous phages) may also undergo the non-bactericidal chronic cycle, in which virions are continuously synthesized and extruded from the bacterial host cell (Sausset et al., [2020](#); Liang & Bushman, [2021](#)).

More importantly, these activities of gut phages have health implications, and virome signatures have been implicated in the development of different diseases (Tiamani et al., [2022](#)). Through the modification of bacterial communities, phages are capable of affecting the physiology of the mammalian host. This has been demonstrated for the induction of ulcerative colitis in mice after receiving virus-like particles from sick patients (Sinha et al., [2022](#)). Another study identified distinct fecal virome profiles, bacterial communities and metabolome linked to differences in dietary intake between South African urban or rural cohorts. This included patterns of gut microbial composition in the latter group, commonly associated with a higher risk of CRC (Ramaboli et al., [2024](#)). The expansion of taxonomic richness of *Caudoviricetes* phages has been associated with IBD in a disease-specific manner (Crohn's disease versus ulcerative colitis), partially through the induction of bacterial dysbiosis (Norman et al.,

[2015](#)). Another study also demonstrated the ability of the virome to regulate the bacterial community in stunted children, such as promoting the increase in Pseudomonadota (previously known as Proteobacteria), leading to the development of the disease (Khan Mirzaei et al., [2020](#)). In the Netherlands, a temperate phage, *Candidatus* order *Heliusvirales* (*Candidatus Heliusviridae* family), was enriched in people with type 1 and type 2 diabetes as well as IBD, with *C. Heliusviridae* linked to urbanized and westernized lifestyles (de Jonge et al., [2022](#), [2024](#)). Other disorders associated with the gut virome include severe acute malnutrition (Reyes et al., [2015](#)) and Parkinson's disease (Tetz et al., [2018](#)).

Taken together, these findings highlight that the virome is associated with different human diseases. Thus, a growing field of research has been dedicated to investigating the interactions between phages and their bacterial hosts, along with their effects on human health. In particular, research efforts have been aimed at exploring the therapeutic potential of phages to combat gut-related diseases, including those related to infections or various metabolic disorders. This chapter provides a panoptic view of phage-bacteria interactions that could be explored as therapeutic interventions for improving and restoring gut health.

23.2 Phage Therapy for Precise Modification of Microbiome Composition

Gut diseases could be caused by invading pathogenic bacteria (infection-related) or commensals that are associated with metabolic or inflammatory diseases (for example, through production of harmful metabolites or PAMPs). A major group of infection-causing pathogenic bacteria belongs to the family Enterobacteriaceae, including *Salmonella enterica*, pathogenic *Escherichia coli*, and *Klebsiella pneumoniae*. In addition, commensal bacteria may also indirectly cause diseases, such as those belonging to phyla Bacillota and Pseudomonadota that are capable of producing trimethylamine N-oxide (TMAO), which has been

linked to atherosclerosis (Bennett et al., [2013](#); Zhu et al., [2014](#)). Thus, targeted elimination of disease-promoting bacteria is of great interest to restore the healthy gut environment. One strategy to achieve this is through the use of virulent phages (strictly lytic phages; see Sect. [23.1](#))—as efficient killing of the target bacteria is paramount, temperate phages are not suitable for this type of phage therapy.

23.2.1 Targeted Elimination of Causative Bacteria in the Gut

A hallmark of phage therapy is host specificity, thus allowing for targeted modification of the gut microbiome. This is in contrast to antibiotic treatments, which are broad-spectrum and susceptible to nonspecific killing of non-target bacteria, potentially leading to the disruption of the microbiome, including dysbiosis in severe cases. For example, *Salmonella* phages fed to piglets did not lead to differential ileal microbiome composition found in groups treated with antibiotics, relative to non-treated controls (Hong et al., [2016](#)). Consistently, the gut microbiome of healthy adults consuming *E. coli* phages remained relatively undisrupted, albeit population-specific changes in butyrate-producing *Eubacterium* and *Clostridium perfringens* were observed (Febvre et al., [2019](#)). Nevertheless, the knock-on effects of phage therapy on gut microbial communities remain largely underexplored (Fazzino et al., [2020](#)), particularly within the context of prolonged phage therapy, and warrant further investigation (Hsu et al., [2019](#)).

Phage therapy could be used to modify gut metabolomes by targeting bacteria producing certain metabolites. For example, the production of two neurotransmitters (tryptamine and tyramine) in the murine gut could be manipulated by targeting *Clostridium sporogenes* and *Enterococcus faecalis*, respectively (Hsu et al., [2019](#)). Similarly, the elimination of intestinal cytolysin-producing *E. faecalis* by phage therapy attenuated the severity of alcoholic liver disease in humanized mice (Duan et al., [2019](#)). Phage-based eradication of high alcohol-producing *K. pneumoniae* in murine gut was also found to relieve non-alcoholic fatty liver disease, including through the regulation of

inflammatory pathways, lipid metabolism, and carbohydrate metabolism (Gan et al., [2023](#)). Furthermore, selective targeting of certain bacteria within the gut microbiome of diabetic mice led to specific changes in the microbial structure and metabolism, resulting in decreased levels of glucose, lipid, and proinflammatory factors as well as increased levels of short-chained fatty acids (Ye et al., [2024](#)). Albeit, to achieve effective modulation of the gut metabolome, studies are needed to better understand the gut microbial networks and functionality of their concomitant metabolites (Khan Mirzaei & Deng, [2022](#)).

Another promising use of phage therapy is for combating infections, particularly those that are caused by antimicrobial-resistant pathogenic bacteria. Several studies have aimed to develop phage therapies against enteric pathogens, with some success reported in the treatment of infections caused by enteroaggregative *E. coli* (EAEC) (Cepko et al., [2020](#)), adherent invasive *E. coli* (AIEC; commonly found in Crohn's disease patients) (Galtier et al., [2017](#); Titecat et al., [2022](#)), *K. pneumoniae* (Federici et al., [2022](#)) and *Vibrio parahaemolyticus* (Jun et al., [2014](#)) in murine models. A collection of phages against enteric pathogenic bacteria, known as the Intesti Bacteriophage, has also been established by the Eliava Institute in Georgia (Kutateladze & Adamia, [2008](#)). Despite the promising in vivo results, data on human clinical applications are sparse, and treatment efficacy varies across studies (Sarker et al., [2016](#), [2017](#); Schooley et al., [2017](#); Febvre et al., [2019](#); Corbellino et al., [2020](#)). Yet, consistent with trials in non-human animal models, evidence shows that phage therapy is generally safe and does not trigger significant changes to the gut microbiome in human patients (Duan et al., [2021](#)).

In addition to the gut infections mentioned above, the emergence of multidrug-resistant *Clostridioides difficile* and *Helicobacter pylori* presents yet another global health threat that calls for an immediate solution (Boyanova et al., [2019](#); Sholeh et al., [2020](#)). *C. difficile* is a causative agent for diarrheal disease and pseudomembranous colitis associated with antibiotic-induced dysbiosis (Antharam et al., [2013](#)).

Phage therapy against *C. difficile* infections is limited by the availability of virulent phages. Intriguingly, one study developed combinations of seven temperate phages against 62 *C. difficile* strains representing 18 epidemic and clinically severe ribotypes, with optimized phage treatment reducing colonization in a hamster model (Nale et al., [2016](#)). This preliminary data warrants further studies on the improvements of phage therapy against *C. difficile*, particularly those aimed at the isolation of virulent phages. *H. pylori* infections have been associated with pre-cancerous lesions (atrophic gastritis and gastric intestinal metaplasia) and gastric cancer (Watari et al., [2014](#)) as well as impaired energy homeostasis, lipid metabolism, and elevated fasting insulin levels and sensitivity (El-Maksoud & Metwaly, [2016](#)). A study combined lactoferrin (LF), an antimicrobial molecule with a proven activity against *H. pylori* infection, with a novel lytic phage (Hp ϕ) to inhibit *H. pylori* colonization and minimize host pro-inflammatory responses in Human Caucasian gastric adenocarcinoma cell lines (Cuomo et al., [2020](#)). LF was complexed with hydroxyapatite (HA) nanoparticles as a scaffold for drug delivery (Li et al., [2019](#)). HA is an inorganic material that is naturally part of human teeth and bones, is biologically inert, and has been shown to reduce the susceptibility of phages to gastric acid, enhancing their stability, resistance, and lytic activity, while also boosting the biological properties of LF (Kruzel et al., [2017](#); Fulgione et al., [2019](#); Papaiani et al., [2020](#)).

To improve the applicability of phage therapy, a systematic approach to characterize specific phage cocktails against a broad range of target bacteria could be undertaken. Genomic surveillance of carbapenem-resistant *Acinetobacter baumannii* was used to identify strains prevalent in different parts of the world. Subsequently, phage cocktails were developed for those bacterial strains prevalent in Eastern Europe, demonstrating the possibility of generating region-specific phage collections (Koncz et al., [2024](#)). Furthermore, a phage bank has been developed for carbapenem-resistant, gut-relevant *K. pneumoniae* strains through screening of host factors required for phage infection in major *Klebsiella* phage families, potentially allowing

for rapid design of phage cocktails with minimal resistance development (Rotman et al., [2024](#)). In addition, *in silico* tools could be deployed to ease phage cocktail design. Bacterial and phage genomic information, such as bacterial adsorption factors, phage receptor binding proteins, and antiphage system, could be used to predict phage-bacteria interactions and subsequently may be fed to machine learning for developing predictive algorithms in phage therapy (Gaborieau et al., [2024](#)). This approach may be combined with other tools, such as Cytoscape-based Exhaustive Search, that design a phage cocktail based on known phage-bacteria infection networks (Menor-Flores et al., [2022](#)). These *in silico* strategies focus the phage screening process to relevant phage-bacteria interactions, expediting the phage cocktail design process and reducing the amount of laboratory work needed.

23.2.2 Fecal Virome Transplant to Treat Gut Disorders

In contrast to traditional phage therapy, which targets specific bacterial pathogens, fecal virome transplantation (FVT)—also known as fecal filtrate transplantation (FFT)—exploits the broader potential of phages and gut virome to restore microbial balance and alleviate the symptoms of gastrointestinal and metabolic diseases associated with dysbiosis. FVT is an extension of the traditional fecal microbiota transplantation (FMT), in which viral components (primarily phages) are transferred from healthy donors to patients. The idea behind FVT is to modify the gut microbiota without introducing foreign bacteria, potentially reducing the risk of complications. However, the risk of transmission of eukaryotic viruses and prophage-encoded virulence factors remains a safety concern (Rasmussen et al., [2020](#)). Nevertheless, phages and eukaryotic viruses could be separated through differences in their characteristics: eukaryotic viruses are typically enveloped RNA viruses, whereas the majority of phages are non-enveloped DNA viruses. Indeed, researchers have applied solvent/detergent-based methods for inactivating enveloped viruses (FVT-SDT), pyronin-Y for inhibiting replication of RNA viruses (FVT-PyT), and a chemostat-propagated virome (FVT-ChP) for removing the majority of eukaryotic viruses by

dilution from fecal filtrates. This processed FVT was found to be effective in controlling *C. difficile* colonization in the murine gut (Rasmussen et al., [2024](#)). A similar approach was used to treat metabolic syndrome in obese mice (Mao et al., [2024](#)).

FVT-induced shift of the gut microbiome has been explored to treat various diseases (Table [23.1](#)). These include gut disorders associated with infections, such as necrotizing enterocolitis (NEC) or *C. difficile*-associated IBD (Ott et al., [2017](#); Brunse et al., [2022](#)). Furthermore, the shift in microbiome could alleviate metabolic disorders, as demonstrated in the alleviation of type 2 diabetes and obesity symptoms in mice fed with a high-fat diet (Rasmussen et al., [2020](#)) or restore the healthy gut microbiome after disruption by antibiotics (Draper et al., [2020](#)). Importantly, the safety of FVT has been demonstrated in a pilot study of 24 patients, where the participants were randomly assigned to receive either FVT from a lean, healthy donor or a placebo. The study found that there were no serious adverse reactions, with only some recipients suffering from mild gastrointestinal side effects. Two days after FVT treatment, the phage composition had changed significantly compared to the placebo group, and more virulent phage-microbe interactions were observed (Wortelboer et al., [2023](#)).

Table 23.1 Transplantation of fecal virome or filtrate against different diseases in in vivo models or clinical setting

Disease	Model	Main finding	References
NEC	Pig model	FVT reduced the incidence and severity of NEC, improved intestinal barrier integrity, and modulated immune responses. The treatment also decreased the NEC pathobionts, particularly <i>Clostridium perfringens</i> and <i>Klebsiella pneumoniae</i>	Brunse et al. (2022); Offersen et al. (2024)
Metabolic syndrome	Murine model	Improved blood glucose tolerance, insulin sensitivity, and liver pathology, and reduced immune cells in adipose tissue (inconsistent response).	Mao et al. (2024)

Disease	Model	Main finding	References
Type 2 diabetes and obesity		Significant decrease in weight gain and glucose tolerance in treated mice, linked to shifts in the composition of the gut microbiome, blood plasma metabolome, and expression of genes associated with obesity and the development of type 2 diabetes	Rasmussen et al. (2020)
Antibiotic-induced dysbiosis		Reshaped gut microbiome towards pre-antibiotic state. Abundance and diversity of phages in the FVT group were maintained at a higher level and persisted for up to 15 days after treatment	Draper et al. (2020)
Obesity		Mice treated with FVT from lean donors had lower weight gain regardless of diet regimes (low-fat or high-fat), possibly due to changes in the relative abundances of certain gut bacteria. On the other hand, mice treated with FVT from obese donors had higher weight gain and reduced α -diversity in their gut microbiome.	Borin et al. (2023)
SIBO		FVT reduced the density of bacteria in HFD-induced dysbiotic mice, as determined by the relative quantification of 16S rRNA gene sequencing	Lin et al. (2019)
Metabolic syndrome	Patients with metabolic syndrome	No significant differences in overall glucose metabolism, FVT was well-tolerated and more virulent phage-microbe interactions in treated patients	Wortelboer et al. (2023)
IBD	IBD patients with chronic-relapsing <i>Clostridium difficile</i> infection	FVT eliminated symptoms of <i>C. difficile</i> infection within days of administration, which was sustained for 6 months to 2 years post-treatment. There were patient-specific shifts in the relative abundance, α -diversity, and β -diversity of the gut bacteria	Ott et al. (2017)
IBD	Patients with ulcerative colitis	Three out of six patients had clinical improvements after undergoing FVT, with stable and patient-specific changes in the gut bacterial community and virome observed	Junca et al. (2025)
Stunting	Stunted and non-stunted children	Bacterial abundance and composition could be regulated in an age-specific manner. Phages from stunted children promoted the growth of Pseudomonadota in younger children, whereas phages from non-stunted children promoted the growth of Bacillota	Khan Mirzaei et al. (2020)

NEC necrotizing enterocolitis, *FVT* fecal virome transplant, *SIBO* small intestinal bacteria overgrowth, *HFD* high-fat diet, *IBD* inflammatory bowel disease

23.3 Prophages, Auxiliary Metabolic Genes, and Gut Microbiome Functionalities

Besides inducing cell lysis, phages impact bacterial communities through horizontal gene transfer (HGT), facilitating the movement of genetic material between bacterial species. In general, the process in which bacteriophages transfer genomic material from one bacterium to another is called transduction (Morse et al., [1956](#)). This gene transfer contributes to bacterial adaptation in the gut, reinforcing the functional diversity and resilience of the microbiome. One example of this is viral-encoded auxiliary metabolic genes (AMGs). In virulent phages, these AMGs are targeted towards manipulating the metabolism of the host bacteria for gaining competitive advantages in challenging growth environments, thus allowing for an increased production of phage progeny (Chevallereau et al., [2021](#)). Conversely, temperate phages typically encode AMGs related to microbial physiology regulations, potentially facilitating virus-host mutualism (Warwick-Dugdale et al., [2019](#)). Virome studies have shown that prophages are involved in microbial processes, such as anaerobic respiration and amino acid, nucleotide, lipid, and xenobiotic metabolisms (Reyes et al., [2010](#)). Prophages also encode genes for polysaccharide and carbohydrate metabolism, vitamins, and cofactors, all of which could enhance bacterial fitness (Modi et al., [2013](#); Mirzaei & Maurice, [2017](#)). More recently, several prophage-associated AMGs, which encoded corrinoid transporters essential for vitamin B₁₂ transport, were shown to confer competitive advantages to Bacteroidetes in colonizing the murine gut (Frye et al., [2021](#)). Analyses of organosulfur AMGs in human gut microbiomes identified genes associated with assimilation of sulfur and degradation of organosulfur into sulfite, including in putative pathogenic bacteria, implying the role of AMGs in regulating dysbiosis

(Kieft et al., [2021](#)). Indeed, the impact of AMGs on human gut health has been demonstrated in rheumatoid arthritis patients, whereby intestinal phage AMGs encoding phosphonate phosphodiesterase were overexpressed, and AMGs encoding various transferases were absent in at-risk individuals, relative to a healthy cohort (Mangalea et al., [2021](#)).

Collectively, these studies highlight the potential use of AMGs as a versatile tool for modifying gut microbiome functionalities. Therapeutic strategies could be developed to utilize prophages for delivering AMGs that enhance metabolic functions beneficial to gut health, such as increased short-chain fatty acid (SCFA) production, improved nutrient cycling, or enhanced vitamin biosynthesis. To this end, the first step required is the identification and characterization of AMGs in silico (Box [23.1](#)). Subsequently, phages could be engineered to carry AMGs as a payload, as it has been demonstrated for clustered regularly interspaced short palindromic repeats (CRISPR)-based microbiome editing (Nethery et al., [2022](#); Brödel et al., [2024](#)).

Box 23.1 Bioinformatics Tools for Studying Prophages

To effectively leverage temperate phages for therapeutic microbiome modulation, identifying and characterizing prophages within bacterial genomes is a crucial first step. This process involves using advanced bioinformatics tools to uncover the presence, functionality, and genetic impact of prophages in gut microbial communities. Key tools, such as PHASTEST, ProphageHunter, and VirSorter, are invaluable for efficiently detecting prophage sequences and assessing whether these prophages are complete, active, and functionally capable of influencing host bacterial dynamics (Roux et al., [2015](#); Song et al., [2019](#); Wishart et al., [2023](#)). After initial identification, deeper genomic and functional analyses could be conducted using tools like ViroProfiler, VIBRANT, and DRAM/DRAM-v (Kieft et al., [2020](#); Shaffer et al., [2020](#); Ru et al., [2023](#)). These pipelines allow for a comprehensive annotation, classification, and examination of viral genomes within a microbial sample, revealing how specific prophages influence bacterial host metabolism.

ViroProfiler, a robust viral metagenomics pipeline, integrates multiple functionalities for the discovery and classification of viral genomes, providing detailed predictions on host-phage interactions (Ru et al., [2023](#)). Through ViroProfiler's combined use of *VIBRANT* for genome annotation and *DRAM/DRAM-v* for AMG profiling, researchers could identify nutrient-cycling genes, SCFA-related pathways, and vitamin synthesis genes that prophages may transfer to bacterial hosts (Kieft et al., [2020](#); Shaffer et al., [2020](#); Ru et al., [2023](#)). These AMGs serve as essential tools for modifying bacterial metabolism and could be targeted to improve gut health outcomes. Together, these tools would enable researchers to identify, classify, and modulate phages in microbiome studies, potentially informing phage-based interventions aimed at enhancing beneficial microbial functions, such as nutrient cycling, short-chain fatty acid production, and vitamin synthesis, particularly within the gut microbiome. In the context of microbiome-based therapy, these bioinformatic tools support the identification of candidate AMGs and prophages, enabling researchers to first pinpoint promising targets and, following any modifications, to monitor their persistence and assess long-term functional outcomes within the gut microbiome.

23.4 Phage-Derived Antibacterials

Several phage-encoded enzymes could be used as antibacterials to treat infections, particularly those that are involved in the lysis of the host bacterial cell in the final stage of phage infection cycle. This lysing process typically involves the subversion of three layers of the bacterial cellular envelope: inner membrane, peptidoglycan layer, and outer membrane (Gram-negative bacteria). To this end, phages encode holins to disrupt the inner membrane, endolysins to degrade the peptidoglycan layer, and spanin complexes to lyse the outer membrane (Young, [2014](#)). In addition, some phages possess virion-associated peptidoglycan hydrolases (VAPGH), which, unlike endolysins, induce a small hole in the peptidoglycan layer through which the phage tail tube

could inject the genetic material at the beginning of the infection cycle (Rodríguez-Rubio et al., [2013](#)). Several phages also encode depolymerases for the degradation of polysaccharides, such as capsular polysaccharide (CPS) or extracellular polysaccharide (EPS) (Knecht et al., [2020](#)).

Endolysins are one of the most commonly used phage-derived antibacterials. Following the induction of micron-scale holes in the inner membrane by holins, endolysins escape from the cytoplasm to degrade the peptidoglycan layer (Young, [2014](#)). Endolysins targeting Gram-positive bacteria have two components, namely the cell wall binding domain (CBD) and the enzymatically active domain (EAD), for substrate recognition and catalytic activity, respectively (Schmelcher et al., [2012a](#)). The CBD module may confer host specificity to an endolysin, as it has been demonstrated in those targeting *Listeria monocytogenes* (on a species or serovar level) (Loessner et al., [2002](#); Kretzer et al., [2007](#); Schmelcher et al., [2010](#); Eugster et al., [2011](#); Eugster & Loessner, [2012](#)). However, some endolysins target a broader range of Gram-positive bacteria (Yoong et al., [2004](#); Nelson et al., [2006](#)). On the other hand, endolysins against Gram-negative bacteria are typically small globular proteins (molecular mass between 15 and 20 kDa) that lack a specific CBD module (Schmelcher et al., [2012a](#)). Furthermore, in murine models, endolysin treatments have been shown to be effective against cytolysin-positive *E. faecalis* within the gut, resulting in the suppression of acute graft-versus-host disease (Fujimoto et al., [2024](#)). Similarly, other studies have demonstrated the use of endolysins against streptococci and *C. difficile* infections (Nelson et al., [2001](#); Jado et al., [2003](#); Fujimoto et al., [2020](#)). Intriguingly, endolysins have been deployed synergistically with other antimicrobials, including with other endolysins or peptidoglycan hydrolases, to increase treatment efficacy against *Streptococcus pneumoniae*, *Staphylococcus aureus*, and *E. faecalis* (Loeffler & Fischetti, [2003](#); Becker et al., [2008](#); Schmelcher et al., [2012b](#); Zhang et al., [2023](#)). In particular, endolysins have been combined with holins into an effective treatment against several Gram-positive and Gram-negative bacteria, albeit the application of holins

alone typically only induces bacteriostatic effects (i.e., no bacterial lysis) (Rajesh et al., [2011](#); Rodas et al., [2011](#); Shi et al., [2012](#); Wang et al., [2022a](#)).

Another group of peptidoglycan hydrolases is VAPGH. These enzymes are produced in the early stages of the infection cycle and are known to cause the phenomenon “lysis from without”, in which bacterial lysis occurs without the production of viral progeny (Abedon, [2011](#); Rodríguez-Rubio et al., [2013](#)). In contrast to endolysins, VAPGH do not contain CBD due to the fact that these enzymes are delivered directly to the peptidoglycan layer by the phage as part of the tail structure. Importantly, while not all phages encode VAPGH in their genome, known VAPGH are present in phages targeting both Gram-positive and Gram-negative bacteria. Purified VAPGH from *Pseudomonas* phage $\phi 6$ (protein P5) and *Pseudomonas aeruginosa* phage Φ KMV (gp35C protein) exhibited activity against a range of Gram-negative, but not against Gram-positive bacteria (Caldentey & Bamford, [1992](#); Lavigne et al., [2004](#)). VAPGH against Gram-positive bacteria has been characterized for *S. aureus*, *Bacillus subtilis* and *Lactococcus lactis* (Kenny et al., [2004](#); Takáč & Bläsi, [2005](#); Rashel et al., [2007](#); Sudiarta et al., [2010](#); Rodríguez et al., [2011](#)). In addition, similar to endolysins, the modular structure of VAPGH allows for protein engineering to modify their bacteriolytic activities. An example of this is the chimeric enzyme P128, which has been tested against *S. aureus* in murine models (Paul et al., [2011](#); Vipra et al., [2012](#)). Another VAPGH from the *S. aureus* bacteriophage vB_SauS-phiPLA88 (HydH5) was fused with lysostaphin and combined with an endolysin (LysH5) to improve the killing efficacy of multiple *S. aureus* and *Staphylococcus epidermidis* strains, relative to the parental enzyme (Rodríguez-Rubio et al., [2012](#)).

Bacterial polysaccharides are physical barriers to phage receptors, necessitating some phages to encode depolymerases for an effective infection. A hallmark of these depolymerase-encoding phages is the formation of haloes around their plaques. Phage-derived depolymerases exist either as a structural protein (typically as part of

the tail fibers) or a soluble protein produced during host cell lysis. Furthermore, these depolymerases could be classified into two main classes: hydrolases (hydrolyzing glycosidic bonds) and lyases (cleaving 1,4-glycosidic bonds by a β -elimination mechanism), which are divided into other subclasses (Pires et al., [2016](#)). It is noteworthy that depolymerases may not induce bacterial killing (bacteriolytic) or growth inhibition (bacteriostatic) alone. Nevertheless, these enzymes could inactivate the polysaccharide protective layers, allowing host immune defenses, antimicrobial agents, or phages to neutralize the target bacteria with higher efficacy. Examples of this include endosialidase E that facilitated the phagocytosis of *E. coli* in mice through the degradation of CPS (Mushtaq et al., [2005](#)), K64 capsule depolymerase that enhanced the serum- or neutrophil-associated killing of *K. pneumoniae* (Pan et al., [2015](#)), and poly-*N*-acetylglucosamine depolymerase DA7 that improved the activities of endolysins and antibiotics against *S. aureus* (Olsen et al., [2018](#); Sumrall et al., [2021](#)). Besides CPS, depolymerases have been demonstrated to degrade EPS, the main component of extracellular polymeric substance in biofilms, in *P. aeruginosa* (Glonti et al., [2010](#)), *Staphylococcus* spp. (Gutiérrez et al., [2012](#), [2015](#)) and *A. baumannii* (Chen et al., [2022](#))—among others (Wang et al., [2024](#)).

23.5 In Vitro and in In Vivo Models for Assessing Phage Therapy

23.5.1 Synthetic Community for Gut Modeling

Microbiome research relies on models that mimic the human gut microbial environment while being straightforward to manipulate. In particular, these models must be able to capture the microbial dynamics in a consistent manner across experimental replicates. Furthermore, variances in the gut microbiome exist across different animal models, including between individuals within the same species. Thus, to mitigate these uncertainties, researchers have developed various synthetic communities based on taxonomic, metabolite, and/or

functional representations of a target microbiome (Table 23.2). In addition, host-specific synthetic communities could also be developed (Zenner et al., 2021; Afrizal et al., 2022; Hitch et al., 2025). The development of a synthetic community is typically achieved by a bottom-up approach, in which single strains of bacteria are individually cultured, characterized, and selected prior to their mixing into a consortium. In contrast, a top-down approach could also be used, in which specific members of a complex microbial community are selectively removed (Jennings & Clavel, 2024). To facilitate the designing of a synthetic community, bioinformatics tools (for example, MDSINE, CoMiDa and MiMiC) could be used to predict microbial functionalities from genomic or metagenomic data, which in turn, may assist with determining the optimal composition of a consortium based on specific metabolic features (Bucci et al., 2016; Eng & Borenstein, 2016; Kumar et al., 2021).

Table 23.2 Microbial composition of selected synthetic microbial community for gut modeling

Consortium (target host)	Bacterial species ^a	Description	References
SIHUMIx (human)	<i>Anaerostipes caccae</i> , <i>Bacteroides thetaiotaomicron</i> , <i>Bifidobacterium longum</i> subsp. <i>longum</i> , <i>Blautia producta</i> , <i>Clostridium butyricum</i> , <i>Clostridium ramosum</i> , <i>Escherichia coli</i> K12, and <i>Lactobacillus plantarum</i>	Consortium design is based on the phyla and metabolite representation of the human gut microbiome. Community structure is resistant to pulsed perturbations, including various transit times. Compositions and metabolites of SIHUMIx are also highly reproducible across different bioreactors. SIHUMIx is stable over time and transferable to the murine offspring	Becker et al. (2011); Schäpe et al. (2019); Krause et al. (2020)

Consortium (target host)	Bacterial species ^a	Description	References
Colitis-SIHUMI (human)	<i>E. coli</i> , <i>E. faecalis</i> , <i>Ruminococcus gnavus</i> , <i>Bacteroides vulgatus</i> , <i>Faecalibacterium prausnitzii</i> , <i>L. plantarum</i> and <i>B. longum</i>	Bacterial strains were determined based on alterations in IBD patients, human origin, availability of genomic sequences, and ability to form a stable community in rodents. Colitis-SIHUMI could form a stable consortium and induce colitis in germ-free and interleukin-10-deficient mice	Eun et al. (2014)
OMM¹² (murine)	<i>Akkermansia muciniphila</i> , <i>Bacteroides caecimuris</i> , <i>Blautia pseudococcoides</i> , <i>Enterocloster clostridioformis</i> , <i>Clostridium innocuum</i> , <i>Enterococcus faecalis</i> , <i>Flavonifractor plautii</i> , <i>Limosilactobacillus reuteri</i> , <i>Turicimonas muris</i> , <i>Acutalibacter muris</i> , and <i>Bifidobacterium animalis</i>	Consortium design is based on the phyla representation and functionalities of the murine gut microbiome. Once established, OMM ¹² confers colonization resistance to mice against <i>Salmonella enterica</i> subsp. <i>enterica</i> serovar Typhimurium	Brugiroux et al. (2016)
GUT-108 (murine)	<i>Bacteroides xylanisolvens</i> , <i>C. butyricum</i> , <i>Clostridium scindens</i> , <i>Intestinimonas butyriciproducens</i> , <i>Eubacterium callanderi</i> , <i>Extibacter</i> sp., <i>Akkermansia</i> sp., <i>Clostridium symbiosum</i> , <i>Bacteroides uniformis</i> , <i>Bitterella massiliensis</i> , <i>Barnesiella</i> sp.	Consortium design is based on functionalities of the human gut microbiome, particularly the production of metabolites (SCFAs, indole, antimicrobial, and siderophores) and deconjugation of bile acids. GUT-108 is capable of colonizing gnotobiotic mice, reversing immune-mediated colitis, and reducing pathobionts in the gut	van der Lelie et al. (2021)
BacMix (murine)	<i>A. muciniphila</i> , <i>Ruthenibacterium lactatiformans</i> , <i>Alistipes timonensis</i> , <i>Muribaculum intestinale</i> and <i>Bacteroides</i> sp.	Murine commensal bacteria are selected based on the ability to forage on mucosal sugars, particularly NeuAC and GlcNAC. BacMix provides murine models with colonization resistance against <i>Clostridioides difficile</i> through competition for mucosal sugars	Pereira et al. (2020)

Consortium (target host)	Bacterial species ^a	Description	References
Bristol microbiota (porcine)	<i>Parabacteroides</i> sp., <i>L. amylovorus</i> and <i>C. glycolicum</i>	The bacterial strains are derived from ASF, a murine intestinal microbiota, which colonized the gut of piglets. Colonization of these piglets with Bristol microbiota led to increased immunoglobulin levels compared to germ-free piglets	Laycock et al. (2012)
CeMbio (<i>Caenorhabditis elegans</i>)	<i>Enterobacter hormaechei</i> , <i>Lelliottia amnigena</i> , <i>Acinetobacter guillouiae</i> , <i>Sphingomonas molluscorum</i> , <i>Stenotrophomonas indicatrix</i> , <i>Pseudomonas lurida</i> , <i>Pseudomonas berkeleyensis</i> , <i>Comamonas piscis</i> , <i>Pantoea nemavictus</i> , <i>Ochrobactrum vermis</i> , <i>Sphingobacterium multivorum</i> and <i>Chryseobacterium scophthalmum</i>	Consortium is designed based on the taxonomic representation of microbial populations typically found in <i>C. elegans</i> . Bacterial strains in CeMbio are capable of colonizing <i>C. elegans</i> as single strains and as a consortium	Dirksen et al. (2020)

SIHUMIx simplified human intestinal microbiota, *OMM*¹² Oligo-Mouse-Microbiota, *SCFAs* short-chain fatty acids, *ASF* altered Schaedler flora, *IBD* inflammatory bowel disease, *NeuAC* *N*-acetylneuraminic acid, *GlcNAC* *N*-acetylglucosamine

^aThis list only mentions the species of the bacteria, but not the specific strains tested for each consortium. Readers are referred to the references for information on the strains

An important type of synthetic community is the simplified human intestinal microbiota (SIHUMI), which is aimed at mimicking the human gut microbiome. Two main SIHUMI consortia have been designed: SIHUMIx and Colitis-SIHUMI (Table [23.2](#)). SIHUMIx is designed to represent a healthy gut microbiome and has been used to test the potential inflammatory-inducing trait of *Akkermansia muciniphila* in gnotobiotic interleukin-10-deficient mice (Ring et al.,

[2019](#)). On the other hand, Colitis-SIHUMI is a disease-specific microbial consortium that induces colitis, typically found in IBD (Eun et al., [2014](#)). Several studies have used Colitis-SIHUMI or its derivatives to study the correlation between the human microbiome and different diseases in vivo (Lengfelder et al., [2019](#); Rogala et al., [2020](#)). Importantly, derivatives of the Colitis-SIHUMI consortium have also been deployed to study the effect of Φ crAss002 phage on *Bacteroides xylanisolvens* within a defined microbial community (Guerin et al., [2021](#)) or the impact of *E. coli* and *Enterococcus faecalis* phages on the cross-feeding of the gut community (Buttimer et al., [2022](#)).

Synthetic communities have also been designed for various animal models used in microbiome studies (Table [23.2](#)). For murine models, Oligo-Mouse-Microbiota (OMM¹²) and its derivatives (OMM¹⁴ and OMM^{19.1}) have been developed based on microbial functionalities at a phylum level (Brugiroux et al., [2016](#); Afrizal et al., [2022](#); von Strempel et al., [2023](#)). One study used OMM¹⁴ to test the effects of phage cocktails targeting *E. coli* and *E. faecalis* on the colonization resistance of the gut microbiome against *Salmonella enterica* subsp. *enterica* serovar Typhimurium (von Strempel et al., [2023](#)). Other murine synthetic communities are available for studying inflammation (GUT-108) and *C. difficile* infection (BacMix) (Pereira et al., [2020](#); van der Lelie et al., [2021](#)). On the other hand, a synthetic community for porcine models is yet to be established, albeit one study has proposed the Bristol microbiota as a consortium capable of colonizing the gut of gnotobiotic piglets (Laycock et al., [2012](#)). Nevertheless, a collection of porcine gut microbiota has been characterized based on their taxonomy and functionalities, allowing for future synthetic communities to be developed (Wylensek et al., [2020](#)). Intriguingly, another study has shown that fecal microbiota communities were better established in germ-free porcine than murine models, highlighting the need for a stable synthetic community for pigs (Aluthge et al., [2020](#)). Furthermore, CeMbio is a consortium that has been established in *Caenorhabditis elegans* (Dirksen et al., [2020](#)), an important invertebrate model (Sect. [23.5.2](#)).

23.5.2 Vertebrate Versus Invertebrate In Vivo Models

There are several in vivo models available for assessing phage therapy, including for potential application within the gut microbiome. The most commonly used animal model is germ-free mice. Studies on phage-based therapeutics in these mice range from the assessment of gut metabolome alteration, cross-feeding dynamics of microbial community, to the facilitation of chemotherapy against colorectal cancer through modulation of gut microbiota (Hsu et al., [2019](#); Zheng et al., [2019](#); Buttner et al., [2022](#)). Pigs are another animal model that could be colonized with human-derived bacterial communities, for example, by means of FMT (Zhang et al., [2013](#)). In regard to phage therapy, the main use of porcine models has been for the assessment of FVT against NEC (Table [23.1](#)) (Brunse et al., [2022](#); Offersen et al., [2024](#)). Other animal models for studying phage therapy have been reviewed elsewhere (Penziner et al., [2021](#)).

An alternative infection model is the larvae of the greater wax moth (*Galleria mellonella*). They lack nociceptors, and thus they are insensitive to pain, easing ethical rules unlike for vertebrate models. Although lacking an adaptive immune system, invertebrates have similar innate immune defenses to their vertebrate counterparts—including mammals—due to an extensive number of orthologous genes that control general functions. To note, the lack of adaptive immunity in invertebrates (absence of T- or B-lymphocytes and no production of antibodies) has been construed as a limitation for studying infections. However, evidence suggests that *G. mellonella* possesses an immune-like memory against foreign microorganisms, known as larval-trained immunity, which is mediated by hemocytes and antimicrobial peptides. Furthermore, during the infection process, soluble effectors in *G. mellonella* bind to the bacterial lipopolysaccharides (LPS) or lipoteichoic acid, triggering a cascade of reactions that leads to the production of melanin. Thus, infected *G. mellonella* larvae could be identified by their color change (melanization) (Ménard et al., [2021](#)). In regard to practical applications, one study used *G. mellonella* as an in vivo model for studying phage therapy against carbapenem-resistant *A.*

baumannii, with the authors also finding a correlation between the results in *G. mellonella* and murine models (Jeon et al., [2019](#)). Other studies have also tested various phage-based therapeutics against *P. aeruginosa*, *Salmonella enterica*, and *K. pneumoniae* in *G. mellonella* (Beeton et al., [2015](#); Wintachai et al., [2020](#); Kosznik-Kwaśnicka et al., [2022](#)). Intriguingly, *G. mellonella* could be colonized by human gut microbiota, further expanding its use as an in vivo gut model (Gooch et al., [2024](#)). Indeed, one study has demonstrated the efficacy of phage therapy against *E. coli* and *K. pneumoniae* in the gut of *G. mellonella* (Mirza et al., [2024](#)). In addition, a standardized method for scoring the efficacy of phage therapy in *G. mellonella* has been described by Nieuwenhuysen and colleagues (Van Nieuwenhuysen et al., [2022](#)).

Another invertebrate infection model is *Caenorhabditis elegans*, a species of roundworm. *C. elegans* is a promising model for studying bacteria-phage interactions, particularly considering that this organism feeds on bacteria. Similar to *G. mellonella*, *C. elegans* possesses an innate immune system, including the conserved mitogen-activated protein kinase (MAPK) signaling pathways for combating bacterial infections, albeit it lacks homologs of the transcription factor NF- κ B or the Toll-like receptor (TLR) adaptor protein MYD88 (Pukkila-Worley & Ausubel, [2012](#); Ermolaeva & Schumacher, [2014](#)). Like other invertebrate models, ethical restrictions are negligible for *C. elegans*. Importantly, *C. elegans* has a short life cycle and high fecundity, which makes it amenable to high-throughput studies on host-bacteria-phage interactions. Furthermore, *C. elegans* can be rendered germ-free by bleaching, implying that there would be a high degree of control over the gut microbial compositions (Zhang et al., [2017](#)). However, most studies on *C. elegans* have utilized the natural microbiome of the organism (Zhang et al., [2017](#), [2021](#); Backes et al., [2021](#)), albeit researchers have also demonstrated the colonization of *C. elegans* gut with human enteric pathogens (Walker et al., [2021](#)) or fecal microbiota (Møller et al., [2023](#)). Nevertheless, further studies are needed on the development of a robust protocol for the efficient colonization of *C. elegans* with human gut-derived bacteria. In regard to phage therapy, a multi-well assay has

been developed to study the interactions between phages and pathogenic bacteria in *C. elegans*, including the physiological impacts on the nematode host (Manohar et al., [2022](#)). This model has been expanded to assess the efficacy of a biofilm-degrading *Pseudomonas* phage (Manohar et al., [2024](#)). A similar study has also shown the use of *C. elegans* for the verification of a phage therapy against *Burkholderia pseudomallei* (Wang et al., [2022b](#)).

23.6 Challenges and Potential Solutions

23.6.1 Bacterial Defense, Phage Antidefense, and Synthetic Biology

Despite the promising therapeutic potential of phage therapy, several challenges remain to be overcome prior to its future application in the human gut. A prominent limitation of phage therapy is the development of bacterial tolerance or resistance against phage infection, leading to reduced efficacy of the therapy. There are several strategies commonly used by bacteria for resisting their phage invaders, such as the prevention of phage adsorption, inactivation of phage genomic materials upon entry into the bacterial cell, and killing of infected cells as a means to protect the bacterial population against widespread phage infection—among others (Labrie et al., [2010](#); Cohen et al., [2019](#); Hampton et al., [2020](#); Bernheim et al., [2020](#); Maestri et al., [2024](#)). On the other hand, phages have developed anti-defense systems to evade bacterial immune responses during phage infection (Sberro et al., [2013](#); Murphy et al., [2013](#); Rusinov et al., [2018](#); Pinilla-Redondo et al., [2020](#); Hobbs et al., [2022](#); Ho et al., [2023](#)). More importantly, these anti-defense systems have technological potential to improve phage therapy. An example of this is the engineering of *E. coli* phage tail fibers to target a broader range of bacterial surface receptors—thus mitigating potential phage resistance associated with impaired phage adsorption—and led to better treatment outcomes in murine models (Yehl et al., [2019](#); Gencay et al., [2023](#)). Furthermore, engineered phages carrying anti-CRISPR genes have been used to enhance phage therapy against *P.*

aeruginosa infection in human cells (HEK293T) and murine models (Qin et al., [2022](#)). These phages with anti-CRISPR genes could also be deployed as a cocktail—including in combination with CRISPR-susceptible phage strains—to increase treatment efficacy (Landsberger et al., [2018](#); Chevallereau et al., [2020](#)). Taken together, these findings highlight the competitive evolutionary relationship between bacteria and phages, which entails important technological considerations during the development and administration of phage therapies (Fig. [23.1](#)).

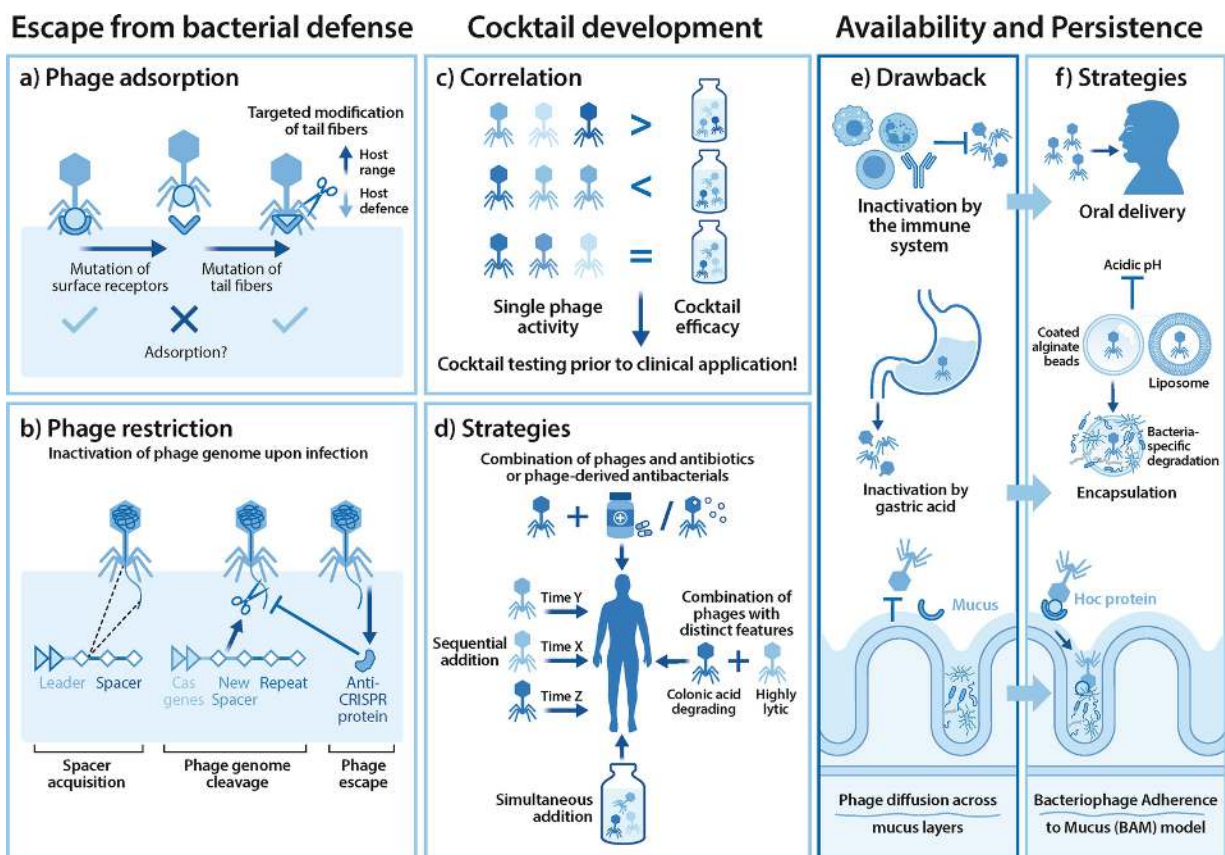


Fig. 23.1 Challenges of phage therapy and strategies to overcome them. A major challenge is related to bacterial antiviral defenses, such as the modification of bacterial surface receptors (**a**) or the inactivation of phage genome (CRISPR-Cas or restriction systems) (**b**)—among others (Bernheim & Sorek, [2019](#)). These challenges can be overcome through the use of synthetic biology, including the mutagenesis of phage tail fibers (**a**) and the engineering of anti-CRISPR gene into the phage genome (**b**). Another challenge is the potential antagonistic relationship of phages in a cocktail, resulting in phage therapy with reduced efficacy. Thus, pre-clinical testing is required for verifying whether the phage cocktail has a lower ($>$), higher ($<$) or equal ($=$) efficacy than the constituent phages applied alone (**c**). Alternatively, strategic application of the phage cocktail, such as sequential versus simultaneous, combination of phage cocktail with other antimicrobial agents

or the selection of phages with distinct features, could be used to optimize phage therapy efficacy (d). Lastly, physiological constraints—for example, inactivation of phages by mammalian immune system or gastric acid (e)—could be overcome through the use of encapsulation and phages that are adherent to mucus (f)

23.6.2 Optimization of Phage Cocktail: Viral Synergism Versus Antagonism

A strategy to administer therapeutic phages is in a cocktail, in which multiple phages (polyphage) are deployed synergistically to achieve a higher bacterial killing, relative to when the phages are used alone (monophage). In particular, phage cocktails have been reported to be effective at suppressing the development of phage resistance in the target bacteria (Gu et al., [2012](#); Chadha et al., [2016](#); Yuan et al., [2019](#)). Indeed, several studies have proposed the use of a phage cocktail to target multiple bacterial receptors to circumvent phage adsorption-associated resistance in *E. coli* O157:H7, *K. pneumoniae*, *P. aeruginosa* and *Salmonella enterica* (Tanji et al., [2004](#); Gu et al., [2012](#); Yang et al., [2020](#); Martinez-Soto et al., [2024](#)). Simultaneous addition of all phages in a cocktail is effective in preventing the development of resistance and the clearance of bacterial infection (Hall et al., [2012](#); Wright et al., [2019](#)). On the other hand, sequential addition of phages targeting different bacterial receptors could be used to minimize cross-resistance to each other (Hall et al., [2012](#)), albeit the specific order of phage addition needs to be verified for limiting the strength of resistance in the target bacteria or imposing the highest fitness costs on the resistant bacterial mutants (Wright et al., [2019](#)). Another strategy is to use a combination of phages with distinct features—for example, a phage with colanic acid-degrading activity was paired with a highly lytic phage to improve the killing of mucoid *E. coli*, with the combination of two phages being more effective than the monophage use of each phage (Schmerer et al., [2014](#)).

However, there remains a caveat concerning the antagonistic relationships between individual phages in a cocktail, potentially impairing the efficacy of phage therapy (Fig. [23.1](#)). This antagonism arises from the finite availability of host resources, resulting in

competition for host receptors or the production of phage repressors (temperate phages). The aforementioned strategy of selecting for phages that target different receptors may circumvent the issue with host receptor competition to some extent (Gordillo Altamirano & Barr, [2021](#)). Nevertheless, it is imperative to verify the bactericidal activity of a phage cocktail, relative to that of the individual phages used as a monophage therapy, prior to clinical application. An example of this is demonstrated by a study showing that individual activities of phages against *E. coli* O157:H7 did not correlate with the efficacy of these phages when combined in multiple cocktail combinations, with different cocktails having a lower, equal, or higher efficacy than the constituent phages applied alone (Niu et al., [2021](#)). To this end, one study has proposed the use of Cocktail, a computer program for mathematical modelling of phage infection kinetics. This program allows for the selection of an optimal phage cocktail against a bacterial host based on specific characteristics, as defined by sixteen parameters for bacteria (for example, growth rate, phage resistance, or biofilm formation) and eight parameters for phages (for example, adsorption rate, latency period, or burst size) (Nilsson, [2022](#)).

23.6.3 Phage Availability: Oral Delivery System and Mucus-Adherent Phages

Besides the inherent ability of bacteria to resist viral infections, another hurdle to a successful phage therapy is the low phage availability and persistence in the human body. A prominent factor contributing to this is the inactivation of phage particles by gastric acid and the innate or adaptive immune systems (Krut & Bekeredjian-Ding, [2018](#); Dąbrowska & Abedon, [2019](#)). Within the context of phage therapy targeted towards the gastrointestinal tract, phage diffusion across the mucus layer and the interactions of therapeutic phages with the gut microbial community (i.e., microbiome and virome) are also aspects to consider (Fig. [23.1](#)).

Beyond infecting their prokaryotic host, therapeutic phages could interact with the mammalian cells through different mechanisms,

particularly by means of endocytosis (Box [23.2](#)). A prominent implication of these interactions is the production of phage-specific neutralizing antibodies (for example, IgG and IgM), which have been found to impair the efficacy of phage therapies targeted against gut pathogens in murine models (Berkson et al., [2024](#)). A clinical study epitomizes this issue, in which the development of phage-specific antibodies led to treatment failure of recurrent *Enterococcus faecium* bacteremia caused by the overgrowth of enterococcal bacteria in a patient's gastrointestinal tract (Stellfox et al., [2024](#)). Intriguingly, oral administration of phage therapy has been associated with minimal and delayed immune response (Bruttin & Brüssow, [2005](#); Sarker et al., [2012](#); McCallin et al., [2013](#); Łusiak-Szelachowska et al., [2014](#); Majewska et al., [2015](#)), thus it is potentially a suitable means of phage delivery to the gastrointestinal tract. To enhance the efficacy of oral delivery, phages can be encapsulated in different carrier materials (for example, liposomes or polymers). Indeed, encapsulated phages have been reported to be more stable than non-encapsulated phages in murine models (Colom et al., [2015](#); Singla et al., [2016](#); Yin et al., [2021](#)).

Box 23.2 Internalization of Phages by Mammalian Immune Cells

There are different means by which phages could be internalized by mammalian cells. Epithelial cells, a prevalent cell type in the gut, could internalize phages through macropinocytosis, involving the intracellular accumulation of functional phages (Bichet et al., [2021](#)). Indeed, phages are capable of undergoing transcytosis across epithelial cells, through transit in the Golgi apparatus (Nguyen et al., [2017](#)). Other means of endocytosis, namely clathrin- or caveolae-mediated, have been reported for the filamentous M13 into different cell types (Tian et al., [2015](#)).

The internalization of phages by mammalian cells, particularly the immune cells, has implications for phage therapy. For example, LPS-free *E. coli* phages could induce the expansion of IFN- γ -producing CD4⁺ and CD8⁺ T-cells in Peyer's patches of germ-free

mice. Dendritic cells, which are phagocytic, incubated with these phages elicited the expression of several cytokines, including interleukin (IL)-12, IL-6, and IL-10, and the production of IFN- γ through Toll-like receptor (TLR) 9-dependent signal. These phage-induced immune responses exacerbated intestinal colitis in murine models (Gogokhia et al., [2019](#)). Furthermore, it has been reported that the internalization of a temperate filamentous phage into immune cells could lead to the production of phage mRNA. This triggered TLR3- and TIR domain-containing adapter-inducing interferon- β (TRIF)-dependent type I interferon production, inhibition of tumor necrosis factor (TNF), and suppression of phagocytosis. Consequently, there was an impaired clearance of *P. aeruginosa* in the phage-treated mice, i.e., persistent infection (Sweere et al., [2019](#)). In contrast, therapeutic phages have been shown to facilitate the intracellular killing of *S. aureus* in murine macrophages (Kaur et al., [2014](#)). Further studies are needed to ascertain the interactions of therapeutic phages and the mammalian cells, along with the concomitant effects on the efficacy and safety of phage therapy.

Beyond protecting phages against external stresses, encapsulation is also a means of delivering phages into a specific target site within the human body (i.e., controlled release). From the limited data available in the current literature, alginate-based encapsulation is the primary delivery technique tested to date. Two studies have demonstrated that an alginate-based delivery system could protect *E. coli* and *Salmonella* phages from acidic conditions and subsequently release these phages in environments with a pH resembling that of the intestines (Silva Batalha et al., [2021](#); Gomez-Garcia et al., [2021](#)). Furthermore, excipients could be added to alginate beads to improve the stability of the carrier material as well as to modify the release mechanism (Malik et al., [2017](#)). An example of this is by coating alginate-calcium chloride (Alg-CaCl₂) microspheres with multilayers of polyethylenimine (PEI) and pectin. Coated Alg-CaCl₂ beads were found to be stable at a low pH of

1.1 and outperformed uncoated beads in delivering a temperate *E. coli* phage into mice. In addition to protection against acid, the pectin within the coating layers also acted as a bacteria-specific degradation mechanism, triggering a cargo release in the microbial-dense area within the murine gut (Hsu et al., [2020](#)). Taken together, these studies highlight the possibility of using alginate-based encapsulation as a protective and tunable delivery system of phages. However, the aforementioned studies only encapsulated one type of phage; thus, further research is needed to determine the feasibility of encapsulation methods for delivering phage cocktails.

The mucous layer of the gut may provide a spatial refuge for bacteria against phage predation, potentially reducing the efficacy of phage therapy (Lourenço et al., [2020](#)). In addition, components of the intestinal mucins could have an inhibitory effect on the activity of phages (Green et al., [2021](#)), further complicating the deployment of phage therapy in the human gut. Nevertheless, a strategy could be devised for selecting phages with the ability to thrive in the gut environment—for example, an *E. coli* phage was found to be capable of binding to human heparan sulfated proteoglycans typically found on mammalian cells and in mucus (Green et al., [2021](#)). Previous bacteriophage adherence to mucus (BAM) model has also proposed the binding of T4 phages to mucin glycoproteins via immunoglobulin (Ig)-like domains of the Hoc protein on their capsids (Barr et al., [2013](#)), and the subsequent subdiffusion of these phages to infect their bacterial host in the mucosal surfaces (Barr et al., [2015](#)). This BAM model has been confirmed in some phages against *P. aeruginosa* (Almeida et al., [2024](#)). More importantly, phages have the ability to coevolve with the mammalian gut mucosal layer (Chin et al., [2022](#)), highlighting the possibility of pre-adapting phages to the gut environment prior to therapeutic application.

23.7 Concluding Remark and Future Outlook

Phages residing in the gut play an important role in maintaining human health. In the era of personalized medicine, phage-based technology arises as a promising tool for specific modification of the gut microbiome, albeit some technical challenges are yet to be overcome (Sect. [23.6](#)). However, there are several technologies available for developing and improving phage-based therapeutics, including a repertoire of omics tools for analyzing microbial community structure within the human gut, flow cytometry-based techniques for characterizing unculturable phages, and various molecular methods for studying phage-bacteria interactions on a single-cell level (Khan Mirzaei & Deng, [2022](#)). The lack of a defined regulatory framework is another hurdle to the clinical application of phage therapy and phage-derived antibacterials, which calls for an urgent solution (Naureen et al., [2020](#); Yang et al., [2023](#)). Phage therapy has been widely used in Eastern Europe, with varying degrees of regulations in different states, and other countries have started to follow suit (Yang et al., [2023](#)). Indeed, collaborative efforts by the global scientific community and health regulatory bodies are required for ensuring the safe and effective implementations of phages to treat gut-related disorders, particularly those associated with multidrug-resistant bacteria.

References

Abedon, S. T. (2011). *Lysis from without*. *Bacteriophage*, 1, 46. <https://doi.org/10.4161/BACT.1.1.13980>
[\[PubMed\]](#)

Afrizal, A., Jennings, S. A. V., Hitch, T. C. A., et al. (2022). Enhanced cultured diversity of the mouse gut microbiota enables custom-made synthetic communities. *Cell Host & Microbe*, 30, 1630–1645.e25. <https://doi.org/10.1016/j.CHOM.2022.09.011>

Ahlgren, N. A., Ren, J., Lu, Y. Y., et al. (2017). Alignment-free d_2^* oligonucleotide frequency dissimilarity measure improves prediction of hosts from metagenomically-derived viral sequences. *Nucleic Acids Research*, 45, 39–53. <https://doi.org/10.1093/NAR/GKW1002>
[\[PubMed\]](#)

Almeida, G. M. d. F., Ravantti, J., Grdzelišvili, N., et al. (2024). Relevance of the bacteriophage adherence to mucus model for *Pseudomonas aeruginosa* phages. *Microbiol Spectr*, 12. https://doi.org/10.1128/SPECTRUM.03520-23/SUPPL_FILE/REVIEWER-COMMENTS.PDF

Aluthge, N. D., Tom, W. A., Bartenslager, A. C., et al. (2020). Differential longitudinal establishment of human fecal bacterial communities in germ-free porcine and murine models. *Communications Biology*, 3, 1–14. <https://doi.org/10.1038/s42003-020-01477-0>

Antharam, V. C., Li, E. C., Ishmael, A., et al. (2013). Intestinal dysbiosis and depletion of butyrogenic bacteria in *Clostridium difficile* infection and nosocomial diarrhea. *Journal of Clinical Microbiology*, 51, 2884–2892. https://doi.org/10.1128/JCM.00845-13/SUPPL_FILE/ZJM999092753S01.PDF
[PubMed]

Backes, C., Martinez-Martinez, D., & Cabreiro, F. (2021). *C. elegans*: A biosensor for host–microbe interactions. *Lab Animal (NY)*, 50, 127–135. <https://doi.org/10.1038/s41684-021-00724-z>

Barr, J. J., Auro, R., Furlan, M., et al. (2013). Bacteriophage adhering to mucus provide a non-host-derived immunity. *Proceedings of the National Academy of Sciences of the United States of America*, 110, 10771–10776. https://doi.org/10.1073/PNAS.1305923110/SUPPL_FILE/PNAS.201305923SI.PDF
[PubMed]

Barr, J. J., Auro, R., Sam-Soon, N., et al. (2015). Subdiffusive motion of bacteriophage in mucosal surfaces increases the frequency of bacterial encounters. *Proceedings of the National Academy of Sciences of the United States of America*, 112, 13675–13680. https://doi.org/10.1073/PNAS.1508355112/SUPPL_FILE/PNAS.1508355112.SAPP.PDF
[PubMed]

Becker, N., Kunath, J., Loh, G., & Blaut, M. (2011). Human intestinal microbiota: Characterization of a simplified and stable gnotobiotic rat model. *Gut Microbes*, 2, 25–33. <https://doi.org/10.4161/GMIC.2.1.14651>
[PubMed]

Becker, S. C., Foster-Frey, J., & Donovan, D. M. (2008). The phage K lytic enzyme LysK and lysostaphin act synergistically to kill MRSA. *FEMS Microbiology Letters*, 287, 185–191. <https://doi.org/10.1111/J.1574-6968.2008.01308.X>
[PubMed]

Beeton, M. L., Alves, D. R., Enright, M. C., & Jenkins, A. T. A. (2015). Assessing phage therapy against *Pseudomonas aeruginosa* using a *Galleria mellonella* infection model. *International Journal of Antimicrobial Agents*, 46, 196–200. <https://doi.org/10.1016/j.ijantimicag.2015.04.005>
[PubMed]

Bennett, B. J., Vallim, T. Q. D. A., Wang, Z., et al. (2013). Trimethylamine-N-Oxide, a metabolite associated with atherosclerosis, exhibits complex genetic and dietary regulation. *Cell Metabolism*, 17, 49–60. <https://doi.org/10.1016/j.cmet.2012.12.011>
[PubMed]

Berkson, J. D., Wate, C. E., Allen, G. B., et al. (2024). Phage-specific immunity impairs efficacy of bacteriophage targeting Vancomycin Resistant Enterococcus in a murine model. *Nature Communications*, 15, 1–14. <https://doi.org/10.1038/s41467-024-47192-w>

Bernheim, A., Millman, A., Ofir, G., et al. (2020). Prokaryotic viperins produce diverse antiviral molecules. *Nature*, 589, 120–124. <https://doi.org/10.1038/s41586-020-2762-2> [PubMed]

Bernheim, A., & Sorek, R. (2019). The pan-immune system of bacteria: Antiviral defence as a community resource. *Nature Reviews. Microbiology*, 18, 113–119. <https://doi.org/10.1038/s41579-019-0278-2> [PubMed]

Bichet, M. C., Chin, W. H., Richards, W., et al. (2021). Bacteriophage uptake by mammalian cell layers represents a potential sink that may impact phage therapy. *iScience*, 24. <https://doi.org/10.1016/j.jisci.2021.102287/ATTACHMENT/14007866-74DD-472A-B043-E97572A438CF/MMC3.ZIP>

Borin, J. M., Liu, R., Wang, Y., et al. (2023). Fecal virome transplantation is sufficient to alter fecal microbiota and drive lean and obese body phenotypes in mice. *Gut Microbes*, 15. <https://doi.org/10.1080/19490976.2023.2236750>

Boyanova, L., Hadzhiyski, P., Kandilarov, N., et al. (2019). Multidrug resistance in *Helicobacter pylori*: Current state and future directions. *Expert Review of Clinical Pharmacology*, 12, 909–915. <https://doi.org/10.1080/17512433.2019.1654858> [PubMed]

Brödel, A. K., Charpenay, L. H., Galtier, M., et al. (2024). In situ targeted base editing of bacteria in the mouse gut. *Nature*, 632, 877–884. <https://doi.org/10.1038/s41586-024-07681-w> [PubMed]

Brugiroux, S., Beutler, M., Pfann, C., et al. (2016). Genome-guided design of a defined mouse microbiota that confers colonization resistance against *Salmonella enterica* serovar Typhimurium. *Nature Microbiology*, 2, 1–12. <https://doi.org/10.1038/nmicrobiol.2016.215>

Brunse, A., Deng, L., Pan, X., et al. (2022). Fecal filtrate transplantation protects against necrotizing enterocolitis. *ISME Journal*, 16, 686–694. <https://doi.org/10.1038/s41396-021-01107-5> [PubMed]

Bruttin, A., & Brüssow, H. (2005). Human volunteers receiving *Escherichia coli* phage T4 orally: A safety test of phage therapy. *Antimicrobial Agents and Chemotherapy*, 49, 2874–2878. <https://doi.org/10.1128/AAC.49.7.2874-2878.2005/ASSET/318FACB2-6305-48A6-A17E-3A7CDD1D4A96/ASSETS/GRAPHIC/ZAC0070550480002.JPEG> [PubMed]

Bucci, V., Tzen, B., Li, N., et al. (2016). MDSINE: Microbial Dynamical Systems INference Engine for microbiome time-series analyses. *Genome Biology*, 17, 1–17. <https://doi.org/10.1186/S13059-016-0980-6/FIGURES/4>

Buttimer, C., Sutton, T., Colom, J., et al. (2022). Impact of a phage cocktail targeting *Escherichia coli* and *enterococcus faecalis* as members of a gut bacterial consortium in vitro and in vivo. *Frontiers in Microbiology*, 13, 2748. <https://doi.org/10.3389/FMICB.2022.936083/BIBTEX>

Caldentey, J., & Bamford, D. H. (1992). The lytic enzyme of the *Pseudomonas* phage $\phi 6$. Purification and biochemical characterization. *Biochimica et Biophysica Acta (BBA) - Protein Structure and Molecular Enzymology*, 1159, 44–50. [https://doi.org/10.1016/0167-4838\(92\)90073-M](https://doi.org/10.1016/0167-4838(92)90073-M)
[PubMed]

Cepko, L. C. S., Garling, E. E., Dinsdale, M. J., et al. (2020). Myoviridae phage PDX kills enteroaggregative *Escherichia coli* without human microbiome dysbiosis. *Journal of Medical Microbiology*, 69, 309–323. <https://doi.org/10.1099/JMM.0.001162/CITE/REFWORKS>
[PubMed]

Chadha, P., Katare, O. P., & Chhibber, S. (2016). In vivo efficacy of single phage versus phage cocktail in resolving burn wound infection in BALB/c mice. *Microbial Pathogenesis*, 99, 68–77. <https://doi.org/10.1016/j.MICPATH.2016.08.001>
[PubMed]

Chen, X., Liu, M., Zhang, P., et al. (2022). Phage-derived depolymerase as an antibiotic adjuvant against multidrug-resistant *Acinetobacter baumannii*. *Frontiers in Microbiology*, 13, 845500. <https://doi.org/10.3389/FMICB.2022.845500/BIBTEX>
[PubMed]

Chevallereau, A., Meaden, S., Fradet, O., et al. (2020). Exploitation of the cooperative behaviors of anti-CRISPR phages. *Cell Host & Microbe*, 27, 189–198.e6. <https://doi.org/10.1016/j.chom.2019.12.004>

Chevallereau, A., Pons, B. J., van Houte, S., & Westra, E. R. (2021). Interactions between bacterial and phage communities in natural environments. *Nature Reviews Microbiology*, 20(1), 49–62. <https://doi.org/10.1038/s41579-021-00602-y>
[PubMed]

Chin, W. H., Kett, C., Cooper, O., et al. (2022). Bacteriophages evolve enhanced persistence to a mucosal surface. *Proceedings of the National Academy of Sciences of the United States of America*, 119, e2116197119. https://doi.org/10.1073/PNAS.2116197119/SUPPL_FILE/PNAS.2116197119.SD06.TXT
[PubMed]

Cohen, D., Melamed, S., Millman, A., et al. (2019). Cyclic GMP–AMP signalling protects bacteria against viral infection. *Nature*, 574, 691–695. <https://doi.org/10.1038/s41586-019-1605-5>
[PubMed]

Colom, J., Cano-Sarabia, M., Otero, J., et al. (2015). Liposome-encapsulated bacteriophages for enhanced oral phage therapy against *Salmonella* spp. *Applied and Environmental Microbiology*, 81, 4841–4849. <https://doi.org/10.1128/AEM.00812-15/ASSET/E0B6EECC-63E9-47CC-BC29-E84C54F5176C/ASSETS/GRAPHIC/ZAM9991164030005.JPEG>
[PubMed]

Corbellino, M., Kieffer, N., Kutateladze, M., et al. (2020). Eradication of a multidrug-resistant, carbapenemase-producing *Klebsiella pneumoniae* isolate following oral and intra-rectal therapy

with a custom made, lytic bacteriophage preparation. *Clinical Infectious Diseases*, 70, 1998–2001. <https://doi.org/10.1093/CID/CIZ782> [PubMed]

Cuomo, P., Papaiani, M., Fulgione, A., et al. (2020). An innovative approach to control H. Pylori-induced persistent inflammation and colonization. *Microorganisms*, 8, 1–13. <https://doi.org/10.3390/microorganisms8081214>

Dąbrowska, K., & Abedon, S. T. (2019). Pharmacologically aware phage therapy: Pharmacodynamic and pharmacokinetic obstacles to phage antibacterial action in animal and human bodies. *Microbiology and Molecular Biology Reviews*, 83, e00012–e00019. <https://doi.org/10.1128/MMBR.00012-19/ASSET/33F9CFB1-8F30-48A5-9EFC-615FF75136DF/ASSETS/GRAPHIC/MMBR.00012-19-F0003.JPEG> [PubMed]

de Jonge, P. A., van den Born, B. J. H., Zwinderman, A. H., et al. (2024). Phylogeny and disease associations of a widespread and ancient intestinal bacteriophage lineage. *Nature Communications*, 15, 1–14. <https://doi.org/10.1038/s41467-024-50777-0>

de Jonge, P. A., Wortelboer, K., Scheithauer, T. P. M., et al. (2022). Gut virome profiling identifies a widespread bacteriophage family associated with metabolic syndrome. *Nature Communications*, 13, 1–15. <https://doi.org/10.1038/s41467-022-31390-5>

Dirksen, P., Assié, A., Zimmermann, J., et al. (2020). CeMbio - The Caenorhabditis elegans microbiome resource. *G3: Genes, Genomes, Genetics*, 10, 3025–3039. <https://doi.org/10.1534/G3.120.401309> [PubMed]

Draper, L. A., Ryan, F. J., Dalmaso, M., et al. (2020). Autochthonous faecal viral transfer (FVT) impacts the murine microbiome after antibiotic perturbation. *BMC Biology*, 18. <https://doi.org/10.1186/s12915-020-00906-0>

Duan, Y., Llorente, C., Lang, S., et al. (2019). Bacteriophage targeting of gut bacterium attenuates alcoholic liver disease. *Nature*, 575, 505–511. <https://doi.org/10.1038/s41586-019-1742-x> [PubMed]

Duan, Y., Young, R., & Schnabl, B. (2021). Bacteriophages and their potential for treatment of gastrointestinal diseases. *Nature Reviews. Gastroenterology & Hepatology*, 19, 135–144. <https://doi.org/10.1038/s41575-021-00536-z>

Dutilh, B. E., Cassman, N., McNair, K., et al. (2014). A highly abundant bacteriophage discovered in the unknown sequences of human faecal metagenomes. *Nature Communications*, 5, 1–11. <https://doi.org/10.1038/ncomms5498>

Džunková, M., Low, S. J., Daly, J. N., et al. (2019). Defining the human gut host–phage network through single-cell viral tagging. *Nature Microbiology*, 12(4), 2192–2203. <https://doi.org/10.1038/s41564-019-0526-2>

El-Maksoud, A., & Metwaly, M. (2016). *Biochemical changes associated with helicobacter pylori*

infection A B S T R A C T.

Eng, A., & Borenstein, E. (2016). An algorithm for designing minimal microbial communities with desired metabolic capacities. *Bioinformatics*, 32, 2008–2016. <https://doi.org/10.1093/BIOINFORMATICS/BTW107>
[PubMed]

Ermolaeva, M. A., & Schumacher, B. (2014). Insights from the worm: The *C. elegans* model for innate immunity. *Seminars in Immunology*, 26, 303–309. <https://doi.org/10.1016/J.SMIM.2014.04.005>
[PubMed]

Eugster, M. R., Haug, M. C., Huwiler, S. G., & Loessner, M. J. (2011). The cell wall binding domain of *Listeria* bacteriophage endolysin PlyP35 recognizes terminal GlcNAc residues in cell wall teichoic acid. *Molecular Microbiology*, 81, 1419–1432. <https://doi.org/10.1111/J.1365-2958.2011.07774.X>
[PubMed]

Eugster, M. R., & Loessner, M. J. (2012). Wall teichoic acids restrict access of bacteriophage endolysin Ply118, Ply511, and Plyp40 cell wall binding domains to the *listeria monocytogenes* peptidoglycan. *Journal of Bacteriology*, 194, 6498–6506. https://doi.org/10.1128/JB.00808-12/SUPPL_FILE/ZJB999092182S01.PDF
[PubMed]

Eun, C. S., Mishima, Y., Wohlgemuth, S., et al. (2014). Induction of bacterial antigen-specific colitis by a simplified human microbiota consortium in gnotobiotic interleukin-10-/- mice. *Infection and Immunity*, 82, 2239–2246. https://doi.org/10.1128/IAI.01513-13/SUPPL_FILE/ZII999090669S01.PDF
[PubMed]

Fazzino, L., Anisman, J., Chacón, J. M., et al. (2020). Lytic bacteriophage have diverse indirect effects in a synthetic cross-feeding community. *The ISME Journal*, 14, 123–134. <https://doi.org/10.1038/S41396-019-0511-Z>
[PubMed]

Febvre, H. P., Rao, S., Gindin, M., et al. (2019). PHAGE study: Effects of supplemental bacteriophage intake on inflammation and gut microbiota in healthy adults. *Nutrients*, 11, 666. <https://doi.org/10.3390/NU11030666>
[PubMed]

Federici, S., Kredo-Russo, S., Valdés-Mas, R., et al. (2022). Targeted suppression of human IBD-associated gut microbiota commensals by phage consortia for treatment of intestinal inflammation. *Cell*, 185, 2879–2898.e24. <https://doi.org/10.1016/J.CELL.2022.07.003/ASSET/531A9B42-ABAC-404D-A712-5128C0F7BD84/MAIN.ASSETS/FIGS9.JPG>
[PubMed]

Frye, K. A., Piamthai, V., Hsiao, A., & Degnan, P. H. (2021). Mobilization of vitamin B12 transporters alters competitive dynamics in a human gut microbe. *Cell Reports*, 37, 110164. <https://doi.org/10.1016/J.CELREP.2021.110164>

[[PubMed](#)]

Fujimoto, K., Hayashi, T., Yamamoto, M., et al. (2024). An enterococcal phage-derived enzyme suppresses graft-versus-host disease. *Nature*, 632, 174–181. <https://doi.org/10.1038/s41586-024-07667-8>

[[PubMed](#)]

Fujimoto, K., Kimura, Y., Shimohigoshi, M., et al. (2020). Metagenome data on intestinal phage-bacteria associations aids the development of phage therapy against pathobionts. *Cell Host & Microbe*, 28, 380–389.e9. <https://doi.org/10.1016/j.CHOM.2020.06.005/ATTACHMENT/0931DB8C-7F50-4B84-8914-0555B82C57FB/MMC2.PDF>

Fulgione, A., Ianniello, F., Papaiani, M., et al. (2019). Biomimetic hydroxyapatite nanocrystals are an active carrier for salmonella bacteriophages. *International Journal of Nanomedicine*, 14, 2219–2232. <https://doi.org/10.2147/IJN.S190188>

[[PubMed](#)]

Gaborieau, B., Vaysset, H., Tesson, F., et al. (2024). Prediction of strain level phage–host interactions across the Escherichia genus using only genomic information. *Nature Microbiology*, 11(9), 2847–2861. <https://doi.org/10.1038/s41564-024-01832-5>

Galtier, M., De Sordi, L., Sivignon, A., et al. (2017). Bacteriophages targeting adherent invasive Escherichia coli strains as a promising new treatment for Crohn's disease. *Journal of Crohn's & Colitis*, 11, 840–847. <https://doi.org/10.1093/ECCO-JCC/JJW224>

Gan, L., Feng, Y., Du, B., et al. (2023). Bacteriophage targeting microbiota alleviates non-alcoholic fatty liver disease induced by high alcohol-producing Klebsiella pneumoniae. *Nature Communications*, 14, 1–15. <https://doi.org/10.1038/s41467-023-39028-w>

Garmaeva, S., Sinha, T., Gulyaeva, A., et al. (2024). Transmission and dynamics of mother-infant gut viruses during pregnancy and early life. *Nature Communications*, 15, 1–19. <https://doi.org/10.1038/s41467-024-45257-4>

Gencay, Y. E., Jasinskytė, D., Robert, C., et al. (2023). Engineered phage with antibacterial CRISPR–Cas selectively reduce E. coli burden in mice. *Nature Biotechnology*, 42, 265–274. <https://doi.org/10.1038/s41587-023-01759-y>

[[PubMed](#)]

Glonti, T., Chanishvili, N., & Taylor, P. W. (2010). Bacteriophage-derived enzyme that depolymerizes the alginic acid capsule associated with cystic fibrosis isolates of Pseudomonas aeruginosa. *Journal of Applied Microbiology*, 108, 695–702. <https://doi.org/10.1111/J.1365-2672.2009.04469.X>

[[PubMed](#)]

Gogokhia, L., Buhrke, K., Bell, R., et al. (2019). Expansion of bacteriophages is linked to aggravated intestinal inflammation and colitis. *Cell Host & Microbe*, 25, 285–299.e8. <https://doi.org/10.1016/j.CHOM.2019.01.008/ATTACHMENT/681E284C-DD30-4006-8752-04493740F6AE/MMC2.PDF>

Gomez-Garcia, J., Chavez-Carbajal, A., Segundo-Arizmendi, N., et al. (2021). Efficacy of salmonella

bacteriophage s1 delivered and released by alginate beads in a chicken model of infection. *Viruses*, 13, 1932. <https://doi.org/10.3390/V13101932/S1>
[PubMed]

Gooch, H. C. C., Labedan, M., Hall, L. J., & Maxwell, A. (2024). Transplanting human infant gut microbiome species into *Galleria mellonella*. *BMC Research Notes*, 17, 1–7. <https://doi.org/10.1186/S13104-024-06785-W/FIGURES/3>

Gordillo Altamirano, F. L., & Barr, J. J. (2021). Unlocking the next generation of phage therapy: The key is in the receptors. *Current Opinion in Biotechnology*, 68, 115–123. <https://doi.org/10.1016/j.COPBIO.2020.10.002>
[PubMed]

Green, S. I., Liu, C. G., Yu, X., et al. (2021). Targeting of mammalian glycans enhances phage predation in the gastrointestinal tract. *MBio*, 12, 1–18. <https://doi.org/10.1128/MBIO.03474-20/ASSET/36769305-1DA4-4DAC-87A7-D76666E69316/ASSETS/IMAGES/MEDIUM/MBIO.03474-20-F0006.GIF>

Gregory, A. C., Zablocki, O., Zayed, A. A., et al. (2020). The gut Virome database reveals age-dependent patterns of Virome diversity in the human gut. *Cell Host & Microbe*, 28, 724–740.e8. <https://doi.org/10.1016/j.CHOM.2020.08.003/ASSET/12C4B866-BA02-4534-9044-1604B74D1EDD/MAIN.ASSETS/GR5.JPG>

Gu, J., Liu, X., Li, Y., et al. (2012). A method for generation phage cocktail with great therapeutic potential. *PLoS One*, 7, e31698. <https://doi.org/10.1371/JOURNAL.PONE.0031698>
[PubMed]

Guerin, E., Shkoporov, A. N., Stockdale, S. R., et al. (2021). Isolation and characterisation of Φ crAss002, a crAss-like phage from the human gut that infects *Bacteroides xylanisolvens*. *Microbiome*, 9, 1–21. <https://doi.org/10.1186/S40168-021-01036-7/FIGURES/1>

Gutiérrez, D., Briers, Y., Rodríguez-Rubio, L., et al. (2015). Role of the pre-neck appendage protein (Dpo7) from phage vB_SepiS-phiPLA7 as an anti-biofilm agent in staphylococcal species. *Frontiers in Microbiology*, 6, 167086. <https://doi.org/10.3389/FMICB.2015.01315/BIBTEX>

Gutiérrez, D., Martínez, B., Rodríguez, A., & García, P. (2012). Genomic characterization of two *Staphylococcus epidermidis* bacteriophages with anti-biofilm potential. *BMC Genomics*, 13, 1–10. <https://doi.org/10.1186/1471-2164-13-228/FIGURES/5>

Hall, A. R., De Vos, D., Friman, V. P., et al. (2012). Effects of sequential and simultaneous applications of bacteriophages on populations of *Pseudomonas aeruginosa* in vitro and in wax moth larvae. *Applied and Environmental Microbiology*, 78, 5646–5652. https://doi.org/10.1128/AEM.00757-12/SUPPL_FILE/ZAM999103522SO1.PDF
[PubMed]

Hampton, H. G., Watson, B. N. J., & Fineran, P. C. (2020). The arms race between bacteria and their phage foes. *Nature*, 577, 327–336. <https://doi.org/10.1038/s41586-019-1894-8>
[PubMed]

Hitch, T. C. A., Masson, J. M., Pauvert, C., et al. (2025). HiBC: A publicly available collection of bacterial strains isolated from the human gut. *Nature Communications*, 16, 4203. <https://doi.org/10.1038/S41467-025-59229-9>
[PubMed]

Ho, P., Chen, Y., Biswas, S., et al. (2023). Bacteriophage antidefense genes that neutralize TIR and STING immune responses. *Cell Reports*, 42, 112305. <https://doi.org/10.1016/j.celrep.2023.112305>
[PubMed]

Hobbs, S. J., Wein, T., Lu, A., et al. (2022). Phage anti-CBASS and anti-Pycsar nucleases subvert bacterial immunity. *Nature*, 605, 522–526. <https://doi.org/10.1038/s41586-022-04716-y>
[PubMed]

Hoffmann, C., Dollive, S., Grunberg, S., et al. (2013). Archaea and fungi of the human gut microbiome: Correlations with diet and bacterial residents. *PLoS One*, 8. <https://doi.org/10.1371/journal.pone.0066019>

Hong, Y., Thimmapuram, J., Zhang, J., et al. (2016). The impact of orally administered phages on host immune response and surrounding microbial communities. *Bacteriophage*, 6, e1211066. <https://doi.org/10.1080/21597081.2016.1211066>
[PubMed]

Hsu, B. B., Gibson, T. E., Yeliseyev, V., et al. (2019). Dynamic modulation of the gut microbiota and metabolome by bacteriophages in a mouse model. *Cell Host & Microbe*, 25, 803–814.e5. <https://doi.org/10.1016/j.CHOM.2019.05.001/ATTACHMENT/7E197EA9-16B7-4DE0-9650-2B443DFDA1B5/MMC2.PDF>

Hsu, B. B., Plant, I. N., Lyon, L., et al. (2020). In situ reprogramming of gut bacteria by oral delivery. *Nature Communications*, 11, 1–11. <https://doi.org/10.1038/s41467-020-18614-2>

Huttenhower, C., Gevers, D., Knight, R., et al. (2012). Structure, function and diversity of the healthy human microbiome. *Nature*, 486, 207–214. <https://doi.org/10.1038/nature11234>

Jado, I., López, R., García, E., et al. (2003). Phage lytic enzymes as therapy for antibiotic-resistant *Streptococcus pneumoniae* infection in a murine sepsis model. *Journal of Antimicrobial Chemotherapy*, 52, 967–973. <https://doi.org/10.1093/JAC/DKG485>
[PubMed]

Jennings, S. A. V., & Clavel, T. (2024). Annual review of animal biosciences synthetic communities of gut microbes for basic research and translational approaches in animal health and nutrition. *Annual Review of Animal Biosciences*, 12, 283–300. <https://doi.org/10.1146/ANNUREV-ANIMAL-021022-025552/CITE/REFWORKS>
[PubMed]

Jeon, J., Park, J. H., & Yong, D. (2019). Efficacy of bacteriophage treatment against carbapenem-resistant *Acinetobacter baumannii* in *Galleria mellonella* larvae and a mouse model of acute pneumonia. *BMC Microbiology*, 19, 1–14. <https://doi.org/10.1186/S12866-019-1443-5/FIGURES/6>

Jun, J. W., Shin, T. H., Kim, J. H., et al. (2014). Bacteriophage therapy of a *Vibrio parahaemolyticus* infection caused by a multiple-antibiotic-resistant O3:K6 pandemic clinical strain. *The Journal of Infectious Diseases*, 210, 72–78. <https://doi.org/10.1093/INFDIS/JIU059> [PubMed]

Junca, H., Steube, A., Mrowietz, S., et al. (2025). Bacterial and viral assemblages in ulcerative colitis patients following fecal microbiota and fecal filtrate transfer. *ISME Communications*, 5, 167. <https://doi.org/10.1093/ISMECO/YCAE167>

Kaur, S., Harjai, K., & Chhibber, S. (2014). Bacteriophage-aided intracellular killing of engulfed methicillin-resistant *Staphylococcus aureus* (MRSA) by murine macrophages. *Applied Microbiology and Biotechnology*, 98, 4653–4661. <https://doi.org/10.1007/S00253-014-5643-5/FIGURES/5> [PubMed]

Kenny, J. G., McGrath, S., Fitzgerald, G. F., & Van Sinderen, D. (2004). Bacteriophage Tuc2009 encodes a tail-associated cell wall-degrading activity. *Journal of Bacteriology*, 186, 3480–3491. <https://doi.org/10.1128/JB.186.11.3480-3491.2004/ASSET/A16010EC-C1C9-42F4-AFC3-A7D77338F50E/ASSETS/GRAPHIC/ZJB0110437330006.JPEG> [PubMed]

Khan Mirzaei, M., & Deng, L. (2022). New technologies for developing phage-based tools to manipulate the human microbiome. *Trends in Microbiology*, 30, 131–142. <https://doi.org/10.1016/j.tim.2021.04.007> [PubMed]

Khan Mirzaei, M., Khan, M. A. A., Ghosh, P., et al. (2020). Bacteriophages isolated from stunted children can regulate gut bacterial communities in an age-specific manner. *Cell Host & Microbe*, 27, 199–212.e5. <https://doi.org/10.1016/J.CHOM.2020.01.004>

Kieft, K., Breister, A. M., Huss, P., et al. (2021). Virus-associated organosulfur metabolism in human and environmental systems. *Cell Reports*, 36. https://doi.org/10.1016/J.CELREP.2021.109471/ASSET/0CEC96D9-7A47-4BCD-9BEB-562B0C105F57/MAIN.ASSETS/GR6_LRG.JPG

Kieft, K., Zhou, Z., & Anantharaman, K. (2020). VIBRANT: Automated recovery, annotation and curation of microbial viruses, and evaluation of viral community function from genomic sequences. *Microbiome*, 8, 1–23. <https://doi.org/10.1186/S40168-020-00867-0/TABLES/2>

Knecht, L. E., Veljkovic, M., & Fieseler, L. (2020). Diversity and function of phage encoded depolymerases. *Frontiers in Microbiology*, 10, 502215. <https://doi.org/10.3389/FMICB.2019.02949/BIBTEX>

Koncz, M., Stirling, T., Hadj Mehdi, H., et al. (2024). Genomic surveillance as a scalable framework for precision phage therapy against antibiotic-resistant pathogens. *Cell*, 187, 5901–5918.e28. <https://doi.org/10.1016/J.CELL.2024.09.009> [PubMed]

Kosznik-Kwaśnicka, K., Stasiłojć, M., Grabowski, Ł., et al. (2022). Efficacy and safety of phage therapy against salmonella enterica serovars typhimurium and enteritidis estimated by using a

battery of in vitro tests and the galleria mellonella animal model. *Microbiological Research*, 261, 127052. <https://doi.org/10.1016/J.MICRES.2022.127052>
[PubMed]

Krause, J. L., Schaepe, S. S., Fritz-Wallace, K., et al. (2020). Following the community development of SIHUMIx–a new intestinal in vitro model for bioreactor use. *Gut Microbes*, 11, 1116–1129. https://doi.org/10.1080/19490976.2019.1702431/SUPPL_FILE/KGMI_A_1702431_SM7376.ZIP
[PubMed]

Kretzer, J. W., Lehmann, R., Schmelcher, M., et al. (2007). Use of high-affinity cell wall-binding domains of bacteriophage endolysins for immobilization and separation of bacterial cells. *Applied and Environmental Microbiology*, 73, 1992–2000. <https://doi.org/10.1128/AEM.02402-06/ASSET/2DFD0776-B980-4B2B-9770-2EDC8722C26B/ASSETS/GRAPHIC/ZAM0060776210006.JPEG>
[PubMed]

Krut, O., & Bekeredjian-Ding, I. (2018). Contribution of the immune response to phage therapy. *The Journal of Immunology*, 200, 3037–3044. <https://doi.org/10.4049/JIMMUNOL.1701745>
[PubMed]

Kruzel, M. L., Zimecki, M., & Actor, J. K. (2017). Lactoferrin in a context of inflammation-induced pathology. *Frontiers in Immunology*, 8.

Kumar, N., Hitch, T. C. A., Haller, D., et al. (2021). MiMiC: A bioinformatic approach for generation of synthetic communities from metagenomes. *Microbial Biotechnology*, 14, 1757–1770. <https://doi.org/10.1111/1751-7915.13845>
[PubMed]

Kutateladze, M., & Adamia, R. (2008). Phage therapy experience at the Eliava Institute. *Médecine et Maladies Infectieuses*, 38, 426–430. <https://doi.org/10.1016/J.MEDMAL.2008.06.023>
[PubMed]

Labrie, S. J., Samson, J. E., & Moineau, S. (2010). Bacteriophage resistance mechanisms. *Nature Reviews. Microbiology*, 8, 317–327. <https://doi.org/10.1038/nrmicro2315>
[PubMed]

Landsberger, M., Gandon, S., Meaden, S., et al. (2018). Anti-CRISPR phages cooperate to overcome CRISPR-cas immunity. *Cell*, 174, 908–916.e12. <https://doi.org/10.1016/j.cell.2018.05.058>
[PubMed]

Lavigne, R., Briers, Y., Hertveldt, K., et al. (2004). Identification and characterization of a highly thermostable bacteriophage lysozyme. *Cellular and Molecular Life Sciences*, 61, 2753–2759. <https://doi.org/10.1007/S00018-004-4301-Y/METRICS>
[PubMed]

Laycock, G., Sait, L., Inman, C., et al. (2012). A defined intestinal colonization microbiota for gnotobiotic pigs. *Veterinary Immunology and Immunopathology*, 149, 216–224. <https://doi.org/10.1016/J.VETIMM.2012.07.004>
[PubMed]

Lengfelder, I., Sava, I. G., Hansen, J. J., et al. (2019). Complex bacterial consortia reprogram the colitogenic activity of enterococcus faecalis in a gnotobiotic mouse model of chronic, immune-mediated colitis. *Frontiers in Immunology*, 10, 1420. <https://doi.org/10.3389/FIMMU.2019.01420/BIBTEX>
[PubMed]

Li, T. T., Ling, L., Lin, M. C., et al. (2019). Properties and mechanism of hydroxyapatite coating prepared by electrodeposition on a braid for biodegradable bone scaffolds. *Nanomaterials*, 9. <https://doi.org/10.3390/nano9050679>

Liang, G., & Bushman, F. D. (2021). The human virome: Assembly, composition and host interactions. *Nature Reviews. Microbiology*, 19, 514–527. <https://doi.org/10.1038/s41579-021-00536-5>
[PubMed]

Lin, D. M., Koskella, B., Ritz, N. L., et al. (2019). Transplanting fecal virus-like particles reduces high-fat diet-induced small intestinal bacterial overgrowth in mice. *Frontiers in Cellular and Infection Microbiology*, 9, 474419. <https://doi.org/10.3389/FCIMB.2019.00348/BIBTEX>

Loeffler, J. M., & Fischetti, V. A. (2003). Synergistic lethal effect of a combination of phage lytic enzymes with different activities on penicillin-sensitive and -resistant *Streptococcus pneumoniae* strains. *Antimicrobial Agents and Chemotherapy*, 47, 375–377. <https://doi.org/10.1128/AAC.47.1.375-377.2003/ASSET/EDFAFFBC-CAE0-4E5F-A16C-8DA904DF2FA2/ASSETS/GRAPHIC/AC0130562003.JPEG>
[PubMed]

Loessner, M. J., Kramer, K., Ebel, F., & Scherer, S. (2002). C-terminal domains of listeria monocytogenes bacteriophage murein hydrolases determine specific recognition and high-affinity binding to bacterial cell wall carbohydrates. *Molecular Microbiology*, 44, 335–349. <https://doi.org/10.1046/j.1365-2958.2002.02889.x>
[PubMed]

Lou, Y. C., Chen, L. X., Borges, A. L., et al. (2024). Infant gut DNA bacteriophage strain persistence during the first 3 years of life. *Cell Host & Microbe*, 32, 35–47.e6. <https://doi.org/10.1016/j.chom.2023.11.015/ASSET/7DA0F7C2-629F-44A5-A796-038E5D11DD7E/MAIN.ASSETS/GR4.JPG>

Lourenço, M., Chaffringeon, L., Lamy-Besnier, Q., et al. (2020). The spatial heterogeneity of the gut limits predation and fosters coexistence of bacteria and bacteriophages. *Cell Host & Microbe*, 28, 390–401.e5. <https://doi.org/10.1016/j.chom.2020.06.002/ATTACHMENT/BB02AB48-FB26-43AF-9C9F-738A6E7D1AF7/MMC3.PDF>

Łusiak-Szelachowska, M., Zaczek, M., Weber-Dabrowska, B., et al. (2014). Phage neutralization by sera of patients receiving phage therapy. *Viral Immunology*, 27, 295. <https://doi.org/10.1089/VIM.2013.0128>
[PubMed]

Maestri, A., Pons, B. J., Pursey, E., et al. (2024). The bacterial defense system MADS interacts with CRISPR-Cas to limit phage infection and escape. *Cell Host & Microbe*, 32. <https://doi.org/10.1016/j.chom.2024.07.005>

Majewska, J., Beta, W., Lecion, D., et al. (2015). Oral application of T4 phage induces weak antibody production in the gut and in the blood. *Viruses*, 7, 4783–4799. <https://doi.org/10.3390/V7082845> [PubMed]

Malik, D. J., Sokolov, I. J., Vinner, G. K., et al. (2017). Formulation, stabilisation and encapsulation of bacteriophage for phage therapy. *Advances in Colloid and Interface Science*, 249, 100–133. <https://doi.org/10.1016/J.CIS.2017.05.014> [PubMed]

Mangalea, M. R., Paez-Espino, D., Kieft, K., et al. (2021). Individuals at risk for rheumatoid arthritis harbor differential intestinal bacteriophage communities with distinct metabolic potential. *Cell Host & Microbe*, 29, 726–739.e5. <https://doi.org/10.1016/J.CHOM.2021.03.020/ASSET/7D6FC15D-3EF6-4064-9BB5-2A9EBBBF867A/MAIN.ASSETS/GR6.JPG>

Manohar, P., Loh, B., Elangovan, N., et al. (2022). A multiwell-plate *Caenorhabditis elegans* assay for assessing the therapeutic potential of bacteriophages against clinical pathogens. *Microbiol Spectr*, 10, e01393–e01321. https://doi.org/10.1128/SPECTRUM.01393-21/SUPPL_FILE/REVIEWER-COMMENTS.PDF [PubMed]

Manohar, P., Loh, B., Turner, D., et al. (2024). In vitro and in vivo evaluation of the biofilm-degrading *Pseudomonas* phage Motto, as a candidate for phage therapy. *Frontiers in Microbiology*, 15, 1344962. <https://doi.org/10.3389/FMICB.2024.1344962/BIBTEX> [PubMed]

Manrique, P., Bolduc, B., Walk, S. T., et al. (2016). Healthy human gut phageome. *Proceedings of the National Academy of Sciences of the United States of America*, 113, 10400–10405. https://doi.org/10.1073/PNAS.1601060113/SUPPL_FILE/PNAS.201601060SI.PDF [PubMed]

Mao, X., Larsen, S. B., Zachariassen, L. S. F., et al. (2024). Transfer of modified gut viromes improves symptoms associated with metabolic syndrome in obese male mice. *Nature Communications*, 15. <https://doi.org/10.1038/s41467-024-49152-w>

Martinez-Soto, C. E., McClelland, M., Kropinski, A. M., et al. (2024). Multireceptor phage cocktail against *Salmonella enterica* to circumvent phage resistance. *microLife*, 5, 3. <https://doi.org/10.1093/FEMSML/UQAE003>

McCallin, S., Alam Sarker, S., Barretto, C., et al. (2013). Safety analysis of a Russian phage cocktail: From MetaGenomic analysis to oral application in healthy human subjects. *Virology*, 443, 187–196. <https://doi.org/10.1016/J.VIROL.2013.05.022> [PubMed]

Ménard, G., Rouillon, A., Cattoir, V., & Donnio, P. Y. (2021). *Galleria mellonella* as a suitable model of bacterial infection: Past, present and future. *Frontiers in Cellular and Infection Microbiology*, 11, 782733. <https://doi.org/10.3389/FCIMB.2021.782733/BIBTEX> [PubMed]

Menor-Flores, M., Vega-Rodríguez, M. A., & Molina, F. (2022). Computational design of phage

cocktails based on phage-bacteria infection networks. *Computers in Biology and Medicine*, 142, 105186. <https://doi.org/10.1016/j.COMPBIOMED.2021.105186>
[PubMed]

Mirza, K. A., Nietzsche, S., Makarewicz, O., et al. (2024). Bacteriophage-mediated decolonization of *Klebsiella pneumoniae* in a novel *Galleria mellonella* gut colonization model with Enterobacteriaceae. *Scientific Reports*, 14, 1–10. <https://doi.org/10.1038/s41598-023-50823-9>

Mirzaei, M. K., & Maurice, C. F. (2017). Ménage à trois in the human gut: Interactions between host, bacteria and phages. *Nature Reviews. Microbiology*, 15, 397–408. <https://doi.org/10.1038/nrmicro.2017.30>
[PubMed]

Modi, S. R., Lee, H. H., Spina, C. S., & Collins, J. J. (2013). Antibiotic treatment expands the resistance reservoir and ecological network of the phage metagenome. *Nature*, 7457(499), 219–222. <https://doi.org/10.1038/nature12212>

Møller, K. V., Wesseltøft, J. B., Malazarte, R., et al. (2023). Usage of cultured human fecal microbiota for colonization of *Caenorhabditis elegans* to study host–microbe interaction. *Applied Microbiology*, 3, 1130–1143. <https://doi.org/10.3390/APPLMICROBIOL3040078/S1>

Morse, M. L., Lederberg, E. M., & Lederberg, J. (1956). Transduction in *Escherichia coli* K-12. *Genetics*, 41, 142. <https://doi.org/10.1093/GENETICS/41.1.142>
[PubMed]

Murphy, J., Mahony, J., Ainsworth, S., et al. (2013). Bacteriophage orphan DNA methyltransferases: Insights from their bacterial origin, function, and occurrence. *Applied and Environmental Microbiology*, 79, 7547–7555. https://doi.org/10.1128/AEM.02229-13/SUPPL_FILE/ZAM999104965S01.PDF
[PubMed]

Mushtaq, N., Redpath, M. B., Luzio, J. P., & Taylor, P. W. (2005). Treatment of experimental *Escherichia coli* infection with recombinant bacteriophage-derived capsule depolymerase. *Journal of Antimicrobial Chemotherapy*, 56, 160–165. <https://doi.org/10.1093/JAC/DKI177>
[PubMed]

Nale, J. Y., Spencer, J., Hargreaves, K. R., et al. (2016). Bacteriophage combinations significantly reduce *Clostridium difficile* growth in vitro and proliferation in vivo. *Antimicrobial Agents and Chemotherapy*, 60, 968–981. <https://doi.org/10.1128/AAC.01774-15>
[PubMed]

Naureen, Z., Malacarne, D., Anpilogov, K., et al. (2020). Comparison between American and European legislation in the therapeutical and alimentary bacteriophage usage. *Acta Bio Medica: Atenei Parmensis*, 91, e2020023. <https://doi.org/10.23750/ABM.V91I113-S.10815>
[PubMed]

Nelson, D., Loomis, L., & Fischetti, V. A. (2001). Prevention and elimination of upper respiratory colonization of mice by group A streptococci by using a bacteriophage lytic enzyme. *Proceedings of the National Academy of Sciences of the United States of America*, 98, 4107–4112. <https://doi.org/10.1073/pnas.010500198>

[org/10.1073/PNAS.061038398/ASSET/4178C8F2-27C3-45D6-80C1-3EE50107DB38/ASSETS/GRAPHIC/PQ0610383003.JPEG](https://doi.org/10.1073/PNAS.061038398/ASSET/4178C8F2-27C3-45D6-80C1-3EE50107DB38/ASSETS/GRAPHIC/PQ0610383003.JPEG)

[PubMed]

Nelson, D., Schuch, R., Chahales, P., et al. (2006). PlyC: A multimeric bacteriophage lysin. *Proceedings of the National Academy of Sciences of the United States of America*, 103, 10765–10770. https://doi.org/10.1073/PNAS.0604521103/SUPPL_FILE/INDEX.HTML

[PubMed]

Nethery, M. A., Hidalgo-Cantabrana, C., Roberts, A., & Barrangou, R. (2022). CRISPR-based engineering of phages for in situ bacterial base editing. *Proceedings of the National Academy of Sciences of the United States of America*, 119, e2206744119. https://doi.org/10.1073/PNAS.2206744119/SUPPL_FILE/PNAS.2206744119.SAPP.PDF

[PubMed]

Nguyen, S., Baker, K., Padman, B. S., et al. (2017). Bacteriophage transcytosis provides a mechanism to cross epithelial cell layers. *MBio*, 8, 10.1128/MBIO.01874-17/ASSET/E014D127-9B0A-4571-933D-3B3E960B1CD0/ASSETS/GRAPHIC/MB00061736010007.JPEG.

[\[https://doi.org/10.1128/mBio.01874-17\]](https://doi.org/10.1128/mBio.01874-17)

Nicholson, J. K., Holmes, E., Kinross, J., et al. (2012). Host-gut microbiota metabolic interactions. *Science* (1979), 336, 1262–1267. https://doi.org/10.1126/SCIENCE.1223813/ASSET/6CB4C145-E6AE-4557-86F1-2BDD196B2064/ASSETS/GRAPHIC/336_1262_F1.JPEG

Nilsson, A. S. (2022). Cocktail, a computer program for modelling bacteriophage infection kinetics. *Viruses*, 14, 2483. <https://doi.org/10.3390/V14112483/S1>

[PubMed]

Niu, Y. D., Liu, H., Du, H., et al. (2021). Efficacy of individual bacteriophages does not predict efficacy of bacteriophage cocktails for control of Escherichia coli O157. *Frontiers in Microbiology*, 12, 616712. <https://doi.org/10.3389/FMICB.2021.616712/BIBTEX>

[PubMed]

Norman, J. M., Handley, S. A., Baldridge, M. T., et al. (2015). Disease-specific alterations in the enteric virome in inflammatory bowel disease. *Cell*, 160, 447–460. <https://doi.org/10.1016/J.CELL.2015.01.002/ATTACHMENT/15DA9038-8C5E-4EDF-9AF5-8823A7819FC9/MMC9.PDF>

[PubMed]

Offersen, S. M., Mao, X., Spiegelhauer, M. R., et al. (2024). Fecal virus-like particles are sufficient to reduce necrotizing enterocolitis. *Gut Microbes*, 16. <https://doi.org/10.1080/19490976.2024.2392876>

Olsen, N. M. C., Thiran, E., Hasler, T., et al. (2018). Synergistic removal of static and dynamic Staphylococcus aureus biofilms by combined treatment with a bacteriophage endolysin and a polysaccharide depolymerase. *Viruses*, 10, 438. <https://doi.org/10.3390/V10080438>

[PubMed]

Ott, S. J., Waetzig, G. H., Rehman, A., et al. (2017). Efficacy of sterile fecal filtrate transfer for treating patients with Clostridium difficile infection. *Gastroenterology*, 152, 799–811.e7. <https://doi.org/10.1053/j.gastro.2017.05.038>

doi.org/10.1053/J.GASTRO.2016.11.010
[PubMed]

Pan, Y. J., Lin, T. L., Lin, Y. T., et al. (2015). Identification of capsular types in carbapenem-resistant *klebsiella pneumoniae* strains by *wzc* sequencing and implications for capsule depolymerase treatment. *Antimicrobial Agents and Chemotherapy*, 59, 1038–1047. https://doi.org/10.1128/AAC.03560-14/SUPPL_FILE/ZAC002153679SO1.PDF
[PubMed]

Papaianni, M., Cuomo, P., Fulgione, A., et al. (2020). Bacteriophages promote metabolic changes in bacteria biofilm. *Microorganisms*, 8. <https://doi.org/10.3390/microorganisms8040480>

Parfrey, L. W., Walters, W. A., & Knight, R. (2011). Microbial eukaryotes in the human microbiome: Ecology, evolution, and future directions. *Frontiers in Microbiology*, 2.

Pargin, E., Roach, M. J., Skye, A., et al. (2023). The human gut virome: Composition, colonization, interactions, and impacts on human health. *Frontiers in Microbiology*, 14.

Paul, V., Rajagopalan, S., Sundarrajan, S., et al. (2011). A novel bacteriophage Tail-Associated Muralytic Enzyme (TAME) from Phage K and its development into a potent antistaphylococcal protein. *BMC Microbiology*, 11, 1–11. <https://doi.org/10.1186/1471-2180-11-226/FIGURES/8>

Penziner, S., Schooley, R. T., & Pride, D. T. (2021). Animal models of phage therapy. *Frontiers in Microbiology*, 12, 631794. <https://doi.org/10.3389/FMICB.2021.631794/BIBTEX>
[PubMed]

Pereira, F. C., Wasmund, K., Cobankovic, I., et al. (2020). Rational design of a microbial consortium of mucosal sugar utilizers reduces *Clostridiodes difficile* colonization. *Nature Communications*, 11, 1–15. <https://doi.org/10.1038/s41467-020-18928-1>

Pinilla-Redondo, R., Shehreen, S., Marino, N. D., et al. (2020). Discovery of multiple anti-CRISPRs highlights anti-defense gene clustering in mobile genetic elements. *Nature Communications*, 11, 1–11. <https://doi.org/10.1038/s41467-020-19415-3>

Pires, D. P., Oliveira, H., Melo, L. D. R., et al. (2016). Bacteriophage-encoded depolymerases: Their diversity and biotechnological applications. *Applied Microbiology and Biotechnology*, 100, 2141–2151. <https://doi.org/10.1007/S00253-015-7247-0>
[PubMed]

Pukkila-Worley, R., & Ausubel, F. M. (2012). Immune defense mechanisms in the *Caenorhabditis elegans* intestinal epithelium. *Current Opinion in Immunology*, 24, 3. <https://doi.org/10.1016/J.COI.2011.10.004>
[PubMed]

Qin, S., Liu, Y., Chen, Y., et al. (2022). Engineered bacteriophages containing anti-CRISPR suppress infection of antibiotic-resistant *P. aeruginosa*. *Microbiol Spectr*, 10, e01602–e01622. https://doi.org/10.1128/SPECTRUM.01602-22/SUPPL_FILE/SPECTRUM.01602-22-S0001.PDF
[PubMed]

Rajesh, T., Anthony, T., Saranya, S., et al. (2011). Functional characterization of a new holin-like antibacterial protein coding gene *tmp1* from goat skin surface metagenome. *Applied Microbiology and Biotechnology*, 89, 1061–1073. <https://doi.org/10.1007/S00253-010-2907-6/FIGURES/8> [PubMed]

Ramaboli, M. C., Ocvirk, S., Khan Mirzaei, M., et al. (2024). Diet changes due to urbanization in South Africa are linked to microbiome and metabolome signatures of Westernization and colorectal cancer. *Nature Communications*, 15, 1–12. <https://doi.org/10.1038/s41467-024-46265-0>

Rashel, M., Uchiyama, J., Ujihara, T., et al. (2007). Efficient elimination of multidrug-resistant *Staphylococcus aureus* by cloned lysin derived from bacteriophage ϕ MR11. *The Journal of Infectious Diseases*, 196, 1237–1247. <https://doi.org/10.1086/521305> [PubMed]

Rasmussen, T. S., Mao, X., Forster, S., et al. (2024). Overcoming donor variability and risks associated with fecal microbiota transplants through bacteriophage-mediated treatments. *Microbiome*, 12, 1–24. <https://doi.org/10.1186/S40168-024-01820-1>

Rasmussen, T. S., Mentzel, C. M. J., Kot, W., et al. (2020). Faecal virome transplantation decreases symptoms of type 2 diabetes and obesity in a murine model. *Gut*, 69, 2122–2130. <https://doi.org/10.1136/gutjnl-2019-320005> [PubMed]

Reyes, A., Blanton, L. V., Cao, S., et al. (2015). Gut DNA viromes of Malawian twins discordant for severe acute malnutrition. *Proceedings of the National Academy of Sciences of the United States of America*, 112, 11941–11946. https://doi.org/10.1073/PNAS.1514285112/SUPPL_FILE/PNAS.1514285112.ST06.XLSX [PubMed]

Reyes, A., Haynes, M., Hanson, N., et al. (2010). Viruses in the faecal microbiota of monozygotic twins and their mothers. *Nature*, 466, 334–338. <https://doi.org/10.1038/nature09199> [PubMed]

Ring, C., Klopfeisch, R., Dahlke, K., et al. (2019). Akkermansia muciniphila strain ATCC BAA-835 does not promote short-term intestinal inflammation in gnotobiotic interleukin-10-deficient mice. *Gut Microbes*, 10, 188–203. https://doi.org/10.1080/19490976.2018.1511663/SUPPL_FILE/KGMI_A_1511663_SM5450.DOCX [PubMed]

Rodas, P. I., Trombert, A. N., & Mora, G. C. (2011). A holin remnant protein encoded by STY1365 is involved in envelope stability of *Salmonella enterica* serovar Typhi. *FEMS Microbiology Letters*, 321, 58–66. <https://doi.org/10.1111/J.1574-6968.2011.02310.X> [PubMed]

Rodríguez, L., Martínez, B., Zhou, Y., et al. (2011). Lytic activity of the virion-associated peptidoglycan hydrolase HydH5 of *Staphylococcus aureus* bacteriophage vB-SauS-phiPLA88. *BMC Microbiology*, 11, 1–11. <https://doi.org/10.1186/1471-2180-11-138/FIGURES/6>

Rodríguez-Rubio, L., Martínez, B., Donovan, D. M., et al. (2013). Bacteriophage virion-associated peptidoglycan hydrolases: Potential new enzybiotics. *Critical Reviews in Microbiology*, 39, 427–434. <https://doi.org/10.3109/1040841X.2012.723675>
[PubMed]

Rodríguez-Rubio, L., Martínez, B., Rodríguez, A., et al. (2012). Enhanced staphylococcal activity of the Staphylococcus aureus bacteriophage vB_SauS-phiIPLA88 HydH5 virion-associated peptidoglycan hydrolase: Fusions, deletions, and synergy with LysH5. *Applied and Environmental Microbiology*, 78, 2241. <https://doi.org/10.1128/AEM.07621-11>
[PubMed]

Rogala, A. R., Oka, A., & Sartor, R. B. (2020). Strategies to dissect host-microbial immune interactions that determine mucosal homeostasis vs. intestinal inflammation in gnotobiotic mice. *Frontiers in Immunology*, 11, 214. <https://doi.org/10.3389/FIMMU.2020.00214/BIBTEX>
[PubMed]

Rotman, E., McClure, S., Glazier, J., et al. (2024). Rapid design of bacteriophage cocktails to suppress the burden and virulence of gut-resident carbapenem-resistant Klebsiella pneumoniae. *Cell Host & Microbe*, 32, 1988–2003.e8. <https://doi.org/10.1016/j.CHOM.2024.09.004>

Roux, S., Enault, F., Hurwitz, B. L., & Sullivan, M. B. (2015). VirSorter: Mining viral signal from microbial genomic data. *PeerJ*, 2015, e985. <https://doi.org/10.7717/PEERJ.985/SUPP-2>

Ru, J., Khan Mirzaei, M., Xue, J., et al. (2023). ViroProfiler: a containerized bioinformatics pipeline for viral metagenomic data analysis. *Gut Microbes*, 15. <https://doi.org/10.1080/19490976.2023.2192522>

Rusinov, I. S., Ershova, A. S., Karyagina, A. S., et al. (2018). Avoidance of recognition sites of restriction-modification systems is a widespread but not universal anti-restriction strategy of prokaryotic viruses. *BMC Genomics*, 19, 1–11. <https://doi.org/10.1186/S12864-018-5324-3/FIGURES/4>

Sarker, S. A., Berger, B., Deng, Y., et al. (2017). Oral application of Escherichia coli bacteriophage: Safety tests in healthy and diarrheal children from Bangladesh. *Environmental Microbiology*, 19, 237–250. <https://doi.org/10.1111/1462-2920.13574>
[PubMed]

Sarker, S. A., McCallin, S., Barretto, C., et al. (2012). Oral T4-like phage cocktail application to healthy adult volunteers from Bangladesh. *Virology*, 434, 222–232. <https://doi.org/10.1016/j.VIROL.2012.09.002>
[PubMed]

Sarker, S. A., Sultana, S., Reuteler, G., et al. (2016). Oral phage therapy of acute bacterial Diarrhea with two coliphage preparations: A randomized trial in children from Bangladesh. *eBioMedicine*, 4, 124–137. <https://doi.org/10.1016/j.ebiom.2015.12.023>
[PubMed]

Sausset, R., Petit, M. A., Gaboriau-Routhiau, V., & De Paepe, M. (2020). New insights into intestinal phages. *Mucosal Immunology*, 13, 205–215. <https://doi.org/10.1038/s41385-019-0250-5>

[PubMed]

Sberro, H., Leavitt, A., Kiro, R., et al. (2013). Discovery of functional toxin/antitoxin systems in bacteria by shotgun cloning. *Molecular Cell*, 50, 136–148. <https://doi.org/10.1016/j.molcel.2013.02.002>

[PubMed]

Schäpe, S. S., Krause, J. L., Engelmann, B., et al. (2019). The simplified human intestinal microbiota (SIHUMIX) shows high structural and functional resistance against changing transit times in in vitro bioreactors. *Microorganisms*, 7, 641. <https://doi.org/10.3390/MICROORGANISMS7120641>

[PubMed]

Schaubeck, M., Clavel, T., Calasan, J., et al. (2016). Dysbiotic gut microbiota causes transmissible Crohn's disease-like ileitis independent of failure in antimicrobial defence. *Gut*, 65, 225–237. <https://doi.org/10.1136/GUTJNL-2015-309333/-/DC1>

[PubMed]

Schmelcher, M., Donovan, D. M., & Loessner, M. J. (2012a). Bacteriophage endolysins as novel antimicrobials. *Future Microbiology*, 7, 1147–1171. <https://doi.org/10.2217/FMB.12.97>

[PubMed]

Schmelcher, M., Powell, A. M., Becker, S. C., et al. (2012b). Chimeric phage lysins act synergistically with lysostaphin to kill mastitis-causing *Staphylococcus aureus* in murine mammary glands.

Applied and Environmental Microbiology, 78, 2297–2305. https://doi.org/10.1128/AEM.07050-11/SUPPL_FILE/AEM-AEM07050-11-S01.PDF

[PubMed]

Schmelcher, M., Shabarova, T., Eugster, M. R., et al. (2010). Rapid multiplex detection and differentiation of listeria cells by use of fluorescent phage endolysin cell wall binding domains.

Applied and Environmental Microbiology, 76, 5745–5756. https://doi.org/10.1128/AEM.00801-10/SUPPL_FILE/AEM00801_10_SCHMELCHER ET AL SUPPLEMENTARY MATERIAL TABLE S1.DOC

[PubMed]

Schmerer, M., Molineux, I. J., & Bull, J. J. (2014). Synergy as a rationale for phage therapy using phage cocktails. *PeerJ*, 2014, e590. <https://doi.org/10.7717/PEERJ.590/SUPP-1>

Schooley, R. T., Biswas, B., Gill, J. J., et al. (2017). Development and use of personalized bacteriophage-based therapeutic cocktails to treat a patient with a disseminated resistant *Acinetobacter baumannii* infection. *Antimicrobial Agents and Chemotherapy*, 61, e00954–e00917. https://doi.org/10.1128/AAC.00954-17/SUPPL_FILE/ZAC010176597S1.PDF

[PubMed]

Shaffer, M., Borton, M. A., McGivern, B. B., et al. (2020). DRAM for distilling microbial metabolism to automate the curation of microbiome function. *Nucleic Acids Research*, 48, 8883–8900. <https://doi.org/10.1093/NAR/GKAA621>

[PubMed]

Shi, Y., Li, N., Yan, Y., et al. (2012). Combined antibacterial activity of phage lytic proteins holin and

lysin from streptococcus suis bacteriophage SMP. *Current Microbiology*, 65, 28–34. <https://doi.org/10.1007/S00284-012-0119-2/FIGURES/6>
[PubMed]

Shkoporov, A. N., Clooney, A. G., Sutton, T. D. S., et al. (2019). The human gut Virome is highly diverse, stable, and individual specific. *Cell Host & Microbe*, 26, 527–541.e5. <https://doi.org/10.1016/J.CHOM.2019.09.009/ATTACHMENT/20A3C693-F51F-4525-89E5-721364CC63D6/MMC5.PDF>

Sholeh, M., Krutova, M., Forouzesh, M., et al. (2020). Antimicrobial resistance in Clostridioides (Clostridium) difficile derived from humans: A systematic review and meta-analysis. *Antimicrobial Resistance and Infection Control*, 9, 1–11. <https://doi.org/10.1186/S13756-020-00815-5/FIGURES/2>

Silva Batalha, L., Pardini Gontijo, M. T., Vianna Novaes de Carvalho Teixeira, A., et al. (2021). Encapsulation in alginate-polymers improves stability and allows controlled release of the UFV-AREG1 bacteriophage. *Food Research International*, 139, 109947. <https://doi.org/10.1016/J.FOODRES.2020.109947>
[PubMed]

Singla, S., Harjai, K., Katare, O. P., & Chhibber, S. (2016). Encapsulation of bacteriophage in liposome accentuates its entry in to macrophage and shields it from neutralizing antibodies. *PLoS One*, 11, e0153777. <https://doi.org/10.1371/JOURNAL.PONE.0153777>
[PubMed]

Sinha, A., Li, Y., Mirzaei, M. K., et al. (2022). Transplantation of bacteriophages from ulcerative colitis patients shifts the gut bacteriome and exacerbates the severity of DSS colitis. *Microbiome*, 10, 1–23. <https://doi.org/10.1186/S40168-022-01275-2>

Song, W., Sun, H. X., Zhang, C., et al. (2019). Prophage hunter: An integrative hunting tool for active prophages. *Nucleic Acids Research*, 47, W74–W80. <https://doi.org/10.1093/NAR/GKZ380>
[PubMed]

Stellfox, M. E., Fernandes, C., Shields, R. K., et al. (2024). Bacteriophage and antibiotic combination therapy for recurrent Enterococcus faecium bacteremia. *MBio*, 15. https://doi.org/10.1128/MBIO.03396-23/SUPPL_FILE/MBIO.03396-23-S0002.XLSX

Sudiarta, I. P., Fukushima, T., & Sekiguchi, J. (2010). Bacillus subtilis CwlP of the Sp-β prophage has two novel peptidoglycan hydrolase domains, muramidase and cross-linkage digesting DD-endopeptidase. *Journal of Biological Chemistry*, 285, 41232–41243. <https://doi.org/10.1074/jbc.M110.156273>
[PubMed]

Sumrall, E. T., Hofstee, M. I., Arens, D., et al. (2021). An enzybiotic regimen for the treatment of methicillin-resistant staphylococcus aureus orthopaedic device-related infection. *Antibiotics*, 10, 1186. <https://doi.org/10.3390/ANTIBIOTICS10101186/S1>
[PubMed]

Sweere, J. M., Van Belleghem, J. D., Ishak, H., et al. (2019). Bacteriophage trigger antiviral immunity

and prevent clearance of bacterial infection. *Science*, 1979, 363. https://doi.org/10.1126/SCIENCE.AAT9691/SUPPL_FILE/AAT9691S1.MOV

Takáč, M., & Bläsi, U. (2005). Phage P68 virion-associated protein 17 displays activity against clinical isolates of *Staphylococcus aureus*. *Antimicrobial Agents and Chemotherapy*, 49, 2934–2940. <https://doi.org/10.1128/AAC.49.7.2934-2940.2005/ASSET/72F6EBEB-6210-4C95-958A-34F78AC7DC31/ASSETS/GRAPHIC/ZAC0070550410005.JPEG>
[PubMed]

Tanji, Y., Shimada, T., Yoichi, M., et al. (2004). Toward rational control of *Escherichia coli* O157:H7 by a phage cocktail. *Applied Microbiology and Biotechnology*, 64, 270–274. <https://doi.org/10.1007/S00253-003-1438-9/FIGURES/3>
[PubMed]

Tetz, G., Brown, S. M., Hao, Y., & Tetz, V. (2018). Parkinson's disease and bacteriophages as its overlooked contributors. *Scientific Reports*, 8, 1–11. <https://doi.org/10.1038/s41598-018-29173-4>

Tiamani, K., Luo, S., Schulz, S., et al. (2022). The role of virome in the gastrointestinal tract and beyond. *FEMS Microbiology Reviews*, 46, 1–12. <https://doi.org/10.1093/FEMSRE/FUAC027>

Tian, Y., Wu, M., Liu, X., et al. (2015). Probing the endocytic pathways of the filamentous bacteriophage in live cells using ratiometric pH fluorescent indicator. *Advanced Healthcare Materials*, 4, 413–419. <https://doi.org/10.1002/ADHM.201400508>
[PubMed]

Titecat, M., Rousseaux, C., Dubuquoy, C., et al. (2022). Safety and efficacy of an AIEC-targeted bacteriophage cocktail in a mice colitis model. *Journal of Crohn's & Colitis*, 16, 1617–1627. <https://doi.org/10.1093/ECCO-JCC/JJAC064>

van der Lelie, D., Oka, A., Taghavi, S., et al. (2021). Rationally designed bacterial consortia to treat chronic immune-mediated colitis and restore intestinal homeostasis. *Nature Communications*, 12, 1–17. <https://doi.org/10.1038/s41467-021-23460-x>

Van Nieuwenhuysse, B., Van der Linden, D., Chatzis, O., et al. (2022). Bacteriophage-antibiotic combination therapy against extensively drug-resistant *Pseudomonas aeruginosa* infection to allow liver transplantation in a toddler. *Nature Communications*, 13, 1–12. <https://doi.org/10.1038/s41467-022-33294-w>

Vipra, A. A., Desai, S. N., Roy, P., et al. (2012). Antistaphylococcal activity of bacteriophage derived chimeric protein P128. *BMC Microbiology*, 12, 1–9. <https://doi.org/10.1186/1471-2180-12-41/TABLES/4>

von Strepel, A., Weiss, A. S., Wittmann, J., et al. (2023). Bacteriophages targeting protective commensals impair resistance against *Salmonella Typhimurium* infection in gnotobiotic mice. *PLoS Pathogens*, 19, e1011600. <https://doi.org/10.1371/JOURNAL.PPAT.1011600>

Walker, A. C., Bhargava, R., Vaziriyan-Sani, A. S., et al. (2021). Colonization of the *Caenorhabditis elegans* gut with human enteric bacterial pathogens leads to proteostasis disruption that is rescued

by butyrate. *PLoS Pathogens*, 17, e1009510. <https://doi.org/10.1371/JOURNAL.PPAT.1009510> [PubMed]

Walters, W. A., Granados, A. C., Ley, C., et al. (2023). Longitudinal comparison of the developing gut virome in infants and their mothers. *Cell Host & Microbe*, 31, 187–198.e3. <https://doi.org/10.1016/j.chom.2023.01.003>

Wang, H., Liu, Y., Bai, C., & Leung, S. S. Y. (2024). Translating bacteriophage-derived depolymerases into antibacterial therapeutics: Challenges and prospects. *Acta Pharmaceutica Sinica B*, 14, 155–169. <https://doi.org/10.1016/j.APSB.2023.08.017> [PubMed]

Wang, X., Han, L., Rong, J., et al. (2022a). Endolysins of bacteriophage vB_Sal-S-S10 can naturally lyse *Salmonella enteritidis*. *BMC Veterinary Research*, 18, 1–11. <https://doi.org/10.1186/S12917-022-03514-Y/FIGURES/7>

Wang, Y., Li, X., Dance, D. A. B., et al. (2022b). A novel lytic phage potentially effective for phage therapy against *Burkholderia pseudomallei* in the tropics. *Infectious Diseases of Poverty*, 11, 1–13. <https://doi.org/10.1186/S40249-022-01012-9/FIGURES/8>

Warwick-Dugdale, J., Buchholz, H. H., Allen, M. J., & Temperton, B. (2019). Host-hijacking and planktonic piracy: How phages command the microbial high seas. *Virology Journal*, 16(1), 1–13. <https://doi.org/10.1186/S12985-019-1120-1>

Watari, J., Chen, N., Amenta, P. S., et al. (2014). *Helicobacter pylori* associated chronic gastritis, clinical syndromes, precancerous lesions, and pathogenesis of gastric cancer development. *World Journal of Gastroenterology*, 20, 5461. <https://doi.org/10.3748/WJG.V20.I18.5461> [PubMed]

Wintachai, P., Naknaen, A., Thammaphet, J., et al. (2020). Characterization of extended-spectrum- β -lactamase producing *Klebsiella pneumoniae* phage KP1801 and evaluation of therapeutic efficacy in vitro and in vivo. *Scientific Reports*, 10, 1–18. <https://doi.org/10.1038/S41598-020-68702-Y/FIGURES/8>

Wishart, D. S., Han, S., Saha, S., et al. (2023). PHASTEST: Faster than PHASTER, better than PHAST. *Nucleic Acids Research*, 51, W443–W450. <https://doi.org/10.1093/NAR/GKAD382> [PubMed]

Wong, C. C., & Yu, J. (2023). Gut microbiota in colorectal cancer development and therapy. *Nature Reviews. Clinical Oncology*, 20, 429–452. <https://doi.org/10.1038/s41571-023-00766-x> [PubMed]

Wortelboer, K., de Jonge, P. A., Scheithauer, T. P. M., et al. (2023). Phage-microbe dynamics after sterile faecal filtrate transplantation in individuals with metabolic syndrome: A double-blind, randomised, placebo-controlled clinical trial assessing efficacy and safety. *Nature Communications*, 14. <https://doi.org/10.1038/s41467-023-41329-z>

Wright, R. C. T., Friman, V. P., Smith, M. C. M., & Brockhurst, M. A. (2019). Resistance evolution against phage combinations depends on the timing and order of exposure. *MBio*, 10. <https://doi.org/10.1128/mbio.01012-19>

[org/10.1128/MBIO.01652-19/SUPPL_FILE/MBIO.01652-19-SF001.PDF](https://doi.org/10.1128/MBIO.01652-19/SUPPL_FILE/MBIO.01652-19-SF001.PDF)

Wylensek, D., Hitch, T. C. A., Riedel, T., et al. (2020). A collection of bacterial isolates from the pig intestine reveals functional and taxonomic diversity. *Nature Communications*, 11, 1–26. <https://doi.org/10.1038/s41467-020-19929-w>

Yang, Q., Le, S., Zhu, T., & Wu, N. (2023). Regulations of phage therapy across the world. *Frontiers in Microbiology*, 14, 1250848. <https://doi.org/10.3389/FMICB.2023.1250848> [PubMed]

Yang, Y., Shen, W., Zhong, Q., et al. (2020). Development of a bacteriophage cocktail to constrain the emergence of phage-resistant *Pseudomonas aeruginosa*. *Frontiers in Microbiology*, 11, 515123. <https://doi.org/10.3389/FMICB.2020.00327/BIBTEX>

Ye, J., Meng, Q., Jin, K., et al. (2024). Phage cocktail alleviated type 2 diabetes by reshaping gut microbiota and decreasing proinflammatory cytokines. *Applied Microbiology and Biotechnology*, 108, 1–14. <https://doi.org/10.1007/S00253-023-12912-7/FIGURES/5>

Yehl, K., Lemire, S., Yang, A. C., et al. (2019). Engineering phage host-range and suppressing bacterial resistance through phage tail fiber mutagenesis. *Cell*, 179, 459–469.e9. <https://doi.org/10.1016/J.CELL.2019.09.015> [PubMed]

Yin, H., Li, J., Huang, H., et al. (2021). Microencapsulated phages show prolonged stability in gastrointestinal environments and high therapeutic efficiency to treat *Escherichia coli* O157:H7 infection. *Veterinary Research*, 52, 118. <https://doi.org/10.1186/S13567-021-00991-1/FIGURES/6> [PubMed]

Yoong, P., Schuch, R., Nelson, D., & Fischetti, V. A. (2004). Identification of a broadly active phage lytic enzyme with lethal activity against antibiotic-resistant *Enterococcus faecalis* and *Enterococcus faecium*. *Journal of Bacteriology*, 186, 4808–4812. <https://doi.org/10.1128/JB.186.14.4808-4812.2004/ASSET/4B5137F5-3F2A-457C-900D-53233C51E18B/ASSETS/GRAPHIC/ZJB0140438430003.JPEG> [PubMed]

Young, R. (2014). Phage lysis: Three steps, three choices, one outcome. *Journal of Microbiology*, 52, 243–258. <https://doi.org/10.1007/S12275-014-4087-Z/METRICS> [PubMed]

Yuan, Y., Wang, L., Li, X., et al. (2019). Efficacy of a phage cocktail in controlling phage resistance development in multidrug resistant *Acinetobacter baumannii*. *Virus Research*, 272, 197734. <https://doi.org/10.1016/J.VIRUSRES.2019.197734> [PubMed]

Zenner, C., Hitch, T. C. A., Riedel, T., et al. (2021). Early-life immune system maturation in chickens using a synthetic community of cultured gut bacteria. *mSystems*, 6. https://doi.org/10.1128/MSYSTEMS.01300-20/SUPPL_FILE/MSYSTEMS.01300-20-ST001.XLSX

Zhang, F., Berg, M., Dierking, K., et al. (2017). *Caenorhabditis elegans* as a model for microbiome research. *Frontiers in Microbiology*, 8, 485. <https://doi.org/10.3389/FMICB.2017.00485>
[PubMed]

Zhang, F., Weckhorst, J. L., Assié, A., et al. (2021). Natural genetic variation drives microbiome selection in the *Caenorhabditis elegans* gut. *Current Biology*, 31, 2603–2618.e9. <https://doi.org/10.1016/J.CUB.2021.04.046>
[PubMed]

Zhang, H., Zhang, X., Liang, S., et al. (2023). Bactericidal synergism between phage endolysin Ply2660 and cathelicidin LL-37 against vancomycin-resistant *Enterococcus faecalis* biofilms. *NPJ Biofilms Microbiomes*, 9, 16. <https://doi.org/10.1038/S41522-023-00385-5>
[PubMed]

Zhang, Q., Widmer, G., & Tzipori, S. (2013). A pig model of the human gastrointestinal tract. *Gut Microbes*, 4, 193–200. <https://doi.org/10.4161/GMIC.23867>
[PubMed]

Zheng, D. W., Dong, X., Pan, P., et al. (2019). Phage-guided modulation of the gut microbiota of mouse models of colorectal cancer augments their responses to chemotherapy. *Nature Biomedical Engineering*, 3, 717–728. <https://doi.org/10.1038/s41551-019-0423-2>
[PubMed]

Zhu, Y., Jameson, E., Crosatti, M., et al. (2014). Carnitine metabolism to trimethylamine by an unusual Rieske-type oxygenase from human microbiota. *Proceedings of the National Academy of Sciences of the United States of America*, 111, 4268–4273. <https://doi.org/10.1073/pnas.1316569111>
[PubMed]