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Microbial genes and pathways in inflammatory bowel disease

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Abstract

Perturbations in the intestinal microbiome are implicated in inflammatory bowel disease (IBD). Studies of treatment-naïve patients have identified microbial taxa associated with disease course and treatment efficacy. In order to gain a mechanistic understanding of how the microbiome impacts gastrointestinal health, we need to move from census to function. Bacteria including those that adhere to epithelial cells as well as several *Clostridium* species can alter differentiation of T helper 17 cells and regulatory T cells. Similarly, microbial products such as short-chain fatty acids and sphingolipids also influence immune responses. Metagenomics and culturomics have identified strains of *Ruminococcus gnavus* and adherent invasive *Escherichia coli* that are linked to IBD and gut inflammation. Integrated analysis of multi-omics data, including metagenomics, metatranscriptomics, and metabolomics, with measurements of host response and culturomics have great potential in understanding the role of the microbiome in IBD. In this Review, we highlight current knowledge of gut microbial factors linked to IBD pathogenesis and discuss how multi-omics data from large-scale population studies in health and disease have been used to identify specific microbial strains, transcriptional changes, and metabolic alterations associated with IBD.

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Author contributions

M.S., A.G., H.V., and R.J.X. conceived and wrote the article. All authors substantially contributed to discussion of content and reviewed/edited the manuscript before submission. M.S. generated Figure 2.

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Introduction

The intestinal microbiota has a major role in human health, including the maturation and education of host immune responses, protection against enteric pathogen proliferation, and response to or modification of specific drugs. Host physiology can be altered at the cellular level by microbiome-induced cell signaling, proliferation and neurotransmitter biosynthesis^{1,2}, leading to mucosal and systemic alterations and thereby affecting homeostasis, barrier function, innate and adaptive immune responses and metabolism²⁻⁴. Through microbial metabolites and their impact on dietary breakdown, the microbiota provides energy and vitamins for the host⁵. Furthermore, it alters therapeutic drug availability⁶ and modifies bile salts impacting a variety of host functions⁶. With such a broad range of effects on host physiology and its role in the induction, education and function of the immune system, it is unsurprising that the microbiota is implicated in gut-related diseases. Examples include inflammatory bowel disease (IBD) and metabolic disorders⁷⁻¹⁰ as well as diseases that affect other systems including atherosclerosis¹¹⁻¹³, autism¹⁴, asthma^{15,16}, and type I diabetes^{17,18}. Although the microbiome is dysregulated in these conditions, causal roles have yet to be determined in most diseases, and whether the microbiome drives disease or is altered in response to disease pathology remains unclear.

IBD, which includes Crohn's disease (CD) and ulcerative colitis (UC), is a model for the study of microbiome-related diseases (Box 1). IBD risk is linked to 200 host genetic loci, most of which are associated with key immunological pathways including innate immunity (for example, the sensor *NOD2* detects bacterial peptidoglycans), immune responses (for example, *IL23R* allele rs11209026 provides protective effect in CD), and autophagy (for example, the product of *ATG16L1* affects autophagy in Paneth cells and goblet cells)¹⁹⁻²¹. Since 1950, a drastic increase of IBD has been observed in Western countries. Incidence rates of IBD have been stable or falling since 1990; however, disease burden remains high with prevalence surpassing 0.3% in the Western world. As many newly industrialized countries are becoming more westernized and urbanized, IBD rates are rising there as well²². These patterns suggest that other factors besides host genetics must be driving changes in disease prevalence. Environmental and gut microbial influences affect the host immune response and have been linked to IBD onset and disease progression. Recent studies found that host factors associated with industrialization, such as body mass index, glycemic response, high-density lipoprotein cholesterol, and lactose consumption, have dominant roles in shaping the microbiota^{23,24}. Effects of specific environmental factors on IBD development have also been examined, including smoking, diet, medications, circadian rhythm, and stress. In particular, increased IBD risk was associated with early childhood exposure to antibiotics²⁵. Interestingly, in some cases disparate effects on CD and UC have been observed, suggesting unresolved heterogeneity in IBD.

In addition to these factors, changes in the gut microbiome influence IBD susceptibility. A lack of early childhood exposure to microorganisms due to cleaner living, urbanization, and the widespread usage of antibiotics affects immune education and maturation (known as the hygiene hypothesis). This lack of early childhood exposure is hypothesized to lead to a loss of negative regulatory pathways resulting in overactive immune responses to the commensal intestinal microbiota. Serological markers are associated with disease course and IBD

phenotypes, providing further evidence for the microbiome's role. A combination of a mannan epitope of *Saccharomyces cerevisiae* (gASCA), the atypical perinuclear anti-neutrophil cytoplasmic antibody (pANCA) and laminaribioside (ALCA) can differentiate between healthy individuals, IBD patients, and patients with non-IBD gastrointestinal inflammation²⁶. Furthermore, differences in gASCA and pANCA levels can distinguish between CD and UC, while increasing antibody responses against gASCA, ALCA, chitobioside, mannobioside, and outer membrane porins are associated with more complicated disease behaviour and surgery requirement in CD^{26,27}. In UC, anti-*S. cerevisiae* antibody (ASCA) immunoglobulin A (IgA), ANCA, anti-flagellin antibodies, and anti-outer membrane porin C (OmpC) are associated with disease severity. Additionally, ASCA IgA and OmpC are associated with the later requirement of colectomy, and higher ASCA IgA levels are linked to refractory disease in UC²⁸. Further evidence supporting a causal role of the microbiome in IBD are the findings that transfer of fecal microbiota from mice with colitis to healthy mice is sufficient to induce colitis^{29–34} and that many genetically susceptible mice do not spontaneously develop colitis in germ-free facilities^{35–37}.

Progress to date indicates that IBD is a polymicrobial disease with a combination of various gut microbial factors, abnormal immune responses and a weakened intestinal mucosal barrier leading to aberrant host–microbial interactions³⁸. In this Review we highlight current knowledge of gut microbial factors linked to IBD pathogenesis. We discuss how multi-omics data from large-scale population studies in health and disease have been used to identify specific microbial strains from metagenomic data, transcriptional changes from metatranscriptomic data, and metabolic alterations from metabolomic data. When integrated with host-derived data, analyses can link these microbial changes to the host in order to predict aberrant host–microbial interactions. Furthermore, much effort is currently being focused on creating large collections of strains isolated from individuals with altered mucosal ecosystems. These collections together with culturomics techniques will be invaluable to validate the colitogenic potential of disease-associated strains as well as to identify pathogenic factors. Thus, the gut microbiome's contribution to disease onset, activity and the development of complications needs to be taken into consideration in order to treat IBD effectively, achieve long-lasting remission and reset the host–microbial balance.

Microbial immunomodulation in IBD

IBD is believed to result from aberrant immune responses to commensal bacteria in genetically susceptible hosts that disrupt the host–microbial balance (Figure 1). The symbiotic relationship between the gut microbiome and the host is foremost protected by the intestinal epithelial barrier: a mucus bilayer and cellular junctions within the intestinal epithelium that form a physical barrier to contain the microbiota. Moreover, secretion of antimicrobial peptides, such as defensins produced by Paneth cells, goblet cells and other types of epithelial cells, creates a chemical barrier against invading microorganisms (Box 2). IgA also has a crucial role in maintaining homeostasis at mucosal surfaces. Two distinct types of humoral immunity were proposed to coexist in the gastrointestinal mucosa, where IgA can elicit high-affinity responses, for example in the context of pathogens and vaccines, or be polyreactive and bind to a broad but taxonomically distinct subset of the microbiota³⁹. IgA can mediate potent anti-inflammatory functions through interaction with the C-type

lectin receptor SIGNR1 on dendritic cells, which induces immune tolerance via regulatory T cell (Treg) expansion. IgA antibodies also have a crucial role in the prevention of tissue damage in autoimmune and inflammatory diseases⁴⁰. The microbiome in turn influences IgA levels in the gut mucosa through degradation, potentially disrupting homeostasis⁴¹. In IBD, there is an increased coating of intestinal microbiota by IgA antibodies.

Defects of the epithelial barrier have been observed in IBD. Although host IBD-associated genes, such as *FUT2* and *C1orf106*⁴², have roles in mucosal barrier function, several mechanisms involve gut microorganisms themselves. For example, short-chain fatty acids (SCFAs), particularly butyrate, produced by gut bacteria promote Treg development and enhance mucus production from goblet cells to strengthen the mucosal barrier^{43,44}. SCFAs activate cells via G protein-coupled receptors (GPCRs), such as GPR41 and GPR43, leading to chemokine and cytokine production which regulate protective immunity and tissue inflammation⁴⁵. Bacterial indole metabolites such as indoleacrylic acid (IA) and indole 3-propionic acid — the latter of which is produced by the intestinal commensal *Clostridium sporogenes* — regulate intestinal barrier function through the xenobiotic sensor pregnane X receptor⁴⁶. Produced by *Peptostreptococcus* species, IA promotes intestinal epithelial barrier function and mitigates inflammatory responses. The biosynthetic gene cluster for IA is decreased in the gut metagenomes of IBD patients, perhaps contributing to barrier dysfunction⁴⁷. Decreased tryptophan metabolism, reduced SCFA levels, and compromised epithelial barrier compound the detrimental effects of IBD.

Microbial communities have an important role in the maturation and education of the host immune system. Infant gut colonization and the early-life microbiome have long-lasting effects^{48,49} and are strongly influenced by delivery mode and feeding. Lower diversity and delayed colonization of Bacteroidetes, for example, are linked to delivery by caesarean section and are associated with reduced T helper 1 (Th1) cell responses in the first two years of life⁵⁰. T cell subtypes have crucial roles in sensing inflammation and ensuring appropriately timed and localized immune responses. The importance of the microbiota for T cell development and immune tolerance has been studied in mouse models. Germ-free mice have fewer CD4⁺ Tregs in their colons than conventional mice⁵¹. CD4⁺ Tregs express the Foxp3 transcription factor and have a crucial role in the maintenance of immune homeostasis. Further, early life exposure to microorganisms is required in mice to prevent an accumulation of invariant natural killer T (iNKT) cells, which predispose mice to increased morbidity in models of IBD and allergic asthma⁵². Specifically, either colonization of neonatal mice with conventional microbiota from specific pathogen-free mice⁵² or monocolonization with a sphingolipid-expressing strain of *Bacteroides fragilis*⁵³ is sufficient to prevent the accumulation of iNKT cells. Exposure of adult germ-free mice to these microorganisms is insufficient to decrease levels of iNKT cells^{52,53}, supporting a body of literature that suggests early life exposure to microorganisms regulates adult immune functions and disease susceptibility^{54,55}. Colonizing germ-free mice with fecal microbiota from individuals with IBD increases the number of intestinal Th17 cells and decreases the number of ROR γ ⁺ Tregs, providing evidence for disease mechanisms involving the gut microbiome in humans. Further, the induced proportions of Th17 and ROR γ ⁺ Tregs accounted for colitis severity in *Rag1*^{-/-} mice lacking adaptive immunity⁵⁶ and were predictive of human disease status.

In mouse models, segmented filamentous bacteria (SFB) are primary drivers of Th17 responses^{34,57}. *Rag1*^{-/-} mice have higher levels of SFB than immunocompetent mice⁵⁸. SFB levels in these immunocompromised mice further increase when the STAT3-dependent innate immune response is compromised, indicating that the murine immune system continues to restrict these closely associated bacteria even in the absence of a functional adaptive immune response⁵⁸. These findings provided a unique framework to translate mouse studies to human disease. One study isolated 20 bacterial strains from a UC patient based on their ability to induce Th17 cells in mice and showed that adhesion to epithelial cells is a common mechanism used by intestinal microorganisms to activate host Th17 responses⁵⁹. One host mechanism that restricts SFB independently of the adaptive immune system is the production of α -defensins by Paneth cells, a cell type whose function is diminished in CD. SFB abundances are increased in mouse models of autoimmune polyendocrinopathy candidiasis ectodermal dystrophy, and the host was additionally seropositive for autoantibodies against an enteric α -defensin⁶⁰. SFBs are typically only found in mice, and the specific human counterpart is unknown.

Microbial factors affect Treg differentiation as well. Tregs are negative regulators of inflammation, and commensal species such as *B. fragilis*⁶¹ and species from *Clostridium* cluster XIVa, IV, and XVIII^{51,62} can stimulate the differentiation of Tregs. Alterations of T cell subtypes can have long-lasting effects, where Treg depletion leads to non-remitting destructive disease even after restoration of normal Treg numbers in mouse models of arthritis. Microbial imbalances in IBD likely disrupt regulatory processes that suppress inflammation. For example, a pronounced depletion of Clostridiales organisms was observed in treatment-naïve patients with new-onset CD and UC^{28,63}.

These studies present evidence that the gut microbiome is a crucial component for a healthy immune system; however, a comprehensive understanding of mechanisms mediating host–microbial interactions is currently incomplete. To this end, several extensive longitudinal IBD studies were initiated with the aim of linking taxonomic and functional changes in the gut microbiome to IBD pathogenesis.

The gut microbiome in IBD pathology

Early studies determined differences in the microbiota of IBD patients based on 16S ribosomal RNA (rRNA) amplicon sequencing of stool and biopsy samples^{28,63,64}. Collectively, these studies found a decrease in gut microbial diversity in IBD patients^{65,66}, including a decrease in Firmicutes with a depletion of *Clostridium* cluster IV and XIV species^{65,67–69} and an increase in *Enterobacteriaceae* species^{70–72} (Figure 1). Some, but not all, studies identified changes in *Bacteroides*, *Bifidobacteria*, or *Lactobacilli* species^{65,67–70,72,73}. In pediatric IBD patients, microbial shifts were detected at earlier time points in biopsies compared to stool⁶³. Studies examining bacteria associated with tissue biopsies from IBD patients found more mucosa-associated bacteria in IBD than in controls^{69,70,73,74} and significant differences in the abundances of tissue-associated bacteria between inflamed and non-inflamed sites^{67,75}. Defining distinct association networks of taxa from intestinal biopsies of CD and UC patients, one study identified *Blautia*, *Faecalibacterium* and *Ruminococcus* species as probable keystone taxa in CD and UC⁷⁶.

They further linked disturbances of Lachnospiraceae and Ruminococcaceae species with relapsing disease, poor response to anti-TNF α therapy and disease recurrence after surgical interventions in CD patients.

16S rRNA amplicon studies have limited taxonomic resolution and predominantly identified family-level or genus-level but rarely species-level associations. Although taxonomic imbalances in IBD have been described, functional disruptions may have a greater impact. However, amplicon studies do not reveal information about the metabolic pathways encoded by the microbial community. To address this possibility and circumvent the limitations described above, metagenomic sequencing of stool samples showed that metabolic pathway abundances were more consistently perturbed. The microbiomes of IBD patients encode more oxidative stress and nutrient transport pathways and fewer pathways related to carbohydrate metabolism and amino acid synthesis^{63,77}. Metagenomic studies have been largely limited to stool samples due to a high host to microbial DNA ratio present in biopsies that makes sequencing total DNA an inefficient way to profile microbial communities. Nevertheless, these early studies indicate that important insights can be gained through metagenomic data.

Several large cohort studies (Table 1) have now used metagenomic sequencing on human stool samples to characterize species and strain-level differences as well as functional alterations in IBD. Comparing IBD, irritable bowel syndrome (IBS), and healthy controls, a study⁷⁸ analyzed metagenomic profiles of stool samples from 1,792 individuals (355 IBD patients from the NLIBD cohort, of which 208 patients were diagnosed with CD, 126 patients with UC, and 21 patients with IBD-unclassified or indeterminate; 412 IBS patients; and 1,025 controls from the LifeLinesDeep cohort). Overall, 219 taxa from various taxonomic levels (including 152 species) were associated with CD, and 102 taxa (including 93 species) were associated with UC. Profiles were similar between both IBD subtypes, with 87 of the UC-associated taxa also associated with CD. Families with the highest number of decreased taxa in CD were Lachnospiraceae (n=21 taxa) and Ruminococcaceae (n=17), whereas the highest number of increased taxa belonged to the Enterobacteriaceae family (n=8). Taxa decreased in UC were largely from the Bacteroidaceae family (n=5), and taxa increased in UC were from the Lachnospiraceae family (n=11). Furthermore, significantly reduced strain diversity in beneficial species such as *Faecalibacterium prausnitzii* and *Roseburia intestinalis* were observed, and bacterial growth rates of *B. fragilis* and *Escherichia coli* were increased in CD patients compared to controls. The abundance of antibiotic resistance genes was increased in IBD and correlated with *Escherichia* species and *Bacteroides* species abundances. Functional differences were observed in the microbiome of IBD patients, in particular in the synthesis of amino acids, neurotransmitters, and vitamins, as well as the regulation of mineral absorption and the degradation of complex carbohydrates. Pathways related to SCFAs and L-arginine synthesis, which have important roles in maintaining intestinal barrier function and inflammation-associated immunosuppression, were perturbed in IBD.

In a study investigating changes in metabolic activity of the IBD microbiota, one study performed metagenomic and untargeted metabolomics profiling of 220 samples from an American discovery cohort (PRISM) and a Dutch validation cohort (LifeLines DEEP and

NLIBD)⁷⁹. Metagenomic analysis revealed a pronounced separation of CD and non-IBD patients, whereas UC patients were more heterogeneous. In total, 50 species were differentially abundant in IBD, of which 35 were elevated in controls indicating a general loss of diversity in IBD patients. In particular, *Roseburia hominis*, *Dorea formicigenerans* and *Ruminococcus obeum* were depleted in IBD. In CD patients, an enrichment of *R. gnavus*, *E. coli* and *Clostridium clostridioforme* was observed, whereas *Bifidobacterium breve* and *Clostridium symbiosum* were uniquely enriched in UC. Functional metagenomic analysis identified 568 differentially abundant enzymes, the majority of which could be ascribed to a single species. For example, *E. coli* dominated 200 of the differentially abundant enzymes, owing in part to the species' strong enrichment in IBD and exceptionally thorough functional annotations. Whereas some predicted functions may indicate mechanistic links between the microbiome and IBD, others may simply indicate changes in the abundances of genomes encoding those functions. Overall, 246 differentially abundant enzymes were not dominated by a single species, suggesting that their enrichment is the result of a community-level shift in functional potential and therefore is of greater mechanistic significance. Examples include a magnesium-importing ATPase and an ethanolamine ammonia-lyase. As metabolites constitute a direct measurement of functional activity, their quantification may be more effective in identifying putative mechanistic associations. Metabolic alterations identified in this and other studies will be discussed further in a later section.

In addition to species-level and functional characterizations of the gut microbiome in IBD patients, temporal changes are important to monitor. Interindividual differences account for ~50% of the variation observed in taxonomic community composition²⁸. Further, less than half of IBD patients respond to conventional treatment strategies, and disease is characterized by periodic bouts of inflammation separated by periods of remission. For these reasons, it is particularly important to conduct longitudinal studies that capture changes of microbial features and loss of response to treatment over time. To date, there have been only a handful of longitudinal, metagenomic studies that associated microbial species with IBD as described in more detail below.

One study focused on a treatment-naïve pediatric CD cohort and monitored patients over the first 8 weeks of treatment with either anti-TNF α therapy or enteral nutrition⁸⁰. Enteral nutrition consists of nutritionally complete liquid diets presenting simpler, often predigested amino acids, fatty acids, and oligosaccharides that can be absorbed quickly by the host and reduce the metabolic activity of the microbiota. Using metagenomic sequencing of gut microbiota from healthy individuals and CD patients, the authors found that changes in microbial genes distinguished between CD patients: decreases in selenocompound metabolism pathways and increases in microbial genes encoding glycerophospholipid metabolism, aminobenzoate degradation, sulfur relay systems, and glutathione metabolism were predictive of more profound microbial shifts within CD patients. By sequencing the entire genetic content of the samples instead of amplifying a specific bacterial gene (such as the 16S rRNA gene), they further showed that IBD was correlated with higher levels of fungal and human DNA. After one week of therapy, the authors were able to predict which patients would achieve remission based on their gut microbiome; however, gut microbial composition of CD patients remained altered over the course of the study⁸⁰.

Another study examined the microbiome of patients with established CD or UC in a meta-analysis of two studies with longitudinal data^{80,81}. In addition to examining taxonomic differences, they binned bacteria by their oxygen utilization capabilities as facultative or obligate anaerobes, which can and cannot utilize oxygen as a terminal electron acceptor. The authors found that facultative anaerobes are overrepresented in IBD patients, and oxygen utilization explained eight out of nine species that were consistently differentially abundant between the two studies. This supports previously published hypotheses that the microbial shifts associated with IBD are in part due to increased aerobicity in the inflamed gut^{63,77}. The only species that was not explained by oxygen utilization was *R. gnavus*, an aerotolerant obligate anaerobe that transiently dominates the IBD microbiota during periods of increased disease severity. Such dynamics of bacterial abundances can only be captured in longitudinal studies. Furthermore, metagenomic data allowed the researchers to distinguish between two strain-specific clades of *R. gnavus*, one of which is found in both controls and IBD patients, whereas the other is only present in the IBD gut⁸¹. As the field transitions from amplicon studies towards metagenomic studies, our knowledge of strain diversity within a given species will increase.

Although these studies identified differentially abundant species in IBD, many microorganisms identified across studies differ in presence or absence, or abundance, making it challenging to identify common patterns. Figure 2 is a phylogenetic representation summarizing all species associations identified across these metagenomic studies. Colored leaves of the phylogenetic tree represent differentially abundant species identified in at least two of the studies. The outer rings summarize study-specific results. While this visualization highlights the broad spectrum of IBD-associated bacterial species from all major taxonomic groups typically found in the gut, it also reveals phylogenetic patterns. Species from the Proteobacteria phylum, such as *E. coli*, are generally increased in IBD. Actinobacteria are generally decreased, with the majority of these associations identified in the NLIBD cohort. Actinobacteria have been observed to be highly prevalent in the gut microbiomes of healthy Dutch individuals compared to American individuals^{23,82}. Most Bacteroidetes species identified in at least two studies were depleted in IBD, in particular several *Alistipes* species. Although Firmicutes include increased and decreased species in IBD, the phylogenetic analysis highlights that patterns are largely clade-specific, with *Eubacteria* species and Ruminococcaceae consistently decreased and *Streptococci* species and *Lactobacilli* species increased. Furthermore, Lachnospiraceae species including *Clostridia* showed opposing trends, suggesting species-specific interactions with the host immune system.

Communication between microorganisms and the host is not unidirectional. Just as microbial products can influence host cell physiology, host factors such as chromogranins and secretogranins appear to influence gut microbial composition⁸³. Overall, large cohort analyses have provided a detailed characterization and indicate substantial changes of gut microbiota composition in IBD based on metagenomics data. Integrating functional microbial measurements, such as metatranscriptomics and metabolomics data, and measurements of host responses will be crucial to generate testable hypothesis and gain mechanistic insights into the role of the microbiome in disease.

Microbial strains implicated in IBD

As highlighted by the example of *R. gnavus* in the previous section, strain-level variation within a species can be important. One of the difficulties in drawing conclusions from amplicon sequencing data is that diversity within a bacterial species is not fully captured. Organisms of the same species are defined to have 70% DNA–DNA hybridization or 95% average nucleotide identity⁸⁴; therefore, strains of the same species can possess important functional differences, including antibiotic resistance or pathogenicity. Most *E. coli* strains are harmless and part of a healthy gut microbiota, but some are pathogenic and can cause serious and fatal diseases by producing, for example, Shiga toxin. Furthermore, specific *E. coli* strains such as adhesive invasive *E. coli* (AIEC) are implicated in IBD. AIEC is a pathobiont and its role in IBD was recently reviewed in detail⁸⁵. Briefly, AIEC is able to evade the immune system and to adhere and invade intestinal epithelial cells and macrophages in genetically susceptible hosts, including IBD patients. In addition to taking advantage of IBD host genetics that impair autophagy mechanisms, AIEC can suppress autophagic processes enabling it to survive and thrive inside cells. First, bacterial translocation allows AIEC to gain access to the lamina propria, where it is engulfed by macrophages but escapes autophagy. Continuous replication within macrophages results in the secretion of high levels of TNF α without inducing host cell death, leading to gut inflammation and AIEC over-colonization. Hyper-motility and increased acetate-consumption are associated with strains of *E. coli* from CD patients compared to those from healthy individuals, suggesting AIEC adaptation within the host⁸⁶. Various strategies for targeting AIEC strains and/or inhibiting AIEC adhesion, including bacteriophages and small molecule drugs, are being investigated as potential therapies for IBD⁸⁷.

In the absence of metagenomic sequencing, studies relied on culture-based methods to distinguish pathogenic strains. Culture-based methods were instrumental in identifying the ability of human oral *Klebsiella* strains to drive inflammation in the colon of genetically susceptible mice (that is, *III10*^{-/-}). Although some oral isolates of *Klebsiella* species from both controls and IBD patients induced murine colon inflammation, this was not consistent across the tested human, mouse, and environmental strains⁸⁸. In particular, the *Klebsiella pneumoniae* 2H7 strain strongly induced accumulation of interferon- γ ⁺ CD4⁺ Th1 cells in the intestinal lamina propria, however, only in genetically susceptible mice. Strain-specific genes associated with Th1 cell induction are predicted to encode hemolysin-coregulated protein and enzymes involved in fructose-, galactitol-, mannose-, and long-chain fatty acid-related uptake and metabolic pathways. Furthermore, the ability of pathobionts to outgrow other bacteria in the inflamed gut is supported by sequencing data, in which *Klebsiella* species and other bacteria associated with the oral cavity are more abundant in the guts of IBD patients compared to controls^{28,88,89}. *Klebsiella* species are also more abundant in mouse models and human cohort studies of other gut diseases^{90,91}. The specific mechanisms involved in bacterial translocation and inflammatory responses, however, remain to be elucidated.

Beneficial species that elicit positive effects and provide protection for the host can also be diverse as evidenced by *F. prausnitzii*, which contains numerous subspecies or phylogroups. Targeted qPCR studies suggested that the presence or absence of *F. prausnitzii* phylogroups

may be indicative of disease⁹². The implication of the presence or absence of different *F. prausnitzii* phylogroups on disease is currently unclear as the full diversity of this group is not apparent in the short hypervariable regions sequenced in most amplicon-based studies. Even when the full 16S rRNA gene is sequenced, many *F. prausnitzii* strains do not fall into any of the identified phylogroups⁹².

Studies of strain-specific phenotypes, such as those outlined here, will likely become more common and will enable a deeper understanding of the role of microorganisms in IBD pathogenesis. As the number of genomes associated with pathogenesis-promoting strains increases, fewer genes will be shared exclusively among these disease-causing strains. This will eventually facilitate the identification of virulence factors (either presence or absence) and enable the comparison of potential virulence genes (including SNP identification) across species to identify commonalities.

Insights from metatranscriptomics

Metagenomics is limited to revealing the functional potential of microorganisms, rather than the actual functional activity. The presence of a gene or pathway does not necessarily mean that it is expressed. In addition to isolating DNA for metagenomics, RNA can be extracted from a sample, reversed transcribed into cDNA and sequenced for metatranscriptomics that measures actual microbial gene expression. Few studies have investigated the functional activity of the gut microbiome to date. One study established the feasibility of metatranscriptomics for fecal samples and found that metatranscriptional profiles varied more between individuals than metagenome functional profiles, highlighting the importance of measuring actual gene expression in addition to functional potential inferred from gene presence for understanding disease-related microbiota changes. This subject-specific, whole-community regulation suggests that bacteria interact with their host in a very specific, individualistic manner⁹³.

Another study further showed that directly measuring functional activity reveals important insight into gut microbial community dynamics, including IBD-specific transcriptional activity that was either more pronounced or only detectable on the RNA level⁹⁴. The authors observed that the distribution of microbial species encoding a pathway can remain fairly constant in a patient over time while pathway transcription can change. For example, in the context of the methylerythritol phosphate pathway, increases in disease severity for a patient co-occurred with *A. putredinis* domination of pathway transcription while no DNA-level changes were observed. Species-specific transcriptional biases in metabolic pathways were also observed. Transcription of many pathways such as the dTDP-L-rhamnose biosynthesis I pathway that produces deoxysugar β -l-rhamnopyranose — a building block of surface glycans that are often targets of the immune system — was dominated by *F. prausnitzii*.

Microbial transcriptional programs can respond rapidly to environmental cues such as changes in inflammation and oxygen levels, which may not necessarily be reflected on the DNA level. However, some caveats apply to fecal metatranscriptomics, such as variation due to subject-specific transit times, and that it only captures extractable, non-degraded RNA restricted to organisms that are present in stool. Additionally, owing to a high ratio of host to microbial DNA and RNA, performing metagenomics or metatranscriptomics on biopsy

samples is not yet cost effective. In the absence of metatranscriptomic data, the ratio of reads located near the chromosomal origin of replication compared to reads near the terminus for a given bacteria can be used to estimate growth dynamics from metagenomic data⁹⁵. Although it does not answer questions about gene expression, this approach cleverly enables one to determine which bacteria are actively dividing and presumably transcribing their genes.

Metabolites associated with health and IBD

Several classes of small molecules produced or modified by the gut microbiota can modulate immune and epithelial cell function (Box 3)^{96,97}. Comparisons between conventional and germ-free or SPF mice show drastic differences in serum and tissue metabolites, underscoring the importance of the microbiome for host metabolism system-wide (reviewed in Ref. ⁹⁸). In mouse models, intestinal inflammation and clinical response to dextran sulfate sodium (DSS)-induced colitis can be altered by postbiotically modulating levels of the microbial metabolites taurine, histamine, and spermine, highlighting the potential clinical relevance of microbial metabolites⁹⁹. A combination of microbial and diet-derived metabolites likely contributes to inflammatory diseases such as IBD⁹⁶.

Several human studies identified metabolite differences in stool^{79,100,101}, serum^{101,102}, or mucosa of IBD patients compared to controls. Taurine and cadaverine levels are increased in UC, and carnosine, ribose, and choline levels correlate with inflammation as measured by fecal calprotectin¹⁰¹. In a twin-pair study of healthy individuals and IBD patients, increases in tryptophan, bile acids, and unsaturated fatty acids were linked to ileal CD¹⁰³. Strong associations between disease-associated microorganisms and metabolites were found by pairing metabolomics with microbial taxonomic analyses, showing increased levels of bile acids, sphingolipids and tryptophan^{79,100,103}. IBD patients with inactive disease displayed similar microbiomes and metabolotypes to their healthy first-degree relatives¹⁰⁰. Although the mechanistic relationship between host disease, microorganisms, and metabolites is becoming clearer, key questions about disease-associated metabolites remain to be answered — whether they are bacterially produced or metabolized, affect bacteria directly or indirectly by altering host physiology, or a combination of these possibilities.

Studies often perform a combination of targeted (known) and untargeted (unknown) metabolomics, but subsequent analyses, as those described above, have focused on the small subset of known molecules. The majority of the gut metabolome is uncharacterized, and untargeted metabolomics has great potential to identify novel disease-associated molecules. Computational approaches will have a crucial role in prioritizing microorganism–metabolite associations for experimental validations. This knowledge can be further used to develop therapeutic approaches that either inhibit disease-associated microbial metabolism and the corresponding microorganisms (for example, tungstate treatment) or augment beneficial metabolites and their respective species (for example, probiotic and postbiotic treatments; Figure 3).

Fungi and viruses in IBD

Most efforts to date have focused on bacterial alterations of the IBD gut microbiome, in part due to the popularity of 16S rRNA amplicon sequencing. However, the gut also harbors a diverse community of fungi and viruses that may have roles in IBD pathogenesis, affecting the host directly or indirectly by affecting bacterial members of the gut microbiota (for example, bacteriophages). Although relatively few studies have investigated IBD-associated fungi, ASCA is elevated in IBD, suggesting a role for fungi in disease pathogenesis¹⁰⁴ (Box 2). One study observed fungal taxonomic shifts in IBD with an increased *Basidiomycota* species to *Ascomycota* species ratio, a decreased proportion of *S. cerevisiae*, and an increased proportion of *Candida albicans* compared to healthy controls¹⁰⁵. Similarly, another study found that disease severity was positively correlated with fungal representation⁸⁰ and hypothesized that the CD-specific gut environment may favor fungi at the expense of bacteria or that antibiotic treatment creates a niche for fungal expansion. Recent studies have started to investigate the interactions between intestinal fungi and immune cells; for example, CX3CR1⁺ mononuclear phagocytes are essential for the initiation of immune responses to intestinal fungi, and a missense mutation in the gene encoding CX3CR1 was associated with impaired antifungal responses in CD patients¹⁰⁶. Furthermore, *S. cerevisiae* enhanced host purine metabolism in murine colitis models, leading to an increase in uric acid production. Treatment with uric acid in turn increased gut permeability in mice.¹⁰⁷ In addition, *Malassezia restricta*, a fungus typically found on the human skin, is enriched in the colonic mucosa of CD patients with a disease-linked polymorphism in *CARD9*, a signaling adaptor important for anti-fungal defense. In mouse models, *M. restricta* exacerbated colitis through mechanisms requiring *Card9*¹⁰⁸.

Enteric viruses are known to trigger disease onset in genetically susceptible mice. An interaction between murine norovirus infection and a mutation in the autophagy gene *Atg16L1*, a known CD susceptibility allele, induces abnormalities in granule packaging and unique patterns of gene expression in Paneth cells, albeit Paneth cells are not infected by the virus¹⁰⁹. CD-like pathologies were observed in response to DSS-induced colitis but were dependent on this virus-plus-susceptibility gene interaction, as mice carrying either alone did not exhibit similar pathologies. Furthermore, pathologies were TNF α -dependent and IFN γ -dependent, and preventable with broad spectrum antibiotics, implicating the commensal gut microbiota. Thus, a combination of environmental factors, commensal bacteria, and a virus-plus-susceptibility gene interaction led to an IBD phenotype. Other studies linked *FUT2* mutations, which lead to asymptomatic norovirus infections, with the pathogenesis of ileal CD¹¹⁰. Recent findings revealed that noroviruses target tuft cells within the intestinal epithelium¹¹¹, and tuft cell-specific gene expression in the colon decreases with broad-spectrum antibiotic treatment. In antibiotic-treated mice, IL-4 and IL-25 stimulation induce tuft cell hyperplasia in the ileum but not in the colon, indicating that both intestinal bacteria and type 2 cytokines regulate tuft cells in a tissue-specific manner.

Several studies have recently investigated the role of the enteric virome in human IBD using virus-like particle enrichment^{112,113}. A significant expansion of *Caudovirales* bacteriophages was detected in CD and UC patients; however, this expansion was cohort-specific and highlights the need for further studies. Analysis of viral sequencing data is

currently challenging as the large majority of reads are of unknown origin and cannot be assigned to reference genomes. Viral classification is further complicated by integration of many virus genomes into the host genome. As more tools are developed and viral reference genomes are sequenced, we will be better able to determine the role of the virome in IBD.

Microbiome-based IBD therapies

Several treatments for IBD are available; however, most of these treatments have remission rates of less than 50%. In some cases, knowledge of a patient's microbiome composition can predict response to specific IBD treatments. For example, the response to anti-integrin treatment¹¹⁴, anti-TNF α therapy¹¹⁵, or ustekinumab therapy can be predicted based on a combination of the gut microbiome and other clinical factors¹¹⁶. Another study examined treatment-naïve pediatric patients, whose treatment was not standardized in the study protocol, and was able to predict a patient treatment response with 76.5% accuracy based on the abundance of six bacterial genera in pre-treatment samples: *Faecalibacterium*, *Veillonella*, *Fusobacterium*, *Coprococcus*, *Akkermansia*, and *Adlercreutzia*¹¹⁷. Although additional replications of these results are required, they highlight the therapeutic potential of the gut microbiome in choosing optimal treatment strategies for IBD patients.

IBD treatments targeting host factors and microorganisms (including microbial physiology and metabolites) that influence disease are in various stages of development (Figures 1 and 3). Current standard treatments suppress the immune system and alter diet, with dietary modification being one of the most common behavioral interventions for IBD. This includes prebiotic effects that shift the microbial composition as a result of changes in nutrient availability; however, the scientific evidence for many dietary modifications is lacking (reviewed in Ref. ¹¹⁸). To date, the strongest clinical evidence was observed with enteral nutrition treatment for CD. Although not effective for UC patients, enteral nutrition has comparable efficacy to corticosteroids in pediatric CD patients. The effect of enteral nutrition is less compelling in adults, which may in part be due to poor compliance^{80,119,120}.

Diet has a strong influence on gut microbial communities¹²¹ and dietary changes have rapid effects on gut microbiota composition independent of inflammation and antibiotics⁸⁰. Moreover, dietary patterns have been associated with IBD risk (recently reviewed in Ref. ¹²²). Many challenges arise when evaluating dietary effects on disease, including accuracy of information on dietary intake, complex interactions between foods consumed, and differences in food metabolism among individuals¹²². Despite these challenges, clinical studies have shown that certain dietary components can promote or prevent intestinal inflammation and influence IBD risk. A prospective study following 170,776 women monitored long-term intake of dietary fiber. Intake of the highest quintile (median of 24.3 g/day) was associated with a 40% reduction of CD risk compared to the lowest quintile (median of 12.7 g/day)¹²³. The protective effect of dietary fiber on CD risk may be mediated through gut microorganisms¹²³ that metabolize fiber into SCFAs, which leads to an increased mucosal immune tolerance through the activation of GPCRs and the subsequent activation of Tregs¹²¹. In addition, interactions between the gut microbiota and dietary concentrations of proteins and fiber can change intestinal permeability and severity of intestinal inflammation in mice¹²⁴. Reduced consumption of red and processed meat,

however, did not decrease the rate of CD flares in a separate study¹²⁵. In the future, engineered diets that restrict deleterious components but supplement beneficial nutrients may be used alone or in combination with other therapies to maintain or prevent disease.

Fecal microbiota transplantation (FMT), in which the stool of a healthy donor is transferred to the intestinal tract of a patient, has been highly effective in treating *Clostridium difficile* infections and has been assessed for the treatment of IBD¹²⁶. Many FMT studies have been limited by small sample number and have employed different methods for administering FMT, making results across studies difficult to compare. Despite these challenges, evidence supports that FMT induces clinical remission in UC, particularly when patients received multiple, lower gastrointestinal infusions¹²⁶. Clinical remission was achieved for 28% of UC patients across four randomized controlled trials¹²⁷. Variable response rates to FMT in UC are likely due to the heterogeneity of the disease. The emerging consensus in the field, however, is that FMT has potential as a treatment for UC if 1) antibiotics are administered prior to FMT treatment; 2) inflammation can be controlled, which would likely result in higher efficacy rates; and 3) a designed cocktail is used that replaces missing and boosts beneficial organisms. Encouragingly, engraftment of species after FMT can be predicted based on the abundance and phylogeny of the bacteria in the donor sample and pre-FMT patient sample, and donor strains engraft in an all-or-nothing manner¹²⁸. Although there is evidence that FMT may induce clinical remission in CD, fewer studies of FMT in CD have been conducted and the confidence interval is broad¹²⁶. Only one study reported endoscopic outcomes, which may not correlate with clinical outcomes in CD described in other studies. In this study, no patient achieved endoscopic remission^{126,129}. The long-term effects of FMT and the ability to use FMT as a maintenance therapy have not yet been examined in IBD.

The microbiome alters host immune function and provides a number of therapeutic leads and targets^{34,41,47,51,57–59,61,62,130,131}. Little evidence supports the efficacy of prebiotic or probiotic treatment for IBD; however, the possibility remains that the most efficacious probiotic bacterial strains have yet to be identified. The adaptive immune response could be modulated by administering microbially-derived metabolites or enzymes in postbiotic therapies. For example, nitrogen scavenging pathways of Proteobacteria are a potential therapeutic target, as Proteobacteria gain a growth advantage from host-derived nitrogen, which exacerbates colitis in mouse models^{132,133}. Host nitrogen sources include nitric oxide produced by immune cells or urea produced as a by-product of host metabolism. In fact, one study showed that tungstate treatment, which specifically targets the molybdenum cofactor-requiring enzymes needed to use nitric oxide for anaerobic respiration, can inhibit Proteobacteria replication and ameliorate colitis in mice¹³⁴. These types of therapies are still in early stages of development; however, increasing our knowledge of mechanisms by which specific microorganisms interact with the immune system will increase our ability to develop directed microbiome-based therapies.

Conclusions and future perspectives

IBD is a complex disease involving host, microbial, and environmental factors. Adopting multi-disciplinary approaches that connect genetic risk factors, microorganisms, and microbial metabolites with altered immune responses and epithelial cell functions will be

essential to fully understand the underlying mechanisms of host–microbiota interactions in disease^{135,136}. Gut commensals contribute important functions for human health that include developing and maintaining a robust immune system, providing colonization resistance against pathogens, maintaining the intestinal mucosal barrier, and regulating host immunity. There is no single causative organism and IBD is a polymicrobial disease with more severe disease linked to reduced gut microbial diversity and blooms of bacteria such as *R. gnavus* and *E. coli*. At the same time, the gut microbiome is required for disease onset, as mouse models in germ-free conditions rarely develop IBD-like phenotypes, and broad-spectrum antibiotics can prevent disease onset in mice. In humans, antibiotics can lead to remission in severe cases, and enteral nutrition reliably induces remission in pediatric CD, albeit reducing gut diversity. Neither of these approaches, however, result in lasting remission or curing the disease. This paradox highlights that in order to develop effective therapies and eventually cure IBD we need to understand the mechanisms underlying aberrant interactions between the host immune system and the gut microbiome.

For future research in the field it will be particularly important to develop an understanding of how microorganisms and microbial products affect the immune status of the host in both health and disease. Functional differences between strains (for example, *R. gnavus* and inflammation-inducing oral *Klebsiella* species) can provide important insights and opportunities for mechanistic studies. Multi-omics technologies have potential to predict disease-relevant host–microbial mechanisms by identifying transcriptional alterations and metabolic changes in IBD. However, the large proportion of microbial genes with unknown function and microorganisms without a sequenced genome need to be taken into account to fully characterize the impact of the microbiome. Fungal and viral communities in the gut have also been implicated in IBD, but are currently largely unexplored. The ability of norovirus to trigger IBD-like phenotypes and the tropism of norovirus for tuft cells suggest that tuft cells may have a yet-unknown role in IBD. Furthermore, because gut microbial composition is highly individualized, we must focus clinical studies on longitudinal tracking of unique patient subsets to understand how normal host–microbiota interactions are shifted in IBD and in response to treatments. Investigating the initial microbiome state of treatment-naïve patients and identifying changes over time implicated in disease progression, such as the development of complications¹³⁵, disease course and treatment efficacy²⁸, will be particularly illuminating. Finally, rather than identifying microorganisms and microbial products that cause inflammation in mice and exploring their relevance in humans, it will be important for future functional studies to focus on human disease-relevant microbial factors and test their effects in mouse models¹³⁷.

All of these research avenues have high clinical value and will reveal important factors implicated in IBD development. One of the biggest limitations of the current standard of care is that it treats disease symptoms, such as chronic inflammation, rather than restores the host–microbial balance that leads to IBD. Microbiome-based therapeutic interventions could augment beneficial bacteria, target pathogenic organisms, use synthetic organisms or leverage microbial bioactive metabolites to reverse specific defects in IBD by restoring community structure and promoting barrier restitution, immune tolerance and tissue healing. Importantly, IBD is a heterogeneous disease with distinct clinical manifestations. As findings are translated to the clinic, significant microbiome-related differences between

disease subtypes will be crucial to consider in order to develop targeted therapies and improve treatment efficacy rates.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Glossary

16S rRNA gene

A gene conserved among bacteria often used for taxonomic classification

Anti-TNF α therapy

Drugs that target TNF α to decrease inflammation are often used to treat autoimmune diseases and IBD

Atypical perinuclear anti-neutrophil cytoplasmic antibody (pANCA)

Anti-neutrophil cytoplasmic antibody (ANCA) is classified based on staining patterns. Cytoplasmic ANCA (cANCA) refers to staining of the entire cytoplasm, and perinuclear ANCA (pANCA) refers to staining of the area around the nucleus. pANCAs have been implicated in IBD; however, their target antigens are unknown, and they are therefore described as atypical pANCA

Autoimmune polyendocrinopathy candidiasis ectodermal dystrophy

Autoimmune disease characterized by destruction of endocrine tissues, chronic mucocutaneous candidiasis and ectodermal disorders

Barrier function

Epithelial cell-cell junctions plus mucosal layer that permit nutrients and prevent luminal contents from accessing the rest of the body

Chitobioside

A building block of the N-acetylglucosamine-based glycan chitin and a component of the cell walls of microorganisms

Chromogranin/Secretogranin

A family of water-soluble acidic glycoproteins that are mainly produced by endocrine cells such as the enteroendocrine cells of the gut. Also known as granins, they are precursors of biologically active peptides involved in inflammation

Colectomy

A surgical procedure removing all or part of the colon

Culturomics

The process of using classical microbiological techniques to culture and identify unknown bacteria that inhabit a given environment

Glycemic response

The glycemic response to food describes its effect on blood glucose levels after consumption

Hygiene hypothesis

According to the hygiene hypothesis, a lack of early childhood microbial exposure affects the development of the immune system. This has been ascribed to the increase of allergic and autoimmune diseases in Western countries

Indole metabolites

Indole metabolites derive from microbial metabolism of tryptophan and can be recognized by several host receptors that regulate host-microbial homeostasis

Irritable bowel syndrome (IBS)

A chronic condition, which affects the large intestine and causes abdominal pain, cramping, and shifts in bowel movement patterns. In contrast to inflammatory bowel disease, IBS is not associated with mucosal inflammation, ulcers or other damage to the bowel

Keystone taxa

Species with high connectivity in microbial networks (built based on statistical associations), suggesting that they are a key component of the ecosystem and their removal would result in drastic changes to the microbial ecosystem

Laminaribioside

A glucose disaccharide building block of laminarin and a component of the cell walls of fungi and algae

Mannan

A mannose polymer and component of fungal and plant cell walls

Mannobioside

A disaccharide of mannose

Metagenome

All of the DNA in an environment

Metatranscriptome

All of the RNA in an environment

Metabolome

All of the metabolites in an environment

Microbiome

The genes, genomes and products of the microbiota

Microbiota

The collection of microorganisms in a particular environment

Non-caseating granulomas

Granulomas are clusters of immune cells that form during infection, inflammation, or in the presence of a foreign substance to prevent a systemic spread. The absence of necrosis is a defining feature of non-caseating granulomas. In Crohn's disease non-caseating granulomas are formed during inflammation without an obvious infectious trigger

Pre-biotics

Certain foods or food components that confer a beneficial effect by providing a competitive advantage to beneficial commensal bacteria capable of metabolizing these substrates or by augmenting the production of metabolic products that result from their fermentation

Pro-biotics

An organism or multiple organisms that confer beneficial effects to the host

Post-biotics

Bacterial metabolic products that mediate benefits to the host

ROR γ t⁺ Tregs

Regulatory T cells that express the transcription factor ROR γ t, a nuclear hormone receptor and critical regulator of anti-microbial immunity

Strain

The classical microbiological definition of strain is a single bacterial isolate. In the context of metagenomic data, it refers to a combination of single nucleotide polymorphisms that are computationally predicted to be linked and originating from an individual strain genome

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Box 1:**Features of inflammatory bowel disease**

Inflammatory bowel disease (IBD) is a term that is used to describe chronic inflammatory disorders of the gastrointestinal (GI) tract that manifest in two major forms — Crohn's disease (CD) and ulcerative colitis (UC). Traditionally, CD and UC are defined by clinical, histological, endoscopic, and radiological features¹³⁸. The location and extent of inflammation along the GI tract distinguishes UC and CD; UC inflammation extends proximally from the rectum and is restricted to the colon, whereas CD may affect any site in the GI tract but commonly occurs in the terminal ileum and may be discontinuous. CD is histologically characterized by macrophage aggregates that often form non-caseating granulomas, whereas UC presents with micro-abscesses composed of neutrophils within the lamina propria and crypts. Macroscopically, extensive mucosal ulcerations develop in UC. Mucosal lesions in CD are often found over Peyer's patches (small masses of lymphatic tissue in the ileum). In addition to these features, main immune cell involvement differs between CD and UC. CD is dominated by a T helper 1 cell phenotype and production of interferon- γ and IL-2, whereas UC is primarily a T helper 2 cell phenotype with production of TGF- β and IL-5 but not IL-4. IBD treatment regimens vary based on disease type, extent and severity.

IBD onset and disease course are associated with a combination of genetic, host (Box 2), and microbial (Box 3) factors. Precise pathophysiologies for IBD, however, remain elusive. Although CD and UC are clinically distinct, it is unknown whether they are also pathologically distinct or are part of a singular spectrum. Recent multi-omics studies such as the ones highlighted in this Review have greatly contributed to a more comprehensive understanding of the pathogenesis of IBD.

Box 2.**Host physiological factors associated with inflammatory bowel disease****Local factors**

Changes in host physiology are observed within the gut during IBD.

Barrier function: The intestinal barrier in inflammatory bowel disease (IBD) is leaky due to changes in epithelial tight junctions, increased rates of apoptosis, erosion events, and ulcerations in the intestinal lining¹³⁹.

Paneth cells: These specialized intestinal epithelial cells produce antimicrobial peptides, which regulate the gut microbiota. Changes in the numbers and function of Paneth cells have been noted in Crohn's disease (CD) patients, and functional consequences of these abnormalities have been demonstrated in mice^{109,140–142}. Paneth cell dysfunction is associated with mutations in the IBD-associated host genes *NOD2* and *Atg16L*¹⁴¹.

Goblet cells: Goblet cells, specialized intestinal epithelial cells that secrete mucin, are commonly depleted in IBD¹⁴³. The mucin layer is thinner in IBD, and mouse mutants lacking *Muc2* develop chronic colitis¹⁴⁴. Goblet cells also secrete cytokines, deliver antigens to dendritic cells, and affect barrier function¹⁴⁵.

Fecal calprotectin: Quantification of this abundant neutrophil protein in stool serves as a readout of gut inflammation. Upon activation or cell death, neutrophils release calprotectin, which is then stable in stool for several days. Fecal calprotectin levels correlate with histological examination and disease severity, particularly in ulcerative colitis (UC).

Systematic factors

Evidence of a host response to IBD is also found systemically, such as in serum.

Microbial antibodies: One of the most commonly measured antibodies, anti-*Saccharomyces cerevisiae* antibody (ASCA), is used to distinguish CD from UC patients during diagnosis, with CD patients being more likely to have higher levels of ASCA than UC patients¹⁰⁴.

Inflammatory markers: The most commonly used inflammatory marker is C-reactive protein (CRP). CRP is produced by the liver and released into the blood in response to inflammatory cytokines accumulating at the site of infection. Due to a short half-life, serum CRP levels rapidly decrease upon the cessation of inflammation. CRP correlates well with disease activity for most patients with UC or CD, although CD patients tend to have higher CRP levels relative to UC patients¹⁴⁶.

Cytokines: Inflammation associated with IBD is largely driven by an imbalance between pro-inflammatory and anti-inflammatory cytokines. The role of cytokines in IBD pathogenesis has been recently reviewed¹⁴⁷.

Box 3.**Microbial immunomodulatory molecules**

Microbial metabolites are critical for host-microbiota interactions and regulate host immune responses.

Short-chain fatty acids (SCFAs), including butyrate, propionate, and acetate, are byproducts of bacterial breakdown of dietary fibers. SCFAs can affect gene expression and cell proliferation during immune responses by modulating histone deacetylases¹⁴⁸. Butyrate and butyrate-producing bacteria are less abundant in the stool of inflammatory bowel disease (IBD) patients²¹.

Tryptophan can be converted into bioactive indole-containing metabolites by gut bacteria. Significant differences in tryptophan metabolite levels have been observed in the serum of germ-free mice compared to conventional mice¹⁴⁹. Indole derivatives can affect the host by activating aryl hydrocarbon receptor, which regulates inflammation¹⁵⁰. Further, indoleacrylic acid, a specific indole derivative produced by the mucus-utilizing bacterium *Peptostreptococcus*, induces mucin gene expression and activates the NRF2 pathway⁴⁷. This further affects host tryptophan metabolism by decreasing tryptophan availability, which reduces host metabolism products such as serotonin¹⁵¹. In IBD patients, bacterial metabolism of tryptophan is reduced.

Bile acid metabolites are generated by bacteria from host-produced bile acids. These secondary bile acids are sensed by host receptors including FXR and TGR5 and can regulate genes involved in immune cell maturation, cytokine release, and microbial defense¹⁵². Production of secondary bile acids is reduced in IBD patients. Taurine, which is cleaved from certain classes of primary bile acids, is an inflammatory metabolite that activates IL-18 expression⁹⁹. Additionally, conjugated bile acids cause CD4⁺ T effector cell-mediated upregulation of the xenobiotic transporter, Mdr1, to maintain homeostasis. Mdr1 function in IBD patients is reduced relative to controls¹⁵³.

Succinate is produced by *Bacteroides* species through the breakdown of dietary fiber, which in turn is metabolized by Clostridium species to produce butyrate and ATP¹⁵⁴. Succinate increases IL-1 β production by stabilizing hypoxia-inducible factor-1 α in macrophages and stimulating dendritic cells via succinate receptor 1¹⁵⁵. Serum succinate levels are increased in hypertension, ischemic heart disease, type 2 diabetes, and obesity¹⁵⁶. Murine colonic succinate levels are affected by dietary fiber concentrations, antibiotics, and chemically induced intestinal motility disturbances^{157,158}.

Sphingolipids, including sphingosine, ceramide, and sphingomyelin, are a class of plasma membrane-associated lipids that are produced by both the host and specific bacteria. Sphingolipids, most prominently ceramide, are closely tied to metabolic, apoptotic, and inflammatory pathways in host cells. In IBD, the cellular levels and distribution of different sphingolipids is significantly different between inflamed and non-inflamed intestinal tissue^{53,159}.

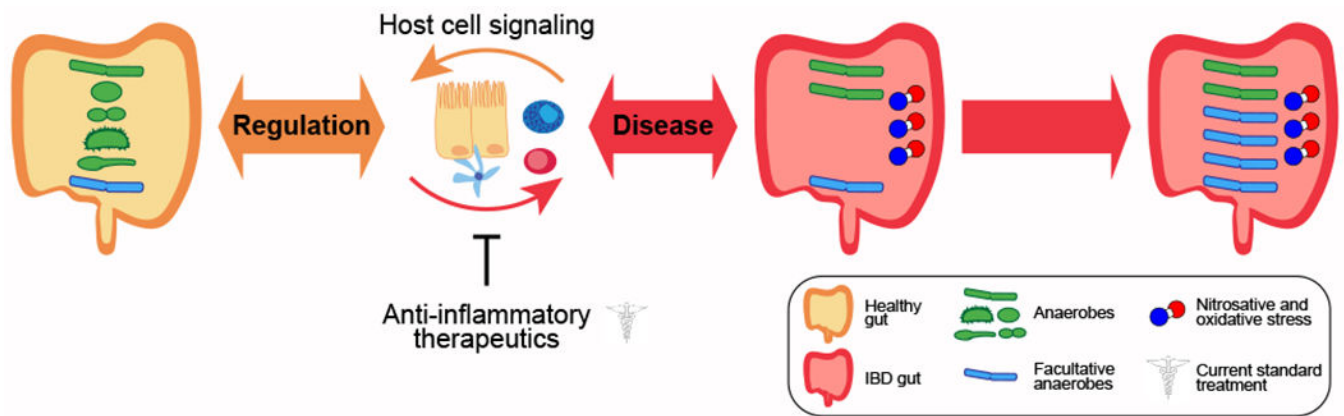


Figure 1: Inflammatory bowel disease and the microbiota.

In health, gut bacterial composition (anaerobes and facultative anaerobes) is maintained in balance with host cell physiology. Alterations in gut microbiome composition during disease include reduced microbial diversity and expansion of facultative anaerobes due to increased nitrosative and oxidative stress in the gut. Current standard treatments, such as 5ASA mesalamine, corticosteroids, immunomodulators, and anti-TNF α biologic therapy, focus on treating and controlling disease symptoms, in particular chronic inflammation.

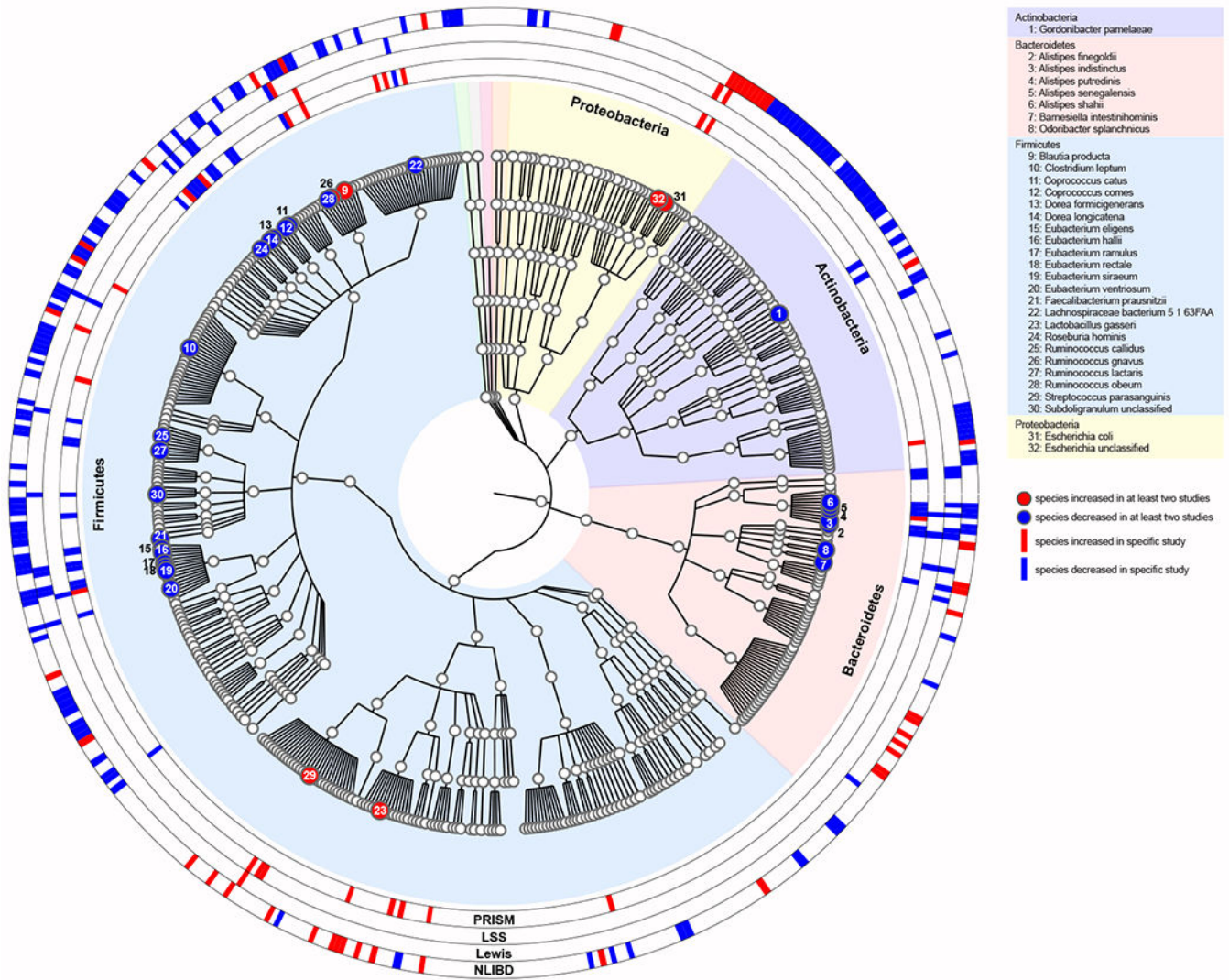


Figure 2. Phylogenetic tree of bacterial species associated with inflammatory bowel disease. Multiple studies have implicated bacterial species in inflammatory bowel disease (IBD); however, results differ between studies. The association results from metagenomic studies have been summarized in a phylogenetic tree to highlight common pattern across studies. The phylogenetic tree (generated using the software GraPhlAn)¹⁶⁰ was constructed based on all bacterial species identified in samples from PRISM⁷⁹ (n=159), LSS⁸¹ (n=271), Lewis^{80,81} (n=368), and NLIBD in combination with LLDeep controls⁷⁸ (n=1,380) and includes all species that were detected in at least 20 samples ($n_{\text{species}}=726$). Colored tree leaves indicate species that were differentially abundant (false discovery rate <0.1) in at least two studies, with 6 increased (red circles) and 26 decreased (blue circles) species. The outer rings indicate study-specific results and highlight all microorganisms identified in the respective study (Supplementary Table 1 lists the differentially abundant species for each study, respectively). Background colours indicate all species that belong to the same phylum.



Microbiome-based therapies	Effects		
	Alters gut microbiome	Alters gut metabolites	Reduces inflammation
Enteral Nutrition	✓	✓	✓
Fecal Microbiota Transplant	✓	✓	✓
Prebiotic	✓	✓	✓
Probiotic	✓	✓	✓
Postbiotic	✓	✓	✓
Inhibition of AIEC adhesion	✓	✓	✓
Tungstate treatment	✓	✓	✓

Figure 3. Microbiome-based therapies for inflammatory bowel disease.

Microbiome-based therapies for inflammatory bowel disease aim to restore the gut microbial balance, which includes increasing microbial diversity, in particular anaerobic bacteria, reducing facultative anaerobes, and reducing gut inflammation (part **a**). Current and developing treatments either alter nutrition, administer microbial organisms and/or metabolites, or directly target microorganisms and/or pathways (part **b**). The effects of these treatments, including reduction of gut inflammation and alterations of the gut microbial

communities and metabolites, are either direct or indirect. AIEC, adhesive invasive *Escherichia coli*.

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Table 1.

Summary of human stool metagenomic studies to date.

Title of paper	Year published	Number of metagenomic or metatranscriptomic datasets	SRA or BioProject identifier	Ref
Dysfunction of the intestinal microbiome in inflammatory bowel disease and treatment	2012	11	PRJNA175224	77
The treatment-naive microbiome in new-onset Crohn's disease	2014	43	PRJNA237362; PRJNA205152	63
Inflammation, antibiotics, and diet as environmental stressors of the gut microbiome in pediatric Crohn's Disease	2015	369	SRP057027	80
Increased intestinal microbial diversity following fecal microbiota transplant for active Crohn's disease	2016	53	PRJNA321058	161
A novel <i>Ruminococcus gnavus</i> clade enriched in inflammatory bowel disease patients	2017	267	PRJNA385949	81
Gut microbiome function predicts response to anti-integrin biologic therapy in inflammatory bowel diseases	2017	175	PRJNA384246	114
Dynamics of metatranscription in the inflammatory bowel disease gut microbiome	2018	300/78	PRJNA389280	94
Gut microbiota composition and functional changes in inflammatory bowel disease and irritable bowel syndrome	2018	1792	LifeLines DEEP (upon request): European Genome-Phenome Archive EGAS00001001704 UMCG IBD (upon request): EGAD00001004194 Maastricht IBS (MIBS): EGAS00001001924	78
Gut microbiome structure and metabolic activity in inflammatory bowel disease	2019	222	PRJNA400072	79

SRA, sequence read archive.