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The Microbiome in Inflammatory Bowel Diseases: Current Status and the Future Ahead

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Abstract

Studies of the roles of microbial communities in the development of inflammatory bowel diseases (IBD) have reached an important milestone. A decade of genome-wide association studies and other genetic analyses have linked IBD with loci that implicate an aberrant immune response to the intestinal microbiota. More recently, profiling studies of the intestinal microbiome have associated pathogenesis of IBD with characteristic shifts in the composition of the intestinal microbiota, reinforcing the view that IBD results from altered interactions between intestinal microbes and the mucosal immune system. Enhanced technologies can increase our understanding of the interactions between the host and its resident microbiota, and their respective roles in IBD, from both a large-scale pathway view and at the metabolic level. We review important microbiome studies of patients with IBD and describe what we have learned about the mechanisms of intestinal microbiota dysfunction. We describe the recent progress in microbiome research from exploratory 16S-based studies, reporting associations of specific organisms with a disease, to more recent studies that have taken a more nuanced view, addressing the function of the microbiota by metagenomic and metabolomic methods. Finally, we propose study designs and methodologies for future investigations of the microbiome in patients with inflammatory gut and autoimmune diseases in general.

Keywords

Microbiota; Crohn’s disease; ulcerative colitis; metagenomics

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Over the past decade, inflammatory bowel diseases (IBD) have emerged as one of the most studied human conditions linked to the gut microbiota.[1, 2] IBD comprises both Crohn's disease (CD) and ulcerative colitis (UC), which together affect over 3.6 million persons.[3] Large scale studies of human genetics across a total of 75,000 cases and controls have revealed 163 host susceptibility loci to date.[4] These loci are enriched for pathways that interact with environmental factors to modulate intestinal homeostasis.[5] The incidence of the disease has been on the rise over the past few decades, further highlighting the role of environmental factors in this disease. IBD was once a very rare disorder, and only began to rise dramatically in incidence in the second half of the 20th century in North America and Europe, at times doubling every decade, and in the last two decades expanded into developing countries, although there are more cases of UC than CD in the developing world.[6] In addition, several twin studies have now shown that the concordance rate for IBD between monozygotic twin pairs is significantly less than 50%, with the least concordance in CD.[7] IBD is thus a multifaceted disorder in which not only germline genetics and the immune system, but also several environmental factors, play an important role.[8] One such factor, the gut microbial community, is gaining increasing attention for its influence on many aspects of health in general,[9] and IBD in particular (Table 1).

The gut microbiota, the largest reservoir of microbes in the body, coexists with its host in variable concentrations throughout the GI tract, reaching an upper level in the colon of 10^{11} or 10^{12} cells/g of luminal contents.[10] This community carries out a range of useful functions for the host, including digesting substrates inaccessible to host enzymes, educating the immune system, and repressing the growth of harmful microorganisms.[11] The extensive use of low-resolution surveys of the microbial community structure in the past, and renewed efforts using next-generation sequencing for a high-resolution description of composition, function, and ecology,[12, 13] have improved our overall understanding of the role of the gut microbiota in health, a prerequisite for the study of disease-related dysbiosis. Several factors can intervene with microbial gut community composition, including genetics, diet, age, drug treatment, smoking, and potentially many more (Figure 1).[14] The relative importance of each of these factors is still unclear, but several of them are directly or indirectly linked to disease state.

Environmental factors affect the microbiome composition

Diet

One of the most important environmental factors impacting microbial composition is dietary preference, which has been demonstrated to determine microbiome composition throughout mammalian evolution.[15] Although no specific diet has been shown to directly cause, prevent, or treat IBD, it is important to take interactions between nutrients and microbiota into account when studying the role of the microbiome in disease. Thus far, only limited information on this topic has been gathered in humans, undoubtedly as a result of the challenge of setting up a large-scale controlled diet study. Wu and colleagues have shown that long-term dietary patterns affect the ratios of *Bacteroides*, *Prevotella*, and *Firmicutes*, and that short-term changes may not have major influences.[16] In addition, Zimmer and colleagues have studied the impact of a strict vegan or vegetarian diet on the microbiota,[17]

and found a significant reduction in *Bacteroides* spp., *Bifidobacterium* spp., and the Enterobacteriaceae, while total bacterial load remain unaltered. Since the Enterobacteriaceae are among the taxa that are consistently found to be increased in patients with IBD (see below), it would be of value to include both short- and long-term dietary patterns in future studies of the role of the microbiome in IBD. Given the complexity of dietary effects, including such information will likely only be feasible in a large cohort study.[18]

Age

There is an age-related variation in the distribution of IBD phenotypes, with three distinct stages of onset. A peak age of onset is typically 15 to 30 years old, with late onset cases occurring closer to 60, and early onset less than 10 years of age. Noticeably, the latter group has seen a significant increase in incidence over the last decade.[19] These stages correspond to phases in which the gut microbiota alters its diversity and stability.[20] Early life is marked by a microbiome of low complexity and low stability, one that is more volatile, is affected by the birth route, and fluctuates with events such as changes in diet (switch from breastfeeding to solid foods), illness, and puberty.[21] It takes until adulthood for the microbial assemblage to reach a maximal stability and complexity, with improved resilience towards perturbations.[22] However, decreased stability has been observed in the elderly (60 years or older).[23] Given these different characteristics of the microbiome at the three distinct stages of disease onset, a different role for the microbiome in disease initiation and progression should be considered.

IBD genetics point to an interplay between the immune system and microbiota in IBD

A potential link between genetics and the microbiome has long been suspected. The first identified CD susceptibility gene was nucleotide-binding oligomerization domain containing 2 (NOD2),[24] which stimulates an immune reaction upon recognizing muramyl dipeptide, a cell wall peptidoglycan constituent of Gram-positive and Gram-negative bacteria. NOD2 is expressed in Paneth cells, which are located predominantly in the terminal ileum at the base of intestinal crypts, and produce antimicrobial defensins.[25] Therefore, it may not be surprising that mutations in NOD2 can have significant effects on the composition of the microbial milieu. Indeed, IBD patients carrying NOD2 mutations have increased numbers of mucosa-adherent bacteria[2] and decreased transcription of the anti-inflammatory cytokine interleukin (IL)-10.[26] IBD patients with NOD2 and autophagy related 16-like 1 (ATG16L1, an IBD susceptibility gene involved in autophagy) risk alleles have significant alterations in the structure of their gut microbiota, including decreased levels of *Faecalibacterium* and increases in *Escherichia*.[27] Individuals homozygous for loss-of-function alleles for fucosyltransferase 2 (FUT2) are "nonsecretors," who do not express ABO antigen on the gastrointestinal mucosa and bodily secretions. Nonsecretors are at increased risk for CD[28] and exhibit substantial alterations in the mucosa-associated microbiota.[29] Host genetics may thus play a strong role in the establishment and shaping of the gut microbiota; indeed, monozygotic twins share more similar microbiomes than non-twin siblings.[30] On the skin, a recent study in primary immunodeficiency patients demonstrated a bi-directional dialogue between the microbiome and the host immune system. The skin of primary

immunodeficiency patients holds an altered population composition compared to immunocompetent subjects, which in turn results in increased susceptibility to infection by altering the immune response towards pathogens.[31] Although currently no genome-wide studies examining the interactions between common human genetic variation and the composition of the microbial ecosystem exist, such a study could hold great value.[5]

An overview of gut microbiome studies in IBD

Many IBD susceptibility loci suggest an impaired response to microbes in disease, but the causality of this relationship is unclear. IBD pathogenesis may result from a dysregulation of the mucosal immune system driving a pathogenic immune response against the commensal gut flora.[32] Some studies show that the gut microbiota is an essential factor in driving inflammation in IBD,[1] and indeed, short-term treatment with enterically-coated antibiotics dramatically reduces intestinal inflammation[33] and has been demonstrated to have some efficacy in IBD, and particularly in pouchitis.[34] Specifically, rifaximin has demonstrated efficacy in recent trials in CD.[35] Additionally, IBD patients show mucosal secretion of IgG antibodies[36] and mucosal T cell responses against commensal microbiota.[37]

The dramatic improvements to DNA sequencing technology and analysis over the last decade have set the stage for investigations of the IBD microbiome. Many studies find structural imbalances, or dysbioses, that occur in IBD since the initial report,[38] and a broad pattern has begun to emerge which includes a reduction in biodiversity, a decreased representation of several taxa within the Firmicutes phylum, and an increase in the Gammaproteobacteria.[27, 39]

Many studies consistently report a decrease in biodiversity, known as alpha-diversity or species richness in ecological terms, a measure of the total number of species in a community. There is a reduced alpha-diversity in the fecal microbiome in CD compared to healthy controls,[40] which was also found in pairs of monozygotic twins discordant for CD.[41] This decreased diversity has been attributed to a reduced diversity specifically within the Firmicutes phylum,[42] and has also been associated with temporal instability in the dominant taxa in both UC and CD.[43] There is a reduced diversity in inflamed versus non-inflamed tissues even within the same patient, and CD patients have lower overall bacterial loads at inflamed regions.[44] The largest IBD-related microbiome study to date, is on new-onset Crohn's disease in a multicenter pediatric cohort.[45] This study analyzed over 1000 treatment-naïve samples, which were collected from multiple concurrent GI locations, from patients representing the variety of disease phenotypes with respect to location, severity, and behavior. In addition to a detailed characterization of the specific organisms either lost or associated with disease, this study indicates that assessing the rectal mucosa-associated microbiome offers unique potential for convenient and early diagnosis of CD.

Other non-bacterial members of the microbiota, namely the fungi, viruses, archaea, and phage may have a significant role in gastrointestinal disease;[46] however, the vast majority of recent studies of the microbiota are based on 16S sequencing, thus largely ignoring these groups of organisms. For example, norovirus infection, in the context of an intact gut microflora and mutated Atg16l1, is required for the development of CD in a mouse model.[47]

A number of studies note a relationship between fungi and IBD[48] including an overall increase of fungal diversity in UC and CD.[49] The relationship between these organisms and IBD will no doubt be explored in more detail in the coming years, as microbiome studies will increasingly be performed by unbiased shotgun sequencing.

Microbes enriched in IBD may potentiate disease

Specific taxonomic shifts have been reported in IBD (Table 1). The Enterobacteriaceae are increased in relative abundance both in IBD patients and in mouse models.[50] *Escherichia coli*, particularly adherent-invasive *E. coli* (AIEC) strains, have been isolated from from ileal CD (iCD) biopsies in culture-based studies,[51] and are enriched in UC patients.[52] This enrichment is more pronounced in mucosal samples compared to fecal samples.[53] The increase in Enterobacteriaceae may indicate the preference of this clade for an inflammatory environment. In fact, treatment with mesalamine, an anti-inflammatory drug used to treat IBD, decreases intestinal inflammation and is associated with a decrease in *Escherichia/Shigella*.[39, 54]

In addition to trends seen in the lumen, a number of studies have observed a shift in microbes that are attached to the intestinal mucus layer. The small intestine has a single layer of mucus, whereas the colon has two mucus layers, a firmly attached inner mucus layer that is essentially sterile, and an outer mucus layer of variable thickness.[55] The mucus layer consists of mucins, trefoil peptides, and secretory IgA.[56] Though host-microbiota interactions are bidirectional, direct contact with the epithelium is limited by the mucus and the production antimicrobial factors such as defensins and RegIII-gamma.[57–59] As long as the mucus layer is relatively healthy and intact, microbes will attach to the mucus and generally do not have direct access to epithelial cells. There is a greater overall density of attached bacteria on the colonic mucus layer in UC patients compared to healthy controls.[2] The AIEC pathovar, in particular, is at higher abundance in mucosal biopsies from CD compared to healthy individuals, and particularly high in ileal specimens.[60] AIEC invades epithelial cells and can replicate within macrophages[61] and induce granuloma formation *in vitro*.[62] In fact, *E.coli* has also been found at higher levels in granulomas from CD relative to other non-CD granulomas.[63]

A second group of adherent and invasive bacteria is the Fusobacteria. The genus *Fusobacterium* is a group of Gram-negative anaerobes that principally colonize the oral cavity, but can also inhabit the gut. *Fusobacterium* spp. have been found to be at higher abundance in the colonic mucosa of patients with UC relative to control individuals,[64, 65] and human isolates of *Fusobacterium varium* have been shown to induce colonic mucosal erosion in mice by rectal enema.[66] The invasive ability of human *Fusobacterium* isolates has a positive correlation with the IBD status of the host,[67] suggesting that invasive *Fusobacterium* spp. may influence IBD pathology. Intriguingly, *Fusobacterium* species have recently been shown to be enriched in tumor versus noninvolved adjacent tissue in colorectal cancer[68] and human *Fusobacterium* isolates have been demonstrated to directly promote tumorigenesis in a mouse model.[69] As IBD is among the highest risk factors for the development of colorectal cancer, *Fusobacterium* spp. may represent a potential link between these diseases.

Protective effects of microbes in IBD

Several lines of evidence suggest that specific groups of gut bacteria may have protective effects against IBD. For example, the colitis phenotype following treatment with dextran sulfate sodium is more severe in mice that are reared germ-free compared to conventionally reared mice.[70] One mechanism by which the commensal microbiota may protect the host is colonization resistance, in which commensals occupy niches within the host and prevent colonization by pathogens[71] and help out-compete pathogenic bacteria[72] (Interestingly, the microbiota can sometimes take on the opposite role and facilitate viral infection.[73]) Commensal microbiota can also have direct functional effects on potential pathogens, for example in dampening virulence-related gene expression.[74] In addition, the gut microbiota plays a role in shaping the mucosal immune system. *Bacteroides* and *Clostridium* species have been shown to induce the expansion of T_{reg} cells, reducing intestinal inflammation.[75] Other members of the microbiota can attenuate mucosal inflammation by regulating nuclear factor (NF)-κB activation.[76]

A number of bacterial species, most notably the *Bifidobacterium, Lactobacillus*, and *Faecalibacterium* genera, may protect the host from mucosal inflammation by several mechanisms, including the down-regulation of inflammatory cytokines[77] or stimulation of IL-10,[78] an anti-inflammatory cytokine. *Faecalibacterium prausnitzii*, one such proposed member of the microbiota with anti-inflammatory properties, is under-represented in IBD.[79] *F. prausnitzii* is depleted in iCD biopsy samples concomitant with an increase in *E. coli* abundance,[80] and low levels of mucosa-associated *F. prausnitzii* is associated with higher risk of recurrent CD following surgery.[78] Conversely, recovery of *F. prausnitzii* after relapse is associated with maintenance of clinical remission of UC.[81]

Several constituents of the gut microbiota ferment dietary fiber, a prebiotic, to produce short-chain fatty acids (SCFAs), which include acetate, propionate, and butyrate. SCFAs are the primary energy source for colonic epithelial cells[82] and have recently been shown to induce the expansion of colonic T_{reg} cells.[75] The Ruminococcaceae, particularly the butyrate-producing genus *Faecalibacterium*,[83] is decreased in IBD, especially in iCD.[38, 39, 42, 78, 80] Other SCFA-producing bacteria including Odoribacter and the Leuconostocaceae are reduced in UC, and *Phascolarctobacterium* and *Roseburia* are reduced in CD.[39] Interestingly, the Ruminococcaceae consume hydrogen and produce acetate that can be utilized by *Roseburia* to produce butyrate,[39] and it is therefore consistent that both groups together are reduced in IBD.

Functional composition of the gut microbiota in IBD

At the phylogenetic level, there is a generally high variability in the human microbiota between and within individuals over time.[13] However, the functional composition (i.e. the functional potential of the gene content of the metagenome) of the gut microbiota is strikingly stable.[13] Metagenomic approaches may therefore provide greater insight to the function of the gut microbiota in disease than taxonomic profiling;[84, 85] indeed, one such metagenomics study of the IBD microbiome found that 12% of metabolic pathways were significantly different between IBD patients and healthy controls compared to just 2% of

genus-level clades.[39] Metagenomic and metaproteomic studies have confirmed a decrease in butanoate and propanoate metabolism genes in iCD[39] and lower overall levels of butyrate and other SCFAs in iCD,[86] consistent with the decreases in SCFA-producing Firmicutes clades seen in taxonomic profiling studies. Another metagenomic trend that has been identified in the IBD microbiome is an increase in functions characteristic of auxotrophic and pathobiont bacteria, such as a decrease in biosynthesis of amino acids, and an increase in amino acid transporter genes.[39] These bacteria generally have a reduced ability to produce their own nutrients, but rather transport them from the environment as they are readily available at sites of inflammation and tissue destruction.[39]

A number of studies note an increase of sulfate-reducing bacteria, such as *Desulfovibrio*, in IBD.[87] Mesalamine, a common treatment for IBD, inhibits fecal sulfide production and, intriguingly, stool samples from patients not treated with mesalamine show higher levels of sulfide.[88] Genes involved in the metabolism of the sulfur-containing amino acid cysteine are increased in IBD, particularly in iCD, and there is increased sulfate transport in both UC and CD.[39] Saturated fat-derived taurine conjugates to bile acids, increasing the availability of free sulfur and causing an expansion of the sulfate-reducing pathobiont *Bilophila wadsworthia*, driving colitis in genetically susceptible *Il10*$^{-/-}$ but not wild-type mice.[89] The IBD metagenome has an increased propensity for managing oxidative stress, a hallmark of an inflammatory environment, as indicated by increased glutathione transport and riboflavin metabolism in UC.[39] There is also an increase in type II secretion systems,[39] which are involved in the secretion of toxins, and an increase in bacterial genes with virulence-related functions[86] in patients with CD, indicative of a shift towards an inflammation-promoting microbiome.

The gut microbiota in related diseases

A number of parallels can be drawn between IBD and related metabolic diseases such as type 2 diabetes (T2D) and obesity. For example, there is an overall decrease in diversity in obesity at both the phylogenetic level (i.e. reduced number of distinct species)[30] and metagenomic gene-count level (i.e. reduced number of distinct genes).[90] Major shifts in clade abundances include a reduction of the Firmicutes and Clostridia in T2D[91] and a significant increase in the Firmicutes-to-Bacteroidetes ratio in obesity in mice;[92] however, it is less clear whether this shift also holds true in human obesity.[30, 93] There is a decrease in *Bifidobacterium* species in obesity and T2D,[93] and *Bifidobacterium* is reduced in children who become overweight,[94] suggesting that it may act as a predictive factor. As in IBD, *Faecalibacterium prausnitzii* is reduced in abundance in T2D.[95] In terms of gene function, there is an enrichment of genes involved in membrane transport,[96] sulphate reduction, and oxidative stress resistance functions, and a decrease for functions involving cofactor and vitamin metabolism and butyrate production.[97] Therefore, many of the same shifts in function of the gut microbiota -- and even specific taxa -- are seen across these diseases, suggesting the existence of generalized features that relate these diseases and the selection for an auxotrophic microbiota that can thrive in an inflammatory environment.

IBD treatments affecting the microbiome

An array of antibiotics have been shown to lead to a bloom of *Escherichia coli*.[98] Since increased *Enterobacteriaceae* is a distinctive feature of intestinal inflammation and oxidative stress, the relationship between microbial composition, inflammation, and antibiotic use forms an important topic for future research. In contrast, some promising data show that antibiotic therapy specifically in IBD does induce remission or prevent relapse, but this topic will require further controlled trials.[99] To better understand the consequences of perturbing the gut microbiota of patients and the role of the microbiota in treatment outcome, studies that monitor the temporal response at the levels of microbial ecology and functional composition will be required.[100] Thus far, several studies of healthy humans briefly exposed to some antibiotics demonstrate the substantial perturbation, and the level of resilience, of the gut microbiota.[101] Repeated exposures to a single antibiotic in healthy individuals results in cumulative and persistent changes to gut microbial composition.[102] Microbial homeostasis is typically disrupted by the loss of species complexity, particularly of protective microbes, thereby potentially resulting in an increased risk of infections,[103] or dysbiosis.[45] Another mechanism by which antibiotics lead to increased gut infections is by causing a thinning of the mucus layer, thereby weakening its barrier function.[104]

Instead of perturbing the existing microbiome by removing diversity through antibiotics, repopulating the gut habitat with a healthy community has gained popularity in the last few years. This will be an exciting new direction for the pharmaceutical industry, expanding the focus beyond traditional small molecules and biologics.[105] The complexity and composition that will be used to repopulate the gut community will be very important. The success of probiotics in the management of IBD ranges from mixed results to considerable potential,[106] and is dependent on the strains used and disease subtype targeted. In contrast, the evidence that fecal microbiota transplantation (FMT) can be highly effective in replenishing our complex microbiota has received considerable attention following a convincing clinical trial for the treatment of relapsing *C. difficile* infection.[107] Related studies have shown that the use of a well-selected community subset rather than whole fecal communities can be sufficient for recovery.[108] The high success rate reported for relapsing *C. difficile* infection has elevated FMT as an emerging treatment for several gastrointestinal and metabolic disorders,[109] and is actively being considered for IBD.[110] So far, the sparse results reported for IBD cases have been variable with regard to the success rate for inducing remission, and well-designed randomized control trials are currently still lacking.[111] Although changes in the composition of the intestinal microbiota were significant, and reductions in Proteobacteria as well as an increase in *Bacteroides* after FMT were observed, reaching or maintaining remission has been less frequent.[112] Changing protocols towards repeated FMT procedures for multiple days in a row seems to increase the chances for achieving clinical remission.[113]

Future directions

Studies thus far have been able to address many aspects of IBD, including genetics, immune responses, microbial dysbiosis, and microbial functional activity. However, because of the complexity of the human microbiome as a dynamically interacting system, only limited data

has been produced to bridge the gap between pathogenesis in a human host, individual microbes, and alterations in microbial metabolism and function. This suggests the need for a more multifaceted approach to the microbiome in IBD (Figure 2). Since the gaps in IBD are arguably the narrowest among diseases in the microbiome field, as indicated by the progress reviewed above, it can be considered as a model for systems-level investigations of human-associated microbial communities and their interactions with the host's immune system. Increasing our mechanistic understanding of host-microbe interactions through such a systems-level approach will provide new opportunities to develop diagnostics and treatments,[114] but is certainly not without challenges.[115] Recent technological advances, including improvements in sequencing and computational biology, are contributing novel methods to study human-associated microbial communities,[116] with an increasing focus on functional follow-up using cultured microbes and germ-free animal models.

Essential to further explorations, more longitudinal surveys of patients before, during, and after treatment, as well as large-scale clinical trials that take into account both microbial and genetic heterogeneity, will need to be performed, and should take a multifaceted approach. One approach to increase sample size is to combine different cohort studies. However, sample type,[117] collection,[118] and extraction[119] can introduce artifactual differences in microbial composition, which unfortunately makes it more challenging to combine datasets produced under different protocols.[120] Streamlining experimental protocols across cohorts will be essential to preserve statistical power for identifying true biological effects in microbiome datasets. This will require growing efforts for standardizing the collection of patient samples and their clinical information. The discussion around the value of uniform data collection has resulted in solutions already,[121] but these need further adaptation for clinical information. Pursuing a true systems biology approach will require a solution for simultaneous measurement of the host state, the microbiome, and the multi-directional signaling between them. Although solutions for sequential isolation of metabolites, RNA, DNA, and proteins from the same unique sample have been described,[122] engineering a solution that can easily be deployed as a self-sampling kit for patients will require further exploration. Such challenges have been addressed with other technologies, for example, in the use of microarrays as a tool for biomarker detection in clinical applications, which led to the establishment of a consortium with a mandate to set up standards and quality measures.[123] Such efforts are now also initiated in the microbiome space with both environmental and clinical relevance (see www.hmpdacc.org, http://www.mbqc.org, and http://www.earthmicrobiome.org), and could eventually allow us to combine several large well-characterized cohorts without the challenge of study-introduced biases.[120]

Despite promising correlations between shifts in microbial composition and disease phenotypes, to date no causative role for the microbiome has been established, and our understanding of the dynamic role of the human microbiome in IBD remains incomplete. The biological questions of interest enabled by prospective, longitudinal studies would be (1) to identify the potential role of the intestinal microbiome in triggering disease; (2) to determine if microbial composition predicts subsequent risk of activity flares; and (3) to examine whether the luminal flora predicts response to therapy. The identification of a correlative microbial pattern in humans that induces antimicrobial defense and ameliorates inflammation would have considerable promise as a novel diagnostic and therapeutic

approach for the management of these complex diseases. Existing correlative genetic-microbial studies have helped to motivate this area, but cannot speak to causality, response to treatment, or risk prediction in the absence of multifaceted longitudinal measurements.

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Abbreviations used in this paper

AIEC	adherent invasive *Escherichia coli*
ATG16L1	autophagy related 16-like 1
CD	Crohn's disease
FMT	fecal microbiota transplantation
FUT2	fucosyltransferase 2
IBD	inflammatory bowel disease
iCD	ileal Crohn's disease
IL	interleukin
NF-κB	nuclear factor-κB
NOD2	nucleotide-binding oligomerization domain containing 2
SCFA	short-chain fatty acid
T2D	type 2 diabetes
UC	ulcerative colitis

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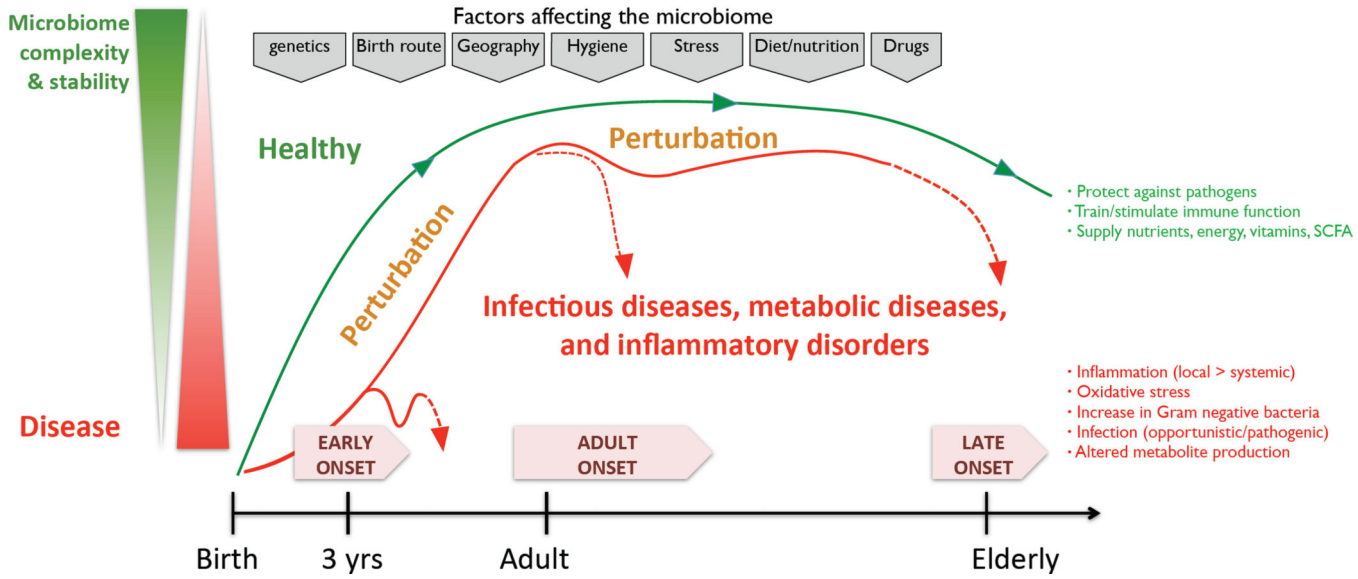

Figure 1. Factors affecting the stability and complexity of the gut microbiome in health and disease

Key characteristics of the microbiome, including stability, resilience, and complexity, are influenced over time from infancy through adulthood and in old age. In the healthy gut, these characteristics contribute to important physiological processes such as protection against pathogens, training of the immune system, and digestion of food to supply energy and nutrients including vitamins and SCFAs. Many factors are indicated to impact the microbiome throughout microbiome development and even established assembly, including genetics, diet, medication, among others (marked in the grey boxes at the top of the figure). Some of these factors can introduce perturbations affecting the complexity and stability of the microbiome, potentially introducing microbial dysbiosis. Features of an imbalanced microbiome include, for example, an increase in Gram-negative bacteria linked to an environment of oxidative stress and inflammation, and metabolite production.

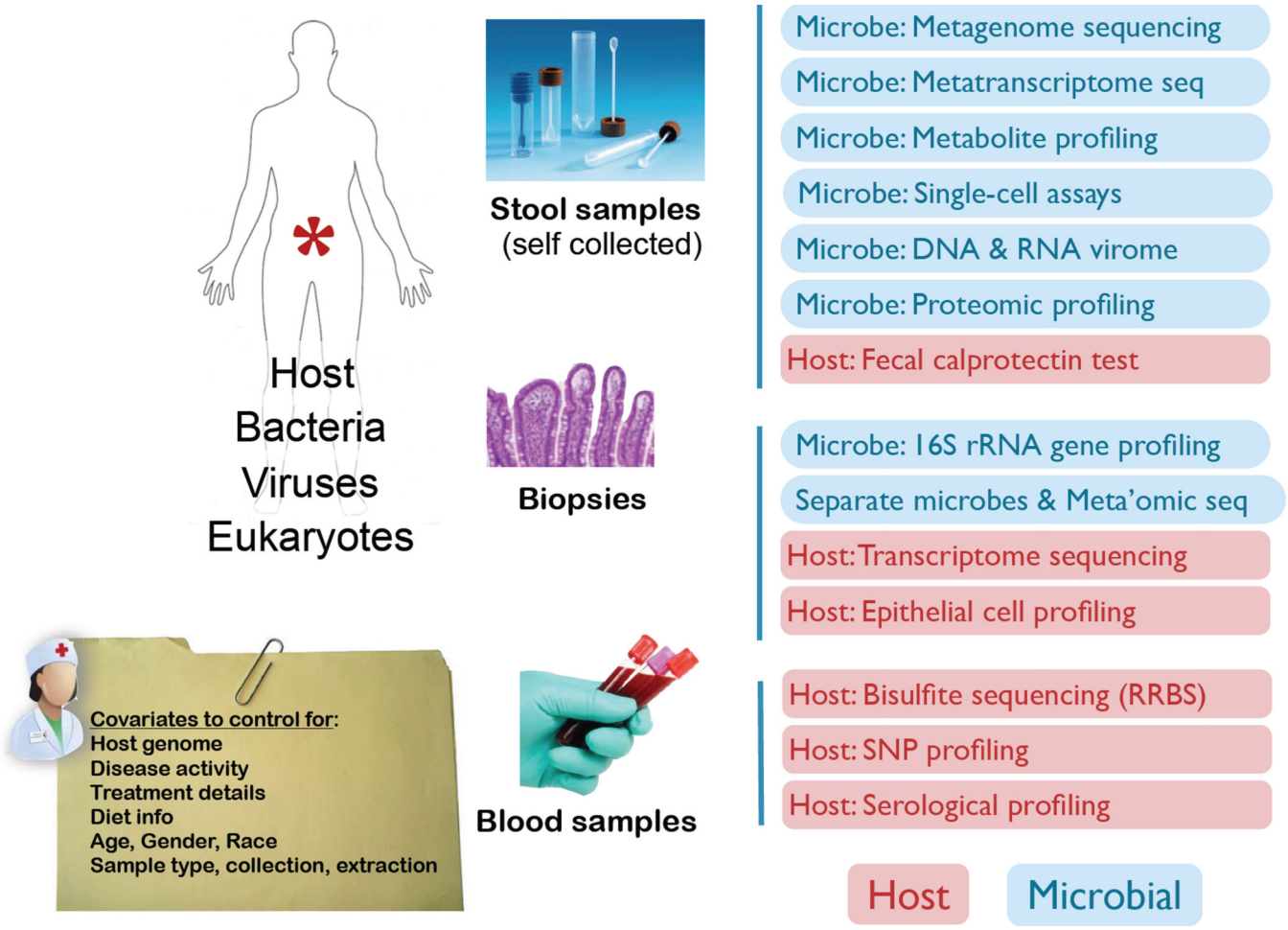

Figure 2. A multifaceted approach to study the role of the microbiome in IBD

Future microbiome studies in the context of disease will shift towards multi-omics approaches in order to study host-microbe relations more comprehensively. Optimized sample collection, detailed clinical annotation, and sample processing will be key to expand data generation far beyond the typical marker gene and shotgun sequencing approaches. A number of assays on the host side (red) and microbial end (blue) will gain increasing attention going forward.

Table 1

Changes in the microbiome linked to Inflammatory Bowel Disease

Microbial composition	Decrease in alpha diversity
	Decrease in Bacteroides and Firmicutes
	Increase in Gammaproteobacteria
	Presence of Escherichia coli, specifically AIEC
	Presence of Fusobacterium
	Decrease in Clostridia, Ruminococcaceae, Bifidobacterium, Lactobacillus
	Decrease in Faecalibacterium prausnitzii
Microbial function	Decrease in Short Chain Fatty Acids (SCFA), butyrate
	Decrease in butanoate and propanoate metabolism
	Decrease in amino acid biosynthesis
	Increase in auxotrophy
	Increase in amino acid transport
	Increase in sulfate transport
	Increased oxidative stress
	Increase in type II secretion system, secretion of toxins