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The Gut Microbiota and Inflammatory Bowel Diseases

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Abstract

Inflammatory bowel diseases (IBD) are chronic diseases of unclear etiology that affect over 1 million individuals in the United States and over 2.5 million people in Europe(1). However, they are also expanding globally, affecting populations in Asia, South America, and the Middle East as they become more industrialized. These diseases are believed to arise from the convergence of genetic, environmental, and microbial factors that trigger aberrant immune and tissue responses, resulting in intestinal inflammation. Advances in cultivation-independent investigations, experimental models, and bioinformatics approaches have improved our understanding of the role of gut microbiota in IBD. However, determining and understanding the functional consequences of gut dysbiosis and altered host-microbiota interactions in IBD remain a challenge due to the limits of current experimental models and difficulty in establishing causal links in human-based investigations. Continued development of new methodologies and improvements in clinical study design are needed to better understand the interplay of genetic, microbial, and immunological factors in IBD. This knowledge can then be applied clinically to improve therapeutic strategies and outcomes for IBD.

Introduction

Inflammatory bowel diseases (IBD) are chronic disorders of unclear typify “Western” disorders, i.e. diseases that were previously uncommon, but now are emerging with alarming frequency over the past century as diet, environment, and social norms have changed in industrialized countries(1). IBD are heterogeneous diseases that likely arise from the convergence of genetic, microbial, and environmental factors. They fall into two main clinical phenotypes, ulcerative colitis (UC) and Crohn’s disease (CD), which were first described in the 19th century and 20th century, respectively(2). UC is characterized by mucosal inflammation in colon, beginning in the rectum and extending proximally in a continuous and circumferential fashion. In contrast, CD can affect any part of the GI tract and its lesions are focal and transmural, progressing to deep ulcerations that can result in development of fistulas, abscesses and strictures. Both types of IBD often involve chronic

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relapsing that can be medically managed, but may require surgical intervention in severe cases.

A role for gut microbes in IBD has been suspected since the early descriptions of potential infectious agents, but no single agent has been proven to cause IBD(2). Many IBD genetic risk variants identified by genome wide association studies, for example, are known or implicated to be involved in mediating host responses to gut microbiota. This has raised the possibility that members of the commensal gut microbiota play a role in the etiopathogenesis of IBD, particularly under conditions that might induce virulence properties. Furthermore, recent advances in experimental models, technology, and computational platforms have shed light on the structure and function of complex microbial communities and host-microbe interactions, creating a framework for understanding potential disease-causing mechanisms.

IBD is clearly associated with intestinal dysbiosis, i.e. the imbalance in the structures and/or functions of gut microbiota that disrupts host-microbe and immune homeostasis(3–7). However, many questions remain unanswered. Is intestinal dysbiosis a cause or consequence of IBD? Are these diseases caused by an aberrant host response to normal gut microbiota and/or to they arise from potentially disease-causing organisms (pathobionts) which are acquired or are part of the commensal microbiota? Are IBD-associated changes in gut microbiota constant or continuously changing throughout the course of disease? How do the environmental, dietary, medications, host, and other factors affect the gut microbiota and IBD development? Are interventions that attempt to correct gut dysbiosis in IBD effective? If not, why? These questions will be addressed by this review, but for many, the answers await further study and inquiry.

Host-genetic factors affecting the development of IBD

Many studies of the genetic basis of IBD, including genome-wide association studies (GWAS), have revealed key insights and improved our understanding of IBD pathogenesis. From the over 200 single-nucleotide polymorphisms (SNPs) associated with increased risk for IBD(8), several pathways and genes have been identified that may either affect the way gut microbes are assembled or how the host is impacted by alterations in the gut microbiome. The latter include functions such as intestinal barrier function, wound healing, autophagy, apoptosis, immune activation, and stress responses(9–13).

Nucleotide-binding oligomerization domain-containing protein 2 (*NOD2*, also known as *CARD15*) was the first gene of the IBD susceptibility genes to be identified that encodes an intracellular receptor for the bacterial peptidoglycan muramyl dipeptide (MDP)(14, 15). The discovery of *NOD2* and other IBD susceptibility genes (e.g. *ATG16L1*, *IRGM*, *CARD9*, and *IL23R*) underscores the importance of innate and adaptive host immune responses to gut microbiota. Furthermore, mutations that potentially alter the function or expression of these genes can affect the gut microbiome in ways that disrupt host-microbe interactions to set the stage for the onset of disease(16, 17). *Nod2*-deficient mice, for instance, exhibit significantly increased representation by Bacteroides, Firmicutes, and Bacilli in their terminal ileum, and decreased ability to clear the pathogenic bacteria, *Helicobacter hepaticus*(18). In addition, CD and UC patients with *NOD2* risk alleles (Leu1007fs, R702W, or G908R) often have

decreased Clostridium Groups XIVa and IV and increased Actinobacteria and Proteobacteria(19). Knights et al. analyzed intestinal biopsy samples from IBD patients and reported the association between *NOD2* risk allele count and increased relative abundance of Enterobacteriaceae. They also suggested that other genetic risk loci (e.g. *TNFSF15* and *IL12B*) appeared to affect the taxonomic structure of the microbiome(20). A study in healthy individuals showed that the *IRGM* mutation (rs11747270 SNP, a CD-risk locus) is associated with increased presence of Prevotella, but negatively correlated with the presence of Bacteroides(21). In turn, gut microbiota appear to affect *Nod2* gene expression, because germ free mice have less *Nod2* expression in the terminal ileum an effect can be restored by conventionalizing the mice with gut microbiota(18).

Not all forms of IBD are equally impacted by changes in gut microbiota. Cases of very early-onset, aggressive IBD, where disease develops soon after birth and before maturation of a gut microbiota, are likely caused by dominant genetic (monogenic) defects(22). For example, IL-10 is known as one of major anti-inflammatory cytokines and the deficiency of IL-10 and IL-10 receptor genes are associated with very early-onset IBD(23–25). Similarly, genetic variants of components of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, required for generation of reactive oxygen species and phagosome function, have been reported in early onset cases(26). Variants in tetratricopeptide repeat domain 7 (*TTC7A*) were also found in patients with severe very early-onset IBD, possibly causing impaired epithelial barrier function.(27)

In the majority of cases, however, genetic susceptibility is insufficient to cause IBD. Even among monozygotic (identical) twins, where the concordance for CD between is between 35% and 58% and for UC between 16% to 18.5%(28–30), the penetrance is not 100%. Thus, non-genetic factors, such as environment and gut microbiota, are important contributors to the pathogenesis of IBD. The T300A variant in *ATG16L1* is associated with ileal CD and with increased endoplasmic reticulum stress in Paneth cell IL-1 β production in response to MDP. However, this gene variant is common in the general population and most subjects carrying it do not develop IBD(31). In addition, mice hypomorphic for the *Atg16l1* mutation that have demonstrated abnormalities of Paneth cells are prone to the development of dextran sodium sulfate (DSS)-induced intestinal inflammation only when concomitantly infected with the replicative murine norovirus(32).

Environmental factors affecting gut microbiota

In the past century, there has been a remarkable increase in incidence and prevalence of IBD on a global scale, especially in industrialized countries where dramatic changes in hygienic conditions, diet, environment, and lifestyle have taken place(33). Over this short evolutionary time scale, the increase in IBD cannot be attributed to genetic drift, and are more likely caused by environmental and societal factors. In this regard, the ever increasing and promiscuous use of antibiotics may have a contributory role by disturbing the gut microbiota. In genetically prone individuals, alterations in critical host-microbe interactions could promote the eventual development of IBD. A cohort study among Danish children from 1995 to 2003 showed a greater relative risk of IBD in antibiotic users compared with non-users(34). A study among children born in 1994–2008 in Finland revealed the risk of

pediatric Crohn's disease increased with the number of antibiotic purchases from birth(35). A retrospective cohort study in the United Kingdom showed that childhood exposure to anaerobe-targeting antibiotics is associated with IBD development(36). A recent meta-analysis also concluded that the exposure to antibiotics is associated with increased risk for CD but not UC, especially amongst children(37).

Rapid changes in environmental factors are also potential contributors to collective shifts of intestinal microbiomes of human populations. A recent population-based cohort study in Canada revealed that there was increased risk of IBD with younger age at time of immigration in individuals from regions with lower prevalence of IBD(38). A cohort study of Danes born in 1973–2008 showed that Cesarean sections moderately increased rates of IBD with onset in childhood(39). Blaser and colleagues showed that mice exposed to sub-therapeutic, low-dose antibiotic therapy after weaning exhibited increased adiposity, changes in hepatic lipid metabolism, and altered immune balance that were associated with significant changes in gut microbial membership and function. This antibiotic treatment increased levels of colonic short-chain fatty acids (SCFAs), products of microbial fermentation that have many bioactive functions affecting the host. They also showed that the transient exposure to antibiotics early in life was sufficient to induce long-lasting effects on body composition. Their observations suggested that the early life exposure to antibiotics impact gut microbiota in ways that can have long-term effects on metabolism and development(40, 41). Deshmukh et al. showed that the antibiotic treatment for pregnant dams cause the alteration of the gut microbiota in their neonates and it was associated with decreased numbers of circulating and bone marrow neutrophils and granulocyte/macrophage-restricted progenitor cells in the bone marrow and increased susceptibility to sepsis of the neonates(42). Finally, Maurice et al. reported that short-term exposure to other medications, such as digoxin and phenacetin, affects the physiology and assemblage of the gut microbiota, which can ultimately change the host ability to metabolize medications(43).

Changes in diet and dietary habits are among the largest shifts that occur with increased industrialization and westernization. In addition to increased daily caloric consumption, diets are higher in fat and refined sugars, and lower in complex carbohydrates and fiber. A controlled-feeding study showed that the composition of gut microbiota is dramatically changed by a high-fat/low-fiber compared to a low-fat/high-fiber diet(44). African children on a high-fiber, plant-based diet exhibit a vastly different gut microbial community than their European counterparts whose dietary intake consists of a diets rich in sugar, fat, and protein(45). A study in wild type and IL-10-deficient mice showed that different types of dietary fat had different effects on gut microbiota. A diet rich in saturated milk fat, for instance, caused shifts in the gut microbiota that favored blooms of Proteobacteria, particularly *Bilophila wadsworthia*, compared to isocaloric, isonitrogenous diets high in polyunsaturated (predominantly n-6) or lard fat. Diet-induced blooms of *B. wadsworthia* increased the incidence and severity of spontaneous colitis in genetically susceptible IL10-deficient mice(46). In addition, David et al. demonstrated in human subjects that an animal-based diet rapidly changed the composition of the gut microbiota and lead to increased levels of bile-tolerant organisms (*Alistipes*, *Bilophila* and *Bacteroides*) including *B. wadsworthia* and decreases in Firmicutes that metabolize plant polysaccharides, such as *Roseburia*, *Eubacterium rectale*, and *Ruminococcus bromii*.(47)

Improvements in hygienic practices and environment conditions over the past century have also been implicated in contributing to the increase incidence and prevalence of IBD. The “hygiene hypothesis” proposes that reduction of important microbial cues necessary to educate the host immune system results in decreased gut microbial diversity and altered host-microbe interactions that can promote disease in susceptible hosts(48). A study in India showed that CD was positively associated with urban living and safe drinking water and negatively with the presence of cattle in the home compound(49). Chung et al. colonized germ free mice with mouse or human microbiota and demonstrated that human microbiota were not sufficient for the proper development of the intestinal immune system due to host-microbe species mismatch. As a consequence, mice colonized with human microbiota were more susceptible to *Salmonella* infection than the mice with mouse microbiota(50). In addition, it has been shown that the maternal microbiota during gestation plays a role in the development of innate immunity in mouse pups, making them less prone to microbial infection and immune activation by microbial signals(51).

Intestinal dysbiosis in IBD patients

Many changes in both structure (composition) and function of gut microbiota are associated with active IBD. Seksik et al. demonstrated that the biodiversity of the microbiota was still high but that Enterobacteria were increased in fecal samples of CD patients(3). Gophna et al. found that increased Proteobacteria and Bacteroidetes and decreased Clostridia in biopsy samples from CD patients(6). Frank et al. reported that resected GI tract tissue samples from CD and UC patients showed depletion of members of Bacteroidetes and Lachnospiraceae (group IV and XIVa Clostridia)(7). Ott et al. and Manicanh et al. found decreased diversity of the gut microbiome in CD and UC using biopsy samples and fecal samples(4, 5). Sokol et al. demonstrated a lower proportion of *F. prausnitzii* in resected ileal specimens were associated with endoscopic recurrence after surgery in CD patients. They also showed that the anti-inflammatory potential of this species and the decrease in fecal samples from active IBD patients(52, 53). Machiels et al. reported a reduced abundance of butyrate-producing *Roseburia hominis* and *Faecalibacterium prausnitzii* in fecal samples of UC patients(54). Gevers et al. analyzed the mucosa-associated microbiota in ileal and rectal biopsy samples from newly-diagnosed pediatric CD patients and found increased abundance in Enterobacteriaceae, Pasteurellaceae, Veillonellaceae, and Fusobacteriaceae, and decreased abundance in Erysipelotrichales, Bacteroidales, and Clostridias(55). In assessing all these findings, the sample type and sites from where they were taken (e.g. fecal or surgical sample, remission or inflamed condition, or which portion of GI tract) have to be considered. The composition and abundance of both luminal and mucosa-associated microbiota vary along the GI tract(56, 57) and can be significantly impacted by intestinal inflammation in a number of different ways. Mucosal-associated microbiota can differ significantly from their luminal counterparts, the latter most likely being the major contributors to fecal microbiota. Thus, the fecal sampling commonly used by many IBD studies may not accurately reflect regional changes in gut microbes, particularly those that are taking place at the mucosal surface at sites of inflammation. Two other challenges to the understanding the role of gut microbes in IBD are related to the bioinformatics analysis of microbial community structure and function. Most methods for analysis do not provide

sufficient resolution to go beyond the genus level(58) and are unable to determine if IBD is caused or contributed to by IBD-related pathobionts or pathogens. However, several new computational platforms have recently been developed that now allow investigators to examine operational taxonomic units at an extremely precise level by utilizing very subtle variations among 16S ribosomal RNA gene sequences(59, 60). For metagenomic and metatranscriptomic analysis to assess functional profiles of microbial communities, an advanced analysis and visualization platform for 'omics data (Anvi'o) now provides an assembly-based metagenomic workflow that integrates, analyzes, and displays sequencing datasets of diverse origins to address a wide variety of questions(61).

Other important members of the gut microbiota that may be relevant to IBD should be mentioned, although they are poorly understood and investigated. Bacteriophage are viruses that infect and replicate within bacteria. They are extremely diverse and abundant, and appear to be as prevalent in the gut as bacteria(62). Temperate phages are bacteriophages which can be either lytic or lysogenic, the former representing virulent phage, whereas the latter integrate into the bacterial genome to be dormant until induction, capable of altering bacterial function and fitness. Bacteriophage also represent an important form of horizontal gene transfer within bacterial populations, the relevance of which in IBD is undetermined. Lepage et al. analyzed virus-like particles (VLPs) in colonic mucosal biopsy samples from CD patients and healthy individuals and detected significantly more bacteriophage in CD patients than in healthy controls. Among samples from CD patients, ulcerated mucosa is less colonized by bacteriophages than non-ulcerated mucosa. They also reported that each individual seemed to harbor one dominant phage family(63). Another study of viral communities in the gut showed a lower diversity of the virome but larger variability among fecal samples from CD patients compared with healthy controls(64). Currently, incomplete inventories of gut phage, technical limitations, and the lack of experimental models prevent us from achieving a more complete annotation of the healthy and IBD virome and in understanding the functional insights of these findings.

Fungi and Archaea are two other groups of the gut microbiota that are poorly understood. Intestinal fungal communities have been shown to interact with the host immune system through the innate immune receptor Dectin-1(65). Dectin-1 recognizes β -1,3-glucans in the fungal cell wall and activates intracellular signals through CARD9 leading to inflammatory cytokine production and induction of Th17 responses. Interestingly, variants in CARD9 are strongly associated with Crohn's disease and ulcerative colitis. In addition, a SNP in the Dectin-1 gene appears to be associated with medically-refractory UC(65). Ott et al. reported that fungal diversity increased in colonic biopsy samples from CD patients compared with healthy controls(66). Li et al. analyzed ileal mucosal specimens and fecal samples from a small patient cohort and showed that fungal diversity, particularly among *Candida albicans*, *Aspergillus clavatus*, and *C. neoformans*, increased in CD patients. They also showed differences in the fungal composition in mucosal samples with and without inflammation(67). On the other hand, Chehoud et al. found a lower diversity of fungal microbiota, although two lineages annotated as *Candida* appeared significantly greater in fecal samples from IBD patients(68). Lewis et al. examined pediatric subjects and showed that five fungal taxa detected in samples (*Saccharomyces cerevisiae*, *Clavispora lusitanae*, *Cyberlindnera jadinii*, *Candida albicans* and *Kluyveromyces marxianus*) were positively

associated with CD(69). Interestingly, anti-Saccharomyces cerevisiae antibodies (ASCA), which are believed to be a response primarily to *C. albicans*, have been used as a serological marker of ileal CD. *C. albicans* was also found to be more abundant in stool samples from CD patients than from healthy controls(70, 71). Even fewer studies have investigated Archaea in IBD. Scanlan et al. found that methanogens are less abundant in fecal samples from IBD patients, possibly impacting methane production and metabolism of the gut microbiota(72). Another study has shown that Archaea are less abundant in children compared to adults and there was no significant differences between pediatric IBD patients and healthy children(68). Similarly, a study of pediatric subjects for the genome of *Methanobrevibacter smithii*, which is the primarily representative of Archaea in the gut, demonstrated that most of children had little or no detectable colonization and there was no significant difference between CD patients and healthy controls(69).

Microbes, mechanisms, and mediators of intestinal dysbiosis that contribute to the pathogenesis of IBD

Intestinal dysbiosis can contribute to the pathogenesis of IBD either by loss of health-promoting or gain of disease-promoting microbes. Mazmanian et al. reported that the human symbiont *Bacteroides fragilis* protect mice from *Helicobacter hepaticus*-induced inflammation through the action of polysaccharide A (PSA). PSA suppressed the production of pro-inflammatory cytokine IL-17 and induced IL-10-producing CD4⁺ T cells(73). Fujiya et al. demonstrated that the quorum-sensing pentapeptide, competence and sporulation factor, of *Bacillus subtilis* is selectively taken up by the membrane organic cation transporter-2 of intestinal epithelial cells, resulting in activation of key survival pathways such as heat shock proteins, Akt, and MAPK(74). Several studies also show that soluble factors produced by probiotics and gut microbes have anti-inflammatory and cytoprotective effects(75, 76).

Short chain fatty acids (SCFAs), which are prodigiously produced by intestinal bacterial fermentation of dietary fiber, appear to be important mediators in health. Their levels are often significantly decreased in IBD which may be a key factor that compromises intestinal and immune homeostasis(54, 77). Maslowski et al. showed that the binding of G-protein-coupled receptor 43 (GPR43, also known as FFAR2) by SCFAs is necessary for the resolution of inflammatory responses(78). Atarashi et al. reported that SCFA-producing bacterial strains in *Clostridia* clusters IV, XIVa, XVII from a healthy human fecal sample induced colonic regulatory T (Treg) cell differentiation, expansion, and function(79, 80). Furthermore, Smith et al. showed that SCFAs regulate the size and function of the colonic Treg pool in a GPR43-dependent manner(81). Thus, inadequate development and function of Treg cells, due to low fiber diet- or dysbiosis-induced decreases in SCFA production can contribute to the severity and chronicity of IBD.

In contrast, the emergence of disease-promoting bacteria (pathobionts and pathogens) that promote immune activation and/or produce factors that negatively affect the host can also potentially contribute the pathogenesis of IBD. For example, increased sulfur-reducing, bile-tolerant bacteria and production of hydrogen sulfide (H₂S) have been reported in UC

patients(82, 83). Many of these types of bacteria have strong anti-oxidant capabilities, which provides a selective advantage to survive in the harsh milieu of intestinal inflammation. H₂S has also be shown to be genotoxic, modulating the expression of genes involved in cell-cycle progression and triggering inflammatory and DNA repair responses(84). H₂S can also impair the oxidation of butyrate(85). The expression of thiosulfate sulfur transferase, one of two enzymes responsible for detoxification of H₂S, has been found to be significantly decreased in active UC, but restored to normal after successful treatment(86). Several reports have implicated Enterobacteriaceae in IBD, largely due to increased observed surges in abundance(3, 6, 87). Two Enterobacteriaceae species, *K. pneumoniae* and *P. mirabilis*, isolated from TRUC mice (mice deficient in innate and adaptive immunity that develop spontaneous UC-like inflammation) are able to cause colitis in wild type mice in concert with the endogenous microbiota(88, 89). Winter et al. showed that host-associated nitrate, derived as a by-product of the inflammatory response, provides increased fitness of *Escherichia coli* to outcompete other bacteria that require fermentation substrates(90). A recent study showed that immunoglobulin A (IgA)-coated intestinal microbes from IBD patients promoted susceptibility to DSS colitis in gnotobiotic mice(91). Another study of undernutrition and enteropathy reported that gut bacteria, particularly Enterobacteriaceae, that promote host IgA responses may interact with other consortium members to produce enteropathy(92). On the other hand, Moon et al. showed that phenotype variations in a same mouse strain at different institutions may be a result of gut microbiota that can degrade the IgA secretory component and IgA itself, causing decreased levels of luminal and fecal IgA(93). They also demonstrated these IgA-low mice exhibited greater susceptibility to DSS-induced colitis than their IgA-high counterparts. Taken together, these findings highlight the complex interplay between gut microbiota and luminal IgA responses in the pathogenesis of intestinal inflammation.

Investigations of bacterial transcriptomes have provided additional insights into microbial virulence and fitness under conditions of inflammation-induced stress. *E. coli* NC101 from *IL-10*^{-/-} mice with colitis showed upregulation of the stress response operon for small heat shock proteins IbpA and IbpB that confer protection from oxidative stress. Increases in IbpA and Ibp was also implicated in reducing host inflammatory responses(94). Other studies of adherent-invasive *E. coli* (AIEC) showed that specific FimH mutations were associated with CD and UC patients(95, 96). FimH facilitates AIEC binding to the mucosa and increased abundance of AIEC has been reported in IBD. Collectively, studies to date have shown that the inflammatory environment induces gene expression and function of certain bacteria in ways that allow them to survive, adapt, and contribute to the pathogenesis of IBD.

Several attempts have been made to characterize and understand the functional properties of gut microbial communities in IBD. Morgan et al. assessed microbial functions based on reference genomes of well represented taxa of fecal samples and intestinal biopsies from IBD patients and healthy subjects, validating some of their findings with shotgun metagenomics(97). They found that 12% of the analyzed functional pathways and 2% of genera changed in IBD patients, suggesting that functional inferences might be more informative than changes in microbial composition. Increases in glycolysis and carbohydrate transport/metabolism and decreases in lipid metabolism and catabolism modules were found, possibly indicating alterations of energy metabolism in microbial communities. In

addition, increased representation of genes involved in secretion systems associated with cell wall degradation and exotoxins as well as microbial adherence/invasion were also observed in ileal CD. Greenblum et al integrated the metagenomic data with an *in silico* systems analysis of metabolic networks(98). They concluded that much of the microbial enzyme-level variation associated with IBD seemed to relate to changes in how the gut microbiome interacts with the host gut environment rather than alterations in core metabolic processes. In the analysis of newly-diagnosed, treatment-naive pediatric CD patients, Gevers et al. employed the PICRUSt algorithm to predict the microbial function with mucosal biopsy samples and also performed shotgun metagenomics sequencing on a subset of fecal samples(55). PICRUSt showed a loss in basic biosynthesis associated with reductions in *Bacteroides* and *Clostridia* and a switch towards a pathobiont-like auxotrophy associated with increases in aerobic or aerotolerant taxa, such as *Proteobacteria* and *Pasteurellaceae*. From their shotgun metagenomics analysis, they concluded that the bacterial species increased in CD contributed to the glycerophospholipid and lipopolysaccharide metabolism pathways thought to cause inflammation and that increased phosphonoacetate hydrolase expression would enhance the ability to utilize novel carbon and phosphate sources.

Challenges to microbiome studies in IBD

Over the past decade, major advances have been made in understanding the role of the intestinal microbiota in IBD pathogenesis. However, it still remains unclear whether IBD-associated dysbiosis is causative, contributory, or consequential to the disease. In all likelihood, all three possibilities occur. The inability to identify with certainty causative microbial organisms is due to limitations in technology, bioinformatics, and clinical study design. Most studies of IBD dysbiosis, for example, have been performed after the onset of disease and interpreted without consideration of clinical metadata other than the clinical phenotype (CD or UC) and location of disease. Most microbial data have been acquired from stool samples, which may not reflect the more disease-relevant changes in regional and mucosa-associated microbiota. The presence of mucosal immune activation and inflammation also exert selective pressures, favoring microorganisms that have the ability to survive and flourish under these harsh conditions and impairing the fitness of most commensal microbes. Thus, the dysbiosis described by most cross-sectional studies is mostly a consequence of the disease process. That said, many of the members of the IBD-associated microbiota are pro-inflammatory which helps sustain the conditions favorable to them, and, at the same time, promote the chronicity and severity of IBD. To identify causative microbes, i.e. those responsible for the initial development of IBD, is difficult, because studies ideally should be done before the onset of disease, which, in most cases, is not possible because we are unable to determine who will develop IBD. The analysis of IBD-associated dysbiotic microbial communities is also limited by the commonly used computational platforms for 16S rRNA gene and metagenomic/metatranscriptome analysis, the former unable to go beyond the genus level and the latter by incomplete inventories of microbial genome/genes to provide insight into community function. However, higher resolution bioinformatics tools are now becoming available that can provide strain- and genome-level information which ultimately may reveal key insights into the pathogenesis of IBD (Minimum Entropy Decomposition and Anvi'o) (59–61).

Cross-sectional studies of gut microbiota in IBD subjects have provided a plethora of descriptive information that often is difficult to interpret, mostly because of large inherent variations in individual gut microbiota and the heterogeneity of study subjects. Future studies should involve a longitudinal design that can be performed on fewer patients because each patient would serve as their own control. Moreover, clinical information collected at each point can be used to interpret corresponding changes in gut microbiota and host functions. These metadata also provide a basis for developing leads that can be followed up experimentally and to develop more informative measures to stratify IBD patients that can help physicians achieve more precise diagnosis, treatment, and better outcomes.

For all its limitations, research using animal models of IBD still represents our best opportunity (currently) to discover the mechanisms of pathogenesis. The use of gnotobiotic animal models, for example, provides the surrogate measure to assess the function of specific microbial communities or evaluate individual strains in the context of a controlled environment, genetics, diet, and disease. As the microbiome field moves away from descriptive studies toward a deeper understanding of microbiota function, the importance of metabolomics studies, in tandem with other meta-'omics (genomes, transcriptomes, proteomes) will be paramount to discovering how host and microbes choreograph their interactions in health and disease. Similarly, metabolomics approaches to studying gut microbiota(99) or IBD patient populations(100) will contribute important knowledge of bioactive molecules that could translate into therapeutic innovations for inflammatory disease.

The pitfalls, potential, and promise of microbiome-base interventions in IBD

If IBD is caused by the convergence of host genetics, environment, and gut microbes, there is a strong rationale for controlling the environment (e.g. smoking, diet, etc) and in restoring a healthy state to the gut microbiota, as we currently have no measures to correct genetic anomalies. Several microbiome-based interventions have been studied in IBD, including pre-, pro-, and postbiotics. With the exception of a few circumstances, few of these agents have shown promise or efficacy(101, 102). The lack of success of these agents is not surprising, given the rather simplistic view on which these approaches are based, i.e. restoring “good” microbes by either sheer mass or through changing conditions that mostly favor fermentative microbes that produce SCFAs. Many of these preparations contain microbial strains that may not be indigenous to the gut or were selected on limited or no evidentiary basis. The notion that these microbes can take hold, supercede the endogenous microbiota of IBD patients, and survive in a hostile inflammatory environment is tenuous. Moreover, it is unlikely that “one size fits all”, given the heterogeneity of IBD patients and their microbiota.

Recently, fecal microbiota transplantation (FMT) has attracted considerable attention as a treatment option for IBD. The rationale for FMT is based on the notion that the donor microbiota has been naturally selected under conditions that promote health. The transplant of this “microbial organ” to a host recipient would replace the functions of the diseased microbial organ, thus restoring intestinal and immune homeostasis. While highly effective for the treatment of *Clostridium difficile* colitis, its efficacy in IBD remains

controversial(103). Interestingly, in studies of FMT for UC patients, Angelberger et al.(104) and Rossen et al.(105) reported that only responders showed successful colonization of donor-derived phylotypes at 12 weeks after FMT, which may be indicative of how difficult it is to “transplant” another person’s microbial organ into an IBD host. Many factors may therefore have to be taken into consideration to increase the success of FMT in IBD, such as the genetic background of donor and recipient, environmental factors, disease states, and the inherent resilience of the recipient’s microbiota to change, which may be difficult to replace because these organisms have been already selected for their ability to survive and flourish in an inflammatory condition. Other factors should also be taken under consideration as well, such as long-term complications and unintended consequences of FMT. A recent study by Chehoud et al. looked at a single healthy adult donor and three pediatric ulcerative colitis recipient patients who received multiple FMT treatments over the course of 6–12 weeks(106). While the initial response to FMT appeared promising, all three subject eventually relapsed and had to be placed on immunomodulators. However, the study also found the transfer of bacteriophage populations and their genetic elements from donor to recipients that persisted. The long-term clinical significance of these findings remains uncertain, but they underscore the importance of developing better tools, more studies at the clinical and experimental level, and longer-term follow-up. Without these pieces of information, we are ill-equipped to fully define the microbiota which in a practical sense impacts the success and failure of FMT.

Today’s therapeutic goal for IBD is to induce and maintain remission, however, the ultimate goals should focus on prevention and cure. With regard to the microbiome, we have the opportunity to gain more knowledge that can be leveraged to achieve these goals.

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Abbreviations

IBD	inflammatory bowel diseases
GI tract	gastrointestinal tract
UC	ulcerative colitis
CD	Crohn’s disease
GWAS	genome-wide association study
SNP	single-nucleotide polymorphism
NADPH	nicotinamide adenine dinucleotide phosphate
MDP	muramyl dipeptide
DSS	dextran sodium sulfate

SCFA	short-chain fatty acids
VLP	virus-like particle
ASCA	anti-Saccharomyces cerevisiae antibody
PSA	polysaccharide A
GPR43	G-protein-coupled receptor 43
Treg	regulatory T cell
IgA	immunoglobulin A
AIEC	adherent-invasive <i>Escherichia coli</i>
FMT	fecal microbiota transplantation

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