The microbial basis of inflammatory bowel diseases

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Inflammatory bowel diseases (IBD) are chronic, progressive diseases characterized by aberrant immune responses to environmental and gut microbial triggers in genetically susceptible hosts. Clinical, genetic, and experimental data support the role of gut microbes in causing and sustaining these diseases. Our understanding of IBD has changed dramatically as the result of advances in cultivation-independent approaches and computational platforms for the analysis of large data sets. However, investigations relevant to clinical observations and the natural history of the diseases will be essential for the development of microbial, genetic, and biological metrics that may be used to individualize assessment of risk and improve clinical outcomes in IBD.

The inflammatory bowel diseases (IBD) are a group of chronic diseases that result from a confluence of genetic and environmental triggers. IBD have two main clinical phenotypes, ulcerative colitis (UC) and Crohn’s disease (CD). UC is characterized by mucosal inflammation that is limited to the colon, beginning in the rectum and extending proximally in a continuous and circumferential fashion. CD, in contrast, can occur at any location in the gastrointestinal tract, from mouth to anus, though each patient’s CD typically involves a specific region, such as the small bowel or colon. CD is characterized by focal lesions that progress to ulcers that can extend through the bowel wall and result in the development of fistulas, abscesses, and strictures.

A microbial basis for IBD has long been suspected, starting with the early descriptions of potential infectious agents associated with UC in the 19th century and CD in the early 20th century (1, 2). Despite these associations, no single agent has been proven to cause IBD. As a result, research and clinical management strategies shifted away from the notion that IBD are infectious diseases, and instead toward understanding and treating the underlying immune and inflammatory processes. However, the development of cultivation-independent approaches to characterize structural and functional profiles of complex microbial communities revealed many new insights into the relationships between host and microbes. At the same time, the pivotal role of indigenous gut microbes in causing IBD became apparent with the first descriptions of IBD susceptibility genes, as many were involved in mediating host responses to gut microbes.

Most experts now recognize that intestinal dysbiosis, or the imbalance in the structural and/or functional properties of the gut microbiota that can disrupt host-microbe homeostasis, is integral to the pathogenesis of IBD (3–5). However, the field remains descriptive, and some of the most basic questions about the role of the gut microbiota in IBD remain unanswered. Is the intestinal dysbiosis a cause or consequence of IBD? Are these diseases caused by the emergence of pathobionts and/or disappearance of symbionts, or merely due to an aberrant host immune response to commensal microbiota? Are the host-microbe disturbances constant or changing throughout the natural history of these diseases? What role do environmental and dietary factors play in determining the risk and course of IBD? Can IBD be prevented or treated by correcting the intestinal dysbiosis? Answering these questions will significantly advance the field and improve the understanding and management of human IBD. The clinical ramifications of the gut dysbiosis in IBD can only be sorted out by relating these findings to the natural history and complexities of these diseases.

Host factors affecting microbial assemblage and IBD etiopathogenesis

The assemblage of gut microbes throughout life is not stochastic, but determined by a combination of host, microbial (i.e., self-selective), and environmental factors. Genome-wide association studies (GWASs) provided some of the first insights into the host-microbe connection in IBD. Over 160 SNPs associated with increased risk for IBD have been identified (6). Of the genes and gene loci known to be associated with IBD susceptibility, many appear to be involved in processes critical for determining how the host responds to microbes, including barrier function, wound healing, autophagy, immune balance, and stress responses (6–8).

Nucleotide-binding oligomerization domain–containing protein 2 (NOD2, also known as CARD15) was the first of these genes to be identified and encodes an intracellular receptor for the bacterial peptidoglycan muramyl dipeptide (MDP) (9, 10). This discovery and that of other IBD susceptibility genes (e.g., ATG16L1, IRGM, CARD9, and IL23R) shed light on fundamental relationships between host genetics with gut microbes, particularly with regard to innate and adaptive immune functions that affect the assemblage of the gut microbiome (8). Mutations in NOD2 and other genes that interact with it, for instance, are implicated in causing the intestinal dysbiosis associated with CD, possibly through altered states of innate and adaptive immune balance, impairment of autophagy, defective Paneth cell function, and perturbations of non-MDP–dependent functions (11, 12). Nod2-deficient mice have significantly increased Bacteroides, Firmicutes, and Bacillus bacterial loads in the terminal ileum, as well as decreased…

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ability to clear a pathogenic bacterial species, *Helicobacter hepaticus* (13). Studies in human patients have also shown that the presence of a *NOD2* risk allele (Leu1007fs, R702W, or G908R) is associated with shifts in microbial composition, such as decreased Clostridium Group XIVa and IV and increased Actinobacteria and Proteobacteria, in surgical specimens from CD and UC patients as compared with controls (14, 15). The luminal bacteria, in turn, appear to feed forward to affect *Nod2* gene expression, because germ-free mice have less *Nod2* expression in the terminal ileum. Conventionalization of these mice with gut microbiota restores *Nod2* expression (13). On the other hand, the dysbiosis associated with *Nod2*-deficient mice appears to confer risk of colitis. Wild-type mice co-housed with *Nod2*-deficient mice developed greater inflammation in a model of chemically induced colitis and exhibited enhanced expression of genes related to apoptosis, necrosis, and tumorigenesis (16). The findings further raise the possibility that the maternal transmission of a disease-promoting microbiota to offspring might contribute to the risk of disease in familial forms of IBD.

While genetic risk is an important factor in IBD and can influence the assemblage of gut microbes in individuals, it is not likely to be sufficient in causing disease except in rare cases of aggressive, early-onset IBD such as the disease caused by deficiency of IL-10 or the IL-10 receptor (17). This partial role for genetics is highlighted by the concordance rate for CD between monozygotic twins, which is between 35% and 58%, while that for UC is only 16% to 18.5% (18–20). In both cases, these rates are much higher than that of the general population and dizygotic twins, which supports a genetic basis for IBD. However, the fact that the penetrance is not 100% strongly suggests that other, non-genetic factors are involved in determining disease, such as the gut microbiome, environmental factors, or epigenetic modifications acquired through life. These types of disease modifiers can potentially trigger the onset of disease and influence the phenotype and clinical course. For example, the T300A variant in *ATG16L1* is associated with ileal CD, which may be related to disturbances in Paneth cell function and increased IL-1β production in response to MDP (21). However, many individuals in the general population carry this variant and remain healthy (21). In fact, the T300A variant increases disease risk by less than 2-fold, which suggests a role for other factors that interact with the gene to confer risk. Along these lines, mice hypomorphic for the *Atg16l1* mutation exhibit Paneth cell abnormalities, such as abnormal lysozyme granule packaging (22). When these mice are infected with murine norovirus and then challenged with the colitis-inducing agent dextran sodium sulfate (DSS), the mice develop an intestinal pathology resembling CD. In contrast, *Atg16l1*-deficient mice infected with a nonreplicative norovirus develop far less severe inflammation.

**Dietary and environmental factors affecting the gut microbiome**

One of the most striking features of complex immune disorders such as IBD is the alarming increase in their incidence and prevalence over the past century, particularly in highly developed urban populations. A systematic review of population-based IBD data revealed that among studies showing a significant rise in incidence, the average annual percentage change ranged from 1.2% to 23.3% in CD and from 2.4% to 18.1% in UC over study periods from 1920 to 2010 (23). The rapid pace of these developments cannot be explained by genetic drift, but is more likely due to shifts in environmental factors and societal norms brought on by cultural westernization. Conditions that have been implicated in changing the gut microbiome of Western societies include the exposure to environmental pollutants and xenobiotics, wide and indiscriminate use of antibiotics by livestock producers and in medical practice, and lifestyle changes (e.g., diet, stress, and sleep disorders). Turnbaugh and colleagues have shown that short-term exposure to a specific medication, digoxin, elicited effects on the physiology, structure, and gene expression of the microbiome, which in turn altered the drug’s action on the host (24). The Blaser group studied the impact of sub-therapeutic antibiotic therapy (STAT) on gut microbes, a practice widely used in livestock to promote weight gain for food production (25). Mice exposed to STAT after weaning exhibited increased adiposity, changes in hepatic lipid metabolism, and altered immune balance that was associated with significant changes in gut microbial membership and function. In addition, STAT increased levels of colonic short-chain fatty acids (SCFAs), products of microbial fermentation that have many trophic biological effects on the host. The authors concluded from these findings that exposure of humans to antibiotics early in life could have long-lasting effects on metabolism and development. A study of Danish children from 1995 to 2003 provides further support for this notion, finding a 1.84-fold greater relative risk for IBD in children that received antibiotics as compared with those who did not (26). Caesarean sections are another common feature of Western medical practice that can affect the acquisition of the gut microbiome and possibly increase the risk of IBD. A Danish cohort study of subjects born from 1973 to 2008 found that Caesarean section was associated with a modest increased risk of IBD in children in the age range of 0 to 14 (27). Assuming causality, the authors estimated 3.2% of IBD cases diagnosed before the age of 15 were attributable to Caesarean section.

The evolution of human dietary patterns over time and the recent popularization of the high-fat, high-sugar Western diet also represent major selective forces in microbiome assembly. 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 Western diet rich in sugar, fat, and protein (28). Such shifts in microbial assemblage have consequences for intestinal health, as was demonstrated in a study of genetically susceptible IL-10-deficient mice fed a diet rich in saturated milk fat. This diet promoted a bloom of *Bilophila wadsworthia*, which increased the incidence and severity of spontaneous colitis in these animals (29). Similarly, Turnbaugh and colleagues placed human subjects on either an animal-based diet of meats, eggs, and cheese, or a plant-based diet of grains, legumes, fruits, and vegetables, and compared microbial community structures in the subjects’ stool (30). The subjects on an animal-based diet had increased levels of bile-tolerant organisms such as *Alstes* and *B. wadsworthia* and decreases in Firmicutes that metabolize plant polysaccharides, such as *Roseburia*, *Eubacterium rectale*, and *Ruminococcus bromii*.
While the highest rates of IBD have been reported in Europe and North America, the incidence has also risen in Asia over the past 50 years, particularly in Westernized nations (23, 31–33). One popular theory for this trend, dubbed the “hygiene hypothesis,” postulates that the adoption of hygienic practices has reduced exposure to important environmental and microbial cues necessary for proper immune development, resulting in perturbations that trigger aberrant immune responses in genetically susceptible hosts (34). For example, one Indian study found higher rates of IBD in homes with safe drinking water, whereas the presence of cattle in the home compound was negatively correlated with IBD (33). Kasper and colleagues proposed that the natural selection of host commensal microbes is necessary for the proper development of the intestinal immune system (35). This group colonized germ-free mice with mouse or human microbiota and found that the latter group exhibited fewer CD4+ and CD8+ T cells, proliferating T cells, and dendritic cells and lower anti-microbial peptide expression. Collectively, these studies suggest that driving factors associated with increased westernization perturb critical, evolutionarily determined host-microbe relationships, triggering diseases such as IBD in individuals unable to compensate for these changes.

**Intestinal dysbiosis in IBD**

Through rapid advances in cultivation-independent technologies and bioinformatics, the intestinal dysbiosis in active human IBD and experimental colitis has been fairly well described. Frank et al. found that CD and UC patients had depletion of members of Bacteroidetes and Lachnospiraceae (group IV and XIVa Clostridia) (4). Several other groups have described decreased biodiversity, with a lower proportion of Firmicutes and increased Gammaproteobacteria and Enterobacteriaceae in UC and CD patients (36–38). UC patients also have increased levels of sulfite-reducing Deltaproteobacteria (39). *Faecalibacterium prausnitzii* is a common intestinal microbial species that secrete metabolites that have antiinflammatory properties (40, 41). In one study, CD patients were observed to have lower levels of *F. prausnitzii* than healthy controls. Furthermore, depletion of this bacterium was predictive of postoperative disease recurrence in patients who had undergone ileal resection (42). A recent analysis of the mucosal-associated bacteria in ileal and rectal biopsies from treatment-naïve, newly diagnosed pediatric CD patients has shown increased abundance in Enterobacteriaceae, Pasteurellaceae, Veillonellaceae, and Fusobacteriaceae and decreased abundance of Erysipelotrichales, Bacteroidales, and Clostridiales (43).

Bacteriophages far outnumber bacteria themselves and may represent another mechanism by which shifts in the bacterial community and dysbiosis occur. Lepage et al. reported on the abundance of virus-like particles (VLPs) from CD and healthy mucosal biopsy samples and found them to be quite abundant, on the order of 1.2 × 10^9 VLPs per mucosal biopsy (44). Each individual appeared to have one dominant phage type. In addition, VLPs appear to be more abundant in CD patients than in healthy controls, and localize more frequently to non-ulcerated areas of mucosa than to ulcerated regions. Another study of phages reported lower phage diversity and greater inter-individual variability among fecal samples from CD patients compared with healthy subjects (45). The functional significance of these findings remains unknown, due to the lack of reference genome inventories needed for identification and functional classification of bacteriophages.

The role of fungi and Archaea in IBD is less well understood. The intestines of several mammalian species, including humans, also harbor fungal communities that interact with the host immune system via receptors such as Dectin-1 (46). Dectin-1 recognizes the β1,3 glucans found in the fungal cell wall and is necessary for phagocytosis and killing of fungi by myeloid phagocytes. Several lines of evidence suggest fungi may be relevant to IBD. Dectin-1 signals through *CARD9*, a gene with many UC-associated variants. A SNP for *CLEC7A* (the gene encoding Dectin-1) appears to be associated with medically refractory UC. Moreover, mice lacking Dectin-1 are more susceptible to DSS-induced colitis. Thus, several plausible links exist between the ability of the host to respond immunologically to intestinal fungi and susceptibility to inflammation. A few studies in humans have also observed increased fungal diversity in patients with CD, particularly in inflamed areas, based on 18S rDNA sequencing (47). While few studies have looked at the Archaea in the intestine, data suggest that CD and UC patients have fewer methanogens as compared with healthy controls (48). Methanogens remove end products of fermentation, and a decrease in their membership could be associated with metabolic dysfunction.

While general trends in gut dysbiosis are observed in IBD, the question now becomes how these changes affect host immune states and responses. The risk of onset or relapse of IBD can also be triggered by decreases in function and/or representation of symbionts of the gut microbiota, such as microorganisms with immunomodulatory cell wall agents such as polysaccharide A (PSA), or that express quorum-sensing molecules such as competence sporulation factor (CSF). Mazmanian et al. showed that the symbiont *Bacteroides fragilis* can protect mice from *H. hepaticus*-induced inflammation in an experimental colitis model through the action of PSA (49). PSA is able to suppress proinflammatory cytokine IL-17 production and provide protection from colitis by inducing protective IL-10 production. *Bacillus subtilis*-derived quorum-sensing pentapeptide CSF is taken up by the membrane transporter OCTN2 to activate key survival pathways such as heat shock proteins in intestinal epithelial cells (50). Studies of probiotic mechanisms have also shown that soluble bacterial components can modulate the host immune system (51–53). These data illustrate that many bacterial components contribute to the maintenance of intestinal and immune homeostasis.

Bacterial fermentation products such as SCFAs can also affect the host immune system. A decrease in the bacterial populations that produce SCFAs from the metabolism of fiber is a common observed trend in IBD. Atarashi et al. isolated a group of SCFA producing bacterial strains within Clostridia cluster IV, XIVa, XVII from human stool and found that these strains induced colonic Treg differentiation, accumulation, and function (54). These strains also reduced the severity of trinitrobenzene sulfonic acid–induced (TNBS-induced) colitis in mice, an action possibly mediated by the increased bioavailability of SCFAs to elicit TGF-β and promote the differentiation and expansion of Tregs. Others have similarly reported decreased SCFAs and Treg function in human IBD and other models of experimental colitis (4, 5, 55).
Garrett and colleagues has shown that SCFAs regulate the size and function of the colonic Treg pool (56). In Western diets low in plant fiber, decreased microbial production of SCFAs potentially reduces colonic Treg development, shifting immune homeostasis to a more proinflammatory state (56). The effects of SCFAs on Treg expression appeared to be in part mediated by the G protein-coupled receptor GPR43 (gene symbol Ffar2), which then promotes epigenetic modifications that may involve the inhibition of histone deacetylase (56, 57).

The loss of beneficial microbes and their products can also provide opportunity for the emergence of disease-promoting microbes that produce microbial metabolites with negative effects on the intestine under inflammatory conditions. Patients with UC have increased representation of sulfur-reducing, bile-tolerant bacteria in their gut microbiota, with concomitant increases in production of toxic, proinflammatory hydrogen sulfide (39, 58). Many have strong antioxidant capabilities, allowing them to survive the hostile host-induced inflammatory environment (39). In non-transformed colonic epithelial cells, H₂S can be genotoxic at concentrations found in the colon. H₂S has also been shown to modulate expression of genes involved in cell cycle progression, trigger immune and inflammatory responses, as well as impair DNA repair (59). H₂S also inhibits SCFA oxidation and utilization properties (60). One of the two enzymes responsible for detoxification of H₂S, thiosulfate sulfur transferase, has recently been described as significantly decreased in patients with active UC and can be restored upon successful medical treatment (61). Enterobacteriaceae have also been implicated in IBD, as the relative abundance of these facultative anaerobes is increased in active disease (36–38, 40). Two Enterobacteriaceae species, Klebsiella pneumoniae and Proteus mirabilis, isolated from TRUC mice (mice deficient in innate and adaptive immunity that develop spontaneous UC-like inflammation) can elicit colitis in wild-type mice in the presence of the endogenous microbiota (62, 63). A recent study showed that Enterobacteriaceae such as E. coli are able to utilize nitrate generated as a by-product of the inflamed gut, and can therefore outcompete bacteria that require fermentation substrates (64). These microorganisms may therefore contribute to the inflammatory process by suppressing commensal microbiota seen in health and inciting mechanisms that activate immune and inflammatory processes.

Assessing the communal function of the gut microbiota has been a challenge. Several functional metagenomic analyses of the inflammation-associated gut microbiota have been reported, although these data have been limited by incomplete inventories of annotated functional gene sets (65). Taking a different approach, Morgan et al. analyzed fecal specimens and intestinal biopsies from individuals with IBD and from healthy subjects to develop inferred microbial functions based on reference genomes of well-represented taxa, with confirmation of some samples via shotgun metagenomic sequencing (66). Twelve percent of total metabolic modules were differentially abundant between patients with IBD and controls, while only 2% of genus-level clades reached this significance threshold. Changes in microbial function therefore appeared more consistent and informative than changes in community structure. Notably, increases in glycolysis and carbohydrate transport/metabolism and decreases in lipid metabolism and catabolism modules were observed in ileal CD, suggesting perturbations in overall microbial energy metabolism. In addition, increased representation of genes involved in secretion systems associated with cell wall degradation and exotoxins as well as microbial adherence/invasion were observed in ileal CD. These data suggest a complex interplay between the host and microbiota in which microbial functions that allow microbes to survive and promote the inflammatory environment are selected. Greenblum et al. utilized a shotgun metagenomic data set from patients with IBD and subjects without IBD, integrating this data with an in silico systems analysis of metabolic networks (67). The microbial enzyme level variation that was associated with IBD appeared to be related to the way in which the microbiome interacted with the host gut environment, rather than with core metabolic processes. Gevers et al. analyzed treatment-naive pediatric patients with CD; they used the PICRUSt algorithm to estimate the functional changes in the CD patient mucosal-associated microbiota and predicted the switch toward auxotrophy with increases in aerobic or aerotolerant taxa such as Proteobacteria and Pasteurellaceae (43). Shotgun metagenomic sequencing of a subset of stool samples from this cohort suggests that the bacterial species that are increased in CD patients contribute components
of the glycerophospholipid and lipopolysaccharide metabolism pathways thought to initiate inflammation and also contribute phosphonoacetate hydrolase, which provides access to novel carbon and phosphate sources.

Studies of individual bacterial species’ transcriptomes have also revealed interesting insights into the effects of inflammation on microbial function and the ability to survive in the inflammatory milieu. Analysis of *E. coli* NC101, a non-pathogenic murine strain isolated from *Il10−/−* mice with colitis, demonstrated upregulation of the stress response operon, including the small heat shock proteins IbpA and IbpB, which participate in protection from oxidative stress (68). Induction of these bacterial stress response proteins in the inflamed intestine may serve to reduce inflammation, as expression of IbpA and IbpB reduced histologic signs of inflammation, secretion of IL-12/23p40 from colon tissue explants, and secretion of interferon-γ by stimulated mesenteric lymph node cells in vitro. IBD patients also have increased numbers of adherent-invasive *E. coli* (AIEC) and increased expression of CEACAM6, to which the FimH component of type I AIEC pili specifically adheres. FimH is subject to amino acid substitutions, and specific FimH mutations have been associated with CD and UC patients and may be a mechanism by which AIEC proliferates (69, 70). Thus, the inflammatory environment may select for expression of certain bacterial proteins that affect microbial survival and the perpetuation of the host’s inflammation.

In summary, despite the large amount of information that describes the gut dysbiosis of IBD, many questions remain about the microbial role and mechanisms that are involved in human IBD. As studies become more focused on the functional consequences of microbial shifts and investigators appreciate the subtleties of host age, medications, diet, and environment on the microbiota, there is every reason to believe that solutions to overcome these challenges will be found.

Closing the gap between clinical and microbial investigations

Clinical investigators who study IBD are often unfamiliar with microbial and ecological concepts, while most microbial ecologists are adept at large-scale surveys but unaware of the nuances of IBD. Thus, bringing the two sides together to leverage their respective expertise represents one of the major challenges at hand. The next phase of research investigation of the gut microbiome in IBD should be guided by specific biological questions relevant to the clinical aspects and natural history of the disease. For instance, the intestinal dysbiosis associated with active IBD was initially felt to be irrelevant to what causes these diseases, i.e., merely a consequence of the aberrant immune and inflammatory process. However, these changes may in fact be important in sustaining these diseases, promoting the development of long-term complications, and contributing to poor responses to certain therapeutic approaches.

The chronicity of many diseases arises from a series of self-reinforcing events that create a new steady state that is difficult to interrupt (Figure 1). In IBD, the inflammatory milieu created by altered immune states selects against commensal microbes associated with health in favor of those that can survive and flourish in this hostile environment. Many of these microbes are also highly proinflammatory (pathobionts), a property that confers a selective advantage over the return of commensal microbes by maintaining the host inflammatory state. In IBD, immune homeostasis cannot be easily restored, because of inherent defects or inability to shut down the immune activation and correct the proinflammatory intestinal dysbiosis. Therefore, interventions aimed at correcting only one facet of this vicious cycle (e.g., solely targeting the host or only targeting gut microbes) are not likely to be successful. Broad-spectrum antibiotics, for instance, have not been useful in IBD as primary therapy because they suppress the return of a
diverse microbiota and do little in mitigating the host inflammatory-immune derangements (71–74). Probiotics and prebiotics are also of limited value because the former cannot survive in an inflammatory environment and the latter require a diverse microbiota associated with health that is not present in the inflammatory state to mediate their actions. Fecal microbiota transplant (FMT) or interventions with defined, complex communities of microbiota are theoretically better than probiotics because the microbial populations are assembled and self-selected under healthy conditions. However, they too are not likely to be effective in moderate or severe cases of IBD in absence of other therapies directed against the host inflammatory and immune dysregulation (75). Even then, the question remains how long the membership, diversity, and function of the transferred microbiota can be sustained under different set of conditions presented by the new host’s genetic, environmental, and physiological factors. The correct strategy in any scenario will likely call for a personalized, multi-pronged approach aimed at halting the chain of events that maintains the disease-sustaining steady state.

IBD are progressive diseases that arise from a wide variety of pathophysiological processes (Figure 2). Changes in gut microbial composition and function are likely to evolve through the course (natural history) of IBD, reflecting transitions in host-microbial relationships arising from disease-intrinsic and confounding factors. Microbial factors that trigger the onset of disease may be quite different from those that sustain the inflammatory process or are a consequence of long-term complications and interventions. The interpretation of gut microbial data in absence of this contextual information can be limited and potentially misleading.

Along the same lines, the same treatment or intervention is unlikely to work for all cases and stages of IBD. Our interventional goals should therefore be guided by a combination of microbial, genetic, and biological metrics that assess risk and states of health of patients on an individual basis. As we approach the era of personalized medicine, this strategy is most likely to yield the best and most sustainable clinical outcomes.

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