Inflammatory bowel disease (IBD) is a chronic immune-mediated disease affecting the gastrointestinal tract. IBD consists of 2 subtypes: ulcerative colitis and Crohn disease. IBD is thought to develop as a result of interactions between environmental, microbial, and immune-mediated factors in a genetically susceptible host. Of late, the potential role of the microbiome in the development, progression, and treatment of IBD has been a subject of considerable interest and enquiry. Indeed, studies in human subjects have shown that the gut microbiome is different in patients with IBD compared with that in healthy control subjects. Other evidence in support of a fundamental role for the microbiome in patients with IBD includes identification of mutations in genes involved in microbiome-immune interactions among patients with IBD and epidemiologic observations implicating such microbiota-modulating risk factors as antibiotic use, cigarette smoking, levels of sanitation, and diet in the pathogenesis of IBD. Consequently, there has been much interest in the possible benefits of microbiome-modulating interventions, such as probiotics, prebiotics, antibiotics, fecal microbiota transplantation, and gene manipulation in the treatment of IBD. In this review, we will discuss the role of the gut microbiome in patients with IBD; our focus will be on human studies. (J Allergy Clin Immunol 2020;145:16-27.)

**Key words:** Inflammatory bowel disease, ulcerative colitis, Crohn disease, microbiome, microbiota, inflammation, genetics, environmental factors, antibiotics, probiotics, prebiotics, fecal microbiota transplantation

The gut microbiome comprises more than 100 trillion different microbial organisms and includes bacteria, fungi, viruses, and protozoa. In fact, the enteric microbiome has more than 100 times more genes within it than are in the genome of its host. The majority of intestinal bacteria belong to 4 phyla, Firmicutes, Bacteroidetes, Proteobacteria, and Actinobacteria, and in healthy adults Firmicutes and Bacteroidetes predominate. The number of bacteria vary throughout the gastrointestinal tract, with the colon containing both the greatest number and diversity of species compared with the stomach and small intestine. The gut microbiome plays a fundamental role in several aspects of host homeostasis: nutrition, immune development, metabolism, and defense against pathogens.5

### THE MICROBIOME AND INFLAMMATORY BOWEL DISEASE

Inflammatory bowel disease (IBD) is a chronic immune-mediated disease affecting the gastrointestinal tract. The disease is thought to develop as a result of interactions between environmental, microbial, and immune-mediated factors in a genetically susceptible host (Fig 1). Several strands of evidence suggest a role for the microbiome in the pathogenesis of IBD (Table 1). Data from a number of animal models provide a convincing argument for a fundamental role of an altered microbiome or an aberrant immune response to the microbiome in the development of intestinal inflammation. Thus a germ-free environment prevents the development of colitis in genetically susceptible mice. In addition, transfer of proinflammatory bacteria or microbiota from diseased mice into healthy mice can induce inflammation, and colonization of mice with intestinal microbiota from donors with IBD exacerbates colitis by altering...
FIG 1. Risk factors for the development of IBD. IBD is thought to develop as a result of complex interactions between the immune system, microbiome, and environment in genetically susceptible hosts.

immune responses. Finally, transfer of naive CD4+ lymphocytes from healthy mice into mice that lack T and B lymphocytes can induce colitis, and the degree of susceptibility to colitis in these mice is associated with differences in the composition of their gut microbiota.

In human subjects several observations support a role for the microbiome in patients with IBD. For example, disease activity is most evident in areas where bacterial populations are highest (the colon) and where there is relative stasis of fecal material (the terminal ileum and rectum). Furthermore, fecal diversion has been an effective strategy in the management of Crohn disease (CD), with remission occurring in the excluded segment of the bowel. After diversion, restoration of continuity and thus re-exposure to the fecal stream is associated with postoperative recurrence of CD. Also, antibiotic therapy has proved useful in the treatment of certain phenotypes of IBD, such as ciprofloxacin and metronidazole use in patients with perianal CD and pouchitis, and metronidazole for the prevention of postoperative recurrence in patients with CD. In addition, many of the genetic markers associated with IBD are related to engagement between the immune system and microbiota. To further support the effect of the microbiome in patients with IBD, recent studies have demonstrated a role for specific microbes in driving immune responses in the gut lamina propria of mice. T300A and WT mice were orally inoculated with human stool from patients with IBD; T300A mice that received stool from patients with active CD had an increase in Bacteroides species compared with WT mice. The T300A mice that received stool from patients with active CD had increased numbers of T H1 and T H17 T cells in the lamina propria of the colon and ileum. However, these changes may occur before disease onset because histology of the colon and small intestine was normal in the T300A mice that received stool from patients with active CD. This study also found that in the setting of IBD with the T300A genotype, a trend toward an increase in Bacteroides species was seen compared with levels seen in the setting of IBD without the T300A genotype. Furthermore, human immune cells that express the T300A variant of ATG16L1 do not induce regulatory T-lymphocyte development when exposed to Bacteroides fragilis. Normally, B fragilis is a commensal bacterium that prevents colitis in mice by interacting with CD4 Foxp3 regulatory T cells to produce IL-10.

Card9 is a protein located within an adaptor protein caspase recruitment domain involved with Dectin-1 (CLEC7A) signaling. Dectin-1 is a pattern recognition receptor that recognizes the components of the fungal cell wall.CARD9 signaling occurs in response to the recognition of fungal ligands by Dectin-1. Alterations in Dectin-1 have been associated with medically refractory ulcerative colitis (UC). Card9 is also required for inflammatory cytokine production in response to specific bacterial stimuli and viral infection. IL-6, TNF-α, and IL-1β are cytokines that are dependent on Card9 function and are protective against fungal infection. Card9 knockout mice are susceptible to Candida albicans, Aspergillus fumigatus, and Cryptococcus neoformans among other fungi. In human subjects inherited Card9 deficiency has been linked to the development of invasive Candida species infections of the central nervous system and digestive tract in otherwise healthy subjects. Loss of Card9 is associated with a loss of TH17 cells, which are involved with mucosal barrier integrity and contribute to pathogen clearance at mucosal
surfaces.\textsuperscript{51} A study by Lamas et al\textsuperscript{52} showed that CARD9 knockout mice had susceptibility to colitis, which was associated with impaired microbial tryptophan metabolism. Tryptophan metabolites play an important role in the balance of gut mucosal reactivity in mice by activating the aryl hydrocarbon receptor (AhR) in lymphoid tissue.\textsuperscript{53} AhR promotes the differentiation of regulatory T cells and is required for IL-22 production.\textsuperscript{54} AhR knockout mice exhibit diminished IL-22 secretion by type 2 innate lymphoid cells and increased susceptibility to intestinal bacterial infections.\textsuperscript{55} Administration of Lactobacillus strains able to metabolize tryptophan into AhRs has been shown to alleviate colitis in CARD9 knockout mice.\textsuperscript{52} Notably, in human subjects with IBD and CARD9 mutations, stool samples were found to lack AHR ligands.\textsuperscript{52}

**Environmental risk factors and the microbiome**

Environmental factors are known to play a role in the development of IBD, as evidenced by the observation that even among identical twins, there is only a 20% to 50% concordance rate for CD.\textsuperscript{76} Many of the risk factors that have been identified for IBD are related to the microbiome (Fig 3). These include the hygiene hypothesis, exposure to gastroenteritis, breast-feeding, early antibiotic use, cigarette smoking, and diet. The hygiene hypothesis contends that a lack of childhood exposure to a range of microbes might have a negative effect on development of the adaptive immune response. The change in exposure to microorganisms is attributed to cleaner living, urbanization, and increased antibiotic use. Evidence to support this theory is the observation that both the temporal and geographic incidence of IBD seem to parallel the industrialization and urbanization of societies. More recent epidemiologic studies suggest that the incidence of IBD has now stabilized in the Western world (United States, Canada, Australia, New Zealand, and Western Europe) but continues to increase in South America, Eastern Europe, Asia, and Africa, regions where there has been rapid recent socioeconomic development.\textsuperscript{57}

Antibiotic use in the years before diagnosis has been associated with the development of IBD and is thought to be related to effects on the commensal microbiota and immune regulation in genetically susceptible subjects. In an animal model neonatal NOD2-deficient mice treated with amoxicillin and then dextran sulfate sodium to induce colitis had more severe colitis and demonstrated delayed recovery of microbial diversity after antibiotic cessation compared with WT mice. The colitis phenotype could also be transferred by fecal transplantation of stool from the NOD2-deficient neonates into germ-free NOD2 recipients.\textsuperscript{58} In human studies use of antibiotics 1 or more times during the first year of life increased the odds of having IBD diagnosed during childhood by 2.9.\textsuperscript{59} Other studies have found that children with IBD were more likely to have had at least 1 episode of otitis media (a likely surrogate for antibiotic use) before diagnosis,\textsuperscript{60} and patients with IBD are more likely to have received antibiotics in the 2 to 5 years before diagnosis than healthy control subjects.\textsuperscript{47} A meta-analysis performed by Ungaro et al\textsuperscript{62} found that any antibiotic use was associated with an odds ratio of 1.74 for patients with CD and 1.08 for patients with UC. This was even higher in children, with an odds ratio for patients with CD of 2.75.\textsuperscript{63} Furthermore, antibiotic use has been shown to amplify the microbiome changes seen with patients with CD.\textsuperscript{63,64}

Additional evidence to support a role for the microbiome in patients with IBD includes the effect of infectious gastroenteritis in patients with IBD. Having had an episode of infectious gastroenteritis has been shown in some studies to increase the risk for the subsequent development of IBD by 40%.\textsuperscript{65} In mice exposed to Salmonella typhimurium to induce acute infectious gastroenteritis, those that were colonized with an adherent-invasive Escherichia coli (AIEC) isolated from a patient with CD before exposure to S typhimurium experienced an expansion of AIEC with increased cellular and proinflammatory responses.\textsuperscript{66} The AIEC-colonized mice also had delayed mucosal epithelial reconstitution after recovery from S typhimurium infection. These findings suggest that an episode of infectious gastroenteritis can trigger an abnormal immune response in a subject with a baseline “susceptible gut microbiota profile.” In subjects with a previous diagnosis of IBS who had an episode of gastroenteritis, the risk of IBD increased 5-fold. Another study conducted in the United Kingdom showed a 2- to 3-fold increased risk of IBD in those who had an episode of gastroenteritis.\textsuperscript{67} In this scenario it is possible that undiagnosed IBD might have existed at the time of exposure to infection or that the infectious process itself can initiate an inflammatory cascade that is either exaggerated, cannot be downregulated, or both. It must also be mentioned that infectious gastroenteritis is often treated with antibiotics, which, as discussed, has been associated with the development of IBD and might contribute to the increased risk of IBD seen after infectious gastroenteritis.

**TABLE I. Evidence from animal and human studies to support a role for the microbiome in the pathogenesis of IBD**

<table>
<thead>
<tr>
<th>Evidence for the role of the microbiome in patients with IBD</th>
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<tbody>
<tr>
<td>Animal studies</td>
</tr>
<tr>
<td>• Germ-free environment prevents colitis in genetically susceptible mice.</td>
</tr>
<tr>
<td>• Transfer of proinflammatory bacteria or microbiota from diseased mice to healthy mice induces inflammation.</td>
</tr>
<tr>
<td>• Transfer of naïve CD4\textsuperscript{+} lymphocytes from healthy mice into mice that lack T and B lymphocytes can induce colitis.</td>
</tr>
<tr>
<td>• Susceptibility to the colitis that occurs in healthy mice through transfer of naïve CD4\textsuperscript{+} lymphocytes depends on differences in composition of their gut microbiota.</td>
</tr>
</tbody>
</table>

| Human studies                                             |
| • Disease activity is most evident in areas where bacterial populations are high (colon) and where there is relative stasis of fecal material (terminal ileum and rectum). |
| • Fecal diversion is an effective strategy in the management of CD, with remission occurring in the excluded segment of the bowel. |
| • Recurrence of disease occurs after restoration of continuity of the gastrointestinal tract and fecal stream. |
| • Antibiotic therapy is effective in some patients with IBD. |
| • Genetic markers associated with IBD are related to engagement of the immune system with the microbiota. |
| • Specific microbes have been found to have roles in driving or suppressing inflammation. |
There is some evidence that breast-feeding is protective against the development of IBD. Previous studies have shown that human breast milk is microbially diverse and has both probiotic and prebiotic effects. Breast milk contains *Lactobacillus rhamnosus*, *Lactobacillus gasseri*, *Lactococcus lactis*, *Leuconostoc mesenteroides*, and *Bifidobacteria*. Microbiota in breast milk promote immune tolerance, prevent infection, and play a role in the maintenance of the epithelial barrier through an immune-mediated influence on intestinal microbiota composition. The oligosaccharides in breast milk have prebiotic effects that contribute to the establishment of the infant gut microbiota. In addition, human milk oligosaccharides have been found to inhibit the adhesion of enteropathogenic *E. coli*, *Vibrio cholerae*, and *Salmonella typhimurium* to epithelial cells. Infants who are breast-fed have a lower incidence of gastrointestinal tract infections. Breast-feeding has been shown to lead to an increased abundance of Firmicutes and Actinobacteria compared with formula-fed infants. A meta-analysis conducted by Xu et al concluded that breast-feeding was protective against the development of IBD, with greater benefits accruing from a longer duration of breast-feeding.

Dietary changes, if sufficiently drastic, can alter the intestinal microbiome in as little as 24 hours. Animal-based diets lead to increased abundance of bile-tolerant bacteria, including *Alistipes*, *Bilophila*, and *Bacteroides* species, and a decreased abundance of Firmicutes. In contrast, plant-based diets lead to increased abundance of Firmicutes. Certain diets have been associated with an increased risk for IBD. A population-based case-control survey conducted by Bernstein et al found that patients with IBD were less likely to have consumed unpasteurized milk or eaten pork. A systematic review by Hou et al found that diets high in total fats, omega-6 fatty acids, and meat were associated with an increased risk of IBD, whereas higher fiber and fruit intakes were associated with a decreased risk for CD, and a high intake of vegetables was associated with a decreased risk for UC. These findings can be explained by diet-induced shifts in the microbiome, such as the decreased abundance of Firmicutes with animal-based diets. Decreased abundance of *Faecalibacterium prausnitzii*, a member of the Firmicutes phylum with anti-inflammatory effects, has been associated with CD. Cigarette smoking has a complex interaction, with IBD being apparently protective against UC but negatively affecting the natural history of CD. In animal models IL-10–deficient mice exposed to cigarette smoke had colitis with increased immune cells in the colon and IFN-γ expression in the ileum, whereas WT mice exposed to cigarette smoke had no apparent signs of disease. Similarly, NOD2-deficient mice exposed to cigarette smoke had colitis with increased immune cells in the colon and IFN-γ expression in the ileum, whereas WT mice exposed to cigarette smoke had no apparent signs of disease.
smoke had ileitis. Although little studied, there is evidence that the gut microbiota of current and former smokers differs from that of nonsmokers, with a relative increase in the numbers of Bacteroidetes and decrease in Firmicutes and Proteobacteria in one of these studies.\textsuperscript{81,82} In a study evaluating the microbiota of cigarette smokers before and after smoking cessation, cigarette smokers had a lower abundance of \textit{Bifidobacterium} species compared with nonsmoking control subjects, and increases in \textit{Bifidobacterium} species were seen after smoking cessation.\textsuperscript{83} There was also a decrease in \textit{Bacteroides} species.
after smoking cessation. Some of the differences seen in the gut microbiota of cigarette smokers mirror those seen in patients with CD and those with UC, suggesting a potential link between smoking, microbiota changes, and development of IBD.

In summary, there is some evidence to suggest that environmental factors, such as early antibiotic use, enteric infections, breast-feeding, diet, and cigarette smoking, affect the gut microbiota and drive immune activation in subjects genetically susceptible to the development of IBD. Although studies have been conducted to evaluate the effect of specific environmental factors on immune responses and microbiota in genetically susceptible animal models, studies in human subjects are rather limited. In the future, human studies designed to evaluate the effects of environmental factors on microbiota-immune interactions in subjects with known genetic risk factors for IBD (eg, NOD2 or ATG16L1) will help better define these relationships.

### Microbial composition and function in patients with IBD

That the fecal stream, which contains bacteria, is relevant to the pathogenesis of IBD was first noted decades ago when surgical diversion was used to induce remission of diseased segments; relapse was inevitable when continuity was restored. More direct evidence for the role of bacteria per se comes from the observation that disease activity in patients with IBD is most evident in those parts of the bowel that harbor the greatest numbers and greatest diversity of bacteria. Numerous studies have described changes in gut microbiota composition related to IBD. These studies have generated much excitement around the diagnostic and prognostic potential of microbiota signatures in patients with IBD. Could such signatures, for example, distinguish between patients with IBD and healthy control subjects or between patients with CD and those with UC? Alternately, could microbiota profiling predict the risk for future complications, extraintestinal disease, and response to therapy?

**Metagenomics of the gut microbiota in patients with IBD.** When compared with the microbiota of healthy subjects, microbiota samples from patients with IBD demonstrate a decrease in overall diversity and a reduced abundance of anti-inflammatory taxa (Table II). There is evidence to support that in patients with IBD, Proteobacteria (particularly adherent invasive E coli), Pasteurellaceae, Veillonellaceae, Fusobacterium species, and Ruminococcus gnavus are increased, and Clostridium groups IV and XIVa, Bacteroides, Sutterella, Roseburia, Bifidobacterium species and F prausnitzii are decreased. However, it has been difficult to ascertain whether changes in the microbiota in patients with IBD are causative or rather a result of inflammation, treatment, or both.

Gevers et al conducted a study in treatment-naive patients with CD to characterize disease-associated microbiota based on sampling of mucosal tissue by endoscopic biopsy and luminal content by fecal sampling. Microbiota from patients with CD featured increased abundance of Pasteurellaceae, Veillonellaceae, Neisseriaceae, Fusobacteriaceae species, and E coli; there was a decreased abundance of Bacteroides, Clostridiales, Faecalibacterium species, Roseburia species, Blautia species, Ruminococcus species and Lachnospiraceae. In this study microbiota changes occurred in a treatment-naive, newly diagnosed population of pediatric patients with CD, suggesting that changes in the microbiota can occur early, perhaps preceding clinical disease, and independent of medical therapy.

CD, in particular, is associated with a more altered and unstable gut microbial composition than UC. In patients with CD, there is a loss of the beneficial butyrate-producing organisms Faecalibacterium species, Christensenellaceae, Methanobrevibacter species, Peptostreptococcaceae species, Anaerostipes species, Methanobrevibacter species, Christensenellaceae, and Collinsella species and increased abundance of Fusobacterium and Escherichia species), which have been used to distinguish samples from patients with CD from those of subjects without CD. Yilmaz et al evaluated the microbial communities of patients with IBD using 16S sequencing of mucosal biopsy specimens. Alterations in microbiota composition correlated with likelihood of treatment response in patients with CD, as well as risk of relapse in post-surgical patients with CD. Specifically, Bifidobacterium species, Collinsella species, Lachnospira species, Lachnospiraceae, Roseburia species, and Eggerthella taxa were associated with responsiveness to treatment with anti-TNF-α therapy. Even among patients with CD with inactive disease after small-bowel resection, there were reductions in Parabacteroides species and Clostridiales and increases in Enterobacteriaceae compared with patients with CD who had not undergone prior surgery. Interestingly, age and body habitus (body mass index) were found to have a greater effect on microbiota composition than were differences attributable to the presence of UC or CD.

To better characterize the long-term behavior of the gut microbiome in patients with IBD, Halfvarson et al followed a cohort of 128 subjects with fecal samples collected every 3 months for 2 years. The microbiomes of the patients with IBD fluctuated more over time than those of healthy control subjects, and patients with ileal CD (particularly those with a history of surgical resection) had the largest deviations from healthy control samples. Patients who received a course of oral corticosteroids for a disease flare had greater microbiome fluctuations than patients who did not require corticosteroid therapy, suggesting that disease activity and inflammation or possibly medical therapy contribute to microbiota changes. Using the microbial changes associated with IBD

<table>
<thead>
<tr>
<th>Increased</th>
<th>Decreased</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bacteria:</strong></td>
<td><strong>Bacteria:</strong></td>
</tr>
<tr>
<td>Fusobacterium species</td>
<td>Bacteroides species</td>
</tr>
<tr>
<td>Pasturellaceae</td>
<td>Bifidobacterium species</td>
</tr>
<tr>
<td>Proteobacteria (adherent invasive Escherichia coli)</td>
<td>Clostridium XIVa, IV</td>
</tr>
<tr>
<td>Ruminococcus gnavus</td>
<td>Faecalibacterium prausnitzii</td>
</tr>
<tr>
<td>Veillonellaceae</td>
<td>Roseburia species</td>
</tr>
<tr>
<td>Sutterella species</td>
<td></td>
</tr>
<tr>
<td><strong>Fungi:</strong></td>
<td><strong>Fungi:</strong></td>
</tr>
<tr>
<td>Candida albicans</td>
<td>Saccharomyces cerevisiae</td>
</tr>
<tr>
<td>Candida tropicalis</td>
<td></td>
</tr>
<tr>
<td>Clavispora lusitaniae</td>
<td></td>
</tr>
<tr>
<td>Cyberlindnera jadinii</td>
<td></td>
</tr>
<tr>
<td>Kluyveromyces marxianus</td>
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</tbody>
</table>

The microbial composition in patients with IBD is altered compared with that in healthy control subjects. Specific changes have been identified in the abundance of various bacteria, fungi, and viruses.
and clinical data collected from this study, patients with IBD subtypes could be accurately distinguished from healthy control subjects in up to 66% of samples, which was similar to previous findings.86,87

Meta-transcriptomics of the gut microbiota in patients with IBD. Other studies have looked at the functional activity (metatranscriptomics) rather than just the functional potential (metagenomics) of the gut microbiota in patients with IBD.88 The functional activity and potential of an organism do not always correlate. For example, R. gnavus has a greatly increased abundance at the RNA level in both patients with UC and those with CD compared with healthy control subjects, with only a small increase in DNA abundance.88 This means that even small changes in R. gnavus abundance at the DNA level can have greater effects in patients with IBD than expected. R. gnavus produces a complex glucorhamnan polysaccharide that potently induces secretion of the inflammatory cytokine TNF-α by dendritic cells.89 On the other hand, in patients with UC, B. fragilis is less abundant in terms of DNA, and RNA abundance is much lower compared with that in healthy control subjects.88 Thus both the functional potential and activity of B. fragilis are decreased in patients with UC compared with those in healthy control subjects.

F. prausnitzii, B. vulgatus, and Alistipes putredinis were found to contribute significantly to metabolic pathway transcription in patients with IBD, even when not the most abundant organisms present.88 F. prausnitzii dominates the dTDP-L-rhamnose biosynthesis I pathway, which produces deoxy sugar β-L-rhamnopyranose. Deoxy sugar β-L-rhamnopyranose is a building block of the glycan component of O-antigens, which contribute to the surface LPS of gram-negative bacteria, a major target of the immune system.90 The methylerythritol pathway, which produces isopentenyl diphosphate, is consistently overtranscribed by A. putredinis. The methylerythritol pathway has been implicated in the intracellular survival of pathogenic bacteria.91

To further support these findings, abundances of Clostridium hathewayi, Clostridium bolteae, and R. gnavus are increased significantly in terms of transcriptional activity in patients with IBD relative to genomic abundance, suggesting that their roles might be more pronounced than suggested solely by differences in genomic abundance.92 These findings highlight differences between the actual functional activity and functional potential of the gut microbiota in patients with IBD and the limitations of studies that do not include either metabolomics or metatranscriptomics.

Metabolomics of the gut microbiota in patients with IBD. Biologically active and functionally important metabolites have been shown in several studies to be depleted in patients with IBD. Short-chain fatty acids, which include acetate, propionate, and butyrate, are important anti-inflammatory bacterial metabolites,84 and bacteria that produce these metabolites are decreased in patients with IBD.93 Short-chain fatty acids are an energy source for colonic epithelial cells and promote the expansion of regulatory T cells in the colon.94,95 In particular, decreased butyrate production has been associated with depletion of Bacteroidetes and Clostridium clusters IV and XIVA, including F. prausnitzii.84 Some metabolites, such as hydrogen sulfide, can block the use of butyrate by colonocytes.96,97 Sulfate-reducing bacteria have been found to be increased in abundance in patients with IBD.98 Lloyd-Price et al92 analyzed mucosal biopsy specimens, blood, and stool samples from 132 patients with and without IBD and found an increase in facultative anaerobes with a decrease in obligate anaerobes, along with disruptions in metabolite pools. Levels of short-chain fatty acids, including butyrate, as well as the secondary bile acids lithocholate and deoxycholate, were found to be reduced in patients with IBD.

Although not typically deficient in the sera of patients with IBD, the vitamins pantothenate (vitamin B5) and nicotinate (vitamin B3) were noted to be particularly depleted in the gut. Nicotinate has known anti-inflammatory and antiapoptotic properties in the gut.99 Notably, other metabolites, such as sphingolipids and carboximidic acids, have been found to be overabundant in patients with CD.100 B. fragilis is known to synthesize sphingolipids, which, because of the structural similarities with invariant natural killer T (iNKT) cell agonists, are able to reduce iNKT cell activation and expansion triggered by self and microbial stimuli in neonatal mice.101 This results in decreased iNKT cell numbers once the neonate reaches adulthood and is protective against experimentally induced colitis. It is critical to appreciate that alterations in gut microbiota composition in patients with IBD could be the result and not the cause of inflammation; inflammation resulting in higher oxygen concentrations might create an environment that is toxic to obligate anaerobes and result in a diminished mucus layer.88,102

The role of F. prausnitzii in patients with IBD. Of the various bacteria described above, F. prausnitzii has attracted special attention in relation to the pathogenesis of IBD. A low abundance of this anti-inflammatory Firmicute has been described in patients with CD and has been associated with an increased risk of postoperative recurrence of ileal disease.78,103 Furthermore, low levels of F. prausnitzii in stool were found to be predictive of CD relapse in patients in remission.104 F. prausnitzii is associated with secretion of metabolites that block nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) activation and IL-8 production by intestinal epithelial cells.103,105,106 In addition to butyrate, F. prausnitzii has also been associated with salicylic acid production.103,106 F. prausnitzii induces a tolerogenic cytokine profile in serum, which includes low IL-12 and IFN-γ and high IL-10 levels.106 Furthermore, F. prausnitzii is protective in dinitrobenzene sulfonic acid induced colitis in mice.105

A unique protein produced by F. prausnitzii called microbial anti-inflammatory molecule (MAM) has been identified.107 This protein has anti-inflammatory effects and inhibits the NF-κB pathway in epithelial cell lines. L. lactis used to deliver a plasmid encoding the MAM protein was successful in the prevention of dinitrobenzene sulfonic acid–induced colitis in mice.78

Fungi and viruses in patients with IBD. The composition of fungi and viruses in the gut microbiome is also altered in patients with IBD.106,109,110 The microbiome of patients with ileal CD in particular appears to feature an increase in fungi at the expense of bacteria, whereas patients with UC and those with CD without ileal involvement exhibit decreased fungal diversity.110 Saccharomyces cerevisiae is decreased, and Candida albicans, Candida tropicalis, Clavispora lusitaniae, Cyberlindnera jadinii, and Kluyveromyces marxianus are increased.84 There is also an increased Basidiomycota/Ascomycota ratio in patients with IBD with inflammation compared with healthy control subjects.110 Malassezia restricta, a fungus that is commonly found on the skin, is abundant in the intestinal mucosa of patients with CD.109 Malassezia species elicit inflammatory cytokine production from innate immune cells that have CARD9 gene mutations typically associated with IBD and exacerbates colitis in mouse models of disease.109
receptors to promote Th1 cell differentiation, improve intestinal barrier function, increase bacterial diversity, and inhibit the growth of potentially pathogenic bacteria, results to date have not been consistent or impressive in patients with IBD. 

Although some positive outcomes have been documented in patients with UC, the application of these findings to everyday practice has been hampered by limitations in study design, availability of effective strains, and issues related to quality control of available probiotic preparations. On the other hand, there is evidence to support the use of probiotics in the primary and secondary (ie, after induction of remission with antibiotics) prevention of pouchitis. The probiotic preparation shown to be effective in pouchitis, VSL#3, a mixture of 4 strains of Lactobacillus species, 3 strains of Bifidobacterium species, and Streptococcus salivarius, was found to increase the number of mucosal regulatory T lymphocytes and decrease the mucosal expression of mRNA of the proinflammatory cytokine IL-1β, effects that would promote tolerance rather than an inflammatory response. There also might be some benefit of probiotics in the maintenance of remission in patients with mild-to-moderate UC, although outcomes have varied. In patients with CD, probiotics have not been as effective.

Unfortunately, studies of probiotics in patients with IBD are quite heterogeneous in terms of the composition of the specific probiotic preparations used. Lack of efficacy of probiotics might reflect our failure to identify the ideal combination, administering treatment for an insufficient length of time or intervening too late in the disease course when the “pathogenic” microbiota is already established. In the future, probiotics designed to target the specific microbial changes associated with a particular IBD phenotype might open the door to a more individualized and personalized approach to treatment.

**Antibiotics.** Antibiotics play a role in the treatment of IBD in specific scenarios, including perianal CD, prevention of postoperative CD, and pouchitis. Although ciprofloxacin and metronidazole are used frequently in clinical practice to promote healing of simple fistulas in patients with perianal CD, evidence to support this practice is limited. Only a few small studies have been performed, including a randomized, double-blind, placebo-controlled pilot study of 25 patients with CD and actively draining perianal fistulas treated with ciprofloxacin, metronidazole, or placebo for 10 weeks. Ciprofloxacin led to remission and response more frequently, but differences were not significant. Several trials have evaluated use of metronidazole in the prevention of postoperative recurrence of CD after small intestinal resection, although it is not as effective as biologic therapy, and the benefit is lost when the antibiotic is stopped. Rutgeerts et al demonstrated that in 60 patients with CD who had undergone ileal resection, 3 months of treatment with metronidazole decreased the rate of recurrent lesions in the neoterminal ileum at 12 weeks and clinical recurrence at 1 year compared with those who received placebo. More recently, a study of a retrospective cohort of 70 patients with CD who had undergone ileal resection found that low-dose metronidazole for 3 months reduced endoscopic recurrence of CD within the first 12 months of surgery compared with patients who did not receive metronidazole.

The microbiome plays a central role in driving inflammation of the ileal pouch after colectomy with ileal pouch–anal anastomosis. Pouchitis only occurs after restoration of the fecal stream through the pouch. Accordingly, the antibiotics

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**TABLE III. Microbiota-based strategies for IBD**

<table>
<thead>
<tr>
<th>Diagnostic</th>
<th>Prognostic</th>
<th>Treatment</th>
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</thead>
<tbody>
<tr>
<td>Use a specific “microbial signature” to diagnose IBD</td>
<td>Predict disease phenotype</td>
<td>Replace Specific anti-inflammatory bacterium (Faecalibacterium prausnitzii)</td>
</tr>
<tr>
<td>Differentiate subtypes, such as UC vs CD</td>
<td>Risk for complications/disease progression</td>
<td>Multiple-bacteria probiotics</td>
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<td></td>
<td>Response to therapy</td>
<td>Specific anti-inflammatory molecule (MAM protein)</td>
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<tr>
<td></td>
<td></td>
<td>Identify and remove specific inflammatory bacteria with a phage</td>
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<td></td>
<td></td>
<td>Antibiotics</td>
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<td></td>
<td></td>
<td>FMT</td>
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<tr>
<td></td>
<td></td>
<td>Redesign Genetically modify organisms to deliver medications or anti-inflammatory molecules</td>
</tr>
</tbody>
</table>

There are multiple approaches that can be taken to modulation of the gut microbiota for therapeutic benefit in patients with IBD.

Fecal samples from patients with IBD have demonstrated that their enteric virome is associated with a significant expansion of Caudovirales bacteriophages. Similarly, rectal mucosal samples in patients with UC had an increased abundance of Caudovirales bacteriophages with a reduction in bacterial diversity, which correlated with intestinal inflammation. Data are lacking in this area, and future studies are needed to examine the effects of fungal and viral alterations in the gut microbiome in patients with IBD.

Several limitations must be taken into account when considering the previously mentioned alterations of microbial composition and function in patients with IBD. First, the changes seen in the mucosal-associated microbiota are not always reflected to the same degree in fecal samples. Second, metagenomics reveals functional potential but might not correlate with actual functional activity. Third, metatranscriptomics measures actual gene expression; however, there are few studies thus far in patients with IBD. Finally, metabolomics measures the actual metabolites produced, but the majority of the gut metabolome is uncharacterized. These findings are important to consider when designing future studies of the microbiome in patients with IBD.

**Microbiota-based strategies in patients with IBD**

Several approaches can be taken to modulation of gut microbiota for therapeutic benefit in patients with IBD (Table III). For example, identification of a deficit in relevant anti-inflammatory bacteria, such as F prausnitzii, could lead to augmentation or administration of anti-inflammatory molecules produced by bacteria, such as MAM protein in the case of F prausnitzii. Probiotics, prebiotics, and synbiotics have been used in attempts to replenish anti-inflammatory bacteria and their substrates. Conversely, finding inflammatory bacteria that are overexpressed or toxic and using antibiotics or phage therapy to achieve their removal provides yet another approach. Fecal microbiota transplantation (FMT) can be used to reset the entire microbiome. In the future, the microbiota could be used to deliver medications, such as genetically modified organisms designed to release anti-inflammatory cytokines or other molecules directly to the site of inflammation. Finally, the microbiome-immune interface has provided multiple targets that can be addressed.

**Probiotics.** Although studies have shown that probiotics are able to alter the mucosal immune system through Toll-like

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GLASSNER, ABRAHAM, AND QUIGLEY
ciprofloxacin and metronidazole are used as first-line therapy for the treatment of pouchitis, although data to support this are quite limited. A small randomized trial of 16 patients treated with ciprofloxacin or metronidazole for 2 weeks found that all patients treated with ciprofloxacin and 67% of those treated with metronidazole had resolution of pouchitis based on pouchitis disease activity scores. In summary, antibiotics are often used in clinical practice in the management of IBD; however, there is surprisingly little evidence in the literature to support this.

Diet. Studies assessing diet in the treatment of IBD have been notoriously difficult because of the effect of confounding factors and patient compliance. In children with CD, exclusive enteral nutrition is as effective as corticosteroids; however, long-term adherence is challenging, and disease recurs after a regular diet is resumed. Recently, a randomized controlled trial of the CD exclusion diet with partial enteral nutrition in children showed comparable rates of remission to those achieved by using exclusive enteral nutrition. The study showed that after returning to a regular diet, there was a rebound effect in the composition of the microbiome to the baseline state. Fecal samples of children who achieved remission showed decreases in abundance of Proteobacteria and increases in Firmicutes, bacteria that are associated with CD.

There is insufficient evidence to support the use of this strategy in adults.

Altenberg et al assessed the effect of a diet low in red and processed meat in preventing symptomatic relapse of CD. There was no difference in relapse (defined as increase in short Crohn disease activity index score by ≥70 points or a total score >150 points, initiation or increase of IBD medications, or surgery) or fecal calprotectin levels among patients with CD on diets high or low in red and processed meats. These findings support existing outcomes in dietary interventions in patients with IBD; only extreme elimination diets have shown significant effects on gut microbiota composition and disease improvement. Larger randomized controlled trials assessing the use of diet in patients with IBD are needed to determine whether a specific diet can induce remission in these patients.

FMT. The evidence supporting FMT in patients with IBD is also rather limited. Although several trials are underway, there are no published randomized controlled trials of FMT for CD.

There is some evidence that FMT might be effective for UC. However, because of the chronic alterations in the microbiome of patients with IBD, repeated FMT might be required. In fact, a subgroup analysis of UC cohort studies demonstrated that patients who received a greater number of FMT infusions were more likely to achieve remission. Similar to probiotics, it is likely that the varying success seen with FMT is related to the specific composition of the donor stool and the correction of the microbiome changes associated with IBD. Supporting this, several of the trials of FMT in patients with UC showed that specific donor samples produced the majority of the treatment benefit.

Furthermore, a recent randomized controlled trial of FMT performed by using initial colonoscopic infusion and then multidonor FMT enemas 5 days a week for 8 weeks in patients with UC demonstrated that patients who achieved remission after FMT had greater microbial diversity and enrichment of *Escherichia coli* and *Roseburia inulinivorans* in fecal and colon samples compared with patients who did not achieve remission. In addition, those who achieved remission had increased levels of short-chain fatty acid biosynthesis and secondary bile acid production. Donor stool that contained *Bacteroides* species was associated with remission, whereas *Streptococcus* species in donor stool was associated with no response to FMT.

As we become better able to select donors to target the microbiome changes in patients with IBD, treatment outcomes might improve, and valuable clues toward effective bacteriotherapy for IBD might well emerge.

CONCLUSIONS

The microbiome in patients with IBD is altered compared with that in healthy control subjects. There is robust evidence from animal models in particular to support a role for the microbiome in disease development and progression. Some, although limited, clinical data support the efficacy of treatment strategies that target the microbiome in patients with IBD. Current evidence supports the use of antibiotics to prevent postoperative recurrence in patients with CD, in the treatment of pouchitis, and in perianal disease. Certain probiotics might help in the prevention of pouchitis and possibly in the maintenance of remission in patients with mild-to-moderate UC. Dietary changes can be effective in patients with IBD, particularly use of exclusively enteral nutrition in children with CD, but additional studies are needed in adults and in patients with UC. There is limited evidence to support a role for FMT in the treatment of UC but not as yet in patients with CD. The microbiome is a rapidly evolving target for diagnosis, prognostication, and treatment of IBD with exciting future potential.

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