Emerging Role of the Gut Microbiome in Irritable Bowel Syndrome

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KEYWORDS

• Microbial dysbiosis • Breath test • Abdominal pain • Diarrhea • Constipation

KEY POINTS

- Epidemiologic studies suggest acute gastroenteritis can trigger the onset of irritable bowel syndrome (IBS), leading to development of postinfection IBS.
- Small intestinal bacterial overgrowth is associated with diarrhea-predominant IBS, whereas increased levels of methanogenic Archaea, specifically *Methanobrevibacter smithii*, are associated with constipation-predominant IBS.
- Fecal and/or gut mucosal microbiome are altered in at least a subset of patients with IBS.
- Alterations in gut microbiome can affect the gut-brain axis, visceral sensitivity, intestinal barrier, intestinal secretion, gut motility. and immune activation, which in turn can cause IBS symptoms.
- Therapies targeting the microbiome, such as probiotics, antibiotics, diet, and fecal microbiota transplant, can improve symptoms in subsets of patients with IBS.

INTRODUCTION

Microorganisms, including bacteria, Archaea, fungi, eukaryotic viruses, and bacteriophages, residing in the human gut are collectively referred to as the gut microbiome. Most of these organisms are commensal. The collection of all gut microbiome genes in an individual represents a genetic repertoire that is significantly more abundant than the human genome. The gut microbiome is influenced by factors related to birth (ie, vaginal delivery vs cesarean section) and early infancy (ie, infant feeding, infections, and antibiotics). The gut microbiome is further modulated in adult life by lifestyle (ie, exercise and diet), gastrointestinal (GI) infections, and antibiotics.

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Recent omics-based epidemiologic, clinical, and translational human studies, along with in vitro and in vivo studies in animals, have shown that gut microbial communities play a key role in the pathogenesis of several GI, as well as non-GI diseases. Despite significant interindividual variation, around 90% of all taxa in the human gut microbiome belong to just 2 phyla: Bacteroidetes and Firmicutes. Other phyla consistently found in the human distal gut are Proteobacteria, Actinobacteria, Fusobacteria, and Verrucomicrobia. Few species of Archaea (mostly *Methanobrevibacter smithii*) are represented. There are important differences between fecal and mucosa-associated communities within the same individual.[1] Bacterial composition in the lumen varies from cecum to rectum with pronounced variability in the microbial composition in the same individual when measured across months, weeks, and even days.[2] Factors such as diet, drug intake, traveling, or colonic transit time can affect microbial composition of fecal samples over time. Fluctuations in the gut microbiome among individuals can be significant, although the microbial pattern tends to return to its baseline over time.[2]

This article focuses on the role of microbiome in irritable bowel syndrome (IBS). IBS is a multifactorial disorder characterized by alterations in gut motility, barrier function, low-grade immune activation, visceral hypersensitivity (VH), and hypervigilance toward gut symptoms.[3] It summarizes the current evidence of microbial dysbiosis in IBS, discusses the literature on the role of microbiome in mediating central and peripheral dysfunctions summarized earlier, and discusses potential microbial components and products leading to these dysfunctions. In addition, it discusses the current state of evidence on therapies targeting microbiome in the management of IBS.

POSTINFECTION IRRITABLE BOWEL SYNDROME

Even before McKendrick and Read[4] reported the first case of IBS following outbreaks of *Salmonella* in the United Kingdom, there were reports in the literature of chronic GI symptoms following gastroenteritis.[4,5] Subsequently, multiple studies have reported what is now known as postinfection IBS, with incidence ranging from 3% to 31%.[6] A recent meta-analysis of 45 studies that prospectively followed infectious outbreaks found that the pooled incidence of IBS was 14.5% at more than 12 months after acute gastroenteritis.[7] Several host-related factors increased the likelihood of developing IBS, including female gender, psychosomatic comorbidities such as presence of anxiety, depression, somatization, and neuroticism.[7] In addition, severity of the infectious gastroenteritis (such as presence of bloody stool, episode lasting >7 days) was also associated with development of postinfection IBS.[7] Various types of infectious gastroenteritis have been implicated, such as bacterial, protozoal, and viral infections.

SMALL INTESTINAL BACTERIAL OVERGROWTH AND IRRITABLE BOWEL SYNDROME

Several studies have reported increased prevalence of small intestinal bacterial overgrowth (SIBO) in patients with IBS compared with healthy controls based on either glucose or lactulose breath testing. Patients with IBS have a 3.5 to 4.7 times higher odds of having an abnormal breath test compared with healthy controls, depending on the criteria used to define a positive test.[8,9] As expected, because of the invasive nature, difficulty in performing, and lack of a clear threshold to define SIBO, few studies have used small bowel cultures to diagnosis SIBO in IBS. Initial studies showed patients with IBS are more likely to have small intestinal bacterial counts of greater than 10^3 colony-forming units (CFU) per milliliter compared with healthy controls.[10] These findings have been validated using newer technologies such as quantitative polymerase chain reaction (PCR).[11]

The predominant archaeon and methane producer in human gut is *M smithii*.[12] Methane has been shown to slow down small bowel transit in animal studies (discussed later), and has been associated with constipation-predominant IBS (IBS-C).[13] Similarly, increased methane excretion on breath testing has been associated with decreases in stool consistency and transit time and an increase in constipation severity.[14–16] However, more recent studies have failed to confirm these associations.[17] In a recent study, Parthasarathy and colleagues[18] found that the fecal microbiota correlated with colonic transit and breath methane production but methane levels did not correlate with colonic transit, going against a link between breath methane levels and slow transit constipation. In this study, constipated patients had a unique profile of colonic mucosal microbiota that discriminated between constipation and health with an accuracy of 94% independent of diet and colonic transit.[18] In addition, treating constipated patients with increased breath methane levels with targeted antibiotics has been shown to improve constipation symptoms.[19] However, it is unclear whether this effect is caused by reduction in methane levels or some other mechanism related or unrelated to microbiome perturbation. Therefore, it is not clear whether methane is caused by constipation or is the cause of constipation.

MICROBIOME ALTERATIONS IN IRRITABLE BOWEL SYNDROME

There are several studies assessing microbiome alterations in patients with IBS (**Table 1**). Most of these studies assessed fecal microbiome, whereas a few have investigated both mucosal and fecal microbiome. Only a few of these studies have shown an IBS-specific microbial signature, whereas others have failed to replicate these findings in larger studies.[20,21] Several, but not all, studies have shown that microbial diversity is reduced in patients with IBS compared with healthy controls.[22] Although there is significant heterogeneity in the findings of these studies, a recent meta-analysis showed an overabundance of the phylum Bacteroidetes and the families of Lactobacillaceae and Enterobacteriaceae in patients with IBS compared with healthy controls.[22] There also seems to be a decreased abundance of genus *Faecalibacterium* and *Bifidobacterium*.[22] Only a few studies have focused solely on diarrhea-predominant IBS (IBS-D), showing an overabundance of phylum Bacteroidetes and a decrease in genus *Bifidobacterium*.[22] Although a few studies have found an association between specific bacteria groups and disease severity, these findings have not been consistently replicated by additional studies,[23] likely because of significant limitations of these studies: heterogeneity of patients with IBS (including subtypes), single-center studies with small sample size, and lack of demographic details on healthy controls (ie, whether they were age or gender matched).

GUT MICROBIOTA AND THE GUT-BRAIN AXIS

The gut-brain axis is a bidirectional communication network involving neural, endocrine, and immune pathways between the central nervous system (CNS) and enteric nervous system (ENS).[24] Studies in germ-free (GF) mice have shown that gut bacterial colonization with commensals is central to development and maturation of both the ENS and CNS.[25,26] Moreover, the gut microbiota also seems to influence stress reactivity, anxietylike behavior, and the development of the hypothalamus-pituitary axis, which regulates stress response.[27–31] In addition, the gut microbiota also modulates the serotoninergic system, because an increase in serotonin turnover and altered levels of related metabolites have been reported in the limbic system of GF animals.[28] Engevik and colleagues[32] found that *Bifidobacterium dentium* and its metabolite, acetate, increased intestinal serotonin concentrations along with expression of serotonin

Table 1
Microbiome analysis in irritable bowel syndrome[a]

Study	Subjects	Sample and Techniques	Findings
Kerckhoffs et al,[96] 2009	41 IBS and 26 healthy controls	Fecal and duodenal mucosa brush samples, FISH analyses for microbiome composition, qPCR for *Bifidobacterium* spp	Lower *Bifidobacterium* counts in duodenum and fecal samples in IBS
Kerckhoffs et al,[97] 2011	37 IBS and 20 healthy controls	Fecal and duodenal mucosa brush samples; bacterial 16S rRNA using DGGE and qPCR	Higher levels of *Pseudomonas aeruginosa* in duodenal mucosa and feces of patients with IBS
Codling et al[98] 2010	47 IBS and 33 healthy controls	Fecal samples using 16S rRNA DGGE	Lower microbial diversity in patients with IBS
Ponnusamy et al,[99] 2011	11 IBS and 8 non-IBS	Fecal samples using 16S rRNA DGGE and qPCR	Higher diversity of total bacteria, Bacteroidetes and *Lactobacillus* in IBS Lower diversity of *Bifidobacterium* and *Clostridium coccoides* in IBS
Rajilić-Stojanović et al,[100] 2011	62 IBS and 46 healthy controls	Fecal samples using phylogenetic microarray and qPCR	2-fold increased ratio of the Firmicutes to Bacteroidetes This resulted from an approximately 1.5-fold increase in numbers of *Dorea*, *Ruminococcus*, and *Clostridium* spp ($P<.005$); a 2-fold decrease in the number of Bacteroidetes ($P<.0001$); a 1.5-fold decrease in numbers of *Bifidobacterium* and *Faecalibacterium* spp ($P<.05$)
Saulnier et al,[60] 2011	22 pediatric IBS and 22 healthy controls	Fecal samples using 16S gene sequencing	Higher levels of Gammaproteobacteria in IBS, including more *Haemophilus parainfluenzae*

Carroll et al,[101] 2012	23 IBS-D and 23 healthy controls	Fecal samples using 16S gene sequencing	Reduced microbial richness in IBS-D Increased levels of Enterobacteriaceae in IBS-D Decreased levels of *Faecalibacterium* in IBS-D
Jeffery et al,[20] 2020	37 IBS and 20 healthy controls	Fecal samples using 16S rRNA gene sequencing	Lower microbial diversity in IBS A subset of patients with IBS with microbial composition different from healthy controls characterized by increased Firmicutes and decreased Bacteroidetes among other findings
Rangel et al,[102] 2015	33 IBS and 16 healthy controls	Fecal and colonic mucosal biopsies using phylogenetic microarray	A significantly lower abundance of the bacterial group uncultured Clostridiales I in the mucosal-associated microbiota in IBS. Many differences in IBS in fecal samples. Notable findings include increases in Actinobacteria, Bacilli, several *Clostridium* clusters, and Proteobacteria, and a decrease in Bacteroidetes
Tap et al,[23] 2017	Cohort 1: 110 IBS, 39 healthy controls Cohort 2: 29 IBS, 17 healthy controls	Fecal samples and mucosal biopsies using 16S rRNA gene sequencing	By using classic approaches, no differences in fecal microbiota abundance or composition Using machine learning approach, signature for severe IBS included presence of methanogens, and enterotypes enriched with Clostridiales or *Prevotella* spp
Maharshak et al,[103] 2018	23 IBS-D and 24 healthy subjects	Fecal samples and mucosal biopsies using 16S rRNA gene sequencing	Decreased richness in IBS fecal samples only *Faecalibacterium* lower in IBS-D. *Dorea* higher in IBS-D

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Study	Subjects	Sample and Techniques	Findings
Vich Vila et al,[104] 2018	181 patients with IBS. The control group were recruited from LifeLine Deep cohort (n = 893) and Maastricht IBS case-control cohort (n = 132) without GI complaints	Metagenomic shotgun sequencing using fecal samples	Increase in several species of phylum Actinobacteria. Decrease in species of Bacteroidetes, Increase in species of Streptococcaceae and Lachnospiraceae families
Dior et al,[89,105] 2016	16 IBS-D, 15 IBS-C and 15 healthy controls	Fecal samples analyzed using RT-PCR	Increase in *Escherichia coli* in patients with IBS-D, and an increase in *Bacteroides* and *Bifidobacterium* in patients with IBS-C
Chung et al,[106] 2016	28 IBS and 19 healthy controls	Fecal and jejunal mucosal samples analyzed using 16s rRNA gene sequencing	Patients with IBS had a higher proportion of Veillonellaceae in stool than controls. Prevotellaceae was more abundant in jejunal mucosa of patients with IBS than in controls
Chassard et al,[107] 2012	14 IBS-C women and 12 sex-matched healthy subject	Feces analyzed using FISH and anaerobic bacterial culture	Butyrate-producing *Roseburia–Eubacterium rectale* group reduced in IBS-C vs controls
Duboc et al,[108] 2012	14 IBS-D and 18 healthy controls	Fecal microbiota composition was assessed by quantitative PCR	There was a significant increase of *E coli* and a significant decrease of *Clostridium leptum* and *Bifidobacterium* in patients with IBS-D
Jalanka-Tuovinen et al,[63] 2014	11 postinfection IBS, 11 postinfection bowel dysfunction, 12 postinfection without bowel dysfunction, 12 IBS-D, and 11 healthy controls	16S rRNA gene phylogenetic microarray analysis with HITChip, 16S rRNA gene qPCR with group and species-specific primers	12-fold increase in Bacteroidetes phylum in IBS, whereas healthy controls had 35-fold more uncultured Clostridia

Pozuelo et al,[109] 2015	113 IBS and 66 healthy controls	Fecal samples using 16S rRNA gene sequencing	Patients with IBS-M and IBS-D had lower relative abundance of butyrate-producing bacteria
Ringel-Kulka et al,[110] 2016	60 IBS and 20 healthy controls	Fecal samples using 16S rRNA gene sequencing	Subjects with IBS showed significantly higher levels of species of *Lactobacillus* and *Streptococcus* with the most significant increase being observed in IBS subjects without bloating Members of the Firmicutes phylum (*Oscillibacter*, *Anaerovorax*, incertae sedis XIII, *Streptococcus*, and Eubacteriaceae) were significantly decreased in IBS-D and M-IBS compared with healthy controls
Shukla et al,[111] 2015	47 IBS and 30 healthy controls	qPCR with group-specific primers in fecal samples	Lower abundance of *Bifidobacterium* and increased abundance of *Ruminococcus*, *Ruminococcus productus–C coccoides*, *Veillonella*, *Bacteroides thetaiotaomicron*, *P aeruginosa*, and gram-negative bacteria in patients with IBS
Tana et al,[112] 2010	26 IBS and 26 healthy controls	16S rRNA gene qPCR with group-specific and species-specific primers, culture, microscopy	Higher counts of *Veillonella* and *Lactobacillus* in subjects with IBS compared with controls

Abbreviations: DGGE, denaturing gradient gel electrophoresis; FISH, fluorescence in situ hybridization; HITChip, human intestinal tract chip; IBS-C, constipation-predominant IBS; IBS-D, diarrhea-predominant IBS; IBS-M, IBS with mixed bowel habits; qPCR, quantitative PCR; rRNA, ribosomal RNA; RT-PCR, reverse transcription PCR.

[a] Modified from Pimentel et al.[113]

receptors, and serotonin transporter in in vivo and/or in vitro models. Moreover, *B dentium*–treated GF mice had higher hippocampal expression of serotonin receptor and showed less repetitive and anxietylike behaviors relative to GF controls. Similarly, modulation of brain regions controlling central processing of emotion and sensation have also been shown in response to ingestion of fermented milk with probiotics (Bifidobacterium, Lactobacillus, and *Streptococcus thermophiles*) in healthy controls.[33] In a landmark study, De Palma and colleagues[34] showed GF mice inoculated with the fecal microbiota from patients with IBS-D, but not the fecal microbiota from healthy controls, showed rapid GI transit, alterations in the intestinal barrier, and anxietylike behavior. Similarly, in a double-blind randomized controlled trial, the probiotic *Bifidobacterium longum* improved depression scores and quality of life in patients with IBS compared with placebo.[35] Interestingly, brain functional MRI studies performed before and after the intervention showed that *B longum* reduced responses to negative emotional stimuli in multiple brain areas, including the amygdala and frontolimbic regions, compared with placebo.[35] One way by which the gut microbiota could alter brain function and behavior is via short-chain fatty acids (SCFAs).[36] As an example, propionic acid produced by gut bacteria readily crosses the blood-brain barrier and influences brain function and behavior in animals.[36] Besides SCFAs, gut microbes such as *Lactobacillus* and *Bifidobacterium* can also generate γ-amino butyric acid (GABA), an inhibitory neurotransmitter in the human brain.[37] These studies highlight the important role of the gut microbiota and its metabolites in modulating the gut-brain axis.

GUT MICROBIOTA AND VISCERAL HYPERSENSITIVITY

VH is defined as enhanced perception of mechanical triggers applied to the bowel, which is reflected clinically as pain and discomfort.[38] Using visceral distension models, the prevalence of VH in patients with IBS varies from 33% to 50%.[38] Evidence to suggest that the gut microbiome plays a key role in mediating VH includes the observation that probiotics and antibiotics alter VH. Verdu and colleagues[39] showed that antibiotics induce VH in mice, whereas *Lactobacillus paracasei* NCC2461 reduces VH. Similar effects of other probiotics, such as *L paracasei* and *Lactobacillus acidophilus* NCFM in normalizing stress-induced VH has also been shown.[40,41] Similarly, the poorly absorbed oral antibiotic rifaximin has been shown to normalize VH in chronic psychological stress rodent models by altering the composition of the ileal microbiota.[42] Furthermore, Crouzet and colleagues[43] recently showed that inoculation of GF mice with the fecal microbiota from patients with IBS induced VH to colorectal distention, whereas microbiota inoculation from healthy volunteers did not. The mechanisms by which the gut microbiome modulates VH in IBS are not well understood but might involve bacterial components such as lipopolysaccharide (LPS), bacterial products such as SCFAs, or gases such as hydrogen sulfide.[44–47]

GUT MICROBIOTA AND GASTROINTESTINAL MOTILITY

GI motility requires complex coordination among neurons, interstitial cells of Cajal, and smooth muscle. Recent studies suggest an interdependent relationship between the gut microbiome and transit. Kashyap and colleagues[48] recently showed that introducing fecal microbiota from a healthy human into GF mice (humanized mice) altered GI transit and colonic contractility. The magnitude and directionality of this effect depended on the type of carbohydrates in the diet, suggesting that the diet plays a significant role in the microbial influence on the GI tract.[48] In contrast, the abundance of gut microbial communities was altered by changes in GI transit.[48] Accelerating or

decelerating GI transit using polyethylene glycol or loperamide, respectively, led to differences in the gut microbiome that were reversed on return to a normal GI transit.[48] Gut microbiota and its metabolites can influence GI motility by either direct effects on enteric neurons or indirect effects on immune cells causing release of bioactive molecules. Bacterial metabolites such as SCFAs and deconjugated bile salts are known to generate potent motor responses in both animals and humans.[49,50] Similar to SCFAs, secondary metabolites from aromatic amino acids such as tryptamine have also been shown to increase contractility in ex vivo preparations of guinea pig ileum by stimulating serotonin release.[51] In contrast, methane produced by bacterial fermentation has been shown to reduce small intestinal transit.[13] Likewise, hydrogen sulfide, which is derived from sulfate-reducing bacteria, has also been shown to inhibit small intestinal and colonic contractility via potassium channels.[52] However, it is unclear whether luminal hydrogen sulfide overcomes the detoxification process present in colonic mucosa and what role (if any) it has in IBS. In addition to bacterial metabolites, bacterial components can also modulate gut motility. This process is best exemplified by LPS derived from gram-negative bacteria, which has been suggested to promote the survival of enteric nitrergic neurons that promote gut motility through Toll-like receptor (TLR) 4 signaling.[53]

GUT MICROBIOTA AND INTESTINAL PERMEABILITY

The GI tract is a semipermeable barrier that allows the absorption of nutrients and immune sensing, while limiting the transport of potentially harmful antigens and microorganisms into the body. The gut barrier is impaired in several GI and non-GI diseases, including IBS. Gut barrier function has been shown to be impaired in about 40% of patients with IBS-D and those with postinfection IBS and seems to correlate with severity of IBS symptoms.[54–56] A recent study showed that up to 40% of patients with postinfection IBS have high fecal proteolytic activity, which in turn increases paracellular permeability by decreasing expression of the tight junction protein occludin, and redistributing occludin from tight junctions to cytosol, decreasing microbial diversity.[57]

The gut microbiome also plays a key role in the pathophysiology of diet-induced IBS symptoms. A diet high in fermentable oligosaccharides, disaccharides, monosaccharides, and polyols (FODMAPs) has been shown to cause intestinal barrier loss in rodent models through an LPS-TLR4 pathway that decreases colonic epithelial tight junction proteins.[44] Interestingly, patients with IBS have higher fecal LPS levels compared with heathy controls and studies have reported increased colonocyte expression of TLR4 receptor in biopsies from patients with IBS.[44] Another possible mechanism by which gut microbiota modulates gut barrier function is via SCFAs. Butyrate, a microbial-derived SCFA, helps maintain gut barrier function by increasing expression of tight junction proteins.[58] However, the role of butyrate or other SCFAs in IBS pathophysiology is not clear because most of the studies are descriptive, have conflicting data on SCFA levels in patients with IBS, have not accounted for confounding factors such as colon transit, and rely on fecal SCFA levels rather than luminal SCFAs (discussed later).

In addition to affecting tight junction proteins, the gut microbiota also regulates mucus production in the intestines. Intestinal mucus forms a barrier between the lumen and the epithelial cells, thereby protecting the epithelial surface from pathogens. Constituents of the microbiota that degrade mucin, such as *Ruminococcus torques*, *Ruminococcus gnavus*, and *Akkermansia muciniphila*, have been reported to be increased and their levels associated with severity of bowel symptoms in patients with IBS.[59,60]

GUT MICROBIOME AND IMMUNE ACTIVATION

Mucosal immune activation underlying IBS has been an important area of investigation, with several studies showing low-grade inflammation and infiltration of inflammatory cells, notably mast cells, in the intestinal mucosa of patients with IBS. Mast cells express pattern recognition receptors, including TLR2 and TLR4. Increased levels of fecal LPS, serum LPS, and increased mucosal TLR4 expression have been observed in patients with IBS.[44,61,62] Increased levels of fecal and serum LPS in IBS can activate mast cells through TLR4, which in turn causes release of inflammatory mediators such as histamine, tryptase, and prostaglandin E_2. These mast cell mediators are increased in mucosa of patients with IBS and lead to barrier loss and VH, and thus may lead to symptom generation in IBS.

Studies have shown that the fecal microbiome of patients with postinfection IBS significantly differs from healthy controls, with increased Bacteroidetes phylum and decreased uncultured Clostridiales.[63] These microbiome changes were associated with mucosal expression of inflammatory cytokines such as interleukin (IL) 1β and IL-6.[63] Moreover, compared with healthy controls, patients with postinfection IBS have been found to have increased mucosal enteroendocrine cells, intraepithelial lymphocytes, and T lymphocytes in the lamina propria.[64,65] Taken together, these findings suggest that low-grade mucosal immune activation in response to microbiome perturbation plays a significant role in the pathophysiology of postinfection IBS.

Besides increased number of mucosal innate immune cells, levels of proinflammatory and antiinflammatory cytokines are also altered in patients with IBS. Several studies have shown an increase in proinflammatory cytokines (tumor necrosis factor-α, IL-1β, IL-6, IL-8) and a decrease in antiinflammatory cytokines such as IL-10.[59] Probiotics have been investigated given their ability to restore cytokine balance in rodent models.[59] In a randomized, double-blind, placebo-controlled, crossover study in older adults without GI symptoms, a probiotic containing *Lactobacillus gasseri* KS-13, *Bifidobacterium bifidum* G9-1, and *B longum* MM2 increased the levels of ex vivo antiinflammatory IL-10 production, possibly via increasing the levels of fecal *Bifidobacterium* and *Lactobacillus* and reducing the levels of *Escherichia coli*.[66] Similarly, a placebo-controlled, randomized controlled trial in patients with IBS showed that a 12-week course of *Bifidobacterium infantis* normalized the abnormal IL-10/IL-12 ratio seen in patients with IBS.[67] In addition to probiotics, bacterial metabolites of dietary nutrients have also been shown to be antiinflammatory. Butyrate produced by dietary fiber fermentation has an antiinflammatory effect, via inhibition of nuclear factor kappa-B and downstream proinflammatory cytokine production from colonic epithelial cells as well as inflammatory cells (such as mast cells).[68,69]

ALTERATION OF MICROBIOME MEDIATORS AFFECTING GUT PHYSIOLOGY IN IRRITABLE BOWEL SYNDROME

Tryptamine

Tryptamine is a tryptophan-derived monoamine that is abundant in human feces. Tryptamine concentrations increase nearly 200-fold in feces following colonization of GF mice with human gut microbiota, suggesting that bacterial metabolism of tryptophan generates luminal tryptamine.[70] Using in vitro and in vivo models, Bhattarai and colleagues[70] showed that tryptamine increases ionic flux across the colonic epithelium and increases fluid via 5-hydroxytryptamine receptor 4 (5-HT_4R) activation, which in turn accelerates colonic transit in mice. In another study, patients with IBS-D were found to have increased tryptamine levels compared with healthy controls.[71] Baseline colonic secretion was also increased in patients with IBS-D, suggesting

either an inherent change in epithelial transport or an increase in metabolites that promote fluid secretion.[71] Application of tryptamine to colonic mucosal biopsies from healthy controls and patients with IBS-D also increased secretion in both groups to similar extents.[71] This finding indicates that the colonic epithelium of patients with IBS and healthy controls is capable of tryptamine-induced fluid secretion, and observed changes could thus be caused by changes in tryptamine abundance. Overall, it is possible that increased fecal tryptamine abundance leads to increased mucosal secretion in a subset of patients with IBS-D; however, this needs to be validated in larger studies.

Short-Chain Fatty Acids

Several studies, including a meta-analysis, have shown decreased fecal levels of butyrate and propionate in the feces of patients with IBS-C and increased levels of butyrate in IBS-D.[72] However, the functional significance of these in vivo changes in fecal SCFA levels are not clear because most of these studies are observational. Moreover, the fermentation and production of SCFAs occur in the proximal colon and most of the SCFAs are absorbed rapidly by the colon epithelial cells, which means that the intestinal transit time affects the fecal SCFA levels.[73] The differences in the fecal levels of different SCFAs between the IBS subtypes may therefore be caused by differences in the intestinal transit time between these subtypes.[73]

Lipopolysaccharide

In rodent models, a high-FODMAP diet led to the development of gram-negative dysbiosis and associated increased levels of fecal LPS. These changes were further shown to cause colonic barrier dysfunction, mast cell recruitment, and VH, findings seen in patients with IBS-D.[44] Translating these findings into human disease, patients with IBS-D had significantly increased fecal LPS levels compared with healthy controls, and LPS levels significantly decreased after introduction of a low-FODMAP diet.[44] In addition, intracolonic administration of fecal supernatants from patients with IBS-D induced VH in rodent models, which was reversed after a low-FODMAP diet or in the presence of an LPS antagonist.[44] This finding suggests fecal LPS plays a key role in VH induced by a high-FODMAP diet in patients with IBS-D. However, it is not clear whether this effect by LPS is mediated via direct stimulation of TLR4 receptor on enteric neurons or indirectly via activation of TLR4 receptors on mast cells (or other immune cells).

Proteases

Fecal as well as mucosal proteolytic activity has been shown to be increased in patients with IBS-D.[74] However, it is not clear whether this increased proteolytic activity is derived from host and/or the microbiome. In a recent study, transplant of GF mice with feces from patients with IBS-D with high fecal proteolytic activity led to ineffective inhibition or, in some cases, an increase in fecal proteolytic activity compared with GF state.[57] This finding suggests either microbial production of proteases or decreased production of protease inhibitors such as siropins, miropins, or elafins in a subset of patients with IBS-D.[57] However, this needs to be further investigated in future studies.

MICROBIOME-DIRECTED THERAPIES

Prebiotics and Probiotics

Prebiotic are nondigestible food ingredients that stimulate the growth and/or activity of health-promoting bacteria (ie, *Lactobacillus* and *Bifidobacterium*). Most prebiotics are carbohydrates (eg, galacto-oligosaccharides, pyrodextrins, lactulose). A recent meta-

analysis of prebiotics in IBS identified only 3 trials that met criteria for inclusion.[75] Two of the trials included in this meta-analysis assessed fructo-oligosaccharides and the third assessed trans-galacto-oligosaccharide.[76–78] Both of the fructo-oligosaccharide trials found no significant improvement compared with placebo, although there seemed to be a trend toward benefit with short-chain fructo-oligosaccharide in 1 of the trials.[76,77] In the third trial, 2 doses of trans-galacto-oligosaccharide were assessed for 4 weeks in 60 patients with IBS using a crossover design.[78] Both doses of trans-galacto-oligosaccharides showed significant improvement in global IBS symptoms but no effect on mean abdominal pain scores.[78] Given the paucity of data, a definitive conclusion on the efficacy of prebiotics in IBS cannot be made at this time.

Probiotics are organisms that confer a health benefit on the host. The popularity of probiotics has increased recently because of the interest in the role of the gut microbiome in health. However, data supporting the use of probiotics in IBS remain controversial because of the lack of large, multicenter, high-quality studies using rigorous end points and clinical outcomes. Although there are many trials with probiotics in IBS, few use the same strain of probiotic or the same combination of probiotics, thereby limiting the ability to pool the data from these studies and make definitive conclusions regarding their efficacy. A recent meta-analysis identified 37 trials using probiotics in IBS that met their entry criteria, which included 4403 subjects.[75] In this meta-analysis, trials that used combination probiotics (n = 21 trials) resulted in a significant pooled effect (relative risk, 0.79; confidence interval, 0.68–0.91) for global symptom improvement. Significant heterogeneity (I^2 = 72%) and publication bias were present in these trials, limiting the confidence in any recommendations that could be offered. In contrast, trials that used single probiotics (n = 16 trials) found no significant pooled effect, with the exception of *Escherichia* spp and *Streptococcus* spp, which did show a significant pooled effect. However, because there were few trials (2 trials with *Escherichia* spp and 1 with *Streptococcus faecium*), with small numbers of subjects (the trial with *Streptococcus* included only 34 subjects), no definitive conclusions could be made as to their efficacy.

A few of the larger, higher-quality trials with probiotics in patients with IBS are worth reviewing in detail. In 1 trial, 362 women with IBS were randomized to 3 different doses of *B infantis* 35624 (10^6, 10^8, 10^{10} CFU/mL) or placebo for 4 weeks.[79] Only patients receiving the 10^8-CFU/mL dose had significant improvement in the primary end point, which was abdominal pain or discomfort.[79] *B infantis* 35624 also improved bloating and bowel-related symptoms (eg, bowel dysfunction, straining). All subtypes of IBS seemed to benefit, although there was a trend toward greater efficacy in IBS-D.

In the second study, 379 patients with IBS were randomized to *Saccharomyces cerevisiae* I-3856 or placebo for 12 weeks.[80] A small numerical, but not statistically significant, improvement was present for the primary end point (32% vs 27%; $P>.05$), which was a greater than 50% reduction in intestinal pain/discomfort for at least 4 out of the last 8 weeks of the study.[80] There was a significant improvement in patients with IBS-C, which may be worth exploring further in the future.[80]

In the third trial, 298 patients with IBS were randomized to receive *E coli* DSM 17252 or placebo for 8 weeks.[81] A greater percentage of patients receiving *E coli* DSM 17252 reported improvement compared with placebo in the primary end points of abdominal pain score (19% vs 7% $P<.05$) and general symptom score (19% vs 5%; $P<.05$).[81]

Probiotics are a potentially important treatment; however, current data are limited and do not conclusively support their general use for IBS at this time. Recent guidelines by both the American College of Gastroenterology and the American Gastrointestinal Association recommend against the use of probiotics for treatment of

IBS.[82,83] Large, multicenter trials with rigorous end points, of at least 12 weeks' duration, are needed to make conclusions on efficacy of specific strains of probiotics.

Antibiotics

Antibiotics, particularly nonabsorbable antibiotics, seem to improve symptoms in some patients with IBS. One of the first trials with antibiotics randomized 111 patients with IBS (Rome I criteria) with varying subtypes to receive neomycin or placebo.[84] Neomycin resulted in a 35% improvement in a composite score of IBS symptoms compared with 11.4% for placebo ($P<.05$).[84] Patients whose lactulose breath test for SIBO normalized following treatment with neomycin were significantly more likely to have improvement in symptoms compared with patients whose breath test did not normalize.

Rifaximin, a nonsystemic derivative of rifamycin, is by far the best studied and only US Food and Drug Administration (FDA)–approved antibiotic for the treatment of IBS.[85] Two identically designed phase III trials (TARGET 1 and 2, N = 1260) randomized patients with IBS without constipation according to ROME II criteria to receive rifaximin 550 mg 3 times a day for 2 weeks or placebo.[85] Patients were followed for an additional 10 weeks, although the primary end point was assessed during the 4 weeks after completion of the treatment. A significantly greater proportion of patients receiving rifaximin reported adequate relief of global IBS symptoms for at least 2 of the first 4 weeks after treatment (40.7% vs 31.7% for placebo, pooled; $P<.001$). In addition, a greater proportion of patients reported relief of IBS-related bloating (40.2% vs 30.3% for placebo, pooled; $P<.001$). Improvement compared with placebo persisted during the 10-week follow-up period, although overall efficacy decreased in both groups and was no longer statistically significantly different at the end of the follow-up period.

To answer questions of whether repeat treatment with rifaximin is effective and safe, a third phase III trial (TARGET 3) was conducted.[86] In this trial, 636 patients with IBS-D according to Rome III who responded to open-label rifaximin and developed recurrence of symptoms during an 18-week follow-up period were randomized to receive 2 courses of rifaximin 550 mg 3 times a day for 2 weeks or placebo separated by 6 weeks.[86] The primary end point was assessed during the 4 weeks after completion of the first retreatment. In total, 1074 patients with IBS-D were enrolled and received open-label rifaximin 550 mg 3 times a day for 2 weeks. Of these patients, 44% had a response to rifaximin; however, 64% (n = 692) of responders had a relapse of symptoms during the 18-week follow-up period and were randomized to receive double-blind rifaximin or placebo. A greater percentage of patients randomized to receive double-blind rifaximin were responders compared with those who received placebo (38% vs 32%; $P = .03$). Abdominal pain, but not stool consistency, was significantly improved with rifaximin versus placebo. Similar results were seen with the second retreatment. The results of this trial support repeat treatment with rifaximin after initial response to treatment, although it should be noted that the improvement compared with placebo was small, which may have been caused, at least in part, by the patients entering into the double-blind phase having lower symptom severity compared with their baseline before receiving open-label rifaximin.

In an effort to better understand the mechanism of action of rifaximin, a subset of patients (N = 93) in the TARGET 3 trial underwent lactulose hydrogen breath testing before and 4 weeks after completion of the initial, open-label treatment with rifaximin.[87] Among patients with a positive breath test at baseline, a greater percentage were responders to rifaximin than those with a negative test (60% vs 26%; $P = .002$). Moreover, patients whose breath test normalized after treatment had a

response rate of nearly 77%. These results support the role of rifaximin in altering the microbiome potentially within the small intestine.

Importantly rifaximin seems to be well tolerated, with an adverse event rate in trials similar between rifaximin and placebo. Patients in the TARGET 3 trial showed no evidence of developing on-going bacterial resistance, nor did there seem to be significant alterations in the microbiome.[88] Likewise, side effects such as diarrhea or *Clostridium difficile* colitis were rare.[89] One case of *C difficile* colitis was reported in the TARGET 3 trial in a patient who was off rifaximin and had used interceding antibiotics. The American College of Gastroenterology guidelines on IBS supports the use of rifaximin to treat global symptoms in patients with IBS-D (strong recommendation; moderate level of evidence).

Diet Modification

The microbiome is heavily influenced by the types of food that is eaten. Further, most patients with IBS report food to be a trigger of their symptoms.[90] Foods such as dairy, wheat, cabbage, caffeine, alcohol, onion, garlic, beans, spices, and fried food are commonly reported as triggers for symptoms in patients with IBS.[90] Not surprisingly, several diets have been studied in IBS. Two of the diets in particular, the low-FODMAP diet and, to a lesser extent, a gluten-free diet, have been studied in randomized controlled trials.[90] These studies are reviewed in detail in the Emily Haller and Kate Scarlata's article, "Diet Interventions for Irritable Bowel Syndrome (IBS): Separating the Wheat from the Chafe," in this issue.

Fecal Microbiota Transplant

Fecal microbiota transplant (FMT) has been proved to be an effective treatment of recurrent *C difficile* colitis. In IBS, the data has been more mixed. Studies have used a variety of routes of administration (eg, oral capsule, nasojejunal infusion, and colonoscopy), formulations (eg, frozen, dried, and fresh), and number and type of donors. A recent meta-analysis of 5 randomized trials that included 267 patients with IBS found colonoscopy delivery of FMT to be effective, whereas nasojejunal tube delivery showed only a trend toward benefit, and oral capsules offered no benefit.[91] Subsequently, a large, single-center trial by El-Salhy and colleagues,[92] which included patients with IBS of all subtypes, assessed the efficacy of FMT (30 g and 60 g) delivered via a gastroscope into the distal duodenum versus placebo (autologous FMT). All FMT was acquired from a single so-called superdonor who was in excellent health (normal body mass index, on no medications, breastfed, healthy diet, and so forth) and had limited lifetime exposure to antibiotics. After 3 months, 76% in the 30-g FMT group and 89% in the 60-g FMT group were responders (as defined as a $\geq$50 decrease in the IBS-symptom severity score) compared with 24% in patients receiving autologous FMT. Similar differences were present using the FDA and European Medicines Agency responder end points. The donor's microbiota profile was particularly richer than average in *Lactobacillus*, Lachnospiraceae, and Verrucomicrobia, and lower in *Shigella* and *Escherichia* spp. Whether this microbiota profile is important for response remains to be determined.

Another recent randomized placebo-controlled trial assessed the efficacy of FMT via nasojejunal infusion in 62 patients with refractory IBS (defined as failure of $\geq$3 conventional therapies) of all subtypes with predominant bloating.[93] After 12 weeks, 56% of patients who received FMT reported improvement in both IBS symptoms scores and quality of life compared with 26% of patients receiving placebo ($P = .03$). No specific taxa were found in the stool of patients who responded to FMT, although responders had higher diversity of microbiomes before receiving FMT than

Table 2
Evidence supporting the role of microbiome in irritable bowel syndrome[a]

Epidemiology	Several Studies Support IBS Can Be Precipitated by Acute Gastroenteritis
Diagnostics	Hydrogen breath tests are more commonly abnormal in IBS suggesting SIBO Duodenal cultures more commonly grow coliforms in IBS suggesting SIBO Stool microbiome analyses in several studies different from healthy controls *M smithii* (and breath methane) increased in IBS-C
Translational studies	Gut-brain axis dysfunction, VH, barrier dysfunction can be transplanted in rodent models using feces from patients with IBS
Probiotics	Conflicting data on its efficacy in IBS. Available data are of low methodological quality and larger, multicenter, well-designed studies of at least 12-wk duration with rigorous end points are needed
Antibiotics	Nonabsorbable antibiotics show short-term benefit in patients with IBS without constipation. A higher proportion of initial responders to rifaximin have symptom improvement with retreatment compared with placebo
Diet	Restricting fermentable carbohydrates (known to modulate microbiome) causes symptomatic improvement in IBS

[a] Modified from Pimentel et al.[113]

nonresponders. Importantly, 21% of patients who received FMT reported improvement in symptoms for longer than 1 year, compared with only 5% of patients who received placebo. A second FMT improved symptoms in 67% of patients who had an initial response but not in patients who did not respond initially to the FMT.

The potential risks of FMT need to be carefully examined in IBS. The study by El-Salhy and colleagues[92] reported adverse effects in 20% of the FMT group versus 2% in the autologous FMT group, including 2 patients who developed diverticulitis in the FMT group and none with diverticulitis in the autologous FMT group. Most side effects associated with FMT are mild and self-limiting. Severe side effects seem to be rare.[94] A recent report of antibiotic-resistant *E coli* bacteremia in 2 immunosuppressed patients, 1 of whom died, highlights the potential for serious complications with FMT.[95]

SUMMARY

There is growing evidence supporting the role of the microbiome in the pathophysiology of IBS (**Table 2**). Studies show that an episode of gastroenteritis can trigger development of postinfection IBS. Observational studies have found that a significant proportion of patients with IBS have SIBO, as shown by abnormal breath test and/or small intestinal culture. Furthermore, studies show that a significant proportion of patients (if not all) with IBS show alterations in mucosal and fecal microbiome compared with healthy controls. Moreover, basic and translational studies suggest that microbial components/products can cause dysfunction of the gut-brain axis, visceral sensitivity, intestinal barrier, motility, intestinal secretion, and mucosal immune activation. In addition, several interventions targeting the gut microbiome, including prebiotics,

probiotics, antibiotics, diet modification, and FMT, have opened up the potential for new treatments for patients with IBS that target the underlying cause rather than focusing only on improving symptoms. This article supports the concept that IBS is, at least in some patients, a microbiome-associated condition. If this is true, it opens the door to the development of novel therapies designed to modulate the gut microbiome. Future research is needed to identify the underlying mechanisms responsible for the link between the gut microbiome and IBS symptoms, develop biomarkers to identify the subset of patients with IBS with a microbiome-based cause of their gut symptoms, and develop and properly validate novel, efficacious therapies targeting the gut microbiome.

CLINICS CARE POINTS

- A subset of patients with diarrhea-predominant IBS have small intestinal bacterial overgrowth and treatment often leads to symptom improvement.
- Constipation-predominant IBS can be associated with methanogenic Archaea and there is some evidence that treating constipated patients with increased breath methane levels with targeted antibiotics can improve constipation symptoms.
- Therapies targeting the microbiome such as prebiotics, probiotics, antibiotics, diet and fecal microbiota transplant can improve symptoms in subset of patients with IBS. Among these therapies, the best evidence is for gut-specific antibiotics such as Rifaximin and dietary interventions such as low FODMAP diet.

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