Intestinal microbiota and immune function in the pathogenesis of irritable bowel syndrome

Yehuda Ringel¹ and Nitsan Maharshak¹,²

¹Division of Gastroenterology and Hepatology, University of North Carolina at Chapel Hill School of Medicine, Chapel Hill, North Carolina; and ²Department of Gastroenterology and Liver Diseases, Tel Aviv Sourasky Medical Center, Affiliated to the Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel

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IRRITABLE BOWEL SYNDROME (IBS) is the most common and best studied condition of a larger group of functional gastrointestinal (GI) disorders. Functional GI disorders refer to the presence of a variable combination of chronic or recurrent GI symptoms not explained by structural or biochemical abnormalities (130, 159). IBS is characterized by chronic or recurring abdominal pain or discomfort that is associated with altered bowel habits (53, 99). The condition is often associated with other GI symptoms (e.g., bloating, distention, and gas), other functional GI disorders (e.g., functional dyspepsia), other non-GI disorders (e.g., fibromyalgia, interstitial cystitis, migraine headache), and psychological disorders (e.g., depression, anxiety, somatization) (96, 146, 167). Traditionally, IBS has been subtyped on the basis of the patient’s predominant symptom/stool pattern (99). These subtypes include diarrhea predominant (IBS-D), constipation predominant (IBS-C), and mixed type (IBS-M), in which patients experience alternating periods of diarrhea and constipation. Unlike other conditions that can be associated with abdominal pain and abnormal bowel patterns such as inflammatory bowel diseases (IBD), IBS is not associated with any overt histopathology, structural, or biochemical abnormalities. IBS has been generally considered to be caused by alterations in the brain-gut axis (the bidirectional communication network involving the enteric nervous system, the autonomic nervous system, and the central nervous system) (60, 108, 125), and the pathophysiological mechanisms behind this condition remain unclear. Traditionally, IBS is considered to be a multifactorial condition in which multiple environmental, genetic, physiological, and psychosocial factors contribute to the development of the disorder through direct or indirect effects on the brain-gut axis (65, 125, 146). Bacterial infection, altered GI microbiota, dysregulated immune function, and low-grade inflammation are additional factors that have been gaining more interest as evidence supporting their contribution to the pathophysiology of IBS continues to emerge. What remains unclear is how these factors converge to cause the observed abnormal GI function and functional GI symptoms reported in patients with IBS. This review focuses on the potential etiological role of these factors, suggests an updated conceptual model for the pathogenesis of the disorder, and identifies areas for future research.

THE INTESTINAL MICROBIOTA

Gastrointestinal bacteria play a critical role in the normal physiological and immunological functions of the GI tract, and alterations in GI microbiota can lead to various GI and non-GI conditions (5, 145, 164). Specifically with regard to IBS, animal studies have shown that alterations in the GI microbiota can lead to changes in GI functions, e.g., altered intestinal motility and visceral hypersensitivity that are often observed in patients with IBS and considered important factors in the pathophysiology of the disorder (12, 20, 25, 43, 80). Additional evidence supporting the role of altered intestinal microbiota in

Address for reprint requests and other correspondence: Y. Ringel, Division of Gastroenterology and Hepatology, Univ. of North Carolina at Chapel Hill School of Medicine, 4107 Bioinformatics Bldg., CB# 7080, 130 Mason Farm Rd., Chapel Hill, NC 27599-7080 (e-mail: ringel@med.unc.edu).

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the pathogenesis of IBS has come from epidemiological observations linking bacterial gastroenteritis and small intestinal bacterial overgrowth (SIBO) to IBS. Microbiology studies describing quantitative and qualitative alterations in the GI microbiota in patients with IBS compared with healthy controls, and clinical studies demonstrating the efficacy of antimicrobial and probiotic treatments in patients with IBS.

Postinfectious IBS

Postinfectious IBS (PI-IBS) refers to the development of IBS following an episode of acute gastroenteritis. PI-IBS occurs in ~10% of patients with acute gastroenteritis, and acute gastroenteritis increases the odds of developing IBS six- to sevenfold (76, 157, 172). The extended duration of IBS symptoms in these patients indicates that the condition persists long after the initial infection has cleared. The precise mechanisms by which IBS symptoms persist are not clear, although researchers have speculated that genetic polymorphisms in genes related to host immune response to microbial pathogens, ongoing altered immune function, or chronic low-grade inflammation may be possible factors (26, 145, 170).

Small Intestinal Bacterial Overgrowth

The symptoms of SIBO and IBS overlap since SIBO can lead to similar symptoms and altered intestinal motility that are often observed in patients with IBS (140). Detecting SIBO in patients with IBS remains a challenge. Historically, SIBO has been defined as ≥10^5 colony-forming units (cfu)/ml intestinal fluid (14). Using this definition, a study of bacterial cultures of aspirates from patients with IBS compared with cultures from healthy volunteers showed no statistically significant differences in the incidence of SIBO (141). However, when a lower cutoff concentration of ≥10^4 cfu/ml was used, significant differences were observed, suggesting increased numbers of bacteria in the small intestine of patients with IBS.

Because of the limitations in obtaining intestinal cultures, SIBO is often diagnosed indirectly by hydrogen breath testing (HBT). A meta-analysis of studies using HBT reported a pooled prevalence of SIBO in patients with IBS of 54–64% (61). However, the wide range of prevalence of SIBO reported in individual studies [from 10% (186) to 84% (130)] reflects the lack of standardization of HBT and the possibility that lactulose HBT results may reflect changes in small bowel transit time rather than the true presence of SIBO (194). These diagnostic limitations led to some controversies regarding the real prevalence and the importance of SIBO in the pathogenesis of IBS.

Nonetheless, the data from meta-analyses indicating increased prevalence of SIBO in IBS, the symptom overlap between the two conditions, and the improvement of symptoms with antibiotic treatment (47, 49, 59, 103, 131, 135, 136, 193) further support the hypothesis that alterations in the intestinal microbiota may have a role in the pathogenesis of IBS, at least in some patients.

Alterations in Intestinal Microbiota

Several studies have investigated and compared the composition of intestinal microbiota of patients with IBS with that of healthy individuals. Although early studies were relatively small with noticeable methodological limitations, their overall findings suggested differences in the intestinal microbiota of patients with IBS and healthy controls (145, 153). These early findings are further supported by recent studies using advanced molecular biology techniques that are on the basis of the bacterial 16S ribosomal RNA (rRNA) gene. These include genetic fingerprinting (e.g., denaturing gradient gel electrophoresis and terminal restriction fragment length polymorphism), 16S rRNA high-throughput sequencing (e.g., 454-pyrosequencing and Illumina), and phylogenetic microarray (e.g., human intestinal tract chip (HITChip) and PhylloChip). These techniques enable detailed qualitative and quantitative information regarding the most abundant bacterial communities in the intestinal microbiota (63). Recent studies using these advanced molecular biology techniques have demonstrated quantitative alterations of specific bacterial groups (33, 34, 81, 85, 142, 154) and reduced diversity in gut microbial populations in patients with IBS compared with healthy controls (33, 40). Limited data also suggest differences in mucosa-associated microbiota (32, 33, 86, 129), reduced stability over time (106, 107), rDNA, and differences between IBS subtypes (81, 104, 129, 142). Overall, it appears that the composition of the intestinal microbiota and the relative abundance of specific bacterial species are altered in patients with IBS. Emerging data demonstrate associations between altered intestinal microbiota and abnormal intestinal functions that contribute to the pathophysiology of IBS as detailed below.

Altered Fermentation Processes

Poorly absorbed short-chain carbohydrates (e.g., fructose and dietary starch) provide substrate for generation of short-chain fatty acids (SCFA) by colonic bacterial fermentation. Fecal SCFA have been shown to be increased in patients with IBS (175). In addition, a recent study reported higher counts of acetic- and propionic-acid-producing bacteria (Veillonella and Lactobacillus) in IBS patients and an association between the higher levels of these SCFA and GI symptoms and quality of life (171). From a mechanistic perspective, animal studies demonstrate that SCFA can initiate high-amplitude propagated colonic contractions (82) and increase intestinal transit and motility via intestinal release of 5-hydroxytryptamine (66). The association between certain dietary products and GI symptoms (e.g., abdominal bloating, distention, and pain) in patients with IBS (161) and the emerging data on the beneficial effects of dietary manipulation, particularly elimination of highly fermentable short-chain carbohydrates (127), further support the importance of altered fermentation processes by the intestinal microbiota in the pathogenesis of IBS.

Microbiota and the Intestinal Barrier

The gut microbiota have an important role in promoting the development, maintenance, and function of the intestinal barrier by increasing IgA production and mucin expression, preventing intestinal epithelial cell apoptosis, inhibiting colonization by enteric pathogens, and promoting physiological immune responses (113, 173, 178). Several in vivo studies in children and adults with IBS (56, 128, 162) and in vitro studies of mucosal samples from IBS patients (132) demonstrated increased intestinal permeability in IBS compared with healthy controls. The mechanisms related to the increased permeability in IBS are not clear but alterations in intestinal microbiota and...
mucosal inflammation have been suggested (28). The increased gut permeability of colonic biopsies from patients with IBS was found to be associated with decreased expression of a tight junction protein, zona occludens-1 (ZO-1). When fecal supernatants from IBS patients were applied to colonic mucosa from mice (67) or to Caco-2 cells (132), the expression of ZO-1 decreased and permeability increased. Furthermore, in patients with D-IBS, this phenomenon was found to be associated with increased bacterial-related protease activity and their epithelial receptor PAR-2 (proteinase activated receptor 2) (67). Additional support for the role of enteric bacteria in maintaining normal barrier function is provided by data that SCFA (e.g., butyrate and acetate) improve intestinal barrier function (64, 84). One small study found that administration of butyrate enemas in healthy volunteers resulted in decreased in visceral perception (180).

**Microbiota and Enteric Sensorimotor Function**

Intestinal microbiota may affect gut motility and pain perception. In mice, perturbation of the microbiota by exposure to antibiotics resulted in increased visceromotor responses to colonic distension (182). Interestingly, in this model, treatment of mice with *Lactobacillus* ameliorated inflammatory indexes in the gut wall and reduced the antibiotic-induced visceromotor response. Direct effects of bacterial products on gut sensorimotor functions have been shown in several in vitro studies. For example, stimulation of smooth muscle cells by supernatants from *Escherichia coli* Nissle 1917 enhances colonic contractility (9) and *Lactobacillus rhamnosus* and *Bifidobacterium lactis* increases intestinal myoelectrical activity in rats (97). In addition, exposure of rats to *L. reuteri* (83) or *L. acidophilus NCFM* (151) reduce their pain response to balloon distension, through targeting ion channels in the enteric nervous system (83, 91) or increasing the expression of μ-opioid and cannabinoid receptors in intestinal epithelial cells (151).

Several recent studies have demonstrated bile acid malabsorption in a subset of patients with IBS (1, 29, 93, 192). Furthermore, reduced ileal absorption and excess of bile acid in the colon can lead to acceleration of colonic transit by stimulutating motility and secretion (8, 109). Since the intestinal microbiota are directly responsible for the transformation of primary into secondary bile acid it is tempting to hypothesize that the intestinal dysbiosis observed in patients with IBS can affect intestinal motility and lead to IBS symptoms through its effect(s) on bile acid metabolism. Indeed, a recent small study in patients with IBS demonstrated increase of primary bile acid in the feces of patients with IBS-D compared with healthy subjects (54). Interestingly, these differences correlated with stool consistency and frequency and were associated with decreased levels of the *Clostridium leptum* group, which contains many of the bacteria that can transform primary into secondary bile acid.

Emerging observations of perturbed composition of the intestinal microbiota and altered microbiota-related fermentation, barrier, and sensorimotor functions in patients with IBS support the hypothesis that the intestinal microbiota contribute to the pathogenesis of the disorder. However, two key questions remained unanswered. First, are the observed alterations in the intestinal microbiota in IBS a cause or a consequence of the disorder? Relatedly, since the majority of the reported studies were done at a single time point, it is not clear whether and how the altered intestinal microbiota correlate with the clinical presentation and the IBS symptoms over time. Second, what are the relative contributions of the abnormal composition vs. function of the intestinal microbiota and mechanisms by which these changes contribute to the pathogenesis of IBS?

Current advances in the area of microbiome research including advance in nucleic acid sequencing, metagenomics, and metaproteomics allow investigation of structural and functional aspects of the intestinal microbiome. Incorporating microbial response variables (e.g., taxonomy, microbial diversity, richness, and stability) into future clinical studies in IBS is crucial to advance this field forward. However, controlling for all the variables that influence the intestinal microbiota (e.g., diet and interpatient variability) poses challenges to the design of clinical studies. Animal models may be useful by controlling for host genotype, baseline microbiota, and diet. Furthermore, since the results from animal models cannot be generalized to humans because of differences in the intestinal microbiota, it is possible to improve mouse models of IBS by colonizing germ-free animals with human-derived microbiota. The ability of these systems to mimic human clinical conditions is unclear at present, but they can be useful to test hypotheses particularly regarding causation.

In addition, the fact that genome sequence data for many of the bacterial species in the human gut microbiota are not available limits taxonomic assignment of the data with clinical, physiological, and metabolomic data. The anticipated reference genome sequences generated by the Human Microbiome Reference Genomes initiative, and the rapidly developing analytic tools and bioinformatic technologies, are required to ensure progress.

**THE INTESTINAL IMMUNE SYSTEM**

**Altered Intestinal Immune Function in IBS**

Although IBS is not generally considered an inflammatory disease, there is growing appreciation for the hypothesis that IBS may be a condition of low-grade inflammation and/or abnormal immune function, despite a lack of detectable inflammation on routine endoscopy or with conventional histology (48). Evidence supporting this hypothesis includes increased concentrations of inflammatory/immune cells in intestinal tissue from IBS patients, altered levels of pro- and anti-inflammatory cytokines in the GI tract and peripheral blood of patients with IBS (Table 1) (11, 13, 24, 36, 46, 50, 51, 62, 73, 75, 89, 90, 94, 98, 102, 118, 121–124, 168, 174, 185, 187), and IBS-associated polymorphisms in genes involved in immune and inflammatory responses (Table 2) (15, 16, 70, 179, 184). This topic has been thoroughly reviewed recently (27).

**Immune cells. MAST CELLS.** Several studies have demonstrated increased concentrations of activated mast cells in patients with IBS compared with controls (11, 13, 24, 46, 89, 94, 121, 187). Interestingly, the activated mast cells were found to be in close proximity to enteric nerves and correlated with abdominal pain/discomfort (11). Other studies have reported elevated levels of histamine and tryptase release in patients with IBS compared with controls (11, 13, 35, 73). Notably, some studies demonstrated an increase in activated mast cells in biopsies from specific regions of the GI tract (i.e., cecum and terminal ileum) but not from other regions (e.g., ascending...
Table 1. Evidence for immune activation in patients with IBS or PI-IBS

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<th>Evidence Change in IBS or PI-IBS vs. Healthy Controls</th>
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<td>Altered concentrations or activation of immune cells</td>
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<td>GI tract</td>
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GI, gastrointestinal; IBS, irritable bowel syndrome; IELs, intraepithelial lymphocytes; IL, interleukin; PI-IBS, postinfectious irritable bowel syndrome; TNF-α, tumor necrosis factor α. *Message RNA levels quantified by reverse-transcriptase polymerase chain reaction.

Table 2. Gene polymorphisms in patients with IBS

<table>
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<th>Gene</th>
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<td>IL-2</td>
<td>15, 16, 70, 179, 184</td>
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<td>IL-4</td>
<td>TLR9</td>
</tr>
<tr>
<td>IL-6</td>
<td>TNF-α</td>
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<tr>
<td>IL-10</td>
<td>Based on Refs. 15, 16, 70, 179, 184. TLR9, Toll-like receptor 9.</td>
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colon, descending colon, rectum) (89, 121, 187), whereas other studies have found activated mast cells in the descending colon (11, 13). The abundance of mast cells in patients with IBS may correlate with some symptoms of IBS, including bloating and abdominal pain (2, 46). However, some studies failed to demonstrate increased activated mast cells in IBS patients (35, 36, 57, 58, 168) and there are no consistent differences in the tissue concentrations of activated mast cells in patients with different IBS subtypes (36, 189).

LYMPHOCYTES. The concentrations of intestinal epithelial lymphocytes (IELs) may be increased in the small intestine (73, 174, 185), colon, and rectum (36, 89) of patients with IBS or PI-IBS. Additionally, increased concentrations of CD3+ T cells in the colon and rectum and in the myenteric plexus of the small intestine were increased in these patients compared with normal controls (36, 46, 57, 58, 89, 94, 133, 168, 174). However, as with mast cells, IELs are not consistently elevated in IBS patients (57, 58, 133). Moreover, CD3+ T cells in the rectum of patients with PI-IBS were not elevated compared with patients who had gastroenteritis but did not develop IBS (57). Similarly, increased levels of CD4+ T helper cells were observed in the colon of patients with IBS in one study (46) but not in another (123), leaving the role for this T cell subtype in IBS unclear. Concentrations of cytotoxic CD8+ T cells, which may also be elevated in the lamina propria of the colon of patients with IBS (46, 123) or PI-IBS (89) although this finding was disputed by others (36). The reasons for the differences in some of the results are unknown, but, overall, it appears that T cell concentrations are increased in patients with IBS or PI-IBS relative to normal controls.

In addition to elevated concentrations of T cells, patients with IBS may also have an increased numbers of activated T cells in the colon (36) and peripheral blood (122) compared with controls. Peripheral blood T cells from patients with IBS express elevated levels of integrin β7 (122, 123), which is important for homing of cells to the intestines. Levels of MadCAM-1, the ligand of integrin β7, are also elevated on colonic endothelial cells (123), creating an environment for enhanced recruitment of CD4+ and CD8+ T cells to the intestines of patients with IBS.

Although no differences in total B cell numbers were observed in the colons of IBS patients (46), a single study reported reduced levels of IgA+ B cells in the ascending but not sigmoid colon compared with controls, whereas levels of IgG+, IgM+, and IgE+ B cells in both the ascending and sigmoid colon were normal (62). This altered pattern of B cells suggests that there may be a modified defense mechanism against pathogens in the gut of patients with IBS. In addition, patients with IBS may have increased expression of IgG and the costimulatory molecules CD80 and CD86 on B cells and an increased frequency of peripheral blood IgG+CD80+ and IgG+CD86+ B cells (124). Furthermore, B cells from patients with IBS had an impaired ability to upregulate CD80 in response to in vitro lipopolysaccharide (LPS) stimulation, suggesting that B cells from patients with IBS may have an altered ability to costimulate and regulate T cells.

Inflammatory cytokines. GI TRACT. In parallel with altered immune cell concentrations and activation, the levels and relative proportions of certain pro- and anti-inflammatory cytokines have been reported to be altered in the small intestine, colon, and rectum of patients with IBS and PI-IBS compared with controls. Levels of interleukin (IL)-1B mRNA may be increased in the small intestine, colon, and rectum of patients with PI-IBS compared with healthy controls (75, 187), supporting the hypothesis that low-grade inflammation is present in patients with PI-IBS. In contrast, a study by Macsharry and colleagues (102) found reduced levels of IL-1β mRNA in colonic biopsies from patients with IBS. However, in this study a decreased level of mRNA encoding the anti-inflammatory IL-10 gene was observed, as well as an altered IL-10:IL-12 ratio in colonic biopsies from patients with IBS compared with controls, supporting the hypothesis that patients with IBS have a dysfunctional immunological response in the mucosa. Results from examination of cytokine protein levels have been
conflicting. In one study, levels of TNF-α were elevated, but levels of IL-6 and interferon-γ were decreased in mucosa of patients with IBS compared with controls (41). Ex vivo examination of cytokine secretion by the colon failed to show elevation of proinflammatory cytokines or chemokines in patients with IBS compared with controls (102).

PERIPHERAL BLOOD. A recent study demonstrated significantly higher levels of high-sensitivity C-reactive protein in patients with IBS-D but not in patients with IBS-C or IBS-M compared with healthy controls (77). IL-6, which is a major regulator of serum C-reactive protein levels, and IL-8 were also found to be increased in the serum of patients with IBS compared with controls, supporting a proinflammatory profile in these patients (50, 51).

Additionally, IL-1β, IL-6, and TNF-α released from peripheral blood mononuclear cells (PBMCs) were elevated in patients with IBS-D but not in patients with IBS-C or IBS-M (98). Additionally, a decreased ratio of IL-10 to IL-12, indicative of a proinflammatory response, was demonstrated in supernatants of cultured PBMCs obtained from patients with IBS compared with controls (118). In this same study, normalization of the IL-10:IL-12 ratio in patients with IBS following probiotic treatment was paralleled by improvement in the symptoms of IBS, thus supporting an association between changes in the GI microbiota, the IL-10:IL-12 ratio, and symptoms in patients with IBS. However, other studies did not observe differences in IL-10 levels between IBS patients and controls (50, 51, 90), leaving the relevance of IL-10 levels in patients with IBS unclear.

Cytokine secretion following in vitro stimulation of peripheral blood lymphocytes/monocytes may also be altered in patients with IBS (90, 98, 122). Increased secretion of TNF-α, IL-1β, and IL-6 by LPS-stimulated PBMCs was observed only in cells from patients with IBS-D but not in patients with IBS-C or IBS-M (98). In contrast, only IL-1β was secreted following LPS stimulation of PBMCs obtained from IBS-C patients (98). Furthermore, increases in the secretion of TNF-α following LPS stimulation of PBMCs and of IL-1β following T-cell anti-CD3/CD28 antibodies stimulation were significantly associated with anxiety (98) and bowel habit dissatisfaction and overall IBS symptoms (122), respectively. Overall, it seems that alterations in cytokine secretion by stimulated peripheral blood cells appear to favor an activated immune state (98, 122), and an altered immune response shifted toward a Th-helper 2 phenotype (90) in IBS patients.

Immune-Related Genetic Alterations in IBS

Gene polymorphisms (188) in TNF-α (15, 179), IL-2 (16), IL-4 (16), IL-6 (15), and IL-10 (16, 70) have been associated with IBS or PI-IBS (Table 2). For example, IBS patients are more likely to carry a combination of polymorphisms that lead to increased expression of TNF-α and decreased expression of IL-10 compared with healthy controls (179). However, the prevalence of specific polymorphisms in the IL-10 gene in patients with IBS are not consistent across studies and may reflect the variety of ethnic groups in which these studies were conducted (16). Genetic variation in promoter regions of the IL-6 and Toll-like receptor 9 (TLR9) genes were also identified as independent risk factors for the development of PI-IBS (26, 184). Altered levels of IL-6, IL-10, and TNF-α in colon tissue from IBS and PI-IBS patients suggest that the genetic polymorphisms in immune genes may have functional consequences in patients with IBS. The presence of polymorphisms in the promoter of TLR9 suggests that patients with PI-IBS may have an inappropriate regulation of the immune response to microbial products.

Immune-Mediated Food Hypersensitivity in IBS

The majority of IBS patients believe that their IBS symptoms are initiated or worsened by certain foods and many of them report intolerance (i.e., hypersensitivity) to a variety of foods (68). The mechanisms by which dietary factors lead to IBS symptoms are currently unknown. However, several studies have documented IgG-mediated food hypersensitivity and demonstrated that food-elimination diets based on IgG antibodies to specific food items improved IBS symptoms (4, 195, 196), perhaps through amelioration of the immune response. In addition, few studies demonstrated increased peripheral blood basophil activation following stimulation with food antigens (30) and elevated levels of fecal tryptase and eosinophil cationic protein (31) in patients with IBS and food hypersensitivity, thus further suggesting possible inflammatory and altered immune etiology.

Although the mechanisms for food hypersensitivity in patients with IBS are not clear, it can be postulated that dysregulated immune responses and possibly altered intestinal mucosal barrier function may be involved.

Inflammation and Sensorimotor Function

The effects of inflammation on sensorimotor enteric function have been demonstrated in IBDs, where patients suffer from abnormal intestinal motor function and enhanced sensory perception, even when inflammation is restricted to the mucosa (44, 143). In addition, human and animal studies have shown anatomical changes in the enteric nervous system (ENS) around inflammatory infiltrates in the gut wall (2, 11, 13). Functionally, intestinal inflammation is associated with increased expression of neuropeptides and mediators that are also involved with gut motility and sensation (e.g., VIP and substance P) [for review, see Vasina et al. (181)]. Similar observations have been made in IBS patients. One study that supports this association demonstrated a strong correlation between number of infiltrated colonic mast cells around nerves and the severity and frequency of abdominal pain in patients with IBS (11).

MICROBIOTA, IMMUNE SYSTEM, AND THE BRAIN-GUT AXIS

The current understanding of IBS as a disorder of malfunction in the bidirectional brain-gut communication suggests that both peripheral (GI) and central nervous system (CNS) factors are involved in the pathophysiology of the disorder. Indeed, at the gut level, the intestinal mucosal neuroimmune system identifies and responds to intestinal luminal food products, nutrients, bacteria, metabolites, and toxins. At the CNS level, the brain-gut axis connects environmental, cognitive, and emotional states with GI functions (191). For example, it has been shown that physical and psychological stress (112), cognition (e.g., attention vs. distraction to aversive stimulus) (55), and early life experience (e.g., sexual and physical abuse) (39, 149) can affect intestinal sensory perception. Furthermore, treat-
ments targeting the CNS, such as antidepressants and psychological interventions, can reduce intestinal sensation/sensitivity and improve GI symptoms in patients with IBS (21, 111, 150, 165).

The Intestinal Microbiota and Brain-Gut Axis

Recent data from animal and human studies have demonstrated that alterations in the intestinal microbiota have substantial effects on the brain-gut axis at both the peripheral and central levels (Fig. 1). In the periphery, intestinal microbiota can affect the ENS directly via release of bacterial substances or metabolites, or indirectly by inducing the release of host-derived immune mediators that in turn impact the ENS (10, 155). For example, production of the SCFA butyrate by bacteria affects gene expression and the phenotype of enteric neurons in a similar manner to that observed in the colons of patients treated with prokinetic agents (166, 169). Gastrointestinal microbiota may also contribute to the affective symptoms associated with GI disorders by impacting the CNS via immune, hormonal, and neural mechanisms (17, 69, 155). Preliminary studies have demonstrated that alterations in the GI microbiota can lead to long-term increased anxiety (69, 115, 116) and altered exploratory behavior in mice (18, 19). Similarly, psychosocial stressors can change the composition of the intestinal microbiota and affect the brain-gut axis function (6, 7, 119). For example, it has been shown that maternal separation, a well-established rodent model of early life stress, can lead to significant alterations in the intestinal microbiota as well as in brain-gut axis function, including increased plasma corticosterone levels, systemic immune responses, and visceral sensation compared with a control group (119).

The Enteric Immune System and Brain-Gut Axis

The connection between altered intestinal immune function and abnormal brain-gut axis in patients with IBS remains unclear. However, some of the suggested mechanisms involve

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**Fig. 1.** Interactions between the intestinal microbiota, immune system and brain-gut axis, and their effects on intestinal function and functional gastrointestinal (GI) symptoms. A number of factors and triggers, from both the environment and the host, combine to drive the complex interactions between the brain-gut axis, intestinal microbiota, and immune system toward altered intestinal functions and functional GI symptoms. Altered intestinal microbiota may affect the brain-gut axis directly via effect on the mucosal barrier and intestinal neuroimmune system or indirectly via generation of bacterial-related metabolites. Immune activation (e.g., increased levels of proinflammatory cytokines) may affect the mucosal barrier, enteric nervous system (ENS) and peripheral nervous system [i.e., autonomic nervous system (ANS), hypothalamic-pituitary-adrenal (HPA) axis, central nervous system (CNS)]. The outcome of these complex interactions may alter the intestinal sensorimotor function and lead to functional GI symptoms.
stress and psychological disturbances and disruption of mucosal barrier function (120). For example, rats exposed to chronic stress exhibited activation of the hypothalamic-pituitary-adrenal (HPA) axis, intestinal mucosal inflammation, increased epithelial permeability, and colonic hyperalgesia (183). At the peripheral level, the close interaction between the immune system and the ENS in IBS is demonstrated by the increased infiltration of mast cells in the colonic mucosa in close proximity to enteric nerve fibers and by the increased number of sensory nerve fibers surrounding the mast cells (2, 11, 13). In addition, several studies support the hypothesis that elevated levels of proinflammatory cytokines may affect the brain-gut axis. For example, in rats elevated IL-1β and TNF-α secretion in response to the bacterial endotoxin LPS leads to visceral hyperalgesia via activation of sensory nerve pathways (42). Others showed that the proinflammatory cytokines IL-6, IL-1, and TNF-α affect the HPA axis through elevated release of arginine vasopressin (AVP), corticotropin-releasing hormone (CRH), and adrenocorticotropic hormone (ACTH) (38, 176). AVP and CRH, by stimulating ACTH secretion, may affect the autonomic nervous system (38) and colonic sensorimotor functions (125, 152). Additionally, CRH may act directly on peripheral receptors of the ENS, independent of alterations in the HPA axis, to impact sensorimotor functions in patients with IBS (152). Thus proinflammatory cytokines (e.g., IL-1β, IL-6, and TNF-α) may interact with the brain-gut axis at different levels, modulate its function, and lead to altered visceral and motility function in patients with IBS (Fig. 1).

Interactions of the Intestinal Microbiota and Immune Function in IBS

The effects of the intestinal microbiota on the development, function, and homeostasis of the enteric immune system are well documented (5, 158). Enteric microbiota are crucial to the host in numerous aspects including maturation of the systemic and enteric immune response, maintenance of the mucosal barrier function, digestion of food, metabolism, and more (for review, see Ref. 78). Although evidence is rapidly accumulating that dysregulated immune responses to normal and “dysbiotic” enteric microbiota contribute to the pathogenesis of IBD (105), there are limited data on the interactions between the intestinal microbiota and the enteric immune system in IBS. However, some recent data suggest that IBS patients also exhibit dysregulated immune responses to commensal and/or pathogenic intestinal bacteria. For example, recognition of bacterial components through Toll-like receptors (TLRs) is increased in IBS patients. Expression of TLR4 (recognizes bacterial LPS) and TLR5 (recognizes flagellin, a common bacterial antigen present in most motile bacteria in the gut) are increased in colons of IBS patients compared with controls. TLRs have a central role in the mucosal innate immune response and their increased expression is also associated with IBDs (23). In this regard, anti-flagellin antibodies were found in almost 30% of IBS patients (mostly in PI-IBS) as opposed to only 7% of healthy controls (156). The importance of TLRs in IBS is further supported by a recent study in a mouse model, indicating that TLR4 stimulation by enteric microbiota can also affect the enteric nervous system and intestinal motility (3). Moreover, the antimicrobial peptide β-defensin-2, which is an innate immune molecule expressed by certain enteric epithelial cells, is elevated in feces collected from IBS patients (similarly to ulcerative colitis) compared with healthy controls (92).

The importance of the interaction between the intestinal microbiota and immune system in IBS patients is demonstrated by a few studies in patients with PI-IBS, indicating activation of the GI immune system following the acute GI infection (57, 58, 75, 89, 94, 124, 168, 170, 187), and the recent animal studies showing that stress-induced intestinal microbial changes are associated with altered immune response (6) and increased susceptibility to an enteric pathogen (183).

Clinical Implications

Manipulation of intestinal microbiota. The possible role of the altered intestinal microbiota, immune function, and the abnormal interaction between the two systems in the pathogenesis of IBS has led to the increased interest in targeting the intestinal microbiota and immune system in the treatment of this disorder. Early studies demonstrated symptom improvement in IBS patients whose SIBO was successfully treated with antibiotics, including neomycin, metronidazole, and the non-absorbable antibiotic rifaximin (47, 49, 59, 103, 131, 135, 136, 193). Other studies have demonstrated beneficial effect of antibiotics in patients with IBS regardless of either the diagnosis of SIBO or the effect of antibiotics on HBT (95, 100, 114, 134, 137–139, 160).

Several systematic reviews and meta-analyses suggest that modulation of intestinal microbiota with probiotics may improve some of the symptoms of IBS (22, 79, 110, 147, 148). The largest reported study was a randomized, double-blinded, placebo-controlled, multicenter study of 362 patients that demonstrated beneficial effects of *Bifidobacterium infantis* 35624 over placebo on IBS composite score and individual symptoms of IBS (e.g., pain and bloating) (190). Although the effects were modest, they were significant. Other smaller studies reported some beneficial effects with other probiotic strains including *Bifidobacterium animalis* DN-173 010 (74), *Bifidobacterium bifidum* MIMBb75 (72), *Lactobacillus plantarum* 299V (117), *Saccharomyces boulardii* (37), and combination of *Lactobacillus acidophilus* NCFM and *Bifidobacterium lactis* Bi-07 (144) and multispecies probiotic mixtures (71, 87, 88). Other approaches targeting the intestinal microbiota that have shown benefit in the treatment of IBS include prebiotics (163) and dietary manipulation (127).

Currently, despite the demonstrated efficacy in subgroups of patients with IBS, antibiotics are not FDA-approved medications for treatment of IBS, and there are no clear guidelines for the use of probiotics, prebiotics, and diets in this disorder. Nevertheless, although the mechanisms of action are unclear, the available data suggest potential therapeutic benefit in targeting the intestinal microbiota in the treatment of IBS. The data underscore the need for further preclinical studies, including the use of various animal model systems and well-designed human trials in this area. Future research should help direct patients and healthcare providers in making evidence-based decisions regarding the selection of patients, preferred products and treatment regimens.

Anti-inflammatory treatment in IBS. An alternative, and perhaps complementary, therapeutic approach is to target the dysregulated immune response in IBS patients. Few studies have investigated the use of anti-inflammatory medications in...
IBS. A small, double-blind, randomized study of 20 IBS patients showed that mesalamine 2.4 g/day for 8 wk failed to show improvement in IBS symptoms despite a reduction in the number of total immune cells in colonic biopsies and improvement in general well-being (45, 177). Similarly, mesalamine 3.2 g/day for 12 wk (168) and prednisolone 30 mg/day for 3 wk (52) were not effective in improving the GI symptoms in patients with PI-IBS. A single small study suggests a possible benefit to targeting mast cells with sodium cromoglicate, a mast cell stabilizer, in patients with IBS and food intolerance (101).

Taken together, the current available data do not support the clinical use of anti-inflammatory agents in the treatment of IBS. However, the documented low-grade inflammation in IBS, the suggestive preliminary data from animal studies (52), and the noticeable paucity of clinical data in this area emphasize the need for further investigation to enable more solid conclusions regarding this treatment option in IBS. Understanding the role of the immune system in IBS pathophysiology may further clarify the potential therapeutic role of targeting the immune system in the treatment of IBS.

CONCLUSIONS

IBS has historically been considered a condition without an identifiable organic etiology. However, accumulating observations in animal models and in patients with IBS suggest a conceptual model in which environmental factors at both the peripheral (e.g., diet, GI infections, antibiotics) and central (e.g., psychological stress, anxiety, depression) levels can affect the intestinal microbiota and enteric immune system, which interact with each other and the brain-gut axis (Fig. 1). Alterations in these systems, or in their interactions, can affect GI function and lead to functional GI symptoms. Future research in this area may provide further insight into the relevance and relative importance of these interactions in the pathogenesis of IBS and improve our understanding of how manipulating relevant environmental factors (e.g., diet and intestinal microbiota), and the immune and inflammatory processes may affect the brain-gut axis and improve patient outcomes.

GRANTS


AUTHOR CONTRIBUTIONS

Y.R. conception and design of research; Y.R. and N.M. prepared figures; Y.R. and N.M. drafted manuscript; Y.R. and N.M. edited and revised manuscript; Y.R. and N.M. approved final version of manuscript.

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