Inflammation and Microflora

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KEYWORDS

• Irritable bowel syndrome • Inflammation • Microflora
• Methanogenic flora

Irritable bowel syndrome (IBS) is a chronic bowel condition characterized by abdominal pain, altered bowel function, and bloating. It is in fact the most common gastrointestinal condition, affecting 10% to 20% of adults in developed countries[1,2] and accounting for 50% of all gastrointestinal office visits.[2] Due to the high prevalence, the health care costs related to IBS are estimated to exceed $30 billion per year.[3] Moreover, this condition has serious implications for quality of life, which have been likened to diabetes or heart disease, in young adults who should otherwise be productive and healthy.[4] However, despite the seriousness of IBS as a health care issue, the underlying causes remain largely unknown.

Although the etiology of IBS has remained unclear, many hypotheses have emerged, based on associations between IBS and stressful life events in the past[5] as well as altered gut sensations.[6] The association between stress, psychological trauma, and findings of lower thresholds for rectal balloon sensation in IBS[7] led to the concept of the brain-gut dysregulation as a hypothesis in IBS.[8] The brain-gut concept has continued to be a fertile area of work in IBS but unfortunately, it is difficult to prove a cause-and-effect relationship between life events and IBS. In fact, the United States householder study suggested that in the community, psychological problems are not more common in subjects with IBS.[9]

The human intestinal tract is composed of more than 500 different species of bacteria that usually function in symbiosis with the host. Although the composition and number of bacteria in the gut depends on many factors,[10–12] by adulthood, if not earlier, most humans reach an established balance of type and numbers of bacteria that is unique to a given individual, much like a fingerprint. Over the last few years, growing evidence has supported a new hypothesis for IBS based on alterations in intestinal bacterial composition. Several nonmutually exclusive mechanisms may explain how altered gut flora can lead to IBS. First, gut microbes interact with the gut mucosal immune system through innate and adaptive mechanisms. Second,

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Gastroenterol Clin N Am 40 (2011) 69–85
doi:10.1016/j.gtc.2010.12.010 **gastro.theclinics.com**
0889-8553/11/$ – see front matter

altered flora can lead to changes in the intestinal epithelial barrier. Third, neuroimmune and pain modulation pathways may be influenced by the flora.[13,14] Fourth, changing flora can increase food fermentation and subsequent intestinal gas production. Finally, bile acid malabsorption can result from expansion of gut flora into the small bowel.[15] For any or all of these reasons, gut flora can produce IBS-like symptoms.

Further epidemiologic and clinical data support this new bacterial concept of IBS. First, there has been growing data linking the development of IBS to an initial episode of acute gastroenteritis[16,17]; this is now termed postinfectious IBS (PI-IBS). The second area of interest in IBS related to gut microbes is the concept that IBS patients have alterations in the balance of fecal flora. It is on this basis that probiotic studies in IBS began to be conducted. The final and most promising area is that of alterations in small intestinal flora. Relevant studies suggest that IBS subjects have excessive coliform bacteria in their small intestine (otherwise known as SIBO). The link to SIBO has led to clinical trials of antibiotics in IBS. In this article the authors review the evidence for a bacterial concept in IBS, and by the end begin to formulate a hypothesis of how these bacterial systems could integrate in a new pathophysiologic mechanism in the development of IBS. In addition, there have been data to suggest an interaction between this gut flora and inflammation in the context of IBS, and this is also be presented.

GUT MICROBES AND IBS

Altered Intestinal Flora Composition and IBS

The effect of gut microflora on gastrointestinal physiology has been most clearly demonstrated in animal experiments under controlled conditions not feasible in human studies. For example, germ-free rats had delayed gastric emptying and intestinal transit, and a prolonged interdigestive migrating motor complex (MMC) as compared with rats with conventional flora.[18–21] Moreover, introduction of normal gut flora to these germ-free rats normalized their motility.[21,22] Of interest, when germ-free rats were mono-associated with either *Lactobacillus acidophilus* or *Bifidobacterium bifidum*, their small intestine transit accelerated and their MMC frequency increased.[22] Hooper and Gordon[23] profiled gene expression patterns in germ-free mice, and showed reduction of several enteric neuron and intestinal smooth muscle genes. Subsequent mono-association with *Bacteroides thetaiotaomicron*, a highly adapted and abundant commensal of the human and murine colon, restored the normal expression pattern. These experiments, which involve profound changes in the gut flora of rodents, imply a critical role of the resident flora in establishing and maintaining normal intestinal function, and suggest that changes in the gut microflora can lead to significant alterations in gastrointestinal function.

Changes in gut flora of patients with gastrointestinal disorders, including IBS, have been sought for decades. Efforts have been hampered by (1) disease heterogeneity and multifactorial pathophysiology; (2) studies not controlling for diet and medication use that can influence flora composition; (3) potential fluctuations in stability of gut flora and topographic/geographic variability, both in “normal” and affected subjects; and (4) inherent limitations in methodologies to assess gut flora composition. The last challenge in this area will be overcome by evolving technology.

Although culture of the bowel flora has been the mainstay of evaluating intestinal bacterial composition, the majority of intestinal flora are nonculturable, based on fastidious requirements and limited understanding of the vast expanse of human colonizers. DNA-based strategies such as high-throughput pyro-sequencing are considered more sensitive and accurate, but are still costly and technology intensive.

Despite these limitations, culture studies have consistently demonstrated a paucity of *Lactobacillus*[24] and *Bifidobacterium*[25,26] species in the feces of IBS patients compared with controls (**Table 1**), with the exception of Tana and colleagues[27] who noted increased *Lactobacillus*. Although the influence of *Lactobacillus*, *Bifidobacterium*, and other so-called beneficial bacteria have been studied extensively based on their effects on the epithelium, host immune response, and other factors, this is beyond the scope of this article. However, one finding is notable. Balb/c mice infected with a probiotic *L acidophilus* strain had elevated expression of several intestinal pain receptors that led to decreased visceral sensitivity.[28]

While these results sparked the use of these specific probiotics in IBS, there were inherent problems with this initial research. The results are difficult to interpret because of the failure of these studies to control for diet. A common finding in the literature related to IBS is the association between IBS and lactose intolerance.[29] The reason for this remains unclear, yet it is recognized that more than 60% of IBS sufferers have dairy intolerance on this basis.[30] Because dairy products are the prebiotic for *Lactobacillus* and *Bifidobacterium* species, not accounting for diet, leaves the finding of reduced counts of these organisms possibly secondary to intrinsic diet issues in IBS subjects. The ideal study of this topic would be to put IBS and controls on an identical diet for 2 weeks followed by stool evaluation. This lack of control may explain the overall failure of *Lactobacillus*-based treatment in IBS, as discussed later.

Table 1
Studies demonstrating altered intestinal flora in IBS subjects

IBS Subjects, #	Methodology	Findings in IBS Subjects	Citation
Unsubtyped, n = 25	Culture	Decreased Bifidobacteria and increased Enterobacteriaceae	Si et al,[25] 2004
IBS-D, n = 12 IBS-C, n = 9 IBS-M, n = 5	Culture PCR-DDGE	Increased coliforms and aerobic bacteria/total bacteria Increased *Clostridium* and decreased *Eubacterium*	Matto et al,[98] 2005
IBS-D, n = 12 IBS-C, n = 9 IBS-M, n = 6	Q-RTPCR	Decreased *Lactobacillus* in IBS-D, increased *Veillonella* in IBS-C	Malinen et al,[24] 2005
IBS-D, n = 7 IBS-C, n = 6 IBS-M, n = 3	PCR-DDGE RTPCR-DDGE	Decreased *Clostridium coccoides-Eubacterium rectale* in IBS-C	Maukonen et al,[99] 2006
IBS-D, n = 10 IBS-C, n = 8 IBS-M, n = 6	Q-RTPCR (nucleic acid fractionation)	Decreased *Collinsella, Clostridium,* and *Coprococcus*	Kassinen et al,[31] 2007
IBS-D, n = 14 IBS-C, n = 11 IBS-M, n = 16	FISH	Decreased *Bifidobacterium*	Kerckhoffs et al,[26] 2009
IBS-D, n = 8 IBS-C, n = 11 IBS-M, n = 7	Culture Q-RTPCR	Increased *Lactobacillus* Increased *Veillonella*	Tana et al,[27] 2010

Abbreviations: DDGE, denaturing gradient gel electrophoresis; FISH, fluorescent in situ hybridization; IBS-C, constipation predominant IBS; IBS-D, diarrhea predominant IBS; IBS-M, mixed IBS; PCR, polymerase chain reaction; Q-RTPCR, quantitative real-time PCR.

Recently, more sophisticated techniques have been used to examine subjects with IBS and their fecal content. In a recent study, molecular techniques were used to determine shifts in flora between IBS and controls.[31] In addition to finding differences categorically, subjects with constipation predominant IBS (C-IBS) also appeared to have unique differences in contrast to diarrhea predominant IBS (D-IBS). Specifically, a lack of *Lactobacillus* and *Collinsella* species were seen in IBS. Of note, C-IBS subjects had an abundance of *Ruminococcus*. In D-IBS, a decrease in *Bifidobacterium* was seen. Even in this sophisticated study, however, diet was not controlled, making interpretation an ongoing issue.

Though not specifically a chronic change in intestinal microflora, acute changes may have an impact on IBS and its development. This process involves the association between IBS and acute gastroenteritis. While this is discussed in detail in this article, animal models used to study PI-IBS further suggest a link between altered gut microflora and IBS. The most characterized postinfectious model of IBS used the organism *Trichinella spiralis*. This parasitic mouse infection model was found to produce reduced gut motility and increased visceral sensitivity to colorectal distention,[32] and has been likened to IBS. However, the stool flora have not been characterized in this model.

Small Intestinal Bacterial Overgrowth and IBS

SIBO is a situation whereby coliform bacterial counts in the small bowel become excessive. Symptoms of SIBO are similar to IBS. In the last decade, growing data have linked SIBO and IBS. Whereas the initial criticism of the work was a consequence of inaccuracies of breath testing as a means of diagnosing SIBO, recent work has begun to confirm the results of breath testing in IBS, supported by small bowel culture.

As early as 2000, work began to emerge suggesting that subjects with IBS have bacterial overgrowth, based on the lactulose breath test.[33] In this initial study, SIBO was suspect in 76% of IBS subjects and although based on a prospective database, appeared to improve after antibiotic therapy using an open-label approach. In the first follow-up study to this work, a higher rate of positive lactulose breath test results (up to 84%) were identifed.[33,34] This rate was noted to be far greater than in healthy control subjects. After this work was published there was a high degree of skepticism, due to the complexities of the breath-testing techniques. Now 10 years later, meta-analyses have been conducted that support the breath test findings in IBS compared with controls. In the first of 2 meta-analyses, Ford and colleagues[35] demonstrated that IBS subjects appear to have a higher prevalence of abnormal breath test results in IBS, but only using the most conservative interpretation of the test compared with controls. The second meta-analysis used a different approach based on simply combining the results of studies using breath testing in IBS versus controls in general.[36] This study demonstrated that IBS patients have a greater likelihood of a positive test compared with controls. When only the best studies were used (age- and sex-matched studies), the odds ratio of a positive test in IBS was 9.64 (confidence interval = 4.26–21.82) compared with controls.[36]

Further validation of the SIBO concept in IBS is based on culture and antibiotic trials. In the largest published study of small bowel culture in IBS, aspirates of jejunal fluid in IBS were found to harbor a greater number of coliform bacteria compared with healthy controls (using >5000 coliforms/mL) ($P<.001$).[37] Studies of antibiotic response also support SIBO in IBS (**Table 2**). Controlled trials in IBS[34,38,39] Pimentel and colleagues,[40] and functional bloating[41] demonstrate successful treatment of IBS with antibiotics based on this excessive flora. Using breath testing as an outcome measure, antibiotic therapy led to improvement of SIBO,[34,41] with a 75% improvement in IBS symptoms observed if normalization of the breath test is seen with antibiotics.[34]

Table 2
Controlled studies demonstrating benefit of antibiotics in IBS

Citation	# of Subjects	Diagnostic Criteria	Antibiotic Used	Length (days)	Primary Outcome Measure	Placebo	Antibiotic
Pimentel et al[34]	111	Rome I	Neomycin 500 mg twice daily	10	Symptom composite	11	35%
Sharara et al[41]	124 (70 IBS)	Rome II	Rifaximin 400 mg twice daily	10	Global symptoms	23	41
Pimentel et al[77]	87	Rome I	Rifaximin 400 mg 3 times a day	10	Global symptoms	21	36
Lembo et al[78]	388	Rome II	Rifaximin 550 mg twice daily	14	Adequate relief of IBS	44	52
Pimentel et al[40]	1260	Rome II[a]	Rifaximin 550 mg 3 times a day	14	Adequate relief of IBS	31.7	40.7

[a] Nonconstipated IBS.

Another controlled trial demonstrated improvement in IBS symptoms that were sustained for a full 10 weeks of follow-up after cessation of antibiotics.[38] Taken together, these findings strongly support a role for the gut microbiome and perhaps SIBO in the pathophysiology of symptoms in a subset of IBS sufferers.

Evidence suggests that SIBO in IBS may be caused by a deficiency in phase III of interdigestive motor activity. During the fasting state, the small bowel cycles through 3 phases of activity, phases I to III.[42] Phase III is a high-amplitude multiphasic motor event, and an absence or reduced frequency of these contractions is known to induce SIBO.[43,44] The authors recently demonstrated that IBS patients with SIBO have significantly reduced phase III frequency, suggesting that attenuated gut motility may underlie the development of SIBO in IBS.[44] Although the physiologic basis for this reduced phase III frequency remains unknown, the interstitial cells of Cajal (ICC) are known to be required for normal intestinal motility (including phase III), and their loss interferes with electrical pacemaker activity, slow-wave propagation, and motor neurotransmission in the gut,[45–54] suggesting that altered ICC function may contribute to altered gut motility in IBS. It has yet to be conclusively demonstrated that changes in ICC are involved in IBS.

Postinfectious IBS

Over the last decade, it has been established that intestinal pathogens play a significant role in the development of IBS. Numerous studies have shown that IBS can be precipitated by an episode of acute gastroenteritis, and that up to 57% of subjects who otherwise had normal bowel function may continue to have altered bowel function for at least 6 years after recovering from the initial acute illness.[55] Based on 2 recent meta-analyses of this research, approximately 10% of subjects who have documented acute gastroenteritis develop IBS, with a summary odds ratio of 6 to 7 for PI-IBS.[16,17] As gastroenteritis is extremely common, so-called PI-IBS may in fact constitute a large proportion of IBS cases. Thus, reducing risk factors for IBS development after acute gastroenteritis may have an impact on the incidence of IBS.

Although the mechanisms of PI-IBS remain unclear, investigators have identified certain risk factors for the development of IBS after gastroenteritis. The 2 most significant of these are duration/severity of gastroenteritis and female sex.[56,57] Stress, manifest as recent traumatic life events, and a neurotic personality trait were also predictors of PI-IBS.[57] Evidence of low-grade inflammation is evident in PI-IBS patients. Rectal biopsies demonstrate mildly elevated intraepithelial lymphocytes and enteroendocrine cells that persisted 12 months after *Campylobacter jejuni* infection.[58] Increased rectal lymphocytes also occur in general IBS patients, but to a lesser degree.[59] Elevated expression of proinflammatory cytokine interleukin (IL)-1β was detected in *C jejuni* PI-IBS rectal biopsies[60] and in *Shigella* PI-IBS rectosigmoid and terminal ileum biopsies.[61] Thus, acute gastroenteritis may increase the risk of developing IBS in a susceptible individual through persistent low-grade activation of the gut immune system, or possibly through establishment of an intestinal dysbiosis, defined as an alteration of the composition of the gut flora. Animal infection models of PI-IBS will play a key role in characterizing the mechanistic pathways and underlying alterations in this process.

The discovery of PI-IBS has led to the development of several animal models. The most comprehensively studied model of PI-IBS developed by Canadian researchers uses *Trichinella spiralis*.[62] This model has now been well characterized.[32,63,64] As *T spiralis* is not a common pathogen in humans and is thus a rare cause of human IBS in the Western hemisphere, other models such as post–*C jejuni* are being developed. One rat model of *C jejuni* infection recapitulates many features of human

PI-IBS including altered stool form, bacterial overgrowth, and increased rectal lymphocytes, observed 3 months after clearance of the initial acute infection.[65] In fact, some of the first descriptions of PI-IBS in humans stem from *C jejuni* gastroenteritis.

ROLE OF METHANOGENIC FLORA IN IBS

An important group of bacteria that colonize the gut are the methanogenic flora. This distinct group grows primarily under anaerobic conditions, and produces methane (CH_4) as a by-product of fermentation. Intestinal methane production has been linked to diseases such as C-IBS and diverticulosis.[66–69] The presence of significant methane production (seen even with fasting) is observed in 15% of normal controls, and is higher in subjects with conditions such as IBS.[34] Methanogenic archaebacteria are unique in that their metabolism increases in the presence of products from other bacteria,[70] and they use hydrogen and ammonia from other bacteria as a substrate for the production of methane.[71–73]

In studies of gut transit, methane has a physiologic effect. In IBS subjects, those with methane on breath test were noted to have constipation as a predominant symptom subtype (**Table 3**).[66,67] In addition, the amount of methane produced was related to the degree of constipation as measured by Bristol Stool Score and frequency of bowel movements.[67] This outcome is likely the direct effect of the methane gas,[74,75] as small intestinal methane infusion using an in vivo animal model leads to slowing of small intestinal transit by 69%.[76]

Antibiotic Treatment of IBS: Support for a Gut Flora Hypothesis

Although antibiotics will be discussed as a therapeutic approach to IBS in a later article, the role of antibiotics is important here because its benefit supports the concept of altered gut microflora. There are now 5 randomized controlled studies examining the effect of antibiotics in IBS, all of which have demonstrated significant improvement in primary outcome measures[34,40,41,77,78]; these are summarized in **Table 2**. The first of these studies used neomycin.[34] While neomycin demonstrated successful improvement in the primary outcome measure of the study, an important component of the result was that the antibiotic most improved the symptoms when subjects had normalization of their breath test findings. In fact, subjects with complete normalization of their breath test had a near 75% improvement in IBS. This finding is supported by another controlled study by Sharara and colleagues,[40] wherein subjects who were deemed responders to a course of the nonabsorbed antibiotic, rifaximin, had a greater reduction in breath hydrogen, indicating a reduction in intestinal flora. However, the most convincing of all these studies are the 2 latest phase 3 trials (TARGET 1 and TARGET 2).[40] In these studies, rifaximin was effective in improving IBS based on abdominal pain, stool consistency, bloating, and the primary outcome measure of global relief.

Although there is some remaining debate as to why antibiotics help improve IBS symptoms, these antibiotic approaches have provided support for the role of altered gut microflora in IBS.

Probiotics in IBS

If alterations in gut microbiota account for a large fraction of IBS, it seems reasonable that probiotics should restore a “healthy” gut microbiota and alleviate IBS symptoms. Unfortunately, the numerous controlled trials of probiotics in IBS have shown mixed results at best. These studies used a variety of probiotic species and strains, with heterogeneity of dosing regimens and clinical end points

Table 3
Support for the association between methanogens and constipation

Subjects	Total N	Methane N	Breath Test	Definition Positive Breath Test	Citation
32 IBS subjects	32	11	Not done	Breath methane concentration at least 1 ppm	Peled et al,[75] 1987
67 encopretic or constipated children, 40 healthy controls	107	35	Not done	Breath methane >3 ppm	Fiedorek et al,[74] 1990
120 C-IBS with positive lactulose breath test 11 D-IBS with positive lactulose breath test	231	35	Lactulose	Breath methane >20 ppm within 90 min of lactulose	Pimentel et al,[34] 2003
12 C-IBS 26 D-IBS 12 IBS-like	50	12	Lactulose	Breath methane >20 ppm or any increase in concentration within 90 min of lactulose	Pimentel et al,[34] 2003
30 C-IBS 149 D-IBS 25 IBS-other	204	32	Glucose	Breath methane >20 ppm when baseline <10 ppm, or any increase of 12 ppm	Majewski et al,[100] 2007
224 IBS 40 healthy controls	224	44	Lactulose	Breath methane $\geq$1 ppm at baseline or any time during test	Bratten et al,[101] 2008
31 C-IBS 51 D-IBS 48 IBS-mixed	130	35	Glucose	Methane excretion >10 ppm at baseline or after glucose	Parodi et al,[102] 2009
24 C-IBS 23 D-IBS 9 IBS-mixed/other	56	28	Lactulose	Any detection of methane >5 ppm	Hwang et al,[103] 2010
96 non-IBS chronic constipation 106 controls	202	87	Glucose	Baseline methane $\geq$3 ppm	Attaluri et al,[104] 2010

(reviewed by Parkes and colleagues[79]). The data are strongest for *Bifidobacterium* and *Lactobacillus* strains. *Bifidobacterium infantis* 35624, *Lactobacillus salivarius* UCC4331, or placebo was given to 77 patients and after 8 weeks, the *B infantis* group had a significant reduction in composite IBS symptom scores and abdominal pain scores versus placebo ($P<.05$). In addition, a decrease in the ratio of IL-10/IL-12 cytokine expression in peripheral mononuclear cells suggested an additional anti-inflammatory effect that was not characterized further.[80] No significant benefit was noted with the *Lactobacillus* strain. A larger multicenter study of 362 women with IBS randomized them to receive *B infantis* (at a dose of 10^6, 10^8, or 10^{10} CFU daily) or placebo for 4 weeks followed by a 2-week washout. Only the middle dose led to statistically significant but modest improvements in abdominal pain, bloating/distention, and IBS composite scores at the end of treatment.[81] The unexpected dose response may have reflected poor capsule dissolution and subsequent lack of bioavailability of the higher-dose probiotic following ingestion. VSL#3 (a probiotic mixture of 8 species of *Bifidobacterium, Lactobacillus,* and *Streptococcus*) or placebo was given to 25 D-IBS patients for 8 weeks. No difference in the primary end point of global symptom relief or gastrointestinal transit was seen. However, the investigators observed a significant reduction in the secondary end point of abdominal bloating.[82] A larger follow-up study did not demonstrate a significant benefit for bloating, but noted a significant decrease in flatulence during the treatment period.[83]

More recently, 3 meta-analyses or systematic reviews identified a small overall beneficial effect of probiotics over placebo.[84–86] The investigators found the studies to be heterogeneous, and that funnel plot asymmetry suggested publication bias. However, several of the trials were of good quality, and these tended to show more modest treatment effects. The clinical trials, as well as animal and translational studies with probiotics, indicate that the myriad species and strains of probiotics are clearly unique, with different biochemical and physiologic effects and possibly highly specific interactions with the mucosal immune and neuroendocrine systems. Therefore, the benefits of one probiotic cannot be extrapolated to another strain without thorough studies. Fortunately, the safety profile and adverse event rate of probiotics has been good. Large, well-designed controlled trials are clearly needed to guide the future use of probiotics in treating IBS.

INFLAMMATION AND IBS

In the past 20 years there has been a growing appreciation of gut mucosal immunology and its role in IBS, particularly the role of lymphocytes, mast cells, and cytokines, which are now discussed.

Lymphocytic Infiltration

In a study of PI-IBS subjects, unprepped patients underwent sigmoidoscopy with rectal biopsy. The mucosal biopsies among these subjects demonstrated an increase in rectal lymphocytes as compared with healthy controls that persisted months after acute *Campylobacter* infection.[58] In the absence of understanding the mechanism of how gastroenteritis led to chronic altered patterns in bowel function, the persistence of low-level inflammation was provocative.

Another finding in examining subjects with IBS is the possibility of chronic inflammation of the enteric nervous system. In a seminal article, 10 D-IBS subjects with severe, refractory symptoms underwent a laparoscopic full-thickness biopsy of the small bowel. In this study, IBS subjects demonstrated evidence of excessive lymphocytes in the ganglia of the myenteric plexus.[87] This ambitious study suggested myenteritis

to be present in selected IBS patients, which perhaps explains the visceral hypersensitivity that has been reported by several groups. However, this study is limited by its very small and highly selective study population.

Further evidence for T-cell activation and trafficking to the gut in IBS was demonstrated by Ohman and colleagues.[88] Peripheral blood lymphocyte expression of integrin beta-7 and endothelial cell expression of mucosal addressin cell adhesion molecule-1 (MAdCAM-1) was comparably elevated in patients with IBS and ulcerative colitis (UC) compared with asymptomatic controls, suggesting greater homing of lymphocytes to the gut mucosa.[88]

Mast Cells

Whether in the context of gut microflora or postulated food hypersensitivity, mast cells have been examined in IBS. The first description of altered mast cells in IBS by Weston and colleagues demonstrated elevation of mast cells in ileal biopsies. Since then, Barbara and colleagues[13] have conducted a series of studies examining the role of mast cells in IBS. In a major study, 44 IBS subjects were found to have elevated mucosal mast cells and tryptase in mucosal biopsies compared with controls. The extension of this study was that in deeper sections, there was a tendency for the mast cells to approximate enteric nerves (by $<5\ \mu m$) in patients with greater abdominal pain. The elevation of small bowel mast cells was later confirmed by Guilarte and colleagues.[89] However, Barbara and colleagues[14] then used extracted material from IBS patients and applied this to a preparation of rat enteric nerves and increased afferent nerve activation. This more recent result has led these investigators to speculate that mast cells and their approximation to enteric nerves could serve to induce visceral hypersensitivity.

Innate Immunity

Another measure of inflammation and immune activation is the balance between proinflammatory and anti-inflammatory cytokines. Although the data on cytokines have been limited to date, a provocative finding in the examination of cytokines in IBS was made during the study of the probiotic *B infantis*.[80] Specifically, this group found a low IL-10:IL-12 ratio at baseline in IBS. This ratio was normalized with the administration of the *B infantis*. The difficulty with this study is that the investigators do not state all the cytokines measured. Furthermore, the ratio of these 2 cytokines as a measure of the "inflammatory state" is unconventional. Finally, this same group has since not found this profile in their more comprehensive cytokine studies. Specifically, a recently published study by Scully and colleagues[90] demonstrated in a large group of IBS subjects that IL-6 and IL-8 were elevated in IBS patients without comorbidities, but not IL-10 or IL-12. Furthermore, IBS with coexisting fibromyalgia, chronic fatigue syndrome, or premenstrual dystrophic disorder were associated with the same profile. However, tumor necrosis factor-α (TNF-α) and IL-1β were also noted to be elevated. Similarly, Liebregts and colleagues[91] demonstrated increased IL-6, IL-1β, and TNF-α levels in D-IBS patients compared with healthy controls, patients with mixed IBS, and C-IBS patients. Rectosigmoid and terminal ileal mucosal tissue from PI-IBS patients have also demonstrated elevated levels of proinflammatory cytokine IL-1β mRNA in 2 studies.

Gut Microbiota and Inflammation

Alterations in the gut microflora are associated with IBS, and studies outlined in this review point to the altered flora as a key determinant in pathogenesis. The near consistent finding of low-grade inflammation and intestinal immune activation in IBS, and PI-IBS in particular, may be driven by the gut flora, just as inflammatory bowel disease (IBD) mucosal inflammation is thought to be the result of microbial stimulation of

a dysregulated immune system in a genetically susceptible host. This proposed mechanism for IBD is supported by the absence of intestinal inflammation in various germ-free animal models.[92] In that case, might IBS simply reside within a spectrum of intestinal inflammation, bound by IBD at one end and normal mucosa at the other?[93] Genetic susceptibility to IBS may manifest in exaggerated or prolonged low-grade inflammatory responses, psychological vulnerabilities, and/or neurochemical alterations. While most of this discussion of IBS pathogenesis is still rather speculative, the evidence for an activated immune system in IBS is ever increasing. However, the level of lymphocyte elevation seen by Spiller and colleagues[57] of little over 1 additional lymphocyte per 100 epithelial cells, although statistically significant compared with controls, had questionable impact in a subject with already concurrent diarrhea. Was the diarrhea causing the subtle inflammation or is this part of the mechanism of PI-IBS? Further work remains to be done to determine the relevance of this finding.

Of interest, the gut appears to demonstrate a response to gut flora through another pathway. Defensins are innate proteins thought to control intestinal flora through antimicrobial properties. Langhorst and colleagues[94] used enzyme-linked immunosorbent assay to measure fecal levels of this antimicrobial peptide; levels in the colon were detected by immunohistochemistry. Surprisingly, D-IBS and UC patients had elevated fecal β-defensin–2, 72.9 ng/g and 104.9 ng/g, respectively, compared with healthy controls (31.0 ng/g) ($P<.005$). This result may provide further indirect support for a microbial immune interaction.

Anti-Inflammatory Therapy

Despite the evidence for chronic low-grade mucosal inflammation in IBS and PI-IBS, anti-inflammatory therapies have been disappointing in treating symptoms and providing compelling support for the significance of this inflammation in IBS. A placebo-controlled, double-blind, randomized 3-week trial using prednisolone to treat PI-IBS did not improve symptom severity even though a drop in rectal T cells was noted in this underpowered study.[95] Data on the anti-inflammatory drug mesalazine appear promising, but only one study has appeared as a full peer-reviewed article; treated patients in this blinded, randomized controlled trial (RCT) had improved symptoms and decreased mast cells compared with placebo-treated participants.[96] Finally, the use of the mast cell stabilizer ketotifen was associated with improved IBS symptoms and reduced visceral hypersensitivity in a blinded RCT,[97] but larger studies are needed to confirm the effect of an anti-inflammatory approach as regards IBS.

SUMMARY

In the last decade, there has been a significant acceleration in our understanding of gut flora as it pertains to IBS. The preceding sections of this review point to qualitative and quantitative alterations in the gut microflora to be strongly associated with IBS. While specific alterations of stool flora have been demonstrated, these results are difficult to interpret because of a lack of diet control in this research.

Evidence is also accumulating, using direct and indirect testing, that a proportion of IBS subjects may have small intestinal bacterial overgrowth. The evidence for altered gut microflora is supported by an ever increasing list of large randomized controlled studies demonstrating the efficacy of antibiotics in IBS.

Two interesting areas in the study of gut microbes and IBS are the association between methanogenic organisms and constipation in IBS (see **Table 3**), and the role of acute gastroenteritis in the precipitation of IBS. In the case of methane and methanogenic microbes, it appears that methane gas produced by these organisms

contributes to a slowing of intestinal transit, which may be responsible for the constipation. In the case of PI-IBS, there is a clear cause-and-effect relationship between acute infection in the gastrointestinal tract and the development of IBS. This breakthrough has led to the development of several animal models in an attempt to characterize the mechanisms of this process.

While there is a growing body of literature examining gut inflammation in IBS, such literature is in its early stages. Mast cells may have an important role in IBS but that role remains to be determined, as studies on cellular infiltrates and cytokines are at this time inconsistent and need validation. Given the complex disease heterogeneity and pathophysiology that is IBS, it is likely unrealistic that any one therapeutic approach, whether antibiotic, probiotic, or anti-inflammatory, will achieve broad efficacy unless patients are first carefully selected and stratified for their underlying pathophysiologic mechanism of IBS.

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