

Sonia Michail and Mun-Wah Ng

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## Introduction

This chapter addresses the relationship between the gut microflora and the neurogastrointestinal system. It is divided into several sections that further dissect the contribution of the bacterial content of the gut towards the development of motility disorders, with a main focus on the most common functional and visceral hypersensitivity disorder—irritable bowel syndrome (IBS).

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## The Effect of the Brain on Gut Environment

Communication between the brain and the gut (see Fig. 5.1) is modulated by the autonomic nervous system, both sympathetic and parasympathetic. This gut–brain axis controls gut functions ranging from gastrointestinal secretions to motility and immune response. In turn, the vitality of the gut microbiome, at least in part, is determined by the gastrointestinal transit and motility which, when impaired, can affect the delivery of nutrients to the

microbiota. For example, impaired intestinal transit caused by disarray of the migrating motor complexes (MMC) can result in the development of small bowel bacterial overgrowth [1]. Disordered MMC contractions are common in IBS where decreased MMC contractions in the small bowel are seen in constipation-predominant IBS, and accelerated intestinal transit in diarrhea-predominant IBS [2]. In addition, the autonomic nervous system modulates gastrointestinal mucus secretion which forms the biofilm, home to the many of the enteric microbiota [3]. It also influences immune activation of the gut, directly, through modulation of the response of the gut immune cells to luminal bacteria, or indirectly, through modification of the ability of luminal bacteria to reach the gut immunocytes. Interestingly, stress and stressful stimuli can enhance the permeability of the intestinal epithelium, which allows bacterial antigens to cross the intestinal epithelium triggering an immune response in the intestinal mucosa [4–9] and causing a significant reduction in the tight junction proteins which leads to a compromise in the epithelial barrier function and the development of leaky gut [10]. Therefore, the brain and its axis can greatly influence the gut milieu.

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S. Michail, M.D. (✉)

The Children's Medical Center, Gastroenterology,  
One Children's Plaza, Dayton, OH 45404, USA  
e-mail: sonia.michail@hotmail.com

M.-W. Ng, M.D.

Pediatrics Department, Gastroenterology & Nutrition  
Division, Children's Hospital Los Angeles,  
Los Angeles, CA, USA

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## Mucosal-Gut Microbial Interaction

Stress can also play an important role in mucosal-microbiome interaction and host protection. For example, secretion of mucosal

defensins, which are antimicrobial peptides, can play an important role in host defense mechanisms against inflammatory and infectious diseases of the gastrointestinal tract [11]. The secretion of such defensins by Paneth cells is enhanced by stress [12]. Stress can also impact the secretion of neuroendocrine signaling molecules such as catecholamines, serotonin, and cytokines, which are secreted by neurons, immune cells, and enterochromaffin cells, into the gut lumen in response to different stress stimuli [13–15]. Furthermore, norepinephrine release in the intestine during stress and trauma induces expression of virulence factors in *Pseudomonas aeruginosa* which contributes to gut-derived sepsis [16], stimulates the growth of several other strains of enteric pathogens, and intensify the virulence of *Campylobacter jejuni* [17]. These reports can help us appreciate the association of stressful events with the development of gastrointestinal disease such gastroenteritis and subsequent development of post infectious IBS [18].

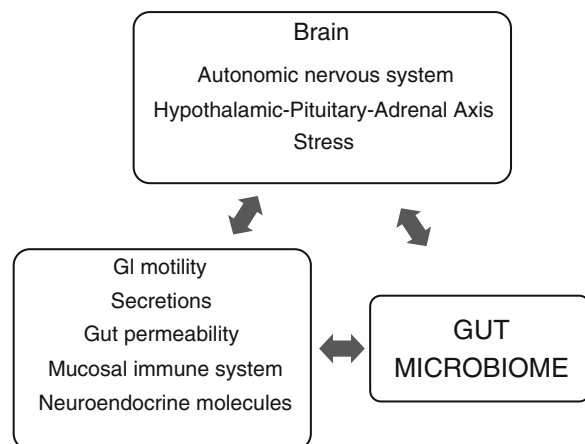
### Bidirectional Signaling

The gut microbiome, much like the enteric nervous system, can affect the intestinal motility. For example, *Lactobacillus acidophilus* and *Bifidobacterium bifidum* are capable of promoting motility, while other members of the gut microbiome such as *Escherichia* species can

inhibit or slow down the intestinal transit [19]. Gut bacteria can also modulate gut transit indirectly through the production of microbial metabolites such as short-chain fatty acids or peptides such as *N*-formylmethionyl-leucine-phenylalanine [20–22]. Disturbance in the intricate balance between different enteric microbial populations might, therefore, predispose the host to altered gut motility and secretion, which results in diarrhea or constipation. These changes are, in turn, likely to influence the balance of enteric microbiota. Therefore, the gut microbiome can directly influence gut homeostasis by the regulation of bowel motility and modulation of visceral pain and immune responses [23–26] (Fig. 5.1).

### Irritable Bowel Syndrome

IBS is a common disorder afflicting millions of adults and children around the world. From the pediatric perspective, it is estimated that IBS affects up to 25% of school-age children and adolescents, accounts for a significant number (2–4%) of office visits to primary care doctors, and represents about 25–50% of all patients who visit a gastroenterologist’s clinic [27]. The past several years have witnessed an emergence of new concepts related to the pathophysiology of IBS, which include alterations in gut motility, small-bowel bacterial overgrowth, microscopic inflammation, visceral hypersensitivity, and changes related to the brain–gut microbial axis.



**Fig. 5.1** Bidirectional relationship of the brain–gut–enteric microbiota axis

## Altered Gut Motility

In IBS, disordered gut motility has been observed along the length of the gastrointestinal tract [28–31]. The major migrating complex (MMC), which consists of periodic, contractions that sweep the luminal contents from the stomach to the colon, becomes disorganized. Several studies have shown that patients with IBS tend to have abnormalities in these contractions. For example, Vassallo et al. measured colonic electronic barostat and perfusion manometry in 16 subjects and demonstrated a greater frequency of prolonged, high amplitude and greater preprandial colonic motility, which may explain the increased perception of pain in these patients. Other studies assessing colonic transit times in IBS, showed a shorter colonic transit in patients with diarrhea-predominant IBS [32, 33] consistent with the presenting symptomatology. More importantly, the dysmotility seen in these patients can predispose them to develop small-bowel bacterial overgrowth, which is another proposed etiology for the development of IBS symptoms.

## The Contribution of the Gut Microbiome Towards IBS

### Small-Bowel Bacterial Overgrowth

Small-bowel bacterial overgrowth has emerged as a possible cause of IBS since Vantrappen and colleagues' work which suggested that it may occur in specific motility disorder such as a reduction in the major migrating complex [1]. Further studies have confirmed this finding in this patient population [34–36]. A large study of 202 patients with IBS found that 78% of these patients had evidence of bacterial overgrowth demonstrated by abnormal lactulose-methane-hydrogen breath testing. In the study, 25 of 47 patients experienced eradication of bacterial overgrowth on follow-up after treatment with antibiotics. Analysis of this subset of patients revealed that those who had successful eradication of bacterial overgrowth reported improvement in their IBS symptoms [37]. Another study by Pimentel showed that 84% of 111 patients

with IBS had abnormal breath testing. These patients were then randomized to receive neomycin or placebo for 1 week. A follow-up questionnaire revealed that patients in the neomycin group reported a 35% reduction in symptomatology as compared to 11.4% in the placebo group [38]. More recently, Peralta and colleagues assessed 97 patients who met Rome II criteria and found that 56% of these patients had positive lactulose breath tests [39].

Although the exact mechanisms by which altered fecal flora induce disease are poorly understood, it has been shown that fecal short chain fatty acids produced by microbiota, which are critical for maintenance of the colonic epithelium, are significantly reduced in children with diarrhea-predominant IBS [40]. Symptoms especially related to gas production are reduced by an exclusion diet, suggesting an alteration in the activity of hydrogen-consuming bacteria and further emphasizing the importance of fermentation in the pathogenesis of IBS [41]. Lactulose breath testing in IBS subjects does not seem to reflect malabsorption but the pattern of hydrogen excretion is suggestive of bacterial overgrowth [42] and suggests that IBS might be associated with rapid excretion of gaseous products of fermentation [43]. On the other hand, increased bacterial methane production was seen with constipation-predominant IBS [44]. Postprandial serotonin release was also blunted [45], suggesting a possible neurochemical basis for impaired motor function. The discovery that specific changes in gut microbiota contribute to IBS pathophysiology could aid in the development of new therapeutic strategies [46, 47].

### The Gut Microbiome in IBS

The gut microbiome is the array of microorganisms that dwell along the human gastrointestinal tract. The human microbiota is estimated to contain as many as  $10^{14}$  bacterial cells—a number that is 10 times greater than the number of human cells present in our bodies [48–50]. The microbiota colonizes every surface in contact with the external environment but the colon is the most heavily colonized and is estimated to contain over 70% of all the organisms in the human body.

The human gut has a large surface area [51] and is rich in nutrients, making it a preferred site for bacterial colonization. The architecture of this population is dynamic and evolves from birth to adulthood. It is influenced by the diet, state of health, external environment and other similar factors. The microbiome is closely associated with many aspects of human health, from nutritional status to immune and stress response. The intricate balance in the make-up of the gut microbial population, as well as the presence or absence of key microbial elements is crucial in ensuring health of the host. Although it is embraced as largely beneficial, it has been postulated that altered bacterial populations or products of bacterial metabolism may contribute to the development of disease in the gastrointestinal tract as well as remote areas of the body. The mechanisms through which microbiota exerts its beneficial or negative influences on the host include the production of signaling pathways and recognition of bacterial proteins by intestinal epithelial and mucosal host immune cells.

Recent data propose a role for the gut microbiota in the development of both central and peripheral neural processes. These interactions, termed the “brain–gut–enteric microbiota axis” [52], as discussed, can be bidirectional, with potential ramifications for disruption of this axis leading to abnormal neurogenic stimulation of the enteric system and the development of disorders such as IBS. The activation of any of the central nervous system and the gut–brain axis has potential in influencing enteric microbiota both directly through interaction between the gut microbiome and the host, and indirectly via changes in their environment [3].

In animal studies, the impact of stress on the composition of the enteric microbiota has become evident [53, 54]. Stress was characterized by transient reductions in the levels of the enteric microbiota in rhesus monkeys. In postnatal, maternal separation-induced stress, reduction in *Lactobacilli* was associated with the appearance of stress-indicative behaviors.

So is there a quantitative difference in the gut bacteria in IBS? Several studies are beginning to address this question. Osipov and colleagues dem-

onstrate that the concentration of *Streptomyces*, *Rhodococci*, and other members of the *Actinomycetales* order become dozen folds higher in quantity [55]. In another study by Malinen, a reduction of *Lactobacilli*, *Clostridium coccoides*, and *Bifidobacterium catenulatum* counts were seen in diarrhea-predominant IBS compared with healthy individuals [56]. Si and colleagues noted a reduction of fecal *Bifidobacteria* and an increase in *Enterobacteriaceae*, as well as lowered resistance to microbial colonization of the bowel in patients with IBS [57]. Taken together, these studies show encouraging associations between the gut microbiome and IBS.

## Intestinal Inflammation

Considerable attention has been recently directed towards the possibility of microscopic inflammation as a contributor to the development of IBS [58, 59]. Low-grade inflammation found in biopsies of different parts of the intestine in subjects with IBS has fueled this concept [47, 58, 60, 61]. The release of certain inflammatory mediators such as cytokines, interleukins, and histamine, may affect nearby enteric nerves, causing alteration in gut function and sensory perception leading to IBS symptomatology [60, 62]. The study by Chadwick et al. led the way to the new concept of IBS as an inflammatory condition. In their study of 77 IBS subjects, 55% were diarrhea predominant; and none had a confirmed infectious origin for IBS [58]. While 38 subjects had normal histology, 31 demonstrated microscopic inflammation and 8 fulfilled histologic criteria for lymphocytic colitis. Interestingly, even in the group with normal histology, immunohistology demonstrated inflammation with increased intraepithelial lymphocytes as well as an increase in CD3+ and CD25+ cells in the lamina propria. Therefore, all subjects had mucosal immune activation.

Additional studies further support the role of inflammation in IBS. Gonsalkorale and colleagues demonstrated that subjects with IBS have a reduction in interleukin-10 (IL-10), which has

an anti-inflammatory effect [63]. Barbara and colleagues' work demonstrate an increase in colonic mast cell degranulation with direct correlation between the proximity of mast cells in the mucosa and clinical pain severity [62]. Furthermore, Tornblom and colleagues examined full-thickness jejunal biopsies in 10 subjects obtained during laparoscopy [64] and noted low-grade infiltration of lymphocytes in the myenteric plexus in all patients and many had evidence of neuronal degeneration, longitudinal muscle hypertrophy and abnormalities in the number and size of interstitial cells of Cajal. There is also evidence to support an alteration in the ratio between the cytokines IL-10 and IL-12 favoring a Th1 response similar to what is seen in peripheral blood mononuclear cells [65]. Spiller further proposed that the inflammatory changes could represent an immune response to an initial enteric infection in individuals who become susceptible by a relative deficiency of anti-inflammatory cytokines [66].

Although embracing this theory broadens therapeutic options, yet efforts to treat the inflammation in an attempt to improve symptoms have been largely unsuccessful. Subjects with post-infectious IBS randomized to either prednisolone 30 mg daily versus placebo showed no improvement in their symptoms even though T lymphocytes decreased by 22% as compared to 11.5% in the placebo group [67]. Therefore, the clinical significance and application of this important concept of IBS being an inflammatory condition are yet to be defined.

### Modulation of Visceral Hypersensitivity

Visceral hypersensitivity is becoming more recognized as a potential contributor to the development of pain in IBS. Studies are now beginning to utilize the concept of the gut microbiome and probiotics to modulate this visceral hypersensitivity. Animal studies using the probiotic *Lactobacillus farciminis* demonstrate significant attenuation of stress-induced proteins during colorectal distension in rats and suggest a link to the epithelial cell cytoskeletal contraction [25, 26].

A study by Verdu showed that administration of *Lactobacillus paracasei* attenuates the antibiotic induced visceral hypersensitivity in mice [68]. Perhaps the most interesting link between gut bacteria and visceral sensitivity is highlighted by Rousseaux et al. [69] establishing that oral administration of *L. acidophilus* induced the expression of mu-opioid and cannabinoid receptors in epithelial cells, and mediated analgesic functions in the intestine in a manner similar to that induced by morphine.

### Potential Therapeutic Applications

The modification of the enteric microbiota to treat subjects with IBS and visceral hypersensitivity is attractive due to its ease, relative safety, and current lack of other effective therapeutic alternatives. Such modifications can be achieved by the administration of antibiotics, probiotics, or prebiotics. Clinical studies have produced variable responses to such treatments and vary depending on age, predominant symptoms, and bowel habits. Our current understanding of IBS pathophysiology remains incomplete, and although the complexity of the network of interactions within the enteric microbiota and visceral hypersensitivity is emerging, some studies have provided evidence for a beneficiary role for enhancement or manipulation of the gut bacteria. The use of nonabsorbable antimicrobial therapies such as Rifaximin has shown some promise and probiotics and prebiotics are also emerging as potential therapies. A recent study by Pimentel [70] validated that rifaximin therapy for 2 weeks provided significant relief of IBS symptoms, bloating, abdominal pain, and diarrhea. In another study, 54 patients with positive lactulose breath tests were treated with a 7-day course of rifaximin. Follow-up after 3 weeks revealed that half of their subjects had a subsequent negative lactulose breath test and a statistically significant improvement in symptoms. These results were similar to those found in another recent study by Majewski and associates, in which a 4-week course of rifaximin led

to improvement in IBS-related symptoms and a negative breath test in patients who previously had positive tests [71]. Although these results are encouraging, other researchers have failed to confirm these findings and further research is needed in this area [72].

An important study by O'Mahony et al. [65] showed that *Bifidobacterium infantis* not only resulted in symptom reduction in IBS, but also correlated with normalization of proinflammatory cytokines, suggesting an immune modulating effect of probiotics. The study by Bazzocchi et al. [73] is the first observation showing a clinical improvement related to changes in the composition of the fecal bacterial flora, fecal biochemistry and colonic motility pattern, all of which was induced by administration of probiotics, in patients with functional diarrhea. In constipation-predominant IBS, Agrawal and colleagues [74] show improvements in abdominal girth and gastrointestinal transit, as well as reduced symptomatology after 4 weeks of *Bifidobacterium lactis* consumption.

Pediatric studies addressing the role of probiotics in IBS have recently emerged (Table 5.1). A study published by Bausserman and Michail [75] designed to determine whether oral administration of the probiotic *Lactobacillus GG* under randomized, placebo-controlled, double-blinded conditions would improve symptoms of IBS in children, showed a lower incidence of perceived abdominal distension but did not alter any of the other parameters. Another double-blinded, randomized controlled trial by Gawronska et al. [76] designed to determine the efficacy of a 4-week therapy with *Lactobacillus rhamnosus GG* (LGG) in treating functional abdominal pain disorders (FAPD) in children showed a higher incidence of treatment success (i.e., no pain) in children with IBS receiving the probiotic. A more recent study by Guandalini and colleagues [77] suggests a beneficial role for VSL#3 (Sigma-Tau Pharmaceuticals, Inc.) in children with IBS. Finally, reports of amelioration of symptoms of bloating and flatulence in patients with IBS when treated with a poorly absorbed antibiotic, Rifaximin [77, 78], and the poorly absorbed antibiotic Neomycin has been

effective in reducing symptoms and decreasing hydrogen and methane production in IBS [38, 79], suggesting major role for intestinal bacteria as a contributor to symptoms of IBS.

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## Conclusions

Strong evidence suggests that the gut microbiome plays an important role in functional gastrointestinal disorders and interactions between the gut and the nervous system influence intestinal motility and inflammation. Although several reports suggest a disruption in the balance of the enteric microbiota in patients with IBS, considerably more data are needed to establish whether these changes are merely seen due to the dysmotility or indeed there is a causative role for these findings. While most of the studies addressing the role of the enteric bacteria rely on traditional culture techniques to identify the microbiome, studies utilizing molecular technology in identifying the microbiota would prove useful in further investigating the role of these bacteria in gastrointestinal symptomatology and disease. Results from a small number of well-designed, randomized, controlled, clinical trials suggest that, not only does regular intake of certain probiotic bacteria help to treat the symptoms of IBS, but their effects go beyond symptoms and are associated with modulation of biological parameters, such as intestinal transit, abdominal girth and inflammatory markers that in turn influence the gut-brain axis.

Further understanding of the gut microbiota will improve our knowledge of their role in health and disease, and allow for improved future therapeutic and prophylactic modalities. Although significant strides have been made in our journey of deciphering the codes of the gut microbiome, our ability to delve deep into this fascinating organ has been hampered by the complexities of its inhabitants. The introduction of non-culture-based molecular techniques that enable quantitative assessment of the entire enteric microbiota coupled with encouraging results from probiotic research continue to improve our understanding in this area.



**Table 5.1** Summary of published pediatric reports of probiotic role in irritable bowel syndrome

| Author                      | Year | Type of probiotic   | Duration in weeks | Population             | Type of trial | Outcome of study  |
|-----------------------------|------|---|-------------------|------------------------|---------------|---|
| Bausserman and Michail [75] | 2005 | LGG ( $10^{10}$ CFU given BID)                            | 6                 | Age: 6–20 ( $n=50$ )   | R, DB, PC     | Lower incidence of perceived abdominal distention in LGG group, but no difference in other parameters         |
| Gawronska [76]              | 2007 | LGG ( $3 \times 10^8$ CFU given BID)                      | 4                 | Age : 6–16 ( $n=104$ ) | R, DB, PC     | LGG group more likely to have no pain, reduced frequency of pain, but not pain severity than controls         |
| Guandalini [77]             | 2010 | VSL#3 (4–11 y/o: 1 sachet daily; 12–18 y/o: 1 sachet BID) | 6                 | Age : 4–18 ( $n=59$ )  | R, DB, PC     | Relief of symptoms, lowered abdominal pain/discomfort, bloating/gassiness, and life disruption in VSL#3 group |

R randomized, PC placebo controlled, DB double-blinded, LGG *Lactobacillus GG*, VSL#3 proprietary mixture of *Bifidobacterium breve*, *Bifidobacterium infantis*, *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Lactobacillus paracasei*, *Lactobacillus bulgaricus*, and *Streptococcus thermophilus* (Sigma-Tau Pharmaceuticals, Inc.)

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