Microflora Modulation of Motility

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That gastrointestinal motility can influence the gut microbiota has been known for decades and the clinical consequences of impaired motility, in terms of the bacterial population of the small intestine, amply illustrated by the syndrome of small intestinal bacterial overgrowth which so commonly accompanies diffuse intestinal motility disorders. As the importance of the microbiota to homeostasis in health and to a variety of disease states is increasingly appreciated and as the full diversity and biology of this “hidden organ” have been revealed by molecular methodologies, the true nature of the interaction between the microbiota and motility is being re-examined and the complexity of this relationship exposed. In health, as well as in disease states, this is a truly bi-directional relationship: not only can gut motor patterns influence the microbiota but changes in the microbiota can exert profound influences on gut sensori-motor function.

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Key Words
Gastrointestinal motility; Irritable bowel syndrome; Metagenome

Introduction

The gut microbiota (formerly referred to as the gut flora) and its interactions, both symbiotic and pathogenic, with the host, have become one of the hottest areas in biomedical science. This explosion in research and knowledge has its origins in both the laboratory and the clinic. While, in the laboratory, the application of modern molecular techniques, such as genomics and metabolomics, has revealed the true size, genetic diversity and metabolic complexity of the bacterial components of the microbiota, clinical observations have highlighted the importance of the microbiota and host-microbe interactions in health and disease. It is now acknowledged that the number of bacteria within the gut is about 10 times that of all of the cells in the human body, that the microbiome contains much more genetic material than the host and that it represents a formidable metabolic “organ,” not only being capable of extracting calories from food components impenetrable to the host but also elaborating a large array of biologically important compounds ranging from short-chain fatty acids and gases to antibiotics and neuro-modulatory substances. Techniques based on 16S rRNA sequences have revealed that the diversity of the human microbiota is much greater than previously thought and that the majority of bacterial sequences correspond to unculturable sequences and novel bacteria.

Motility-Microbiota Interactions: The Basics —

The Development of the Microbiota

Interactions between the microbiota and motility may be evident from the earliest stages of life. The developmental aspects of...
the microbiota are also of clinical interest, given the importance of early life events to the pathophysiology of disorders such as irritable bowel syndrome (IBS). At birth, the entire intestinal tract is sterile; bacteria enter the gut with the first feed.\(^6\) Following infancy, the composition of the intestinal microbiota remains relatively constant thereafter. Motor patterns, motor responses and enteric neuromuscular physiology also evolve over this time period.\(^6,7\) That the phenomena of motor and microbiota maturation are related is most vividly illustrated by studies in germ-free animals;\(^8\) not only are motor patterns disrupted in these animals but enteric neural and muscular function and morphology are abnormal. When disturbed, the microbiota has a remarkable capacity to restore itself and to return to exactly the same state as it was before.\(^9\) Whether this restorative capacity is equally evident in the neonate and infant remains to be defined; it is possible that disturbances to the microbiota at this formative stage in its evolution may have more permanent consequences. It has recently been suggested, for example, that antibiotic use in early childhood is a significant risk factor for the development of inflammatory bowel disease in later life.\(^10\)

**Motor Patterns and the Microbiota**

Relationships between motility and the microbiota are evident in the normal intestine. Indeed, it is because of the presence of such normal motility patterns as peristalsis and the migrating motor complex, acting together with the antimicrobial effects of gastric acid, that the stomach and proximal small intestine contain relatively small numbers of bacteria in healthy subjects; jejunal cultures may not detect any bacteria in as many as 33%.\(^11,12\) The relationship between the integrity of the migrating motor complex and the absence of small intestinal bacterial overgrowth (SIBO) was, in fact, first described in man almost 35 years ago by Vantrappen et al.\(^13\) The microbiology of the terminal ileum represents a transition zone between the jejunum containing predominantly facultative anaerobes and the dense population of anaerobes found in the colon. Bacterial colony counts may be as high as \(1 \times 10^9\) CFU/mL in the terminal ileum immediately proximal to the ileocecal valve, with a predominance of Gram-negative organisms and anaerobes. On crossing into the colon, the bacterial concentration and variety of the enteric microbiota change dramatically. Specialized motor patterns of the terminal ileum\(^14,15\) (where the migrating motor complex is less evident in man\(^15\)), as well as the physiological and bio-mechanical properties of the ileo-colonic junction,\(^16,17\) no doubt contribute to these ileo-colonic bacterial gradients. Concentrations as high as \(1 \times 10^{12}\) CFU/mL may be found in the colon; comprised mainly of anaerobes such as *Bacteroides*, *Porphyromonas*, *Bifidobacterium*, *Lactobacillus* and *Clostridium*, with anaerobic bacteria outnumbering aerobic bacteria by a factor of 100-1,000:1.\(^4\) The predominance of anaerobes in the colon reflects the fact that oxygen concentrations in the colon are very low; the microbiota has simply adapted to survive in this hostile environment!

**Luminal Versus Mucosal Microbiota**

At any given level of the gut, the composition of the microbiota also demonstrates variation along its diameter with certain bacteria tending to be adherent to the mucosal surface while others predominate in the lumen;\(^18\) studies which rely on the analysis of the fecal microbiota alone may miss the impact of an important population of organisms: those closely adherent to the mucosa.\(^5\) Indeed, there is considerable interest in the potential role of these juxta-mucosal organisms in the initiation and/or perpetuation of inflammation in inflammatory bowel disease.\(^19,20\) Motility, including the contractile activity of both the longitudinal and circular muscle layers could well be seen to play a role in determining the relative numbers and diversity of bacteria in the center and at the periphery of the lumen.

**“Normal” Variations in the Microbiota**

In humans, the composition of the microbiota is also influenced by age, diet and socio-economic conditions and, above all, by the use of antibiotics; factors that may also influence motor patterns. Whether these changes in the microbiota and motility are related or independent events remains to be defined. There is a suggestion that SIBO occurring in the elderly in the absence of any other identifiable cause may be consequent upon age-related changes in small intestinal motility.\(^21\) Given these “normal” variations in the microbiota, studies purporting to identify alterations in the microbiota in disease states must, accordingly, be interpreted with great care and some degree of skepticism.

**Motility and the Microbiota; Who Is in Charge?**

Motility and the microbiota are clearly associated; the question is which is calling the tune? The conventional doctrine is that motor patterns influence the size, location and diversity of the microbiota; thus the clinical association between a variety of motor disorders and SIBO. That the microbiota can influence motor patterns has been vividly illustrated, not only by the aforementioned studies in the germ-free animal, but also in experimental models of the short bowel syndrome where resection
of extensive portions of normal, more distal small intestine result in profound motor disruption in the more proximal remnant. These dramatic motor changes occur in the context of a dramatic increase in the bacterial population, as well as the concentration of a bacterial product, short chain fatty acids, in the remnant.

These observations make teleological sense: motility seeks to rid the gut of a presence that it senses to be pathogenic. Extensive studies in a murine model of enteric nematode infestation have revealed, in great detail, the physiological, morphological and ultrastructural changes that can result from transient infestation and that appear to be mediated via the immune response; the net effect being the expulsion of the invader.

Barbara et al., in their elegant review, identified 3 mechanisms whereby the microbiota could influence gut motor function: (1) through the release of bacterial substances or end-products of bacterial fermentation, (2) via intestinal neuroendocrine factors and (3) indirectly through the effects of mediators released by the gut immune response.

A variety of bacterial components and products have been shown to affect motility. Indeed, the potential for short-chain fatty acids and another bacterial metabolite, deconjugated bile salts, to generate potent motor responses has been amply demonstrated in both animal models and man. The bile salt effects may well be relevant to bile salt diarrhea, a phenomenon that has recently enjoyed a renaissance as a factor in the etiology of diarrhea-predominant IBS and has even led to the exploration of a prebiotic which selectively increased the numbers of bifidobacteria, reduced colonic fermentation. Changes in fermentation influence motor function, not just in the colon, but at sites as distant as the lower esophageal sphincter and stomach.

While microbial effects on gut mucosal neuroendocrine function have been recognized for some time, it has only been recently that the ability of components of the microbiota to elicit and sensory function has been recognized. Certain species have been shown to elaborate neurotransmitter peptides, as well as the inhibitory neurotransmitter gas, nitric oxide. Perturbation of the flora by an antibiotic has been shown to increase substance P immunoreactivity and induce hypersensitivity in the colon; in contrast, certain commensal strains have the capacity to modulate intestinal pain through the induction of opioid and cannabinoid receptors.

The microbiota and the host enjoy many complex interactions which include influences on the gut epithelium and its integrity, the mucosal and systemic immune systems and the gut-brain axis. Each of these areas has been the subject of extensive research; suffice it to say that effects of the microbiota on the mucosal barrier, immune responses and neuroendocrine responses could have direct and indirect impacts on the function and, even, the morphology of gut muscle and nerve. Such studies have illustrated relationships between mucosal inflammation and gut motor and sensory functions, the ability of modifications of the microbiota to influence gut barrier function and the consequences to the host of deficits in the latter. Given the current focus on the possible contributions of inflammation to motor dysfunction in a variety of dysmotility and functional disorders, these microbe-immune responses have attracted considerable attention.

Motility-Microbiota Interactions: Clinical Implications

While the list of gastrointestinal and non-gastrointestinal disorders that may be related to some disturbance in the microbiota and/or the microbiota-host interaction is lengthy, I will illustrate the bidirectionality of this relationship, so evident in laboratory studies, with 2 clinical entities: SIBO and IBS.

Small Intestinal Bacterial Overgrowth

Although SIBO is usually defined in quantitative terms, as the number of colony forming units per mL, the interpretation of such definitions must be mindful, firstly, of the location, in the intestine, from where the sample was obtained and, secondly, of the fact that the majority of bacterial species in the gut remain un culturable. Alternatively, the diagnosis may be based on the presence of such consequences of SIBO as malabsorption, com-
Combined with a positive result from such non-invasive diagnostic methods as breath tests. These reservations notwithstanding, SIBO is usually, and pending the validation of a more accurate methodology based on molecular microbiology, defined as the presence of > 1 × 10⁵ CFU per mL of bacteria in the proximal small bowel.⁴⁹

Dysmotility predisposes to an increase in colonic bacteria in the small intestine; diseases resulting in impaired intestinal motility are, therefore, likely to have SIBO as a complication. Scleroderma and diabetic gastroenteropathy are among the more common disorders that exemplify the occurrence of SIBO in the context of impaired motility; indeed, SIBO may complicate any of the many conditions that lead to the syndrome of chronic intestinal pseudo-obstruction. Reflecting the predilection of small intestinal myopathic disorders to SIBO, this complication has been documented in 43%-56% of patients with scleroderma.⁵⁰,⁵¹ The prevalence of SIBO was higher among those with a higher global symptom score and, especially, among those with a high score for digestive symptoms. Other risk factors for SIBO included the presence of diarrhea and constipation. Eradication of SIBO, successful in 52%-73%, resulted in symptom improvement.⁵⁰,⁵¹ SIBO is associated with small intestinal diverticulosis. Diverticula in the jejunum occur in 0.07% to 2% of the population, are twice as frequent in men and are observed predominantly among those over 60 years of age. Morphological studies suggest that disorders of intestinal motility such as progressive systemic sclerosis, visceral myopathies and neuropathies play an important role in the formation of the small bowel diverticula.⁵²

However, by far the greatest controversy related to SIBO over the past decade and a half has been the proposal that SIBO is linked to IBS. The initial reports from Pimentel et al., using the lactulose breath test, documented SIBO in 84% of their patients with IBS. Normalization of the lactulose breath test in this group, by use of neomycin, resulted in a significant improvement in IBS symptoms. Furthermore, methan excretion, on breath testing, was highly associated with a constipation-predominant subgroup of IBS. The same group found that their IBS patients with SIBO exhibited both a lower number and duration of phase III of the MMC, on antroduodenal manometry, in comparison to control subjects. Significant concerns have been raised regarding the validity of this association.⁵³-⁵⁵ In an important systematic review and meta-analysis of the link between SIBO and IBS, Ford et al. drew attention to the impact of test modality on SIBO prevalence, the average prevalence of SIBO on breath tests being 54%; in sharp contrast to a mean prevalence of just 4% for jejunal aspirate cultures.⁶⁰ These authors also drew attention to the impact of diagnostic criteria, which varied considerably between studies, on study outcome.⁶⁰ To muddy the waters further it has been suggested that the apparent link between SIBO and IBS may represent the impact of the proton pump inhibitors which are prescribed so readily in this patient population.⁶⁵ My personal opinion is that, while some patients with SIBO may present with IBS-type symptoms, SIBO is not a major contributor to the pathogenesis of IBS, in general. Furthermore, I believe that the modest improvement in IBS symptoms that has now been reported with some consistency in IBS with antibiotic therapy may owe more to the effects of these agents on the colonic flora rather than SIBO.⁵²-⁶⁴ This is supported both by the presence, in the study by Sharara et al., of a symptomatic response to antibiotic therapy among subjects with a normal baseline breath test and by the very recent demonstration that accelerated small bowel transit, and not SIBO, was the major contributor to positive lactulose breath tests in IBS.⁶⁶ These and other considerations have led to a serious re-evaluation of the status of SIBO in IBS.⁶⁷

**Irritable Bowel Syndrome**

Approximately 10%-20% of adults in the West have symptoms consistent with IBS. A combination of visceral hyper-sensitivity, smooth muscle spasm and impairment of central pain processing likely contribute to the pain associated with IBS, while altered intestinal motility underlies the disordered defecation experienced by some patients with IBS.

Over the decades, various theories have been advanced to explain the pathogenesis of symptoms in the IBS patient, including dysmotility, visceral hypersensitivity and the psyche. The concept of the gut brain axis, emphasizing the interactivity at sensory, motor and neuro-endocrine levels, between the brain and the gut has provided a useful paradigm to encompass these diverse factors. This axis has been extended, by some, to include interaction between the gut flora (or microbiota), the immune system (both mucosal and systemic), the gut and the brain (the gut-brain-immune-microbial axis). In this scenario, interactions between the flora (be it normal and disturbed) and the mucosal immune system (gut-, or mucosa-associated lymphoid tissue) lead to the release of peptides and other neuro-active substances which generate, both locally and systemically, the neuro-muscular events that typify IBS and generate the patient's symptoms. One of the most convincing pieces of evidence supporting the involvement of the microbiota in IBS is the phenomenon of post-infectious IBS. We are now beginning to see real data to directly support
the concept of post-infectious IBS. The occurrence of IBS following episodes of bacteriologically-confirmed gastroenteritis has now been documented in several studies. Thabane et al concluded that the overall risk for the development of IBS was increased 6-fold following an episode of bacterial gastro-enteritis with younger subjects, those who have prolonged fever during the episode of gastroenteritis and those who suffer from anxiety or depression being at greatest risk.

Post-infectious IBS may explain only a minority of cases of IBS but it does illustrate a clear link between exposure to an environmental agent, inflammation and IBS in predisposed individuals. Thus, Gwee et al demonstrated an increase in the number of chronic inflammatory cells in the rectal mucosa among those patients exposed to bacterial gastroenteritis who had developed IBS. Others have demonstrated a persisting increase in rectal mucosal enteroendocrine cells, T lymphocytes and gut permeability in patients with post-dysenteric IBS. These observations are important as they indicate a relationship between perturbations of the microbiota, mucosal inflammation and IBS, an hypothesis that is amply supported by data from studies in experimental animal models. In IBS, in general, evidence for a role for the microbiota can also be inferred from observations suggesting the presence of immune activation and subtle levels of inflammation in IBS. Such evidence was first provided by Chadwick et al who demonstrated microscopic inflammation or immune activation in among diarrhea-predominant IBS. Subsequent studies have provided further evidence of T lymphocyte and mast cell activation in the mucosa in IBS; others have demonstrated an extension of inflammation into the myoneural compartments and others still cytokine profiles in peripheral blood mononuclear cells and serum compatible with a pro-inflammatory state.

It is attractive to suggest that these immunological changes could result from exposure to an exogenous (such as bacterial) antigen challenge. That IBS patients may be predisposed to an, albeit contained, inflammatory response to luminal triggers is, indeed, supported by the finding of polymorphisms in genes that encode for the production of anti-inflammatory cytokines among IBS patients and by the very recent description of high titers of anti-flagellin antibodies in serum derived from IBS patients. Further, more direct, support for this hypothesis comes from the demonstration of elevated levels of defensins in fecal fluid and of upregulation of Toll-like receptor 4 in IBS.

So is there direct evidence for changes in the microbiota in IBS? While, in the past, various studies had suggested the presence of qualitative changes in the colonic flora in IBS patients; a relative decrease in the population of bifidobacteria being the most consistent finding. Several factors limit the interpretability of these studies, including the unrepresentative nature of the fecal flora, a failure to describe those bacterial populations that may be adherent to the mucosal surface and, above all, the recognition that a very significant proportion of the colonic microbiota cannot be identified by conventional culture methods. Molecular methods are now being applied to this complex issue and have, indeed, confirmed that IBS patients, regardless of sub-type, do exhibit a fecal flora that is clearly different from control subjects. The precise nature of these differences and their potential to disturb mucosal or myoneural function, in the gut wall, or induce local or systemic immune responses, remains to be defined.

Conclusion

In the past, the relationship between motility and the microbiota was viewed as unidirectional with motility maintaining the sterility of the upper gastrointestinal tract and dysmotility predisposing to small intestinal bacterial overgrowth. This narrow concept has been cast aside with the recognition of the ability of the microbiota to influence the development of normal motor patterns and the potential for disturbances in the microbiota to elicit motor responses or, if sustained, to lead to sensori-motor dysfunction and motility and, perhaps, functional disorders such as IBS. Accordingly, therapeutic approaches which modify the microbiota, including prebiotics, probiotics and antibiotics are being explored in IBS.

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