EDITORIAL

Gastrointestinal neuromuscular apparatus: An underestimated target of gut microbiota

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Abstract

Over the last few years, the importance of the resident intestinal microbiota in the pathogenesis of several gastrointestinal diseases has been largely investigated. Growing evidence suggest that microbiota can influence gastrointestinal motility. The current working hypothesis is that dysbiosis-driven mucosal alterations induce the production of several inflammatory/immune mediators which affect gut neuro-muscular functions. Besides these indirect mucosal-mediated effects, the present review highlights that recent evidence suggests that microbiota can directly affect enteric nerves and smooth muscle cells functions through its metabolic products or bacterial molecular components translocated from the intestinal lumen. Toll-like receptors, the bacterial recognition receptors, are expressed both on enteric nerves and smooth muscle and are emerging as potential mediators between microbiota and the enteric neuromuscular apparatus. Furthermore, the ongoing studies on probiotics support the hypothesis that the neuromuscular apparatus may represent a target of intervention, thus opening new physiopathological and therapeutic scenarios.

Key words: Microbiota; Gastrointestinal motility; Smooth muscle; Enteric nervous system; Probiotics; Irritable bowel syndrome

Core tip: This article reviews the current evidence of gut microbiota and neuromuscular apparatus connection that results to be both direct and indirect. Besides dysbiosis-driven mucosal inflammatory mediators, recent evidence suggests that gut neuromuscular apparatus can be modulated directly by microbiota metabolic products or circulating bacterial molecular components translocated from the intestinal lumen.

INTRODUCTION

Microbiota and gut motility are clearly associated, but it's difficult to establish what plays the major role in influencing the other. According to the classical theory, gastrointestinal (GI) motility can affect the microbiota in terms of amount, location and diversity. This concept is mainly supported by the association between different GI motility disorders and small intestinal bacterial overgrowth (SIBO)[1,2]. GI motility disorders and alterations of migrating motor complex (MMC), that eliminates residual content through the GI tract during periods of fasting, predispose to SIBO because bacteria are not swept from the small bowel into the colon, as reported in experimental models and specific clinical conditions[3-5]. Neuropathic and myopathic diseases, such as scleroderma and polymyositis, seem to be associated with SIBO[1,6] as well as conditions associated to long-standing diabetes, such as gastroparesis[7].

On the other hand, both *in vivo* and *in vitro* evidence highlights that microbiota can affect GI motility[8,9]. In studies conducted on germ-free animals, impairment of neural and motor functions of the GI tract due to reduced expression of neurotransmitters and contractile proteins, were reversed by gut colonization[10]. Moreover, probiotics have been shown to affect GI motility *in vivo* and *in vitro*. Prebiotic or probiotic therapies are associated with a significant clinical improvement in irritable bowel syndrome (IBS)[11,12] and animal studies suggest that the neuromuscular apparatus could represent a target for probiotics[13-15]. Finally, dysbiosis is associated with significant alterations in intestinal transit time[16].

By interacting directly with mucosal environment, the microbiota impacts intestinal mucosal functions and permeability, and influences local and systemic inflammatory activity[12]. In normal conditions neuromuscular apparatus is not in contact with the luminal content and quite inaccessible by the luminal microbes. However, dysbiotic conditions cause an increase in mucosal inflammation and intestinal paracellular permeability[17,18] (Figure 1) with possible translocation of pathogens, toxins, antigens and bacteria in the circulatory system[16,19,20]. GI motility might then be affected by microbiota essentially by two mechanisms: an indirect mechanism driven by the inflammatory mediators released by the mucosal immune system and a direct mechanism driven both by the release of end products of bacterial fermentation and bacterial substances.

INDIRECT EFFECTS

The potential for the microbiota to produce inflammatory alterations in the gut microenvironment deranging gastrointestinal motor function prompts to a unifying hypothesis for the role of the microbiota in the pathogenesis of IBS. To support a role of the microbiota in IBD pathophysiology is the evidence that an acute episode of gastroenteritis precedes the onset of IBS, a specific condition called post-infectious IBS (PI-IBS)[11,21,22]. PI-IBS is characterized by persistent abdominal discomfort, bloating and diarrhea, despite the elimination of the causative pathogen. In this condition, the imbalance in microbiota composition leads to low-grade inflammation followed by alteration of the sensory and motor bowel functions. An increased amount of immune cells in the colonic, ileal, and jejunal mucosa of IBS patients has been largely reported[23,24]. The persistent inflammatory state is also characterized by increased mucosal interleukin 1β levels and mast cells count, as well as activation of entero-endocrine cells (EC), mainly those producing serotonin (5-HT)[25-28]. The interesting data is that most of these mucosal alterations persist for over a year and thus could contribute to the persistence of a PI-IBS. Therefore, the mucosal inflammation resulting from an acute infection can lead to a dysfunction of intestinal motility and 5-HT could play a pivotal role as its release increases motility and secretion, features which may explain diarrheal symptoms frequent in PI-IBS patients[29]. With an experimental model of primary infection with *Trichinella spiralis*, that causes hypercontractility of intestinal muscle persisting for over 20 d after the infection was cleared, it was shown that chronic immune response may extend to smooth muscle layers[30]. In this model, the levels of Th2 cytokines (interleukins 4, 5, and 13) resulted increased during the acute infection but not thereafter, whereas cyclooxygenase-2 (COX-2) and relative enzymatic activity localized to muscle remained significantly increased. These effects did not occur in athymic mice, suggesting a crucial role of T cells in the impairment of intestinal muscle function in post-infective disorders[30]. The role of COX-2 in muscle impairment during inflammation has been reported both in animal and humans. During severe mucosal inflammatory conditions, it has been shown in colonic muscle cells an altered expression of contractile key-signaling molecules and an increase in nuclear factor NF-κB DNA binding, which is low or absent in normal colonic muscle cells[31-33]. In human colonic smooth muscle, NF-κB activation leads to inflammatory gene expression of COX-2 and to production of prostaglandin E, both widely considered responsible for muscle cell impairment[34-37].
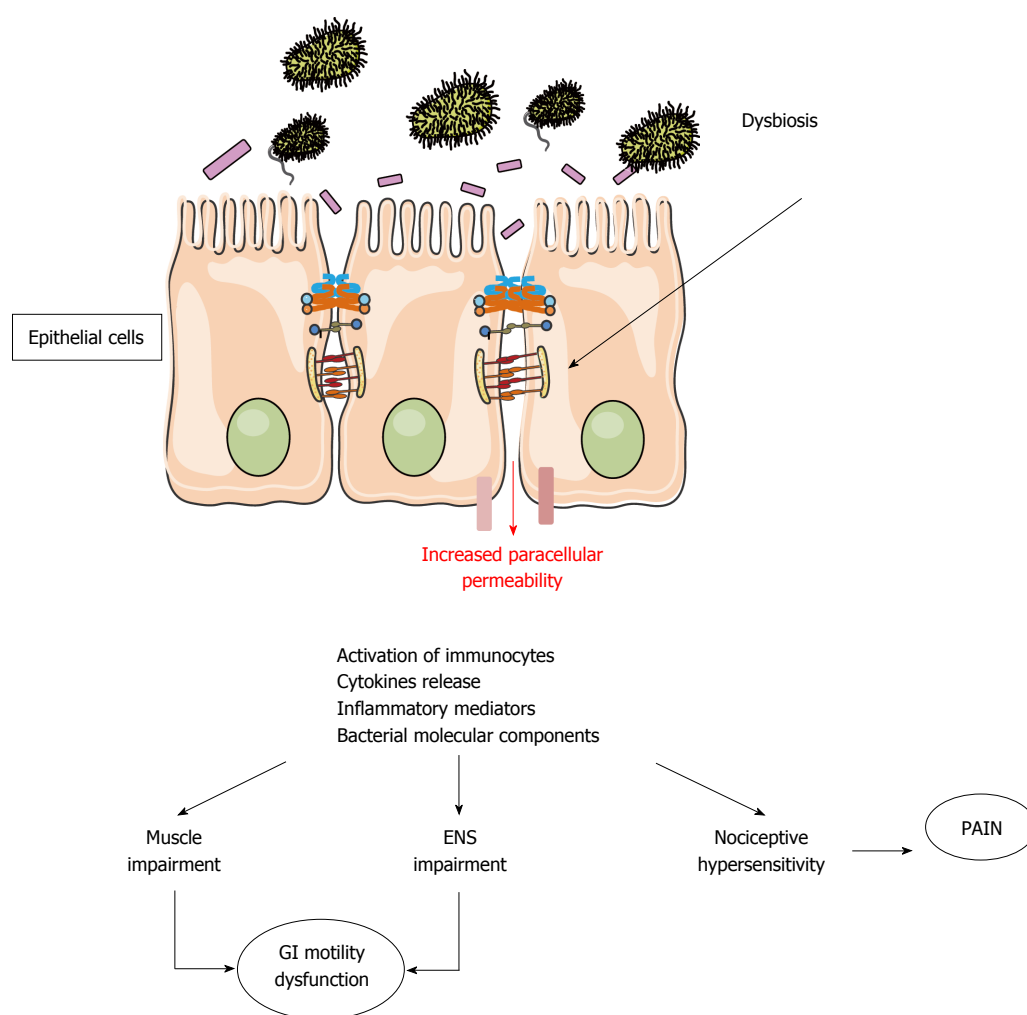
Figure 1 Dysbiosis and intestinal motility disorders. One hypothesis regarding the pathogenesis of functional intestinal disorders suggests that dysbiosis increases paracellular permeability leading to translocation of luminal contents with activation of immunocytes, cytokines and inflammatory mediators release. The activation of this state of inflammation and the presence of bacterial components, such as LPS, lead to nociceptive hypersensitivity, thus explaining the pain, and to enteric nervous system (ENS) or muscle impairment, thus explaining the intestinal motor disorders. LPS: Lipopolysaccharide.

Mediators released by the colonic mucosa of IBS patients are able to activate aberrant responses in the enteric nervous system[38,39] and to impair contractility of human colonic smooth muscle likely through a receptor-dependent mechanism[40]. Histamine and proteases, two soluble inflammatory products obtained from IBS biopsy supernatants, are able to excite visceral afferents neurons and to cause hyperalgesia and allodynia when introduced into the colon of mice[41,42]. Beside increased visceral sensory activation, the soluble products found in supernatants derived from the colon of IBS patients have been shown to evoke excitatory cholinergic longitudinal muscle contractions in the guinea pig ileum[43]. This effect correlates with the number of mast cells and the activation of the nerve fibers appears to be mediated by the activation of different receptors, including transient receptor potential vanilloid subfamily member 1 (TRPV1), purinergic and prostanoids receptors[43].

Many studies have been conducted in attempt to identify a specific pattern of intestinal faecal microbiota in IBS patients and, although heterogeneity of IBS patients, qualitative and quantitative alterations in intestinal microflora have been found. Differently from traditional microbial culture-based techniques, studies using DNA-based techniques showed that specific fecal and mucosal microbiota composition are associated with different subgroups of IBS patients, even if these investigations have produced non univocal results. Some studies reported increased abundance of *Proteobacteria* and *Firmicutes* and reduction in *Actinobacteria* and *Bacteroidetes* in patients with IBS[11,44] while others reported a decreased amount of *Lactobacilli* and *Bifidobacteria*[45]. A very recent meta-analysis demonstrated that composition of IBS patients microbiota vary across geographical regions. The study reported a decreased numbers of *Bifidobacteria* and *Lactobacillus* and increased numbers of *Escherichia coli* and *Enterobacterium* in Chinese IBS patients with no significant differences in the abundance of *Bacteroides* and *Enterococcus*. On the other hand, a decreased numbers of *Bifidobacteria* and increased numbers of

Bacteroides were found in IBS patients from other regions of the world[46]. The strict relationship between dysbiosis and GI motility in IBS need to be further elucidated as one of the major challenges in IBS is the absence of an animal model that fully represent this condition.

DIRECT EFFECTS

New physiopathologic and therapeutic scenarios have arisen by the recent evidence highlighting that microbiota metabolic products or bacterial molecular components can directly affect enteric nerves and smooth muscle cells functions.

Fermentation products

The microbiota is a formidable metabolic "organ", not only able to capture calories from food but also to elaborate a large amount of compounds such as short-chain fatty acids (SCFAs), neurotransmitters homologs and gases that can act directly with the enteric neuromuscular apparatus[47].

SCFAs such as acetate, propionate, and butyrate are produced by bacterial fermentation of dietary fibers. SCFAs exert multiple beneficial effects and act both as signal transduction molecules, *via* G-protein coupled free fatty acid receptors (FFAR2, FFAR3, OLFR78, GPR109A) and regulators of gene expression[48]. Besides improving the intestinal environment, SCFAs directly affect various host peripheral tissues, generate potent motor responses and have a considerable role in regulating the propulsive activity of the gut, both in animal models and in humans. SCFAs, when administered into the human terminal ileum, have been shown to increase parietal tone and stimulate ileal propulsive contractions[49,50]. This compounds are suggested to act *via* either extrinsic or intrinsic afferent neurons which can ultimately stimulate myenteric cholinergic neurons[51]. Most of these responses are not observed in mucosal free preparations, suggesting that SCFAs receptors are located on mucosal EC cells. In particular, propionate acts on receptors in the mucosa causing the release of 5-HT from EC cells that activates, through 5-HT4 receptors on the endings of intrinsic primary afferent neurons, the enteric peristaltic reflex pathways[51]. In the rat distal colon, propionate causes also tonic contraction *via* prostaglandin release[52]. Similarly, butyrate and acetate may also affect GI motility through several mechanisms including direct effects on smooth muscle and myenteric neurons[53] and production of mucosal 5-HT[54]. SCFAs receptors have been also localized in mucosal EC cells containing peptide YY that might represent another important messenger in transducing this contractile signal[55]. However, the effect of these metabolites still remain controversial; a recent human study found no significant differences in global motility index after intracolonic infusion of SCFAs[56].

Deconjugated bile salts, another bacterial metabolite[57], have also been reported to affect gastrointestinal motility through activation of transmembrane G-protein coupled receptor (TGR5)[58]. In animals, TGR5 have been detected in inhibitory intestinal motor neurons and on gallbladder smooth muscle cells[59]. The direct activation of TGR5 causes relaxation of the smooth muscle cells and inhibition of gallbladder contractility resulting in gallbladder filling. In humans, treating normal gallbladder muscle cells with a hydrophobic bile acid, the tauro-chenodeoxycholic acid, results in impairment of contraction to cholecystokinin due to a significant reduction in receptor binding and an increase in inflammatory mediators and oxidative stress[60,61]. These latter abnormalities, observed also in gallstone patients, are prevented by treatment with the hydrophilic ursodeoxycholic acid[61,62].

Among microbiota compounds that might influence GI motility, there is tryptamine, a secondary metabolite resulting from the transformation of the aromatic amino acid tryptophan, that mimics the serotonin stimulatory effects on motility in *ex vivo* preparations of guinea pig ileum[63]. It is of note that most genes encoding amino-acid-metabolizing enzymes involved in the synthesis of neurotransmitters (catecholamines, serotonin/melatonin, acetylcholine) are present in the microbiota genome[64]. Commensal bacteria have also been shown to be a significant source of nitric oxide (NO), a key molecule in the control of gut motor functions[65].

Finally, fermentation by the anaerobic flora of the undigested polysaccharide fraction of certain carbohydrates generates gases, mostly hydrogen (H_2) and methane (CH_4). Even if clinical studies are still controversial, experimental evidence has been provided that methane is not an inert intestinal gas since it can affect the intestinal neuromuscular function[66]. In animal models, it has been shown that intestinal methane infusion slowed down small intestinal transit time and augmented ileal circular muscle contractile activity[66,67]. In turn, in an *ex vivo* experiment on guinea pig gut, H_2 by itself has been reported to significantly shorten colonic transit times, this effect being restored by methane[68]. Finally, the resident sulfate-reducing bacteria produce hydrogen sulfide (H_2S) that inhibits intestinal contractile activity acting on interstitial cells of Cajal and enteric extrinsic neurons[66]. The effects of fermentation products on GI motility are summarized in Table 1.

Bacterial molecular components

One of the main mechanisms of bacterial recognition are toll-like receptors (TLRs) a family of pattern recognition receptors that are emerging as potential mediators between microbiota and the enteric neuromuscular apparatus. TLR-dependent signaling regulates structural integrity in both the myenteric and submucosal plexus[69,70]. The mRNA encoding for TLRs

Fermentation product	Effect on GI motility	Mechanism	Ref.
Short-chain fatty acids	Increase of ileal tone and propulsive contractions	Activation of G-protein coupled free fatty acid receptors (FFAR2, FFAR3, OLFR78, GPR109A)	[48-55]
	Smooth muscle and myenteric neurons activation	Release of 5-HT from EC cells	
		Release of prostaglandins	
Deconjugated bile salts	Relaxation of gallbladder smooth muscle cells	Activation of transmembrane G-protein coupled receptor	[58-61]
	Inhibition of gallbladder contractility	Reduction in cholecystokinin receptor binding	
		Increase of inflammatory mediators and oxidative stress	
Tryptamine	Stimulation of ileum motility	Synthesis of neurotransmitters	[63-65]
Gases	Decrease of small intestinal transit time	Methane (CH_4) production	[66,67]
	Augmented ileal circular muscle contractile activity		
	Shortening of colonic transit times	Hydrogen (H_2) production	[68]
	Inhibition of intestinal contractile activity	Hydrogen sulfide (H_2S) production	[66]

Table 1 Direct effect of bacterial fermentation products on gastrointestinal motility

GI: Gastrointestinal.

have been detected on neurons[71], glial[72] and smooth muscle cells[73]. TLR-2 activation on smooth muscle leads to the production of neurotrophins that enhance the structural and functional integrity of the enteric nervous system[74].

In acute inflammatory conditions an excessive increase of mucosal permeability leads to luminal bacteria/endotoxins translocation[75]. Bacteria or bacterial products can migrate from the intestinal lumen to mesenteric lymph nodes, or the circulation, due to the disruption of the normal host/flora equilibrium as reported in cirrhosis[76], inflammatory bowel diseases[77] and recently in diarrhea-predominant IBS patients[78].

Most evidence on the effects of bacterial components on the neuromuscular apparatus derives from studies on lipopolysaccharide (LPS), the major component of the outer membrane of Gram-negative bacteria. Although the exact mechanisms whereby LPS is able to impair muscle contractility are still to be established, various targets have been demonstrated. LPS can directly activate muscular TLR4 inducing a time- and concentration-dependent impairment of contractility associated to cytoskeleton alterations, together with an intracellular oxidative imbalance as shown on human colonic smooth muscle cells[79] (Figure 2). Many of these effects persisted even after LPS withdrawn suggesting that motility dysfunction might play a pivotal role both during an acute infective process and after its resolution. In an experimental model that enables to stimulate human intestinal mucosa in a polarized fashion with LPS[37], it has been shown that LPS affects enteric contractility both through translocation from the mucosa and submucosa, with subsequent activation of TLR expressed in muscle, and through mucosal production of oxygen free radicals. LPS effects on human smooth muscle were reversed by the H_2O_2 scavenger catalase, by NFκB transcription inhibitors and by indomethacin, which blocks activation of COX2[37]. Besides, LPS can directly activate macrophages embedded within the intestinal muscularis externa that produce inflammatory mediators that indirectly alter smooth muscle contractility[80,81].

Interestingly, the expression of multiple TLRs receptors subtypes differentially activated by bacterial antigens on the enteric neuromuscular apparatus seems to allow a discrimination between pathogens and probiotics, as reported for both human enteric glial[72], smooth muscle cells[73,82]. The crosstalk between TLRs subtypes is emerging as an important regulatory defense mechanism also in neuromuscular apparatus[83]. On human colonic smooth muscle cells, it has been observed that the activation of TLR2, whose ligands are the components of the outer membrane of Gram-positive bacteria, prevents LPS-induced muscular alterations. By interacting with this receptor, *Lactobacillus rhamnosus* GG (LGG) is able to reduce LPS-induced NFκB activation and inflammatory IL6 secretion cytokine and to restore the levels of secretion of anti-inflammatory cytokine IL10[82]. These *in vitro* studies support the recent evidence that indicates the neuromuscular apparatus as possible target for probiotics[13-15]. *Escherichia coli* strain Nissle 1917 specifically modulates contractility of human colonic muscle strips[84], *Lactobacillus* species regulate jejunal motility[14], colonic neuron excitability[15] and attenuate post-infective muscle hypercontractility[85]. *Bifidobacterium* and *Lactobacillus* also alleviate visceral hypersensitivity and recover intestinal barrier function as well as inflammation[86]. Also in humans recent evidence further suggests that probiotics might be effective in neuro-motor disorders[87,88].

CONCLUSION

In summary, the current working hypothesis is that dysbiosis-driven mucosal alterations induce the production of several inflammatory/immune mediators which affect gut neuro-muscular functions suggesting a potential for disturbances in the microbiota to elicit directly intestinal dismotility or, if sustained,
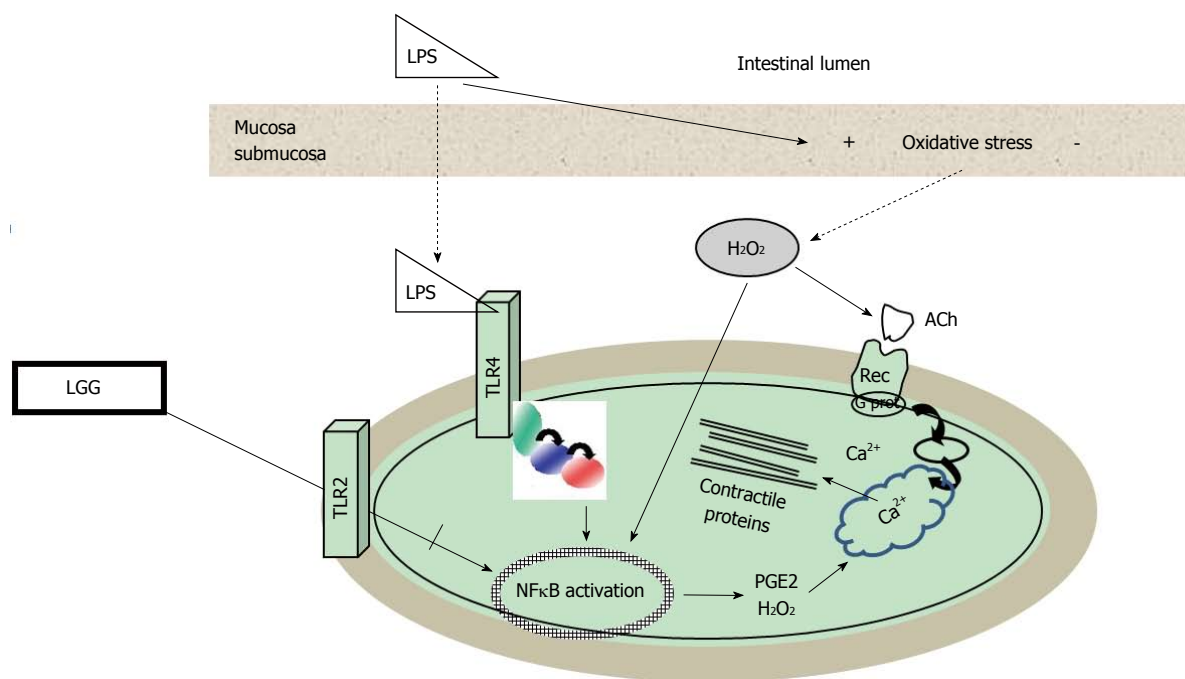
Figure 2 Role of toll-like receptors on human colonic smooth muscle cells. LPS affects intestinal contractility by activating oxidative stress in the mucosa and, once translocated, by activating TLR4 expressed in colonic muscle cells. Activation of muscular TLR4 impairs cell contractility by activation of the nuclear factor kB transcription with intracellular increase of oxidative stress and by prostaglandin E2 (PGE2) that block intracellular calcium release. The oxygen free radicals, produced in the mucosa, impair cell contractility with a similar mechanism and also by de-regulation of contractile receptors. The activation of TLR2, whose ligands are the components of the outer membrane of Gram-positive bacteria, such Lactobacillus rhamnosus GG (LGG), prevents LPS-induced muscular alterations. TLR4: Toll-like receptor 4; LPS: Lipopolysaccharide.

to lead to chronic sensory-motor dysfunction. The understanding in these fields would hopefully open new therapeutic scenarios in GI disease with underlying neuromuscular disorders as manipulation of gut microbiota composition could also correct the mechanisms promoting development and maintenance of symptoms.

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