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Bacterial modulation of visceral sensation: mediators and mechanisms

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Abstract

The potential role of the intestinal microbiota in modulating visceral pain has received increasing attention during recent years. This has led to the identification of signaling pathways that have been implicated in communication between gut bacteria and peripheral pain pathways. In addition to the well-characterised impact of the microbiota on the immune system, which in turn affects nociceptor excitability, bacteria can modulate visceral afferent pathways by effects on enterocytes, enteroendocrine cells and the neurons themselves. Proteases produced by bacteria, or by host cells in response to bacteria, can increase or decrease the excitability of nociceptive dorsal root ganglion (DRG) neurons depending on the receptor activated. Short chain fatty acids generated by colonic bacteria are involved in gut-brain communication, and intracolonic short chain fatty acids have pro-nociceptive effects in rodents but may be anti-nociceptive in humans. Gut bacteria modulate the synthesis and release of enteroendocrine cell mediators including serotonin and glucagon-like peptide-1, which activate extrinsic afferent neurons. Deciphering the complex interactions between visceral afferent neurons and the gut microbiota may lead to the development of improved probiotic therapies for visceral pain.

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Introduction

Visceral pain is a common and debilitating symptom of many digestive diseases, including inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS) (17). Visceral pain is often resistant to conventional analgesics and can sometimes be exacerbated by opioid drugs (45, 55). In light of this, new therapeutics to relieve visceral pain are urgently needed. Progress towards this goal will be accelerated by a more complete understanding of the peripheral signaling molecules that modulate nociception in the gut.

The perception of pain is accomplished by neural pathways that connect the gut to the brain via the spinal cord. The first neurons in this chain have cell bodies in dorsal root ganglia (DRG), project sensory axons into the gut and form excitatory synapses in the dorsal horn of the spinal cord. A subpopulation of these neurons, called nociceptors, detects noxious stimuli and activates pain circuits in the brain. Host-derived mediators from biopsies of IBS and IBD patients induce hyperexcitability in nociceptive DRG neurons, leading to an exaggerated response to stimuli such as distension or a bowel movement (16, 26, 60). This change in nociceptor sensitivity is a major driver of visceral pain. Superimposed upon these peripheral changes are changes in central nervous system (CNS) circuits that amplify synaptic inputs from the periphery (17, 20). Thus, visceral pain results from a combination of peripheral sensitisation and central plasticity. Combating these pro-nociceptive influences are host-derived analgesic substances including endogenous opioids and cannabinoids (22, 124). This balance between pro-nociceptive and anti-nociceptive influences on DRG neuron excitability dictates the transmission of pain stimuli to the CNS and the perception of pain. Recent investigations have identified the gut microbiota as an additional factor in pain modulation, capable of either worsening or ameliorating pain (8, 88). Microbial modulation of visceral pain may have translational

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relevance given the changes in microbiota composition associated with IBD and IBS. Although intestinal fungi may also play important roles in modulating visceral pain (21), in this review, we discuss the potential mediators of bacterial modulation of peripheral visceral pain pathways.

A potential role for gut bacteria in visceral pain signalling

The mutualistic relationship that has evolved between bacteria and eukaryotes includes the ability of commensal bacteria in the gut to influence behavior and pain (24, 40, 88, 96, 122). Although probiotics have been marketed for the treatment of visceral pain for over a decade, there is a lack of mechanistic insight into which bacteria, bacterial metabolites, or signaling pathways are most important. To date, much of the evidence in support of a role for the microbiota in regulating pain is derived from *in vivo* studies demonstrating that germ-free mice, or mice treated with antibiotics that alter the microbiota early in life, have heightened pain sensitivity (39-41, 74, 88, 90, 98). However, changes to pain sensitivity in germ-free mice may not be due solely to direct microbial-neuronal interaction, as germ-free mice exhibit a number of potentially confounding developmental changes to the immune system. Similarly, a study of visceral pain sensitivity in mice treated with a cocktail of antibiotics reported an increase in visceral pain accompanied by an increase in colonic myeloperoxidase activity, which is indicative of immune system activation (126). This suggested a role for inflammatory changes in nociceptive effects of modulating the microbiota. Although there is potential for bacterial products to directly activate nociceptive neurons, the evidence until recently, largely supported a role for epithelial and immune cells in mediating many of the effects of the gut microbiota on pain pathways *in vivo* (Table 1) (5, 80, 84, 131).

Bacteria as a source of host modulatory factors

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There is a growing appreciation that the gut microbiota can be considered an endocrine organ, having the capability to directly or indirectly regulate different gastrointestinal and stress hormones, which may modify host physiological function (33). Intriguingly, the transfer of faecal matter from IBS patients is sufficient to evoke visceral hypersensitivity in gnotobiotic rats. This is not due to changes in mucosal permeability or immune activation, raising the possibility that bacterial metabolites in IBS patient stool directly modify gut-brain signalling (35). DRG neurons are capable of "sensing" the presence of microbes. They express functional microbial pattern recognition molecules, including toll like receptors and nucleotide-binding oligomerization domains 1 and 2 (91), whose activation can modulate neuronal excitability. Furthermore, the pathogenic bacterium *Staphylococcus aureus* directly excites DRG neurons through a toxin that forms cation-permeable pores in DRG neuronal membranes and through secretion of N-formylated peptides (32). In contrast to the pro-nociceptive effects of this skin pathogen however, the commensal gut microbes studied to date have inhibitory effects on DRG neuron excitability (88, 93, 109). Given the potential importance of the microbiota as a modulator of visceral pain, identification of the specific species involved and mediators responsible will be particularly important. Gut microbes produce a plethora of neuro-active compounds such as proteases (116), short chain fatty acids (SCFA) (99) and also classical neurotransmitters such as γ-amino butyric acid (GABA), dopamine and norepinephrine (94). We will consider the available evidence in support of a role for specific bacterial mediators in terms of their capability to directly access and act upon nerve circuits to modulate their function (39, 88, 137). We will also discuss microbe-mediated modulation of visceral pain pathways by using immune cells and enterocytes as cellular transducers (Figure 1).

Direct signalling by bacterial metabolites

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Proteases

Extracellular proteases, in particular serine and cysteine proteases, are important modulators of visceral pain (127). Proteases are released from many eukaryotic cell types, including mast cells, neutrophils and enterocytes (97, 104). Recent *in vivo* and *in vitro* work has identified the gut microbiota as an important source of proteases (116) capable of affecting peripheral pain pathways (8, 81, 109). Pain regulation by proteases most often occurs through the activation of protease activated receptors (PARs). PARs are a family of four G-protein coupled receptors that lack conventional ligand binding sites and are instead activated via protease-mediated hydrolysis of amino acid residues. Upon protease cleavage, a tethered ligand within the receptor is revealed that activates intracellular signaling pathways (97). The net effect of receptor signaling depends not just on the PAR subtype involved but the specific amino acids hydrolysed (97). A consistent finding from numerous laboratories is that PAR-2 activation causes sustained hyperexcitability of DRG neurons (6, 34, 51, 136). Indeed, activation of nociceptor PAR-2 by mast cell tryptase and enterocyte derived trypsin-3 (85, 104) has been implicated in visceral pain (12, 63). However, nociceptive neurons also express PAR-1 and PAR-4. Activation of PAR-1 and PAR-4 reduces DRG neuron excitability and is anti-nociceptive (10, 11, 66, 104). PAR-2 activation *in vivo* by cysteine proteases in fecal supernatants from IBS patients enhanced the visceromotor response to colorectal distension in rats, an *in vivo* assay of visceral pain. In contrast, activation of PAR-4 by commensal microbes has an analgesic effect *in vivo* and *in vitro* (81, 109). The opposing effects of PAR-2, PAR-1 and -4 suggest that the balance between PAR-2, and PAR-1 - 4 activation could be a critical determinant of nociception.

While it seems clear that activation of PARs by proteases derived from the microbiota can modulate pain, an important unresolved issue is whether these proteases exert this influence

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via actions on mucosal cells, immune cells or directly on DRG nerve terminals. The intestinal barrier is comprised of a mucus-coated epithelial monolayer whose integrity is maintained by tight junction proteins, which regulate the paracellular movement of luminal molecules. Beneath the epithelial layer, intrinsic and extrinsic neurons relay neural information both within the GI tract but also between the gut and the CNS. However, evidence that this communication system extends beyond the epithelial barrier to the microbially-dominated environment of the gut lumen, has resulted in it being referred to as the microbiota-gut-brain axis (19, 47, 76). It appears that at least in some circumstances, the impact of PAR activation on visceral pain is due to modulation of epithelial barrier function. Using a model of IBS in rodents, Miquel and colleagues found that proteases derived from *Faecalibacterium prausnitzii* inhibited the increase in visceral pain that results from neonatal maternal separation. In this case, the decrease in visceral pain was ascribed to PAR-4 mediated reversal of the increase in mucosal permeability in this model of visceral pain (81). Faecal supernatants from patients with chronic ulcerative colitis led to a decrease in visceromotor response to colorectal distention due to activation of PAR-4 (8). In a separate study, serine proteases from *Faecalibacterium prausnitzii* acted directly on nerve terminals to inhibit colonic sensory nerve spike discharge and reduced the excitability of colon-projecting DRG neurons via PAR-4 activation (109). Furthermore, these proteases reversed DRG neuronal hyperexcitability caused by the dextran sulphate sodium model of colitis in mice (109).

Opposite findings have been reported for microbial activation of PAR-2. Luminal administration of faecal supernatants from patients with diarrhea-predominant IBS increased visceral pain sensitivity and impaired mucosal barrier function *in vivo* via PAR-2 activation (49). Consistent with the ability of luminal proteases to have pronociceptive effects, luminal administration of the PAR-2 activating serine protease, cathepsin S, was sufficient to increase

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visceromotor responses in mice in a PAR-2-dependent manner (27). Similarly, activation of PAR-2 by host derived proteases causes a sustained increase in the excitability of mouse DRG neurons (67). Thus, although there is abundant evidence that activation of neuronal PAR-2 has pro-nociceptive effects, it remains unclear whether neuronal PAR-2, in addition to mucosal PAR-2, participates in the pro-nociceptive effects of bacterial proteases. Cell-specific receptor knockout strategies will be important tools in identifying which PAR-expressing cells are most important to visceral pain modulation *in vivo*.

In addition to microbial-derived proteases, the microbiota is a rich source of protease inhibitors (54) including siropins, which has been shown to mitigate the effect of host-derived proteases implicated in IBD pathogenesis (82). A recent study using a rodent model of post-inflammatory hypersensitivity provided valuable evidence that synthetic protease inhibitors can mitigate the pro-nociceptive effects of proteases in this model (28). It therefore appears that the balance between the activity of proteases and protease inhibitors can influence visceral perception and may be an important target for novel therapeutics (128).

Short chain fatty acids

Short chain fatty acids (SCFAs) are produced by the fermentation of dietary polysaccharides that are metabolized by the anaerobic bacteria found in the cecum and colon. Formate, acetate, butyrate, and propionate are the major byproducts of this fermentation process (83). Earlier reports have identified *Fecalibacterium prausnitzii, Eubacterium rectale, Eubacterium hallii and Roseburia faecis* as bacteria capable of producing butyrate. Likewise, acetate and pyruvate are produced by enteric bacteria such as *Blautia hydrogenotrophica*; propionate, on the other hand, can be produced by *Bacteroidetes* and *Firmicutes* (72).

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A well-established effect of butyrate is inhibition of bowel inflammation and enhancement of mucosal repair, which would have an indirect effect on inflammatory visceral pain (103). SCFAs also modulate the enteric nervous system (113) and have been posited as an important mediator of microbiota-gut-brain communication (88). Microbial dysbiosis, due to the administration of antibiotics or due to modulation of diet, led to a decrease in SCFA and an increase in visceral sensitivity (38, 90, 100, 112). This suggests an association between SCFA and visceral pain modulation but does not directly establish a causal relationship. Contrary to these studies, when SCFAs were administered to control rats and rats with TNBS-induced colitis, visceral hypersensitivity was not improved by any of the SCFAs (acetate, propionate and butyrate) used (121). In fact, butyrate administration decreased the noxious pressure threshold in rats, indicating a pronociceptive effect; this phenomenon was more pronounced in control rats than in TNBS- treated rats. This observation is supported by a report that rectal administration of sodium butyrate induced colonic hypersensitivity in rats (133). This pronociceptive effect was associated with neuronal activation of extracellular signal related kinase (ERK)1/2 and an enhancement of DRG neuronal excitability. However, a study of healthy human volunteers concluded that butyrate treatment induced a dose-dependent reduction of visceral sensitivity (125). In summary, despite evidence implicating SCFAs in mediating gut-brain communication in general, there are conflicting findings regarding the role of SCFAs in modulating visceral pain.

Microbial neurotransmitters and neurotrophic factors

Microbial depletion and recolonization studies have linked microbial modification of neuroactive compounds in the gut-brain communication axis to diseases of the peripheral and central nervous system (119). Germ-free studies illustrate the crucial role of microbes in the development of

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200 brain function and expression of central neurochemicals (15, 23) however, antibiotic treatment in
201 mature animals can avoid the confounding developmental effects of early-life microbial
202 alterations. Hoban and colleagues reported modification of central monoamines, serotonin and
203 brain derived neurotrophic factor (BDNF) following sustained antibiotic administration to adult
204 rats. These changes were accompanied by altered behaviors and diminished visceral pain
205 sensitivity to colorectal distension (58). Interestingly, antibiotic-related alterations in
206 neurotransmitters can be long-lasting and have different functional outcomes when administered
207 early in life. A gender-specific increase in visceral sensitivity, which was linked to decreases in
208 spinal cord expression of transient receptor potential (TRP)V1, α2A adrenergic receptors and
209 cholecystokinin B receptors, was noted in male rats treated with vancomycin from postnatal days
210 4-13 (90).

211 In addition to modification of host neurotransmitters, microbes also exhibit the capacity
212 to secrete functional neurotransmitters and neurotrophins. GABA, the major inhibitory
213 neurotransmitter, is synthesized by several *Lactobacilli and Bifidobacteria* (14, 129). As GABA
214 receptor agonists can suppress visceral pain responses to colorectal distension (56) and
215 inflammation-induced pain signals (73), this may contribute to nociceptive signaling from the
216 gut (62). Dopamine and norepinephrine, which have reported anti-nociceptive effects of visceral
217 pain sensitivity (37, 92), are also produced by several gut bacterial species, including *Bacilli* and
218 *Escherichia* (94, 129). BDNF, an important neurotrophic regulator of synaptic plasticity and
219 neurogenesis, is purported to be a hallmark of altered microbiota-gut-brain axis signaling, given
220 that its expression is altered in germ-free mice (87, 120) and in antibiotic- (58) and prebiotic-
221 treated mice (107). Moreover, BDNF is expressed on TRPV1-expressing nociceptive DRG
222 neurons (132) and neutralizing BDNF blocked visceral hypersensitivity in inflammatory colonic

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hypersensitivity (42). In IBS patients, increased expression of nerve growth factor (NGF) correlated with visceral pain sensitivity (134), which may be due to sensitization of pro-nociceptive receptors on primary afferent neurons. Indeed, NGF increases TRPV1 expression in DRGs (110). In the context of microbial modification of host molecules, an *in vitro* study demonstrated that *Lactobacillus rhamnosus* induces anti-inflammatory effects in human epithelial cells which is mediated by NGF (75). Although intriguing, evidence that gut bacteria have the capacity to secrete neurotransmitters and neurotrophins, does not explain how neuromodulatory molecules in the external environment of the gut lumen can modify gut-to-brain nociceptive signalling. As afferent nerves do not reach through the epithelium into the gut lumen, further mechanistic studies are needed to determine how bacterially-derived neuromodulatory factors can cross the gut barrier to influence gut-brain signalling.

Indirect signaling

Serotonin secretion from Enterochromaffin cells

Serotonin has long been recognised as a critical regulator of gut function, inflammation and pain (50, 77). Accordingly, the release of serotonin from enterochromaffin (EC) cells and its sites of action are important therapeutic targets for visceral pain. Two recent independent reports delineated the ability of microbes to modulate serotonin synthesis by EC cells. One study reported an increase in serotonin production in mice colonised with human fecal microbiota, compared to germ-free mice (99). This was associated with an increase in expression of tryptophan hydroxylase 1 (TPH1), the rate limiting enzyme for serotonin synthesis in EC cells. Consistent with the ability of microbial metabolites to increase TPH1 expression, the SCFAs, sodium acetate and sodium butyrate, increased TPH1 expression in a human-derived EC cell

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line. The second study identified spore-forming bacteria as important modulators of serotonin production by EC cells, and revealed that this effect occurred in the colon but not the small intestine (135). Furthermore, EC cell serotonin modulation by microbiota was also observed in RAG1 knockout mice which lack T and B cells, suggesting a direct action on EC cells rather than an indirect effect via immunomodulation. SCFAs were also implicated as modulators of EC cell function, which may be an important mechanism of pain modulation by microbiota. Other bacterial metabolites, such as bile acids and p-aminobenzoate, have also been implicated in regulating serotonin production. From these findings it appears that several bacterial signaling pathways depend on the release of serotonin from EC cell as a means of modulating gut function, inflammation and visceral pain. In addition to microbial modulation of serotonin release, Kwon and colleagues have recently (69) demonstrated that host-derived serotonin has direct and species-specific effects on the growth of commensal microbes *in vivo* and *in vitro*. Furthermore, the secretion of the anti-microbial peptide α-defensin from the HT-29 epithelial cell line was inhibited by serotonin (69). These findings illustrate the complex and bidirectional nature of the interactions between gut microbes and enterochromaffin cells.

GLP-1 secretion from L-cells

Similar to EC cells, GLP-1-secreting L-cells may act as chemosensory sentinels, conveying information about the luminal environment to the host. L-cells are polarised, electrically excitable enteroendocrine cells (31), which sense the arrival of nutrients, such as glucose and amino acids, in the small intestine. Despite the reduced probability of nutrients being present, the abundance of GLP-1-secreting L-cells increases towards the distal end of the GI tract (117). Consistent with the contents of the colonic lumen, L-cells in this region express receptors for SCFAs and bile acids (101, 123). Moreover, dietary supplementation with SCFAs (123), the

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introduction of specific commensal strains (9, 118) or antibiotic treatment (61) increased GLP-1 levels. Somewhat counter-intuitively, one study determined that serum GLP-1 was also elevated in germ-free mice (108), although other researchers found that germ-free mice exhibited a strong state of GLP-1 resistance, with impaired GLP-1 evoked gut-brain signalling and enteric nervous system function (52). A clinical trial in IBS patients found that administration of a GLP-1 mimetic reduced acute abdominal pain in patients (57). GLP-1 can act as a classical endocrine hormone, however GLP-1 also has direct neurostimulatory actions on vagal afferent neurons (78). Furthermore, there is evidence of direct, physical contact between a pseudopod-like elongation of L-cells and afferent nerve fibres (18), providing for a potential neural signalling pathway in the modification of GI function. Thus, L-cells are appropriately positioned to facilitate cross-barrier signalling from the gut lumen to the host peripheral nervous system and on to the CNS, and should be investigated as a potential modulator of visceral pain.

Histamine release from mast cells

Histamine, which is mainly secreted by mast cells, promotes allergic inflammation but also appears to play a role in visceral nociception. Indeed, histamine-containing secretions from IBS patient mucosal mast cells have been shown to excite rat nociceptive visceral afferent nerves, and are thus likely to participate in relaying visceral pain signals (13). Of the four histamine receptor subtypes, H1R and H2R are most prevalent in the gut. Similar to the opposing actions of PAR subtypes described earlier, activation of H1R promotes pro-inflammatory pathways (30), whereas H2R suppresses inflammation (111). In patients with IBD, reduced expression of H2R may underlie decreased suppression of TLR-induced cytokine secretion in this patient population (111). H1R antagonists decreased abdominal pain in IBS patients (68) and in a rat model of visceral hypersensitivity (115). Moreover, IBS patient biopsies display increased expression of

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H1R (106). Histamine may also be secreted by bacterial species such as *Lactobacillus reuteri* 6475, a commonly-used probiotic (114), which can reduce intestinal inflammation (48) and may also have an impact of visceral pain sensitivity.

Vagal afferent pathways

Vagal afferent neurons may also participate in the sensory arm of gut-brain nociceptive signaling. Although electrical stimulation of abdominal vagal afferents does not induce pain *per se*, nociceptive signaling may be modulated by vagal activity (7). Vagal nerve activation may in fact, induce an inhibitory modulation of chemically or mechanically-provoked insults (29, 53), as noted in a rat model of visceral pain where vagal nerve stimulation had an anti-nociceptive effect (138). Vagal afferent terminals are located within enteric ganglia, and in the smooth muscle and mucosal layers, where they are well-positioned to sense chemo-nociceptive signals (70, 95, 130). Given the essential role of the vagus nerve in mediating microbe-gut-brain communication (15, 23), future work should address whether modulation of vagal afferent pathways by bacteria impacts visceral pain.

Conclusions

There is abundant evidence that the microbiota is capable of modifying visceral pain *in vivo*. However, clinical trials of probiotics as therapies for visceral pain have yielded equivocal results. This may reflect patient heterogeneity, patient compliance, or the variety of probiotic formulations used, which is in turn reflects a relative paucity of mechanistic work identifying the most important microbial species and mediators to target for clinical benefit. A number of issues remain unresolved in bridging the gaps between our present state of knowledge and successful manipulation of the gut microbiota to alleviate pain. For example, the detection of high threshold noxious stimuli in rodents is accomplished by visceral afferent neurons with terminals

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that lie along serosal and mesenteric blood vessels (25). Furthermore, based on a limited number of recordings from visceral afferent neurons from human bowel, the majority of afferent terminals that have been characterized to date have been located in the muscle and vasculature. Thus, it appears that luminal mediators from the microbiota may have traverse the epithelial barrier and enter the circulation to access and modulate gut nociceptive terminals. Future studies of full-thickness resected bowel preparations from patients may provide insight into how the luminal microbiota accesses these terminals. Another potential caveat when translating findings from rodents to patients is that signaling mechanisms that are inhibitory in rodents may be excitatory in patients, and vice versa. A recent Ca^{2+} imaging study of PAR activation in human DRG neurons reported that PAR-1 activation in human neurons is excitatory (43), whereas PAR-1 is inhibitory in rodents (10). By increasing mechanistic insights into the interplay between the microbiota and peripheral pain pathways, particularly using patient microbiota and human DRG neurons (59), improved therapies that harness the analgesic properties of the microbiota may soon be on the horizon.

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791 **Table 1: *In vivo* studies of the effects of probiotics on visceral pain.**

Probiotic strain	Reference	Main finding	Proposed mechanism
Lactobacillus rhamnosus and/or prebiotics polydextrose/ galactooligosaccharide	(65)	Neonatal zymosan-treated rats treated with probiotic did not exhibit visceral hyperalgesia in response to CRD in adulthood	Altered CNS neurotransmitters
Lactobacillus reuteri	(93)	Inhibited the bradycardia induced by painful gastric distension in rats	TRPV1 modulation
Lactobacillus paracasei	(126)	Normalized visceral sensitivity to CRD in antibiotic treated mice in mice	Immunomodulation
	(46)	Prevented the maternal deprivation increased visceral sensitivity in response to CRD in rats	Epithelial barrier regulation
Lactobacillus acidophilus	(105)	Normalized visceral pain responses to CRD in mice and rats	Altered epithelial expression of opioid and cannabinoid receptors
	(102)	Reduced bloating symptoms in patients with functional bowel diseases experiencing abdominal pain in females	Modulated μ-opioid receptor expression and activity
Lactobacillus farciminis	(3)	Reversed visceral hypersensitivity induced by partial restraint stress (PRS) in rats	Epithelial barrier regulation
	(2)	Inhibited Fos protein expression at spinal and supraspinal levels as a marker of visceral pain in response to CRD in rats after PRS	None specified
Bifidobacterium infantis	(64)	Reversed post-inflammatory (TNBS) visceral hypersensitivity in rats	Immunomodulation
Bifidobacterium lactis	(1)	Inhibited PRS-induced visceral hypersensitivity in rats	Epithelial barrier regulation
Bifidobacterium longum and Lactobacillus helveticus	(4)	Reduced chronic stress-induced visceral hypersensitivity in mice	Regulation of hypothalamic-pituitary-adrenal axis

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Bifidobacterium infantis,Lactobacillus salivarius,Bifidobacterium breve	(79)	Reduced CRD-induced visceral pain behaviours in rats	None specified
Bifidobacterium infantis or Lactobacillus salivarius	(89)	*Bifidobacterium infantis* decreased visceral pain more than *Lactobacillus salivarius* or placebo in IBS patients	Immunomodulation
Lactibiane Tolerance®: Lactobacillus acidophilus, Lactobacillus plantarum, Lactobacillus salivarius Bifidobacterium lactis	(86)	Reversed visceral hypersensitivity induced by water-avoidance stress or IBS fecal supernatant administration in mice	Epithelial barrier regulation
VSL#3 Bifidobacterium (B. longum, B. infantis and B. breve); Lactobacillus (L. acidophilus, L. casei, L. delbrueckii ssp. bulgaricus and L. plantarum); and Streptococcus salivarius ssp. Thermophilus	(44)	Early life administration of VSL#3 reduced visceral pain perception in a model of IBS in rats	Altered colonic expression of genes influencing pain and inflammation
	(36)	Decreases acetic-acid-induced visceral hypersensitivity in rats	Epithelial barrier regulation
	(71)	Decreases acetic-acid-induced visceral hypersensitivity in rats	Immunomodulation
Faecalibacterium prausnitzii	(81)	Decreased colonic hypersensitivity induced by either NMS in mice or partial restraint stress in rats	Epithelial barrier regulation

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Figure 1: Microbial modulation of visceral afferent pathways

The figure illustrates potential mechanisms by which microbes in the gut lumen could modify afferent signaling from the gut to the central nervous system. The microbiota can affect the sensitivity of peripheral pain pathways by direct effects on the peripheral terminals of DRG neurons or indirectly by changing mediator release from enteroendocrine cells, immune cells or enterocytes. NTS: nucleus tractus solitarius, DRG: dorsal root ganglion, ENS: enteric nervous system, ECC: enterochromaffin cell, TLRs: Toll-like receptors.

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Figure 1

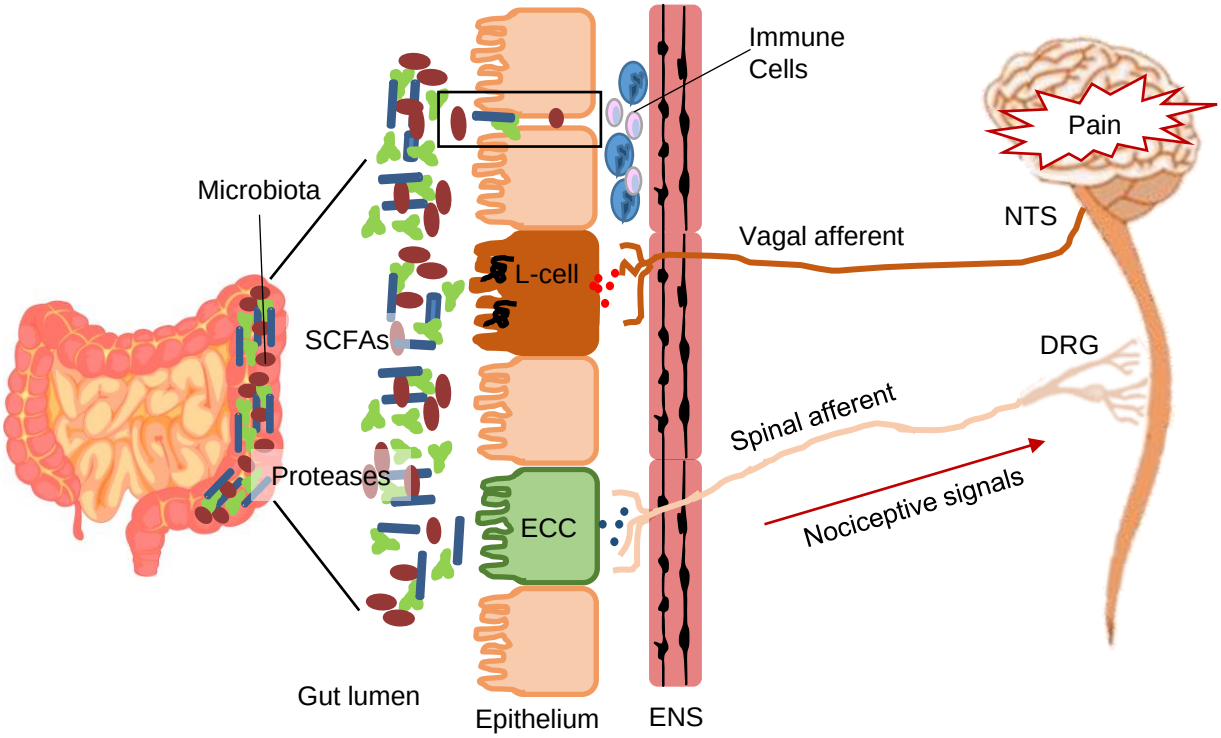

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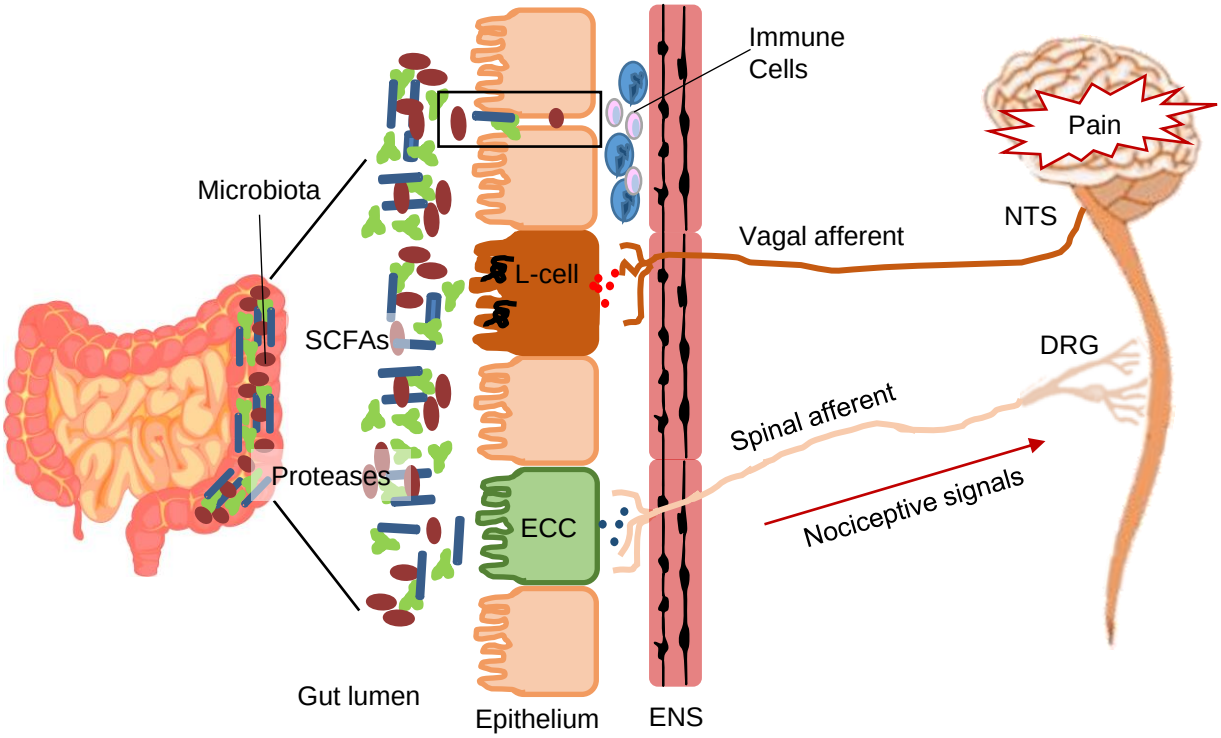
Figure 1



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