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1 2	Bacterial modulation of visceral sensation: mediators and mechanisms
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25 Abstract

26 The potential role of the intestinal microbiota in modulating visceral pain has received increasing 27 attention during recent years. This has led to the identification of signaling pathways that have 28 been implicated in communication between gut bacteria and peripheral pain pathways. In 29 addition to the well-characterised impact of the microbiota on the immune system, which in turn 30 affects nociceptor excitability, bacteria can modulate visceral afferent pathways by effects on enterocytes, enteroendocrine cells and the neurons themselves. Proteases produced by bacteria, 31 or by host cells in response to bacteria, can increase or decrease the excitability of nociceptive 32 33 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 34 chain fatty acids have pro-nociceptive effects in rodents but may be anti-nociceptive in humans. 35 36 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 37 complex interactions between visceral afferent neurons and the gut microbiota may lead to the 38 39 development of improved probiotic therapies for visceral pain.

41 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.

48 The perception of pain is accomplished by neural pathways that connect the gut to the 49 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 50 spinal cord. A subpopulation of these neurons, called nociceptors, detects noxious stimuli and 51 52 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 53 54 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 55 changes are changes in central nervous system (CNS) circuits that amplify synaptic inputs from 56 57 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 58 substances including endogenous opioids and cannabinoids (22, 124). This balance between pro-59 60 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 61 62 gut microbiota as an additional factor in pain modulation, capable of either worsening or 63 ameliorating pain (8, 88). Microbial modulation of visceral pain may have translational

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.

67

7 A potential role for gut bacteria in visceral pain signalling

The mutualistic relationship that has evolved between bacteria and eukaryotes includes 68 69 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, 70 there is a lack of mechanistic insight into which bacteria, bacterial metabolites, or signaling 71 72 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, 73 74 or mice treated with antibiotics that alter the microbiota early in life, have heightened pain 75 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 76 77 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 78 79 visceral pain accompanied by an increase in colonic myeloperoxidase activity, which is 80 indicative of immune system activation (126). This suggested a role for inflammatory changes in 81 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 82 83 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). 84

85 Bacteria as a source of host modulatory factors

86 There is a growing appreciation that the gut microbiota can be considered an endocrine 87 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 88 89 faecal matter from IBS patients is sufficient to evoke visceral hypersensitivity in gnotobiotic rats. 90 This is not due to changes in mucosal permeability or immune activation, raising the possibility 91 that bacterial metabolites in IBS patient stool directly modify gut-brain signalling (35). DRG 92 neurons are capable of "sensing" the presence of microbes. They express functional microbial pattern recognition molecules, including toll like receptors and nucleotide-binding 93 94 oligomerization domains 1 and 2 (91), whose activation can modulate neuronal excitability. Furthermore, the pathogenic bacterium *Staphylococcus aureus* directly excites DRG neurons 95 through a toxin that forms cation-permeable pores in DRG neuronal membranes and through 96 97 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 98 99 neuron excitability (88, 93, 109). Given the potential importance of the microbiota as a 100 modulator of visceral pain, identification of the specific species involved and mediators 101 responsible will be particularly important. Gut microbes produce a plethora of neuro-active 102 compounds such as proteases (116), short chain fatty acids (SCFA) (99) and also classical 103 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 104 105 of their capability to directly access and act upon nerve circuits to modulate their function (39, 106 88, 137). We will also discuss microbe-mediated modulation of visceral pain pathways by using 107 immune cells and enterocytes as cellular transducers (Figure 1).

108 Direct signalling by bacterial metabolites

109 *Proteases*

110 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, 111 112 neutrophils and enterocytes (97, 104). Recent in vivo and in vitro work has identified the gut 113 microbiota as an important source of proteases (116) capable of affecting peripheral pain 114 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 115 lack conventional ligand binding sites and are instead activated via protease-mediated hydrolysis 116 117 of amino acid residues. Upon protease cleavage, a tethered ligand within the receptor is revealed 118 that activates intracellular signaling pathways (97). The net effect of receptor signaling depends 119 not just on the PAR subtype involved but the specific amino acids hydrolysed (97). A consistent 120 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 121 122 and enterocyte derived trypsin-3 (85, 104) has been implicated in visceral pain (12, 63). 123 However, nociceptive neurons also express PAR-1 and PAR-4. Activation of PAR-1 and PAR-4 124 reduces DRG neuron excitability and is anti-nociceptive (10, 11, 66, 104). PAR-2 activation in 125 vivo by cysteine proteases in fecal supernatants from IBS patients enhanced the visceromotor 126 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 127 128 opposing effects of PAR-2, PAR-1 and -4 suggest that the balance between PAR-2, and PAR-1 -129 4 activation could be a critical determinant of nociception.

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

132 via actions on mucosal cells, immune cells or directly on DRG nerve terminals. The intestinal 133 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 134 135 the epithelial layer, intrinsic and extrinsic neurons relay neural information both within the GI 136 tract but also between the gut and the CNS. However, evidence that this communication system 137 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 138 least in some circumstances, the impact of PAR activation on visceral pain is due to modulation 139 140 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 141 results from neonatal maternal separation. In this case, the decrease in visceral pain was ascribed 142 143 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 144 visceromotor response to colorectal distention due to activation of PAR-4 (8). In a separate 145 146 study, serine proteases from *Faecalibacterium prausnitzii* acted directly on nerve terminals to 147 inhibit colonic sensory nerve spike discharge and reduced the excitability of colon-projecting 148 DRG neurons via PAR-4 activation (109). Furthermore, these proteases reversed DRG neuronal 149 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 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 postinflammatory 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).

169 Short chain fatty acids

Short chain fatty acids (SCFAs) are produced by the fermentation of dietary polysaccharides thatare 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
- 173 reports have identified Fecalibacterium prausnitzii, Eubacterium rectale, Eubacterium hallii and
- 174 *Roseburia faecis* as bacteria capable of producing butyrate. Likewise, acetate and pyruvate are
- 175 produced by enteric bacteria such as *Blautia hydrogenotrophica*; propionate, on the other hand,

176 can be produced by *Bacteroidetes* and *Firmicutes* (72).

177 A well-established effect of butyrate is inhibition of bowel inflammation and 178 enhancement of mucosal repair, which would have an indirect effect on inflammatory visceral 179 pain (103). SCFAs also modulate the enteric nervous system (113) and have been posited as an 180 important mediator of microbiota-gut-brain communication (88). Microbial dysbiosis, due to the 181 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 182 and visceral pain modulation but does not directly establish a causal relationship. Contrary to 183 these studies, when SCFAs were administered to control rats and rats with TNBS-induced colitis, 184 185 visceral hypersensitivity was not improved by any of the SCFAs (acetate, propionate and 186 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 187 188 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 189 190 associated with neuronal activation of extracellular signal related kinase (ERK)1/2 and an 191 enhancement of DRG neuronal excitability. However, a study of healthy human volunteers 192 concluded that butyrate treatment induced a dose-dependent reduction of visceral sensitivity 193 (125). In summary, despite evidence implicating SCFAs in mediating gut-brain communication 194 in general, there are conflicting findings regarding the role of SCFAs in modulating visceral 195 pain.

196 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

brain function and expression of central neurochemicals (15, 23) however, antibiotic treatment in 200 201 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 to secrete functional neurotransmitters and neurotrophins. GABA, the major inhibitory 212 213 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 Escherichia (94, 129). BDNF, an important neurotrophic regulator of synaptic plasticity and 218 219 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

223 hypersensitivity (42). In IBS patients, increased expression of nerve growth factor (NGF) 224 correlated with visceral pain sensitivity (134), which may be due to sensitization of pro-225 nociceptive receptors on primary afferent neurons. Indeed, NGF increases TRPV1 expression in 226 DRGs (110). In the context of microbial modification of host molecules, an *in vitro* study 227 demonstrated that Lactobacillus rhamnosus induces anti-inflammatory effects in human epithelial cells which is mediated by NGF (75). Although intriguing, evidence that gut bacteria 228 229 have the capacity to secrete neurotransmitters and neurotrophins, does not explain how 230 neuromodulatory molecules in the external environment of the gut lumen can modify gut-to-231 brain nociceptive signalling. As afferent nerves do not reach through the epithelium into the gut 232 lumen, further mechanistic studies are needed to determine how bacterially-derived 233 neuromodulatory factors can cross the gut barrier to influence gut-brain signalling.

234

235 Indirect signaling

236 Serotonin secretion from Enterochromaffin cells

237 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 238 action are important therapeutic targets for visceral pain. Two recent independent reports 239 240 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, 241 242 compared to germ-free mice (99). This was associated with an increase in expression of 243 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, 244 245 sodium acetate and sodium butyrate, increased TPH1 expression in a human-derived EC cell

246 line. The second study identified spore-forming bacteria as important modulators of serotonin 247 production by EC cells, and revealed that this effect occurred in the colon but not the small 248 intestine (135). Furthermore, EC cell serotonin modulation by microbiota was also observed in 249 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 250 251 cell function, which may be an important mechanism of pain modulation by microbiota. Other 252 bacterial metabolites, such as bile acids and p-aminobenzoate, have also been implicated in 253 regulating serotonin production. From these findings it appears that several bacterial signaling 254 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 255 256 and colleagues have recently (69) demonstrated that host-derived serotonin has direct and 257 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 258 259 inhibited by serotonin (69). These findings illustrate the complex and bidirectional nature of the 260 interactions between gut microbes and enterochromaffin cells.

261 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

269 introduction of specific commensal strains (9, 118) or antibiotic treatment (61) increased GLP-1 270 levels. Somewhat counter-intuitively, one study determined that serum GLP-1 was also elevated 271 in germ-free mice (108), although other researchers found that germ-free mice exhibited a strong 272 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-1 273 274 mimetic reduced acute abdominal pain in patients (57). GLP-1 can act as a classical endocrine 275 hormone, however GLP-1 also has direct neurostimulatory actions on vagal afferent neurons 276 (78). Furthermore, there is evidence of direct, physical contact between a pseudopod-like 277 elongation of L-cells and afferent nerve fibres (18), providing for a potential neural signalling 278 pathway in the modification of GI function. Thus, L-cells are appropriately positioned to 279 facilitate cross-barrier signalling from the gut lumen to the host peripheral nervous system and 280 on to the CNS, and should be investigated as a potential modulator of visceral pain.

281 Histamine release from mast cells

282 Histamine, which is mainly secreted by mast cells, promotes allergic inflammation but also 283 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, 284 285 and are thus likely to participate in relaying visceral pain signals (13). Of the four histamine 286 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), 287 288 whereas H2R suppresses inflammation (111). In patients with IBD, reduced expression of H2R 289 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 290 291 visceral hypersensitivity (115). Moreover, IBS patient biopsies display increased expression of

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.

295 Vagal afferent pathways

296 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 297 298 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 299 300 noted in a rat model of visceral pain where vagal nerve stimulation had an anti-nociceptive effect 301 (138). Vagal afferent terminals are located within enteric ganglia, and in the smooth muscle and 302 mucosal layers, where they are well-positioned to sense chemo-nociceptive signals (70, 95, 130). 303 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 304 305 impacts visceral pain.

306 Conclusions

There is abundant evidence that the microbiota is capable of modifying visceral pain in vivo. 307 However, clinical trials of probiotics as therapies for visceral pain have yielded equivocal results. 308 309 This may reflect patient heterogeneity, patient compliance, or the variety of probiotic 310 formulations used, which is in turn reflects a relative paucity of mechanistic work identifying the 311 most important microbial species and mediators to target for clinical benefit. A number of issues 312 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 313 314 threshold noxious stimuli in rodents is accomplished by visceral afferent neurons with terminals

315 that lie along serosal and mesenteric blood vessels (25). Furthermore, based on a limited number 316 of recordings from visceral afferent neurons from human bowel, the majority of afferent 317 terminals that have been characterized to date have been located in the muscle and vasculature. 318 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 319 320 of full-thickness resected bowel preparations from patients may provide insight into how the 321 luminal microbiota accesses these terminals. Another potential caveat when translating findings 322 from rodents to patients is that signaling mechanisms that are inhibitory in rodents may be excitatory in patients, and vice versa. A recent Ca²⁺ imaging study of PAR activation in human 323 324 DRG neurons reported that PAR-1 activation in human neurons is excitatory (43), whereas PAR-325 1 is inhibitory in rodents (10). By increasing mechanistic insights into the interplay between 326 the microbiota and peripheral pain pathways, particularly using patient microbiota and human 327 DRG neurons (59), improved therapies that harness the analgesic properties of the microbiota 328 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
	(126)	Normalized visceral sensitivity to CRD in antibiotic treated mice in mice	Immunomodulation
Lactobacilius paracasei	(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

Bifidobacterium infantis,Lactobacillus salivarius,Bifidobacterium breve	(79)	Reduced CRD-induced visceral pain behaviours in rats	None specified
Bifidobacterium infantis or Lactobacillus salivarius	(89)	<i>Bifidobacterium infantis</i> decreased visceral pain more than <i>Lactobacillus salivarius</i> 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):	(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
Lactobacillus (L. acidophilus, L. casei, L. delbrueckii ssp. bulgaricus and L.	(36)	Decreases acetic-acid-induced visceral hypersensitivity in rats	Epithelial barrier regulation
plantarum); and Streptococcus salivarius ssp. Thermophilus	(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

793 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.

Figure 1



Figure 1

