Visceral Pain and Gastrointestinal Microbiome

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A complex set of interactions between the microbiome, gut and brain modulate responses to visceral pain. These interactions occur at the level of the gastrointestinal mucosa, and via local neural, endocrine or immune activity; as well as by the production of factors transported through the circulatory system, like bacterial metabolites or hormones. Various psychological, infectious and other stressors can disrupt this harmonious relationship and alter both the microbiome and visceral pain responses. There are critical sensitive periods that can impact visceral pain responses in adulthood. In this review we provide a brief background of the intestinal microbiome and emerging concepts of the bidirectional interactions between the microbiome, gut and brain. We also discuss recent work in animal models, and human clinical trials using prebiotics and probiotics that alter the microbiome with resultant alterations in visceral pain responses.

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Key Words
Microbiome; Prebiotics; Probiotics; Visceral pain

Introduction

Visceral pain resulting from distension, ischemia or inflammation of abdominal viscera is mediated through peripheral pathways and the central nervous system. Factors that modulate each individual’s perception of a stimulus as pain are beginning to be unraveled. Among these, emerging data show that there is an interaction between the intestinal microbiome and pathways mediating visceral pain. The absence of gastrointestinal (GI) bacteria, such as which occurs in germ free (GF) mice, is associated with reduced perception of pain following different inflammatory stimuli.1 Furthermore, modulation of the intestinal microbiome by administration of various probiotics also has been shown to alter pain responses.2,3 This review provides a brief background of the intestinal microbiome and emerging concepts of the bidirectional interactions between the microbiome, gut, and brain. Because changes in early life have been shown to impact lifelong visceral pain responses, further details are provided regarding both development of the microbiome and the impact of early stress on the microbiome. Finally, we discuss recent work in animal models and human clinical trials that show how agents altering the microbiome has potential therapeutic value to modulate visceral pain responses, and suggest that these effects may be...
helpful in prevention of long-term sequelae of pain experienced early in development.

The Intestinal Microbiome

Modern molecular techniques have allowed a deeper understanding of the development, metabolic activity and potential interactions between the GI microbiome and the host organism. In the adult the gut microbiota is composed of $10^{13}-10^{14}$ microorganisms, and contains 100 times as many genes as the whole human genome. The composition of the microbiota varies according to the region of the GI tract. Gram-positive facultative anaerobic bacteria predominate in the proximal small intestine, while gram-negative anaerobes do so in the distal small intestine. In the colon, obligate anaerobes outnumber facultative anaerobes. The microbiota produce a large range of metabolic substances that vary among the different bacteria composing each individual’s microbiome, and with the substrates supplied in the diet. These include endogenous vitamins such as folate and biotin; short chain fatty acids such as propionate, butyrate, and acetate, and a variety of neuroactive metabolites such as serotonin and gamma-butyric acid.

Development of the Gastrointestinal Microbiome

The gut is colonized by bacteria from the moment of birth, rapidly achieving concentrations of up to $10^{12}$ organisms per gram of luminal contents in the colon. Many factors influence the composition of the infant gut microbiota and the potential functional outcomes following colonization (reviewed in Borre et al). For example, infants born by vaginal delivery are colonized by the maternal microbiome (eg, fecal and vaginal bacteria), whereas infants born by cesarean delivery are exposed to different commensals from the skin and hospital environment. Infant diet also impacts the microbiome such that the diet of the breast fed infant differs from those fed with infant formula. The gut microbiome contributes to the early programming of epithelial barrier function, angiogenesis, and innate and host immune function. Delayed intestinal bacterial colonization such as occurs in GF mice can have prolonged, lifelong influence on the immune system with aberrant development of the innate immune system and altered immunoregulatory responses later in life. Similarly, in humans, alterations in the pattern of GI colonization are thought to have long-term consequences on immune function.

The brain may also modulate the composition of the gut microbiota. Restraint stress is shown to disrupt the microbiome in mice leading to an increase in colonization by *Citrobacter rodentium*, possibly by altering the local mucosal microenvironment, so that bacterial adherence patterns change. More recently, it was demonstrated that exposure to as little as 2 hours of a social stressor (placement of a young C57BL/6 mouse in a cage with an

Microbiota-Gut-Brain Axis

The microbiome can influence both peripheral and central neurological activity by a variety of mechanisms (Figure). It has long been recognized that the presence of bacteria within the GI lumen can influence myoelectric activity. The introduction of bacteria to GF rats increases slow wave frequency and promotes aboral propagation of the migrating motor complex. Bacterial-derived endotoxins, such as lipopolysaccharides, alter gut motility by activating the enteric nervous system, and bacterial derived peptides such as formyl-methionyl-leucyl-phenalanine have been shown to stimulate primary afferent nerves. Some bacterial derived products may have direct effects on enteric nerves but epithelial cell interactions may also be required for activity. For example, microvesicles derived from *Bacteroides fragilis* increase excitability of myenteric intrinsic primary afferent neurons when applied to the mucosal surface but have no effect when applied directly to the myenteric plexus neurons indicating that bacteria or their components may communicate with local neurons indirectly through signals generated in the epithelium.

Bacterial products have also been shown to have profound effects on behavior. In mice, maternal immune activation (MIA) with the viral mimic poly I:C (polyinosinic-polyycytidlic acid) during pregnancy yields offsprings that exhibit behavioral symptoms of autism. Treatment with *B. fragilis* ameliorates some of these behavioral changes in the offspring. Identification of changes in the serum metabolome associated with such treatment led to the identification of several bacterial metabolites that could explain this effect. Administration of one candidate bacterial metabolite, 4-ethylphenylsulfate to naive mice induced anxiety-like behaviors, similar to those in the mice from the MIA mothers. This suggests that circulating bacterial metabolites may mediate changes in behavioral state.

Children born by caesarean section or who receive antibiotics during infancy have a higher incidence of allergy. The infant transitions towards a more typical adult microbiome with weaning and by 2 years of age the microbiome is similar to that of adults.
This figure illustrates the interactions between microbiome, gut and brain which modulate responses to visceral pain. These interactions occur at the level of the gastrointestinal mucosa, and via local neural, endocrine or immune activity, as well as by the production of factors transported through the circulatory system, like bacterial metabolites or hormones. Endocrine Factors: in germ free mice, chemical changes were associated with an exaggerated hypothalamic pituitary stress response, eg, elevation of plasma adrenocorticotropic hormone and corticosterone. Immune Pathway: the intestinal microbiota secretes factors that alter the mucosal permeability and macrophage release of IL-10. Neural Pathway: while the visceral pain results from the activation of nociceptors in the abdominal viscera, the visceral nociceptive afferent fibers further project onto spinal nociceptive neurons located in the superficial laminae, the lateral neck of the dorsal horn and lamina X of spinal cord that convey information to supraspinal centers. Gut: the microbiota produces a large range of metabolites which include short chain fatty acids (SCFA) and a variety of neuroactive metabolites such as serotonin. It is hypothesized that hydrogen and hydrogen sulfide may be bacterial metabolites responsible for visceral hypersensitivity. For example, in mice, hydrogen sulfide directly triggers visceral nociceptive behavior through sensitization and activation of T-type channels in the primary afferents.
aggressive older CD-1 mouse) altered composition of the colonic mucosa-associated microbiota.\textsuperscript{26} The mechanisms causing these changes are yet to be elucidated but such a rapid change is likely mediated by changes in bacterial adhesion, mediated by either endocrine or neuronal effects on the GI mucosa (Figure).

In summary, there are complex interactions between the microbiome, gut and brain increasingly discussed in the context of the “microbiota-gut-brain axis.” Bidirectional communications occur where the microbiota influences the host and the host alters microbiota composition. These interactions are modulated at the level of the GI mucosa, and via the modulation of local neural, endocrine, or immune activity; as well as by the production of factors transported through the circulatory system (Figure).

**Microbiome and Neurodevelopment**

The early neonatal period is a critical time for the development of the nervous system, including the enteric nervous system.\textsuperscript{27,28} Recent studies comparing the development of the enteric nervous system in GF mice and specific pathogen-free mice suggest that the intestinal microbiota plays an important role in shaping this process.\textsuperscript{9} In GF mice the myenteric plexus of the jejunum and ileum show a decrease in nerve density and the number of neuronal cell bodies per ganglion but an increase in nitricergic neurons. The frequency and amplitude of muscle contractions also were fewer in GF mice.\textsuperscript{29} GF mice also differ from conventional mice in the expression of brain-derived neurotrophic factor in the cortex and hippocampus, and synaptophysin and post-synaptic density protein-95 in the striatum, relative to specific pathogen free mice. These quantifiable chemical changes were associated with an exaggerated hypothalamic pituitary stress response (elevation of plasma adrenocorticotropic hormone and corticosterone) in the GF mice. Reconstitution with *Bifidobacterium infantis* during early development, but not later in development, lessened these differences. In contrast, the reconstitution with *Escherichia coli* enhanced the stress response.\textsuperscript{30,31} This suggests that during the early neonatal period, there is a critical window at which the microbial colonization of the GI tract influences the development of both the peripheral and central nervous system.

As in older animals, stressor exposure early in life alters the types and abundance of bacteria found in the intestines. The stress of separating infant monkeys from their mothers reduces the number of total fecal lactobacilli.\textsuperscript{32} Similarly, separation of rat pups from their mothers during the first 14 days of life alters the GI microbiome.\textsuperscript{33} These changes in microbiome may be associated with exaggerated visceral pain responses that persist through adulthood in rats following maternal separation.\textsuperscript{33}

**Visceral Pain During Development Alter Pain Responses in Adulthood**

Visceral pain results from the activation of nociceptors in the abdominal viscera. Visceral nociceptive afferent fibers project onto spinal nociceptive neurons located in the superficial laminae, the lateral neck of the dorsal horn and lamina X of spinal cord that convey information to supraspinal centers (Figure).\textsuperscript{34} Brain regions that generate pain perception and modulate response to painful stimuli through descending inhibition at the spinal level include the cingulate cortex, medial thalamus, amygdala, hypothalamus, periaqueductal gray, and the solitary tract.\textsuperscript{35}

Visceral hypersensitivity refers to a decreased pain threshold following nociceptor activation, or to an exaggerated response to the painful stimulus. The mechanisms underlying this increased responsiveness might include (1) sensitization of primary sensory afferents innervating the viscera, (2) hyperexcitability of spinal ascending neurons (central sensitization) receiving synaptic input from the viscera, (3) dysregulation of descending pathways that modulate spinal nociceptive transmission, and (4) changes in the central perception of a painful stimulus.\textsuperscript{36}

As with the development of the microbiome, the early neonatal period is a key critical sensitive period for the development of the neural nociceptive pathways and sensory nerves. Stress experienced in early life triggers long-term changes in visceral sensitivity to noxious stimuli. A well-established experimental model of visceral hypersensitivity utilizes moderate periods of maternal separation as a stressor in neonatal rats. As adults, the stressed animals demonstrate increased visceral hypersensitivity as assessed by the response to colorectal distension.\textsuperscript{37} Similar findings of visceral hypersensitivity are observed in adult rats following recurrent neonatal somatic pain such as that induced by acid injection into a muscle or nasogastric suctioning. The adult pain hypersensitivity can be blocked by preemptive administration of glutamate receptor antagonists (given at the time of pain induction) in the case of acid injection,\textsuperscript{38} or corticotrophin releasing factor 1 antagonist in case of nasogastric suctioning.\textsuperscript{39} These findings and many others indicate that the changes in visceral pain responses induced during infancy can be modified by changing the neurochemical milieu during the painful experience.
Alterations in the Microbiome and Visceral Pain Responses in Animal Models

Animal models have been useful to demonstrate potential mechanisms by which the microbiome can modulate visceral pain responses. In GF mice, contact with commensal microbiota is necessary for mice to develop pain sensitivity, possibly in a Toll-like receptor (TLR)-dependent manner. Inflammatory hypernociception induced by diverse stimuli, including lipopolysaccharide (LPS) and Interleukin (IL)-1β is reduced in GF mice. The intestinal microbiota secrete factors that among other actions, alter the mucosal permeability, macrophage release of IL-10, T-regulatory cell differentiation, dendritic cell release of transforming growth factor beta (TGF-β) and other cytokines, and induction of Th17 cells. It is known that immune cell secretions including cytokines alter visceral pain responses. For example, administration of the cytokine inhibitor, diacerein, reduces acetic acid-induced nociception in mice, while inhibiting production of IL-1β and tumor necrosis factor alpha (TNF-α). Thus, it is reasonable to assume that some of the effects of the microbiota on visceral pain responses are mediated via microbiota-neuroimmune interactions. Administration of antibiotics to mice for 2 weeks attenuated the visceral pain-related responses to intraperitoneal acetic acid or intra-colonic capsaicin. These changes were associated with increased levels of secretory IgA, upregulation of the antimicrobial peptide resistin-like molecule beta and TLR5, and upregulation of the cannabinoid 1 receptor and downregulation of the mu-opioid receptor. Thus, alterations in the gut microbiota are associated with changes in a variety of pain-related pathways. Changes in mucosal permeability, mucosal immune system composition and the microbiome populations are also described in humans with irritable bowel syndrome (IBS) suggesting that microbiota-neuroimmune interactions play a key role in some visceral pain syndromes.

Administration of probiotic bacteria has impact on neuronal excitability and motility in animal models. Administration of Lactobacillus rhamnosus increases enteric neuron excitability and modulates colonic motility in rodents. Moreover, it has been suggested that Lactobacillus farciminis exerts antinociceptive effects by altering central sensitization. McKernan and colleagues compared the efficacy of 3 probiotics (Lactobacillus salivarius UCC118, B. infantis 35624, and Bifidobacterium breve UCC2003) on the abdominal response to colorectal distension using visceral normosensitive rats (Spargue-Daweley) and visceral hypersensitive rats (Wistar Kyoto). In that study, B. infantis 35624 reduced the colorectal distension-induced pain behavior in both rat strains. Interestingly, in a clinical trial, comparing an 8 week treatment by B. infantis 35624 and L. salivarius UCC118 in IBS patients, only B. infantis 35624-treated patients experienced a reduction in composite and individual scores for abdominal pain and discomfort, bloating and distension. Lactobacillus spp. was implicated in the modulation of visceral pain; for example, it has been shown that excitability of dorsal root ganglia in response to colorectal distension is prevented by L. rhamnosus treatment. In another study, treatment with Lactobacillus species upregulated cannabinoid receptor 2 expression in rats and mice, leading to the induction of visceral analgesia.

Bravo et al have reported that the vagus nerve is possibly the major modulatory nervous pathway between the probiotic bacteria in the gut and the brain (Figure). Other studies have shown that anxiety-related behavior was reduced after probiotic treatment, as long as vagus nerve integrity was maintained. In the study by Bercik and colleagues mice with experimentally induced chronic colitis showed anxiety-like behavior. Treatment with Bifidobacterium longum abolished such behavior. However, the anxiolytic effect of B. longum was absent in vagotomized mice, suggesting that the effect was transmitted to the central nervous system by activating vagal pathways at the level of the enteric nervous system. In addition, a recent experimental study demonstrated that L. rhamnosus effects on emotional behavior and the central gamma-aminobutyric acid (GABA)-ergic system in mice are regulated via the vagus nerve. Stress causes a release of central corticotrophin-releasing factor which stimulates the vagal efferents that alter intestinal permeability and gut motor function. These observations support a view that the gut microbiota play a fundamental role in modulating visceral pain responses via neural, immune, and endocrine interactions (Figure).

Altered Microbiome During Early Life Critical Sensitive Periods Impacts Visceral Pain in Adulthood

As discussed above, there are critical sensitive periods that can impact visceral pain responses in adulthood. Antibiotics profoundly alter the GI microbiota, and different antibiotics may result in dissimilar patterns of dysbiosis. Aguilera and colleagues studied the impact of broad spectrum antibiotics (bacitracin/neomycin) on the visceral hyperalgesia observed after psychological stress induced by water avoidance in mice. Antibiotic treatment
was associated with a decrease in luminal bacteria, and a dysbiosis with increased bacterial adherence. There was an upregulation of cannabinoid receptors 1 and 2, and a reduction in visceral hypersensitivity suggesting one possible mechanism by which microbial alterations can blunt the visceral pain response. In non-stressed animals, antibiotics had no effects on visceral pain responses. Verdú et al demonstrated that in rodents, antibiotic treatment was associated with increased inflammation in the colon which was also associated with increased substance P immunoreactivity, and visceral hypersensitivity. Disruption of the microbiome during early life by administration of vancomycin for postnatal day 4-13 led to visceral hypersensitivity to colorectal distension in the adult rats, even though the dysbiosis had resolved. Interestingly, vancomycin treatment did not impact cognitive or anxiety-related behaviors, growth or other parameters in the adult animals. Recently, Kannampali and colleagues used the rat chronic visceral hypersensitivity model to test the effect of probiotics and prebiotics on ameliorating the severity of neonatal stress-induced hypersensitivity. In that model, introduction of zymosan into the colon during the neonatal period produces short-term inflammation and subsequent long-term colonic hypersensitivity. Those researchers reported that L. rhamnosus ATCC 53103 (Lactobacillus GG; LGG) attenuates chronic visceral hypersensitivity after animals were exposed to early life painful stimulus. The prebiotic mix (galactooligosaccharides and polydextrose) also expressed significant analgesic effect, but to a less extent compared to LGG. Furthermore, LGG was found to alter the levels of brain neurotransmitters, like serotonin, noradrenaline, and dopamine which are known to be involved in pain modulation. In a study by Kamiya et al, treatment with live and killed Lactobacillus reuteri prevented the pain response to colorectal distension by decreasing of the dorsal root ganglion single unit activity to distension. In another study, L. reuteri was found to reduce sensation of pain via the enteric nerve in a model of visceral pain induced by colorectal distension. A decrease of normal visceral perception and chronic colonic hypersensitivity, elicited by butyrate was also observed after an oral treatment by L. acidophilus NCFM.

Alterations of the Microbiome in Visceral Pain Disorders

IBS is characterized by chronic abdominal pain and discomfort. Growing evidences suggest that IBS patients have a dysbiotic intestinal microbiota. Approximately 8% of children experience recurrent functional abdominal pain and about 61% of these children continue to report abdominal pain or IBS. In childhood, recurrent abdominal pain (RAP) consists of pain symptoms similar and often indistinguishable from those in IBS suggesting that there are similar underlying pathophysiologies. Although infantile colic has not been linked directly to visceral pain, it is generally assumed that the correlation exists.

There are several possible etiologies for the sensory abnormalities in IBS, RAP and colic such as the receptors in the gut wall, the primary sensory afferent neurons, the spinal cord and the brain itself. The developmental timing at which these alterations in responsiveness appear (ie, during infancy, childhood, and adulthood) remains unclear but many IBS patients have histories of early life stressors. The link between IBS and increased intestinal sensitivity has been previously described and visceral hypersensitivity and visceral pain are the important pathophysiologic factors in IBS. These factors are abnormal in patients suffering from IBS and about 35% of the IBS patients have chronic pelvic pain. Mayer et al reported that IBS patients exhibit increased activation of brain regions that are linked to perception of rectal distension and Mertz et al showed differences in brain activation patterns in response to a painful rectal stimulus in IBS patients compared to controls. Many studies have also shown that those with IBS have amplified visceral sensitivity in response to stress or food intake. For example, patients with IBS had enhanced modulation of visceral perception when subjected to auditory or mental stress. Patients with IBS frequently have accompanying psychological disorders, such as anxiety or depression, and those with psychological stress are more likely to develop post-infectious IBS. Abnormal bowel gas accumulation may be linked to bacterial metabolism and abdominal bloating and may be further associated with visceral hypersensitivity and the impaired gas handling observed in some IBS patients. For example, Serra et al reported that IBS patients have impaired transit and altered tolerance of intestinal gas. Koide and colleagues demonstrated excessive bowel gas volume among IBS subjects; however, in their symptoms did not correlate with abnormal bowel gas accumulation suggesting that gas production is not the only factor impacting symptom elicitation.

IBS patients show an altered profile of gut microbiota composition. Earlier studies found that the intestinal microbiota in IBS patients differs from that in healthy individuals, with a consistent decrease in the Bifidobacterium spp. population and an increase in the Enterobacter population. Other studies in patients with IBS have shown alterations in the microbiota, such as an increased ratio of Firmicutes to Bacteroidetes and a reduction in Lacto-
bacillus species. Symptoms of IBS may be linked to those alterations. Similarly, in children with IBS the microbiome differs from normal children with a greater percentage of class gammaproteobacteria and a novel Ruminococcus-like microbe being found in the IBS patients. Recent research has focused on the role of gut microbiota in the pathophysiological pathway to infant colic, with numerous studies revealing differences in the gut microbiota between infants with and without colic. One study of L. reuteri ATCC 5573022 and two studies of L. reuteri DSM 17938. In breastfed infants with colic were effective, but a subsequent study of both breastfed and formula-fed infants with colic indicated L. reuteri DSM17938 to be ineffective. Thus, the effectiveness of pre- or probiotic therapies may vary depending upon the diet and other poorly defined factors including the pre-therapy microbiome composition.

Treatment trials for IBS with probiotics have had varied results which are likely explained by the diversity of study populations, antibiotics or probiotics used, and the small size of most studies. Treatment with the probiotic L. acidophilus SDC 2012 for 4 weeks improved symptoms scores among IBS patients when compared to placebo treatment. Treatment of IBS with E. coli DSM 17252 for 8 weeks was reported to dramatically reduce abdominal pain. L. plantarum 299v has shown reductions in abdominal pain in 2 intervention studies. Whorwell and colleagues reported that B. infantis 35624 improved pain in 437 subjects with IBS symptoms. Treatment with the antibiotic rifaximin appears to benefit some patients with IBS but the population that benefits and efficacy of antibiotic treatment remains controversial. A study by Jeffery and colleagues showed dysbiosis (e.g. increased Firmicutes/Bacteroides ratio) in a subgroup of IBS patients, while the remaining patients had normal-like microbiota composition. In that study, microbiota analysis identified several clear associations with clinical data; however a distinct subset of IBS patients with alterations in their microbiota did not correspond to IBS subtypes. It is thus possible that normal-like microbiota group might be less responsive to the agents aimed at modulation of the microbiota and individualized characterization of the GI microbiota may direct treatment strategies in the future.

The mechanisms by which altering the microbiome in the humans modifies visceral pain response remain underdetermined but likely are similar to those described in animal models of visceral pain as discussed above. In a recent study of healthy women, consumption of a mixture of probiotic bacteria in a fermented milk product affected the activity of brain regions that control the central processing of emotion and sensation. The ingestion of the ferment milk product had no discernible effect on microbiota composition in the study suggesting that the effects on central nervous system were either induced by altered vagal afferent signaling or by systemic metabolic changes related to probiotic intake. IBS subjects have been shown to have higher concentrations of Firmicutes and decrease of Bacteroides-associated taxa which are butyrate producers. The abundance of Faecalibacterium species, which produce butyrate was reduced in IBS patients. Butyrate has been shown to decrease rectal pain perception in healthy individuals so it is possible that reductions in butyrate producing bacteria increases pain responses in IBS subjects.

One of the most fascinating observations used the GF animal model to evaluate the effect of the human IBS microbiome on pain responses. Crouzet et al inoculated GF rats with IBS fecal suspension and showed an increased visceral sensitivity in response to colorectal distension compared with GF rats inoculated with fecal suspensions from healthy control group. In the same study, GF rats inoculated with IBS microbiota showed abnormal gut fermentation with increased hydrogen excretion and sulfides production vs controls. This led the authors to hypothesize that hydrogen and hydrogen sulfide may be bacterial metabolites responsible for visceral hypersensitivity. They note that, in mice, hydrogen sulfide directly triggers visceral nociceptive behavior through sensitization and activation of T-type channels in the primary afferents. Further work using combinations of bacterial metabolic pathway analysis and metabolomic analysis are likely to yield an increased understanding of the underlying mechanisms by which the microbiome may alter visceral pain responses in humans.

Summary

The microbiome, gut and brain have a complex set of interactions that modulate responses to visceral pain. Various psychological, infectious and other stressors can disrupt this harmonious relationship and alter both the microbiome and visceral pain responses. Various approaches (probiotics and prebiotics) to restoring a less pathogenic microbiome appear to have promise to treat functional bowel disorders. It is possible that better management of dysbiosis during early life may prevent the development of life long changes in pain responsiveness. However, further research to better define the underlying mechanisms by which these effects are mediated is needed. This will likely lead to improved approaches for treating visceral pain by modifying the micro-
biome or preventing chronic pain by preemptive maintenance of a healthy microbiome.

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