INTRODUCTION

Irritable bowel syndrome (IBS) is characterized by frequent abdominal pain, often related to altered defecation patterns. This functional bowel disorder affects 10–20% of the general population and presents without gross abnormalities within the gastrointestinal tract, such as inflammation or structural defects (16). Pain is the major indicative symptom in the diagnosis of IBS, per the Rome IV diagnostic criteria: “recurrent abdominal pain on average at least one day per week in the last three months” (50). In addition, patients often display psychological comorbidities. It is widely acknowledged that (early life) stress contributes to the etiology of IBS (37). The pathophysiology of IBS is not fully understood; yet, visceral hypersensitivity of the lower gastrointestinal tract, i.e., an increased response to colorectal distension, frequently prompts episodes of debilitating abdominal pain in irritable bowel syndrome (IBS). Although the pathophysiology of IBS is not yet fully elucidated, it is well known that stress is a major risk factor for development and acts as a trigger of pain sensation. Stress modulates both immune responses as well as the gut microbiota and vice versa. Additionally, either microbes themselves or through involvement of the immune system, activate or sensitize afferent nociceptors. In this paper, we review current knowledge on the influence of stress along the gut-brain-microbiota axis and exemplify relevant neuroimmune cross talk mechanisms in visceral hypersensitivity, working toward understanding how gut microbiota-neuroimmune cross talk contributes to visceral pain sensation in IBS patients.

Brain-gut-microbiota; irritable bowel syndrome; microbiome; stress

Microbiota-neuroimmune cross talk in stress-induced visceral hypersensitivity of the bowel

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brain and gut is influenced by interacting microbiota and immune responses (Fig. 1), which further complicate research on the role of each of the respective elements in IBS patients.

In this review, we aim to provide a brief overview of the role of stress in bidirectional communication within the brain-gut-microbiota axis known thus far and discuss the connecting role of stress in bidirectional communication within the brain-gut-microbiota axis. We will address modulation of immune responses, pain perception, and microbiota under the influence of stress; in the second part, we will outline the influence of the gut microbiota on psychological symptoms and pain perception.

**Descending Interactions: Influence of Stress Along Gut-Brain-Microbiota Axis**

Psychological stress plays a crucial role in IBS through alterations of intestinal physiology. Stress is commonly defined as physical or emotional occurrences that cause mental or physical deviations from what is considered to be ordinary. Under normal conditions, the brain can inhibit ascending nociceptive afferent pathways by activation of descending inhibitory pathways. This endogenous pain inhibition, assessed by using conditioned pain modulation paradigms, is significantly diminished in IBS (3). Because a possible correlation with stress was only addressed in a limited set of studies (47), we refer to two recent IBS-focused meta-analyses for further details on the possible role of such descending pain-modulating pathways (3, 47).

Since (early life) stress is regarded as an important trigger for visceral hypersensitivity in IBS (65), this is frequently employed in IBS-like animal models: the maternal separation model relies on neonatal stress, although other models (e.g., restraint stress, chronic and repeated stress) employ stress at the adult age (44). In these models, the animals display stress-related IBS-like symptoms, including visceral hypersensitivity, motility disturbances, and anxiety-like behaviors. Upon stress, many crucial mediators are released, including corticotropin-releasing factor (CRF), adrenocorticotropic hormone (ACTH), and cortisol (or corticosterone in rodents) (60, 67). The hypothalamic-pituitary-adrenal (HPA) axis is hyperresponsive in IBS-like rodent models, reflected through increased serum corticosterone levels in animals which underwent neonatal maternal separation with respect to their non-handled controls (2, 44). Although hyperactivation of the HPA signaling between the central nervous system (CNS), the enteric nervous system (ENS), immune cells, and the microbiota (24). Direct extrinsic control over the intestine is exerted through the autonomic nervous system, maintaining physiologic functioning of the gut through modulation of secretory factors and motility. The distal colonic segment and rectum are innervated by the inferior mesenteric ganglia and pelvic ganglia, respectively. Moreover, the autonomic nervous system connects with the intrinsic nervous system of the intestine, the ENS, and is thereby also able to influence immune modulation in the intestinal wall (extensively reviewed in Ref. 12). Sensory afferent neurons connect the gut to the brain to relay information on gut luminal contents to the CNS. Vagal afferents reside in the nodose/jugular ganglia and signal to the brain stem, being responsible for nutrient sensation and physiological signaling; spinal afferents reside in the dorsal root ganglia (DRG) and signal to the spinal cord, responding to mechanical, thermal, and noxious stimuli to mediate pain signaling (13, 36).
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axis is clearly displayed in these rodent models, there is no consensus whether this is an accurate reflection of IBS patients. Even though IBS prevalence is higher for women than for men, the majority of animal investigations are restricted to males, only to avoid effects of the estrous cycle. Because of this practice, the translational value of preclinical HPA-axis results is questionable. In the largest study characterizing the HPA axis in IBS patients thus far, intravenous administration of ACTH showed increased cortisol response in men, but blunted response in women compared with sex-matched healthy volunteers (75). These results do not support the hypothesis that IBS is associated with an augmented HPA axis response, and also emphasize that restricting preclinical IBS studies to males should be avoided whenever possible.

The molecules involved in the HPA axis have widespread effects on the gastrointestinal tract, as readily reflected in increased colonic motility and visceral hypersensitivity in rats upon intracerebroventricular administration of CRF. In addition to central CRF, peripheral CRF was also shown to mediate important effects of stress-induced changes in gut motility, barrier function, and visceral sensitivity, partially via mast cell activation (as extensively reviewed in Refs. 60 and 65). Although most of these CRF-related observations were made in animal models, iv CRF administration was shown to change intestinal motility in healthy volunteers and to a bigger extent in IBS patients (33). CRF also exerts its functions through additional stress-related molecular pathways, such as substance P and nerve growth factor (NGF) (8, 76). Both molecules are able to modulate physiological functioning of the intestine. Substance P acts via enteric nerves and mast cells to regulate intestinal ion secretion and barrier function (76). Also, increased levels of NGF have been observed in the cerebrospinal fluid (21) and mucosa (79) of IBS-like rats compared with control animals. Signaling of NGF through tropomyosin receptor kinase A (TrkA) has been associated with epithelial barrier dysfunction (79) and enhanced visceral sensitivity (7, 71). Recent observations in rodents indicated that inhibition of NGF can reverse early life stress-induced enterochromaffin cell hyperplasia, increased serotonin production, and visceral hypersensitivity, all of which are IBS characteristics (79). These same investigators also showed a NGF-dependent increase in Paneth cell numbers of maternal separated rats. This could be highly relevant because these cells are capable of modulating the gut microbiome to induce visceral hypersensitivity (58).

As a result of the multitude of CRF-induced gastrointestinal manifestations, CRF antagonism was attempted as a therapeutic target in IBS. Yet, two clinical trials with CRF1 receptor antagonists showed no patient benefit (30, 66). Failure may be explained by dose-limiting side effects or the antagonists not being potent enough. Another explanation may be found in the design of preclinical investigations that instigated these clinical trials. Almost without exception, these animal experiments were restricted to (successful) prestress administration of CRF receptor antagonists. One investigation, however, compared two different treatment protocols using the CRF1 receptor antagonist α-helical CRF. In maternal-separated rats, antagonist administration before acute stress at the adult age prevented stress-induced visceral hypersensitivity, and a similar result was obtained by the use of a mast cell stabilizer. These results confirmed earlier reports. However, poststress antagonist treatment was unable to reverse existing hypersensitivity to distension whereas mast cell stabilization was still effective (72). Thus, in contrast to the acute phase, chronic symptoms may be fueled by factors other than CRF alone. In a later study, using the same rat model, it was shown that yeast or yeast antigens are a driving force for continued poststress mast cell degranulation and chronic abdominal pain complaints (11). Taken together, these two studies showed the relevance of multidirectional microbiota-neuroimmune interactions for visceral hypersensitivity.

Microbiota are altered upon stressful events. Multiple rodent studies have shown the effect of stress on the gut microbiome, including in models of chronic, unpredicted stress (35, 48), repeated stress (32), and early life stress (26). The latter study provided conclusive evidence for the relevance of stress-induced changes by colonizing adult germ-free maternal separated and germ-free control mice with the same microbiota. This not only led to distinct microbiome profiles in recipient groups, but also to anxiety-like behavior (a psychiatric comorbidity in IBS) in maternal separated mice, but not in normal controls. Using the maternal separation model in rat, it was also shown that early life stress changes the composition of the fungal microbiome (i.e., mycobiome). Moreover, fecal transfer experiments indicated that only the maternal separation mycobiome was capable of conferring the visceral hypersensitivity phenotype to recipient fungicide-treated maternal separation rats (11). These two articles not only confirmed that stress alters the gut microbiome but also showed that these changes are relevant to the observed IBS-like phenotype. Webner and colleagues (77) recently showed that upon stress, microbiota composition and its metagenome can shift toward a more virulent composition, even leading to increased translocation of bacteria into lymph nodes to trigger increased immune responses. Additional stress-induced alterations were shown to include increased presence of inflammation-inducing microbes such as Helicobacter and Streptococcus (35), a decrease in microbial diversity (52), and decreased Lactobacillus (48). The exact mechanisms leading to these microbiota alterations have not yet been elucidated, but may involve changes in gut motility and related substrate availability (34). Moreover, stress is also known to impact the immune system (25), and innate as well as adaptive immune cells were shown to affect gut microbiota (31, 81). Importantly, most of the studies concerning the microbiota-gut-brain axis employ rodent experiments. Whether these observations hold true for humans as well, is still under debate. Early in 2020, Jarbrink-Sehgal and Andreasson (40) authored a publication in which research regarding mental health status and microbiome composition in humans, published during the last two years, was reviewed. It was suggested that confounding factors, large interstudy variation, small sample size, and multiple comparisons preclude conclusions regarding causality and differences in microbiota composition. To further the field, the need for well-designed and larger longitudinal studies was emphasized.

Modulation of systemic and local immune responses by stress. Psychological stress is known to impact (systemic) immunity. Although conflicting data exist, there is evidence to suggest that chronic stress, which is thought to play an important role in IBS, is immunosuppressive (62). This may explain why, despite gut barrier dysfunction (55), in IBS there are reports of low-grade inflammation only (9). Importantly, stress will activate the HPA axis as well as the sympathetic-adren-
medullary axis. Although catecholamines released during the latter not only affect the immune system but the microbiota as well, these hormones were suggested to be mostly responsible for immune effects of acute stressors (61, 62). In consequence, most of the IBS-related literature has a focus on the role of the HPA axis and its related hormones.

Throughout the gastrointestinal tract, many immune cells express CRF receptors, potentiating the role of stress-signaling molecules in intestinal immune responses. A well-known example of the connectivity between brain, gut, and immune response is stress-initiated mast cell signaling in IBS. Colonic mucosal mast cells are activated by CRF (54), inducing release of mast cell mediators such as histamine, proteases, and cytokines. These molecules are able to activate sensory neurons isolated from the dorsal root ganglia (DRG) (15). Secreted molecules from IBS biopsies, but not from healthy volunteers, are also able to activate neurons through protease-associated receptors 1 and 2 (PAR1, PAR2) or histamine-1 receptor activation (14, 15, 18, 27). Especially neuronal histamine signaling plays a crucial role in visceral nociception in IBS patients: histamine signals through the histamine-1 receptor (H1R), to potentiate the transient receptor potential vanilloid 1 (TRPV1) ion channel (71, 80). Corroborating the importance of histamine, both stabilization of mast cells and H1R antagonism successfully reduce abdominal pain in IBS patients and visceral hypersensitivity in a rat model of stress-induced visceral hypersensitivity (43, 64, 80).

In IBS, mechanisms including mast cell degranulation and subsequent afferent activation are clearly involved in mediating visceral hypersensitivity (43, 80). Yet, possibly due to the lack of methodological standardization and differences in patient selection, opposing results were reported concerning low-grade mucosal inflammation. A recently published study, involving 171 IBS patients and 127 healthy volunteers, reported on the expression of 36 immune-related genes in mucosal biopsies. This included markers for inflammatory mediators and mast-cell related genes. Only 33% of patients showed some signs if immune activation, and this was not related to clinical symptoms (1). Nevertheless, in a 2017 systemic review and meta-analysis of case-control studies evaluating immune cell counts, it was concluded that mast cells and CD3+ T cells are increased in colonic patient biopsies (9). Interestingly, results of this meta-analysis showed higher mast cell numbers in diarrhea as well as constipation-predominant patient groups. This may explain why authors suggested that “the diagnostic value of the quantification of colonic mucosal cells in IBS requires further investigation.”

Ascending Interactions: Influence of Microbiota on Immune Responses and Visceral Afferent Sensation

Microbial dysbiosis is associated with IBS symptoms. Increasing evidence fortifies the role of microbiota in visceral hypersensitivity and associated symptoms in IBS. Transfer of human IBS feces into rats induces visceral hypersensitivity (22), which has also been shown in rat-to-rat fecal transfer experiments (11). Although (dysbiotic) microbiota are associated with visceral hypersensitivity, complete eradication of commensals through generation of germ-free animals also leads to enhanced colonic sensitivity (46). Together, these findings indicate that a balance in microbiota is essential for normal perception of visceral stimuli, and presumably other IBS-related symptoms.

Many efforts have been put into defining an “IBS-associated” microbial signature associated with presence of IBS complaints, but both inter- and intrastudy variability complicate the definition hereof. In a recent Swedish study, no IBS microbiota signature was defined compared with healthy volunteers, neither based on microbiota analysis of sigmoid biopsy samples (n = 313 healthy vs. n = 63 IBS) or fecal samples (n = 153 healthy vs. n = 32 IBS) (38). In another recently evaluated cohort (n = 1,025 healthy vs. n = 412 IBS), a microbiotal signature for IBS was identified that is characterized by a small decrease in Faecalibacterium prausnitzii and an increase in Streptococcus spp (74). The microbiota of diarrhea-predominant IBS patients and postinfectious IBS are alike, with both containing more Bacteroidetes spp but less Clostridia spp compared with healthy volunteers (39). Because of relatively small sample sizes, differences in microbial composition may be obscured and thus require more advanced analytical approaches. For example, no differences in fecal microbiota abundance or compositions were found by using a classic approach, although a microbial signature associated with severe IBS was revealed using machine-based learning techniques (n = 110 healthy vs. n = 39 IBS subjects) (69).

Although findings vary between studies, the majority indicates that IBS-associated microbiota show a lower diversity in both bacterial and fungal communities (11, 74). Moreover, a systematic review mainly revealed a decrease in Bifidobacterium and an increase in Lactobacillaceae compared with healthy controls (57).

Influence of microbiota on psychological stress and pain perception. Increasing attention is being given to the role of the gut microbiota in psychological status. Many of the mechanisms through which microbes are able to communicate with the brain, and hence influence psychological status, have extensively been reviewed by Cryan and Dinan (23). The importance of the microbiota in psychological well-being has also been demonstrated by fecal microbiota transfer from anxious mice into germ-free mice, which displayed anxiety-like behavior upon introduction of the microbiota (26). One of the mechanisms through which the microbiota influences the brain is through secretion of their metabolites. Short-chain fatty acids (SCFA), products of bacterial metabolism, have a widespread function in gut functioning. However, the role of SCFA in IBS is controversial, as butyrate enemas appear to have opposing effects on visceral perception. Several studies report that butyrate may decrease pain sensation, both in IBS-like rodents and IBS patients, while other studies report enhanced visceral sensitivity (41). Nevertheless, recent studies indicated that treatment of IBS-like rats with the butyrate-producing probiotic Lachnospiraceae decreases stress-induced visceral hypersensitivity (82). Moreover, SCFA supplementation in psychological stress mouse experiments alleviated stress-induced intestinal permeability and corticosterone (70). In addition to SCFAs, bacteria produce gases such as methane and hydrogen, which have been related to visceral perception, IBS severity, and CNS symptoms like nausea, headache, and tiredness (78).

Microbial products are able to induce neuronal activation or sensitization through direct or indirect pathways. Stress is known to cause mast cell-dependent gut barrier dysfunction in

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it remains to be determined whether bacteria-neuron interactions are involved in visceral pain. In addition, recent investigations showed that fungi may act on nociceptors. Comparable to bacteria, Candidalysin is used for enhanced virulence, being able to induce epithelial responses leading to an increase in proinflammatory mediators (53). Although the same mechanism for neuronal activation was assumed for αHL and Candidalysin, exposure of neurons to the latter is not essential to increases in allodynia in vivo (49). Earlier investigations suggested that yeast dependent visceral hypersensitivity in IBS-like maternal separated rats depends on immune cells and their fungal recognition through a Dectin-1/Syk pathway to subsequently release histamine to activate sensory afferents (11). Again, whether direct fungal-neuron interactions or indirect mycobiome-mediated neuronal activation pathways are relevant in IBS, remains to be determined.

Conclusion and Future Perspectives

Psychological stressors cause exacerbation of IBS-associated symptoms such as abdominal pain, often caused by visceral hypersensitivity, i.e., the enhanced sensitivity toward colonic stimuli. Although not all molecular and cellular mechanisms underlying this altered sensitivity have been fully elucidated, it is clear that complex communication systems along the gut-brain axis play a central role. Neuroimmune interactions and cross talk with the microbiota are of importance in initiation and/or maintenance of nociception.

To decrease altered visceral sensations, modulation of either the stress responses or microbiota could be viable options. Modulation of stress responses may be considered as a therapeutic option, as has been attempted before with blockage of CRFR1. However, the widespread effects of the HPA-axis-derived molecules make it complicated to intervene with this system, as off-target effects may disturb general homeostasis.

As IBS patients often display an altered microbiota composition compared with healthy individuals, it is likely that visceral pain complaints are a result of aberrant responses to these luminal microbes. Moreover, the gut microbiota may have a crucial role in symptoms of IBS patients: the microbiota alters stress responses, immunity, and visceral perception through multiple mechanisms. Because of this central role, we suggest targeting and modulating the microbiota as a whole may qualify as a safe and suitable therapeutic option. Several approaches have already been tested with positive outcomes in rodent models and/or IBS patients, including natural herb oils, medical diets, antibiotics, pre- and probiotics, and fecal microbiota transplantation. Further development of therapeutics or treatment strategies targeting the microbial composition in IBS is favorable, as microbiota modulation may ultimately restore neuroimmune interactions and hence homeostatic communication along the gut-brain-microbiota axis.

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DISCLOSURES

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AUTHOR CONTRIBUTIONS

I.A.M.V.T. prepared figures; I.A.M.V.T. drafted manuscript; I.A.M.V.T., W.J.D.J., I.M.C., and R.M.v.d.W. edited and revised manuscript; I.A.M.V.T., W.J.D.J., I.M.C., and R.M.v.d.W. approved final version of manuscript.
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