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Neuroimmune factors in FGIDs

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Abstract

Abnormal abdominal pain perception is the most bothersome and difficult to treat symptom of functional gastrointestinal disorders (FGIDs). Visceral pain stimuli are perceived and transmitted by afferent neurons residing in the dorsal root ganglia that have sensory nerve endings in the gut wall and mesentery. Accumulating evidence indicates that peripheral activation and sensitization of these sensory nerve endings by bioactive mediators released by activated immune cells, in particular mast cells, can lead to aberrant neuro-immune interactions and the development and maintenance of visceral hypersensitivity. Besides direct neuronal activation, low concentrations of proteases, histamine and serotonin can chronically sensitize nociceptors such as TRP channels, leading to persistent aberrant pain perception. This review discusses the potential mechanisms underlying aberrant neuro-immune interactions in peripheral sensitization of sensory nerves. A better understanding of the cells, mediators and molecular mechanisms triggering persistent aberrant neuro-immune interactions brings new insights into their contribution to the physiology and pathophysiology of visceral pain perception and provides novel opportunities for more efficient therapeutic treatments for these disorders.

Keywords: Pain, TRP channels, (low-grade) inflammation, food antigens

Functional gastrointestinal (GI) disorders (FGIDs) affect the esophagus, gastroduodenum, small and large bowel and are characterized by recurring GI symptoms that cannot be attributed to structural or biochemical abnormalities [1]. Currently, applied classifications of functional GI disorders (FGIDs) are almost entirely symptom based and diagnosed by Rome IV criteria (overview classification system for FGIDs in [2]). The most common symptoms reported by this largest group of GI patients include abnormal pain perception, altered bowel habit or stool form, defecatory dysfunction, abdominal distension/bloating, nausea, early satiety, epigastric pain, postprandial fullness and heartburn. So far, FGIDs are extremely difficult to treat due to our limited understanding of their biological basis explaining why current therapies are simply symptomatic but do not actually cure the disease.

Of all symptoms, aberrant abdominal pain perception is the most bothersome and difficult to treat symptom. The gold standard to assess visceral sensitivity and pain is the barostat technique where a balloon is inserted in the lumen of the intestinal segment of interest. Patients rate their pain score during gradual inflation of the balloon on a visual analogue score. Depending on the threshold used, at least 50% of FGID patients suffer from mechanical hypersensitivity to balloon distention [3-6]. Not only sensitivity to mechanical but also to chemical stimuli such as lipids, acid or capsaicin, which activate the nociceptor transient receptor potential cation channel subfamily V member 1 (TrpV1), can be abnormal. For example, we recently demonstrated that 48% of IBS patients display an increased pain response to rectal application of capsaicin [7] while patients with functional dyspepsia reported more pain following acid infusion directly into the stomach [8] compared to healthy volunteers.

Visceral (nociceptive) sensations from the gut are provided by distinct populations of neurons. Nociceptive signals originating in the gut are transmitted via first order sensory neurons with their cell body in the dorsal root ganglion and synapsing with second order neurons in the dorsal horn of the spinal cord. This sensory information is subsequently transmitted to autonomic and satiety centers in the thalamus and the brain stem and third order neurons leading to conscious perception.

Based on the current scientific evidence, the mechanisms underlying visceral hypersensitivity include (1) peripheral activation and sensitization of visceral afferent neurons; (2) the sensitization of spinal cord dorsal horn neurons; (3) altered brain processing and/or impaired descending inhibitory pathways from the brain. The exact contribution of these mechanisms in visceral hypersensitivity and how they may interact is still unclear, but most likely they are rather complementary than mutually exclusive. This review will mainly focus on peripheral mechanisms, in particular on the role of neuro-immune interactions, in the pathophysiology of IBS.

Peripheral alterations underlying visceral hypersensitivity in IBS preclinical models

As stated above, nerve terminals of extrinsic afferents reside within the gut wall and the mesentery where they sense and mediate transmission of (pain) signals to the brain. They are equipped with a wide range of either activating or inhibiting cell surface receptors and channels [9] that determine the final activation and signaling properties of these nociceptive afferents. These nerve terminals reside in a complex signaling environment in which they are subjected to mechanical distortion during distention or contraction and are exposed to a continuously changing milieu containing a mixture of neuroactive signaling molecules. Changes in this local environment by f.e. aberrant immune activation can not only trigger activation of these nociceptive nerve endings but also alter their sensitivity, leading to long-term aberrant pain perception and abdominal symptoms [9, 10].

Our current insight in the role of neuro-immune mechanisms in visceral hypersensitivity is mainly obtained from preclinical IBS models [9]. In these models, a variety of triggers have been implied to trigger abnormal pain perception, including chronic exposure to stress [11, 12], infection [13-16], inflammation [17-19], neonatal colonic irritation [20, 21] or maternal separation [22]. To date, mainly changes in activation of voltage gated sodium channels [20, 23, 24], ATP-gated and acid-sensing ion channels [17, 21, 25-31], protease-activated and histamine receptors [18, 32-34] and transient reporter potential channels [35-40] have been proposed to be involved in visceral hypersensitivity. Especially mast cell activation and persistent low grade inflammation leading to the release of mast cell mediators, cytokines and opioids changing the performance of the above mentioned receptors and ion channels have been identified as main mechanisms involved in visceral hypersensitivity in these preclinical models. However, even though these preclinical models have provided important insight in the mechanisms and the role of immune activation in the maintenance of chronic visceral hypersensitivity (for excellent reviews, see [41-43]), these mechanisms may be dependent on the model used and not necessarily translate to the human situation. Therefore, this review will discuss the current knowledge on the molecular triggers and specific immune cell types involved in the development and maintenance of visceral hypersensitivity in man.

Evidence for low grade inflammation in IBS

Although the exact etiology of IBS is very heterogeneous and not fully understood, the best studied triggers for the development of IBS are adverse early life events, chronic exposure to stress and infectious gastroenteritis (post-infectious IBS, PI-IBS). Numerous clinical studies attribute IBS symptom development in 3-36% of individuals to gastrointestinal infection with bacteria, viruses or parasites [44, 45]. As the onset of IBS is best defined after an infectious gastroenteritis, this subgroup of patients is considered as a more homogenous group with similar underlying mechanisms that allows studying the pathogenesis of IBS. As such, the most compelling evidence for chronic low-grade inflammation, long after the initial infection has cleared, has been obtained in PI-IBS patients, who had no significant prior gut symptoms and in whom gut function was assumed to be normal before a defined episode of bacterial gastroenteritis [46-49]. Significant increases in the number of T-lymphocytes, macrophages and mast cells, enteroendocrine cells and IL-1β mRNA expression were observed in submucosal biopsies of PI-IBS patients [48-51] compared to healthy volunteers. But data on immune cell infiltration are conflicting as others found no evidence for inflammatory cell infiltrates or increased cytokine expression in the colonic mucosa of PI-IBS patients compared to post-infected individuals who did not develop IBS symptoms [52, 53].

Also in classical or non PI-IBS, evidence has been reported suggesting an association with persistent low-grade inflammation within the gut wall. Increased numbers of mucosal mast cells, eosinophils and (intra-epithelial) T lymphocytes were detected in intestinal tissues of adult and pediatric IBS patients compared to healthy volunteers [54-57]. The increased numbers of T and mast cells in mucosal biopsies of IBS patients significantly correlated with abdominal bloating frequency and symptoms of dysmotility-like dyspepsia [54, 55]. Additionally, mast cells were found to be located in closer proximity to nerve fibers in IBS patients versus controls while the number of mast cells in close proximity to nerves significantly correlated with the severity and frequency of abdominal pain and discomfort [54].

Although the evidence for aberrant immune activation seems overwhelming in (PI-)IBS, care must be taken when interpreting these results as the evidence supporting persistent immune cell infiltration or low-grade inflammation in IBS is conflicting. Various research groups found no differences in

immune cell numbers or cytokine mRNA expression in mucosal biopsies of PI-IBS [52, 53] and IBS [58-63] patients compared to healthy volunteers. Braak et al. even reported decreased numbers of mast cells, macrophages, T cells and λFLC-positive mast cells in the colonic mucosa of 66 IBS patients compared to 20 HV [58]. Also at mRNA level, decreased levels of genes linked to chemokine function or IL-10 were detected among IBS patients [60, 61]. More recently, Bennet et al. found no differences in cytokine mRNA expression levels in sigmoid colon biopsies when analyzing 109 IBS patients versus healthy volunteers [62]. Not only do these reports fail to demonstrate immune infiltration in the colorectal mucosa of (PI-)IBS patients, they also fail to demonstrate a correlation between immune infiltration and visceral pain perception [58]. For example, in PI-IBS, Mearin et al. found no differences in the number of T lymphocytes or pro-inflammatory cytokines between PI-IBS patients and infected control subjects three years after a Salmonella infection, even though only PI-IBS patients were hypersensitive to rectal distention [53]. Also in non PI-IBS, we found no evidence for submucosal immune infiltration while submucosal neurons in rectal biopsies of IBS patients but not healthy volunteers revealed increased sensitivity to capsaicin [64].

In summary, the current evidence supporting mucosal immune infiltration in IBS is conflicting and potentially reflects patient selection bias, geographical differences or differences in diet. The discrepancies observed for mucosal infiltration of immune cells in the gut wall of PI-IBS patients may reflect differences in the time between infection and sample analysis (varies between 3 months up to 8 years post-infection) or differences in the characteristics of the initiating pathogen. Nevertheless, these study results clearly question the role of mucosal immune infiltration as a causal factor for visceral hypersensitivity.

Role for aberrant neuro-immune interactions in visceral hypersensitivity

Based on recent data, it seems more plausible that the activation status of immune cells in the mucosa, rather than an increase in their absolute number, plays a crucial role in the maintenance of visceral hypersensitivity. Electron microscopy studies demonstrated that the number of degranulated mast cells in close proximity to enteric nerves was significantly increased in the descending colon of IBS patients when compared to healthy controls [54], implying a functional interaction between mucosal mast cells, enteric nerves and extrinsic sensory neurons. As such, inflammatory mediators potentially contribute to the mechanisms underlying abdominal pain and dysmotility in patients with IBS. Mucosal intestinal biopsy supernatants serve as an excellent tool to assess the effect of alterations in the local micro-environment on peripheral nociceptive signaling mechanisms in IBS patients [65-69]. IBS mucosal biopsy supernatants produce an enhanced calcium influx after acute application to isolated primary afferents [69, 70] or submucosal enteric neurons [66-68], or enhanced firing rates in rat mesenteric afferents upon injection into mesenteric arteries [69]. Of note, supernatants from IBS patients who are hypersensitive to colorectal distention caused stronger activation of guinea pig enteric and mouse DRG neurons compared to supernatants of normosensitive patients [68], indicating that neuronal activation responses *in vitro* seem to correlate with the individual pain threshold pressure values.

Analysis of the supernatants obtained after incubation of mucosal biopsies revealed that the supernatants from IBS patients contained increased amounts of pro-inflammatory mediators such as histamine, serotonin, polyunsaturated fatty acid metabolites [36, 54, 66, 71] and proteases [70, 71]. Each of these mediators can contribute to aberrant visceral pain perception as in vitro studies revealed that the marked increase in intracellular calcium in rat DRG neurons was at least partially inhibited by the

application of a 5-HT3 antagonist, histamine 1 or 2 receptor antagonists and a protease antagonist [66, 67] (for review, see [65]). Cenac et al. recently provided breaking evidence for the role of polyunsaturated fatty acid metabolites in visceral hypersensitivity. In particular 5,6-EET was upregulated in colonic biopsies but not supernatants from patients with IBS and acts as an endogenous agonist of TRPV4 to induce hypersensitivity. Of interest, upon exposure to supernatants from IBS biopsies, mouse sensory neurons produced 5,6-EET via a mechanism that involved the proteinase-activated receptor-2 (PAR-2) and cytochrome epoxygenase (Fig. 1). 5,6 EET then stimulates TRPV4 on sensory neurons to generate visceral hypersensitivity [71]. Finally, *in vivo*, supernatants from colonic biopsies of IBS patients, but not controls, caused somatic and visceral hyperalgesia and allodynia in mice, when administered into the paw or colon respectively [70]. This pro-nociceptive effect was inhibited by serine protease inhibitors and a PAR-2 antagonist and was absent in PAR-2-deficient mice [70], underscoring the role of protease in the maintenance of visceral hypersensitivity. Besides direct neuronal activation, Dothel et al. recently reported that mucosal biopsy supernatants from patients with IBS trigger nerve sprouting of primary enteric neurons and enteric cell lines [72], potentially contributing to neuronal plasticity and visceral hypersensitivity. More research assessing neuronal markers or nociceptor expression are required to explore this hypothesis.

Others suggested that changes in immune parameters are also evident in peripheral blood mononuclear cell (PBMC), as opposed to biopsy supernatants, from patients with IBS. In particular, evidence indicates increased activation of T cells [73, 74] and correlation of pain with levels of several pro-inflammatory cytokines including in TNF-α, IL-1β and IL-6 in the supernatants of PBMCs from diarrhea predominant IBS patients [73, 75]. PBMC supernatants from IBS patients evoked mechanical hypersensitivity of colonic afferents from mice [75], an effect that is most likely mediated by TNF-α in diarrhea predominant IBS patients or IL-1β in constipation predominant IBS patients [75]. However, additional evidence on systemic immune activation is conflicting as others found no differences in cytokine levels in PBMC supernatant (basal and stimulated, [52]) or serum cytokine levels [62]. It should also be pointed out that the immune subsets in PBMCs have a completely different phenotype and are actually not representative for the immune cells residing in intestinal tissue [76]. Finally, it is not clear why a systemic increased release of cytokines would only sensitize visceral afferents in IBS patients while leaving other organs unaffected.

Altogether there is a general consensus on the role of bio-active mediators released in the colorectal micro-environment on sensory neuron activation and subsequent increased visceral pain perception in IBS patients. More studies are warranted to explore the potential role of systemically released bioactive mediators in visceral hypersensitivity.

Evidence for peripheral sensitization in IBS

Besides direct neuronal activation, the bio-active mediators that are chronically released in the gut wall may also act as neuronal sensitizers. Basically, the same mediators do not only acutely activate sensory nerves but also trigger downstream signaling cascades favoring increased nociceptor excitability as has been shown for kinins, e.g. bradykinin, histamine and serotonin, prostanoids, e.g. prostaglandin E2 growth factors (NGF and GDNF), proteases, chemokines and cytokines as well as reduction in pH and increase in ATP [77].

Previously, we showed increased pain responses to rectal application of the TRPV1 agonist capsaicin in IBS patients that are hypersensitive for barostat distention, but we failed to detect upregulation of TRPV1 mRNA or protein expression [7], indicating that sensitization rather than up-regulation of TRPV1 may occur in IBS. We then directly studied intestinal neuronal activity in IBS patients by performing live calcium imaging of rectal biopsies [64, 78]. Exposure of submucosal neurons of IBS patients (rectal biopsies) to the TRPV1 agonist capsaicin induced greater intracellular calcium (Ca^{2+}) responses and activated more neurons compared with healthy control while TRPV1 messenger RNA or protein levels were comparable [64], providing functional evidence for local neuronal sensitization in IBS.

Although the majority of studies using supernatants obtained from biopsies focused on direct neuronal activation (see above), to date only very few studies assessed the mechanisms underlying nociceptor sensitization following chronic exposure to IBS supernatants. Overnight incubation of murine sensory dorsal root ganglion neurons with supernatants from IBS-D patients elicited a marked increase in neuronal excitability compared with controls, underscoring the capacity of IBS supernatants to increase the intrinsic excitability of sensory neurons. The increased excitability seen with IBS-D supernatant was dependent on proteases and activation of the protease receptor PAR-2 [13].

Besides proteases, also pre-incubation of human submucosal neurons and murine DRG neurons with the mast cell mediator histamine (nM range) potentiated TRPV1 responses via activation of the histamine 1 receptor (Hrh1) [64]. This increased neuronal response was blocked by the Hrh1 receptor antagonist pyrilamine or in mice deficient for Hrh1, further underscoring the role of histamine and Hrh1 activation in TRPV1 and neuronal sensitization. Of note, no differences were detected when assessing histamine concentrations in colonic biopsy supernatant of IBS patients versus healthy volunteers. Instead, the histamine metabolite and Hrh1 agonist imidazole acetaldehyde also sensitized TRPV1, even though no differences in imidazole acetaldehyde levels were found between supernatants of biopsies from patients with IBS and controls. Based on these data, one can speculate that the TRPV1 sensitizing agent may be present at extremely low levels, is another downstream metabolite, or is a mixture of various bio-active mediators and metabolites that are only marginally increased. Examples of neurosensitizing agents include proteases, oxidized linoleic acid metabolites or arachidonic acid metabolites which can all sensitize TRP channels. More studies are needed in IBS patients to assess if a panel of bio-active compounds rather than one mediator should be quantified to identify subgroups of IBS patients.

Besides excitation of viscerosensory nerves by immune derived mediators, visceral hypersensitivity can result from diminished neuronal inhibition by opioids, in particular in IBS-C patients [75]. Interestingly, Hughes et al. showed that the supernatant of PBMCs isolated from healthy controls dampens mechanosensation of visceral afferents, an effect mediated by b-endorphins. Of note, this inhibitory effect of PBMC supernatants is lost in IBS-C patients, potentially contributing to a dysbalance between pro- and nociceptive signals leading to visceral hypersensitivity [75, 79]. The source of b-endorphins was shown to be predominantly in monocytes and macrophages relative to T or B cells in human PBMCs and colonic lamina propria. In the lamina propria of colonic biopsies, nearly all CD68 macrophages expressed b-endorphin. In patients with IBS, however, b-endorphin levels in unstimulated monocytes were lower than in healthy controls, while the number of CD68 cells in the lamina propria of IBS biopsies were lower compared to controls. These data would suggest that a reduction in endogenous b-endorphin levels in IBS patients (IBS-C only?) could contribute to

abnormal pain perception. Of interest, the same authors noted that k-opioid receptor expression is increased in a mouse model of chronic visceral hypersensitivity, leading to a more pronounced analgesic effect of PBMC supernatant. This finding is of interest as this suggests that k-opioid agonists can be particularly effective dependent on the expression of this receptor, as shown by the dose dependently inhibition of colonic nociceptors with asimadoline, a peripherally restricted selective k-opioid agonist [80]. These studies introduce a new and interesting concept that abnormalities in the secretion of endogenous opioids by immune cells may contribute to visceral hypersensitivity in IBS, a concept that definitely deserves further study.

Current therapies

Current therapeutic options for IBS are restricted to symptomatic treatment leaving visceral hypersensitivity untreated. Although there is some evidence for improvement with antidiarrhoeals, antispasmodics, bulking agents, laxatives, tricyclic antidepressants and behavioural therapy [81-83], we will only highlight therapies targeting peripheral neuro-immune dysfunction.

Based on the hypothesis that microscopic inflammation may be involved in the pathogenesis of IBS, two larger clinical trials have been recently performed evaluating mesalazine. This compound is an anti-inflammatory drug used to treat patients with inflammatory bowel disease. Although initial studies suggested it may be beneficial to patients with diarrhoea-predominant IBS [84], these findings were not confirmed in these 2 larger trials [85, 86]. Also prednisone treatment in a reasonably large cohort of PI-IBS patients decreased the lamina propria T lymphocyte counts and probably other inflammatory cells but failed to improve abdominal pain, diarrhea, or urgency. Taken together, these clinical trials all strongly argue against the hypothesis that low-grade inflammation is the underlying factor in pain and IBS symptoms [47].

More promising results were obtained with the mast cell stabilizer ketotifen [59]. Although this compound improved symptoms and visceral pain perception in IBS, it possess central side effects and is therefore a less attractive therapeutic option. Moreover, as no mast cell stabilizing effects were observed in this trial, we further evaluated if its histamine receptor 1 (Hrh1) antagonistic properties could have mediated the beneficial effect. In a preclinical model, similar to ketotifen, Hrh1 antagonism reversed visceral hypersensitivity in a rat model of maternal separation [34]. Based on these observations, we designed a proof-of-concept clinical trial evaluating the effect of 12 week treatment with the Hrh1 antagonist ebastine in 55 IBS patients. We showed that ebastine decreased visceral hypersensitivity, increased symptom relief and reduced abdominal pain scores compared with placebo, an effect that was lost during the washout period [64].

Besides histamine receptor antagonists, also serotonin (5-hydroxytryptamine [5-HT])3 receptor antagonists are effective for the treatment of diarrhea-predominant irritable bowel syndrome [87], showing efficacy in abdominal pain, discomfort, urgency, stool frequency and consistency. However, significant constipation occurred in approximately 25% of patients, leading to withdrawal in up to 10% of patients in clinical trials. Attenuation of 5-HT3 activity without completely abolishing its function may normalize diarrhea without leading to severe constipation, and thus is of great interest to further explore [87, 88].

On the other hand, linaclotide is a drug with proven visceral analgesic properties in constipation predominant IBS patients. In phase II and III studies, linaclotide accelerated colonic transit and improved abdominal pain and constipation associated with constipation predominant IBS [89-92]. In addition, linaclotide has been shown to elicit analgesic effects in several animal models of visceral pain [93]. Castro et al. identified the unique analgesic mechanism of linaclotide using *in vitro* and *in vivo* studies. It acts as a guanylate cyclase C (GC-C) agonist expressed on mucosal epithelial cells, resulting in the production and release of cyclic GMP. This extracellular cGMP inhibits nociceptors, thereby reducing nociception [94].

Finally, several phase 2 and 3 clinical trials assessed the efficacy and safety of compounds that modulate opioid receptor (μ, δ and κ) activity. The mechanism of action of opioid agonists is complex because of various receptor subtypes and central versus peripheral action sites, but these agents generally mediate inhibitory effects that interrupt neuro-neuronal and neuro-effector transmission. Peripherally restricted μ-opioid receptor agonists such as loperamide are powerful antidiarrheal agents but do not show convincing analgesic activity [95] and are associated with constipation [96]. These agents are therefore less attractive in the treatment of hyperalgesia in IBS-D. The κ-opioid receptor agonist asimadoline on the other hand produces both analgesic and antidiarrheal effects, presumably via a peripheral action [97, 98]. An on-demand dosis schedule of asimadoline was not effective in reducing the severity of abdominal pain in a single-center study in females with IBS [99], but post-hoc analysis suggested asimadoline was effective in IBS-mixed. It should be noted that the experimental design used does not exclude the potential benefit of asimadoline if administered daily over a longer term. More recently, Lembo et al. evaluated the clinical response of IBS-D patients to eluxadoline, a mixed peripherally acting μ-opioid receptor agonist, δ-opioid receptor antagonist and k-opioid receptor agonist with minimal oral bioavailability [100]. A total of 2427 IBS-D patients were enrolled and received 75 or 100 mg eluxadoline twice daily for 26 or 52 weeks. Patients who received eluxadoline reported a decrease in stool frequency and urgency while no significant improvement was seen in the mean scores for the worst abdominal pain or in the percentage of patients who reported improvement of 30% or more in the score for the worst abdominal pain [100]. Altogether, there is some evidence for efficacy of opioid receptor agonists in IBS patients with diarrhea or alternating bowel function. Further studies are warranted to explore the subpopulations of patients that may benefit the most from these compounds.

Despite promising evidence that some pharmaceutical agents benefit the treatment of IBS in the short term, there is no medical intervention that has been proven to alter the long-term natural history of this condition. Novel insight into the molecular neuro-immune mechanisms underlying visceral hypersensitivity may potentially lead to the identification of novel therapeutic pathways that may even cure IBS.

Perspectives

Accepting that aberrant mast cell activation and increased neuro-immune interactions at the submucosal level trigger and maintain afferent neuronal activation and sensitization, it remains to be elucidated which triggers can lead to chronic mast cell activation. Central factors such as psychological stress can modulate mast cell activation (Fig. 1). In preclinical studies, several types of

stresses and stress mediators such as corticotrophin releasing hormone indeed have been shown to modulate gastro-intestinal permeability and concomitantly colorectal sensitivity [101-103].

Besides stress, an aberrant adaptive immune response targeting food or microbial antigens may sensitize mast cells leading to their activation upon re-exposure. This hypothesis is supported by various reports documenting increased numbers and activation of B or plasma cells in IBS patients [104, 105]. In addition, IBS patients have increased serum antibody titers directed against certain components of the microbiome, such as flagellin (the primary structural component of bacterial flagella), an observation that was even more pronounced in PI-IBS patients [106, 107]. Finally, when candidate food antigens are directly applied to the duodenal mucosa of IBS patients with suspected food intolerance through an endoscope, it caused immediate breaks, increased intervillous spaces and an increase in intraepithelial lymphocytes in the intestinal mucosa and an individualized exclusion diet improved symptoms in 74% of patients at 1 year follow-up [108]. These data support the concept that humoral immune reactivity to luminal antigens may have a putative role in the development of IBS symptoms (Fig. 1). Evidence for aberrant immune responses targeting food antigens has also recently been provided in a somewhat different patient population, i.e. patients reporting sensitivity to wheat in the absence of celiac disease, but presenting with very similar abdominal complaints [109]. These individuals also display antibody reactivity to bacterial LPS and flagellin in conjunction with a compromised intestinal epithelium. Of note, all individuals reported symptom improvement and immune activation markers returned to normal 6 months after initiation of a diet free of wheat and related cereals [109]. To what extent similar mechanisms also play a role in IBS and whether genetically predisposed individuals may become sensitized to food and microbial antigens during an acute insult of the intestinal barrier (f.e. during an infectious gastroenteritis, inflammation or an episode of stress) remains to be established [110].

As current therapies are merely symptomatic, understanding the complex interaction between food and microbial antigens in immune activation and functional symptoms may lead to better therapeutic strategies that may potentially even cure IBS. Exclusion diets such as a FODMAP diet or a standard exclusion diet frequently recommended for patients with IBS (ie, a regular meal pattern; avoidance of large meals; and reduced intake of fat, insoluble fibers, caffeine, and gas-producing foods, such as beans, cabbage, and onions) reduce IBS symptoms [111]. Metabolic profiling of urine showed that patients with IBS on a low FODMAP diet for 3 weeks differed significantly after the diet. Amongst others, the mast cell mediator histamine was reduced eightfold in the low FODMAP group [112], indicating that diet can alter mast cell activation. However, the exact underlying mechanisms and potential role of mast cell activation have not been explored yet. The challenge therefore will be to i) identify the subgroup of patients that suffer from persistent immune activation by food antigens and ii) to identify a panel of antigens of interest together with immune markers to identify affected individuals and to monitor the response to specific treatment strategies.

Conclusions

Our insight in the pathophysiology of visceral hypersensitivity has significantly expanded over recent years. Accumulating evidence unequivocally supports the contention that peripheral mechanisms can contribute to IBS. A variety of preclinical models based on diverse triggers of visceral hypersensitivity, each with their own limitations, already provided some insight into the receptors, cells and molecular pathways involved in aberrant neuro-immune interactions and visceral hypersensitivity. In man, not immune cell numbers but rather the release of pro-inflammatory

mediators by persistently activated immune cells such as mast cells seems crucial in the development of visceral hypersensitivity. As such, increased levels of bioactive mediators and their metabolites are released in the submucosa that do not only activate but also sensitize sensory neurons, leading to visceral hypersensitivity. To better understand the mechanistic pathways involved, more detailed phenotyping of patients and longitudinal studies will be important in future studies using IBS mucosal biopsies. Another and potentially more important remaining gap is the identification of the triggers that maintain this condition of aberrant immune activation. A better understanding of the factors (diet, stress, infection) involved may provide novel therapeutic strategies that not only elevate symptoms but may even cure a subpopulation of patients with IBS.

Figure 1. Cartoon on potential mechanisms underlying visceral hypersensitivity

Figure 1. Mast cell activation in response to chronic stress or cross-linking of IgG through food or microbial antigens triggers the release of pro-inflammatory mediators that directly activate sensory neurons. In addition, these mediators may act as neurosensitizers via activation of a cellular signaling cascade downstream of their respective nociceptive G protein coupled receptor (GPCR) that subsequently activate, translocate or sensitize nociceptors, leading to visceral hypersensitivity. The best studied nociceptor is TRPV1 but other TRP or ion channels can be involved. Furthermore, proteases deriving from the microbiome, pancreatic juice, epithelium or activated mast cells activate PAR2, leading to direct neuronal activation, sensitization and the release of 5,6EET leading to neuronal activation in a TRPV4 dependent manner. By contrast, activation of another set of channels and/or receptors by f.e. opioids can result in reduced neuronal excitability and subsequent anti-nociceptive effects (analgesic GPCR). In IBS, pro-nociceptive mechanisms seem to be upregulated while anti-nociceptive mechanisms are downregulated.

Abbreviations: corticotrophin releasing hormone (CRH); mast cell (MC); phospholipase C (PLC), adenylate cyclase (AC); protein kinase A and C (PKA, PKC), polyunsaturated fatty acids (PUFA); transient receptor potential (TRP); proteinase-activated receptor-2 (PAR-2)

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Disclosures

The authors have nothing to disclose

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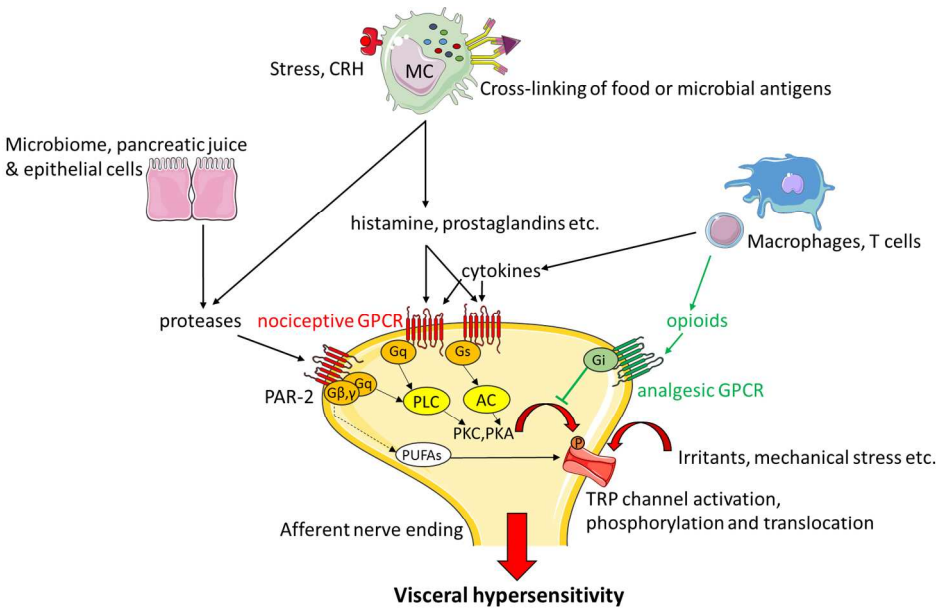

Cartoon on potential mechanisms underlying visceral hypersensitivity

646x396mm (72 x 72 DPI)