Potential neuro-immune therapeutic targets in irritable bowel syndrome

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Abstract: Irritable bowel syndrome (IBS) is a functional gastrointestinal (GI) disorder characterized by recurring abdominal pain and disturbed bowel habits. The aetiology of IBS is unknown but there is evidence that genetic, environmental and immunological factors together contribute to the development of the disease. Current treatment of IBS includes lifestyle and dietary interventions, laxatives or antimotility drugs, probiotics, antispasmodics and antidepressant medication. The gut–brain axis comprises the central nervous system, the hypothalamic pituitary axis, the autonomic nervous system and the enteric nervous system. Within the intestinal mucosa there are close connections between immune cells and nerve fibres of the enteric nervous system, and signalling between, for example, mast cells and nerves has shown to be of great importance during GI disorders such as IBS. Communication between the gut and the brain is most importantly routed *via* the vagus nerve, where signals are transmitted by neuropeptides. It is evident that IBS is a disease of a gut–brain axis dysregulation, involving altered signalling between immune cells and neurotransmitters. In this review, we analyse the most novel and distinct neuro-immune interactions within the IBS mucosa in association with already existing and potential therapeutic targets.

Keywords: IBS, immune cells, neuro-immune interactions, neuropeptides, therapeutic targets

Introduction

Irritable bowel syndrome (IBS) is a functional gastrointestinal (GI) disorder characterized by chronically recurring abdominal pain and disturbed bowel habits. Currently, IBS affects 10–15% of the general population in western countries, with a 2:1 female predominance.[1] The pathophysiology of IBS is still not completely understood; however, genes, lifestyle, psychosocial factors, brain–gut axis dysfunction, microbiota composition, immune disruption and intestinal barrier dysfunction are all factors known to contribute to the development of the disease.[2] Patients with IBS can be divided into subgroups according to the predominant bowel habit; IBS-mixed, IBS-constipation and IBS-diarrhoea,[3] and treatment for IBS is usually based on the predominant symptoms, with options for each subgroup. The current treatment includes lifestyle and dietary interventions, laxatives for IBS-constipation, antimotility drugs for IBS-diarrhoea, probiotics, antispasmodics to relieve pain and antidepressant medication to relieve symptoms of depression and anxiety.[4] The gut–brain axis comprises the central nervous system (CNS), the hypothalamic pituitary axis (HPA), the autonomic nervous system and the enteric nervous system (ENS). Within the intestinal mucosa, there are close connections between immune cells and nerve fibres that have been shown to be of great importance during stress and during GI disorders such as IBS.[5–10] Communication between the brain and the gut is routed *via* the spinal and the enteric nerves, but most importantly *via* the vagus nerve,[11] which interacts with the immune system by neurotransmitters such as acetylcholine and serotonin.[12] It has become evident that IBS is a disease of gut–brain axis dysregulation, which involves altered signalling between these entities. Recent studies have demonstrated a novel

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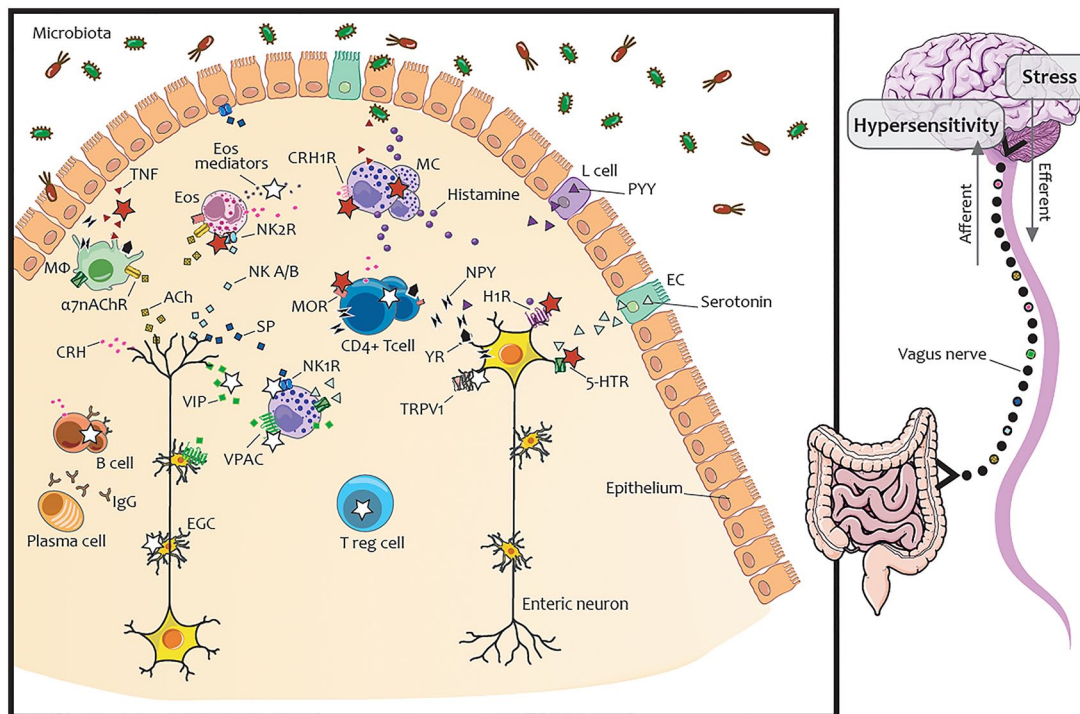
Figure 1. A schematic overview of neuro-immune interactions within the mucosa of IBS. Central stress can lead to the stimulation of efferent nerves, causing the local release in the intestinal mucosa of ACh, NK and SP, which interact with α7nAChR, $NK_{1,2}R$, expressed mainly on immune cells, and MOR. Some neurotransmitters, such as CRH, VIP and NPY, can be secreted by both immune cells and enteric nerves and interact with their corresponding receptors, CRH_1R, VPAC and YR. It is well known that the activation of immune cells like Eos, MC, MΦ and T cells, often located in close proximity to enteric neurons, can lead to inflammation and increased bacterial passage following secretion of histamine and TNF, among other cytokines. Although it may be important, very little is known about the neuro-immune role of EGC, T regs, B and plasma cells in IBS. L cells and EC, located in the intestinal epithelium, are the main sources of PYY and serotonin, respectively, which interact with their receptors, YR and 5-HTR. Those two and other nociceptive receptors such as TRPV1, and H1R are expressed mainly in enteric neurons and are related to hypersensitivity in IBS. Furthermore, the figure illustrates both targets of existing therapies (★) and potential interactions (☆) that could be targeted for therapeutic strategies in IBS.

α7nAChR, α7 nicotinic acetylcholine receptor; ACh, acetylcholine; CRH, corticotropin-releasing hormone; CRH_1R, CRH receptor 1; EC, enterochromaffin cells; EGC, enteric glial cells; Eos, eosinophils; H1R, histamine receptors 1; 5-HTR, 5-hydroxytryptamine (serotonin) receptors; IBS, irritable bowel syndrome; MΦ, macrophages; MC, mast cells; MOR, μ opioid receptor; NK, neurokinin; $NK_{1,2}R$, NK receptors 1 and 2; NPY, neuropeptide Y; PYY, peptide Y; SP, substance P; TNF, tumour necrosis factor; T regs, T regulatory cells; TRPV1, transient receptor potential vanilloid type 1; VIP, vasoactive intestinal polypeptide; VPAC, VIP receptor; YR, receptors Y.

therapeutic potential of mechanisms involving interactions between enteric nerves, neuropeptides and immune cells,[13] as well as their roles in manifestations of IBS clinical symptoms.[14] This review will, as summarized in Figure 1, discuss existing and potential therapies in IBS involving neuro-immune interactions as targets.

The immune system: targeting immune cells

The involvement of an immune activation in IBS clinical symptoms remains unclear. Although the role of a neuro-immune activation in IBS is not fully proven and there are some inconsistent data, there is evidence for immune activation and low-grade mucosal inflammation, characterized by increased amounts of immune cells such as mast cells and T cells, in the colon and ileum of IBS patients.[15] Below we will discuss some of the immune cells that could be of importance.

Mast cells

Intestinal mast cells are granulated cells located mainly in the lamina propria and the submucosa of the intestine. Mast cells can regulate

permeability, secretion, peristalsis, nociception, innate and adaptive immunity.[16] Increased numbers of mast cells have been found in IBS patients compared with healthy individuals, both in the colon and in the small bowel.[10,17] In IBS, mast cell cellularity of the colonic mucosa correlates with fatigue, depression and visceral hypersensitivity.[18,19] Besides the number, their activation level has also been proved increased in IBS patients.[20] The degranulation of mast cells causes the release of inflammatory mediators like histamine, serotonin and proteases that can activate enteric neurons.[21] In addition, studies have shown that the number of mucosal mast cells located in close proximity to enteric nerves correlates to severity and frequency of abdominal pain.[22] In rats, mediators from IBS patients, but not controls, activated mesenteric nerves that were inhibited by histamine H_1-receptors blockade and serine protease inactivation.[23] Histamine is an amine produced and stored mainly by mast cells able to modulate GI motility, gastric acid production and ion secretion. Moreover, it interacts and modulates the immune system regulating cytokine production,[24] upregulates T-helper cell (Th) 1 proliferation and downregulates Th2.[25] Levels of histamine are increased in IBS patients compared with healthy controls,[21] and it participates, together with tryptase and serotonin, in the activation of visceral nociceptive sensory pathways.[26] Histamine receptors 1 (H_1R), H_2R and H_4R, but not H_3R, are expressed in the GI tract; of those, the expression of H_1R and H_2R have been found to be upregulated in the intestinal mucosa of IBS patients.[27] These findings together indicate that mast cells, and especially histamine and its receptors, should be considered as relevant targets in the treatment for IBS. So far, attempts to target mast cells involve oral treatment with the anti-inflammatory drug mesalazine, which was shown initially to reduce mast cell infiltration.[28] However, a recent meta-analysis demonstrated that mesalazine is not superior to placebo in relieving abdominal pain or bloating, defecation frequency, or immune cell infiltration.[29] More successfully, the H_1R antagonist ebastine is a phase IV drug that has been shown to reduce symptoms and visceral hypersensitivity in IBS patients.[30] An interventional clinical trial is further evaluating its efficacy as IBS treatment (ID: NCT01908465). We previously showed that, when pretreating colonic biopsies from patients with IBS *ex vivo* with the mast cell stabilizer and H_1R antagonist ketotifen, that increased bacterial translocation through IBS mucosa was inhibited, suggesting a direct role of mast cells in regulating the intestinal barrier.[10] Ketotifen further reduced symptoms of visceral perception after oral administration to patients with IBS.[31] Treatment with disodium cromoglycate, another mast cell stabilizer, has been tested in clinical trials, resulting in reduced mast cell activation and clinical implications such as improved symptoms in IBS-diarrhoea patients.[20] A more novel way to target mast cells would be to aim for receptors on the surface, such as receptors for vasoactive intestinal polypeptide (VIP), corticotropin-releasing hormone (CRH) and substance P (SP), which will be discussed further below.

Eosinophils

Eosinophils protect the host from infectious agents by secreting toxic inflammatory mediators that are stored in preformed vesicles and also synthesised *de novo* following activation.[32] The major secreted mediators are eosinophil protein X (EPX), eosinophilic cationic protein (ECP), major basic protein (MBP), eosinophil derived neuroendotoxin, and eosinophil peroxidase.[32] In addition to releasing proteins/mediators, eosinophils can secrete CRH in response to, for example, stress.[33] In turn, CRH activates nearby mast cells, that start secreting mediators, with harmful effects on the mucosa and the intestinal barrier. Therapies targeting CRH will be discussed further below. For IBS, studies have shown inconsistent results regarding eosinophil counts in IBS colon[34,35]; however, we recently demonstrated increased numbers of eosinophils in IBS colon compared with healthy colon, which correlated with increased intestinal permeability.[36] Interestingly, activation of eosinophils has been demonstrated in functional dyspepsia,[37] another functional GI disorder that often overlaps with IBS. Blocking of eosinophils, thereby inhibiting secretion of their mediators, may be a potential biological therapy to target the improvement of IBS. Though, considering the diverse roles of eosinophils, being both pro-inflammatory and repairing, it will be critical to reach a balance of inhibiting the inflammatory effects without interfering with the repair mechanisms. Experiments involving blocking of eosinophil mediators have been explored only in experimental models and it remains to be investigated if such blockers will also be useful in the treatment of GI disorders such as IBS.[38]

Monocytes-macrophages

Monocytes are the largest type of leukocytes. Monocytes circulate through the blood before migrating into tissues and differentiating into macrophages. Interestingly, the initial phase of monocyte/macrophage maturation was described as more advanced and activated in IBS compared with controls, suggesting a baseline immune activation.[39] Macrophages are found adjacent to the ENS and possess different functions depending on exact localization.[40] Subtypes of macrophages respond rapidly to threatening pathogens by migrating into the intestinal lumen, thereby hindering bacteria from breaching and passing through the barrier.[41] Macrophages produce and secrete tumour necrosis factor (TNF, former known as TNF-α), and the release can be reduced by cholinergic neural signals that attenuate immune activation through activation of α7 nicotinic acetylcholine receptor (α7nAChR).[42] Anti-TNF antibodies can induce regulatory macrophages in patients with inflammatory bowel disease (IBD), which promote wound repair[43]; however, this treatment has so far not been that successful in IBS, probably because levels of TNF are less elevated, even though there is an underlying mucosal immune activation in many patients, with slightly elevated pro-inflammatory cytokines such as TNF.[15]

B cells and plasma cells

Antigen presentation, cytokine secretion and antibody production are mediated by B cells and plasma cells. Very little has been reported about these cells in IBS; however, humoral immunity has been described as distinctive in IBS patients compared with healthy controls. The number of immunoglobulin (Ig)-A-positive B cells in the colon has shown to be lower in IBS patients.[44] In the small bowel of IBS-diarrhoea patients, activation of B cells and IgG production was increased and correlated with bowel movements, stool form and depression.[17] Further, there is evidence that B cells can secrete neuropeptides such as CRH in response to stress,[45] which indicates that B cells should be relevant as therapeutic targets in IBS treatment, though additional studies are needed to evaluate this.

T cells

T cells are one of the main components of the adaptive immune system defending against intracellular pathogens. Moreover, T cells play an important role in communication with the ENS driven by noradrenaline released by the sympathetic splenic nerve, triggering the production of acetylcholine by T cells expressing β2-adrenergic receptors, and, consequently, switching the production of pro-inflammatory to anti-inflammatory cytokines.[46] Other neurotransmitters and their receptors related with these cells will be discussed below. Moreover, as for B cells, there is also evidence that T cells can secrete CRH in response to stress, which suggests T cells as relevant therapeutic targets in IBS treatment.[45] A recent meta-analysis showed that CD3+ and CD4+, but not CD8+ T cells, are increased in IBS patients.[47] The current involvement of T cells in IBS is conflicting. Dong and colleagues showed that stress-induced diarrhoea can cause an increase in proliferation and number of T cells in the small intestine and ileum of mice.[48] On the other hand, Nasser and colleagues reported an increased activation of CD4+ T cells in blood of IBS patients that did not correlate with GI or physiological symptoms.[49] Immune therapies involving suppressing T-regulatory cells has shown promising results in patients with IBD,[50] but, to our knowledge, no T-cell therapies have been applied in IBS. Additional studies are currently ongoing, and therapies involving T cells, and in particular T-regulatory cells might, in the future, also be relevant for IBS patients.

Cytokines and toll-like receptors

Cytokines are proteins, often produced in a cascade and secreted mostly by immune cells, that have a specific effect on the communication and coordination of immunological response, as well as the control of intestinal motility and visceral pain.[51] Evidence proving low-grade inflammation in the pathogenesis of IBS is increasing. A systematic review and meta-analysis of cytokines in IBS patients revealed an imbalance of pro-inflammatory TNF and anti-inflammatory IL-10.[52] Serum levels of IL-10 (mainly from Th and T-regulatory cells) were found to be lower. On the contrary, IL-5 (from Th2, mast cells and eosinophils), IL-6 (from macrophages and monocytes), IL-17 (from Th17) and TNF (from macrophages) levels were higher in IBS patients compared with controls, correlating with GI symptoms and IBS subtypes; the IBS-diarrhoea inflammatory cytokine profile was the most significantly different from that of healthy controls.[53]

The regulation of inflammatory cytokines in IBS-diarrhoea seems to be partially regulated by nitric oxide synthase in mast cells, being upregulated along with tryptase.[54] Stress upregulates IL-6 mRNA expression in the hypothalamus and its release in plasma,[55] while anxiety-depression status may cause an increase of IL-1β and a decrease of IL-10 levels, leading to the occurrence or perpetuation of IBS.[56] At the same time, pro-inflammatory cytokines can affect the ENS, causing altered pain threshold and visceral hypersensitivity.[57] There are not many studies involving direct manipulation of cytokines, but recently, interest in the therapeutic potential of IL-10 in IBS has been increasing.[58] Martín and colleagues tested a new approach where a recombinant strain of *Lactococcus lactis* secreting active anti-inflammatory IL-10 locally at the mucosal surface of IBS animal models provided protective effects regarding permeability, immune activation and gut-function parameters.[59]

Closely related to cytokines are the toll-like receptors (TLRs). TLRs are single-membrane proteins expressed by several innate immune cells, fibroblasts and epithelial cells. Although TLRs have been described in IBD,[60] their involvement in the pathophysiology of IBS remains unclear. Experiments with IBS animal models have shown a significant increase in the expression of TLR3, 4, 5, 7, 8, 9 and 10, but not TLR2 or 6, in the colonic mucosa of rats as consequence of chronic stress.[61] TLR4 has been suggested to be modulated by CRH levels,[62] and downregulation of TLR4 expression has been related to a decrease in the levels of pro-inflammatory cytokines released by mast cells, ameliorating visceral hypersensitivity and intestinal permeability as a result.[63] However, more studies are needed before TLR4 can be considered as a therapeutic target in IBS treatment.

The ENS: targeting neuropeptides and their receptors

There is continuous ongoing signalling between immune cells and nerves of the ENS within the intestinal mucosa. This signalling involves neurotransmitters secreted either from nerve endings or from the immune cells themselves.[12] The most common neurotransmitters related to GI disorders are VIP, SP, CRH, acetylcholine and serotonin. Several immune cells express receptors for the neuropeptides mentioned, such as SP, VIP

and CRH.[8,64,65] The GI tract is innervated by the vagus nerve, which connects the CNS with visceral organs. The main neurotransmitter of the vagus nerve is acetylcholine, which is known to reduce secretion of pro-inflammatory cytokines from immune cells. The cholinergic anti-inflammatory pathway to the GI tract is divided into two distinct routes. The first involves efferent vagus neurons directly targeting the myenteric plexus in the intestinal wall. The second route involves efferent vagus neurons that synapse functionally in the celiac plexus. All signals are transmitted by the adrenergic splenic nerve and further conveyed by T cells secreting acetylcholine to immune cells in the spleen expressing α7nAChR. Both pathways probably contribute to a reduced release of pro-inflammatory cytokines *via* the activation of α7nAChR.[66]

Within the ENS, an intriguing population of astrocyte-like cells called enteric glial cells (EGC) are found, forming an extensive network within the GI mucosa. Previously, they were regarded as passive support cells, but recent reports confirm their active involvement in the regulation of motility, immune activation and epithelial barrier integrity through the release of soluble mediators.[67,68] EGC actively sense and propagate signals, both to and from nearby enteric neurons and epithelial cells. There is limited knowledge about the activities of EGC in GI disorders, such as IBS; however, Lilli and colleagues recently demonstrated alterations in EGC in patients with IBS compared with healthy subjects that correlated to the frequency and intensity of pain and bloating.[69] A VIP-regulated neuro-EGC circuit has further been demonstrated in the mouse submucosal plexus,[70] and VIP can promote neuroprotection by inducing EGC to release neurotrophic mediators, thereby inhibiting EGC-derived inflammatory cytokines.[71] Together, this indicates that targeting EGC represents a potential novel therapeutic approach that may have important clinical implications for patients suffering from IBS; however, more studies are needed.

VIP and VIP receptors

The neuropeptide VIP is found in immune cells such as mast cells and lymphocytes, and, mostly, in enteric neurons of the GI tract.[8,72] VIP-containing neurons innervate the intestinal epithelium, regulating epithelial homeostasis and fluid and ion secretion.[73] Studies have suggested

VIP in the regulation of intestinal permeability,[74] and we have previously shown that VIP, *via* mast cells, regulates the intestinal barrier of healthy humans and of patients with IBS,[8,10] suggesting VIP or its receptors (VPACs) as potential therapeutic targets. When going through the literature, it is difficult to find studies directly investigating VIP as a potential candidate for IBS treatment since the few studies present were performed in animal models. However, Wu and colleagues demonstrated in a rat model of IBS that electroacupuncture inhibited colonic mast cell degranulation and secretion of VIP (and also SP that will be further discussed below) and concluded that oversecretion of neuropeptides such as SP and VIP, and their receptors, could be one of key mechanisms of IBS aetiology.[75] Moreover, the inhibition of SP and VIP secretion could be the major effect of electroacupuncture when treating rats with IBS. In a study by Del Valle-Pinero and colleagues,[76] the role of VIP in IBS was demonstrated by showing increased plasma levels of VIP in rats with trinitrobenzene sulphonic acid-induced colitis as compared with naive animals. Furthermore, VIP gene expression from peripheral whole blood was significantly upregulated in IBS patients when compared with healthy controls.[76] These findings highlight the importance of VIP in IBS pathophysiology, and suggest VIP and VPACs as potential therapeutic targets.

Tachykinins and neurokinin receptors

Tachykinins are neuropeptides synthesized primarily by neurons that regulate intestinal motility, secretion and vascular functions. The tachykinin family includes, for example, SP and neurokinins A and B.[77] The biological activity of tachykinins is mediated through neurokinin receptors 1 (NK_1R), NK_2R and NK_3R, and it is known that tachykinin receptors on immune cells are activated during gut inflammation.[78] SP is expressed widely throughout the GI tract and mediates its effects through the NK_1R found throughout the ENS and expressed on, for example, enterocytes and immune cells. Neurons containing SP are found adjacent to mucosal mast cells,[79] suggesting a relationship between extrinsic afferents (vagal or spinal) and epithelial- and immune cells of the mucosa. Animal studies have shown that SR140333, an antagonist of NK_1R, alleviated visceral hyperalgesia induced by stress.[80] Further, we previously showed that intestinal barrier dysfunction caused by chronic stress in rats was inhibited by blocking NK_1R, and that SP acts in a discriminatory manner on intestinal permeability.[64] In contrast to SP, neurokinin A and B binds to NK_2R, which is expressed mostly in the muscularis mucosa and on enterocytes and immune cells. Preclinical studies and clinical trials have shown that NK_2R inhibition may constitute a novel option in the treatment of IBS. Among others, nepadutant and ibodutant are two NK_2R-antagonists that have shown promising clinical implications, such as reduced gut motility and pain.[81,82] For more reading on tachykinins, and in particular NK_2R antagonists in IBS treatment, the reader is referred to a recently published review article by Szymaskiewicz and colleagues.[77]

CRH and CRH receptors

CRH, also known as corticotrophin releasing factor, is an endocrine hormone produced in response to stress, activating the HPA axis that results in enhanced levels of adrenocorticotropic hormone (ACTH) and cortisol. In IBS-diarrhoea, levels of both basal and stimulated cortisol were higher in patients, and correlated with anxiety but not IBS symptoms.[83] Nevertheless, peripheral administration of the nonselective CRH receptor antagonist, α-helical CRH, in IBS patients demonstrated an improvement in GI motility and visceral perception.[84] As recently reviewed by Chatoo and colleagues,[85] CRH might be a key molecule involving immune and intestinal motility (together with its receptors) *via* the gut–brain axis in IBS. Activation of CRH receptors is also related to barrier permeability and inflammation. We previously showed that barrier dysfunction caused by chronic stress in rats, or by exposure of human biopsies to stressors, was inhibited by blocking CRH-receptors.[8,64,65] An evident role for neuro-immune signalling *via* mast cells and CRH_1R was described as a positive modulator of stimuli-induced degranulation and *in vivo* pathophysiologic responses to immunologic and psychologic stress.[86] Stress increased the number of mucosal mast cells and intestinal permeability in rats under chronic stress, but these effects were not present in mast cell deficient rats.[87] Kano and colleagues investigated the HPA-axis, colonic motility and autonomic responses to CRH-administration as well as brain activity alterations in IBS patients.[88] Their results showed greater ACTH responses to CRH in IBS compared with healthy controls. A negative association between

ACTH response to CRH and activity in the pregenual anterior cingulate cortex of the brain during rectal distention was identified in controls but not in IBS patients. Impaired top-down inhibitory input from the pregenual anterior cingulate cortex to the HPA-axis may lead to altered neuroendocrine and GI responses to CRH. These authors suggested that centrally acting treatments may dampen stress-induced physical symptoms in IBS. CRH_1R antagonists have been tested in IBS animal models, restoring stress-induced defecation and hypersensitivity[89,90]; however, clinical trials using CRHR antagonists have not been that successful.[91]

Serotonin

Only about 5% of the human body's serotonin, or 5-hydroxytryptamine (5-HT), is produced in the brain. Serotonin is produced mainly by the enterochromaffin cells of the intestinal mucosa (90%) as well as by subgroups of enteric neurons (5%). From the immune system, mast cells can also synthesize and release serotonin.[92] It is further known that mast cells, dendritic cells and macrophages/monocytes have receptors for serotonin, suggesting that serotonin can influence the activity of innate immune cells.[93] Serotonin can cause bloating, nausea and vomiting,[94] and targeting serotonin receptors as well as serotonin uptake mechanisms could play a key role in the advance of effective therapies in visceral sensitivity associated disorders like IBS. A recent study in mice showed a direct link between stress, serotonin and changes in the immune system.[48] Alosetron and ramosetron are both selective serotonin antagonists of 5-HT type 3 receptor with clinical implications such as improved abdominal pain, stool consistency, GI motility and quality of life in patients with IBS-diarrhoea.[95–97] Although no studies have yet evaluated efficacy, the highly selective 5-HT type 4 receptor agonist prucalopride has shown to reduce bloating, straining, abdominal discomfort and painful bowel movements in patients with IBS-constipation.[98] Furthermore, duloxetine is an anti-depressive inhibitor of reuptake of serotonin and noradrenaline used in several cases of depression. Clinical studies have shown that duloxetine is associated with effects on improvement of pain,[99] severity of illness, quality of life, loose stool, work and family disability, and anxiety.[100] Moreover, citalopram, the most selective inhibitor of serotonin reuptake, was shown to improve IBS symptoms,[101] but later it was demonstrated that citalopram is not effective for nondepressed IBS patients,[102] suggesting that other mechanisms besides serotonin are behind the IBS symptoms.

Transient receptor potential vanilloid type I

Transient receptor potential vanilloid type 1 ($TRPV_1$) is a very well-studied pain-producer heat ion channel expressed mainly in small-to-medium sized somatic and visceral sensory neurons.[103] It is widely accepted that its activation contributes to neuro-immune inflammation,[104] which can be mediated by capsaicin, heat ($>42°C$) and acidosis ($pH < 6$), among others.[105] Upregulation of $TRPV_1$ and hyperalgesia have been reported in animal models and in IBS-diarrhoea patients.[106] Although Luo and colleagues did not find a correlation between $TRPV_1$ levels and IBS-diarrhoea abdominal pain,[107] in other studies the expression of $TRPV_1$ in nerve fibres was increased in IBS patients and in quiescent IBD with IBS symptoms,[108,109] correlating with pain severity. Furthermore, a deficiency of $TRPV_1$ is related to reduced sensitivity to colonic distension.[110] On the contrary, the sensitization of $TRPV_1$ by inflammatory factors, such as SP and histamine,[30,111] can decrease its activation threshold and increase immunoreactivity, leading to hyperalgesia.[112] There is a strong motivation to target $TRPV_1$ for drug discovery in relation to visceral hypersensitivity. Capsazepine is the $TRPV_1$ antagonist most tested in gastroenterology. Although it showed promising results in animal pancreatitis,[113] results obtained so far in humans have not been good enough. In 2007, the British pharmaceutical company GlaxoSmithKline finished phase II of a randomized clinical trial (ID: NCT00461682) where the efficiency of the $TRPV_1$ antagonist SB-705498 was tested in IBS patients. Unfortunately, no results were reported since the study objectives were not met due to the early study termination and withdrawal of participants. Surprisingly, a recent study in rats showed that milefosine, a drug used in Leishmaniasis, reversed post-stress hypersensitivity by reducing $TRPV_1$ activation, becoming a new interesting candidate for IBS treatment.[114]

Opioid receptors

Opioid receptors (OR), including the three major subtypes δ (DOR), κ (KOR) and μ (MOR), are G protein-coupled receptors expressed in myenteric

and submucosal plexus neurons of the ENS. Their main role is to modulate GI motility, secretion and visceral sensation.[115] OR are expressed by immune cells, mostly T cells, suggesting that the opioid system also plays an immune-related role.[116] It is important to remember that pain and inflammation are linked and closed related. Basso and colleagues demonstrated that the OR desensitization involves the activation of $TRPV_1$, suggesting that the dysregulation of the $TRPV_1$ axis may thus contribute to the transition from acute to chronic pain.[117] Patients with major depression present higher KOR and MOR compared with healthy controls, which intercorrelates with increased IL-6 and IL-10 levels.[118] Increased expression of MOR with immunoreactivity in CD4+ and CD31+ T cells and eosinophils, but not mast cells, has been reported in IBS patients.[119] Interestingly, it has been shown that blocking of MOR and DOR expression in the cell membrane of CD8+ T cells inhibits their proliferation,[120] and that CD4+ T cells not only produce opioids, but also help in the endogenous regulation of visceral pain and inflammation with the production of IL-4.[121,122] The use of opioid agonists has been suggested in IBS treatment. OR subtypes differ in their responsiveness, but, in general, opioids reduce gut transit by inhibiting colonic motility, resulting in constipation.[123] Asimadoline is a selective KOR agonist that has shown promising clinical implications in IBS-diarrhoea patients, inducing adequate relief of IBS pain, discomfort and symptoms, and anti-diarrhoeal effects.[124] Similarly, loperamide, a MOR agonist, and eluxadoline, a MOR agonist and DOR antagonist, are two approved drugs used for the treatment of IBS-diarrhoea.[125] Despite inducing potent analgesia, clinical utility of opioids is limited by several adverse effects such as tolerance and paradoxical hyperalgesia.[126] A recent study in mice showed that the novel multi-target opioid MCRT had a potent analgesic effect with less GI dysmotility mediated by DOR, and MCRT was thus suggested as a safer opioid analgesic.[127] Berberine is a chemical found in plants used in Chinese and Ayurvedic medicine for thousands of years.[128] Several studies have indicated the benefits of berberine in GI disorders in reducing intestinal motility, pain[129] and the expression of pro-inflammatory cytokines, and, consequently, inflammation[130] mediated by MOR[131] and DOR.[129]

Neuropeptide Y and peptide YY

Neuropeptide Y (NPY) and peptide YY (PYY) are biologically active peptides. NPY is one of the most abundant neuropeptides within the brain. In the gut, not only enteroendocrine cells but also enteric neurons, monocytes, macrophages, lymphocytes and dendritic cells can synthesize NPY. In contrast to NPY, PYY is expressed almost exclusively in the digestive system, and it is secreted mostly by enteroendocrine L cells. NPY and PYY exert their effects *via* five Y receptors, Y1 and Y2 being the most abundant and expressed in immune cells and along the gut–brain signalling pathway.[132] It has been shown that PYY and NPY influence the GI motor and secretory activity accelerating or inhibiting colonic transit after stimulation of Y1 or Y2, respectively.[133] These neuropeptides, besides playing a role in visceral nociception, can influence the immune system and serve as pleiotropic modulators, depending on the context. Increasing evidence proves that NPY promotes GI inflammation[134]; more specifically, Y1 receptor inactivation reduces TNF and IL-12 production by macrophages and decreases the number of T cells.[135] Although there are not many studies related to these neuropeptides in IBS, it was recently reported that concentrations of NPY are increased while concentrations of PYY are remarkably decreased in IBS patients,[136,137] with NPY being suggested as a clinically effective biomarker. These findings underscore an important role of NPY, PYY and their receptors, and support the hypothesis that they are worthwhile targets for IBS therapy.

Conclusion

Current treatment for IBS includes mostly interventions for lifestyle, diet, stool consistency, probiotics, pain relievers and antidepressants. Low-grade mucosal inflammation is present in many IBS patients. Therapies involving immune cells might therefore be a good strategy in the development of novel potential therapeutic options for treating IBS, even if not all patients have obvious immune activation. Moreover, the approach of targeting neuropeptides and their receptors may lead to a more directed pharmacological therapy for patients with IBS and other GI disorders in the future. Some neuro-immune target therapies are already in use, but other potential approaches are summarized in Figure 1. More studies are needed to confirm the more novel

neuro-immune targets, their direct effects on IBS symptoms and disease improvement, and their potential use in IBS treatment.

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