Understanding neuroimmune interactions in disorders of gut–brain interaction: from functional to immune-mediated disorders

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ABSTRACT

Functional gastrointestinal disorders—recently renamed into disorders of gut–brain interaction—such as irritable bowel syndrome and functional dyspepsia are highly prevalent conditions with bothersome abdominal symptoms in the absence of structural abnormalities. While traditionally considered as motility disorders or even psychosomatic conditions, our understanding of the pathophysiology has evolved significantly over the last two decades. Initial observations of subtle mucosal infiltration with immune cells, especially mast cells and eosinophils, are since recently being backed up by mechanistic evidence demonstrating increased release of nociceptive mediators by immune cells and the intestinal epithelium. These mediators can activate sensitised neurons leading to visceral hypersensitivity with bothersome symptoms. The interaction between immune activation and an impaired barrier function of the gut is most likely a bidirectional one with alterations in the microbiota, psychological stress and food components as upstream players in the pathophysiology. Only few immune-targeting treatments are currently available, but an improved understanding through a multidisciplinary scientific approach will hopefully identify novel, more precise treatment targets with ultimately better outcomes.

INTRODUCTION

Functional gastrointestinal (GI) disorders are defined by abdominal symptoms in the absence of a structural abnormality explaining the problems. Among the most frequent conditions are irritable bowel syndrome (IBS) characterised by abdominal pain in association with an altered stool pattern and functional dyspepsia (FD) with upper abdominal symptoms (figure 1).[1 2] FD symptoms can be triggered by a meal in the postprandial distress syndrome (PDS) or present as epigastric pain or burning not related to food intake in case of the epigastric pain syndrome.[2] In the absence of a biomarker, diagnosis relies on symptom criteria of which the Rome IV criteria are the most recent and best validated.[3] In a recent large-scale population study by the Rome foundation, the prevalence of IBS and FD was estimated at 4.1 and 7.2%, respectively, using the strict Rome criteria,[4] even if for IBS the current criteria have been criticised for being too restrictive in terms of symptom description (only pain, not discomfort) and frequency.[5]

The research focus on the pathophysiology of functional GI disorders has significantly evolved over the last 50 years. Functional GI disorders were initially considered to be purely psychosomatic disorders without a disease substrate in the GI tract. Subsequently, with the development of new tools to examine the function of the GI tract in the 70s and 80s including manometry, GI scintigraphy and breath testing, attention shifted to changes in GI motility including an exaggerated gastrocolonic reflex in IBS and delayed gastric emptying in FD.[6–8] Disappointingly, the link between symptoms and disordered motility is limited and has not translated into efficacious treatment options. For example, even if delayed gastric emptying can be found in up to 25% of patients with FD, the correlation to symptoms is inconsistent[9–11] and the efficacy of currently available prokinetics is suboptimal and therefore not recommended as first-line therapy in the American, Canadian and European guidelines.[12–15] The recognition of the interplay between central (eg, psychological comorbidity including stress and anxiety, altered brain processing) and peripheral, GI alterations (eg, disordered motility, visceral hypersensitivity, immune activation, …) led to the renaming of 'functional gastrointestinal disorders' to 'disorders of gut-brain interaction' (DGBI) in the Rome IV consensus definition (figure 2).[3] In the past 20 years, visceral hypersensitivity, rather than dysmotility, has emerged as a central theme in the pathophysiology of DGBI. Visceral hypersensitivity,
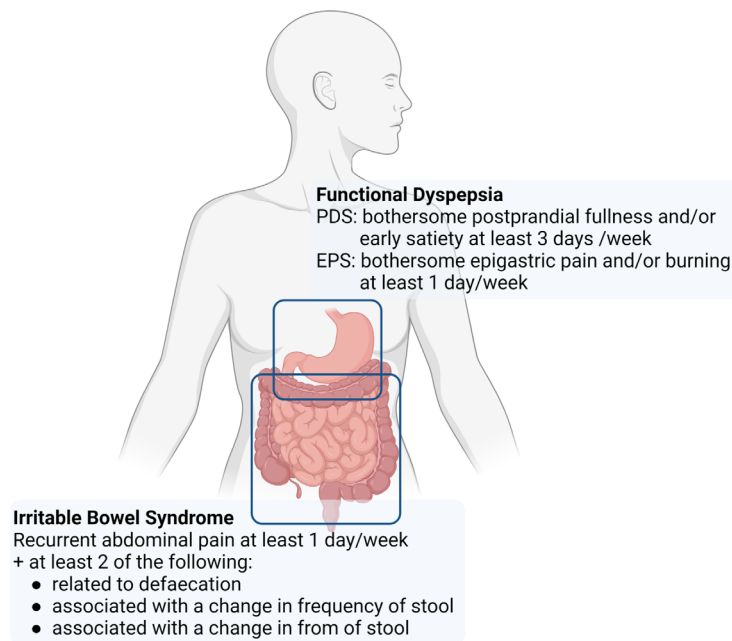
Figure 1 Rome IV criteria of functional dyspepsia and irritable bowel syndrome. EPS, epigastric pain syndrome; PDS, postprandial distress syndrome.

that is, abnormal pain signalling to chemical stimuli and/or mechanical distention, has been reported in a variety of patient populations ranging from patients suffering from non-cardiac chest pain,[16] gastro-oesophageal reflux disease,[17 18] FD[19–22] and IBS.[23–25] In the current review, we will discuss recent advances in basic and translational research in humans which help us to understand how changes in the GI microenvironment contribute to the development of visceral hypersensitivity in IBS and FD, with a focus on immune activation and neuroimmune interactions. In particular, we will critically discuss the literature on potential mechanisms leading to activation of eosinophils and mast cells, including impaired barrier function, reactions to food, diet–microbiome interactions and psychological comorbidity,

and how improved insight in the pathophysiology could be potentially translated into novel treatments.

Visceral pain signalling and visceral hypersensitivity

Although sensory neurons located in the enteric nervous system represent key sensors in local neural circuits involved in secretion, absorption, motility and so on, stimuli triggering visceral sensations are mainly conveyed by extrinsic sensory pathways that link the gut to the central nervous system.[26] These extrinsic nerve fibres have their cell body in the dorsal root ganglia, synapse in the dorsal horn of the spinal cord, and can be classified into different subtypes of sensory endings depending on
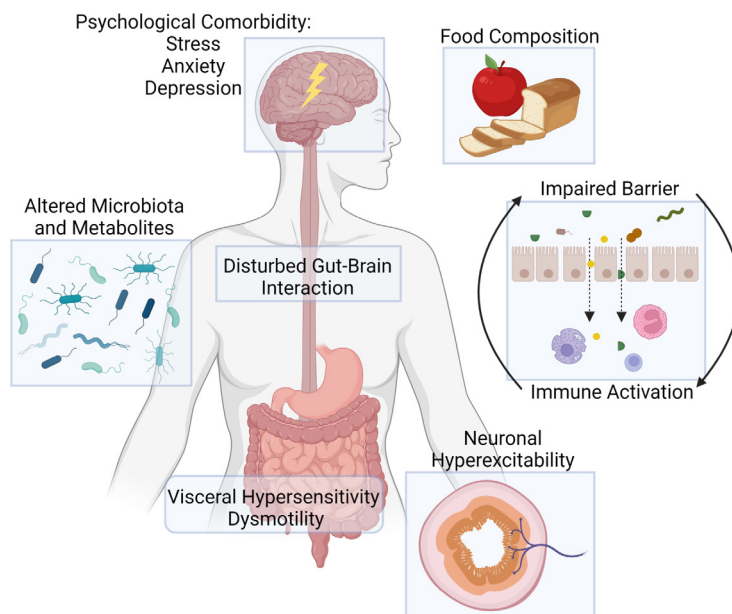
Figure 2 Pathophysiological mechanisms in disorders of gut–brain interaction.
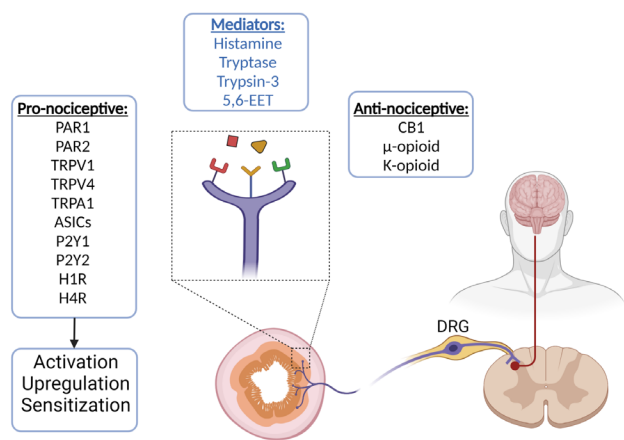
Figure 3 Neuronal mechanisms of visceral hypersensitivity. 5,6-EET, 5,6-epoxyeicosatrienoic acid; ASIC, acid-sensing ion channel; CB1, cannabinoid receptor 1; DRG, dorsal root ganglion; H1R, histamine 1 receptor; P2Y1/2, purinergic receptor P2Y1/2; PAR1/2, protease-activated receptor 1/2; TRPA1, transient receptor potential ankyrin 1; TRPV1/4, transient receptor potential vanilloid 1/4.

the layer they innervate; mucosal, muscular, muscular/mucosal, vascular, serosal and mesenteric afferents.[27 28] Visceral pain in particular is detected by pain sensing neurons or nociceptors dedicated to detect harmful thermal, mechanical or chemical stimuli. To this end, their peripheral nerve terminals are equipped with a variety of receptors and ion channels that can be classified into pronociceptive (or excitatory) and antinociceptive (or inhibitory) (figure 3). Transient receptor potential (TRP) channels (TRPV1, TRPV4), acid-sensing ion channels, purinergic and histaminergic receptors, protease activated receptors (PAR1-2) are examples of pronociceptive receptors while typical examples of antinociceptive receptors are opioid and cannabinoid receptors. Depending on the balance between pronociceptive and antinociceptive signals, the nociceptor will signal to the spinal cord transmitting pain to the brain stem, thalamus and cortex. While acute pain is a physiological mechanism of protection informing the organism on a potentially harmful condition or injury, chronic visceral pain is debilitating and can result from either peripheral or central mechanisms affecting pain signalling. Central mechanisms include sensitisation at the level of the spinal cord and abnormal pain processing in the brain. The latter has been elegantly demonstrated mainly by functional MRI revealing abnormal local and global connectivity in the areas related to pain processing.[29] In this review, we will focus on peripheral mechanisms affecting the function of visceral nociceptors in patients suffering from functional GI disorders.

Visceral hypersensitivity consists of a painful response to a normally innocuous stimulus (allodynia) and/or an exaggerated response to a painful stimulus (hyperalgesia). This condition can result from increased levels of pronociceptive or decreased levels of antinociceptive mediators in the tissue, upregulation of excitatory receptors or sensitisation of these receptors located on nerve terminals.

Increased release of soluble mediators
Although decreased release by peripheral blood mononuclear cells (PBMCs) of antinociceptive mediators, in particular opioids, has been described in patients with constipation-predominant IBS,[30] further evidence supporting this mechanism of aberrant pain signalling is rather limited. In contrast, increased levels of

pronociceptive mediators activating sensory neurons has been repeatedly reported in supernatant of biopsies collected from patients with IBS. IBS supernatant indeed contains more histamine and tryptase than supernatant of healthy control biopsies and activates murine visceral afferents or human submucosal neurons, a phenomenon blocked by histamine antagonists and serine protease inhibition.[31–33] Intestinal epithelial cells of patients with IBS produce and release increased levels of the active protease trypsin-3, able to signal to enteric neurons and induce visceral hypersensitivity.[34] Of interest, increased levels of the TRPV4 agonist 5,6-epoxyeicosatrienoic acid, a metabolite of polyunsaturated fatty acids, have also been reported in IBS biopsies (figure 3).[35] The evidence indicating altered synthesis and release of pronociceptive mediators in FD is limited, although increased spontaneous release of histamine and serotonin by gastric biopsies has been reported, in particular in patients suffering from postinfectious FD.[36] Besides immune cells, enteroendocrine cells (EEC) represent another source of released mediators such as serotonin. Increased numbers of EEC have been reported in colonic biopsies of patients with diarrhoea-predominant IBS (IBS-D) which was linked to symptom severity, but evidence on EEC activation in DGBI is missing.[37 38] Besides production of soluble mediators, subgroups of EECs, labelled as neuropod cells, have been demonstrated to synapse directly with neurons.[39] These neuropod cells may function as sensors for luminal signals, but their role in pathophysiology and symptom generation in DGBI remains to be investigated.

Not only in the gut, but also increased release of pronociceptive mediators in peripheral blood of patients has been reported. Medium collected from cultured PBMCs from patients with IBS-D or FD contains more interleukin (IL)−1b, IL-10, tumor-necrosis factor (TNF)-a and IL-6 than that of healthy controls.[30 40] Of note, murine visceral afferents respond with direct activation to IL-6 and IL-10, not to TNF-a.[30] To what extent peripheral blood cells behave similar to tissue resident immune cells remains however to be confirmed.

Increase excitability of pronociceptive receptors
Increased firing of visceral afferent can also result from increased expression (upregulation) of pronociceptive receptors or ion channels in the plasma membrane or sensitisation of these sensors. Increased expression of TRPV1 and TRPV2 has been reported in gastric biopsies of patients with FD,[41] while TRPV1[42–44] and the purinoreceptors P2Y1 and P2Y2[42] are increased in rectosigmoid biopsies of patients with IBS (figure 3). A recent study also found elevated expression of TRPV1 and TRPV3 in duodenal biopsies of patients with IBS which correlated with abdominal pain scores in case of TRPV1.[45] Inflammatory mediators such as nerve growth factor or inflammatory cytokines can induce increased ion channel expression and transport to the cell membrane of visceral afferents. Of interest, also exposure of DRG neurons to serotonin or histamine results in increased expression of TRPV4 receptors at the plasma membrane surface. This effect was already observed 5 min after treatment and blocked by a specific mitogen-activated protein kinase (MAPK) inhibitor indicating that this effect was not due to new synthesis of TRPV4 but rather to redistribution to the plasma membrane, a phenomenon involving a MAPK pathway.[46]

Sensitisation of nociceptors involves a reduction in the threshold of activation associated with an increase in the response to noxious stimuli. Using live Ca^{2+} imaging, we previously demonstrated that submucosal neurons in rectal biopsies from patients with IBS were significantly more excited by

TRPA1, TRPV1 and TRPV4 agonists than neurons of healthy controls. Of note, gene expression of these receptors was similar between patients with IBS and controls, indicating that the respective TRP channels are sensitised rather than upregulated,[47 48] and may contribute to aberrant pain signalling in IBS. In contrast to IBS, structural abnormalities and decreased responses of duodenal submucosal neurons to general stimuli, including high concentrations of potassium and electrical stimulation, were noted in FD, which correlated with the degree of duodenal eosinophil and mast cell infiltration.[49]

Besides neurons, enteric glial cells are a second important cell type of the enteric nervous system and are involved in the modulation of neuronal function, but also in epithelial barrier function.[50] Activation of enteric glial cells (ie, gliosis) was shown in FD[49] and IBS,[51] although an earlier study found decreased glial cell responses during Ca^{2+} imaging on exposure to IBS-D supernatant which was dependent on activation of the histamine receptor 1 (H1R).[52]

Clearly, as noted above, submucosal enteric neurons are not transmitting nociceptive information to the central nervous system. However, TRP channel sensitisation of submucosal neurons strongly suggests that the microenvironment in which these neurons reside contains mediators with sensitising properties and thus can equally affect neighbouring nerve terminals of extrinsic visceral afferents. Of note, incubation of biopsies from healthy controls with histamine results in sensitisation of TRPV1 on submucosal neurons, suggesting the possible involvement of histamine in the hyperexcitability of pain signalling pathways.[47] Obviously, other mediators such as cytokines, serotonin, proteases and metabolites of polyunsaturated fatty acids, present in supernatant of colonic/rectal biopsies can all contribute.

Aberrant visceral pain signalling induced by biopsy supernatant

Finally, preclinical studies involving both in vivo assessment of visceral pain and in vitro recording of visceral afferents have provided convincing evidence that supernatant, in particular collected from IBS biopsies, contains mediators that excite nociceptors and induce visceral hypersensitivity.[28] For example, Cenac et al elegantly demonstrated increased trypsin and tryptase expression and release in colonic biopsies of patients with IBS and sensitisation of murine sensory neurons in vitro.[53] Interestingly, administration of IBS supernatant into the colon of mice resulted in increased pain responses evoked by colorectal distension. These pronociceptive effects were blocked by serine protease inhibitors and a PAR2 antagonist and absent in PAR2-deficient mice, clearly demonstrating the involvement of proteases. Along the same line, application of IBS supernatant to murine visceral nociceptive afferents[32 54] or human submucosal neurons[33] results in increased neuronal firing, effects mediated by histamine, serotonin and proteases.

Taken together, the above evidence clearly indicates that the microenvironment in the intestine of IBS and most likely also of functional dyspeptic patients possesses pronociceptive properties increasing pain signalling in these patients.

IMMUNE ACTIVATION

The most likely source of nociceptive mediators in the intestinal microenvironment described above is the mucosal immune system. Even if obvious macroscopic or microscopic inflammation is not compatible with a diagnosis of a functional GI disorder following the Rome criteria, cumulative evidence from the last two decades suggests an important role for mucosal immune activation in both IBS and FD.

Immune cell infiltration and activation

Immune cell infiltration in DGBI was first demonstrated in patients who developed IBS after an infectious gastroenteritis with a persistently elevated number of mononuclear cells and T lymphocytes in rectal biopsies.[55 56] Since then, several groups reported similar findings and a meta-analysis confirmed increased T-cells in the lamina propria of the rectosigmoid.[57] In contrast, gastroduodenal lymphocyte infiltration, including intra-epithelial lymphocyte counts, does not come up as a consistent feature of FD,[58 59] even if two small studies reported elevated CD8+T cell counts and aggregates in postinfectious FD.[60 61] Peripheral blood lymphocytes expressing gut-homing markers were increased in FD[40] and IBS.[62–64] Whether the circulating gut-homing lymphocytes have a role in the pathogenesis of the disease, including GI immune activation remains to be demonstrated.

More importantly, the most reproducible finding in the literature relates to the involvement of mast cells and eosinophils in the pathophysiology of IBS and FD, respectively (figure 4). In 2004, the Bologna group was the first to report increased mast cell numbers in rectal biopsies of 44 patients with IBS.[31] These findings were corroborated by several other studies, mainly in the left hemicolon and to a similar extent in the different IBS subtypes, with some studies demonstrating an association between mast cell numbers and symptom.[57 65] Nevertheless, the mast cell infiltration is not an undisputed finding as some groups have not detected a difference or even lower mast cell counts.[57 66 67] These differences may relate to the patient selection, location of the biopsy but also the quantification methodology (eg, slide preparation, staining, counting). Moreover, it is unlikely that the subtle increase in immune cell numbers is relevant for symptom induction and may be an epiphenomenon in the context of immune cell activation. Indeed, mast cell activation—rather than just the numbers—demonstrated by degranulation and release of mast cell mediators such as histamine and tryptase in supernatant emerges as a more consistent finding with more relevance to symptom generation, based on a correlation between symptom severity and the distance of the mast cells to mucosal nerves.[31 68] Moreover, as discussed above, IBS supernatant-induced hyperreactivity of neurons which could be blocked by histamine receptor H1 antagonists and protease inhibitors, underscoring the importance of mast cell derived soluble mediators in neuronal hyperreactivity.[32 33 47] The role of mast cells in FD is less established, although a few studies have shown increased duodenal mast cell counts.[59 69] Mast cell activation in FD is suggested by a study using electron microscopy showing more heterogeneous granules in duodenal mast cells in FD and evidence of increased release of histamine and tryptase from gastric biopsies.[36 70] Similar to IBS, mast cells were more likely to be localised near mucosal nerves in the stomach and the duodenum.[36 71]

In FD, however, a more prominent role has been attributed to duodenal eosinophil infiltration which has been reported by multiple groups (figure 4).[69] Duodenal eosinophils as well as their activation and degranulation were often also linked to symptoms, although only based on statistical correlations and mechanistic evidence is still largely lacking.[59 69] In the initial reports, it was suggested that duodenal eosinophilia was a feature of PDS,[72] but a recent meta-analysis showed similar counts in the FD subgroups.[69] Nevertheless—and similar to data on mast cell
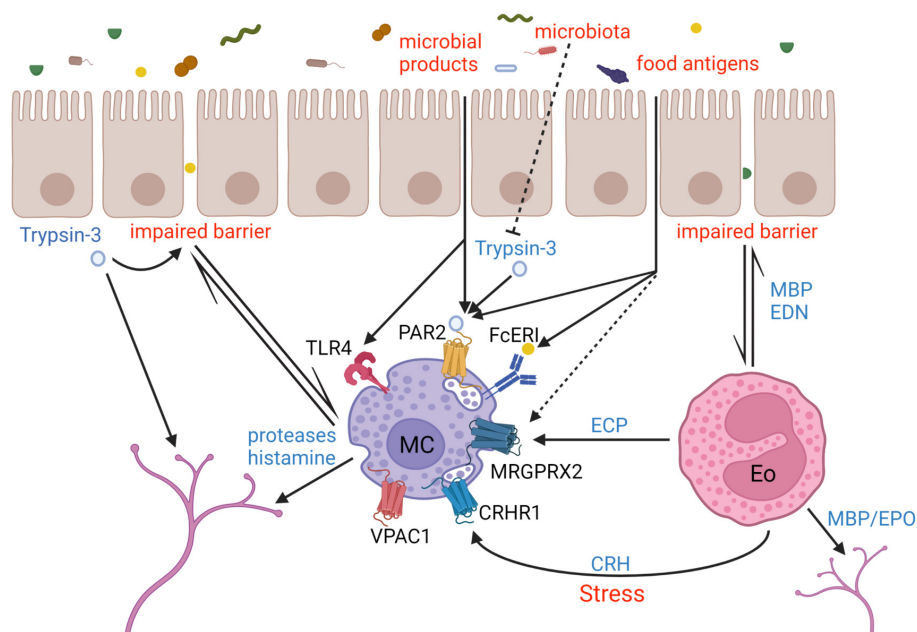
Figure 4 Immune activation in irritable bowel syndrome and functional dyspepsia. Food and microbiota-derived antigens gain access to the subepithelial space through an impaired intestinal barrier function. These antigens and epithelium-derived proteases such as trypsin-3 can activate mast cells and eosinophils through a variety of mechanisms. Mediators from activated mast cells and eosinophils can subsequently activate sensory neurons. Finally, in conditions of psychological stress locally secreted CRH from eosinophils can activate mast cells. CRH, corticotropin-releasing hormone; CRHR1, CRH receptor 1; ECP, eosinophil cationic protein; EDN, eosinophil-derived neurotoxin; Eo, eosinophil; EPO, eosinophil peroxidase; FcERI, high-affinity IgE receptor; MBP, major basic protein; MC, mast cell; MRGPRX2, Mas-related G-protein coupled receptor member X2; NK1/2, neurokinin receptor 1/2; PAR2, Protease-activated receptor 2; SP, Substance P; TLR4, Toll-like receptor 4; VPAC1, vasoactive intestinal peptide receptor type 1. Dashed line indicates a potential link.

counts in IBS—elevated duodenal eosinophil counts were not detected in all cohorts: in the largest study to date, Järbrink-Seghal *et al* found no association between duodenal eosinophil counts and FD in 178 patients and 258 controls, while degranulation, that is, activation, was associated to FD and symptoms of early satiety.[73] These discrepancies in the literature may again come down to patient selection, the variability in quantification methodology and stress that research should focus on activation of immune cells rather than quantification of cell numbers, similar to IBS. Another important confounder in the FD literature is also the use of comedication, most notably proton-pump inhibitors (PPIs), which is not appropriately addressed in most studies. In a recent prospective study, we have shown that the use of a PPI, which is still the first-line treatment for FD as recommended by guidelines,[14] lowered duodenal eosinophil and mast cell counts in the short term, although prolonged treatment may provoke opposite, proinflammatory effects, potentially through duodenal dysbiosis by overgrowth of oropharyngeal flora.[74 75] Indeed, the increased numbers of duodenal eosinophils after longer term PPI in patients without symptomatic benefit of the PPI and in healthy individuals treated with PPI for 4 weeks, were linked to increased abundance of Streptococci in duodenal brushings.[75] Increased duodenal abundance of streptococci has recently been linked to symptom severity in FD.[76] In IBS, a few studies have found eosinophil infiltration in the colon[77] and a recent Swedish study found increased levels of faecal eosinophil-derived neurotoxin in patients with IBS on top of mucosal infiltration and degranulation as shown by histology.[78] Surprisingly, few data are available on gastric immune activation in FD with only one study reporting higher mast cell counts in gastric biopsies of patients with FD.[36]

Unfortunately, the large majority studies described above have only considered histological cell counts rather than immune cell activation which is most likely the critical factor in DGBI. For example, healthy subjects treated with PPI also displayed increased duodenal eosinophil counts in the absence of abdominal symptoms,[74] highlighting that the reported limited increases in cell counts cannot be hold accountable for symptoms just by their presence and that future research should focus on activation of immune cells and the produced mediators.

Circulating and tissue cytokines

The data on systemic and mucosal cytokine expression and release are contradictory and interpretation is hampered by small patient numbers and in most cases selective analysis of a limited number of cytokines. In a meta-analysis, a tendency towards increased circulating TNF-α and lower colonic release of IL-10 was the only signal that emerged in IBS, although the magnitude was limited.[79] Another possible reason for the lack of a clear immune signal is the heterogeneity of the condition itself which is only defined by subjective symptoms. It is to be expected that only a subgroup of DGBI patients will exhibit a mild immune activation signature. Indeed, in several larger studies, 1/5 to 1/3 of patients with IBS demonstrated an 'immuno-active' phenotype in terms of systemic release or local expression of inflammatory mediators.[80–82] In a recent study, we demonstrated that 1/3 of patients with IBS exhibited signs of immune activation in biopsies from the descending colon.[82] This group showed higher expression of IL1β, prostaglandin synthase PTGS2 and the G-protein coupled receptor Mas-related G-protein coupled receptor member X2. The latter is of interest since it is a receptor involved in mast

cell activation and eosinophil-mast cell interaction,[83] even if it was only expressed in a minority of the biopsies.[82] Nevertheless, in none of the mentioned studies, a link between symptom pattern or severity and the immune phenotype could be established. Similarly, large studies are lacking in FD and even if mildly elevated IL-1 and IL6 expression in the duodenal and gastric mucosa have been reported, the findings are limited to single studies with a small sample size.[59] One small study found a higher production of the Th2 related cytokines IL-5 and IL-13 by stimulated PBMCs, which is of interest based on the eosinophil-predominant immune signal in FD, although confirmatory data are needed.[84] Finally, discretely increased high-sensitive C-reactive protein levels in patients with FD in our recent study, which normalised after PPI treatment, also suggest a systemic immune signal although the relevance to symptom generation of these minute systemic changes is most likely limited.[74]

Aetiology of GI immune activation

We selected four potential mechanisms leading to immune activation for which reasonable evidence is available in the current literature demonstrating a mechanism of action, beyond the existence of an associative link with immune activation.

Impaired barrier function

The intestinal barrier represents the largest interface between the environment and the internal milieu of the organism. The barrier has a dual function of protecting the organism from unwanted penetration of luminal, potentially noxious substances including pathogens and their secreted products, while at the same time allowing absorption of fluid and (micro)nutrients. The barrier also plays a critical role in suppressing immune activation towards orally ingested innocuous antigens, mainly food, in a process defined as oral tolerance.[85] To achieve oral tolerance, luminal antigens are sampled by microfold (M) cells in Peyer patches, mucosal macrophages or goblet cells, subsequently acquired by tolerogenic dendritic cells which then migrate to mesenteric lymph nodes with induction of immunosuppressive regulatory T-cells.

Disruption of this tightly regulated barrier function has been described in a variety of conditions, including coeliac disease, inflammatory bowel disease, food allergy and functional GI disorders.[86] However, measurement of intestinal barrier function is complex and contributes to the variability of the reported data. Direct measurement of permeability of endoscopic biopsies in Ussing chambers is still the gold standard, but less frequently used.[86 87]

In a recent systematic review, most of the 66 analysed studies in IBS found a reduced small intestinal or colonic barrier function, which was mainly present in postinfectious and diarrhoea-predominant IBS and to a lesser extent in constipation predominant IBS.[88] Positive associations with altered stool pattern and pain were identified in the majority of the studies, although an inverse association has been reported as well.[89] Similarly, multiple studies have reported increased duodenal permeability in FD using in vivo techniques (urinary sugar excretion tests, mucosal impedance and confocal laser endomicroscopy (CLE)) and biopsies in Ussing chambers, although a link with symptom severity was absent in most studies.[87]

The expression of several tight junction related proteins, which are the key determinants of epithelial barrier function, in different regions of the GI tract was shown to be altered in IBS and FD, although the reported changes were not consistent between studies.[87 88] Colonic infusion of faecal supernatant of

patients with IBS in mice or application of biopsy supernatant on cell lines resulted in lower barrier function, indicating the involvement of luminal mediators.[90–93] The identity of these mediators is still unclear, but proteases, microbial products, food and bile acids are the main candidates (figure 4). Currently, most of the evidence supports a role for proteases. Faecal supernatant of patients with IBS contains increased levels of serine and cysteine proteases which were associated with impaired barrier function and altered tight junction expression.[90 91 94 95] Several studies demonstrated that these proteases are from human origin, including the epithelium-derived trypsin-3.[34 93 95] A recent elegant study demonstrated that high proteolytic activity in patients with postinfectious IBS was related to impaired suppression of the host-derived proteases by the microbiota.[93]

It is often hypothesised that the impaired barrier function allows uncontrolled penetration of antigens in the lamina propria, inciting an immune response. However, whether the increase permeability plays a causal role in these conditions or is rather a consequence of the immune activation remains a topic of ongoing controversy. In support of the last option, tryptase released from mast cells[96 97] and major basic protein from eosinophils[98] have been shown to impair permeability in cell lines. Moreover, stabilising mast cells by ketotifen or blocking the receptors for vasoactive intestinal polypeptide on mast cells reduced transcellular passage of commensal and pathogenic bacteria in colonic biopsies of patients with IBS.[99] We previously demonstrated a correlation between duodenal eosinophil levels and decreased protein expression of phosphorylated occludin and E-cadherin, but mechanistic data in humans with FD are lacking.[100]

Interpretation of the relevance of intestinal barrier function in the pathophysiology of DGBI is hampered by the lack of treatments which can stabilise the barrier. A possible exception is glutamine which improved symptoms and normalised barrier function in patients with postinfectious IBS,[101] possibly through an upregulation of claudin 1.[102] However, replication of these data in a larger multicentric study is warranted.

Food

It is well documented that a majority of patients with FD and IBS link their symptoms to food intake (figure 2).[103 104] Especially the role of FODMAPs or fermentable oligosaccharides, disaccharides, monosaccharides and polyols in symptom generation is well established in IBS and is thought to mainly result from their osmotic effects and fermentation in the colon with gaseous distension.[105] Of interest, recent studies have provided exciting evidence that certain foods can also trigger immune-mediated reactions in the GI tract. Bischoff et al injected food antigen extracts in the caecal mucosa of patients with chronic abdominal symptoms and suspected food allergy, 77% of whom reacted to at least one injection with a weal and flare reaction, which was associated with local eosinophil and mast cell activation.[106] This concept was further developed by studies using CLE, using in vivo fluorescence microscopy after intravenous fluorescein administration, with food extract (wheat, yeast, gluten, cow's milk) application to the duodenal mucosa. Frischer-Ravens et al demonstrated a positive reaction, with increases in intraepithelial lymphocytes, formation of interepithelial gaps and cell shedding, in 2/3 of patients in 2 cohorts of patients with IBS without elevated systemic IgE levels.[107 108] Wheat was the trigger in more than half of the patients. Patients with a positive reaction had a favourable response to an exclusion diet, although a sham diet and a diet in the CLE negative group were lacking, hampering
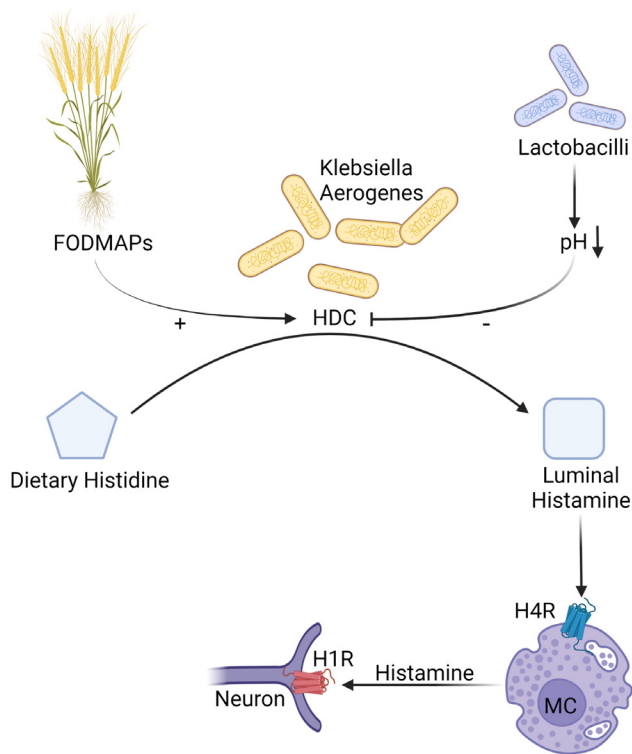
Figure 5 Food–microbiota interaction in visceral hypersensitivity. Bacterial histidine decarboxylase (HDC) in high-histamine producing species such as *Klebsiella aerogenes* metabolises dietary histidine into histamine which is stimulated by high intake of fermentable carbohydrates. Higher abundance of Lactobacilli reduces histamine production by lowering luminal pH through production of lactic acid. Bacteria-derived histamine can subsequently activate H4R on mast cells contributing to visceral hypersensitivity. FODMAPs, Fermentable Oligo-, Di-, Monosaccharides and Polyols; H1R, histamine 1 receptor; H4R, histamine 4 receptor; HDC, histidine decarboxylase; MC, mast cell; SCFA, short-chain fatty acids.

interpretation of the relevance of the test-based diet in clinical practice. Of interest, even if the total number of eosinophils and mast cells were not different in patients with IBS with or without a positive reaction versus healthy controls, a positive reaction was associated with release of eosinophil-cationic protein, highlighting again the importance of released mediators rather than cell counts.[108] This observation is somewhat surprising as the mucosal immune system is educated not to react against innocuous antigens such as dietary antigens, that is, oral tolerance.[28 85] Only failure to develop or loss of oral tolerance to food antigens, for example, following an infection will lead to the development of diseases such as food allergy and coeliac disease.[109–112] In case of food allergy, antigen-specific IgE antibodies directed against dietary antigens can be detected in the systemic circulation. These antibodies bind to IgE receptors on mast cells resulting in mast cell sensitisation and ultimately activation when crosslinked by binding of the respective antigen.[112] Notably, increased levels of systemic IgE antibodies against food epitopes can however not be detected in patients with IBS or FD.[113 114] These findings suggest that either non-IgE mediated eosinophil/mast cell activation or an IgE-mediated reaction confined to the GI mucosa is involved.

Recently, Aguilera-Lizarraga *et al* elegantly highlighted a role of local but not systemic IgE antibodies against a dietary antigen

in an animal model of visceral hypersensitivity.[68] Feeding of the dietary antigen ovalbumin during an infectious colitis (*Citrobacter rodentium*) resulted in loss of oral tolerance resulting in the local production of ovalbumin-specific IgE antibodies in the colon. Feeding of ovalbumin after clearance of the infection resulted in IgE-dependent mast cell activation and sensitisation of visceral afferents leading to abnormal pain signalling. These data are in line with the clinical observation that an infectious gastroenteritis is a major risk factor to develop IBS.[115] Moreover, and similar as in the studies described above, injection of food antigens in the rectosigmoid mucosa of patients with IBS elicited a mucosal reaction. Finally, IgE positive mast cells were more prevalent in patients with IBS and located at a shorter distance from nerve endings, correlating to severity of symptoms.[68] Taken together, these data indicate a role for local IgE antibodies against dietary antigens in food-induced symptoms in IBS and introduce a new mechanism explaining mast cell activation in IBS. We propose that IBS may be part of a spectrum of food-induced disorders mediated by mast cell activation with systemic food allergy at the extreme end of the spectrum. Similar data in FD are currently still lacking.

Diet–microbiome interactions
Altered microbial composition and metabolomic profiles have been found in patients with IBS compared with healthy controls.[116–118] It is unclear, whether these changes are consequence or cause of altered bowel function, diet and symptoms, but accumulating data suggest that at least in some patients, the symptoms of IBS could be attributed to specific microbial neuro-active metabolites, such as serotonin or tryptamine.[118 119] This is not surprising as gut bacteria can produce many neurochemical mediators that are found in mammals using their dietary precursors.[120]

Histamine, previously linked to visceral hypersensitivity, increased permeability and altered motility, can be produced by the microbiome as many bacteria possess the enzyme histidine decarboxylase (HDC) that converts dietary histidine into histamine (figure 5).[121 122] There are two main types of bacterial HDC, pyridoxal phosphate-dependent and pyruvoyl-dependent ones.[122] While the former is found in Gram-negative bacteria (such as *Enterobacter aerogenes*, *Raoultella ornytolytica* or *Morganella morganii*), the latter is encountered in Gram-positive bacteria, especially in lactic acid producing strains such as Lactobacilli.

Bacterial histamine has been identified as a culprit in food poisoning, for example, after consumption of certain fish, which can contain high histamine producing bacteria.[121] However, histamine producers can be a permanent part of the human microbiome, as recent studies found increased incidence of histamine producing bacteria in the gut of patients with inflammatory bowel disease,[123] as well as those with asthma.[124]

Several studies identified histamine as one of the key compounds that separates urine metabolomic profiles, which result from the metabolic activity of both the host and the microbiome, between patients with IBS and healthy controls.[125 126] Furthermore, levels of urinary histamine seem to correlate with levels of IBS symptoms and abdominal pain.[126]

In a dietary intervention study, GI symptoms and pain improved in patients with IBS after reducing intake of FODMAPs, which was associated with decrease in urinary histamine and changes in gut microbiota composition.[125] A subsequent translational study, which used faecal microbiome from those patients to colonise germ-free mice, found that mice colonised with microbiota from
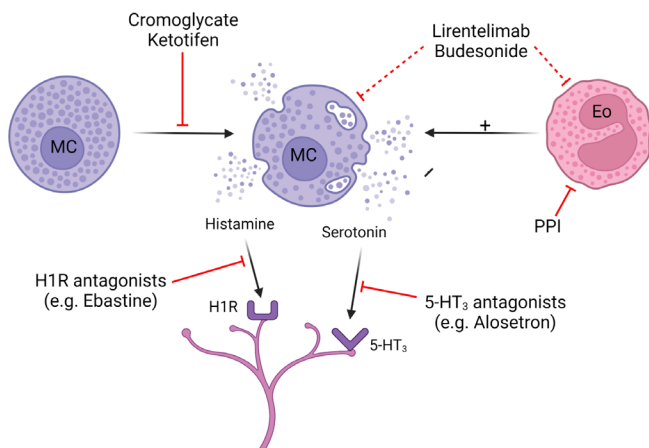
Figure 6 Treatments targeting immune activation. Solid lines: a demonstrated benefit. Dashed lines: potential benefit, but not formally tested. 5-HT$_3$, Serotonin 3 receptor; Eo, eosinophil; H1R, histamine 1 receptor; MC, mast cell; PPI, proton-pump inhibitor.

patients with high urinary histamine developed visceral hyperalgesia compared with mice colonised with microbiota from patients with low urinary histamine or healthy controls.[127] The increased visceral sensitivity was associated with colonic mast cell hyperplasia, increased density of neural fibres and frequent colocalisation of mast cells with neural cells, findings previously observed in patients with IBS.[31] The microbiota from patients with high urinary histamine produced large amount of histamine, and a specific strain of *Klebsiella aerogenes* was identified as the main histamine producer, with ability to generate 100× more histamine than other isolated bacterial strains. Histamine production was highly pH dependent, suggesting that acidity of the colonic milieu, which is largely determined by bacterial fermentation of dietary fibre, can regulate bacterial histamine production (figure 5). Indeed, placing mice colonised with high histamine producing microbiota on a low fermentable diet, which was associated with changes in luminal lactic acid, decreased histamine production.

Mice colonised with high histamine producing bacteria had increased expression of histamine 4 receptors (H4R), which was detected in multiple cell types, including mast cells. H4R is known to mediate mast cell migration and recruitment,[128] as well as regulate visceral sensitivity.[129] Administration of H4R antagonists to mice colonised with histamine producing microbiota prevented mast cell hyperplasia and visceral hyperalgesia.[127] Thus, while in inflammation induced food hypersensitivity the H1R pathways are key,[68] in case of bacterial histamine-to-host signalling the H4R dependent pathways play a major role. Bacterial histamine is therefore a likely trigger in a subset of patients with IBS, and increased urinary histamine or high abundance of bacterial histidine decarboxylase gene may serve as biomarkers to identify those subjects.

Altered microbiome in patients with IBS, who are on high fermentable diet, may also lead to production of other immunomodulatory mediators. Recent studies found that levels of faecal and serum lipopolysaccharide (LPS) are elevated in patients with IBS-D,[130 131] and that faecal LPS decreases when the patients are placed on a low FODMAP diet.[132] A mouse study then showed that faecal LPS induces impairment of the barrier function and mast cell activation through TLR-4 pathways, thus demonstrating another pathway by which microbiome could trigger symptoms of IBS.[132]

Also in FD changes in the composition of the gastric and duodenal microbiota have been described, although the findings across studies are quite diverse.[75 76 133 134] Increased abundance of *Streptococci* has been described by several groups, but this finding may be confounded by the effect of PPI treatment.[75 76] In a recent study from our group, a lower abundance of duodenal mucosa-associated *Porphyromonas* correlated with increased symptoms and eosinophils, although causality remains to be demonstrated.[75]

Psychological comorbidity

Psychological comorbidity, including stress, anxiety and depression, is common in patients with DGBI and affects symptoms and outcome (figure 2).[135–138] Two studies have suggested a link between anxiety and depression on the one hand and duodenal eosinophilia[139] and mast cell degranulation[140] on the other hand, although at this stage it is unclear whether this association was confounded by the presence of FD itself, which is known to be linked to both.[69 141]

Stress may impact the intestinal barrier through a mast-cell dependent mechanism.[142 143] A key mediator of this response is corticotropin-releasing hormone (CRH) which is not only secreted in the hypothalamus as part of the classical hypothalamo–pituitary–adrenal axis activation, but also by eosinophils in the GI tract which can then subsequently activate mast cells.[144 145] CRH release by eosinophils is triggered by psychological stress, possibly through activation of NK1/2 purinergic receptors on eosinophils by nerve-derived Substance P.[144] Moreover, increased expression of CRH in eosinophils correlated with symptom severity and life stress in patients with IBS-D.[146] Mast cells carry CRH receptors and exogenous CRH has been shown to increase intestinal permeability in healthy individuals which was blocked by mast cell stabilisation and vagal nerve stimulation.[142 147 148] Additionally, reduced expression of the antiinflammatory, antinociceptive and barrier-stabilising CRH receptor 2 (CRHR2) in duodenal biopsies of patients with FD was recently reported and may also contribute to stress-related mast cell activation in FD.[149 150] Polymorphisms in both CRHR1 and 2 have been associated with IBS and anxiety, but how this relates to mucosal immune activation is unexplored.[151 152]

TREATMENTS TARGETING THE GI IMMUNE MICROENVIRONMENT

Despite the available evidence for mucosal immune activation in IBS and FD, there is a paucity of therapies targeting this mechanism (figure 6).

Previous attempts with broad antiinflammatory agents in IBS have been disappointing. Prednisolone 30 mg for 3 weeks did not improve symptoms in a placebo-controlled study with 29 patients with postinfectious IBS and there was no significant effect on rectal lymphocytes or mast cells.[153] Similarly, a liquid formulation of budesonide, a corticosteroid with a high first pass metabolism was not effective in lowering duodenal eosinophil counts or improving symptoms in a small pilot study in FD.[154]

Based on the observation that 5-aminosalicylic acid was able to reduce release of histamine and prostaglandin D2 from mast cell cultures[155] and reduced mast cell counts and histamine release in the rectum of patients with IBS,[156] mesalazine was evaluated in two large multicentre trials in patients with IBS, which were both negative.[157 158] A post-hoc analysis of the British study also failed to demonstrate increased mast cell counts or a gene expression signature indicative of inflammation in patients with IBS in comparison to healthy controls.[159]

Because mediator release from specific immune cells is more likely to be involved in symptom generation than a classic inflammatory reaction as seen in, for example, inflammatory bowel disease, more specific mast-cell targeted therapies were trialled in IBS. The mast cell stabiliser disodium cromoglycate reduced abdominal pain and improved stool consistency in a small pilot study in patients with IBS-D, which was also associated with reduced signs of mast cell activation.[160] Ketotifen, another mast cell stabiliser, decreased visceral hypersensitivity to rectal distention and improved symptoms, but had no impact on mast cell counts or measured mediator release from rectal biopsies.[66] These results were confirmed in a more recent study from China with similar improvement of symptoms and reduced mast cell counts in the terminal ileum but not in the colon.[161] More specific eosinophil depleting and mast cell inhibiting therapies have not been evaluated in IBS and FD yet, but a study of lirentelimab, a monoclonal antibody against Siglec 8, in eosinophilic gastritis and duodenitis, showed promising results.[162] Notably, case reports describing clinical improvement of IBS symptoms in patients with severe asthma treated with the anti-IgE antibody omalizumab may indirectly support the role of IgE-mediated mast cell activation in IBS.[163]

An alternative approach to prevent mast cell or eosinophil activation is to block the nociceptive effect of their released mediators. Based on the observations that blocking the histamine receptor H1 prevented the neuronal excitation by supernatant from IBS biopsies, the histamine receptor H1 antagonist ebastine was evaluated in a pilot study showing improved symptom relief and reduced abdominal pain.[47] Blockade of serotonin, another mast cell mediator known to activate and sensitise visceral afferents, is another interesting approach to treat abdominal pain. 5-HT3 antagonists such as alosetron and ramosetron have indeed been repeatedly shown to be effective as treatment of diarrhoea-predominant IBS, but possibly also involves an effect on colonic transit.[164] Protease inhibitors have proven efficacy in preclinical models of visceral hypersensitivity, but clinical studies are lacking.[165]

In eosinophilic esophagitis, PPI have been shown to exert direct anti-inflammatory effects, independent of the acid-suppressive effects, based on a reduction of eotaxin-3 expression through inhibition of binding of STAT6 to its promotor, resulting in reduced eosinophil numbers.[166] Starting from this observation, we investigated the effect of a 4-week treatment with 40 mg pantoprazole in patients with FD.[74] Upper abdominal symptoms and duodenal eosinophils and barrier function improved. Of interest, the effect of pantoprazole on symptoms was mediated by the reduced eosinophil counts and not by changes in luminal pH, suggesting anti-inflammatory effects similar to its mechanism in eosinophilic esophagitis. Nevertheless, the effect of PPI on duodenal eosinophil activation—besides reduction in cell counts—has not been reported to date. However, in the longer term, the beneficial effect of PPI may be overshadowed by induction of dysbiosis through duodenal overgrowth of oropharyngeal flora (cf. supra).[75]

CONCLUSION

Recent insights in the mechanisms underlying aberrant pain signalling in patients with FD and IBS indicate a role for immune activation in the GI tract, but clearly we have just started to unravel the complex pathophysiology of functional bowel disorders. Accumulating evidence suggests immune activation of these conditions, but the underlying causes remain a hot topic for further research. Moreover, the therapeutic tools to address this immune activation in DGBI remain scarce. Intense collaboration between immunologists, neurogastroenterologists, neuroscientists, microbiologists, psychologists, bioinformaticians and many more is undoubtedly needed to be successful. Only with this multidisciplinary scientific approach, new targets can be discovered to improve clinical management of these challenging conditions.

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REFERENCES

1 Lacy BE, Mearin F, Chang L, *et al*. Bowel disorders. *Gastroenterology* 2016;150:1393–407.
2 Stanghellini V, Talley NJ, Chan F. Rome IV - Gastroduodenal Disorders. *Gastroenterology* 2016;150:1380–92.
3 Drossman DA, Hasler WL. Rome IV—Functional Gi disorders: disorders of gut-brain interaction. *Gastroenterology* 2016;150:1257–61.
4 Sperber AD, Bangdiwala SI, Drossman DA, *et al*. Worldwide prevalence and burden of functional gastrointestinal disorders, results of Rome Foundation global study. *Gastroenterology* 2021;160:e3:99–114.
5 Oka P, Parr H, Barberio B, *et al*. Global prevalence of irritable bowel syndrome according to Rome III or IV criteria: a systematic review and meta-analysis. *Lancet Gastroenterol Hepatol* 2020;5:908–17.
6 Rees WD, Miller LJ, Malagelada JR. Dyspepsia, antral motor dysfunction, and gastric stasis of solids. *Gastroenterology* 1980;78:360–5.
7 Sarnelli G, Caenepeel P, Geypens B, *et al*. Symptoms associated with impaired gastric emptying of solids and liquids in functional dyspepsia. *Am J Gastroenterol* 2003;98:783–8.
8 Rogers J, Henry MM, Misiewicz JJ. Increased segmental activity and intraluminal pressures in the sigmoid colon of patients with the irritable bowel syndrome. *Gut* 1989;30:634–41.
9 Stanghellini V, Tack J. Gastroparesis: separate entity or just a part of dyspepsia? *Gut* 2014;63:1972–8.
10 Vijayvargiya P, Jameie-Oskooei S, Camilleri M, *et al*. Association between delayed gastric emptying and upper gastrointestinal symptoms: a systematic review and meta-analysis. *Gut* 2019;68:804–13.
11 Pasricha PJ, Grover M, Yates KP, *et al*. Functional dyspepsia and gastroparesis in tertiary care are interchangeable syndromes with common clinical and pathologic features. *Gastroenterology* 2021;160:2006–17.
12 Pittayanon R, Yuan Y, Bollegala NP, *et al*. Prokinetics for functional dyspepsia: a systematic review and meta-analysis of randomized control trials. *Am J Gastroenterol* 2019;114:233–43.
13 Tack J, Van den Houte K, Carbone F. The unfulfilled promise of Prokinetics for functional Dyspepsia/Postprandial distress syndrome. *Am J Gastroenterol* 2019;114:204–6.
14 Wauters L, Dickman R, Drug V, *et al*. United European gastroenterology (UEG) and European Society for neurogastroenterology and motility (ESNM) consensus on functional dyspepsia. *United European Gastroenterol J* 2021;9:307–31.
15 Moayyedi P, Lacy BE, Andrews CN, *et al*. ACG and CAG clinical guideline: management of dyspepsia. *Am J Gastroenterol* 2017;112:988–1013.

16 Sarkar S, Aziz Q, Woolf CJ, *et al*. Contribution of central sensitisation to the development of non-cardiac chest pain. *Lancet* 2000;356:1154–9.

17 Weijenborg PW, Smout AJPM, Verseijden C, *et al*. Hypersensitivity to acid is associated with impaired esophageal mucosal integrity in patients with gastroesophageal reflux disease with and without esophagitis. *Am J Physiol Gastrointest Liver Physiol* 2014;307:G323–9.

18 Rohof WO, Bennink RJ, de Jonge H, *et al*. Increased proximal reflux in a hypersensitive esophagus might explain symptoms resistant to proton pump inhibitors in patients with gastroesophageal reflux disease. *Clin Gastroenterol Hepatol* 2014;12:1647–55.

19 Barbera R, Feinle C, Read NW. Abnormal sensitivity to duodenal lipid infusion in patients with functional dyspepsia. *Eur J Gastroenterol Hepatol* 1995;7:1051–7.

20 Boeckxstaens GE, Hirsch DP, van den Elzen BD, *et al*. Impaired drinking capacity in patients with functional dyspepsia: relationship with proximal stomach function. *Gastroenterology* 2001;121:1054–63.

21 Tack J, Caenepeel P, Fischler B, *et al*. Symptoms associated with hypersensitivity to gastric distention in functional dyspepsia. *Gastroenterology* 2001;121:526–35.

22 Hammer J. Identification of individuals with functional dyspepsia with a simple, minimally invasive test: a single center cohort study of the oral capsaicin test. *Am J Gastroenterol* 2018;113:584–92.

23 Ritchie J. Pain from distension of the pelvic colon by inflating a balloon in the irritable colon syndrome. *Gut* 1973;14:125–32.

24 Mertz H, Naliboff B, Munakata J, *et al*. Altered rectal perception is a biological marker of patients with irritable bowel syndrome. *Gastroenterology* 1995;109:40–52.

25 Kuiken SD, Lindeboom R, Tytgat GN, *et al*. Relationship between symptoms and hypersensitivity to rectal distension in patients with irritable bowel syndrome. *Aliment Pharmacol Ther* 2005;22:157–64.

26 Brookes SJH, Spencer NJ, Costa M, *et al*. Extrinsic primary afferent signalling in the gut. *Nat Rev Gastroenterol Hepatol* 2013;10:286–96.

27 Brierley SM, Linden DR. Neuroplasticity and dysfunction after gastrointestinal inflammation. *Nat Rev Gastroenterol Hepatol* 2014;11:611–27.

28 Aguilera-Lizarraga J, Hussein K, Boeckxstaens GE. Immune activation in irritable bowel syndrome: what is the evidence? *Nat Rev Immunol* 2022;22:674–86.

29 Kano M, Dupont P, Aziz Q, *et al*. Understanding neurogastroenterology from neuroimaging perspective: a comprehensive review of functional and structural brain imaging in functional gastrointestinal disorders. *J Neurogastroenterol Motil* 2018;24:512–27.

30 Hughes PA, Harrington AM, Castro J, *et al*. Sensory neuro-immune interactions differ between irritable bowel syndrome subtypes. *Gut* 2013;62:1456–65.

31 Barbara G, Stanghellini V, De Giorgio R, *et al*. Activated mast cells in proximity to colonic nerves correlate with abdominal pain in irritable bowel syndrome. *Gastroenterology* 2004;126:693–702.

32 Barbara G, Wang B, Stanghellini V, *et al*. Mast cell-dependent excitation of visceral-nociceptive sensory neurons in irritable bowel syndrome. *Gastroenterology* 2007;132:26–37.

33 Buhner S, Li Q, Vignali S, *et al*. Activation of human enteric neurons by supernatants of colonic biopsy specimens from patients with irritable bowel syndrome. *Gastroenterology* 2009;137:1425–34.

34 Rolland-Fourcade C, Denadai-Souza A, Cirillo C, *et al*. Epithelial expression and function of trypsin-3 in irritable bowel syndrome. *Gut* 2017;66:1767–78.

35 Cenac N, Bautzova T, Le Faouder P, *et al*. Quantification and potential functions of endogenous agonists of transient receptor potential channels in patients with irritable bowel syndrome. *Gastroenterology* 2015;149:433–44.

36 Li X, Chen H, Lu H, *et al*. The study on the role of inflammatory cells and mediators in post-infectious functional dyspepsia. *Scand J Gastroenterol* 2010;45:573–81.

37 El-Salhy M, Hausken T, Gilja OH, *et al*. The possible role of gastrointestinal endocrine cells in the pathophysiology of irritable bowel syndrome. *Expert Rev Gastroenterol Hepatol* 2017;11:139–48.

38 Park JH, Rhee P-L, Kim G, *et al*. Enteroendocrine cell counts correlate with visceral hypersensitivity in patients with diarrhoea-predominant irritable bowel syndrome. *Neurogastroenterol Motil* 2006;18:539–46.

39 Kaelberer MM, Rupprecht LE, Liu WW, *et al*. Neuropod cells: the emerging biology of gut-brain sensory transduction. *Annu Rev Neurosci* 2020;43:337–53.

40 Liebregts T, Adam B, Bredack C, *et al*. Small bowel homing T cells are associated with symptoms and delayed gastric emptying in functional dyspepsia. *Am J Gastroenterol* 2011;106:1089–98.

41 Cheung CKY, Lan LL, Kyaw M, *et al*. Up-regulation of transient receptor potential vanilloid (TRPV) and down-regulation of brain-derived neurotrophic factor (BDNF) expression in patients with functional dyspepsia (FD). *Neurogastroenterol Motil* 2018;30. doi:10.1111/nmo.13176. [Epub ahead of print: 07 08 2017].

42 Luo Y, Feng C, Wu J, *et al*. P2Y1, P2Y2, and TRPV1 receptors are increased in diarrhea-predominant irritable bowel syndrome and P2Y2 correlates with abdominal pain. *Dig Dis Sci* 2016;61:2878–86.

43 Zhou Q, Yang L, Larson S, *et al*. Decreased miR-199 augments visceral pain in patients with IBS through translational upregulation of TRPV1. *Gut* 2016;65:797–805.

44 Akbar A, Yiangou Y, Facer P, *et al*. Increased capsaicin receptor TRPV1-expressing sensory fibres in irritable bowel syndrome and their correlation with abdominal pain. *Gut* 2008;57:923–9.

45 Grover M, Berumen A, Peters S, *et al*. Intestinal chemosensitivity in irritable bowel syndrome associates with small intestinal TRPV channel expression. *Aliment Pharmacol Ther* 2021;54:1179–92.

46 Cenac N, Altier C, Motta J-P, *et al*. Potentiation of TRPV4 signalling by histamine and serotonin: an important mechanism for visceral hypersensitivity. *Gut* 2010;59:481–8.

47 Wouters MM, Balemans D, Van Wanrooy S, *et al*. Histamine receptor H1-mediated sensitization of TRPV1 mediates visceral hypersensitivity and symptoms in patients with irritable bowel syndrome. *Gastroenterology* 2016;150:875–87.

48 Balemans D, Aguilera-Lizarraga J, Florens MV, *et al*. Histamine-mediated potentiation of transient receptor potential (TRP) ankyrin 1 and TRP vanilloid 4 signaling in submucosal neurons in patients with irritable bowel syndrome. *Am J Physiol Gastrointest Liver Physiol* 2019;316:G338–49.

49 Cirillo C, Bessissow T, Desmet A-S, *et al*. Evidence for neuronal and structural changes in submucous ganglia of patients with functional dyspepsia. *Am J Gastroenterol* 2015;110:1205–15.

50 Neunlist M, Rolli-Derkinderen M, Latorre R, *et al*. Enteric glial cells: recent developments and future directions. *Gastroenterology* 2014;147:1230–7.

51 Meira de-Faria F, Casado-Bedmar M, Mårten Lindqvist C, *et al*. Altered interaction between enteric glial cells and mast cells in the colon of women with irritable bowel syndrome. *Neurogastroenterol Motil* 2021;33:e14130.

52 Lilli NL, Quénéhervé L, Haddara S, *et al*. Glioplasticity in irritable bowel syndrome. *Neurogastroenterol Motil* 2018;30:e13232.

53 Cenac N, Andrews CN, Holzhausen M, *et al*. Role for protease activity in visceral pain in irritable bowel syndrome. *J Clin Invest* 2007;117:636–47.

54 Balemans D, Mondelaers SU, Cibert-Goton V, *et al*. Evidence for long-term sensitization of the bowel in patients with post-infectious-IBS. *Sci Rep* 2017;7:13606.

55 Gwee KA, Leong YL, Graham C, *et al*. The role of psychological and biological factors in postinfective gut dysfunction. *Gut* 1999;44:400–6.

56 Spiller RC, Jenkins D, Thornley JP, *et al*. Increased rectal mucosal enteroendocrine cells, T lymphocytes, and increased gut permeability following acute Campylobacter enteritis and in post-dysenteric irritable bowel syndrome. *Gut* 2000;47:804–11.

57 Bashashati M, Moossavi S, Cremon C, *et al*. Colonic immune cells in irritable bowel syndrome: a systematic review and meta-analysis. *Neurogastroenterol Motil* 2018;30:e13192.

58 Burns G, Carroll G, Mathe A, *et al*. Evidence for local and systemic immune activation in functional dyspepsia and the irritable bowel syndrome: a systematic review. *Am J Gastroenterol* 2019;114:429–36.

59 Ceulemans M, Jacobs I, Wauters L, *et al*. Immune activation in functional dyspepsia: bystander becoming the suspect. *Front Neurosci* 2022;16:831761.

60 Kindt S, Tertychnyy A, de Hertogh G, *et al*. Intestinal immune activation in presumed post-infectious functional dyspepsia. *Neurogastroenterol Motil* 2009;21:832–56.

61 Gargala G, Lecleire S, Francois A. Duodenal intraepithelial T lymphocytes in patients with functional dyspepsia. *WJG* 2007;13:2333–8.

62 Ohman L, Isaksson S, Lundgren A, *et al*. A controlled study of colonic immune activity and beta7+ blood T lymphocytes in patients with irritable bowel syndrome. *Clin Gastroenterol Hepatol* 2005;3:980–6.

63 Ohman L, Isaksson S, Lindmark A-C, *et al*. T-Cell activation in patients with irritable bowel syndrome. *Am J Gastroenterol* 2009;104:1205–12.

64 Nasser Y, Petes C, Simmers C, *et al*. Activation of peripheral blood CD4+ T-cells in IBS is not associated with gastrointestinal or psychological symptoms. *Sci Rep* 2019;9:3710.

65 Krammer L, Sowa AS, Lorentz A. Mast cells in irritable bowel syndrome: a systematic review. *J Gastrointestin Liver Dis* 2019;28:463–72.

66 Klooker TK, Braak B, Koopman KE, *et al*. The mast cell stabiliser ketotifen decreases visceral hypersensitivity and improves intestinal symptoms in patients with irritable bowel syndrome. *Gut* 2010;59:1213–21.

67 Braak B, Klooker TK, Wouters MM, *et al*. Mucosal immune cell numbers and visceral sensitivity in patients with irritable bowel syndrome: is there any relationship? *Am J Gastroenterol* 2012;107:715–26.

68 Aguilera-Lizarraga J, Florens MV, Viola MF, *et al*. Local immune response to food antigens drives meal-induced abdominal pain. *Nature* 2021;590:151–6.

69 Shah A, Fairlie T, Brown G, *et al*. Duodenal eosinophils and mast cells in functional dyspepsia: a systematic review and meta-analysis of case-control studies. *Clin Gastroenterol Hepatol* 2022;20:2229–42.

70 Vanheel H, Vicario M, Boesmans W, *et al*. Activation of eosinophils and mast cells in functional dyspepsia: an ultrastructural evaluation. *Sci Rep* 2018;8:5383.

71 Giancola F, Volta U, Repossi R, *et al*. Mast cell-nerve interactions correlate with bloating and abdominal pain severity in patients with non-celiac gluten / wheat sensitivity. *Neurogastroenterol Motil* 2020;32:e13814.

72 Talley NJ, Walker MM, Aro P, *et al*. Non-ulcer dyspepsia and duodenal eosinophilia: an adult endoscopic population-based case-control study. *Clin Gastroenterol Hepatol* 2007;5:1175–83.

73 Järbrink-Sehgal ME, Sparkman J, Damron A, *et al*. Functional dyspepsia and duodenal eosinophil count and degranulation: a multiethnic us veteran cohort study. *Dig Dis Sci* 2021;66:3482–9.

74 Wauters L, Ceulemans M, Frings D, *et al*. Proton pump inhibitors reduce duodenal eosinophilia, mast cells, and permeability in patients with functional dyspepsia. *Gastroenterology* 2021;160:1521–31.

75 Wauters L, Tito RY, Ceulemans M, *et al*. Duodenal dysbiosis and relation to the efficacy of proton pump inhibitors in functional dyspepsia. *Int J Mol Sci* 2021;22. doi:10.3390/ijms222413609. [Epub ahead of print: 19 Dec 2021].

76 Shanahan ER, Kang S, Staudacher H, *et al*. Alterations to the duodenal microbiota are linked to gastric emptying and symptoms in functional dyspepsia. *Gut* 2022;396:gutjnl-2021-326158.

77 Burns GL, Talley NJ, Keely S. Immune responses in the irritable bowel syndromes: time to consider the small intestine. *BMC Med* 2022;20:115.

78 Casado-Bedmar M, de-Faria FM, Biskou O, *et al*. Elevated F-EDN correlates with mucosal eosinophil degranulation in patients with IBS—A possible association with microbiota? *J Leukoc Biol* 2022;111:655–65.

79 Bashashati M, Rezaei N, Shafieyoun A, *et al*. Cytokine imbalance in irritable bowel syndrome: a systematic review and meta-analysis. *Neurogastroenterol Motil* 2014;26:1036–48.

80 Bennet SMP, Palsson O, Whitehead WE, *et al*. Systemic cytokines are elevated in a subset of patients with irritable bowel syndrome but largely unrelated to symptom characteristics. *Neurogastroenterol Motil* 2018;30:e13378.

81 Bennet SMP, Polster A, Törnblom H, *et al*. Global cytokine profiles and association with clinical characteristics in patients with irritable bowel syndrome. *Am J Gastroenterol* 2016;111:1165–76.

82 Aguilera-Lizarraga J, Florens MV, Van Brussel T, *et al*. Expression of immune-related genes in rectum and colon *descendens* of Irritable bowel syndrome patients is unrelated to clinical symptoms. *Neurogastroenterology Motil* 2019;31:e13579.

83 Ogasawara H, Noguchi M. Therapeutic potential of MRGPRX2 inhibitors on mast cells. *Cells* 2021;10:2906.

84 Kindt S, Van Oudenhove L, Broekaert D, *et al*. Immune dysfunction in patients with functional gastrointestinal disorders. *Neurogastroenterol Motil* 2009;21:389–98.

85 Liu EG, Yin X, Swaminathan A, *et al*. Antigen-presenting cells in food tolerance and allergy. *Front Immunol* 2020;11:616020.

86 Vanuytsel T, Tack J, Farre R. The role of intestinal permeability in gastrointestinal disorders and current methods of evaluation. *Front Nutr* 2021;8:717925.

87 Wauters L, Ceulemans M, Schol J, *et al*. The role of leaky gut in functional dyspepsia. *Front Neurosci* 2022;16:851012.

88 Hanning N, Edwinson AL, Ceuleers H, *et al*. Intestinal barrier dysfunction in irritable bowel syndrome: a systematic review. *Therap Adv Gastroenterol* 2021;14:1756284821993586.

89 Witt ST, Bednarska O, Keita AV, *et al*. Interactions between gut permeability and brain structure and function in health and irritable bowel syndrome. *Neuroimage Clin* 2019;21:101602.

90 Annaházi A, Ferrier L, Bézirard V, *et al*. Luminal cysteine-proteases degrade colonic tight junction structure and are responsible for abdominal pain in constipation-predominant IBS. *Am J Gastroenterol* 2013;108:1322–31.

91 Gecse K, Roka R, Ferrier L, *et al*. Increased faecal serine protease activity in diarrhoeic IBS patients: a colonic lumenal factor impairing colonic permeability and sensitivity. *Gut* 2008;57:591–9.

92 Piche T, Barbara G, Aubert P, *et al*. Impaired intestinal barrier integrity in the colon of patients with irritable bowel syndrome: involvement of soluble mediators. *Gut* 2009;58:196–201.

93 Edwinson AL, Yang L, Peters S, *et al*. Gut microbial β-glucuronidases regulate host luminal proteases and are depleted in irritable bowel syndrome. *Nat Microbiol* 2022;7:680–94.

94 Edogawa S, Edwinson AL, Peters SA, *et al*. Serine proteases as luminal mediators of intestinal barrier dysfunction and symptom severity in IBS. *Gut* 2020;69:62–73.

95 Tooth D, Garsed K, Singh G, *et al*. Characterisation of faecal protease activity in irritable bowel syndrome with diarrhoea: origin and effect of gut transit. *Gut* 2014;63:753–60.

96 Jacob C, Yang P-C, Darmoul D, *et al*. Mast cell tryptase controls paracellular permeability of the intestine. Role of protease-activated receptor 2 and beta-arrestins. *J Biol Chem* 2005;280:31936–48.

97 Wilcz-Villega EM, McClean S, O'Sullivan MA. Mast cell tryptase reduces junctional adhesion molecule-A (JAM-A) expression in intestinal epithelial cells: implications for the mechanisms of barrier dysfunction in irritable bowel syndrome. *Am J Gastroenterol* 2013;108:1140–51.

98 Furuta GT, Nieuwenhuis EES, Karhausen J, *et al*. Eosinophils alter colonic epithelial barrier function: role for major basic protein. *Am J Physiol Gastrointest Liver Physiol* 2005;289:G890–7.

99 Bednarska O, Walter SA, Casado-Bedmar M, *et al*. Vasoactive intestinal polypeptide and mast cells regulate increased passage of colonic bacteria in patients with irritable bowel syndrome. *Gastroenterology* 2017;153:948–60.

100 Vanheel H, Vicario M, Vanuytsel T, *et al*. Impaired duodenal mucosal integrity and low-grade inflammation in functional dyspepsia. *Gut* 2014;63:262–71.

101 Zhou Q, Verne ML, Fields JZ, *et al*. Randomised placebo-controlled trial of dietary glutamine supplements for postinfectious irritable bowel syndrome. *Gut* 2019;68:996–1002.

102 Bertrand J, Ghouzali I, Guérin C, *et al*. Glutamine restores tight junction protein claudin-1 expression in colonic mucosa of patients with diarrhea-predominant irritable bowel syndrome. *JPEN J Parenter Enteral Nutr* 2016;40:1170–6.

103 Simrén M, Månsson A, Langkilde AM, *et al*. Food-related gastrointestinal symptoms in the irritable bowel syndrome. *Digestion* 2001;63:108–15.

104 Böhn L, Störsrud S, Törnblom H, *et al*. Self-reported food-related gastrointestinal symptoms in IBS are common and associated with more severe symptoms and reduced quality of life. *Am J Gastroenterol* 2013;108:634–41.

105 Spiller R. Impact of diet on symptoms of the irritable bowel syndrome. *Nutrients* 2021;13:575.

106 Bischoff SC, Mayer J, Wedemeyer J, *et al*. Colonoscopic allergen provocation (COLAP): a new diagnostic approach for gastrointestinal food allergy. *Gut* 1997;40:745–53.

107 Fritscher-Ravens A, Schuppan D, Ellrichmann M, *et al*. Confocal endomicroscopy shows food-associated changes in the intestinal mucosa of patients with irritable bowel syndrome. *Gastroenterology* 2014;147:1012–20.

108 Fritscher-Ravens A, Pflaum T, Mösinger M, *et al*. Many patients with irritable bowel syndrome have atypical food allergies not associated with immunoglobulin E. *Gastroenterology* 2019;157:109–18.

109 Caminero A, Meisel M, Jabri B, *et al*. Mechanisms by which gut microorganisms influence food sensitivities. *Nat Rev Gastroenterol Hepatol* 2019;16:7–18.

110 Bouziat R, Hinterleitner R, Brown JJ, *et al*. Reovirus infection triggers inflammatory responses to dietary antigens and development of celiac disease. *Science* 2017;356:44–50.

111 Fonseca DMda, Hand TW, Han S-J, *et al*. Microbiota-Dependent sequelae of acute infection compromise tissue-specific immunity. *Cell* 2015;163:354–66.

112 Yu W, Freeland DMH, Nadeau KC. Food allergy: immune mechanisms, diagnosis and immunotherapy. *Nat Rev Immunol* 2016;16:751–65.

113 Ismail FW, Abid S, Awan S, *et al*. Frequency of food hypersensitivity in patients with functional gastrointestinal disorders. *Acta Gastroenterol Belg* 2018;81:253–6.

114 Nybacka S, Öhman L, Störsrud S, *et al*. Neither self-reported atopy nor IgE-mediated allergy are linked to gastrointestinal symptoms in patients with irritable bowel syndrome. *Neurogastroenterol Motil* 2018;30:e13379.

115 Spiller R, Garsed K. Postinfectious irritable bowel syndrome. *Gastroenterology* 2009;136:1979–88.

116 Pittayanon R, Lau JT, Yuan Y, *et al*. Gut microbiota in patients with irritable bowel Syndrome—A systematic review. *Gastroenterology* 2019;157:97–108.

117 Jeffery IB, Das A, O'Herlihy E, *et al*. Differences in fecal Microbiomes and metabolomes of people with vs without irritable bowel syndrome and bile acid malabsorption. *Gastroenterology* 2020;158:1016–28.

118 Mars RAT, Yang Y, Ward T, *et al*. Longitudinal multi-omics reveals subset-specific mechanisms underlying irritable bowel syndrome. *Cell* 2020;182:e17:1460–73.

119 Mujagic Z, Kasapi M, Jonkers DMAE, *et al*. Integrated fecal microbiome–metabolome signatures reflect stress and serotonin metabolism in irritable bowel syndrome. *Gut Microbes* 2022;14:2063016.

120 Lyte M. Microbial endocrinology: Host-microbiota neuroendocrine interactions influencing brain and behavior. *Gut Microbes* 2014;5:381–9.

121 Kim SH, Field KG, Morrissey MT, *et al*. Source and identification of histamine-producing bacteria from fresh and temperature-abused albacore. *J Food Prot* 2001;64:1035–44.

122 Landete JM, De las Rivas B, Marcobal A, *et al*. Updated molecular knowledge about histamine biosynthesis by bacteria. *Crit Rev Food Sci Nutr* 2008;48:697–714.

123 Mou Z, Yang Y, Hall AB, *et al*. The taxonomic distribution of histamine-secreting bacteria in the human gut microbiome. *BMC Genomics* 2021;22:695.

124 Barcik W, Pugin B, Westermann P, *et al*. Histamine-secreting microbes are increased in the gut of adult asthma patients. *J Allergy Clin Immunol* 2016;138:1491–4.

125 McIntosh K, Reed DE, Schneider T, *et al*. FODMAPs alter symptoms and the metabolome of patients with IBS: a randomised controlled trial. *Gut* 2017;66:1241–51.

126 Keshteli AH, Madsen KL, Mandal R, *et al*. Comparison of the metabolomic profiles of irritable bowel syndrome patients with ulcerative colitis patients and healthy controls: new insights into pathophysiology and potential biomarkers. *Aliment Pharmacol Ther* 2019;49:723–32.

127 De Palma G, Shimbori C, Reed DE, *et al*. Histamine production by the gut microbiota induces visceral hyperalgesia through histamine 4 receptor signaling in mice. *Sci Transl Med* 2022;14:eabj1895.

128 Godot V, Arock M, Garcia G, *et al*. H4 histamine receptor mediates optimal migration of mast cell precursors to CXCL12. *J Allergy Clin Immunol* 2007;120:827–34.

129 Deiteren A, De Man JG, Ruyssers NE, *et al*. Histamine H4 and H1 receptors contribute to postinflammatory visceral hypersensitivity. *Gut* 2014;63:1873–82.

130 Dlugosz A, Nowak P, D'Amato M, *et al*. Increased serum levels of lipopolysaccharide and antiflagellin antibodies in patients with diarrhea-predominant irritable bowel syndrome. *Neurogastroenterol Motil* 2015;27:1747–54.

131 Zhou S-Y, Gillilland M, Wu X, *et al*. FODMAP diet modulates visceral nociception by lipopolysaccharide-mediated intestinal inflammation and barrier dysfunction. *J Clin Invest* 2018;128:267–80.

132 Singh P, Grabauskas G, Zhou S-Y, *et al*. High FODMAP diet causes barrier loss via lipopolysaccharide-mediated mast cell activation. In: *JCI insight*. 6, 2021.

133 Zhong L, Shanahan ER, Raj A, *et al*. Dyspepsia and the microbiome: time to focus on the small intestine. *Gut* 2017;66:1168–9.

134 Zhou L, Zeng Y, Zhang H, *et al*. The role of gastrointestinal microbiota in functional dyspepsia: a review. *Front Physiol* 2022;13:910568.

135 Faresjö Å, Grodzinsky E, Johansson S, *et al*. Psychosocial factors at work and in every day life are associated with irritable bowel syndrome. *Eur J Epidemiol* 2007;22:473–80.

136 Aro P, Talley NJ, Ronkainen J, *et al*. Anxiety is associated with uninvestigated and functional dyspepsia (Rome III criteria) in a Swedish population-based study. *Gastroenterology* 2009;137:94–100.

137 Klaassen T, Vork L, Smeets FGM, *et al*. The interplay between stress and fullness in patients with functional dyspepsia and healthy controls: an exploratory experience sampling method study. *Psychosom Med* 2022;84:306–12.

138 Schaper SJ, Stengel A. Emotional stress responsivity of patients with IBS - a systematic review. *J Psychosom Res* 2022;153:110694.

139 Ronkainen J, Aro P, Jones M, *et al*. Duodenal eosinophilia and the link to anxiety: a population-based endoscopic study. *Neurogastroenterology & Motility* 2021;33:e14109.

140 Yuan H-P, Li Z, Zhang Y, *et al*. Anxiety and depression are associated with increased counts and degranulation of duodenal mast cells in functional dyspepsia. *Int J Clin Exp Med* 2015;8:8010–4.

141 Koloski NA, Jones M, Talley NJ. Evidence that independent gut-to-brain and brain-to-gut pathways operate in the irritable bowel syndrome and functional dyspepsia: a 1-year population-based prospective study. *Aliment Pharmacol Ther* 2016;44:592–600.

142 Vanuytsel T, van Wanrooy S, Vanheel H, *et al*. Psychological stress and corticotropin-releasing hormone increase intestinal permeability in humans by a mast cell-dependent mechanism. *Gut* 2014;63:1293–9.

143 Alonso C, Guilarte M, Vicario M, *et al*. Maladaptive intestinal epithelial responses to life stress may predispose healthy women to gut mucosal inflammation. *Gastroenterology* 2008;135:163–72.

144 Zheng P-Y, Feng B-S, Oluwole C, *et al*. Psychological stress induces eosinophils to produce corticotrophin releasing hormone in the intestine. *Gut* 2009;58:1473–9.

145 Wallon C, Persborn M, Jönsson M, *et al*. Eosinophils express muscarinic receptors and corticotropin-releasing factor to disrupt the mucosal barrier in ulcerative colitis. *Gastroenterology* 2011;140:1597–607.

146 Salvo-Romero E, Martínez C, Lobo B, *et al*. Overexpression of corticotropin-releasing factor in intestinal mucosal eosinophils is associated with clinical severity in diarrhea-predominant irritable bowel syndrome. *Sci Rep* 2020;10:20706.

147 Mogilevski T, Rosella S, Aziz Q, *et al*. Transcutaneous vagal nerve stimulation protects against stress-induced intestinal barrier dysfunction in healthy adults. *Neurogastroenterol Motil* 2022;34:e14382.

148 Wallon C, Yang P-C, Keita AV, *et al*. Corticotropin-releasing hormone (CRH) regulates macromolecular permeability via mast cells in normal human colonic biopsies in vitro. *Gut* 2008;57:50–8.

149 Bruce JK, Burns GL, Sinn Soh W, *et al*. Defects in NLRP6, autophagy and goblet cell homeostasis are associated with reduced duodenal CRH receptor 2 expression in patients with functional dyspepsia. *Brain Behav Immun* 2022;101:335–45.

150 Fukudo S. Role of corticotropin-releasing hormone in irritable bowel syndrome and intestinal inflammation. *J Gastroenterol* 2007;42 Suppl 17:48–51.

151 Orand A, Naliboff B, Gadd M, *et al*. Corticotropin-Releasing hormone receptor 1 (CRH-R1) polymorphisms are associated with irritable bowel syndrome and acoustic startle response. *Psychoneuroendocrinology* 2016;73:133–41.

152 Komuro H, Sato N, Sasaki A, *et al*. Corticotropin-releasing hormone receptor 2 gene variants in irritable bowel syndrome. *PLoS One* 2016;11:e0147817.

153 Dunlop SP, Jenkins D, Neal KR, *et al*. Randomized, double-blind, placebo-controlled trial of prednisolone in post-infectious irritable bowel syndrome. *Aliment Pharmacol Ther* 2003;18:77–84.

154 Talley NJ, Walker MM, Jones M, *et al*. Letter: budesonide for functional dyspepsia with duodenal eosinophilia—randomised, double-blind, placebo-controlled parallel-group trial. *Aliment Pharmacol Ther* 2021;53:1332–3.

155 Fox CC, Moore WC, Lichtenstein LM. Modulation of mediator release from human intestinal mast cells by sulfasalazine and 5-aminosalicylic acid. *Dig Dis Sci* 1991;36:179–84.

156 Corinaldesi R, Stanghellini V, Cremon C, *et al*. Effect of mesalazine on mucosal immune biomarkers in irritable bowel syndrome: a randomized controlled proof-of-concept study. *Aliment Pharmacol Ther* 2009;30:245–52.

157 Lam C, Tan W, Leighton M, *et al*. A mechanistic multicentre, parallel group, randomised placebo-controlled trial of mesalazine for the treatment of IBS with diarrhoea (IBS-D). *Gut* 2016;65:91–9.

158 Barbara G, Cremon C, Annese V, *et al*. Randomised controlled trial of mesalazine in IBS. *Gut* 2016;65:82–90.

159 Jalanka J, Lam C, Bennett A, *et al*. Colonic gene expression and fecal microbiota in diarrhea-predominant irritable bowel syndrome: increased Toll-like receptor 4 but minimal inflammation and NO response to mesalazine. *J Neurogastroenterol Motil* 2021;27:279–91.

160 Lobo B, Ramos L, Martínez C, *et al*. Downregulation of mucosal mast cell activation and immune response in diarrhoea-irritable bowel syndrome by oral disodium cromoglycate: a pilot study. *United European Gastroenterol J* 2017;5:887–97.

161 Wang J, Wang Y, Zhou H, *et al*. Clinical efficacy and safety of ketotifen in treating irritable bowel syndrome with diarrhea. *Eur J Gastroenterol Hepatol* 2020;32:706–12.

162 Dellon ES, Peterson KA, Murray JA, *et al*. Anti–siglec-8 antibody for eosinophilic gastritis and duodenitis. *N Engl J Med Overseas Ed* 2020;383:1624–34.

163 Pearson JS, Niven RM, Meng J, *et al*. Immunoglobulin E in irritable bowel syndrome: another target for treatment? A case report and literature review. *Therap Adv Gastroenterol* 2015;8:270–7.

164 Black CJ, Burr NE, Camilleri M, *et al*. Efficacy of pharmacological therapies in patients with IBS with diarrhoea or mixed stool pattern: systematic review and network meta-analysis. *Gut* 2020;69:74–82.

165 Ceuleers H, Hanning N, Heirbaut J, *et al*. Newly developed serine protease inhibitors decrease visceral hypersensitivity in a post-inflammatory rat model for irritable bowel syndrome. *Br J Pharmacol* 2018;175:3516–33.

166 Odiase E, Zhang X, Chang Y, *et al*. In esophageal squamous cells from eosinophilic esophagitis patients, Th2 cytokines increase eotaxin-3 secretion through effects on intracellular calcium and a non-gastric proton pump. *Gastroenterology* 2021;160:2072–88.