Crosstalk at the mucosal border: importance of the gut microenvironment in IBS

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Abstract | The aetiology and pathology of IBS, a functional bowel disorder thought to lack an organic cause, is largely unknown. However, studies suggest that various features, such as altered composition of the gut microbiota, together with increased intestinal permeability, a changed balance in the enteroendocrine system and a dysregulated immune system in the gut, most likely have an important role in IBS. Exactly how these entities act together and give rise to symptoms is still unknown, but an altered gut microbiota composition could lead to dysregulation of the intestinal barrier as well as the enteroendocrine and the immune systems, which (through interactions with the nervous system) might generate symptoms. This Review highlights the crosstalk between the gut microbiota, the enteroendocrine system, the immune system and the role of intestinal permeability in patients with IBS.

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Introduction

The key symptoms of IBS—that is, abdominal pain or discomfort in combination with an abnormal bowel habit that are of long-standing character[1]—are common worldwide.[2] Our lack of understanding regarding mechanisms of importance for the generation of IBS symptoms has hampered the development of efficient treatments. Most researchers agree that there will probably not be a unifying factor that explains the development of this disorder, rather a number of different factors interacting with varying effects upon individuals living in highly variable psychological and physical milieus.

Early observations put emphasis upon psychological features of patients with IBS, such as neuroticism and anxiety, but at the same time diagnostic labels involving "colitis" were used implying that some kind of local irritation or inflammation might be of importance despite seemingly normal findings at examination.[3] Even if social and psychological events are still regarded as important factors involved in the pathophysiology of IBS,[4] an increasing number of observations that a physical insult (such as a severe bout of gastroenteritis) is associated with an increased risk of developing long-standing symptoms compatible with IBS[5] indicate that psychological and somatic factors might interact in the development of functional gastrointestinal symptoms.

The ability to measure gastrointestinal physiology has resulted in some pathophysiological insights. For instance, gastrointestinal dysmotility seems to be a widespread, nonconsistent and noncharacteristic feature of IBS;[6] that is, a huge overlap exists between individuals with IBS and those without gastrointestinal complaints regarding the motility patterns observed. Moreover, the association between disturbed motor function and symptoms in IBS are weak at best and mainly associated with the abnormal bowel habit present in these patients.[7] Another pathophysiological abnormality considered to be of central importance in IBS is visceral hypersensitivity,[8,9] a mechanism that involves decreased sensory thresholds when stimulating the gastrointestinal tract at different anatomical levels. However, the association between visceral hypersensitivity and symptoms in IBS is modest.[10] Potential mechanisms to induce this phenomenon are peripheral sensitization in the gut, amplification of sensory signalling along its transmission to the central nervous system (CNS) or a central amplification.[6] Moreover, insights into CNS function gained by functional brain imaging have rapidly expanded our understanding of central and peripheral sensory processing, how psychological factors can affect these processes and, ultimately, how CNS factors might be of importance in the pathophysiology of IBS.[11]

Our rapidly growing ability to characterize gut microbiota composition, intestinal barrier function, enteroendocrine and immune function, as well as nervous signalling have put the intraluminal milieu of the intestine itself in focus in the latest pathophysiological studies in IBS.[12–14] The potential of multiple and complex local interactions as major pathogenetic factors are therefore gaining more interest and several lines of evidence now suggest that local abnormalities occur in the gut in

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Key points

- Altered gut microbiota composition, aberrant expression pattern and function of enterochromaffin cells, abnormal gut permeability and dysregulated immune activity have been found in at least subgroups of patients with IBS
- The complex interaction between these systems has been demonstrated in different animal models of IBS
- The association between these abnormalities and the symptom profile in patients with IBS has been demonstrated
- Targeting these alterations in the development of new therapies for IBS seems promising

patients with IBS. Moreover, ample experimental evidence also indicates bidirectional crosstalk between the gut and its microenvironment, and the CNS, which has been covered in detail elsewhere.[15–18] This topic will therefore only be briefly mentioned in this Review, despite it being widely accepted that the biopsychosocial model of IBS highlighting brain–gut interactions is of major importance for symptom generation in IBS.[19] This Review therefore concentrates on the presentation of the current knowledge regarding the crosstalk locally at the gut mucosal border, and how abnormalities can contribute to symptom generation in IBS.

Crosstalk at the mucosal border

The gastrointestinal mucosa represents the most important barrier between the inner and outer environment at which cells from the nervous, enteroendocrine and immune systems are strategically placed to maintain its integrity (Figure 1). A single layer of epithelial cells separates the luminal contents, including approximately 1×10^{14} bacteria, from the underlying tissue. The gut microbiota has a crucial role in the development and functionality of innate and adaptive immune responses, but also in regulating gut motility and intestinal barrier homeostasis.[20,21] This microbiota normally has a balanced composition that confers health, and disruption of this balance (dysbiosis) confers disease susceptibility.[22] Moreover, several animal models,[23–28] as well as clinical observations,[29–32] have demonstrated that altered immune function and inflammation in the gastrointestinal tract (as well as gastrointestinal infections and gastrointestinal dysbiosis) affect motility and sensitivity of the gut, two of the key pathophysiological factors in IBS.[23–32] Intestinal endocrine cells regulate gut motility and secretion and also modulate activity of immune cells by secreting bioactive molecules.[33,34] Because of the critical role of the immune system in the maintenance of gut homeostasis, the interactions between enteroendocrine cells and immune cells probably play a key part in the maintenance of the integrity of the gut mucosal barrier.

Bearing in mind the complexity of the gastrointestinal tract, diseases such as IBS are probably multifactorial conditions and not caused by one single mechanism, which might explain why little progress has been made in revealing the aetiology and pathology of this disease and the development of therapeutic options for IBS despite major efforts. Thus, to deepen the understanding of the underlying mechanisms of IBS we need a multifactorial research approach, addressing and correlating different aspects of this disease in a large number of patients. Therefore, it is of great importance to define how the gut microbiota, enteroendocrine system, immune system and the epithelial barrier act together and potentially contribute to the development of functional gastrointestinal disorders in general and IBS in particular.

Gut microbiota in IBS

In the human body a complex community of microbes is present (collectively referred to as the microbiota), and the vast majority of these can be found in the gastrointestinal tract.[35,36] The concentration of microbes increases continuously along the gut, ranging from 1×10^{1}–1×10^{3} cells per gram content in the upper parts to 1×10^{11}–1×10^{12} bacteria per gram content distally.[35,37] The composition also differs, with predominantly Gram-positive bacteria in the upper gastrointestinal tract and mainly Gram-negative microorganisms and anaerobes in the colon, where the microbiota composition is totally dominated by three phyla (Firmicutes, Bacteroidetes and Actinobacteria).[38–40] Moreover, major differences exist between the microbiota present in the gut lumen and the microbiota attached to and embedded in the mucus layer of the gastrointestinal tract.[41] A problem for research has been that the majority of the diversity of the microbiota cannot be demonstrated by using standard culturing techniques. Culture-independent techniques have now dramatically increased the possibilities to study the role of the gut microbiota in health and disease.[40,42]

Several lines of evidence suggest an important role of bacteria in the pathogenesis and pathophysiology of functional gastrointestinal disorders in general and IBS in particular.[14,43,44] Perhaps the strongest evidence arises from epidemiological and clinical observations that a substantial number of patients with IBS report onset of their chronic gastrointestinal symptoms after a bout of gastroenteritis.[45] In fact, meta-analyses demonstrated a sixfold to sevenfold increased risk of developing IBS after a gastroenteritis episode, which makes gastrointestinal infections the best-characterized and probably strongest known risk factor for development of IBS.[46,47] Moreover, a study published in 2014 suggested that having gastroenteritis owing to *Salmonella* infection during childhood was an important risk factor for the development of long-standing IBS symptoms in adulthood.[48] Perhaps more controversial is the suggestion that small intestinal bacterial overgrowth explains IBS symptoms in a sizeable proportion of patients with IBS; however, studies with positive and negative findings exist.[49–52] Moreover, indirect evidence from treatment studies demonstrates that different ways of modulating the gut microbiota, such as prebiotics, probiotics and antibiotics, as well as dietary changes, can improve symptoms in patients with IBS.[14,53–55]

By using modern culture-independent techniques, several research groups have demonstrated alterations in the gut microbiota composition in faecal samples from patients with IBS compared with healthy individuals as controls[14,56–81] (Table 1). A small number of studies have demonstrated differences in composition of mucosa-adherent gut microbiota between patients with IBS and

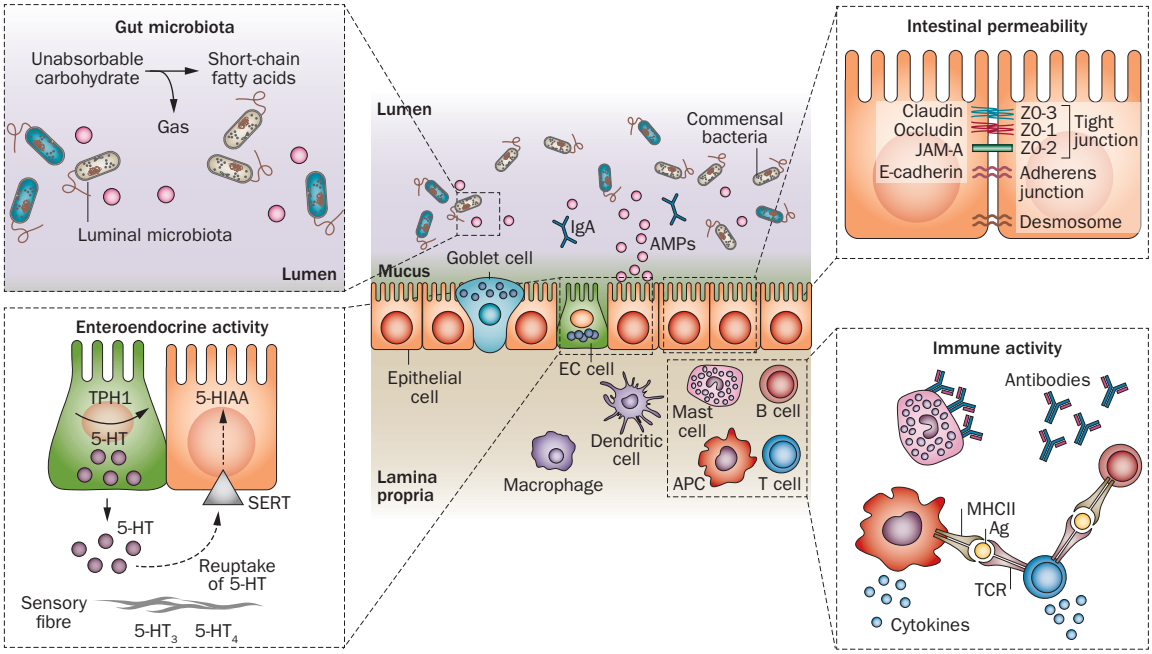

Figure 1 | Peripheral factors involved in the pathophysiology of IBS. Various factors, such as altered gut microbiota composition, together with increased intestinal permeability, a changed balance in the enteroendocrine system, and a dysregulated immune system, in the gut probably have important roles in the development of IBS. Exactly how these entities act together and give rise to symptoms is still unknown, but an altered gut microbiota composition could lead to dysregulation of the intestinal barrier as well as the enteroendocrine and the immune systems, which—through interactions with the nervous system—might lead to the generation of symptoms. Abbreviations: 5-HIAA, 5-hydroxyindole acetic acid; 5-HT, 5-hydroxytryptamine; Ag, antigen; AMP; antimicrobial peptide; APC, antigen presenting cell; IgA, immunoglobulin A; JAM-A, junctional adhesion molecule-A; SERT; 5-hydroxytryptamine transporter; TCR, T-cell receptor; TPH1, tryptophan hydroxylase 1; ZO, zonula occludens.

healthy controls.[56,63,65,67,79,82] However, the link to symptoms and other pathogenetic and pathophysiological factors is unclear in the majority of these studies (although some of the latest studies have demonstrated associations) (Table 1). Moreover, whether these alterations are linked to disease *per se,* or are merely consequences of other factors with known effects on gut microbiota composition, such as diet,[83–86] use of drugs or changes in gastrointestinal transit (reflecting abnormalities in gastrointestinal motility),[85–88] is still poorly defined and needs to be addressed in future studies.

A study by Rajilic-Stojanovic *et al.*[61] demonstrated clear differences in the intestinal microbiota composition between patients with IBS and healthy controls, whereby the microbiotas from patients were characterized by a twofold increased ratio of Firmicutes:Bacteroidetes, and the authors also noted an association between microbial groups and IBS symptom scores. In line with this finding, Jeffery *et al.*[62] also found an increase in Firmicutes-associated taxa and a decrease in Bacterioidetes-associated taxa in a subgroup of patients with IBS, whereas one group of patients with IBS demonstrated a microbiota composition similar to healthy controls. An association between the microbial signature and the clinical phenotype in a subset of patients with IBS was also demonstrated, with depression being more common in the patients with normal gut microbiota composition. This finding might indicate that there could be subgroups of patients with IBS who have a predominantly peripheral cause of their symptoms, such as microbe–host immune interactions, as opposed to those with a stronger CNS basis for their symptoms.[89] This theory has gained support in findings from a study investigating patients with IBS with and without onset of their symptoms after a gastroenteritis episode.[73] However, these findings are not in line with evidence from animal studies, in which a bidirectional association between gut dysbiosis and behavioural changes and/or mood disorders has been suggested,[90,91] or with a clinical study of patients with depression that demonstrated an association between certain bacterial taxa and depression.[92] More studies are therefore needed to clarify the role of alterations in the gut microbiota for psychological, as well as other symptoms, in IBS and other diseases. In the previously mentioned study investigating patients with postinfectious IBS, a microbial profile of 27 genus-like groups provided an Index of Microbial Dysbiosis (IMD) that could separate patients with IBS from controls.[73] Moreover, this IMD was associated with the expression of several host gene pathways, including amino acid synthesis, cell junction integrity and inflammatory response, suggesting an impaired epithelial barrier function in IBS, and the IMD was associated with the gastrointestinal symptom profile, but not with psychological symptoms. This study highlights potential mechanisms through which gut microbiota alterations can affect symptoms in IBS, namely by affecting barrier and immune function in the gut.[93]

Table 1 | Summary of gut microbiota studies in IBS

Study	Study participants*	Sample	Method	Main finding
Balsari *et al.* (1982)[78]	IBS (*n* = 20) Controls (*n* = 20)	Faeces	Culture	IBS: ↓ Coliform bacteria; ↓ *Lactobacillus* spp.; ↓ *Bifidobacterium* spp.
Si *et al.* (2004)[81]	IBS (*n* = 25) Controls (*n* = 25)	Faeces	Culture	IBS: ↓ *Bifidobacterium*; ↑ Enterobacteriaceae; ↓ *Clostridium perfringens*
Malinen *et al.* (2005)[60]	IBS (*n* = 27) Controls (*n* = 22)	Faeces	qPCR	IBS: ↓ *B. catenulatum*; ↓ *C. coccoides* group IBS-D: ↓ *Lactobacillus* spp. IBS-C: ↑ *Veillonella* spp.; ↑ *Lactobacillus* spp.
Mättö *et al.* (2005)[57]	IBS (*n* = 26) Controls (*n* = 25)	Faeces	Culture; PCR-DGGE	IBS: ↑ Coliform bacteria; ↑ aerobe:anaerobe ratio; ↓ temporal stability
Maukonen *et al.* (2006)[58]	IBS (*n* = 24) Controls (*n* = 16)	Faeces	PCR-DGGE; Affinity capture	IBS: ↓ temporal stability IBS-C: ↓ *C. coccoides* group
Kassinen *et al.* (2007)[66]	IBS (*n* = 24) Controls (*n* = 23)	Faeces	G+C-profiling + sequencing of 16S rRNA genes; qPCR	IBS: ↓ *Collinsella aerofaciens*; ↓ *C. cocleatum*; ↓ *Coprococcus eutactus* Subgroup differences (IBS-D,IBS-C, IBS-M)
Kerckhoffs *et al.* (2009)[67]	IBS (*n* = 41) Controls (*n* = 26)	Faeces; duodenal mucosa	FISH; qPCR	IBS: ↓ *Bifidobacterium* spp.; ↓ *B. catenulatum*
Krogius-Kurikka *et al.* (2009)[67]	IBS-D (*n* = 10) Controls (*n* = 23)	Faeces	G+C-profiling + sequencing of 16S rRNA genes	IBS-D: ↑ Proteobacteria; ↑ Firmicutes; ↓ Actinobacteria; ↓ Bacteroidetes
Lyra *et al.* (2009)[69]	IBS (*n* = 20) Controls (*n* = 15)	Faeces	qPCR	IBS-D: ↑ *Ruminococcus torques* 94%; ↓ *C. thermosuccinogenes* 85% IBS-C: ↑ *R. bromii*-like IBS-A: ↓ *R. torques* 93%; ↑ *C. thermosuccinogenes* (85%)
Tana *et al.* (2010)[72]	IBS (*n* = 26) Controls (*n* = 26)	Faeces	Culture; qPCR	IBS: ↑ *Veillonella* spp.; ↑ *Lactobacillus* spp.
Codling *et al.* (2010)[65]	IBS (*n* = 41) Controls (*n* = 33)	Faeces; colonic mucosa	PCR-DGGE	IBS: ↑ temporal stability; no significant difference between findings for faecal and mucosal microbiota
Carroll *et al.* (2010)[63]	IBS-D (*n* = 10) Controls (*n* = 10)	Faeces; colonic biopsies	Culture; qPCR	IBS-D: ↓ aerobic bacteria; ↑ *Lactobacillus* spp.
Noor *et al.* (2010)[70]	IBS (*n* = 11) Controls (*n* = 22) Ulcerative colitis (*n* = 13)	Faeces	PCR-DGGE + sequencing of 16S rRNA genes	IBS: ↓ bacterial species; ↓ biodiversity; ↑ biological variability of predominant bacteria
Malinen *et al.* (2010)[59]	IBS (*n* = 44)	Faeces	qPCR	*R. torques* 94% associated symptom severity Other phylotypes had negative association
Ponnusamy *et al.* (2011)[75]	IBS (*n* = 11) Controls (*n* = 8)	Faeces	DGGE + qPCR of 16S rRNA genes	IBS: ↑ diversity in Bacteroidetes and lactobacilli; ↑ levels of alanine and pyroglutamic acid and phenolic compounds
Rinttila *et al.* (2011)[80]	IBS (*n* = 96) Controls (*n* = 23)	Faeces	qPCR	IBS: *Staphylococcus aureus* (17%)
Saulnier *et al.* (2011)[71]	IBS (*n* = 22) Controls (*n* = 22) (Children)	Faeces	16S metagenomic sequencing; DNA microarray	IBS: ↑ Gammaproteobacteria Classified IBS subtypes using sets of discriminant bacterial species
Rajilic-Stojanovic *et al.* (2011)[61]	IBS (*n* = 62) Controls (*n* = 42)	Faeces	Phylogenetic 16S rRNA microarray; qPCR	IBS: ↑ Proteobacteria and specific Firmicutes; ↓ other Firmicutes, Bacteroidetes and bifidobacteria; association with symptom profile
Carroll *et al.* (2011)[56]	IBS-D (*n* = 16) Controls (*n* = 21)	Faeces; colonic mucosa	T-RFLP fingerprinting of 16S rRNA; PCR	IBS-D: diminished microbial biodiversity in faecal samples
Parkes *et al.* (2012)[82]	IBS-D (*n* = 27) IBS-C (*n* = 26) Controls (*n* = 26)	Colonic mucosa	FISH; confocal microscopy	IBS: expansion of mucosa-associated microbiota; mainly *Bacteroides* and *Clostridium*; association with IBS subgroups and symptoms
Jeffery *et al.* (2012)[62]	IBS (*n* = 37) Controls (*n* = 20)	Faeces	Pyrosequencing 16S rRNA	Clustering of patients with IBS with normal-like vs abnormal microbiota composition (increased ratio of Firmicutes to Bacteroidetes); association with symptom profile
Carroll *et al.* (2012)[64]	IBS-D (*n* = 23) Controls (*n* = 23)	Faeces	16S rRNA; PCR	IBS-D: ↑ Enterobacteriaceae; ↓ *Faecalibacterium* genera; ↓ microbial richness

Table 1 (Cont.) | Summary of gut microbiota studies in IBS

Study	Study participants*	Sample	Method	Main finding
Rigsbee *et al.* (2012)[74]	IBS ($n=22$) Controls ($n=22$) Adolescents	Faeces	16S rRNA; qPCR; FISH	IBS: ↑ *Veillonella*, *Prevotella*, *Lactobacillus* and *Parasporobacterium*; ↓ members of *Bifidobacterium* and *Verrucomicrobium*
Durban *et al.* (2012)[79]	IBS ($n=16$) Controls ($n=9$)	Faeces; colonic biopsies	Sequencing of 16S rRNA	↓ diversity in IBS; more differences between faecal and mucosal samples than between IBS and controls
Durban *et al.* (2013)[77]	IBS-D ($n=2$) Control ($n=1$) Serial samples over 6–8 weeks	Faeces	16S rRNA; metagenomics; metatranscriptomics	IBS and fluctuating symptoms (diarrhoea): ↑ instability in the fraction of active microbiota
Carroll *et al.* (2013)[76]	IBS ($n=30$) Controls ($n=24$)	Faeces	16S rRNA; faecal protease activity	Associations between specific intestinal bacterial groups and faecal protease activity
Jalanka-Tuovinen *et al.* (2014)[73]	Postinfectious IBS ($n=11$) IBS-D ($n=12$) Postinfectious bowel dysfunction ($n=11$) Postinfectious non-bowel dysfunction ($n=12$) Controls ($n=11$)	Faeces	16S rRNA, phylogenetic microarray; qPCR	A bacterial profile of 27 genus-like groups (providing an IMD) separated patient groups and controls Correlations between the IMD and expression of several host gene pathways, including amino acid synthesis, cell junction integrity and inflammatory response

*Healthy individuals as controls. Abbreviations: DGGE, denaturing gradient gel electrophoresis; FISH, fluorescence *in situ* hybridization; G+C, guanosine plus cytosine; IBS-A, alternating-type IBS; IBS-C, IBS with constipation; IBS-D, IBS with diarrhoea; IBS-M, mixed-type IBS; IMD, index of microbial dysbiosis; qPCR, quantitative PCR; T-RFLP, terminal restriction fragment length polymorphism. Adapted and modified from Simrén *et al. Gut* **62**, 159–176 (2013)[14] with permission from BMJ Publishing Group Ltd ©.

Additionally, from a theoretical point of view, luminal and mucosal colonic microbiota might generate symptoms through different mechanisms. The luminal microbiota has the potential to affect symptoms through carbohydrate fermentation and gas production, whereas the mucosa-associated microbiota might affect symptoms through interaction with immune and nerve cells in the gut wall.[94] Even though bacteria *per se* could affect gut function, accumulating evidence suggests that it is not which bacteria that are there that is of importance, but rather what they do. Patients with IBS have, for instance, abnormal levels of faecal short-chain fatty acids, the major end product of bacterial fermentation, and these levels seem to be associated with the symptom profile of the patients.[72] This observation and other studies support the importance of microbial metabolites for symptom generation in IBS.

Enteroendocrine system in IBS

Physiology of the enteroendocrine system

Enteroendocrine cells, identified using immunohistochemistry by their intracellular protein content, are dispersed throughout the gastrointestinal tract. These cells produce hormones that are stored in secretory granules and are released on the luminal or basal side of the cell in response to mechanical, chemical or neural interactions. At least 15 subtypes of enteroendocrine cells exist, secreting multiple peptide hormones that control physiological and homeostatic functions,[95] in particular postprandial secretion and motility, and also having local and systemic effects on the enteric nervous system and on the immune system of the gastrointestinal tract.

Enterochromaffin cells (EC cells) are the most abundant enteroendocrine cell subtype of the colon and rectum.[96] They are the main producers of 5-hydroxytryptamine (5-HT), the most explored hormone in IBS, as well as chromogranin (Cg) A. The most potent stimulus for EC cell degranulation seems to be shear forces induced by gut contractions as exemplified by the *in vitro* effects of mucosal stroking in strips of human jejunum.[97] An interaction with the mucosal immune system also occurs; subpopulations of T cells that can increase the number of EC cells and levels of IL-13 have been suggested to mediate this effect.[98] Chemical stimulants are probably of regional importance as exemplified by the ability of short-chain fatty acids (products of colonic microbiota in humans) to stimulate 5-HT release.[99] Locally released 5-HT acts on specific receptors, and the development of agonists and antagonists has revealed some of the complex physiological effects in humans in terms of motility, secretion and sensory function.[100] Stimulation of both 5-HT_3 and 5-HT_4 receptors[101,102] has been shown to shorten the gastrointestinal transit time mediated by neuronal acetylcholine release, whereas blockage of 5-HT_3 receptors prolongs gastrointestinal transit[103,104] as well as having antiemetic effects.[105] Locally released 5-HT is recycled into the intracellular compartments again by the 5-hydroxytryptamine transporter (SERT) situated on neurons, enterocytes, vascular endothelial cells and platelets.[106] Intracellular catabolism of 5-HT by monoamine oxidase to 5-hydroxyindole acetic acid (5-HIAA) follows without any extracellular catabolic steps involved. As the majority of 5-HT in human peripheral blood originates from the gut, platelet-depleted plasma is supposed to reflect the gastrointestinal release.[107]

Enteroendocrine system—studies in IBS

In IBS, the first report of 5-HT abnormalities originates from 1998 when Bearcroft *et al.*[108] showed increased postprandial platelet-depleted plasma levels of 5-HT in a small number of patients with diarrhoea-predominant

IBS (IBS-D). This finding was later confirmed to be of relevance both in the specific situation of postinfectious IBS[109] and in patients with IBS-D.[110] On the contrary, patients with constipation-predominant IBS (IBS-C) were shown to have a low concentration of platelet-depleted plasma 5-HT after meal intake, which was also reported in patients with functional constipation, without any notable difference compared with IBS-C.[111] When analysing the 5-HIAA:5-HT ratio in mucosal biopsy samples as a measurement of 5-HT turnover, it seems that this value is reduced in IBS-D[109] and increased in IBS-C.[110,112,113] A plausible explanation for this finding could be impaired local 5-HT uptake in patients with IBS-D and impaired 5-HT release in patients with constipation regardless of whether their clinical diagnosis is IBS-C or functional constipation. Delayed transit time in itself does not seem to be the factor causing these abnormalities in mucosal 5-HT content as opiate-induced constipation does not affect 5-HT release.[114]

A possible interaction between the enteroendocrine system and the local immune system in IBS was first highlighted in studies of postinfectious IBS in which the development of long-standing gastrointestinal symptoms compatible with an IBS diagnosis was associated with an increased number of rectal EC cells and T cells.[115] Moreover, indirect evidence supporting these interactions stems from a study demonstrating that IBS-D also shares some features with IBD regarding mucosal 5-HT turnover, at least in the context of ulcerative colitis.[116] Paediatric studies did not find evidence of alterations in 5-HT signalling in functional dyspepsia, but confirmed such abnormalities in IBS when analysing colonic or gastric mucosal specimens for 5-HT content, SERT mRNA and levels of tryptophan hydroxylase-1 (the rate-limiting enzyme in 5-HT synthesis).[117]

A potential surrogate marker for EC cell activity is to use measurement of chromogranins and secretogranins (Sg). Cromogranins and secretogranins are a group of acidic proteins present in the secretory granules of a wide variety of endocrine, neuronal and neuroendocrine cells and are used as a surrogate marker for EC cell activity in other disease conditions. The number of cells with CgA content has been reported to be reduced in the duodenum, ileum and colon of patients with IBS, whereas the number of CgA-positive cells in the rectum of patients with IBS seems to be intact.[118–120] Other chromogranins and secretogranins (such as CgB, SgI and SgII) are co-produced in many EC cells, and these proteins might be complementary. Patients with IBS have been suggested to have increased levels of faecal CgA and SgII, which was associated with colonic transit time and gastrointestinal symptoms.[121] Follow-up studies confirming these findings are needed. Abnormalities in other enteroendocrine cell types, such as those producing cholecystokinin, somatostatin, or peptide YY have been reported in both the small and large intestine of patients with IBS.[122]

Interestingly, research within the field of so-called microbial endocrinology suggests that bidirectional communication between the gut microbiota and the enteroendocrine system takes place. The gut microbiota might be responsible for regulating enteroendocrine activity and immune activity of the host.[123] Moreover, stress hormones can affect microbial expression of colonisation and virulence factors.[124] Thus, it could be hypothesized that altered enteroendocrine activity in patients with IBS can be related to the chronic disease condition *per se,* or the microbiota composition alone, or a combination of these. Furthermore, associations between 5-HT and inflammation and immune activity within the gastrointestinal tract have been demonstrated,[13] with different inflammatory conditions being associated with altered levels of 5-HT, both in humans[116,125,126] and animal studies.[127,128] An intriguing proinflammatory effect of 5-HT has also been demonstrated in various animal models of colitis,[129,130] and data also suggest involvement of chromogranins in immune activation and inflammation,[131–133] augmenting the importance of the enteroendocrine system in inflammatory events in the gastrointestinal tract.[34] EC cells are also important bidirectional transducers that regulate communication between the gut lumen and the enteric nervous system and thereby the CNS as well.[134]

In conclusion, a role for the enteroendocrine system as a pathogenetic factor in IBS has been highlighted during the past decade. 5-HT has been the main focus with the most robust data linked to IBS-D or IBS-C. Pharmacological interventions with 5-HT receptor agonists and antagonists in IBS have been promising, but numbers needed to treat to have one patient with satisfactory symptom relief have been too high to expect this mechanism to have more than a partial role in the generation of IBS symptoms. Markers of EC cell activity to identify subgroups of patients for whom the enteroendocrine system is of pathogenetic importance, as well as to provide further understanding of the interaction with microbiota, might provide further insights into the complex pathophysiology of IBS.

Intestinal permeability in IBS

Barrier function

The inherent property of the gut to act as a semipermeable barrier is crucial for the maintenance of health. The most obvious part of the barrier consists of a single layer of mucosal epithelial cells that are interconnected by tight junctions that allows passage of small particles. Apart from this last line of defence, the mucus layer covering the intestinal mucosa as well as the gut microbiota and products from the immune system (such as defensins and secreted antibodies) have important roles in maintaining gut integrity.[135]

Between 12% and 50% of patients with IBS have been reported to have altered intestinal permeability in research studies[136] using various methods to reflect gut permeability at different parts of the gastrointestinal tract,[137–139] and both postinfectious IBS as well as non-selected groups of patients with IBS have been investigated. An acute bacterial infection results in a transient increase in intestinal permeability.[140,141] This phenomenon seems to be highly persistent in patients who develop postinfectious IBS,[141,142] but altered intestinal permeability

does not seem to be confined to postinfectious IBS alone, as the different subtypes of IBS all seem to have a proportion of patients with increased gut permeability.[143] As an example, both patients with IBS-D who had postinfectious IBS as well as those with onset unrelated to an infectious event have been reported to have increased small intestinal permeability.[142,144] However, a somewhat unexpected finding in one study was that patients with IBS without a history of postinfectious IBS had an even more-severe defect in intestinal barrier function than patients with postinfectious IBS,[144] which could indicate that the barrier dysfunction is of more importance for symptom generation in these patients and an infectious event is not a necessary trigger.

The mechanisms underlying increased permeability in IBS have not been fully established, but the impaired expression of epithelial tight junctions and adherence-junction-associated proteins is probably involved. For example, studies demonstrating low expression of the tight junction protein zonula occludens 1,[145–147] junctional adhesion molecule-A (JAM-A) and E-cadherin[147] in IBS imply a dysfunctional mucosal epithelium in these individuals. However, whether the alteration in permeability precedes onset of IBS, maybe as a result of luminal or host factors, or whether it merely reflects alterations associated with the disorder is unknown. In favour of the former hypothesis is that patients with IBD who are in long-standing remission also have increased gut permeability,[148] although it cannot be ruled out that this phenomenon is a result of previous inflammatory reactions. Interestingly, increased intestinal permeability in patients with IBD in remission is associated with IBS-like symptoms.[149] Furthermore, in patients with IBS, increased permeability has also been linked to more-severe IBS symptoms in general,[146,150] as well as with more-intense abdominal pain,[143] which suggests that structural and functional abnormalities of the mucosal barrier might be involved in symptom generation in IBS and IBD.

Putative causes of altered gut permeability

The identification of triggers that precede increased intestinal permeability could be a key factor for the development of effective therapies and could perhaps even prevent the development of IBS in certain clinical situations. For the moment, several candidate triggers are highly relevant, such as factors in the luminal content, factors within the mucosa itself, exposure to stress and infectious agents, as well as genetic susceptibility.

Faecal supernatants from patients with IBS are able to increase colonic paracellular permeability in mice, possibly mediated by the protease content that has been shown to be increased in IBS.[151,152] Different proteases seems to be of importance in different clinical situations: serine proteases have been found to be elevated in IBS-D,[152] and cysteine proteases in patients with IBS-C.[151] Cysteine proteases have a degrading effect on occludin in mice and also in the human colonic epithelial T84 cell line, and this pathogenetic mechanism can be supported by the finding of decreased occludin levels in mucosal biopsy samples from patients with IBS-C.[151]

A steadily increasing number of studies support that increased intestinal permeability in patients with IBS is linked to enhanced activity of the immune system, and a link with food allergy has been suggested.[153] Experiments in colon explants from mice have shown that the interaction between TNF and neuropeptide Y results in increased intestinal permeability.[154] Proinflammatory cytokines such as TNF and IFN-γ increase intestinal permeability by downregulation of claudin and zonula occludens proteins, probably via regulation of the transcription factor complex nuclear factor κ B.[155] Several reports indicate that mast cell activity, and thus the release of mast cell mediators, might also be a central factor for altered gut permeability. For instance, increased numbers of mucosal mast cells have been associated with increased rectal permeability in patients with IBS-D,[156] and mast cell tryptase was demonstrated to reduce expression of JAM-A, leading to altered caecal epithelial permeability.[157] In addition, jejunal mast cell activation correlates with intestinal permeability, for which regulation of the expression of zonula occludens proteins and intercellular apical junction complex is central.[146,158] The effects of corticoliberin (also known as corticotropin-releasing factor) on permeability also involves an induction of the release of mast cell proteases and TNF in animal models.[159] Moreover, corticoliberin has been shown to mediate transcellular transport via subepithelial mast cells in the human colonic mucosa.[160] From this point of view, the association between stress and gut permeability could also be explained by factors involving corticoliberin and mast cell activation. Acute psychological stress, as well as administration of corticoliberin to mimic the stress response, have both been shown to result in increased intestinal permeability in healthy volunteers.[161] The observation that the use of the mast cell stabilizer disodium cromoglycate can antagonize this effect further supports the central role this cell type might have. On the basis of experiments in rats, another potential mediator of the mast cell stress effects on permeability is vasoactive intestinal peptide.[162]

As not all patients with IBS have signs of altered gut permeability, it might be suggested that the subgroup of patients with IBS in whom altered gut permeability is found might have a genetic predisposition. Investigation of functional variants of genes with products involved in intestinal epithelial barrier function has demonstrated that *CDH1*, which encodes the tight junction protein E-cadherin, was associated with postinfectous IBS, and persisted as an independent risk factor for postinfectious IBS when controlling for previously identified clinical risk factors.[163] Another study demonstrating that HLA-DQ2-positive patients, in contrast to HLA-DQ2-negative patients, with IBS-D, had increased small bowel permeability that was linked to reduced mRNA expression of tight junction proteins,[164] further underlining the possibility of a genetic predisposition for impaired mucosal border function in IBS.

Immunity in IBS

Evidence is accumulating that IBS symptoms might, in at least certain subgroups of patients, be the result of an

exogenous or endogenous trigger that leads to increased immune activity. In general, immune activity in IBS is often reported to involve mast cell activation as well as increased activity of innate and adaptive immunity.

Innate immunity

Increased numbers of mucosal mast cells in close proximity to nerves in the colonic mucosa is one of the most frequently reported features of immune activity in IBS.[165,166] Also, levels of mast cell mediators secreted by activated mast cells, such as tryptase and histamine, are increased in the colon of patients with IBS.[165] The mucosal expression of mast cells and their mediators is especially interesting in the context of maintaining homeostasis at the mucosal barrier. As described earlier, mast cell tryptase reduces the expression of JAM-A expression in the human Caco2 intestinal epithelial cell line, which results in increased epithelial permeability.[157] Furthermore, increased mucosal permeability (induced by acute stress) was blocked by mast cell inhibitors, suggesting that mast cell mediators mediate stress-evoked changes in gut permeability in healthy individuals.[161]

Several reports have focused on Toll-like receptors (TLR),[167–169] which are immune-cell receptors that recognize microbial 'danger' signals. To summarize, the RNA levels of TLR2, TLR4 and TLR5 have been found to be increased, whereas TLR7 and TLR8 are reduced in the colonic mucosa of patients with IBS, and these findings have also been confirmed at the protein level using immunohistochemistry.[170,171] Increased expression of TLR2 in blood monocytes from patients with IBS has also been reported.[172] The expression of TLRs is upregulated by exposure to structures of bacteria and viruses, which means that these findings in IBS fit with the hypothesis of an altered microbiota composition or exposure in this patient group.

Another often reported immune-related feature in patients with IBS is increased levels of circulating proinflammatory cytokines such as IL-6, IL-8, TNF and IL-1β,[173,174] although a large overlap exists in cytokine levels between patients and healthy individuals. By contrast, circulating levels of the anti-inflammatory cytokine IL-10 are reported to be similar in patients with IBS and healthy individuals.[175–177] A meta-analysis published in 2014 reported an imbalance in serum levels of proinflammatory TNF and anti-inflammatory IL-10 in IBS.[178] Moreover, the proinflammatory cytokines IL-6, IL-1β, and TNF[179] are increased in nonstimulated or lipopolysaccharide-stimulated peripheral blood mononuclear cell cultures from patients with IBS. Fewer data on the mucosal cytokine pattern in IBS are available, but RNA levels of IL-10 are reported to be lower in female patients with IBS as compared to healthy women, whereas the levels of several other cytokines did not differ between patients and healthy individuals.[174] Furthermore, increased protein levels of the proinflammatory cytokines IL-8 and IL-1β have been recorded in *ex vivo* biopsy explants from patients with IBS compared with samples from healthy individuals.[171] Also, individuals who developed IBS after an acute episode of infectious gastroenteritis had higher expression of IL-1β mRNA than individuals who did not develop IBS after the infection.[180]

How might the increased local and systemic levels of proinflammatory cytokines contribute to IBS? As previously mentioned, proinflammatory cytokines might have an effect on epithelial barrier function. For example, it has been demonstrated in animal models that anti-TNF antibodies inhibit corticoliberin-mediated intestinal barrier dysfunction,[159] and permeability and colonic transepithelial ion transport can be modulated by IL-6.[181] Thus, even a fairly small increase in levels of proinflammatory cytokines at the epithelial barrier might lead to increased intestinal permeability, and thereby altered homeostasis at the mucosal border.

Given that altered gastrointestinal motility is of relevance for the disturbance of bowel habit in patients with IBS,[7] the well-established interaction between the nervous and immune system during inflammation[182] is therefore of potential interest for IBS, as is the crosstalk between luminal microbiota and the intestinal immune system that influences gastrointestinal motility (as demonstrated in animal models). For instance, lack of TLR4—a receptor commonly expressed on innate immune cells that recognizes lipopolysaccharide from Gram-negative bacteria—results in substantial reduction in gastrointestinal motility in mouse models.[183] Moreover, intestinal macrophages regulate peristaltic activity of the colon by changing the pattern of smooth muscle cell contractions in the presence of luminal microbiota.[184,185]

Adaptive immunity

Increased numbers of T cells within the epithelial layer, lamina propria or in the myenteric plexus of full-thickness or mucosal biopsy samples from the small and large intestine of patients with IBS have been reported in a number of publications.[141,186–189] An immune response induced by type 1 T helper (T_H1) cells with increased IFN-γ and reduced IL-10 levels has been reported in postinfectious IBS,[190] whereas evidence of increased type 2 T helper (T_H2) cell activity has been reported in several functional gastrointestinal disorders.[177] The finding of normal frequencies of apparently functional blood and colonic $CD25^+$ regulatory T cells, with the ability to suppress effector T-cell proliferation,[191] indicates that IBS is not associated with defective regulation of activated mucosal T cells. Increased numbers of T cells have also been linked to increased density of endocrine cells and gut permeability in postinfectious IBS.[141]

Furthermore, B cells isolated from the blood of patients with IBS display an amplified activation level as demonstrated by increased cell surface expression of IgG and the co-stimulatory molecules CD80 and CD86, indicating that B cells in the blood of patients with IBS have a more-activated phenotype.[192] A higher number and activation of jejunal mucosal B cells and plasma cells and increased mucosal immunoglobulin production[193] than normal have also been reported. Along the same lines, increased levels of serum antibodies against flagellin, a bacterial-derived structure, have been observed in patients with

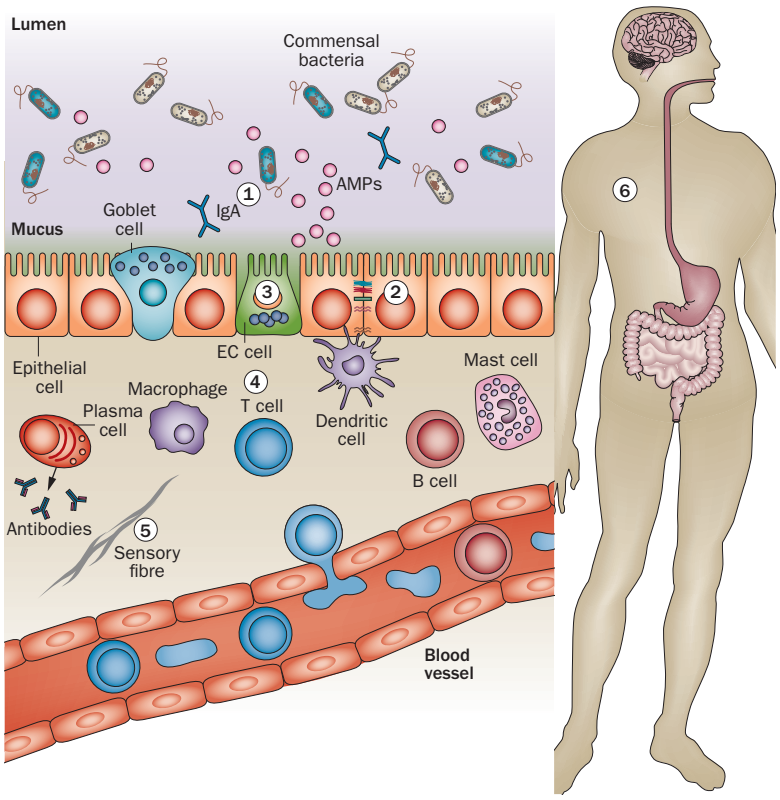

Figure 2 | Different structural abnormalities can interact with each other and contribute to the generation of symptoms in IBS. An altered composition of the gut microbiota (1), or the mediators produced by these gut microbiota, might influence epithelial permeability, possibly by degrading epithelial tight junction proteins such as occludin (2). Also, the gut microbiota can influence the activity of enteroendocrine cells, especially EC cells dispersed between the epithelial cells, inducing altered secretion or re-uptake of 5-hydroxytryptamine (3). Additionally, gut microbiota, or their metabolites, can directly affect the cells of the immune system, inducing increased immune activity (4). However, increased immune activity caused by triggers other than the gut microbiota could potentially directly influence epithelial permeability, endocrine activity and stimulate sensory nerve fibres in the mucosa such as TRPV-1 (5). Cytokines and other mediators secreted by immune cells, as well as mediators secreted by the gut microbiota and enteroendocrine cells, can leave the mucosa via the blood stream and have systemic effects (6). Abbreviations: AMP, antimicrobial peptide; EC cell, enterochromaffin cell; IgA, immunoglobulin A; TRPV-1, transient receptor potential cation channel subfamily V member 1.

postinfectious IBS.[194] Collectively, these findings support the notion that specific T cells and B cells of the adaptive immune system are activated and produce cytokines and antibodies, although at discrete (as in, low) levels, in response to antigens. Although our understanding of the origin of these antigens is limited, they might well derive from the gut. Furthermore, how these changes lead to gastrointestinal symptoms in IBS is not known, but animal studies suggest that crosstalk between the motility apparatus of the gastrointestinal tract and altered adaptive immune function could be relevant. In the postinfectious *Trichinella spiralis* model, smooth muscle hyperactivity has been demonstrated to be mediated by T_H2 cytokines, such as IL-4 and IL-13.[23] Additionally, another report suggests that the cytokine IL-17A also induces gastrointestinal hypermotility, as demonstrated in a T-cell-mediated mouse model of enteritis.[195] By contrast, T_H1-related cytokines have been shown to cause hypocontractility of inflamed intestinal smooth muscle in humans.[196]

Genetic evidence of altered immunity

During the past few years, several studies have reported immune-related alterations in patients with IBS based on gene associations, that is, polymorphisms of immune-associated genes. A meta-analysis indicated a role for *IL10* polymorphisms in IBS in general, and that *TNF* polymorphisms might be important in Asian populations with IBS.[197] Also, the genotype combination of high-producer *TNF* and low-producer *IL-10* gene expression has been demonstrated to be more prevalent in patients with IBS than healthy individuals,[198] as well as polymorphisms of the gene encoding the proinflammatory cytokine IL-8.[199] Two independent studies have reported that IBS is associated with genetic polymorphisms of *TNFSF15*, which encodes the inflammation-related protein TNF ligand superfamily member 15, supporting a role for immune activation in IBS.[200,201] In 2014, it was also demonstrated, using next-generation pair-end sequencing, that patients with IBS-D had decreased mRNA expression of *TNFSF15*, further confirming the importance of altered expression of this gene in IBS.[202]

Neuroimmune interactions in IBS

Exactly how altered immune function might lead to symptoms in patients with IBS is not altogether understood, but several studies now support the relevance of neuroimmune interactions for symptom generation in IBS and other functional gastrointestinal disorders.[167] An anatomical basis for this theory stems from studies that have demonstrated that patients with IBS have increased numbers of sensory nerve fibres in the rectosigmoid area that express the capsaicin receptor TRPV1,[203] a greater number of mast cells in close vicinity to nerves in the colon[165] and an increased density of intestinal mucosal nerve fibres appearing in clusters surrounding mast cells.[204] Moreover, in these studies, positive associations between the severity of abdominal pain and the number of nerves fibres expressing the TRPV1 receptor,[203] as well as with the number of mast cells in close vicinity to colonic nerves,[165] were noted. The anatomical basis for an interaction between mast cells and nerves at different intestinal sites was also demonstrated in a paediatric IBS cohort and, again, the number of mast cells in close vicinity to nerves was related to the intensity and frequency of abdominal pain,[205] further highlighting the relevance of neuroimmune interactions for symptom generation in IBS.

Other studies have used supernatants from colonic biopsy samples from patients with IBS and healthy controls to perform functional studies using different nerve preparations to investigate neuroimmune interactions (reviewed by Nasser *et al.*[206]). Taken together, these studies collectively and strongly support the relevance of neuroimmune interactions in IBS through different mechanisms, including release of histamine, proteases and 5-HT.[207–213] Moreover, the effects of supernatants from peripheral blood mononuclear cells from patients

with IBS and controls on sensory nerves have been investigated. This study showed distinct patterns of immune dysfunction and interaction with sensory pathways through different intracellular pathways that was different not only between patients with IBS and controls, but also between different IBS subgroups.[214] Furthermore, besides the above-mentioned evidence suggesting that interactions between the immune system and nerves in the gut might be of relevance for symptom generation in IBS, bidirectional brain–visceral interactions could also be of importance as interoceptive input is encoded by a network of transducers in the gut and conveyed to the brain via vagal and spinal afferents, immune mediators and endocrine signals, as reviewed elsewhere.[17]

Conclusions

An increasing number of reports have provided good evidence of altered gut microbiota composition, aberrant expression pattern and function of EC cells, abnormal gut permeability and increased immune activity in at least subgroups of patients with IBS (Figure 2). However, understanding the relative importance of each of these factors and their interactions is needed to better comprehend the complex pathophysiology of IBS. It might be proposed that altered composition of the gut microbiota community impairs epithelial permeability, possibly by degrading epithelial tight junction proteins. Moreover, the gut microbiota might also influence the activity of enteroendocrine cells, resulting in an altered hormonal milieu in the gut and affecting immune cells, causing increased immune activity. However, increased activity of immune cells, caused by triggers other than gut microbiota, could potentially harm epithelial integrity and the endocrine activity of the gut, and thereby facilitate microbiota adherence to the gut mucosa. Furthermore, cytokines and other mediators secreted by immune cells, as well as mediators secreted by the gut microbiota and enteroendocrine cells, could have effects beyond the gut, giving rise to extraintestinal symptoms and events. Finally, to determine factors linking the proposed pathogenetic events to symptoms compatible with functional gastrointestinal disorders, probably through interactions with the enteric nervous system and CNS, is a major challenge.

Our increasing knowledge of the prominence of the communication and homeostasis at the mucosal border has the potential to lead to new treatment strategies, possibly by demonstrating how gut microbiota could be used as a therapeutic target, modulating the enteroendocrine and immune system but also the epithelial barrier integrity of the gut. Thus, improved understanding of the complex crosstalk at the mucosal borders will provide improved health and quality of life for a large patient group, for whom treatment options are limited today.

Review criteria

This Review is based on literature searches performed in the PubMed database in January and August 2014 using the search terms: “irritable bowel syndrome”; “functional bowel disorder”; “postinfectious”; “mucosa”; “biopsies”; “inflammation”; “immune function”; “permeability”; “tight junctions”; “microbiota”; “enteroendocrine”; “enterochromaffin cells”; “5-hydroxytryptamine”; “serotonin”; and “chromogranins”. The reference lists of identified articles or linked articles were searched for further papers. English-language original research and review articles were considered. No publication date restrictions were applied. The Review is also based on the authors’ personal knowledge of research groups active in this field.

1. Longstreth, G. F. *et al.* Functional bowel disorders. *Gastroenterology* **130**, 1480–1491 (2006).
2. Lovell, R. M & Ford, A. C. Global prevalence of and risk factors for irritable bowel syndrome: a meta-analysis. *Clin. Gastroenterol. Hepatol.* **10**, 712–721.e4 (2012).
3. White, B. C. J. Mucous colitis: a delineation of the syndrome with certain observations on its mechanisms and on the role of emotional tension as a precipitating factor. *Ann. Int. Med.* **14**, 854–872 (1940).
4. Whitehead, W. E., Palsson, O. & Jones, K. R. Systematic review of the comorbidity of irritable bowel syndrome with other disorders: what are the causes and implications? *Gastroenterology* **122**, 1140–1156 (2002).
5. Chaudhary, N. A. & Truelove, S. C. The irritable colon syndrome. A study of the clinical features, predisposing causes, and prognosis in 130 cases. *Q. J. Med.* **31**, 307–322 (1962).
6. Spiller, R. *et al.* Guidelines on the irritable bowel syndrome: mechanisms and practical management. *Gut* **56**, 1770–1798 (2007).
7. Törnblom, H. *et al.* Colonic transit time and IBS symptoms: what’s the link? *Am. J. Gastroenterol.* **107**, 754–760 (2012).
8. Ritchie, J. Pain from distension of the pelvic colon by inflating a balloon in the irritable colon syndrome. *Gut* **14**, 125–132 (1973).
9. Whitehead, W. E. *et al.* Tolerance for rectosigmoid distention in irritable bowel syndrome. *Gastroenterology* **98**, 1187–1192 (1990).
10. Posserud, I. *et al.* Altered rectal perception in irritable bowel syndrome is associated with symptom severity. *Gastroenterology* **133**, 1113–1123 (2007).
11. Tillisch, K., Mayer, E. A. & Labus, J. S. Quantitative meta-analysis identifies brain regions activated during rectal distension in irritable bowel syndrome. *Gastroenterology* **140**, 91–100 (2011).
12. Furness, J. B. *et al.* The gut as a sensory organ. *Nat. Rev. Gastroenterol. Hepatol.* **10**, 729–740 (2013).
13. Mawe, G. M. & Hoffman, J. M. Serotonin signalling in the gut—functions, dysfunctions and therapeutic targets. *Nat. Rev. Gastroenterol. Hepatol.* **10**, 473–486 (2013).
14. Simren, M. *et al.* Intestinal microbiota in functional bowel disorders: a Rome foundation report. *Gut* **62**, 159–176 (2013).
15. Bercik, P. & Collins, S. M. The effects of inflammation, infection and antibiotics on the microbiota–gut–brain axis. *Adv. Exp. Med. Biol.* **817**, 279–289 (2014).
16. De Palma, G., Collins, S. M. & Bercik, P. The microbiota–gut–brain axis in functional gastrointestinal disorders. *Gut Microbes* **5**, 419–429 (2014).
17. Mayer, E. A. & Tillisch, K. The brain-gut axis in abdominal pain syndromes. *Annu. Rev. Med.* **62**, 381–396 (2011).
18. Mayer, E. A., Savidge, T. & Shulman, R. J. Brain–gut microbiome interactions and functional bowel disorders. *Gastroenterology* **146**, 1500–1512 (2014).
19. Tanaka, Y. *et al.* Biopsychosocial model of irritable bowel syndrome. *J. Neurogastroenterol. Motil.* **17**, 131–139 (2011).
20. Sommer, F. & Backhed, F. The gut microbiota —masters of host development and physiology. *Nat. Rev. Microbiol.* **11**, 227–238 (2013).
21. Peterson, L. W. & Artis, D. Intestinal epithelial cells: regulators of barrier function and immune homeostasis. *Nat. Rev. Immunol.* **14**, 141–153 (2014).
22. Gibson, M. K., Pesesky, M. W. & Dantas, G. The yin and yang of bacterial resilience in the human gut microbiota. *J. Mol. Biol.* http://dx.doi.org/10.1016/j.jmb.2014.05.029.
23. Akiho, H. *et al.* Mechanisms underlying the maintenance of muscle hypercontractility in a model of postinfective gut dysfunction. *Gastroenterology* **129**, 131–141 (2005).
24. Bercik, P. *et al.* Visceral hyperalgesia and intestinal dysmotility in a mouse model of postinfective gut dysfunction. *Gastroenterology* **127**, 179–187 (2004).

25. Gwee, K. A. *et al.* The role of psychological and biological factors in postinfective gut dysfunction. *Gut* **44**, 400–406 (1999).
26. Kanazawa, M. *et al.* Motility response to colonic distention is increased in postinfectious irritable bowel syndrome (PI-IBS). *Neurogastroenterol. Motil.* **26**, 696–704 (2014).
27. Kimball, E. S. *et al.* Acute colitis induction by oil of mustard results in later development of an IBS-like accelerated upper GI transit in mice. *Am. J. Physiol. Gastrointest. Liver Physiol.* **288**, G1266–G1273 (2005).
28. Rao, S. S. *et al.* Studies on the mechanism of bowel disturbance in ulcerative colitis. *Gastroenterology* **93**, 934–940 (1987).
29. Rao, S. S. *et al.* Anorectal sensitivity and responses to rectal distention in patients with ulcerative colitis. *Gastroenterology* **93**, 1270–1275 (1987).
30. Verdu, E. F. *et al. Lactobacillus paracasei* normalizes muscle hypercontractility in a murine model of postinfective gut dysfunction. *Gastroenterology* **127**, 826–837 (2004).
31. Verdu, E. F. *et al.* Specific probiotic therapy attenuates antibiotic induced visceral hypersensitivity in mice. *Gut* **55**, 182–90 (2006).
32. Verma-Gandhu, M. *et al.* Visceral pain perception is determined by the duration of colitis and associated neuropeptide expression in the mouse. *Gut* **56**, 358–364 (2007).
33. Wu, T. *et al.* Gut motility and enteroendocrine secretion. *Curr. Opin. Pharmacol.* **13**, 928–934 (2013).
34. Khan, W. I. & Ghia, J. E. Gut hormones: emerging role in immune activation and inflammation. *Clin. Exp. Immunol.* **161**, 19–27 (2010).
35. Sekirov, I. *et al.* Gut microbiota in health and disease. *Physiol. Rev.* **90**, 859–904 (2010).
36. Young, V. B. & Schmidt, T. M. Overview of the gastrointestinal microbiota. *Adv. Exp. Med. Biol.* **635**, 29–40 (2008).
37. O'Hara, A. M. & Shanahan, F. The gut flora as a forgotten organ. *EMBO Rep.* **7**, 688–693 (2006).
38. Frank, D. N. *et al.* Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases. *Proc. Natl Acad. Sci. USA* **104**, 13780–13785 (2007).
39. Dethlefsen, L., McFall-Ngai M & Relman, D. A. An ecological and evolutionary perspective on human–microbe mutualism and disease. *Nature* **449**, 811–818 (2007).
40. Zoetendal, E. G., Rajilic-Stojanovic, M. & de Vos, W. M. High-throughput diversity and functionality analysis of the gastrointestinal tract microbiota. *Gut* **57**, 1605–1615 (2008).
41. Swidsinski, A., Loening-Baucke, V., Lochs, H. & Hale, L. P. Spatial organization of bacterial flora in normal and inflamed intestine: a fluorescence in situ hybridization study in mice. *World J. Gastroenterol.* **11**, 113–1140 (2005).
42. Wu, G. D. & Lewis, J. D. Analysis of the human gut microbiome and association with disease. *Clin. Gastroenterol. Hepatol.* **11**, 774–777 (2013).
43. Hyland, N. P., Quigley, E. M. & Brint, E. Microbiota–host interactions in irritable bowel syndrome: epithelial barrier, immune regulation and brain-gut interactions. *World J. Gastroenterol.* **20**, 8859–8866 (2014).
44. Lee, K. N. & Lee, O. Y. Intestinal microbiota in pathophysiology and management of irritable bowel syndrome. *World J. Gastroenterol.* **20**, 8886–8897 (2014).
45. Spiller, R. & Garsed, K. Postinfectious irritable bowel syndrome. *Gastroenterology* **136**, 1979–1988 (2009).
46. Thabane, M., Kottachchi, D. T. & Marshall, J. K. Systematic review and meta-analysis: the incidence and prognosis of post-infectious irritable bowel syndrome. *Aliment. Pharmacol. Ther.* **26**, 535–544 (2007).
47. Halvorson, H. A., Schlett, C. D. & Riddle, M. S. Postinfectious irritable bowel syndrome—a meta-analysis. *Am. J. Gastroenterol.* **101**, 1894–1899 (2006).
48. Cremon, C. *et al.* Salmonella gastroenteritis during childhood is a risk factor for irritable bowel syndrome in adulthood. *Gastroenterology* **147**, 69–77 (2014).
49. Pimentel, M., Chow, E. J. & Lin, H. C. Eradication of small intestinal bacterial overgrowth reduces symptoms of irritable bowel syndrome. *Am. J. Gastroenterol.* **95**, 3503–3506 (2000).
50. Posserud, I. *et al.* Small intestinal bacterial overgrowth in patients with irritable bowel syndrome. *Gut* **56**, 802–808 (2007).
51. Walters, B. & Vanner, S. J. Detection of bacterial overgrowth in IBS using the lactulose H2 breath test: comparison with 14C-D-xylose and healthy controls. *Am. J. Gastroenterol.* **100**, 1566–1570 (2005).
52. Vanner, S. The small intestinal bacterial overgrowth. Irritable bowel syndrome hypothesis: implications for treatment. *Gut* **57**, 1315–1321 (2008).
53. Halmos, E. P. *et al.* A diet low in FODMAPs reduces symptoms of irritable bowel syndrome. *Gastroenterology* **146**, 67–75.e5 (2014).
54. Moayyedi, P. *et al.* The efficacy of probiotics in the treatment of irritable bowel syndrome: a systematic review. *Gut* **59**, 325–32 (2010).
55. Schoenfeld, P. *et al.* Safety and tolerability of rifaximin for the treatment of irritable bowel syndrome without constipation: a pooled analysis of randomised, double-blind, placebo-controlled trials. *Aliment. Pharmacol. Ther.* **39**, 1161–1168 (2014).
56. Carroll, I. M. *et al.* Molecular analysis of the luminal- and mucosal-associated intestinal microbiota in diarrhea-predominant irritable bowel syndrome. *Am. J. Physiol. Gastrointest. Liver Physiol.* **301**, G799–G807 (2011).
57. Matto, J. *et al.* Composition and temporal stability of gastrointestinal microbiota in irritable bowel syndrome—a longitudinal study in IBS and control subjects. *FEMS Immunol. Med. Microbiol.* **43**, 213–222 (2005).
58. Maukonen, J. *et al.* Prevalence and temporal stability of selected clostridial groups in irritable bowel syndrome in relation to predominant faecal bacteria. *J. Med. Microbiol.* **55**, 625–633 (2006).
59. Malinen, E. *et al.* Association of symptoms with gastrointestinal microbiota in irritable bowel syndrome. *World J. Gastroenterol.* **16**, 4532–4540 (2010).
60. Malinen, E. *et al.* Analysis of the fecal microbiota of irritable bowel syndrome patients and healthy controls with real-time PCR. *Am. J. Gastroenterol.* **100**, 373–382 (2005).
61. Rajilic-Stojanovic, M. *et al.* Global and deep molecular analysis of microbiota signatures in fecal samples from patients with irritable bowel syndrome. *Gastroenterology* **141**, 1792–1801 (2011).
62. Jeffery, I. B. *et al.* An irritable bowel syndrome subtype defined by species-specific alterations in faecal microbiota. *Gut* **61**, 997–1006 (2012).
63. Carroll, I. M. *et al.* Luminal and mucosal-associated intestinal microbiota in patients with diarrhea-predominant irritable bowel syndrome. *Gut Pathog.* **2**, 19 (2010).
64. Carroll, I. M. *et al.* Alterations in composition and diversity of the intestinal microbiota in patients with diarrhea-predominant irritable bowel syndrome. *Neurogastroenterol. Motil.* **24**, 521–530.e248 (2012).
65. Codling, C. *et al.* A molecular analysis of fecal and mucosal bacterial communities in irritable bowel syndrome. *Dig. Dis. Sci.* **55**, 392–397 (2010).
66. Kassinen, A. *et al.* The fecal microbiota of irritable bowel syndrome patients differs significantly from that of healthy subjects. *Gastroenterology* **133**, 24–33 (2007).
67. Kerckhoffs, A. P. *et al.* Lower Bifidobacteria counts in both duodenal mucosa-associated and fecal microbiota in irritable bowel syndrome patients. *World J. Gastroenterol.* **15**, 2887–2892 (2009).
68. Krogius-Kurikka, L. *et al.* Microbial community analysis reveals high level phylogenetic alterations in the overall gastrointestinal microbiota of diarrhoea-predominant irritable bowel syndrome sufferers. *BMC Gastroenterol.* **9**, 95 (2009).
69. Lyra, A. *et al.* Diarrhoea-predominant irritable bowel syndrome distinguishable by 16S rRNA gene phylotype quantification. *World J. Gastroenterol.* **15**, 5936–5945 (2009).
70. Noor, S. O. *et al.* Ulcerative colitis and irritable bowel patients exhibit distinct abnormalities of the gut microbiota. *BMC Gastroenterol.* **10**, 134 (2010).
71. Saulnier, D. M. *et al.* Gastrointestinal microbiome signatures of pediatric patients with irritable bowel syndrome. *Gastroenterology* **141**, 1782–1791 (2011).
72. Tana, C. *et al.* Altered profiles of intestinal microbiota and organic acids may be the origin of symptoms in irritable bowel syndrome. *Neurogastroenterol. Motil.* **22**, 512–519.e114–115 (2010).
73. Jalanka-Tuovinen, J. *et al.* Faecal microbiota composition and host-microbe cross-talk following gastroenteritis and in postinfectious irritable bowel syndrome. *Gut* **63**, 1737–1745 (2014).
74. Rigsbee, L. *et al.* Quantitative profiling of gut microbiota of children with diarrhea-predominant irritable bowel syndrome. *Am. J. Gastroenterol.* **107**, 1740–1751 (2012).
75. Ponnusamy, K. *et al.* Microbial community and metabolomic comparison of irritable bowel syndrome faeces. *J. Med. Microbiol.* **60**, 817–827 (2011).
76. Carroll, I. M. *et al.* Fecal protease activity is associated with compositional alterations in the intestinal microbiota. *PLoS ONE* **8**, e78017 (2013).
77. Durban, A. *et al.* Instability of the faecal microbiota in diarrhoea-predominant irritable bowel syndrome. *FEMS Microbiol. Ecol.* **86**, 581–589 (2013).
78. Balsari, A. *et al.* The fecal microbial population in the irritable bowel syndrome. *Microbiologica* **5**, 185–194 (1982).
79. Durban, A. *et al.* Structural alterations of faecal and mucosa-associated bacterial communities in irritable bowel syndrome. *Environ. Microbiol. Rep.* **4**, 242–247 (2012).
80. Rinttila, T. *et al.* Real-time PCR analysis of enteric pathogens from fecal samples of irritable bowel syndrome subjects. *Gut Pathog.* **3**, 6 (2011).
81. Si, J. M. *et al.* Intestinal microecology and quality of life in irritable bowel syndrome patients. *World J. Gastroenterol.* **10**, 1802–1805 (2004).
82. Parkes, G. C. *et al.* Distinct microbial populations exist in the mucosa-associated microbiota of sub-groups of irritable bowel syndrome. *Neurogastroenterol. Motil.* **24**, 31–39 (2012).

83. De Filippo, C. *et al.* Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. *Proc. Natl Acad. Sci. USA* **107**, 14691–14696 (2010).
84. Wu, G. D. *et al.* Linking long-term dietary patterns with gut microbial enterotypes. *Science* **334**, 105–108 (2011).
85. Kashyap, P. C. *et al.* Complex interactions among diet, gastrointestinal transit, and gut microbiota in humanized mice. *Gastroenterology* **144**, 967–977 (2013).
86. Halmos, E. P. *et al.* Diets that differ in their FODMAP content alter the colonic luminal microenvironment. *Gut* http://dx.doi.org/10.1136/gutjnl-2014-307264.
87. Lewis, S. & Cochrane, S. Alteration of sulfate and hydrogen metabolism in the human colon by changing intestinal transit rate. *Am. J. Gastroenterol.* **102**, 624–633 (2007).
88. Oufir, L. E. *et al.* Relationships between transit time in man and *in vitro* fermentation of dietary fiber by fecal bacteria. *Eur. J. Clin. Nutr.* **54**, 603–609 (2000).
89. Jeffery, I. B. *et al.* The microbiota link to irritable bowel syndrome: an emerging story. *Gut Microbes* **3**, 572–576 (2012).
90. Bercik, P. *et al.* The intestinal microbiota affect central levels of brain-derived neurotropic factor and behavior in mice. *Gastroenterology* **141**, 599–609.e1–3 (2011).
91. Park, A. J. *et al.* Altered colonic function and microbiota profile in a mouse model of chronic depression. *Neurogastroenterol. Motil.* **25**, 733–e575 (2013).
92. Naseribafrouei, A. *et al.* Correlation between the human fecal microbiota and depression. *Neurogastroenterol. Motil.* **26**, 1155–1162 (2014).
93. Simrén, M. IBS with intestinal microbial dysbiosis: a new and clinically relevant subgroup? *Gut* **63**, 1685–1686 (2014).
94. Parkes, G. C. *et al.* Gastrointestinal microbiota in irritable bowel syndrome: their role in its pathogenesis and treatment. *Am. J. Gastroenterol.* **103**, 1557–1567 (2008).
95. May, C. L. & Kaestner, K. H. Gut endocrine cell development. *Mol. Cell Endocrinol.* **323**, 70–75 (2010).
96. Gunawardene, A. R., Corfe, B. M. & Staton, C. A. Classification and functions of enteroendocrine cells of the lower gastrointestinal tract. *Int. J. Exp. Pathol.* **92**, 219–231 (2011).
97. Kellum, J. M. *et al.* Stroking human jejunal mucosa induces 5-HT release and Cl- secretion via afferent neurons and 5-HT4 receptors. *Am. J. Physiol.* **277**, G515–G520 (1999).
98. Wang, H. *et al.* $CD4^+$ T cell-mediated immunological control of enterochromaffin cell hyperplasia and 5-hydroxytryptamine production in enteric infection. *Gut* **56**, 949–957 (2007).
99. Fukumoto, S. *et al.* Short-chain fatty acids stimulate colonic transit via intraluminal 5-HT release in rats. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **284**, R1269–R1276 (2003).
100. Gershon, M. D. & Tack, J. The serotonin signaling system: from basic understanding to drug development for functional GI disorders. *Gastroenterology* **132**, 397–414 (2007).
101. Fujita, T. *et al.* Effect of MKC-733, a 5-HT receptor partial agonist, on bowel motility and symptoms in subjects with constipation: an exploratory study. *J. Clin. Pharm. Ther.* **30**, 611–622 (2005).
102. Camilleri, M. *et al.* A placebo-controlled trial of prucalopride for severe chronic constipation. *N. Engl. J. Med.* **358**, 2344–2354 (2008).
103. Talley, N. J. *et al.* GR 38032F (ondansetron), a selective 5HT3 receptor antagonist, slows colonic transit in healthy man. *Dig. Dis. Sci.* **35**, 477–480 (1990).
104. Houghton, L. A., Foster, J. M. & Whorwell, P. J. Alosetron, a 5-HT3 receptor antagonist, delays colonic transit in patients with irritable bowel syndrome and healthy volunteers. *Aliment. Pharmacol. Ther.* **14**, 775–782 (2000).
105. Gregory, R. E. & Ettinger, D. S. 5-HT3 receptor antagonists for the prevention of chemotherapy-induced nausea and vomiting. A comparison of their pharmacology and clinical efficacy. *Drugs* **55**, 173–189 (1998).
106. Fuller, R. W. & Wong, D. T. Serotonin uptake and serotonin uptake inhibition. *Ann. N. Y. Acad. Sci.* **600**, 68–78 (1990).
107. Erspamer, V. & Testini, A. Observations on the release and turnover rate of 5 hydroxytryptamine in the gastrointestinal tract. *J. Pharm. Pharmacol.* **11**, 618–623 (1959).
108. Bearcroft, C. P., Perrett D & Farthing, M. J. Postprandial plasma 5-hydroxytryptamine in diarrhoea predominant irritable bowel syndrome: a pilot study. *Gut* **42**, 42–46 (1998).
109. Dunlop, S. P. *et al.* Abnormalities of 5-hydroxytryptamine metabolism in irritable bowel syndrome. *Clin. Gastroenterol. Hepatol.* **3**, 349–357 (2005).
110. Atkinson, W. *et al.* Altered 5-hydroxytryptamine signaling in patients with constipation- and diarrhea-predominant irritable bowel syndrome. *Gastroenterology* **130**, 34–43 (2006).
111. Shekhar, C. *et al.* Rome III functional constipation and irritable bowel syndrome with constipation are similar disorders within a spectrum of sensitization, regulated by serotonin. *Gastroenterology* **145**, 749–757 (2013).
112. Miwa, J. *et al.* Patients with constipation-predominant irritable bowel syndrome (IBS) may have elevated serotonin concentrations in colonic mucosa as compared with diarrhea-predominant patients and subjects with normal bowel habits. *Digestion* **63**, 188–194 (2001).
113. Kerckhoffs, A. P. *et al.* Trypsinogen IV, serotonin transporter transcript levels and serotonin content are increased in small intestine of irritable bowel syndrome patients. *Neurogastroenterol. Motil.* **20**, 900–907 (2008).
114. Costedio, M. M. *et al.* Mucosal serotonin signaling is altered in chronic constipation but not in opiate-induced constipation. *Am. J. Gastroenterol.* **105**, 1173–1180 (2010).
115. Dunlop, S. P. *et al.* Relative importance of enterochromaffin cell hyperplasia, anxiety, and depression in postinfectious IBS. *Gastroenterology* **125**, 1651–1659 (2003).
116. Coates, M. D. *et al.* Molecular defects in mucosal serotonin content and decreased serotonin reuptake transporter in ulcerative colitis and irritable bowel syndrome. *Gastroenterology* **126**, 1657–1664 (2004).
117. Faure, C. *et al.* Serotonin signaling is altered in irritable bowel syndrome with diarrhea but not in functional dyspepsia in pediatric age patients. *Gastroenterology* **139**, 249–258 (2010).
118. El-Salhy, M. *et al.* Chromogranin A cell density in the rectum of patients with irritable bowel syndrome. *Mol. Med. Report* **6**, 1223–1225 (2012).
119. El-Salhy, M., Lomholt-Beck, B. & Hausken, T. Chromogranin A as a possible tool in the diagnosis of irritable bowel syndrome. *Scand. J. Gastroenterol.* **45**, 1435–1439 (2010).
120. El-Salhy, M., Wendelbo, I. H. & Gundersen, D. Reduced chromogranin A cell density in the ileum of patients with irritable bowel syndrome. *Mol. Med. Report* **7**, 1241–1244 (2013).
121. Öhman, L. *et al.* Altered levels of fecal chromogranins and secretogranins in IBS: relevance for pathophysiology and symptoms? *Am. J. Gastroenterol.* **107**, 440–447 (2012).
122. El-Salhy, M. *et al.* Irritable bowel syndrome: the role of gut neuroendocrine peptides. *Front. Biosci. (Elite Ed.)* **4**, 2783–2800 (2012).
123. Lyte, M. Microbial endocrinology and infectious disease in the 21st century. *Trends Microbiol.* **12**, 14–20 (2004).
124. Freestone, P. Communication between bacteria and their hosts. *Scientifica (Cairo)* **2013**, 361073 (2013).
125. Coleman, N. S. *et al.* Abnormalities of serotonin metabolism and their relation to symptoms in untreated celiac disease. *Clin. Gastroenterol. Hepatol.* **4**, 874–881 (2006).
126. Foley, S. *et al.* Impaired uptake of serotonin by platelets from patients with irritable bowel syndrome correlates with duodenal immune activation. *Gastroenterology* **140**, 1434–1443.e1 (2011).
127. Linden, D. R. *et al.* Serotonin availability is increased in mucosa of guinea pigs with TNBS-induced colitis. *Am. J. Physiol. Gastrointest. Liver Physiol.* **285**, G207–G216 (2003).
128. Linden, D. R. *et al.* Serotonin transporter function and expression are reduced in mice with TNBS-induced colitis. *Neurogastroenterol. Motil.* **17**, 565–574 (2005).
129. Ghia, J. E. *et al.* Serotonin has a key role in pathogenesis of experimental colitis. *Gastroenterology* **137**, 1649–1660 (2009).
130. Haub, S. *et al.* Enhancement of intestinal inflammation in mice lacking interleukin 10 by deletion of the serotonin reuptake transporter. *Neurogastroenterol. Motil.* **22**, 826–834.e229 (2010).
131. Feistritzer, C. *et al.* Effects of the neuropeptide secretoneurin on natural killer cell migration and cytokine release. *Regul. Pept.* **126**, 195–201 (2005).
132. Shooshtarizadeh, P. *et al.* The antimicrobial peptides derived from chromogranin/secretogranin family, new actors of innate immunity. *Regul. Pept.* **165**, 102–110 (2010).
133. Zhang, D. *et al.* Two chromogranin a-derived peptides induce calcium entry in human neutrophils by calmodulin-regulated calcium independent phospholipase A2. *PLoS ONE* **4**, e4501 (2009).
134. Rhee, S. H., Pothoulakis, C. & Mayer, E. A. Principles and clinical implications of the brain-gut-enteric microbiota axis. *Nat. Rev. Gastroenterol. Hepatol.* **6**, 306–214 (2009).
135. Johansson, M. E., Sjovall, H. & Hansson, G. C. The gastrointestinal mucus system in health and disease. *Nat. Rev. Gastroenterol. Hepatol.* **10**, 352–361 (2013).
136. Scaldaferri, F. *et al.* The gut barrier: new acquisitions and therapeutic approaches. *J. Clin. Gastroenterol.* **46** (Suppl.), S12–S17 (2012).
137. Camilleri, M. *et al.* Intestinal barrier function in health and gastrointestinal disease. *Neurogastroenterol. Motil.* **24**, 503–512 (2012).
138. Rao, A. S. *et al.* Urine sugars for *in vivo* gut permeability: validation and comparisons in irritable bowel syndrome-diarrhea and controls. *Am. J. Physiol. Gastrointest. Liver Physiol.* **301**, G919–G928 (2011).
139. Camilleri, M. *et al.* Understanding measurements of intestinal permeability in healthy humans with urine lactulose and mannitol excretion. *Neurogastroenterol. Motil.* **22**, e15–e26 (2010).
140. Zuckerman, M. J. *et al.* Intestinal permeability to [51Cr]EDTA in infectious diarrhea. *Dig. Dis. Sci.* **38**, 1651–1657 (1993).
141. Spiller, R. C. *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* **47**, 804–811 (2000).
142. Marshall, J. K. *et al.* Intestinal permeability in patients with irritable bowel syndrome after a waterborne outbreak of acute gastroenteritis in Walkerton, Ontario. *Aliment. Pharmacol. Ther.* **20**, 1317–1322 (2004).
143. Piche, T. *et al.* Impaired intestinal barrier integrity in the colon of patients with irritable bowel syndrome: involvement of soluble mediators. *Gut* **58**, 196–201 (2009).
144. Dunlop, S. P. *et al.* Abnormal intestinal permeability in subgroups of diarrhea-predominant irritable bowel syndromes. *Am. J. Gastroenterol.* **101**, 1288–1294 (2006).
145. Bertiaux-Vandaele, N. *et al.* The expression and the cellular distribution of the tight junction proteins are altered in irritable bowel syndrome patients with differences according to the disease subtype. *Am. J. Gastroenterol.* **106**, 2165–2173 (2011).
146. Martinez, C. *et al.* Diarrhoea-predominant irritable bowel syndrome: an organic disorder with structural abnormalities in the jejunal epithelial barrier. *Gut* **62**, 1160–1168 (2013).
147. Wilcz-Villega, E., McClean S. & O'Sullivan, M. Reduced E-cadherin expression is associated with abdominal pain and symptom duration in a study of alternating and diarrhea predominant IBS. *Neurogastroenterol. Motil.* **26**, 316–325 (2014).
148. Gustafsson, J. K., Hansson, G. C. & Sjovall, H. Ulcerative colitis patients in remission have an altered secretory capacity in the proximal colon despite macroscopically normal mucosa. *Neurogastroenterol. Motil.* **24**, e381–e391 (2012).
149. Vivinus-Nebot, M. *et al.* Functional bowel symptoms in quiescent inflammatory bowel diseases: role of epithelial barrier disruption and low-grade inflammation. *Gut* **63**, 744–752 (2014).
150. Zhou, Q., Zhang, B. & Verne, G. N. Intestinal membrane permeability and hypersensitivity in the irritable bowel syndrome. *Pain* **146**, 41–46 (2009).
151. Annahazi, A. *et al.* Luminal cysteine-proteases degrade colonic tight junction structure and are responsible for abdominal pain in constipation-predominant IBS. *Am. J. Gastroenterol.* **108**, 1322–1331 (2013).
152. Gecse, K. *et al.* Increased faecal serine protease activity in diarrhoeic IBS patients: a colonic lumenal factor impairing colonic permeability and sensitivity. *Gut* **57**, 591–599 (2008).
153. Vivinus-Nebot, M. *et al.* Combination of allergic factors can worsen diarrheic irritable bowel syndrome: role of barrier defects and mast cells. *Am. J. Gastroenterol.* **107**, 75–81 (2012).
154. Chandrasekharan, B. *et al.* Tumor necrosis factor-neuropeptide Y cross talk regulates inflammation, epithelial barrier functions, and colonic motility. *Inflamm. Bowel Dis.* **19**, 2535–2546 (2013).
155. Hu, Y. J. *et al.* Regulation of paracellular permeability: factors and mechanisms. *Mol. Biol. Rep.* **40**, 6123–6142 (2013).
156. Lee, H. *et al.* Mucosal mast cell count is associated with intestinal permeability in patients with diarrhea predominant irritable bowel syndrome. *J. Neurogastroenterol. Motil.* **19**, 244–250 (2013).
157. Wilcz-Villega, E. M., McClean, S. & O'Sullivan, M. A. 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.* **108**, 1140–1151 (2013).
158. Martinez, C. *et al.* The jejunum of diarrhea-predominant irritable bowel syndrome shows molecular alterations in the tight junction signaling pathway that are associated with mucosal pathobiology and clinical manifestations. *Am. J. Gastroenterol.* **107**, 736–746 (2012).
159. Overman, E. L., Rivier, J. E. & Moeser, A. J. CRF induces intestinal epithelial barrier injury via the release of mast cell proteases and TNF-α. *PLoS ONE* **7**, e39935 (2012).
160. Wallon, C. *et al.* Corticotropin-releasing hormone (CRH) regulates macromolecular permeability via mast cells in normal human colonic biopsies *in vitro*. *Gut* **57**, 50–58 (2008).
161. Vanuytsel, T. *et al.* Psychological stress and corticotropin-releasing hormone increase intestinal permeability in humans by a mast cell-dependent mechanism. *Gut* **63**, 1293–1299 (2014).
162. Keita, A. V. *et al.* Vasoactive intestinal polypeptide regulates barrier function via mast cells in human intestinal follicle-associated epithelium and during stress in rats. *Neurogastroenterol. Motil.* **25**, e406–e417 (2013).
163. Villani, A. C. *et al.* Genetic risk factors for post-infectious irritable bowel syndrome following a waterborne outbreak of gastroenteritis. *Gastroenterology* **138**, 1502–1513 (2010).
164. Vazquez-Roque, M. I. *et al.* Association of HLA-DQ gene with bowel transit, barrier function, and inflammation in irritable bowel syndrome with diarrhea. *Am. J. Physiol. Gastrointest. Liver Physiol.* **303**, G1262–G1269 (2012).
165. Barbara, G. *et al.* Activated mast cells in proximity to colonic nerves correlate with abdominal pain in irritable bowel syndrome. *Gastroenterology* **126**, 693–702 (2004).
166. Park, C. H. *et al.* Activated mast cells infiltrate in close proximity to enteric nerves in diarrhea-predominant irritable bowel syndrome. *J. Korean Med. Sci.* **18**, 204–210 (2003).
167. Öhman, L & Simren, M. Pathogenesis of IBS: role of inflammation, immunity and neuroimmune interactions. *Nat. Rev. Gastroenterol. Hepatol.* **7**, 163–173 (2010).
168. Ohman, L. *et al.* T-cell activation in patients with irritable bowel syndrome. *Am. J. Gastroenterol.* **104**, 1205–1212 (2009).
169. Öhman, L. *et al.* A controlled study of colonic immune activity and $\beta7^+$ blood T lymphocytes in patients with irritable bowel syndrome. *Clin. Gastroenterol. Hepatol.* **3**, 980–986 (2005).
170. Brint, E. K. *et al.* Differential expression of toll-like receptors in patients with irritable bowel syndrome. *Am. J. Gastroenterol.* **106**, 329–336 (2011).
171. Belmonte, L. *et al.* Role of toll like receptors in irritable bowel syndrome: differential mucosal immune activation according to the disease subtype. *PLoS ONE* **7**, e42777 (2012).
172. Öhman, L. *et al.* Increased TLR2 expression on blood monocytes in irritable bowel syndrome patients. *Eur. J. Gastroenterol. Hepatol.* **24**, 398–405 (2012).
173. Scully, P. *et al.* Plasma cytokine profiles in females with irritable bowel syndrome and extra-intestinal co-morbidity. *Am. J. Gastroenterol.* **105**, 2235–2243 (2010).
174. Chang, L. *et al.* Serum and colonic mucosal immune markers in irritable bowel syndrome. *Am. J. Gastroenterol.* **107**, 262–272 (2012).
175. Dinan, T. G. *et al.* Enhanced cholinergic-mediated increase in the pro-inflammatory cytokine IL-6 in irritable bowel syndrome: role of muscarinic receptors. *Am. J. Gastroenterol.* **103**, 2570–2576 (2008).
176. Dinan, T. G. *et al.* Hypothalamic–pituitary–gut axis dysregulation in irritable bowel syndrome: plasma cytokines as a potential biomarker? *Gastroenterology* **130**, 304–311 (2006).
177. Kindt, S. *et al. Immune* dysfunction in patients with functional gastrointestinal disorders. *Neurogastroenterol. Motil.* **21**, 389–398 (2009).
178. Bashashati, M. *et al.* Cytokine imbalance in irritable bowel syndrome: a systematic review and meta-analysis. *Neurogastroenterol. Motil.* **26**, 1036–1048 (2014).
179. Liebregts, T. *et al.* Immune activation in patients with irritable bowel syndrome. *Gastroenterology* **132**, 913–920 (2007).
180. Gwee, K. A. *et al.* Increased rectal mucosal expression of interleukin 1beta in recently acquired post-infectious irritable bowel syndrome. *Gut* **52**, 523–526 (2003).
181. O'Malley, D., Dinan, T. G. & Cryan, J. F. Interleukin-6 modulates colonic transepithelial ion transport in the stress-sensitive wistar kyoto rat. *Front. Pharmacol.* **3**, 190 (2012).
182. Olofsson, P. S. *et al.* Rethinking inflammation: neural circuits in the regulation of immunity. *Immunol. Rev.* **248**, 188–204 (2012).
183. Anitha, M. *et al.* Gut microbial products regulate murine gastrointestinal motility via Toll-like receptor 4 signaling. *Gastroenterology* **143**, 1006–1016.e4 (2012).
184. Muller, P. A. *et al.* Crosstalk between muscularis macrophages and enteric neurons regulates gastrointestinal motility. *Cell* **158**, 300–313 (2014).
185. Robinette, M. L. & Colonna, M. GI motility: microbiota and macrophages join forces. *Cell* **158**, 239–240 (2014).
186. Tornblom, H. *et al.* Full-thickness biopsy of the jejunum reveals inflammation and enteric neuropathy in irritable bowel syndrome. *Gastroenterology* **123**, 1972–1979 (2002).
187. Chadwick, V. S. *et al.* Activation of the mucosal immune system in irritable bowel syndrome. *Gastroenterology* **122**, 1778–1783 (2002).
188. Cremon, C. *et al.* Mucosal immune activation in irritable bowel syndrome: gender-dependence and association with digestive symptoms. *Am. J. Gastroenterol.* **104**, 392–400 (2009).
189. Sundin, J. *et al.* Aberrant mucosal lymphocyte number and subsets in the colon of post-infectious irritable bowel syndrome patients. *Scand. J. Gastroenterol.* **49**, 1068–1075 (2014).
190. Chen, J., Zhang, Y. & Deng, Z. Imbalanced shift of cytokine expression between T helper 1 and T helper 2 (Th1/Th2) in intestinal mucosa of patients with post-infectious irritable bowel syndrome. *BMC Gastroenterol.* **12**, 91 (2012).
191. Holmen, N. *et al.* $CD4^+CD25^+$ regulatory T cells in irritable bowel syndrome patients. *Neurogastroenterol. Motil.* **19**, 119–125 (2007).
192. Ohman, L. *et al.* B-cell activation in patients with irritable bowel syndrome (IBS). *Neurogastroenterol. Motil.* **21**, 644–650.e27 (2009).
193. Vicario, M. *et al.* Increased humoral immunity in the jejunum of diarrhoea-predominant irritable bowel syndrome associated with clinical manifestations. *Gut* http://dx.doi.org/10.1136/gutjnl-2013-306236.
194. Schoepfer, A. M. *et al.* Antibodies to flagellin indicate reactivity to bacterial antigens in IBS patients. *Neurogastroenterol. Motil.* **20**, 1110–1118 (2008).
195. Akiho, H. *et al.* Involvement of interleukin-17A-induced hypercontractility of intestinal smooth muscle cells in persistent gut motor dysfunction. *PLoS ONE* **9**, e92960 (2014).

196. Akiho, H., Ihara, E., Motomura, Y. & Nakamura, K. Cytokine-induced alterations of gastrointestinal motility in gastrointestinal disorders. *World J. Gastrointest. Pathophysiol.* **2**, 72–81 (2011).
197. Bashashati, M. *et al.* Cytokine gene polymorphisms are associated with irritable bowel syndrome: a systematic review and meta-analysis. *Neurogastroenterol. Motil.* **24**, 1102–e566 (2012).
198. van der Veek, P. P. *et al.* Role of tumor necrosis factor-alpha and interleukin-10 gene polymorphisms in irritable bowel syndrome. *Am. J. Gastroenterol.* **100**, 2510–2516 (2005).
199. Romero-Valdovinos, M. *et al.* Interleukin-8 and -10 gene polymorphisms in irritable bowel syndrome. *Mol. Biol. Rep.* **39**, 8837–8843 (2012).
200. Swan, C. *et al.* Identifying and testing candidate genetic polymorphisms in the irritable bowel syndrome (IBS): association with *TNFSF15* and TNFα. *Gut* **63**, 985–994 (2013).
201. Zucchelli, M. *et al.* Association of *TNFSF15* polymorphism with irritable bowel syndrome. *Gut* **60**, 1671–1677 (2011).
202. Camilleri, M. *et al.* RNA sequencing shows transcriptomic changes in rectosigmoid mucosa in patients with irritable bowel syndrome-diarrhea: a pilot case–control study. *Am. J. Physiol. Gastrointest. Liver Physiol.* **306**, G1089–G1098 (2014).
203. Akbar, A. *et al.* Increased capsaicin receptor TRPV1-expressing sensory fibres in irritable bowel syndrome and their correlation with abdominal pain. *Gut* **57**, 923–929 (2008).
204. Wang, L. H., Fang, X. C. & Pan, G. Z. Bacillary dysentery as a causative factor of irritable bowel syndrome and its pathogenesis. *Gut* **53**, 1096–1101 (2004).
205. Di Nardo, G. *et al.* Neuroimmune interactions at different intestinal sites are related to abdominal pain symptoms in children with IBS. *Neurogastroenterol. Motil.* **26**, 196–204 (2014).
206. Nasser, Y. *et al.* Using human intestinal biopsies to study the pathogenesis of irritable bowel syndrome. *Neurogastroenterol. Motil.* **26**, 455–469 (2014).
207. Barbara, G. *et al.* Mast cell-dependent excitation of visceral-nociceptive sensory neurons in irritable bowel syndrome. *Gastroenterology* **132**, 26–37 (2007).
208. Buhner, S. *et al.* Activation of human enteric neurons by supernatants of colonic biopsy specimens from patients with irritable bowel syndrome. *Gastroenterology* **137**, 1425–1434 (2009).
209. Balestra, B. *et al.* Colonic mucosal mediators from patients with irritable bowel syndrome excite enteric cholinergic motor neurons. *Neurogastroenterol. Motil.* **24**, 1118–e570 (2012).
210. Cenac, N. *et al.* Role for protease activity in visceral pain in irritable bowel syndrome. *J. Clin. Invest.* **117**, 636–647 (2007).
211. Cremon, C. *et al.* Intestinal serotonin release, sensory neuron activation, and abdominal pain in irritable bowel syndrome. *Am. J. Gastroenterol.* **106**, 1290–1298 (2011).
212. Buhner, S. *et al.* Submucous rather than myenteric neurons are activated by mucosal biopsy supernatants from irritable bowel syndrome patients. *Neurogastroenterol. Motil.* **24**, 1134–e572 (2012).
213. Valdez-Morales, E. E. *et al.* Sensitization of peripheral sensory nerves by mediators from colonic biopsies of diarrhea-predominant irritable bowel syndrome patients: a role for PAR2. *Am. J. Gastroenterol.* **108**, 1634–1643 (2013).
214. Hughes, P. A. *et al.* Sensory neuro-immune interactions differ between irritable bowel syndrome subtypes. *Gut* **62**, 1456–1465 (2013).

Author contributions

All authors contributed equally to all aspects of this manuscript.