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Current insights into the innate immune system dysfunction in irritable bowel syndrome

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Abstract

Irritable bowel syndrome (IBS) is a functional bowel disorder associated with abdominal pain and alterations in bowel habits. The presence of IBS greatly impairs patients' quality of life and imposes a high economic burden on the community; thus, there is intense pressure to reveal its elusive pathogenesis. Many etiological mechanisms have been implicated, but the pathophysiology of the syndrome remains unclear. As a result, novel drug development has been slow and no pharmacological intervention is universally accepted. A growing evidence implicates the role of low-grade inflammation and innate immune system dysfunction, although contradictory results have frequently been presented. Mast cells (MC), eosinophils and other key immune cells together with their mediators seem to play an important role, at least in subgroups of IBS patients. Cytokine imbalance in the systematic circulation and in the intestinal mucosa may also characterize IBS presentation. Toll-like receptors and their emerging role in pathogen recognition have also been highlighted recently, as dysregulation has been reported to occur in patients with IBS. This review summarizes the current knowledge regarding the involvement of any immunological alteration in the development of IBS. There is substantial evidence to support innate immune system dysfunction in several IBS phenotypes, but additional studies are required to better clarify the underlying pathogenetic pathways. IBS heterogeneity could potentially be attributed to multiple causes that lead to different disease phenotypes, thus explaining the variability found between study results.

Keywords Irritable bowel syndrome, low-grade inflammation, innate immunity, cytokines, toll-like receptors

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Introduction

Irritable bowel syndrome (IBS) is a common functional gastrointestinal disease characterized by recurrent abdominal pain that is associated with defecation or an alteration in bowel habits. IBS can be classified into four subtypes based on the predominant stool pattern (IBS with diarrhea [IBS-D], IBS with constipation [IBS-C], IBS with mixed bowel habits [IBS-M], and unclassified IBS) [1].

IBS is a very common diagnosis worldwide, with a calculated prevalence of 10-15%, although there are variations depending on the country and the criteria used to define IBS [2-4]. There is a slight predominance of female patients and the onset of symptoms usually occurs at a young age [3]. Over the last years, recognition of IBS has improved, together with the increasing credibility of the diagnostic criteria developed by the Rome consensus [5,6]; however, clinical experience is still broadly exercised by physicians [7].

The socioeconomic impact of IBS is large across continents. Annual direct medical costs in the USA alone vary from 2-10 billion US dollars, while estimates of indirect medical costs, as patients are frequently absent from work, are even greater [8,9]. Therapeutic interventions are still ineffective and unable to relieve the burden on patients' quality of life [10].

The pathophysiology of the syndrome is poorly understood, while a number of possible mechanisms, such as visceral hypersensitivity, abnormal gut motility, and irregular brain-gut interaction, are implicated in its development (Fig. 1) [11]. Chronic low-grade intestinal mucosal inflammation is another of these potential etiological factors. A number of studies have documented alterations in MC, T lymphocytes, B lymphocytes, and cytokine concentrations in the colonic mucosa or the systemic circulation in IBS patients compared with healthy

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Conflict of Interest: None

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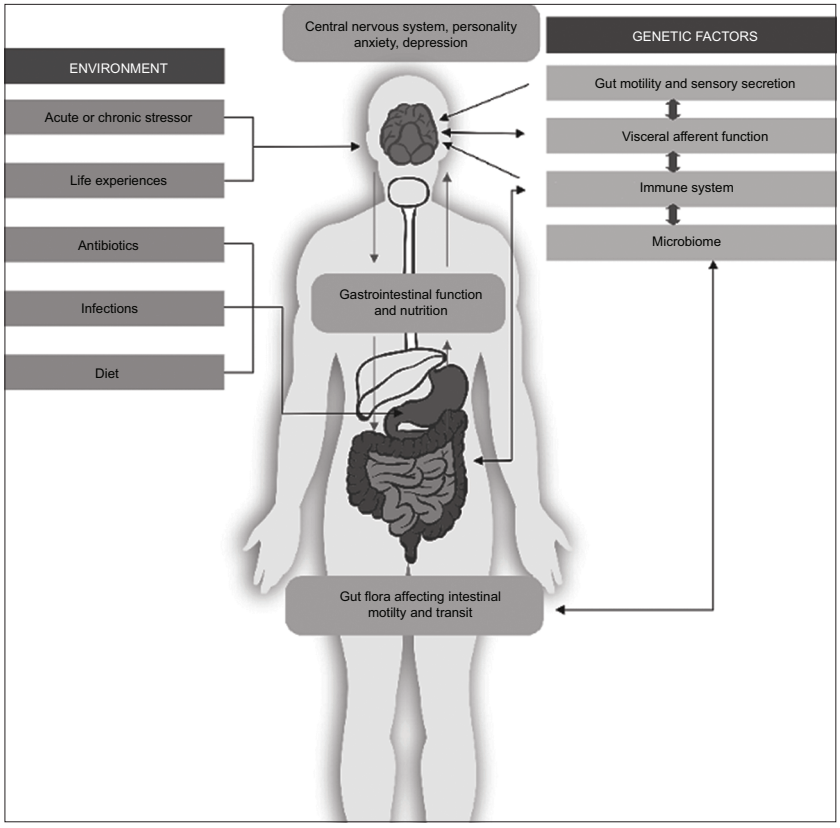

Figure 1 Schematic illustration of the putative factors involved in the pathogenesis of irritable bowel syndrome (IBS). Multiple mechanisms interact in the development of IBS symptoms in each individual. Both environmental parameters and genes could potentially contribute to the manifestation of IBS

controls [12]. This review summarizes the current knowledge regarding the role of immune activation in the pathogenesis and the development of IBS.

Low-grade inflammation

IBS was regarded as an exclusively functional disorder, but recent studies have demonstrated evidence of low-grade mucosal inflammation in some IBS patients. Mechanisms associated with post-infectious alterations [13], dysfunctional epithelial permeability [14], abnormalities in gut microbiota [15], and elevated stress levels [16] can enhance or stimulate abnormal immune responses. A combination of low-grade inflammation of the intestinal mucosa with impaired bowel motility and visceral sensitivity could explain the underlying etiology and pathology of IBS.

The hypothesis of the importance of immune dysfunction in the pathogenesis of IBS was initially based on the observation that IBS symptoms developed following episodes of infectious gastroenteritis elicited by bacteria, viruses or parasites. This syndrome was defined as post-infectious IBS (PI-IBS) [17]. Several studies verified the association of infectious enteritis and the risk of developing IBS, and a meta-analysis reported an estimated sevenfold increase compared with the controls groups [18]. Researchers reported that female sex and young age, anxiety, depression and a protracted period of the initial infection with fever, are risk factors for the development of PI-IBS [13,19]. Moreover, dysbiotic microbiota caused by an infectious gastroenteritis could promote inflammation and alter the innate immune reaction in PI-IBS patients [20]. Intestinal mucosal biopsies from patients with PI-IBS revealed elevated numbers of immunity cells, such as lymphocytes and MC [21-23]. Notably, patients with PI-IBS also have increased cytokine production compared with healthy controls or compared with patients who have fully recovered from infectious enteritis [24,25].

Additionally, IBS and inflammatory bowel disease (IBD) studies have revealed overlapping pathways between the two diseases, such as dysregulated immune activation and abnormalities of the enteric nerve function [26,27]. IBD patients in clinical remission report IBS-like symptoms combined with impaired quality of life and psychological symptoms [28]. Likewise, an increased number of patients with celiac disease and microscopic colitis develop symptoms resembling those of IBS compared with controls [29,30]. Furthermore, data suggest that IBS-like symptoms precede the diagnosis of IBD [31]. The overlap of symptoms in IBS and IBD could thus be explained by similarities in their etiology

and the underlying mechanisms. Specifically, the presence of low-grade mucosal inflammation and mild immune activation in IBS patients may account for the common clinical features also present in patients with IBD.

Molecular genetics studies have indicated a positive association between IBS pathophysiology and genetic factors, although these results were not always replicated [32]. Numerous gene variants related to serotonin metabolism, intestinal barrier and visceral perception were examined. Furthermore, single nucleotide polymorphisms (SNPs) in the *TNFSF15* gene were investigated regarding their potential contribution to immune modulation, low-grade inflammation and cytokine production [33]. Nevertheless, one meta-analysis identified only a moderate association between rs4263839 *TNFSF15* and IBS-C patients, while none of the other 16 SNPs studied showed any relevance with IBS symptom phenotypes [34].

Emerging data from experimental studies in IBS pathogenesis are inconclusive, which probably reflects the heterogeneity of the disorder and the differences between the syndrome subgroups. Yet, preliminary results provide evidence for a link between gut microbiota and host immune response in the development of IBS.

Innate immune activation

Low-grade inflammation in IBS patients can be assessed by alterations in the immune cell populations and mediators. The role of MC, monocytes, neutrophils, natural killer (NK) cells and eosinophils has been evaluated. Cytokine production and activity has been investigated in numerous studies, along with other proinflammatory mediators. Earlier studies mainly focused on systemic immune activation through the role of immune cells and their mediators in serum and plasma of IBS patients. More recent studies have also examined mucosal samples as a more accurate indicator of immune activity and have identified mucosal infiltration of immunocytes and elevated proinflammatory cytokines levels in IBS patients [35,36]. Results from biopsy samples might provide a better understanding of the relevant mechanisms in IBS pathophysiology and help identify potential disease indicators [37].

The potential relationship between innate immune dysregulation in IBS patients and perceived symptoms has also been explored [38]. In a recent study, tumor necrosis factor (TNF)-α and interleukin (IL)-17 serum levels were correlated with discomfort and severity of symptoms in IBS patients [39]. Nevertheless, measurement of cytokine levels in the serum and intestinal mucosa in another study with 144 IBS patients and 42 healthy controls provided no correlation between the overall symptom severity and the cytokine expression, although IL-6 and IL-8 levels were slightly increased in the IBS group [40]. Several studies imply this imperceptible link between low-grade immune activity and IBS symptoms [41-43], raising the hope of future therapeutic possibilities for IBS patients. So far, anti-inflammatory therapies have proven unsuccessful in IBS patients, but better selected subgroups might indeed benefit from these type of treatments [44,45].

MC and their mediators

MC are long-lived granulated cells that circulate in the blood and are also found in tissues; they can release tryptase, histamine and chymase as a result of their activation [46,47]. The involvement of MC in the pathogenesis of IBS is attributed to their mediators, which can alter enteric nerve and motor function [48]. MC counts and density vary among different studies and among different segments of the intestine, though the majority of studies report greater numbers and volume in IBS patients compared with healthy controls (Table 1) [23,41,49-65].

Indications for the role of MC in IBS pathogenesis were firstly considered when elevated number of MC were reported in mucosal biopsies of the terminal ileum and then confirmed in several studies, mainly in patients with IBS-D or PI-IBS [23,58,60,61]. Interestingly, one study with 50 predominantly female IBS patients (Rome III criteria) reported no differences in MC numbers between controls and all IBS patients, independently of bowel habit subtype [50]. Furthermore, a recent assessment of 66 Rome II IBS patients and 20 controls found that the former group had lower numbers of MC in biopsies of the descending colon [52]. In addition, a Spanish group found similar MC numbers in jejunal biopsies in 49 Rome III IBS-D patients compared with controls. Nevertheless, the MC density was higher in IBS patients and the proximity of MC to plasma cells was significantly lower in the jejunal mucosa [62].

Conflicting data about MC counts could be of little importance, because the increased presence of MC is not a prerequisite for inflammation. The activation and degranulation of MC appear to correlate with the presentation of IBS. In addition, the proximity of MC to intestinal nerves is considered to be directly associated with the presentation and severity of IBS symptoms [55,66], although not all studies have confirmed the proximity of MC and nerve fibers [67]. MC activation has been associated with structural changes and thus dysfunction in the apical junctional complex integrity in jejunal mucosa, leading to motor and intestinal barrier dysfunction in IBS-D patients [49,64]. Histamine and tryptase as MC mediators can alter nociceptive visceral sensory nerve function and cause stimulation and hypersensitivity [68,69]. Furthermore tryptase may downregulate the expression of junctional adhesion molecule-A *in vitro* and, therefore, increase epithelial permeability in IBS [70]. Augmented secretion of tryptase and histamine has been documented in duodenal, colon and rectum biopsies in some IBS studies [54,63,68,71-73]. However, rectal biopsies from 29 IBS patients and 22 healthy controls revealed no difference in tryptase release, while MC numbers were smaller in IBS patients. In the same study, histamine release in all patients with IBS was significantly increased compared with healthy volunteers. Administration of ketotifen, an MC stabilizer, did not affect the release of

Table 1 Mast cell counts in studies with at least 50 participants (IBS and controls)

Author	Number of participants (IBS: controls)	Diagnostic criteria	Site of biopsy	Results	Subtype	Patients' sex
Chadwick 2002 [41]	77: 28	Rome I	Ascending, transverse, descending colon and rectum	Increased numbers	35 IBS-D 10 IBS-C 20 IBS-M 4 IBS-U	53 female 16 male
Dunlop 2003 [145]	28:34	Rome I	Rectum	No difference in numbers	28 PI-IBS	17 female 11 male
Dunlop 2003 [146]	75:36	Rome II	Rectum	Increased numbers in non PI-IBS (a trend to be elevated P=0.054)	23 PI-IBS 52 non PI-IBS	53 female 22 male
Wang 2004 [57]	56:12	Rome II	Terminal ileum and rectosigmoid junction	Increased numbers only at the terminal ileum	56 IBS-D	31 female 25 male
Barbara 2004 [55]	44:22	Rome II	Descending colon	Increased numbers Increased activation (degranulating mast cells)	22 IBS-D 22 IBS-C	31 female 13 male
Wang 2007 [56]	38:20	Rome III	Terminal ileum, duodenum and jejunum	Increased numbers only at the terminal ileum	20 IBS-D 18 IBS-C	21 female 17 male
Piche 2008 [79]	50:21	Rome II	Cecum	Increased numbers	21 IBS-D 29 IBS-C	41 female 9 male
Cremon 2009 [51]	48:24	Rome II	Descending colon	Increased numbers	27 IBS-D 21 IBS-C	35 female 13 male
Walker 2009 [77]	41:48	Sweden questionnaire similar to Rome I	Duodenum	Numerically increased cell counts	20 IBS-D 21 IBS-C	20 female 21 male
Goral 2010 [60]	72:50	Rome III	Cecum and rectum	Increased numbers	40 IBS-D 32 IBS-C	
Bhuiyan 2010 [147]	50:10	---	Sigmoid colon	Increased numbers	18 PI-IBS 32 non PI-IBS	
Chang 2012 [65]	45:41	Rome II	Sigmoid colon	No difference in numbers	16 IBS-C 15 IBS-D 14 IBS-M	26 female 19 male
Braak 2012 [52]	66:20	Rome II	Ascending and descending colon	Decreased numbers	15 IBS-D 15 IBS-C 36 IBS-A	49 female 17 male
De Silva 2012 [61]	49:14	Rome III	Ileum, cecum, ascending, transverse, descending colon and rectum	Increased numbers in all sites apart from transverse colon	49 IBS-D	13 female 36 male
Martinez 2013 [49]	45:30	Rome II	Jejunum	Increased numbers and activation	45 IBS-D	34 female 11 male
El-Salhy 2013 [50]	50:27	Rome III	Colon and rectum	No difference in numbers nor density	30 IBS-D 20 IBS-C	42 female 8 male
Vicario 2015 [62]	49:30	Rome III	Jejunum	No difference in numbers Increased density	49 IBS-D	33 female 16 male

IBS, irritable bowel syndrome; IBS-C, constipation predominant; IBS-D, diarrhea predominant; IBS-M, mixed; IBS-U, unsubtyped; IBS-A, with alternating stool pattern; PI-IBS, post-infectious

either histamine or tryptase, but effectively ameliorated visceral hypersensitivity and IBS symptoms [72]. Contrariwise, ebastine, an antagonist of histamine receptor H1, improved both visceral hypersensitivity and symptom discomfort in a proof-of-principle pilot study with 55 Rome III IBS patients, compared with placebo [69].

Eosinophils, basophils

Eosinophils are potent immune cells found in the intestinal mucosa; they participate in host immunity and maintain homeostasis of the intestinal barrier. The exact etiology of eosinophil migration from the blood to the mucosa is unclear, but seems to be associated with inflammation [74,75]. A recent study found increased eosinophil counts in 23 of 42 IBS Rome II patients in colonic biopsies [76], while the number of eosinophil cells was significantly higher in the cecum and in the right colon of 49 IBS-D patients from Sri Lanka [61]. However, other researchers found no differences in eosinophils counts in the duodenum [77], jejunum [59,64], ileum [78], cecum [78-80], ascending and descending colon, and rectum [78,80]. Additionally, no alterations in eosinophil numbers in rectal biopsies were observed in children with IBS [67]. Eosinophils can secrete cytotoxic granule cationic proteins implicated in tissue damage [74]. Studies in IBS patients revealed no difference in eosinophil cationic protein (ECP) [81] or eosinophil protein X (EPX) [82] compared with controls. Measurements of fecal EPX also presented inconclusive results [83,84], while positive skin prick tests and high blood immunoglobulin E and ECP values were more common in IBS patients [85].

The role of basophils is scarcely investigated in IBS patients. A recent study of non-celiac wheat sensitivity utilized IBS Rome II patients as the control group and assessed *in vitro* basophil activation with a flow cytometric test. Basophil activation was less pronounced in the blood samples of IBS patients [86,87]. Likewise, a Turkish study documented no difference in systemic basophil counts between 30 IBS Rome III patients and 30 controls [88].

NK cells

There are only a few data concerning the role of NK cells in IBS. A few studies with small groups of IBS patients measured NK cell numbers in blood samples and found them to be decreased or unaltered [89-92]. Another large study with 77 IBS patients and 28 controls reported no differences in *lamina propria* NK cell numbers, during histologic assessment of colon and rectum biopsies [41].

Neutrophils

Neutrophils are phagocytic cells of the immune system and migrate to the gastrointestinal mucosa when activated during inflammation. In rectal biopsies of children with IBS, no difference in neutrophil numbers was evident compared to healthy controls [67]. Furthermore, in a study of 49 IBS-D patients from Sri Lanka, neutrophil counts were similar to those of controls in biopsies from the ileum, cecum, colon, and rectum [61]. Similar results were derived from multiple cecum, colon and rectum biopsies of IBS Rome I & II patients in two other studies [79,80]. Conversely, in another study a subgroup of IBS patients reported elevated numbers of neutrophils in mucosal biopsies of the colon [41]. Inflammatory mediators of neutrophils, such as myeloperoxidase, human neutrophil lipocalin and lactoferrin, have been measured in stool but results are inconclusive as only one study showed increased myeloperoxidase levels [81,83,93]. Neutrophil gelatinase-associated lipocalin was measured in blood and urine samples with no significant difference between 41 Rome III IBS patients and 82 controls [94]. Finally, fecal lactoferrin, calprotectin and human β-defensin-2 (HBD-2) were measured in stool specimens of 46 IBS patients and 24 controls. While no difference was evident for lactoferrin and calprotectin, HBD-2 was elevated in the IBS group [95]. HBD-2 has antimicrobial potential [96] and is usually elevated in patients with IBD [97]; it thus provides a further indication of the presence of intestinal inflammation in IBS.

Dendritic cells (DCs)

Another cell type that has not been thoroughly investigated for its potential involvement in inflammation is DC. Like neutrophils, DCs are phagocytic cells with a key role in both innate and adaptive immunity and promote inflammatory responses to pathogens [98]. Animal studies have highlighted the role of DCs in IBS pathogenesis through induced visceral hypersensitivity and MC activation [99] and prolonged intestinal activation [100]. In addition, DCs may secrete corticotropin-releasing factor that can induce alterations in intestinal motility and visceral hypersensitivity. Two studies with mouse DCs and human peripheral blood monocyte-derived DCs presented increased levels of corticotropin-releasing factor after stimulation by some commensal bacterial strains [101,102]. In a Scandinavian study, 10 patients with self-reported food hypersensitivity were recruited and 9 of them tested positive for IBS according to the Rome II criteria. Although DC blood counts were not significantly different from those of the 10 healthy controls, when monocyte-derived DCs from patients were stimulated with lipopolysaccharide (LPS), they produced more IL-10 compared with controls [103].

Cytokines

Immune cells can produce a number of cytokines involved in the host response and some have proinflammatory actions, while others suppress inflammation. Various studies have investigated systemic and mucosal levels of cytokines in IBS patients. *In vitro* experiments using peripheral blood mononuclear cell (PBMC) cultures have yielded contradictory results regarding the expression of certain ILs before and after stimulation (Table 2). Secretion of IL-10, an anti-inflammatory cytokine, was found to be depressed [104-106] at baseline, while IL-1β [42], IL-6 [42], IL-8 [106], IL-12 [104] and TNF-α [42,106,107] were elevated. In some studies, when PBMCs were stimulated with *Escherichia coli* LPS, IL-10 expression was reduced [92,105] and IL-6 expression was increased compared with healthy controls [42]. Those results were not always

Table 2 Cytokine expression in cell cultures

Author	Number of participants (IBS: controls)	Diagnostic criteria	Site	Results	Subtype	Patients' sex	Notes
Elsenbruch 2004 [91]	15:15	Rome I	Whole blood cultures	TNF-α decreased in LPS stimulated supernatant IL-6 no difference	3 IBS-C 9 IBS-D 3 IBS-M	15 female	
O'Mahony 2005 [104]	75:20	Rome II	PBMC	Higher IL-12 and lower IL-10 levels	26% IBS-C 28% IBS-D 45% IBS-M	48 female 27 male	
Liebregts 2007 [42]	55:36	Rome II	PBMC	Higher baseline TNF-α IL-1β, IL-6 overall (in particular IBS-D patients) Higher IL-6 after LPS stimulation overall (TNF-α, IL-1β increased in IBS-D)	17 IBS-C 20 IBS-D 18 IBS-M	33 female 22 male	
Kindt 2009 [92]	30:32	Rome II	PBMC	Reduced stimulated monocytic IL-12 expression Borderline significant decrease in IL-10 (LPS)		24 female 6 male	Patients were significantly older than control subjects
Ohman 2009 [43]	74:30	Rome II	PBMC	IL-1β increased after stimulation by anti-CD3/CD28. IFN-γ, IL-10 and IL-2 no difference after stimulation with controls		52 female 22 male	
Hua 2011 [109]	35:25	Rome II	PBMC	IL-10 lower levels at baseline and after LPS stimulation IL-6 and TNF-α have increased levels at both measurements but without achieving statistical significance	7 IBS-C 17 IBS-D 11 IBS-M	15 girls 20 boys	Pediatric patients
Ohman 2012 [108]	74:30	Rome II	PBMC	IL-1β, IL-12 and IL-10 no differences after LPS stimulation	11 IBS-C 26 IBS-D 37 IBS-M	52 female 22 male	
Swan 2013 [107]	55:26	Rome II	PBMC	IL-10 increased. TNF-α increased if 3 outliers were excluded	18 IBS-C 37 IBS-D	41 female 14 male	
Hua 2013 [105]	94:102	Rome II	PBMC	IL-10 levels decreased at baseline and after LPS stimulation	33 IBS-C 32 IBS-D 29 IBS-M	49 female 45 male	
Zhen 2015 [106]	42:20	Rome III	RBMC	IL-8 and TNF-α increased. IL-10 decreased	42 IBS-D	27 female 15 male	IL-8 and TNF-α were positively correlated with worse abdominal symptoms. IL-10 was negatively correlated

IBS, irritable bowel syndrome; IBS-C, constipation predominant; IBS-D, diarrhea predominant; IBS-M, mixed; IL, interleukin; IFN, interferon; LPS, lipopolysaccharide; PBMC, peripheral blood mononuclear cell; TNF, tumor necrosis factor

duplicated and secretion of IL-1β, IL-12 and IL-10 showed no differences in LPS-stimulated PBMCs of 72 Rome II IBS patients [108]. In addition, PBMCs of patients belonging to the IBS-D subgroup showed a significantly increased release of TNF-α and IL-1β after LPS stimulation [42]. Moreover, anti-CD3/CD28-stimulated PBMCs in 74 Rome II patients showed increased IL-1β production, though interferon (IFN)-γ, IL-2 and IL-10 secretion was not different than controls [43]. In a recent study of 35 children with Rome II IBS, IL-10 levels were elevated both before and after LPS stimulation of PBMCs, while IL-6 and TNF-α levels were higher at baseline and after stimulation but without achieving statistical significance [109].

The majority of studies report cytokine levels in the systemic circulation but it is evident that there is great discrepancy among the results, possibly attributable to differences in cytokine measurement assays or the timing of specimen collection (Table 3). Nevertheless, most studies found IL-6 and IL-8 to be elevated in IBS patients [65,92,108,110-116], whereas IL-10 levels were lower or showed no difference compared with healthy controls [39,65,92,108,110-115,117,118]. Secretion of TNF-α is frequently reported without differences between patients and controls [65,110-112], but four studies with a large number of participants all reported that IBS patients had higher levels of TNF-α in serum [39,113,116,117]. Other cytokines, such as IL-1β, IL-2, IL-4, IL-5, IL-12, IL-13 and IFN-γ, were not measured in many studies, and when they were no differences were reported [65,108,110,111,118]. Two studies were published in 2016, each with more than 100 IBS patients. In the first, which was conducted in China and included 102 Rome III patients, the investigators reported higher IL-6 mRNA and lower IL-10 mRNA expression compared with controls. Furthermore, IBS patients with depression had a higher IL-6 level than those without depression [115]. However, in the latter group, which included 144 Rome III patients, no differences were detected in IL-5, IL-6, IL-8, IL-10, IL-12p70, IL-13, IL-17A, TNF or IFN-γ serum levels; IL-6 and IL-8 were higher and IFN-γ lower, but the difference did not reach statistical significance [40]. In addition, a recent study of 90 Rome III IBS patients and 90 healthy controls revealed that IBS patients had higher serum levels of TNF-α and IL-17 and lower IL-10 levels. Moreover, these inflammatory cytokines had a significant positive correlation with the severity of digestive symptoms and were negatively correlated with quality-of-life scores [39]. Interestingly, 100 IBS Rome II patients, with comorbidities that included fibromyalgia, premenstrual dysmorphic disorder and chronic fatigue syndrome, had higher levels of both IL-1β

Table 3 Cytokine expression in systemic circulation (serum and plasma)

Author	Number of participants (IBS: controls)	Diagnostic criteria	Site	Results	Subtype	Patients' sex	Notes
Dinan 2006 [112]	49:48	Rome II	serum	IL-6, IL-8 increased. IL-10 no difference. TNF-αno difference	13% IBS-C 47% IBS-D 40% IBS-M	66% female 34% male	
Dinan 2008 [114]	37:37	Rome II	blood	IL-6, IL-8 increased. IL-10 no difference	5 IBS-C 18 IBS-D 14 IBS-M	24 female 13 male	
Kindt 2009 [92]	30:32	Rome II	serum	No difference in basal plasma IL-6, IL-10		24 female 6 male	Patients were significantly older than controls
Scully 2010 [110]	21:54	Rome II	plasma	IL-6, IL-8 increased. IL-1β, IL-10, IL-12p70, IL-13, TNF-α and IFN-γ no difference			Plus 100 female IBS patients with co-morbidities as fibromyalgia, premenstrual dysmorphic disorder and chronic fatigue syndrome. The co-morbidities groups had increased also IL-1β and TNF-α.
Del Valle-Pinero 2011 [120]	12:12	Rome III	plasma	CCL-16 protein increased but not statistically significant	4 IBS-C 8 IBS-D	7 female 5 male	
McKernan 2011 [111]	30:30	Rome II	plasma	IL-6, IL-8 increased. IL-1β, IL-2, IL-4, IL-5, IL-10, IL-12, IL-13, TNF-α and IFN-γ no difference	10 IBS-C 9 IBS-D 11 IBS-M	25 female 5 male	No PI-IBS

(Contd...)

Table 3 (*Continued*)

Author	Number of participants (IBS: controls)	Diagnostic criteria	Site	Results	Subtype	Patients' sex	Notes
Ohman 2012 [108]	74:30	Rome II	serum	IL-1β, IL-6 and IL-10 no differences. IL-10 increased but without statistical significance	11 IBS-C 26 IBS-D 37 IBS-M	52 female 22 male	
Chang 2012 [65]	39:34	Rome II	serum	IL-1β, IL-6, IL-8, IL-10, IL-12 and TNF-α no differences	35.5% IBS-C 33.3% IBS-D 31.1% IBS-M	22 female 17 male	No PI-IBS
Rana 2012 [113]	63:62	Rome II	serum	IL-6 and TNF-α increased. IL-10 no difference	63 IBS-D	26 female 37 male	
Schmulson 2012 [117]	62:116	Rome II	serum	IL-10 decreased. TNF-α increased	16 IBS-C 15 IBS-D 31 IBS-M	34 female 28 male	
Gao 2013 [118]	28:15	Rome III	blood	IL-1β increased. IL-10 decreased	28 IBS-D	16 female 12 male	
Jizhong 2016 [115]	102:60	Rome III	Peripheral blood	IL-6 mRNA expression increased. IL-10 mRNA expression decreased	30 IBS-C 44 IBS-D 28 IBS-M/A	39 female 63 male	
Seyedmirzaee 2016 [116]	74:75	Rome III	Serum	IL-6, IL-8 and TNF-α increased	29 IBS-C 34 IBS-D 11 IBS-M	46 female 28 male	
Bennet 2016 [40]	144:42	Rome III	Serum	IL-5, IL-6, IL-8, IL-10, IL-12p70, IL-13, IL-17A, TNF and IFN-γ no differences. IL-6 and IL-8 tended to be increased and IFN-γ tended to be decreased	35 IBS-C 58 IBS-D 51 IBS-nonC-nonD	69% female 31% male	
Choghakhori 2017 [39]	90:90	Rome III	Serum	TNF-α and IL-17 increased. IL-10 decreased	30 IBS-C 24 IBS-D 36 IBS-A	61 female 29 male	

CCL, chemokine C-C motif ligand; IBS, irritable bowel syndrome; IBS-C, constipation predominant; IBS-D, diarrhea predominant; IBS-M, mixed; IBS-A, with alternating stool pattern; IL, interleukin; IFN, interferon; PI-IBS, post-infectious; TNF, tumor necrosis factor

and TNF-α compared with the control group [110]. In a recent meta-analysis, sex-related analysis of cytokine imbalance in IBS demonstrated significantly lower levels of IL-10 in systemic circulation in male patients and significantly higher levels of TNF-α in female patients [119].

In addition, in some studies, cytokines such as chemokine (C-C motif) ligand-16 (CCL-16), macrophage migration inhibitory factor and monocyte chemotactin protein-1 were found to be elevated in the serum of IBS patients compared with controls, although the difference did not always reach statistical significance [88,120]. The gene expression level of CCL-16 in plasma was also shown to be greater, especially in IBS-C patients, compared with healthy controls or those in the IBS-D subgroup [120].

Cytokine imbalance in mucosal biopsies may present more reliable insight into low-grade inflammation in IBS patients (Table 4). In two studies, tissue levels of cytokines such as IL-4, IL-6, IFN-γ and TNF-α measured from intestinal mucosal biopsies presented no differences between 38 IBS Rome III patients and 48 controls [121,122]. However, IFN-γ levels were higher in the PI-IBS subgroup, but without statistical significance [122]. IL-10 levels appeared to be depressed [118,122] and IL-1β levels elevated in the IBS-D and IBS-M subgroups [121] and in the total IBS population [118]. Additionally, soluble

IL-2 receptor showed higher levels in IBS-D patients [60] and IL-8 was elevated in the IBS-D and IBS-M subgroups [121]. In *ex vivo* cultures of biopsy tissue from 28 non-PI-IBS Rome II patients, IL-1β, IL-6 and TNF-α secretion showed no differences compared to controls, while secretion of IL-8 was lower [123]. Cytokine expression measured by mRNA levels in mucosal biopsies exhibited no significant difference in IL-1 [63], IL-1β [65,107], IL-2 [122], IL-4 [122], IL-6 [63,65], IL-8 [40,65], IL-12 [65], IL-13 [63] or TNF-α [40,65,107]. In other studies, IL-1β [25,57], IL-8 [61], and IFN-γ [122] mRNA expression was elevated; however, in all three studies the majority of the patient population was identified as PI-IBS or IBS-D. Furthermore Chen *et al* showed no differences in IFN-γ mRNA levels between a non-PI-IBS population and controls in mucosal biopsies of colon and rectum [122]. Expression of IL-10 mRNA was reported to be significantly depressed in three studies with a total of 126 IBS patients [61,65,122], although in one of these the difference was seen only in the female population [65]. These results were contradictory, as other studies at the same time reported no differences in IL-10 mRNA levels [63,107]. A recent study with sigmoidal colon biopsies from 109 Rome III patients found that IL-10 mRNA levels were lower, but without reaching statistical significance [40]. Finally, *ex vivo* stimulation of cultures of anaerobic bacteria derived from the colonic mucosa of 11 PI-IBS patients provided some interesting results. At baseline, only IL-13 release was significantly lower

Table 4 Cytokine expression in biopsies from the intestinal mucosa in IBS patients

Author	Number of participants (IBS: controls)	Diagnostic criteria	Site	Results	Subtype	Patients' sex	Notes
Gwee 2003 [25]	8:18	Rome I	Rectal biopsies	IL-1β mRNA expression increased	PI-IBS	4 female 4 male	
Wang 2004 [57]	30:12	Rome II	Terminal ileum and rectosigmoid	IL-1β mRNA expression increased in terminal ileum and rectosigmoid mucosa in PI-IBS patients	15 PI-IBS 15 non PI-IBS		
Macsharry 2008 [123]	28:10	Rome II	Sigmoid colon and rectum	In *ex vivo* cultures. IL-1β, IL-6 and TNFα secretion no differences. Reduced secretion of IL-8	19% IBS-C 29% IBS-D 52% IBS-M	29 female	No PI-IBS
Goral 2010 [60]	72:50	Rome III	Cecum and rectum	sIL-2 receptor levels increased in IBS-D	32 IBS-C 40 IBS-D		
Foley 2011 [63]	20:29	Rome III	Duodenal	IL-1, IL-6, IL-10 and IL-13 mRNA numerically higher but without statistical significance	20 IBS-D	13 female 7 male	
De Silva 2012 [61]	49:14	Rome III	Ileum, cecum, ascending, transverse, descending colon and rectum	Expression of IL-8 mRNA was increased and expression of IL-10 mRNA decreased	49 IBS-D	13 female 36 male	
Belmonte 2012 [121]	48:31	Rome III	Descending colon	IL-8 and IL-1β mucosal concentration was increased in IBS-D and IBS-M. No changes in IFN-γ TNF-α and IL-6	14 IBS-C 20 IBS-D 14 IBS-M	37 female 11 male	

(Contd...)

Table 4 (*Continued*)

Author	Number of participants (IBS: controls)	Diagnostic criteria	Site	Results	Subtype	Patients' sex	Notes
Chang 2012 [65]	39:34	Rome II	Sigmoid colon	mRNA levels of IL-1β, IL-6, IL-8, IL-12 and TNF-α no differences. Female patients had lower IL-10 than female controls	35.5% IBS-C 33.3% IBS-D 31.1% IBS-M	22 female 17 male	
Chen 2012 [122]	38:20	Rome III	Ascending, descending colon and rectum	IFN-γ mRNA levels increased in PI-IBS. IL-10 mRNA levels decreased in PI-IBS. IL-12 and IL-4 mRNA no differences. In non PI-IBS no differences with controls. IFN-γ protein expression was up regulated in PI-IBS but not statistically important. IL-12, IL-4 protein expression no difference. IL-10 expression was significantly lower in PI-IBS	20 PI-IBS 18 non PI-IBS	17 female 21 male	
Gao 2013 [118]	28:15	Rome III	Sigmoid colon	IL-1β levels were increased. IL-10 was reduced	28 IBS-D	16 female 12 male	
Swan 2013 [107]	55:26	Rome II	Rectum	IL-10, IL-1β and TNF-α mRNA expression had no differences	18 IBS-C 37 IBS-D	41 female 14 male	
Sundin 2015 [124]	11:10	Rome III	Sigmoid colon	*Ex vivo* cultures. At baseline IL-13 release was significantly decreased. IL-1β, IL-2, IL-8, IL-10, IL-17, TNF-α and IFN-γ did not differ significantly. Mixed results after bacteria stimulation. IL-1β was increased after bacteria stimulation with *Subdoligranulum variabi*l	10 IBS-D 1 IBS-M	7 female 4 male	

(Contd...)

Table 4 (*Continued*)

Author	Number of participants (IBS: controls)	Diagnostic criteria	Site	Results	Subtype	Patients' sex	Notes
Bennet 2016 [40]	109:36	Rome III	Sigmoid colon	Mucosal mRNA levels of IL-8, IL-10 and TNF had no differences. IL-10 mRNA tended to be decreased	31 IBS-C 42 IBS-D 36 IBS-nonCnonD	69% female 31% male	

IBS, irritable bowel syndrome; IBS-C, constipation-predominant; IBS-D, diarrhea-predominant; IBS-M, mixed; IL, interleukin; IFN, interferon; PI-IBS, post-infectious; TNF, tumor necrosis factor

compared to healthy controls, while IL-1β, IL-2, IL-8, IL-10, IL-17, TNF-α, and IFN-γ did not differ significantly. The results after stimulation were diverse and levels of cytokine release mainly remained unaltered. However, IL-1β was increased after bacterial stimulation with *Subdoligranulum variabile* in IBS patients [124].

The genetic predisposition of IBS patients to produce increased or decreased levels of cytokines has been subjected to some investigation. Polymorphisms in the cytokine-encoding genes and alterations in allele and genotype frequencies have been reported in IBS patients and may result in higher or lower production of some cytokines [125-127]. Moreover, SNPs in the IL-6 region were associated with an increased risk of developing PI-IBS [128]. Assessment of the IL-10 genotype was performed in blood samples from 230 Rome I IBS patients and 450 healthy controls. Interestingly, fewer IBS patients had the high-producer genotype for IL-10 compared with controls. Conversely, genotype frequencies for transforming growth factor-β were not altered in a smaller group of participants [129]. In addition, in 111 Rome II IBS patients, the heterozygous TNF-α genotype was significantly more common compared with 162 healthy controls. No differences were found in IL-10 genotypes and allele frequencies between patients and controls [130]. Altered gene expression of cytokines IL-10, IL-1β, and transforming growth factor β were also observed in colonic tissue [123]. IL-10 polymorphisms were analyzed in blood samples from 94 children and 102 controls, and genotype, allele or haplotype frequencies remained similar between the two groups [105].

Toll-like receptors (TLRs) and inflammasomes

TLRs are a family of transmembrane innate immune receptors that recognize various bacterial and viral cell components, known as pathogen-associated molecular patterns, and regulate the immune response [131,132]. In animal models, TLRs appear upregulated in response to stress and this upregulation seems to contribute to visceral hypersensitivity [133,134]. Furthermore, evaluation of potential genetic involvement in PI-IBS pathogenesis, studied after an outbreak of acute gastroenteritis, found two gene variants located in the TLR9 region. Moreover those SNPs were identified as independent risk factors for developing PI-IBS [128]. Hence, research focusing on the role of TLRs and alterations in TLR signaling in dysregulated innate immune response could inform investigations into the pathogenesis of IBS (Table 5).

Expression of TLRs on blood monocytes of 74 Rome II IBS patients demonstrated higher levels of TLR2 expression compared to controls, while no differences were evident in TLR4 expression. No correlation between TLR expression and IBS subtypes according to bowel habits could be established [108]. Another study measured cytokine production after TLR agonist-induced stimulation in whole blood cultures. Interestingly, agonists for TLR2-5 and TLR7-8 enhanced cytokine levels in 30 Rome II IBS patients compared with controls. The TLR4 agonist LPS and the TLR5 agonist flagellin increased IL-1β and TNF-α release. In addition the TLR8 agonist ssRNA40 (single-stranded RNA oligonucleotide) stimulated the expression of all relevant cytokines (IL-1β, IL-6, IL-8, and TNF-α) in whole blood supernatants. No differences were found in cytokine production after stimulation with TLR1, TLR6, and TLR9 agonists [111].

In a recent large study with 102 IBS patients, both TLR2 and TLR4 mRNA levels were elevated in peripheral blood samples compared to controls. Furthermore, when depression scores were used to divide different categories of IBS patients, the subgroup defined as IBS with depression had significantly higher levels of TLR mRNA compared to the non-depressed subgroup [115]. The kynurenine:tryptophan ratio has been evaluated in whole blood cultures stimulated with TLR agonists. Researchers demonstrated a different tryptophan degradation profile induced by some TLR agonists and this may implicate a possible TLR dysfunction in low-grade inflammation, caused by either increased expression or enhanced sensitivity [135].

Increases in TLR levels in systemic circulation strengthen the hypothesis for the potential role of innate immune dysfunction in IBS pathophysiology, although increased serum levels of LPS and antiflagellin antibodies have also been found [136]. Nevertheless, similar results would need to be documented in the intestinal mucosa. A study with 26 Rome II IBS female patients investigated TLR1-10 expression using quantitative real-time polymerase chain reaction in biopsies from sigmoid colon and rectum. Expression of TLR4 and TLR5 was elevated, while both TLR7 and TLR8 expression were significantly lower compared to controls. Interestingly, expression of TLR4 was fourfold higher compared with controls, while this increase was lower than that observed in IBD patients (even

Table 5 TLR expression in blood and biopsies from the intestinal mucosa of IBS patients

Author	Number of participants (IBS: controls)	Diagnostic criteria	Site	Results	Subtype	Patients' sex	extra
McKernan 2011 [111]	30:30	Rome II	Whole blood cultures	TLR1, TLR6 and TRL9 agonist induce no difference in cytokine levels. TLR2 agonist increases TNF-α. TLR3 & TLR7 agonist increases IL-8. TLR4 & TLR5 increases IL-1β and TNF-α. TLR8 agonist increases IL6, IL-8, IL-1β and TNF-α	10 IBS-C 9 IBS-D 11 IBS-A	25 female 5 male	Non PI-IBS
Clarke 2012 [135]	25:37	Rome II	Whole blood cultures	Plasma tryptophan levels no difference. Plasma kynurenine levels elevated. TLRs induced alteration in tryptophan degradation	9 IBS-C 8 IBS-D 8 IBS-A	20 female 5 male	Non PI-IBS
Ohman 2012 [108]	74:30	Rome II	Blood monocytes	Increased expression of TLR2. No difference in TLR4	11 IBS-C 26 IBS-D 31 IBS-M	52 female 22 male	
Jizhong 2016 [115]	102:60	Rome III	Peripheral blood	IL-6 mRNA expression increased. IL-10 mRNA expression decreased	30 IBS-C 44 IBS-D 28 IBS-M/A	39 female 63 male	Depressed IBS patients enhanced results than non depressed.
Brint 2011 [137]	26:19	Rome II	Sigmoid colon and rectum	RT-PCR analysis. Increased expression of TLR4 & TLR5. Decreased of TLR7 & TLR8	19% IBS-C 29% IBS-D 52% IBS-A/M	26 female	
Brint 2011 [137]	9:8	Rome II	Sigmoid colon and rectum	Microarray analysis. Increase in TLR4 expression. In microarray gene expression of TLR genes	2 IBS-C 2 IBS-D 5 IBS-A	9 female	Population from previous study of MacSharry 2008 [123]
Belmonte 2012 [121]	48:31	Rome III	Descending colon	No difference in TLR2 and TLR4 mRNA expression. Increased TLR2 and TLR4 mRNA expression in IBS-M subgroup	14 IBS-C 20 IBS-D 14 IBS-M	37 female 11 male	
Kocak 2016 [138]	51:15	Rome II	Sigmoid colon and rectum	Increased colonic tissue levels of TLR4. Increased colonic tissue levels of TLR2 only for IBS-D	31 IBS-C 20 IBS-D	43 female 8 male	

IBS, irritable bowel syndrome; IBS-A, with alternating stool pattern; IBS-C, constipation predominant; IBS-D, diarrhea predominant; IBS-M, mixed; IL, interleukin; PI-IBS, post-infectious; TLR, toll-like receptor; TNF, tumor necrosis factor

from those with active disease). In the same study, other TLR regulatory proteins (Tollip, IL-1 receptor-associated kinase) were measured; however, they showed no major differences when compared with controls, with the exception of single immunoglobulin IL-1 receptor-related molecule, which was upregulated [137].

Another study with 48 Rome III IBS patients evaluated the mRNA expression of TLR2 and TLR4 in mucosal biopsies from the descending colon and showed no significant difference compared with controls. When mRNA expression was analyzed in the IBS subgroups, both TLR2 and TLR4 levels were significantly elevated in IBS-M patients. In addition, TLR2 and TLR4 expression had a positive correlation with the duration of symptoms in IBS patients and in the IBS-M group [121]. Finally, in a very recent study where colonic tissue samples from 51 Rome II IBS patients were analyzed, higher TLR4 levels were found. TLR2 levels were also elevated, though without statistical significance in the total IBS population. In the IBS-D subgroup of patients, the colonic tissue levels of TLR2 were significantly higher than those of controls [138].

The innate immune system is activated through a vast variety of receptors, which recognize potential threats and trigger immune responses. Another complex of pattern recognition receptors is called inflammasomes; these are a cytoplasmic multi-protein family that activate caspase-1 and induce secretion of IL-1β and IL-18, resulting in cell death by pyroptosis. Nucleotide-binding domain and leucine-rich repeat-containing proteins assemble canonical inflammasomes (NLRPs) that promote caspase-1 activation [139,140]. A role for NLR inflammasome modulation and caspase-1 activation in mucosal inflammation has been implicated in IBD and IBS pathophysiology [141]. Recently, a small study with 7 IBD and 6 IBS-D Rome III patients, compared with 13 healthy controls, reported elevated intestinal epithelial cell counts with activated caspase-1 staining [142]. In animal studies, stress-induced small bowel inflammation was associated with NLRP-6 downregulation and alterations in gut microbiota. Interestingly, probiotic therapy ameliorated the induced enteritis, reinforcing the correlation between mild inflammation, gut dysbiosis and appearance of IBS or IBS-like symptoms [143]. Furthermore, both NLRP-3 and caspase-1 were significantly more expressed in the terminal ileal tissues of mice with PI-IBS from *Trichinella spiralis* compared with normal control mice. Elevated expression of IL-1β and IL-18 was also documented in PI-IBS mice [144]. Additional studies are required in order to elucidate the possible role of inflammasomes in IBS pathogenesis.

Concluding remarks

A large number of studies have explored the role of innate immune response dysfunction in the pathophysiology of IBS and the potential implication of low-grade inflammation in both systemic circulation and intestinal mucosa. There is some evidence to support a predominantly inflammatory process occurring locally, in the intestinal mucosa, and perhaps systemically. However, many of the studies, which have focused on certain cell types, cytokines or pathogen recognition receptors, have yielded conflicting results. Hence, the underlining mechanisms are still unclear and the specific role of immune cells and mediators is elusive. To better understand the complex IBS pathogenesis, specific immune response pathways should be investigated and understood in depth in well-designed experimental or translational studies. Interactions between gut microbiota, immune activation and dysfunction of the enteric nervous system need to be more clearly delineated, to reach a convincing hypothesis regarding the pathophysiologic mechanism of IBS. Further comprehension of these factors could broaden therapeutic possibilities and ameliorate the burden of the disease.

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