

Immune biomarkers in irritable bowel syndrome: a review

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Abstract: Irritable bowel syndrome (IBS) is a chronic functional gastrointestinal disorder that affects about 9%–13% of the general population. IBS is one of the main reasons to consult a primary care physician, and nearly 30% of visits to a gastroenterologist are for IBS. The diagnosis of IBS relies on subjective, patient-reported symptoms, thus making urgent the need for IBS-specific biomarkers. The same biomarkers, or perhaps different ones, can also be used to monitor disease evolution and response to treatment. A significant number of studies have looked in the immune system for establishing IBS biomarkers, based on the concept that IBS might represent a condition of immune dysregulation somewhere in the spectrum between health and inflammatory bowel disease. Such biomarkers can be detected in blood, intestinal biopsies, or luminal contents. Overall, results are rarely consistent between studies; small sample size, patient and disease heterogeneity, presence of comorbidities, and variation in sampling might contribute to these discrepancies. So far, studies have failed to provide a diagnostic immune biomarker for IBS, but they have considerably advanced our understanding of the disease pathophysiology, including the role of the individual's genetic make-up, and of the host–microbial interactions. High throughput analysis of a large number of well characterized patients holds promise for developing appropriate biomarkers for IBS.

Keywords: neuroimmune interactions, mast cells, genetic polymorphisms, cytokines, toll-like receptors

Introduction

Irritable bowel syndrome (IBS) is a chronic functional gastrointestinal disorder characterized by recurrent abdominal pain or discomfort and change in bowel habits.^{1,2} IBS is clinically classified into different subgroups: IBS with constipation predominance (IBS-C), IBS with diarrhea predominance (IBS-D), mixed or alternating IBS (IBS-M), and unsubtyped IBS (IBS-U).¹

Post-infectious IBS (PI-IBS) is one special subgroup that meets the Rome criteria for IBS-D or IBS-M. In 6%–7% cases, IBS develops following an episode of viral or bacterial gastroenteritis.^{3–5} More toxigenic organisms increase the risk elevenfold, as does an initial illness lasting more than 3 weeks. Other risk factors for developing PI-IBS include severity of the initial insult, female sex, anxiety, and depression.^{6,7} In general, PI-IBS has a better prognosis, with a decrease or resolution of the symptoms within 10 years after the diagnosis.⁸

According to a recent meta-analysis of 80 studies including a total of 260,960 patients, the prevalence of IBS is estimated at 11.2% (95% confidence interval [CI], 9.8%–12.8%). Females are at slightly higher risk for IBS compared with males (OR, 1.67;

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95% CI, 1.53–1.82).⁹ IBS is one of the top reasons to consult a physician: it accounts for 10%–15% of primary care visits and 25%–50% of gastroenterology referral visits.^{10,11} In the United States alone, US\$1.7–10 billion in direct medical costs per year (ie, primary and specialist physician visits, diagnostic tests, etc) have been associated with IBS, excluding prescription and over-the-counter drug costs;^{12,13} and up to US\$20 billion in indirect costs (eg, productivity loss).¹⁴ Further, the disease has a negative impact on health-related quality of life parameters both in IBS patients and their family members.¹⁵

Diagnosis of IBS as defined by the Rome III criteria is based on patient reported symptoms, which are mainly subjective and prone to recall bias.^{1,2,16} Often, the lack of findings by endoscopy or laboratory tests in these patients has led to the concept of “diagnosis by exclusion”. Furthermore, it drives high expenses in the diagnostic process to exclude other organic conditions that can present with similar symptoms, including gastrointestinal infections or inflammatory bowel disease (IBD). Therefore, the development of objective criteria for disease diagnosis and/or monitoring response to treatment in IBS remains an unmet need.

A disease biological marker, or biomarker, is defined as a change in the physical composition of an organism that can be objectively measured. It indicates the presence of an illness, can reflect the activity of the disease, or may be used to evaluate the pharmacological response to a therapeutic intervention.^{17,18} Biomarkers can be developed based on known biological pathways implicated in disease pathogenesis, or from unbiased high-throughput screening of well defined clinical populations.¹⁹ In that case, the identified biomarkers can also provide insight into potential disease mechanisms.

Cellular biomarkers in IBS

The prevailing notion is that IBS represents a state of chronic low-grade inflammation of the intestinal mucosa, being somewhere in the spectrum between healthy mucosa and IBD.^{20,21} While some studies demonstrate increased numbers of immune cells, particularly T-lymphocytes, in the colonic mucosa of patients with IBS, consensus is lacking, since a significant number of other reports fail to show any differences compared with healthy controls (Table 1). Cremon et al²² studied 48 patients with IBS and 24 healthy controls (HC) and found a greater number of immune cells infiltrating the colonic mucosa in patients with IBS. These cells appear to be primarily T-lymphocytes (CD3⁺, CD4⁺, CD8⁺).²² Similar findings are presented by Piche et al,²³ who studied 50 patients with IBS and 21 HCs. Furthermore, Chadwick et al²⁴ reported an increase in CD3⁺CD25⁺ activated T-cells in 38 patients with IBS, despite the lack of histologically confirmed

active inflammation. Similarly, Ohman et al^{25,26} described an increase in circulating CD4⁺ and CD8⁺ T-cells expressing activation markers such as CD69⁺ and β 7⁺HLADR⁺, but not CD25⁺. However, Holmen et al²⁷ did not find any changes in the number of CD4⁺CD25⁺ cells, either in the colonic mucosa or blood of 34 patients with IBS. Moreover, mRNA colonic expression of FoxP3, a marker for regulatory T-cells was comparable between IBS patients and controls.²⁸ The group from Nottingham led by Spiller focused on patients with PI-IBS and found a significant increase of CD3⁺ cells, as well as CD8⁺ cells in the rectal mucosa of patients with PI-IBS.^{29,30} Akbar et al³¹ reported analogous results in a study of 23 patients with IBS.

Among the studies with negative results, Kindt et al³² and Ohman et al²⁵ found comparable numbers of total lymphocytes, CD4⁺ cells, and CD8⁺ cells in the blood of patients with IBS and controls. Likewise, Chang et al³³ reported no differences in the number of CD3⁺ cells, CD4⁺ cells, or CD8⁺ cells when they examined biopsies from sigma of 45 patients with IBS. Similar findings are reported by Park et al,³⁴ O’Sullivan et al,³⁵ and Lee et al.³⁶ Interestingly, Braak et al²⁸ described a lower number of CD8⁺ cells in the colon of 66 patients with IBS, while the total number of CD3⁺ cells remained unaltered.

Fewer studies have examined the role of B-cells in IBS. Forshammar et al³⁷ found decreased IgA⁺ cells in the colon of 12 patients with IBS, while Ohman et al³⁸ reported a significant increase in IgG⁺ cells in the blood of 74 patients with IBS, as well as in activated B-cells (CD80⁺ or CD86⁺). However, Cremon et al²² found no differences in CD79⁺ B-cells in the colonic mucosa of 48 patients with IBS, and Kindt et al³² in the number of B-cells in the blood of 30 patients with IBS. Consistent with the above were the studies of Park et al³⁴ and O’Sullivan et al.³⁵

The number of eosinophils in the colonic mucosa of patients with IBS was found consistently to be similar to that of controls in four studies,^{23,34,35,39} while neutrophils are reported unaltered,^{23,34,39} increased²⁴ or decreased³⁵ in different studies. Notably, two studies report a decrease in CD68⁺-macrophages in the colonic mucosa of 66 patients with IBS²⁸ and in the rectal mucosa of 16 patients with PI-IBS.³⁰

Mast cells are also an important component of the immune system. In the intestinal mucosa, they are mainly found surrounding vessels and nerves. When activated, they release a number of biologically active substances primarily histamine, serotonin, and diverse proteases. They can also release cytokines and several arachidonic acid metabolites including prostaglandins and leukotriens.⁴⁰

Table 1 Immune activation in IBS

Study	N ^a	Anatomic location	T-CD3 ⁺ b,c	T-CD4 ⁺ b,c	T-CD8 ⁺ b,c	B-cells ^{b,c}	Other ^{b,c}
Foley et al ⁴⁵	20 IBS-D ^{III} 29 HC	Duodenum					↑** intraepithelial lymphocytes
Guilarte et al ⁴⁶	20 IBS-D ^I 14 HC	Jejunum	↑**				
Park et al ³⁴	18 IBS-D ^{II} 15 HC	Ileum Colon Rectum					↔ lymphocytes ↔ plasma cells ↔ neutrophils ↔ eosinophils
De Silva et al ³⁹	39 IBS-D ^{III} 13 CCR controls	Ileum Colon Rectum					↔ neutrophils ↔ eosinophils
Cremon et al ²²	48 IBS ^I 24 HC	Colon	↑***	↑*	↑***	↔ B-CD79 ⁺	↑*** total immune cells
O'Sullivan et al ³⁵	14 IBS ^I 8 HC	Colon					↔ lymphocytes ↔ plasma cells ↓*** neutrophils ↔ eosinophils
Piche et al ²³	50 IBS ^{II} 21 HC	Colon					↑** total immune cells ↑** lymphocytes ↔ neutrophils ↔ eosinophils
Chadwick et al ²⁴	38 IBS ^I 28 HC	Colon	↑*** ↑*** CD25 ⁺				↑*** neutrophils
Braak et al ²⁸	66 IBS ^{II} 20 HC	Colon	↔		↓*		↓** CD68 ⁺ macrophages ↔ CD163 ⁺ macrophages
Forshammar et al ³⁷	12 IBS ^{II} 11 HC	Colon				↓* IgA ⁺	
Chang et al ³³	45 IBS ^{II} 41 HC	Sigmoid	↔	↔	↔		
Akbar et al ³¹	23 IBS ^{II} 22 HC	Rectosigmoid	↑*	↔			
Spiller et al ³⁰	16 PI-IBS 12 HC	Rectum	↑***		↑***		↓ ^{NS} CD68 ⁺ macrophages
Dunlop et al ²⁹	28 PI-IBS 34 HC	Rectum	↑**				
Lee et al ³⁶	17 IBS-D ^{III} 12 HC	Rectum	↔				
Holmen et al ²⁷	34 IBS ^{II} 26 HC	Colon Blood		↔ CD25 ⁺ ↔ CD25 ⁺			↔ FOXP3
Kindt et al ³²	30 IBS ^{II} 32 HC	Blood		↔	↔	↔	↔ total lymphocytes ↔ NK-cells
Ohman et al ²⁶ and Ohman et al ³⁸	74 IBS ^{II} 30 HC	Blood		↔ ↑*** CD69 ⁺ ↑*** β7+HLA-DR ⁺ ↔ CD25 ⁺	↔ ↑*** CD69 ⁺ ↑* β7+HLA-DR ⁺ ↔ CD25 ⁺	↔ ↑*** IgG ⁺ ↑** CD80 ⁺ ↑** CD86 ⁺ ↑** β7+CD80 ⁺ ↑*** β7+CD86 ⁺ ↓** HLA-DR ⁺ ↓** CD40 ⁺	↔ total lymphocytes

Notes: ^aDiagnostic criteria (Rome I, ^{II}Rome II, ^{III}Rome III); ^bexpression (↑ Increased, ↓ Decreased, ↔ no difference; compared with HC); ^csignificance (*P < 0.05; **P < 0.01; ***P < 0.001, ^{NS}not significant).

Abbreviations: CCR controls, individuals with family history of colorectal cancer; HC, healthy control; IBS, irritable bowel syndrome; IBS-D, IBS with diarrhea predominance; PI-IBS, post-infectious IBS.

The study of mast cells in IBS by various groups has produced rather consistent results, suggesting a role of these cells in the pathobiology of IBS (Table 2). Specifically, a greater number of mast cells have been described primarily in patients with IBS-D.³⁴ Mast cells are primarily localized

in the duodenum, jejunum, and terminal ileum, followed by the rectum, while in the colon they are less abundant. This distribution might explain why some studies failed to detect an increase in mast cells in the colonic mucosa of patients with IBS^{33,35,41} while other studies succeeded.^{22–24,34,39,42,43} In

Table 2 Mast cells and IBS

Study	N ^a	Anatomic location	Mast cells ^{b,c}	EE/EC cells ^{b,c}	Soluble mediators ^{b,c}	Correlation with symptoms ^{b,c}
Foley et al ⁴⁵	20 IBS-D ^{III} 29 HC	Duodenum	↑***	↔	↑* tryptase	↑* depression ↑* anxiety
Guilarte et al ⁴⁶	20 IBS-D ^{II} 14 HC	Jejunum	↑***		↑** tryptase	
Martinez et al ⁴⁸	25 IBS-D ^{II} 23 HC	Jejunum	↑**		↑*** tryptase	↔ abdominal pain ↑* bowel movements ↑* stool consistency
Wang et al ⁵⁴	38 IBS ^{III} 18 HC	Terminal ileum	↑***	↔		
De Silva et al ³⁹	39 IBS-D ^{III} 13 CCR controls	Ileum Cecum Colon Rectum	↑*** ↑*** ↑* ↑***			
Park et al ³⁴	18 IBS-D ^{II} 15 HC	Terminal ileum Colon Rectum	↑** ↑** ↑**			↓* rectal sensitivity
Weston et al ⁴¹	20 IBS ^{IV} 15 HC	Terminal ileum Colon	↑*** ↔			
Cremon et al ²²	48 IBS ^I 24 HC	Colon	↑***			↑* abdominal bloating ↑*** dyspepsia
Cremon et al ⁴⁷	25 IBS ^I 12 HC	Colon	↑**	↑*	↑*** serotonin	↑* abdominal pain severity
Piche et al ²³	50 IBS ^I 21 HC	Colon	↑***			↑*** fatigue ↑* depression
Chadwick et al ²⁴	38 IBS ^I 28 HC	Colon	↑***			
Buhner et al ⁴²	18 IBS ^I 7 HC	Colon	↑**		↑*** tryptase ↑** histamine ↔ serotonin	
Barbara et al ⁴³ and Barbara et al ⁵²	44 IBS ^I 22 HC	Colon	↑		↑* tryptase ↑* histamine	↑** abdominal pain when proximal to nerves
Braak et al ²⁸	66 IBS ^I 20 HC	Colon	↓***			↔ rectal sensitivity
Klooker et al ⁴⁴	60 IBS ^I 22 HC	Rectum	↓*		↔ tryptase ↔ histamine	↔ rectal sensitivity
Chang et al ³³	45 IBS ^I 41 HC	Sigmoid	↔	↔		↔
O'Sullivan et al ³⁵	14 IBS ^I 7 HC	Colon Rectum	↔ ↔			
Akbar et al ³¹	23 IBS ^I 22 HC	Rectosigmoid	↑*			
Spiller et al ³⁰	16 PI-IBS 12 HC	Rectum	↔	↑***		
Dunlop et al ²⁹	28 PI-IBS 34 HC	Rectum	↔	↑**		↑* depression
Lee et al ³⁶	17 IBS-D ^{III} 12 HC	Rectum	↑*	↔		↔ anxiety ↔ depression
Corinaldesi et al ⁸⁸	10 IBS ^I -placebo group 10 IBS-mesalazine group	Colon				↓** response to treatment

Notes: ^aDiagnostic criteria (Rome I, ^{II}Rome II, ^{III}Rome III, ^{IV}Manning); ^bexpression (↑ Increased, ↓ Decreased, ↔ no difference; compared with HC); ^csignificance (**P* < 0.05; ***P* < 0.01; ****P* < 0.001).

Abbreviations: CCR controls, individuals with family history of colorectal cancer; EE, enteroendocrine; EC, enterochromaffin; HC, healthy control; IBS, irritable bowel syndrome; IBS-D, IBS with diarrhea predominance; PI-IBS, post-infectious IBS.

contrast to the rest of the studies, Braak et al²⁸ and Klooker et al⁴⁴ reported a decrease in the number of mast cells in the colonic and rectal mucosa, respectively, of patients with IBS.

In several reports, the increase in mast cells was associated with higher release of their soluble mediators tryptase, histamine and serotonin.^{42,43,45-47} Moreover, there were some positive correlations between mast cells and depression, anxiety, fatigue²³ and IBS symptoms such as abdominal pain, bloating, and bowel habits,⁴⁸ in particular when activated mast cells were found in proximity to the enteric neurons.^{34,43} However, studies examining the number of mast cells in relation to visceral sensitivity, as measured by the barostat test, found a negative correlation with visceral hypersensitivity.^{34,44}

Mediators secreted by mast cells in feces or the luminal fluid have also been proposed as biomarkers for IBS. In a group of 38 IBS patients, Roka et al⁴⁹ found that compared to 15 healthy controls, fecal serine-protease activity was threefold higher in the IBS-D subgroup, but not in IBS-C or IBS-M. However, this increase did not correlate with the frequency of bowel movements in a subsequent study.⁵⁰ In contrast, Lettesjo et al⁵¹ found that fecal tryptase levels were not different between a group of 46 IBS patients and 20 healthy controls. The significance of mast cell mediators in IBS is better illustrated by studies in which supernatants from colonic mucosa biopsies of patients with IBS, but not controls, were able to activate rat, mouse, and human sensory neurons in vitro^{42,52} and induce somatic and visceral hyperalgesia and allodynia when administered into the colon of mice.⁵³ The use of pharmacological antagonists in the above mentioned studies revealed that these effects were mediated primarily by proteases, and also by histamine and serotonin.

Some of the studies that report increased numbers of mast cells in the intestinal mucosa of patients with IBS determined also the number of enterochromaffin and enteroendocrine cells and found them unaltered^{36,45,54} or increased.²² Interestingly, Spiller et al³⁰ found an increased number of enteroendocrine cells, and Dunlop et al⁵⁵ an increased number of enterochromaffin (5-HT positive) cells in the rectal mucosa of patients with PI-IBS, albeit a similar number of mast cells.

In an analogy to the mast cell mediators, members of the chromogranin family (chromogranins A [CgA] and B [CgB] and secretogranins) are the major components found in the secretory granules of enteroendocrine cells.⁵⁶ Based on the study of 82 patients with IBS and 29 controls by Ohman et al,⁵⁷ while fecal levels of CgB were lower

than controls, fecal levels of CgA, SgII, and SgIII were significantly increased in IBS patients and had a positive correlation with perceived severity of abdominal pain and a negative correlation with colonic transit times.

Cytokines as biomarkers in IBS

In addition to their immunomodulatory role, various cytokines are also involved in the control of gastrointestinal functions, including motility and visceral sensitivity, although their specific role in IBS is not fully elucidated.⁵⁸⁻⁶¹ Intestinal mucosal biopsies, serum, and blood cells isolated and cultured ex vivo, have been used to examine the significance of cytokines in IBS (Table 3). Overall, results are not consistent between the various studies, primarily due to differences in the source of cytokines (mucosa versus serum versus peripheral blood cells), the type of IBS (most changes in cytokines have been described in PI-IBS and in patients with IBS-D), and the presence of various comorbidities. In the plasma of IBS patients, upregulation of interleukin (IL)-6 and IL-8 levels were reported in four different studies.⁶²⁻⁶⁴ Importantly, Scully et al⁶² found increased plasma levels of IL-1 β and tumor necrosis factor (TNF)- α in a group of women with IBS and comorbidities such as fibromyalgia, premenstrual dysmorphic disorder, or chronic fatigue syndrome. Other groups report increased secretion of IL-1 β , IL-8, and TNF- α by peripheral blood mononuclear cells (PBMCs) of patients with IBS.^{26,64-66}

Chen et al⁶⁷ studied the mRNA levels of several cytokines in the colonic mucosa of patients with PI-IBS and found upregulation of interferon- γ and downregulation of IL-10, while Chang et al³³ found lower IL-10 expression only in female patients with IBS. Likewise, McSharry et al⁶⁸ reported lower levels of IL-10 in female patients with IBS but also decreased IL-1 β . In conclusion, the most consistent finding in the studies of mucosal cytokine expression appears to be lower IL-10.^{33,67,68}

More recent studies have associated polymorphisms in several cytokine genes with susceptibility to IBS.^{69,70} Among them, a single nucleotide polymorphism (SNP) in the *TL1A* gene (*TNFSF15*) increases the risk for IBS-D and IBS-C by 1.4- and 1.8-fold, respectively according to two independent case-control studies of a total of 1992 individuals.⁷¹ These findings have been replicated by different groups in a smaller number of patients with IBS.^{72,73} Accordingly, increased *TL1A* expression has been described in the mucosa of patients with IBS compared with controls.⁷³ Associations of two *TNF- α* SNPs and susceptibility to IBS have also been described

Table 3 Cytokines as biomarkers in IBS

Study	N ^a	IL-1β ^{b,c}	IL-2 ^{b,c}	IL-4 ^{b,c}	IL-5 ^{b,c}	IL-6 ^{b,c}	IL-8 ^{b,c}	IL-10 ^{b,c}	IL-12 ^{b,c}	IL-13 ^{b,c}	TNF-α ^{b,c}	IFN-γ ^{b,c}
Serum/plasma												
Chang et al ³³	45 IBS ^{II} 41 HC	↔				↔	↔	↔	↔		↔	
Scully et al ⁶²	21 IBS ^{II} 19 HC	↔				↑**	↑**	↔	↔	↔	↔	↔
Scully et al ⁶²	100 IBS-F ^{II} with comorbidities	↑***				↑***	↑***	↔	↔	↔	↑***	↔
Dinan et al ⁶³	35 HC-F 49 IBS ^{II}					↑***	↑***	↔			↔	
McKernan et al ⁶⁴	48 HC 30 IBS ^{II} 30 HC	↔	↔	↔	↔	↑***	↑*	↔	↔	↔	↔	↔
PBMCs												
Hughes et al ⁶⁵	20 IBS-D ^{II} 36 HC	↑**				↑**	↑**	↑*	↑**	↔	↑***	
Liebrechts et al ⁶⁶	20 IBS-D ^{II} 36 HC	↑*				↑*					↑*	
PBMCs stimulated												
McKernan et al ⁶⁴	30 IBS ^{II} 30 HC	↑***				↔	↑***				↑***	
Ohman et al ²⁶	74 IBS ^{II} 30 HC	↑*					↔	↔				↔
Kindt et al ³²	30 IBS ^{II} 32 HC		↑*			↔	↔	↔	↔	↑*	↔	↔
Colonic biopsies												
Chang et al ³³	45 IBS ^{II} 41 HC	↔				↔	↔	↔	↔	↔	↔	
Chen et al ⁶⁷	18 IBS ^{II} 20 HC			↔			↔	↔	↔	↔		↔
Chen et al ⁶⁷	20 PI-IBS 20 HC			↔			↔	↔	↔	↔		↑*
Belmonte et al ⁸⁰	15 IBS-D ^{III} 15 IBS-M ^{III} 15 IBS-C ^{III}	↑*				↔	↑***				↔	↔
	25 HC					↔	↑*				↔	↔
Macsharry et al ⁶⁸	59 IBS-F 39 HC-F	↓***				↔	↔	↔	↔		↔	↔
Genetic polymorphisms												
Villani et al ⁷⁶	227 PI-IBS 574 HC					-174C*						-308
Romero-Valdivinos et al ⁷⁷	45 IBS 137 HC						396G*	-1082 AA***				

Barkhordari et al ⁷³	71 IBS ^{III} 140 HC	-174C**	-238 A***
van der Veek et al ⁷⁴	111 IBS ^{II} 162 HC	-1082 -819	-308 A*
Swan et al ⁷⁵	301 IBS-D ^{II} 179 HC		-308
Gonsalkorale et al ⁷⁸	230 IBS ^I 450 HC	-1082 GG**	

Notes: ^IDiagnostic criteria (Rome I, ^{II}Rome II, ^{III}Rome III); [↑]Increased, [↓]Decreased, [↔] no difference; compared with HC); *significance ($P < 0.05$; ** $P < 0.01$; *** $P < 0.001$).

Abbreviations: F, female; HC, healthy control; IBS, irritable bowel syndrome; IBS-C, IBS with constipation predominance; IBS-D, IBS with diarrhea predominance; IBS-M, mixed or alternating IBS; PI-IBS, post-infectious IBS; IFN, interferon; IL, interleukin; TNF, tumor necrosis factor.

in a Dutch⁷⁴ and an Iranian⁷⁵ population. Polymorphisms in additional cytokine genes, including *IL-6*,^{75,76} *IL-8*,⁷⁷ and *IL-10*^{74,77,78} have been linked to IBS in various studies, each of which, however, included a relative small number of IBS patients and controls. Notably, some of the above SNPs, ie, in *IL-10*, *TNF- α* , and *TL1A* are associated with susceptibility to Crohn's disease and ulcerative colitis, perhaps suggesting disease pathophysiology mechanisms shared by IBS and IBD.^{72,73}

Non-cytokine immune modulators as biomarkers in IBS

Toll-like receptors (TLRs) are a family of pathogen-recognition molecules with a central role in mucosal innate immune responses. In IBS patients, Brint et al⁷⁹ described increased colonic expression of TLR4 and TLR5, lower expression of TLR7 and TLR8, and no differences in the rest of the TLR receptors examined (Table 4). In the study by Belmonte et al,⁸⁰ TLR2 and TLR4 upregulation was found predominantly in the IBS-M subgroup. In a larger cohort of IBS patients, Ohman et al⁸¹ examined TLR2 and TLR4 expression in blood monocytes and found only TLR2 to be increased. Finally, in the Walkerton Health Study for clinical and genetic predictors of developing IBS within 2–3 years following an episode of acute gastroenteritis, Villani et al⁷⁶ identified the rs5743936:T variant of TLR9 (the receptor which mediates the recognition of bacterial unmethylated CpG dinucleotides) as an independent risk factor for PI-IBS. In a different population, rs5743936:T was found to be a risk allele for IBS-D by Camilleri et al.⁷² McKernan et al⁶⁴ studied IL-1 β , IL-6, IL-8, and TNF- α release from PBMCs in response to stimulation with specific TLR agonists. Their results indirectly suggested increased TLR 2, 3, 4, 5, 7, and in particular TLR8 signaling activity, but no differences in TLR1, TLR6, or TLR9 (Table 4). Taken together, the above observations further support a role of host-microbial interactions in the pathogenesis of IBS.

From a different perspective, Schoepfer et al⁸² found an increased frequency of antibodies against flagellin, the structural protein in bacterial flagellae that is recognized by TLR5, in the serum of 112 patients with IBS when compared with 43 controls.

In addition to bacterial recognition through TLRs, an important host defense mechanism relies on the secretion by intestinal epithelial cells of various antimicrobial peptides such as defensins.⁸³ Human beta-defensin-2 (hBD2)

Table 4 Toll-like receptors in IBS

Study	N ^a	Source	TLR1 ^{b,c}	TLR2 ^{b,c}	TLR3 ^{b,c}	TLR4 ^{b,c}	TLR5 ^{b,c}	TLR6 ^{b,c}	TLR7 ^{b,c}	TLR8 ^{b,c}	TLR9 ^{b,c}	TLR10 ^{b,c}
Brint et al ⁷⁹	26-IBS-F 19-HC-F	Colonic mucosa	↔	↔	↔	↑***	↑**	↔	↓***	↓**	↔	↔
Belmonte et al ⁸⁰	15 IBS-D ³ 14 IBS-M ³ 13 IBS-C ³ 25 HC	Colonic mucosa		↔		↔	↑*					
Ohman et al ⁸¹	74 IBS ² 30 HC	Monocytes		↑*		↔						
Villani et al ⁷⁶	227 PI-IBS 574 HC	SNPs										-1237 T*
Camilleri et al ⁷²	175 IBS-D ² 233 HC	SNPs										-1237 T*

Notes: ^aDiagnostic criteria (^{II}Rome II, ^{III}Rome III); ^bexpression (↑ Increased, ↓ Decreased, ↔ no difference; compared with HC); ^csignificance (**P* < 0.05; ***P* < 0.01; ****P* < 0.001).

Abbreviations: F, female; HC, healthy control; IBS, irritable bowel syndrome; IBS-C, IBS with constipation predominance; IBS-D, IBS with diarrhea predominance; IBS-M, mixed or alternating IBS; PI-IBS, post-infectious IBS; SNP, single nucleotide polymorphism; TLR, Toll-like receptor.

is constitutively expressed, while hBD2, -3 and -4, only in response to inflammation or infection.^{84,85} Langhorst et al⁸³ described increased levels of beta defensin-2 (hBD2) in the feces of 46 patients with IBS (24 with IBS-D and 22 with IBS-M) compared with 24 controls.

Biomarkers of response to treatment in IBS

The choice of a primary endpoint for a clinical trial is one of the most important determinants of the ability to demonstrate therapeutic efficacy.⁸⁶ This is even more so for diseases like IBS, which currently depend on patient-reported subjective outcomes. Only few studies have addressed immune biomarkers in relation to response to treatment in IBS. O'Mahony et al⁸⁷ reported changes in IL-10 and IL-12 secretion from unstimulated PBMCs in IBS patients treated with a probiotic. In the study by Corinaldesi et al,⁸⁸ treatment of ten patients with IBS with mesalazine resulted in a significant reduction of mast cells in their colonic mucosa compared with placebo-treated patients, suggesting that mast cells could perhaps represent a marker for disease monitoring as well as a therapeutic target in IBS. Indeed, in another report treatment of 60 IBS patients with the mast cell stabilizer ketotifen decreased visceral hypersensitivity and improved their clinical symptoms.⁴⁴ Our own studies revealed an association between changes in serum levels of osteoprotegerin, TNF-like weak inducer of apoptosis (TWEAK), and symptom improvement in patients with IBS who received a placebo-acupuncture intervention.⁸⁹ Osteoprotegerin and TWEAK are both members of the TNF superfamily (TNFRSF11B and TNFSF12, respectively) with pleiotropic immunomodulatory effects.

Summary of findings

The major points of this review are summarized as follows:

- Several studies have demonstrated increased number or activation of mast cells in the intestine of patients with IBS, in particular in those with IBS-D.
- A chronic state of low -grade subclinical inflammation characterizes IBS. This might be the result of an imbalanced host response to the gut microbiome, triggered by a pathogen, as is the case of PI-IBS, and accentuated by genetic factors. This notion is supported by altered expression in IBS of various cytokines (IL-1 β , IL-6, IL-8, IL-10, TNF- α) and toll-like receptors. Single nucleotide polymorphisms in the above genes have been associated with altered susceptibility to IBS.

Conclusion and perspectives

The main conclusions of this review are the following:

- There is not enough evidence for immune biomarkers described so far in IBS studies to be used as diagnostic or disease monitoring tools. Lack of consensus among the various studies might be attributed to small number of patients included, disease heterogeneity, presence of comorbidities, focus on intestinal versus fecal versus blood biomarkers, and variations in tissue sampling and experimental design.
- Immune biomarkers have greatly contributed to our understanding of various aspects of IBS pathophysiology. Moreover, certain immune biomarkers correlate with disease subgroups, raising the intriguing possibility of distinct mechanisms underlying the different IBS subtypes.

- In some cases the identified biomarkers have suggested new therapeutic approaches for IBS, for instance, treatment with probiotics or mast cell stabilizers.

It is our belief that recent advances in high throughput technologies, including genomics, proteomics, metabolomics, and microbiome analysis, or their various combinations, corroborated by powerful bioinformatics approaches and when applied to a large number of well-characterized IBS patients, will lead to the unbiased development of robust biomarkers for disease diagnosis and treatment outcomes. Moreover, categorization of patients according to certain immune/genetic profiles might lead to more effective and personalized treatments.

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Disclosure

The authors report no conflicts of interest in this work.

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