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**REVIEW ARTICLE** 

# Immune activation in irritable bowel syndrome: from basic to clinic

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#### RESUMEN

El síndrome de intestino irritable (SII) es un trastorno funcional intestinal común con una etiología no identificada. La activación de bajo grado del sistema autoinmunitario ha sido considerada la fisiopatología de este trastorno. En los pacientes que lo padecen, las concentraciones de citocinas están alteradas y las células inmunitarias se infiltran en el intestino. Además, en los pacientes con SII se ha observado la activación de estas células inmunitarias y la presencia de alergias alimenticias. Sin embargo, las características del perfil inmunológico de los pacientes con SII pueden ser objeto de debate en la práctica clínica, dado que no existen criterios bien establecidos para diferenciar el SII en controles sanitarios basados en estos parámetros. Esta revisión plantea el rol del sistema inmunitario en la fisiopatología del SII y explora los potenciales roles de estos biomarcadores durante el tratamiento clínico de estos pacientes. (NeuroGastroLatam Rev. 2017;1:116-127)

**Palabras clave:** Síndrome de intestino irritable. Inmunología. Mastocito. Linfocitos. Citocinas.

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#### ABSTRACT

Irritable bowel syndrome (IBS) is a common functional gastrointestinal disorder with unidentified etiology. Low-grade activation of the immune system has been considered in the pathophysiology of this disorder. The levels of cytokines are altered in these patients and the immune cells infiltrate their intestine. Moreover, activation of the immune cells and features of food allergy are observed in IBS patients. Whether these changes in the immunological profile of IBS patients could be translated to clinical practice is under debate, as there is not clear cut-off for differentiating IBS from healthy controls based on immunological parameters. This review discusses the role of immune system in the pathophysiology of IBS and explores the potential roles of these biomarkers during the clinical management of these patients.

Key words: Irritable bowel syndrome. Immunology. Mast cell. Lymphocyte. Cytokine.

#### INTRODUCTION

Irritable bowel syndrome (IBS) is a functional gastrointestinal (GI) disorder of unknown etiology. With a prevalence of 1.1-35%, IBS has one of the highest economic and health burdens among GI diseases<sup>1</sup>.

Conventionally, IBS is considered a disease of brain-gut dysfunction affecting visceral sensation, conception of pain, and secretomotor function of the GI tract. Psychological distress, dysbiosis, GI infection, and female gender are associated with IBS<sup>2,3</sup>.

Different studies have demonstrated the role of immune system in the development of IBS. Measuring immune signaling molecules such as cytokines and counting the immune cells in the intestine of IBS patients have provided some evidence for the activation of immune system in these patients<sup>4-6</sup>. Studies have demonstrated prominent changes in immune system signaling in IBS in the presence of psychological and central nervous system comorbidities<sup>7-9</sup>. Moreover, changes in the immune signaling during post-infectious IBS (PI-IBS) support the crucial role of immune system dysregulation and inflammation in IBS, where there is a shift in the phenotype of circulating immune cells to a T helper-1 predominating<sup>10</sup>.

Whether IBS is a disorder within the spectrum of inflammatory bowel disease (IBD) is not proven, as all changes in the immune system of IBS patients are at molecular levels and no gross inflammation is observed in these patients. Disorders such as microscopic colitis<sup>11</sup>, GI mastocytosis, mast cell activation<sup>12</sup>, eosinophilic gastroenteritis<sup>13</sup>, celiac disease, and small intestinal bacterial overgrowth manifest with symptoms of IBS, and all share a dysregulated immune system in their pathophysiology, at least at the level of the GI tract. Studies, mostly in children, have revealed controversial findings about the potential effects of food allergy or intolerance in IBS14. It is not clear whether food allergy in IBS is a feature of immune activation.

The current review: (a) covers literature on the role of immune system, (b) outlines the potential therapeutic and diagnostic approaches through involving the patients' immunological profile, and (c) highlights the future directions of research on the role of immune system in IBS.

## **Cytokines in IBS**

Cytokines are small protein molecules majorly released by the immune cells. Cytokines regulate differentiation, communications, and interactions between cells. T-cells, macrophages, and mast cells are the major sources of cytokines. Cytokines are also released by the immune cells which reside in the peripheral and central nervous system as well as the enteric neuronal plexus. Cytokines released by the Schwann cells and endothelial cells regulate pain, vascular permeability, and inflammation<sup>15</sup>.

Cytokines include lymphokines and monokines which are produced by lymphocytes and monocytes, respectively. A form of cytokine which has chemotactic activities is called chemokine. Interleukins (ILs) are cytokines which are produced by one leukocyte and act on other leukocytes<sup>15</sup>. Cytokines are divided into pro- and anti-inflammatory from the functional perspectives. Tumor necrosis factor alpha (TNF-α), interferon-γ, IL-1, IL-2, IL-6, IL-8, IL-12, IL-17, and IL-18 are pro-inflammatory, while IL-4, IL-10, and transforming growth factor beta (TGF-β) act as anti-inflammatory<sup>5,15</sup>. Table 1 is a summary of major pro- and anti-inflammatory cytokines and their roles as the regulator of the immune system.

Studies on circulating or mucosal cytokine levels in IBS patients versus controls had inconsistent results and were not all methodologically comparable. While some of these studies measured the serum/plasma or mucosal levels/expression (Table 2)<sup>17-26</sup>, others have focused on cytokine production by monocyte or lymphocyte supernatants in the presence or absence of stimulators such as lipopolysaccharides (LPSs)<sup>9,27-30</sup>.

Extraintestinal and psychiatric comorbidities as well as other potential confounders such as dominant symptom and the severity of symptoms have not been sufficiently considered in the design of many of these studies, and the sample sizes are usually small. On top of these, the findings are usually inconsistent between studies. To partially overcome this inconsistency, we performed a meta-analysis on serum/plasma cytokine levels in IBS versus controls which indicated that circulating TNF- $\alpha$  tends to be higher in IBS and reaches significance in female IBS patients and based on IBS subtypes. Circulating IL-10 is significantly lower in male patients with IBS, and colonic IL-10 mRNA has a significantly lower expression in IBS compared to controls in the overall meta-analysis.<sup>6</sup> In a meta-analysis, we have shown that circulating IL-6 levels are significantly increased in IBS compared to controls. This significance was sustained in IBS-D versus controls, while the IL-6 levels were comparable in the other IBS subtypes compared to controls<sup>36</sup>. Based on these findings, the overall picture of cytokine profile in IBS favors a more pro-inflammatory phenotype, although these findings need to be confirmed in larger multicenter studies after adjusting the study subjects on confounding variables such based

Pro-inflammatory	Anti-inflammatory
IFN- $\gamma$ : increases MHC $^{\dagger}$ class I and class II expression and activates macrophages	IL-4: suppresses macrophage cytotoxicity, antagonizes IFN- $\gamma$ expression, stimulates B/T-cell proliferation, and suppresses the development of Th1 cells
IL-1 $\alpha$ and IL-1 $\beta$ : helps with the differentiation of CD4 T-cell, histamine release from mast cells, and proliferation of mature B-cells, PGE2 <sup>tt</sup> synthesis and increases IL-2 expression	IL-10: inhibits IFN- $\gamma$ production, suppresses Th1 response, and inhibits synthesis of IL-1 $\beta$ and NFKB nuclear translocation
IL-2: activates T-cells, B-cells, and NK cells	IL-11: inhibits pro-inflammatory cytokines and promotes Th2 response
IL-6: stimulates acute phase protein synthesis, and promotes activation and differentiation of plasma cells and T-cells	IL-13: inhibits inflammatory cytokine production and attenuates the activation of macrophages
IL-8: chemoattracts leukocytes	$TGF\mathcal{GF}\beta$ : inhibits monocyte/macrophage MHC class II expression and the synthesis of pro-inflammatory cytokines
IL-16: chemoattracts CD4 T lymphocytes	
IL-17: stimulates the expression of IL-6, IL-8, and ICAM $^{\dagger\dagger\dagger}$	
IL-23: activates CD4 memory T-cells and induces IL-17 and IL-6	
$\text{TNF-}\alpha\text{:}$ stimulates PGE2 release as well as acute and chronic inflammation and induces apoptosis and the release of acute phase reactant proteins	
Retrieved and modified from Darkoh et al. <sup>16</sup> <sup>1</sup> Major histocompatibility complex; <sup>11</sup> Prostaglandin E2; <sup>111</sup> Intercellular adhesion molecule; TNF- <i>c</i> : tumor necrosis factor alpha; IL: interleukins	

#### TABLE 1. The functional aspects of pro- and anti-inflammatory cytokines

as psychiatric comorbidities, diet, and genetic backgrounds. The absolute cytokine levels in different studies are hugely variable, suggesting that the laboratory methods and kits should also be standardized. Alternatively, these variabilities might be due to population differences<sup>31,37</sup>.

One may argue that changes in cytokines in IBS should be an acquired phenomenon. On the other hand, there are case-control studies on cytokine gene polymorphisms in IBS versus controls, suggesting that these cytokine changes in the IBS patients are somehow driven by a genetic background. A list of studies on cytokine gene polymorphisms in IBS can be found in two published reviews<sup>5,38</sup>. Moreover, Villani et al.<sup>39</sup> showed that the IL-6 gene polymorphism is associated with PI-IBS, but

at the same time we have to consider that all of these patients were also exposed to a GI infection. Therefore, a combination of acquired and genetic factors may be translated to changes in circulating or mucosal cytokine levels.

The same as studies on cytokine levels, studies on cytokine gene polymorphisms in IBS are not methodologically consistent. As the polymerase chain reaction method for the detection of single nucleotide polymorphisms (SNPs) is a standardized one and the results are in the form of qualitative variables, the discrepancy may go back to the source populations or potential differences in the selection of cases and controls. We recently performed a meta-analysis on some cytokine gene polymorphisms in IBS. Accordingly, the

Study	Sample	Cytokines in IBS versus control	
Barbaro <sup>32</sup>	Colon biopsy	IFN-γ↑	
Chang <sup>17</sup>	Serum	TNF- $\alpha \rightarrow$ , IL-1 $\beta \rightarrow$ , IL-6 $\rightarrow$ , IL-8 $\rightarrow$ , IL-12 $\rightarrow$ , IL-10 $\rightarrow$	
Darkoh <sup>16</sup>	Serum	TNF-α↑	
Dinan <sup>7</sup>	Serum	IL-6↑, IL-8↑, TNF- $\alpha$ →, IL-10→	
Gwee <sup>††33</sup>	Colon biopsy	IL-1β↑	
Macsharry <sup>26</sup>	Colon biopsy	$\begin{array}{l} TNF-\alpha \rightarrow \text{, } IL-1\beta \downarrow \text{, } IL-6 \rightarrow \text{,} \\ IL-8\downarrow \text{, } IL-12 \rightarrow \text{, } TGF-\beta \downarrow \text{, } IL-10\downarrow \end{array}$	
Rana <sup>22</sup>	Serum	TNF-α↑, IL-6↑	
Schmulson <sup>23</sup>	Serum	IL-10↓, TNF-α↑	
Scully <sup>34</sup>	Plasma	$\begin{array}{l} IFN\text{-}\gamma {\rightarrow},  TNF\text{-}\alpha^{\dagger}\uparrow,  IL\text{-}1\beta^{\dagger}\uparrow, \\ IL\text{-}6\uparrow,  IL\text{-}8\uparrow,  IL\text{-}12{\rightarrow},  IL\text{-}13{\rightarrow}, \\ IL\text{-}10{\rightarrow} \end{array}$	
Seyedmirzaee <sup>24</sup>	Serum	TNF-α↑, IL-6↑, IL-8↑	
Vazquez-Frias <sup>35</sup>	Plasma	$TNF-\alpha \rightarrow$	

TABLE 2. Selected studies on cytokine levels in patients with	1
irritable bowel syndrome <sup>5,31</sup>	

No difference →; increased ↑; decreased ↓; N.A: not available; <sup>†</sup>With extraintestinal comorbidities; <sup>††</sup>in post-infectious irritable bowel syndrome;

TNF-α: tumor necrosis factor alpha;

IL: interleukins

high producer IL-10 (-1082 G/G) was associated with a decreased odds of IBS. Moreover, carriers of the intermediate producer TGF- $\beta$ 1 (+915 G/C) genotype had a tendency toward having less IBS. The analysis of Asian studies revealed an association between the TNF (-308 G/A and G/G) genotypes and IBS38. On the other hand, the meta-analysis of IL-6 (-G174C) polymorphism revealed no differences in the distribution of the studied genotypes or alleles in the IBS compared to controls<sup>36</sup>.

Source population, environmental factors, and methodological/technical variables in the measurement of cytokines may induce variability in the results of original studies. It is also important to mention that as IBS affects the GI tract with a low-grade inflammation at cellular/molecular levels, the scale of immune activation is not sufficient to be detected systemically. Therefore, it is difficult to find a consistent cytokine profile in the blood of heterogeneous IBS patients who are at different phases of the disease and may also have other comorbidities. In summary, while the exact cytokine profile in IBS has not been defined, there is an imbalance favoring a pro-inflammatory phenotype.

The studied gene polymorphisms do not necessarily predict the cytokine profile, suggesting that some of the changes are acquired or mediated by other SNPs which have not been sufficiently studied. Genome-wide association studies may help in understanding many of these phenomena. Based on a GWAS study published in 2015, the 7p22.1 locus, which includes the KDEL endoplasmic reticulum protein retention receptor-2 (KDELR2) and glutamate receptor, ionotropic, delta 2 interacting protein (GRID2IP) encoding genes, showed consistent risk effects for IBS and was associated with a trend for increased mucosal KDLER2 mRNA expression. In addition, the rs3917265 in the (IL-1 receptor type 1) IL1R1 and rs2243250 in the IL-4 genes were associated with IBS as well. The future studies should address whether these findings could be replicated or could be linked with the mRNA expression of the studied genes<sup>40</sup>. To understand the underlying mechanism of the pro-inflammatory phenotype of IBS which is represented by the imbalance of cytokine levels and production, it is crucial to study the potential changes in immune cell counts and function in these patients.

TABLE 3. Colonic immune cell counts in irritable bowel

### Immune cells in IBS

The majority of studies on immune cells in IBS have focused on counting them in intestinal biopsies of patients with IBS and compared them to that of controls. Mast cells and lymphocytes are the major cells which have been counted in IBS patients. Many of these studies are focused on the colon<sup>17,41-59</sup>, while a few studies have counted immune cells in the small bowel<sup>56,60-64</sup>. Table 3 lists some selected studies that have analyzed mast cells and lymphocytes in the colonic mucosa of patients with IBS.

Recently, we conducted a meta-analysis on mast cell and lymphocyte counts in the colonic mucosal layer and lamina propria of IBS patients versus controls<sup>67</sup>. This meta-analysis showed an increased in the mast cells of the rectosigmoid and descending colon of IBS patients. This observation was found both in constipation (IBS-C) and diarrhea (IBS-D) predominant IBS. Based on this meta-analysis, CD3+ T-cells were increased in the rectosigmoid and the descending colon of patients with IBS. CD4+ but not CD8+ T-cells were also increased in the colon of IBS patients.

In addition to studies which counted immune cells in intestinal biopsies of IBS patients, few studies have examined the activation of the immune cells in IBS through looking at their phenotype. Ohman et al. showed that both blood B-cells and T-cells are overactivated in IBS patients. In brief, IBS patients had increased T-cells expressing CD69 and integrin beta7/HLA-DR, while anti-CD3/CD28-stimulated T-cells in blood and colonic tissues from IBS patients proliferated less compared to controls. Moreover, B-cells

Study	Counted cells	Region
Ahn et al.41	Mast cells; T-cells	AC, DC, RS
Akbar et al.42	Mast cells; T-cells	RS
Bian et al.65	Mast cells	DC
Balestra et al.43	Mast cells	DC
Barbara et al.44	Mast cells	DC
Barbara et al.45	Mast cells	DC
Braak et al.46	Mast cells; T-cells	AC, DC
Cenac <sup>47</sup>	Mast cells	AC, RS
Chadwick et al.48	Mast cells; T-cells	AC, DC, RS
Chang et al. <sup>17</sup>	Mast cells; T-cells	RS
Coeffier et al.49	Mast cells	DC
Cremon et al. <sup>51</sup>	Mast cells	DC
Cremon et al. <sup>50</sup>	Mast cells	DC
Dunlop et al. <sup>52</sup>	Mast cells; T-cells	RS
El-Salhy et al. <sup>53</sup>	Mast cells	RS
Kerckhoffs et al.54	Mast cells	RS
Lee <sup>66</sup>	Mast cells	RS
Ohman et al. <sup>59</sup>	T-cells	RS
O'Sullivan <sup>55</sup>	Mast cells	AC
Park <sup>56</sup>	Mast cells	AC, RS
Sohn et al.57	Mast cells	RS
Sundin et al.58	T-cells	RS

RS: rectosigmoid/rectal

isolated from the blood of IBS patients had an increased cell surface expression of immunoglobulin G, CD80, and CD86. The ability of B-cells to increase expression of CD80 in response to LPS was impaired in IBS68,69. In a recent study, Rodriguez-Fandino et al. showed that M1-Early cells without LPS stimulation had lower CD11c expression, while the percentage of intermediate (CD-11c+CD206+CX3CR1+) cells in cultures without LPS was higher in IBS compared to

controls suggesting a more advanced maturation phenotype of peripheral blood monocytes/macrophages in IBS patients<sup>70</sup>. Regional mast cell activation has also been shown in IBS. Tryptase, a marker of mast cell activation, is increased in the jejunal fluid of IBS-D patients compared to controls and mast cells spontaneously release more tryptase and histamine in these patients<sup>44,61</sup>.

In summary, immune cell counts are altered in intestinal biopsies of IBS patients. More specifically, mucosal mast cells and lymphocytes are increased in the colon of IBS patients compared to controls. Moreover, blood B-cells and T-cells and intestinal mast cells are overactivated in IBS. In addition, advance maturation of macrophages in IBS patients suggests a functional role for these cells in this disorder. Of note, matured macrophages could be a source for immune signaling molecules such as TNF- $\alpha$  in these patients.<sup>70</sup> Therefore, there is a very mild form of inflammation in IBS. We need to understand whether this form of inflammation could be translated into non-invasive inflammatory biomarkers such as acute-phase reactants in the blood or stool of IBS patients.

# Biomarkers of inflammation

Whether commercially available biomarkers of inflammation could be used to differentiate IBS from healthy controls is of a great clinical value. This concept has been extensively reviewed by Menees et al. C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), fecal calprotectin, and fecal lactoferrin were 4 biomarkers that were reviewed by these researchers. Increased IL-1 and IL-6 enhances CRP levels through targeting CRP production site, i.e. hepatocytes. As there is an increase in pro-inflammatory cytokines in IBS, increased CRP might be found in these patients as well. ESR also increases through the systematic effects of inflammation on the liver and immune cells. The overproduction of proteins involved in the aggregation of red blood cells increases ESR. Two other markers of intestinal inflammation are lactoferrin and calprotectin which are secreted by activated neutrophils into the feces<sup>71</sup>.

After including 12 studies in their meta-analysis, Menees et al. found no role for CRP, ESR, fecal lactoferrin, and calprotectin in distinguishing IBS from healthy controls; however, a CRP ≤0.5 or calprotectin ≤40µg/g could differentiate IBS from IBD71. The findings of this meta-analysis as well as original studies should be interpreted with caution. The number of included studies in this meta-analysis was small for individual biomarker comparisons, and not all studies have the 3 comparison groups, i.e. IBS, IBD, and healthy controls. Careful analysis of Menees et al.'s study<sup>71</sup> (Figs. 2-5) shows that the predictive probability of being a healthy control or having IBS at different levels do not have a real overlap at least numerically, suggesting that there might be a difference between IBS and healthy controls based on these biomarkers. All these biomarkers in differentiating IBS patients from healthy people should be compared in larger well-controlled studies.

Some studies have evaluated the role of high-sensitive (HS) CRP as a marker of inflammation in IBS. These studies indicated that HS-CRP is higher in IBS patients compared to healthy controls and these levels were highest in IBS-D and in those patients with greater IBS severity<sup>72,73</sup>. These findings also need to be confirmed in larger studies. Based on the latest publication<sup>73</sup>, the median HS-CRP levels in the IBS group and healthy controls were 1.80 and 1.20 mg/L, respectively, with large interquartile ranges questioning the clinical value of HS-CRP in differentiating IBS from healthy controls.

While immune biomarkers might be helpful in differentiating IBS from diseases like IBD which have a much visible pathology and a pro-inflammatory profile, the future studies should focus on finding potential immune-based biomarkers including acutephase reactants of IBS which may help us with the diagnosis of this functional GI disorder as well as the prediction of response to anti-inflammatory therapies.

# Food allergy in IBS patients

Non-celiac gluten sensitivity (NCGS) is a disease which presents with IBS-like symptoms. Furthermore, there has been an increasing interest in gluten-free and low fermentable oligo-di-mono-saccharides and polyols diets in treating IBS patients<sup>14,74</sup>. Whether these food sensitivities are mediated by the immune system is still under debate. Food antigens may activate mast cells; however, skin tests for food allergy are not sufficiently sensitive and may give negative results despite the therapeutic value of eliminating specific food elements from the diet in IBS patients<sup>74</sup>. Direct administration of diluted food antigens into the duodenal mucosa increases intraepithelial lymphocytes in some IBS patients<sup>75</sup>. Moreover, in vitro exposure of the basophils to dietary proteins may or may not activate these immune cells<sup>75,76</sup>. More studies are needed to confirm the role of food allergy and concomitant immune activation in IBS.

# Anti-inflammatory medications for IBS

By considering IBS, a disorder with low-grade inflammation, it would be crucial to examine whether anti-inflammatory medications are helpful in this condition.

In a pilot randomized, double-blind, placebo-controlled study, mesalazine (800 mg, 3 times daily for 8 weeks) significantly reduced colonic mast cell infiltration in IBS and increased patients general well-being but had no substantial effects on IBS symptoms<sup>77</sup>. The same group extended their study through conducting a phase 3 multicentre trial with the same dose of mesalazine but for 12 weeks. With a total of 185 IBS patients included, this study showed that mesalazine was not superior to placebo in relieving abdominal pain/discomfort for at least 50% of the weeks of the treatment period. However, when the authors considered affirmative answers to the question "Did you have satisfactory relief of your abdominal discomfort or pain during the last week?" within ≥75% of the treatment time as "positive response," mesalazine was reported as being helpful in IBS<sup>78</sup>. Another randomized clinical trial with mesalazine (2 g twice daily for 12 weeks) in 136 IBS-D patients showed no improvement of abdominal pain, stool consistency/frequency, or percentage of patients with satisfactory relief of IBS symptoms<sup>79</sup>. In a study by Andrews et al., 8 of 14 IBS patients had a satisfactory response to mesalazine with substantial decrease in days with discomfort or disturbed bowel movement<sup>80</sup>. Studies on mesalazine in PI-IBS are underpowered and controversial. While some of them show it is effective<sup>79,81</sup>, others have shown no substantial improvement in the global symptoms or quality of life with mesalazine in these patients<sup>82</sup>.

Based on a randomized, double-blind, placebo-controlled trial on 29 PI-IBS patients with prednisolone (30 mg/day), lamina propria T-lymphocytes significantly decreased after prednisolone; however, no significant improvement in symptoms including abdominal pain, diarrhea, stool frequency, and urgency was observed<sup>83</sup>.

Increased mast cells in IBS may be associated with higher histamine release in IBS patients. Therefore, antihistamines or mast cell stabilizers could be beneficial in IBS. Cromolyn sodium appears to be antidiarrheal in IBS<sup>84-86</sup>. Another study showed that cromolyn sodium decreases abdominal pain in IBS without affecting the bowel movements<sup>87</sup>. Based on another study, the mast cell-stabilizer ketotifen decreased abdominal pain and improved the quality of life of the IBS patients<sup>88</sup>. Moreover, the histamine 1 (H1)-receptor blocker ebastine decreases abdominal pain scores and improves symptom in IBS<sup>89</sup>.

Therefore, the body of literature does not always support anti-inflammatory and anti-allergic medications as potential therapies for IBS. The number of studies and their sample size are small. Categorizing the data based on IBS-subtypes, comorbidities, presence of PI-IBS, and immunological profiles such as intestinal immune cell counts or blood/ mucosal cytokine levels would change this conclusion.

#### CONCLUSIONS AND FUTURE DIRECTIONS

Cytokines are imbalanced favoring mostly a pro-inflammatory profile in IBS. Intestinal mast cell and lymphocyte counts are modestly increased in IBS supporting a low-grade inflammation in these patients. All these changes are not substantial, and there is always an overlap in the cytokine profile or intestinal immune cell counts of IBS patients and their healthy controls. Therefore, a clear cutoff limit could not be easily defined for any of these parameters. This overlap could be eliminated by categorizing IBS patients based on the bowel habit subtypes, comorbidities, diet, gender, genotype, or other confounding factors such as the presence of a previous enteric infection (i.e. PI-IBS). Stress, depression, and psychiatric comorbidities are common in IBS and are linked with the immunological profile of these patients. Therefore, neuroimmune interaction should always be considered when interpreting the results of studies on immunological profile of IBS. The concept of PI-IBS is very important and may substantially be linked with changes in the immunological profile of IBS patients. As discussed, the immunological changes of IBS are not just limited to IBS-D and should also be studied in other IBS subtypes.

With regard to the acute phase proteins and the biomarkers of inflammation in IBS, there are no sufficient data to support any of them in differentiating IBS from healthy controls. The future studies on the immunological profile of IBS patients should appropriately exclude patients with diseases such as microscopic colitis, NCGS, or intestinal mastocytosis. The controls should appropriately represent the healthy population and match with the cases. What recommended for the future is categorizing IBS patients based on parameters such as cytokine, mast cells, or hsCRP levels. By considering a right diagnostic immunology panel, a new diagnosis such as "low-grade inflammatory IBS" should be considered for patients who fulfil the diagnostic criteria of IBS.

The value of subcategorizing IBS patients based on their immunological profile would be more important when anti-inflammatory medications are considered as a treatment option in these patients. Overall, no promising response to medications such as mesalamine could be based on combining data of low-grade inflammatory with non-inflamed IBS patients. A potential hypothesis which should be tested is whether patients with low-grade inflammatory IBS have a better response to anti-inflammatory medications compared to IBS patients without inflammation.

Finally, the field of immune system activation in IBS is not conclusive at this step. Larger studies conducted by an international consortium would help to answer many unknown questions in the field.

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