

IRRITABLE BOWEL SYNDROME: PERIPHERAL MECHANISMS AND THERAPEUTIC IMPLICATIONS

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Abstract

Irritable bowel syndrome (IBS) is the most common functional gastrointestinal disorder, affecting about 10 to 20% of the population in developed countries. The mechanisms underlying the symptoms of this condition are poorly understood. Considered initially as the consequence of abnormal gut motility, visceral hypersensitivity, psychosocial factors and brain-gut axis dysfunction, IBS is now acknowledged as a multifactorial disorder. Specific peripheral mechanisms are involved, including mucosal immune activation, increased intestinal permeability, entero-endocrine cell products, an excess of bile acids, gut dysbiosis. A better understanding of these mechanisms could help develop new and specific therapeutic pathways in patients suffering from IBS.

Keywords: irritable bowel syndrome, pathogenesis, peripheral mechanisms.

Background

Irritable bowel syndrome (IBS) is a functional disorder of the gastrointestinal tract that is characterized by abdominal pain or discomfort associated with a change in bowel habit [1]. IBS is a common disease, being responsible of 25 to 50% of all referrals to gastroenterologists [2]. However, not only gastroenterologists are sought for medical attention regarding this condition. A study on IBS conducted in two counties of Romania showed that the majority (66%) of general practitioners estimated the prevalence of this condition as 1 to 10% in their practice [3].

A recent meta-analysis which included 80 studies and 206,960 patients revealed a global prevalence of IBS of 11.2%, depending on the country and the diagnostic criteria used [4]. In Romania the estimated prevalence is high, between 10 to 14.9% [4], emphasizing the importance of understanding the disease. The seriousness of IBS and other functional gastrointestinal disorders is also highlighted by their significant economic impact on health care [5].

Even though this condition has long been of interest in the literature, its pathogenesis is still unknown. IBS is considered to be the result of the interaction between psychological and physiological factors (Figure 1). This article intends to provide a short insight into the peripheral mechanisms involved in the pathogenesis of IBS and their therapeutic implications.

Conventional pathogenic mechanisms

The three pathogenic theories traditionally considered to be involved are abnormal gut motility, visceral hypersensitivity and psychosocial factors. Intestinal dysmotility represents the first anomaly identified in IBS. Scintigraphic studies have showed that about 32% of IBS patients have an abnormal intestinal transit [6]. A quarter of the patients with constipation-predominant IBS (IBS-C) have slow colonic transit, while 15 to 45% of diarrhea-predominant IBS (IBS-D) have accelerated intestinal transit [6], the latter also presenting a greater frequency of high amplitude propagating contractions as compared to healthy controls [7]. Despite the altered transit, the colon transit time does not seem to correlate with abdominal pain, bloating or flatulence, as shown by Törnblom et al [8].

Visceral hypersensitivity, a key mechanism in IBS, is thought to be the consequence of an abnormal interaction of the central and peripheral nervous system [9]. It was first documented in 1973 by Ritchie, who reported that rectal distention provoked pain in 55% of IBS patients compared to 6% of controls [10]. According to some authors, the IBS population is heterogeneous: 80-90% of patients have visceral hypersensitivity, 30-50% present both visceral and somatic hypersensitivity and 10-20% have a normal sensitivity threshold [11]. A dysfunction of the brain-gut axis, comprised of the enteric nervous system (ENS) in the periphery, central nervous system (CNS) and of hypothalamic-pituitary-adrenal axis, is also incriminated in the pathogenesis of IBS [12]. However, none of these mechanisms can completely explain the pathogenesis

of IBS, leading to other specific peripheral factors to be unveiled.

Inflammation and immune activation

Although initially considered a purely functional disease with no structural abnormalities, recent growing body of evidence demonstrates the presence of intestinal mucosal inflammation in IBS patients, raising the question whether IBS is in fact a low-grade inflammatory bowel disease. It is hypothesized that intestinal immune activation, characterized by inflammatory cells infiltration and production of mediators that sensitize primary afferent neurons, leads to visceral hypersensitivity and, therefore, pain in IBS subjects [13].

Particular emphasis has been placed on *mast cells* ever since the early 1960s, when Hiatt and Katz reported an increased number of mast cells in the colonic muscularis propria of patients with “spastic” colitis [14]. Mast cells are innate immune cells that undergo a degranulation process, releasing compounds such as histamine, tryptase, chymase, serotonin, which are thought to stimulate the neurons of the ENS, thus determining muscle contractility [15]. Barbara et al [16] showed not only that there was an increased mast cell number in the colonic mucosa of IBS patients compared to controls, but also that the closer the mast cells were to the colonic nerve endings, the more intense and more frequent was the pain or discomfort. Moreover, there seems to be a positive correlation between the number of mucosal mast cells and intestinal permeability in patients with IBS-D [17], suggesting a link between these two pathogenic mechanisms.

Lymphocytes are key components of the adaptive immune response to pathogens, playing important roles in the organism, including the activation of other types of cells

and stimulation of mucosal healing after injuries. There are discrepancies in the literature regarding increases in gut mucosal lymphocyte numbers. Ohman et al [18] found an increased number of peripheral blood CD4+ and CD8+ T cells expressing elevated levels of gut homing integrin $\beta 7$, as well as more frequent lamina propria CD8 + T cells in the colonic mucosa of IBS patients compared with controls. The authors of a landmark study [19] analyzing full-thickness biopsy specimens of proximal jejunum obtained through laparoscopy from IBS patients reported low-grade infiltration of lymphocytes in the myenteric plexus in 9 out of 10 patients. Moreover, in 6 out of 10 patients neuron degeneration was noticed, highlighting the interaction between the immune and enteric nervous systems.

Data regarding *macrophages* and *eosinophils* are scarce. An increased number of duodenal eosinophils were reported in patients with functional dyspepsia, while a higher frequency of mast cells and intraepithelial lymphocytes was observed in IBS patients [20].

The hypothesis of inflammation and immune activation in IBS is supported by an imbalance in *pro- and antiinflammatory cytokines* [21]. The roles of cytokines IBS are poorly understood [22]. Cytokines may influence gut motility, visceral hypersensitivity, intestinal mucosal permeability and the brain-gut axis. There seems to be a Th-1 proinflammatory state in IBS patients, indicated by a reduced IL-10/IL-12 ratio [23]. This ratio was normalized by treatment with *Bifidobacterium infantis* 35624, which also alleviated the symptoms of patients with IBS [23]. Cytokine gene polymorphisms have also been associated with IBS. Patients with this condition are more likely to present a combination of polymorphisms that result in increased expression of TNF- α and decreased expression of antiinflammatory cytokine IL-10 compared with control

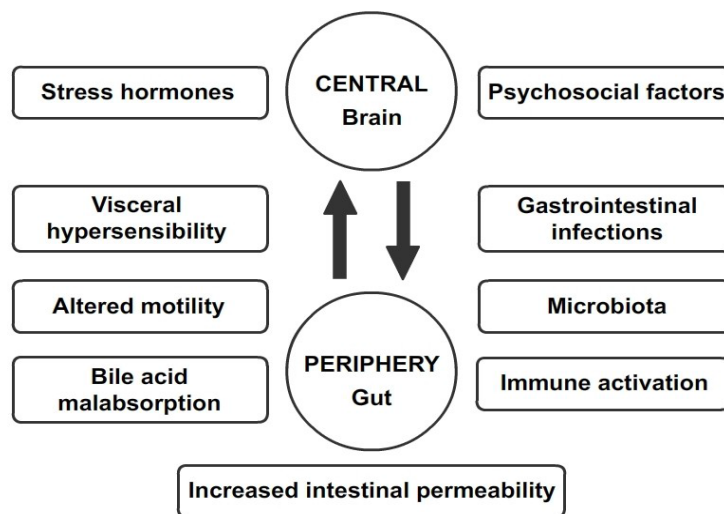


Figure 1. Pathophysiology of irritable bowel syndrome

subjects [24]. In a recent study, an increased expression of the mucosal proinflammatory cytokines IL-8 and IL1 β was positively correlated with an increased Toll-Like Receptors TLR2 and TLR4 expression in IBS-mixed (IBS-M) subtype [25], providing evidence for an inappropriate immune response to gut microbiota alterations. Even more, anxiety and stress are able to stimulate the release of proinflammatory cytokines [26], supporting the concept of brain-gut axis malfunction in IBS.

Enterochromaffin cells and serotonin

Enterochromaffin (EC) cells are entero-endocrine cells of the gastrointestinal tract responsible for 90% of serotonin (5-hydroxytryptamine or 5HT) production in the human body [27]. Serotonin is a key mediator for gastrointestinal secretion and motility and it can also activate afferent nerve endings. Findings regarding an alteration of EC cell number in IBS patients are inconsistent. According to some authors, EC cells significantly increase in the colonic and rectal mucosa of post-infectious IBS (PI IBS) [28,29]. Cremon et al [30] found an increased number of 5HT-positive cells in colonic biopsies from IBS patients compared with controls. Compared with healthy volunteers, serotonin release was 10-fold higher in IBS patients and significantly correlated with mast cell counts and the severity of abdominal pain. These results suggest that an increased serotonin level could contribute to pain development in IBS through mucosal immune activation. However, other authors did not report a difference in EC cell counts in the small intestine and colon of IBS patients compared with controls [31]. More recent work indicated a decreased expression of the 5HT transporter (SERT) in IBS [32-34], suggesting that there might be an alteration in 5HT mucosal signaling in patients suffering from IBS.

Increased intestinal permeability

Intestinal barrier dysfunction has proved to be an important pathogenic mechanism in functional disorders such as IBS [35]. Increased small bowel or colonic mucosal permeability has been documented *in vivo*, in biopsy specimens *in vitro* or in Caco-2 monolayers using various methods. It is believed that an increased intestinal permeability enables bacterial or alimentary antigens to penetrate the intestinal barrier, triggering an inflammatory response, characterized by an influx of immunocompetent cells and release of mediators that are able, in turn, to sensitize primary afferent neurons and determine visceral hypersensitivity. Factors associated with increased mucosal permeability in IBS include atopic disease, cow's milk allergy, gastrointestinal infections, stress, bile salts [36]. An increase in intestinal membrane permeability can be observed in almost 40% of patients with IBS-D [37]. Mast cells and exposure to mast cell tryptase could be responsible for alterations in intestinal mucosal permeability and integrity [17,38]. This increase in intestinal permeability

might also be explained by a reduced expression of the tight junction proteins occludin, claudin-1, zonula occludens-1 in the colonic mucosa of IBS patients [39-41].

Gut microbiota alterations

Gut host-microbial interactions are perceived to be involved in the pathogenesis of IBS. The human microbiota contains about 10^{14} cells, most of which are found in the gastrointestinal tract, especially in the colon [42]. The fact that previously healthy patients develop IBS following exposure to gastroenteritis supports the concept of PI IBS [43]. The mechanisms through which the gut microbiome is involved in IBS are unknown. It is believed that it might alter intestinal motility, secretion, barrier function or the brain-gut axis [44]. There are significant differences in the fecal microbiota of IBS patients compared with controls [45,46]. Jeffery et al [45] reported an increase of Firmicutes-associated taxa and a depletion of Bacteroidetes-related taxa in IBS patients and this alteration correlated with an increase in colon transit time and depression. Quantitative changes in gut microbiota were documented in some studies [47,48], but an association between small intestinal bacterial overgrowth (SIBO) and IBS is still controversial. There are currently no recommendations whether clinicians should routinely test for SIBO in IBS patients. Furthermore, the role of gut microbiota in the pathogenesis of IBS is reinforced by studies that show encouraging results after treatment with rifaximin [49,50] or probiotics [51] in patients with IBS.

Role of toll-like receptors in IBS

Recent findings have shown that Toll-like receptors (TLRs) could be involved in the relationship microbiota-host immune activation in IBS patients. TLRs, members of the pattern recognition receptor family, are transmembrane receptors that recognize pathogen-associated molecular patterns and play an important part in the initiation of innate immune response to a variety of microbial pathogens. The human TLR family comprises of ten TLRs expressed on mucosal surfaces, including on intestinal epithelial cells [52]. The activation of TLRs by endogenous and exogenous ligands can lead to intestinal mucosal inflammation through recruitment of inflammatory cells and inflammatory cytokines. Alterations in the intestinal epithelial cell expression of different TLRs have been documented in inflammatory bowel disease [53]. Given the evidence for low level intestinal inflammation and involvement of dysbiosis in IBS, it has been hypothesized that TLRs could also play a role in the pathogenesis of IBS. Brint et al [54] reported an increased expression of TLR4 and TLR5 and a decreased expression of TLR7 and TLR8 in colonic biopsy samples of IBS patients compared with controls. In another work, significant up-regulation of TLR2 and TLR4 in the epithelial colonic cells was observed in IBS-mixed subtype [25]. In addition, this increased TLR expression in IBS-M

was associated with an enhanced production of the mucosal proinflammatory cytokines IL-8 and IL1 β , supporting the link between microflora and intestinal inflammation. Furthermore, alterations in the expression of TLRs in the colonic mucosa were found in animals with stress-induced IBS symptoms [55], suggesting that stress can modulate innate immune responses in IBS.

Increased levels of intraluminal bile acids

According to a systematic review of the literature, bile acid malabsorption is responsible for approximately 30% of cases of diarrhea-predominant IBS [56]. An excess of intracolonic bile acids appears to be the consequence of an imbalance between the production and absorption of bile acids [57]. An increased level of bile acid in the colon can stimulate motility and secretion, leading to diarrhea. Patients with IBS-D synthesize and excrete higher levels of bile acids than IBS-C patients and controls [58]. One explanation for this observation is a deficiency in fibroblast growth factor (FGF) 19, a hormone produced by ileal enterocytes that downregulates bile acid synthesis [59]. Even more, there might be a genetic predisposition to bile acid malabsorption [58].

Therapeutic implications

IBS is no longer regarded as a purely functional bowel dysfunction. Despite the intensive study of more or less conventional therapies, there is currently no known cure for IBS. Dietary factors are considered to be involved in the pathogenesis of IBS. In a recent study conducted in a county of Romania [60] the authors found a significant association between IBS and the consumption of certain foods such as milk, processed meat, canned food, legumes, whole cereals, herb tea. Food allergy, lactose and gluten intolerance and fermentable oligosaccharides, disaccharides, monosaccharides and polyols (FODMAPs) have all been incriminated in inducing symptoms of IBS.

Dietary recommendations have been made in this respect, even though data are scarce. A single-blind, crossover intervention trial showed that patients on a high FODMAPs diet had a significant increase in gastrointestinal symptoms and lethargy, while healthy volunteers only reported increased flatus production [61]. A randomized controlled four-week trial of gluten-containing diet or gluten-free diet in patients with IBS-D showed that subjects receiving gluten had an increased stool frequency and small bowel permeability, as well as a significant decrease in the expression of tight-junction proteins zonula occludens 1, claudin-1, and occludin in bowel mucosa [62], results that support the link between diet and development of IBS.

Diet, fiber, laxatives and antispasmodics are the most frequently used therapies in IBS. However, with studies providing new insights into the pathophysiology of IBS, other treatments have been developed. **Serotonergic agents** are one of these new therapies. There are seven 5-HT

receptor subtypes [63], but the effects of 5-HT₃ and 5-HT₄ receptor stimulation on gastrointestinal functions have been more extensively studied. Since 5-HT₃ receptor antagonists delay intestinal transit, trials focused on nonconstipated IBS patients. Alosetron and cilansetron proved to be superior to placebo and mebeverine in achieving global improvement in IBS and relief of abdominal pain or discomfort [64]. Alosetron is approved for use and indicated in women with severe IBS and diarrhea only when conventional therapies have failed, because of the drug's association with ischemic colitis and severe constipation. A novel potent and selective 5-HT₃-receptor antagonist in development for use in patients suffering from IBS-D is Ramosetron [65], a drug associated with less side effects than Alosetron and which is already available in Asian countries.

Agonists of 5-HT₄ receptor stimulate the release of neurotransmitters such as acetylcholine, and increase mucosal secretion and colonic motility. Therefore, studies focused on their use in patients with IBS with constipation. The prototype of 5-HT₄ receptor agonist is tegaserod, which significantly improved symptoms in women with IBS-C [66]. However, important cardiovascular side effects of tegaserod led to the development of new 5-HT₄ agonists with more specificity for intestinal 5-HT₄ receptors, such as prucalopride, velusetrag and naronapride [67].

Since evidence suggests that gut microflora plays an important role in the pathophysiology of IBS and given the beneficial effect of the nonsystemic oral antibiotic **rifaximin** in SIBO [68], studies focused on rifaximin also as a treatment in IBS. Two phase III, double-blind, placebo-controlled trials (TARGET 1 and TARGET 2) that included IBS patients without constipation randomly assigned to rifaximin or placebo showed that rifaximin significantly improved symptoms like abdominal pain, bloating and diarrhea [69]. Furthermore, in a recent study oral rifaximin not only altered the composition of ileum microflora in rats, leading to an abundance of *Lactobacillus* species, but also prevented mucosal inflammation, intestinal barrier abnormalities and visceral hyperalgesia in response to chronic stress [70].

Probiotics are defined as live microorganisms, which, when administered in certain amounts, confer a health benefit on the host. The rationale behind the use of probiotics in IBS is provided by the observations that intestinal microflora significantly differs in patients with IBS compared to healthy controls [44-46,71]. Probiotics might exert beneficial effects through various actions like inhibition of pathogen binding to epithelial cells, enhancement of barrier function and reduction of intestinal permeability, anti-inflammatory effects, influence on colonic motility and visceral hypersensitivity [72]. A recent evidence-based international guide on the use of probiotics in lower gastrointestinal complaints [73] concluded that specified probiotics should be tried in alleviating overall symptoms and abdominal pain in IBS, especially in the

subtype with diarrhea. Nevertheless, the grade of evidence for the effects of probiotics on flatulence, bloating, distention or in the IBS subtype with constipation is low to moderate [73].

Anti-inflammatory agents were also studied in the treatment of IBS. A small randomized, double-blind, placebo controlled trial [74] found a significant reduction in colonic mucosal immune cells and mast cells after 800 mg mesalazine three times daily for 8 weeks, with an increase in general well-being in IBS patients, even though no significant effect was noticed on abdominal pain and bloating. A recent study that evaluated the effects of mesalazine alone, mesalazine with probiotic *Saccharomyces boulardii* or *Saccharomyces boulardii* alone on symptoms of IBS-D patients showed a significant improvement in symptom score in all three treatment groups [75]. However, the improvement of the symptom score was greater with mesalazine alone or combined with *Saccharomyces boulardii* as compared with *Saccharomyces boulardii* treatment alone. These results support the immune activation hypothesis in the pathogenesis of IBS. Moreover, other authors found that mast cell stabilizer ketotifen increased the threshold for discomfort by rectal balloon distention in patients with IBS with visceral hypersensitivity, reduced IBS symptoms and improved health-related quality of life, remaining to be investigated whether this effect was secondary to the mast cell stabilizing properties of ketotifen or histamine H-1 receptor blockade [76].

In view of recent pathogenic theories regarding bile acid malabsorption in IBS, **bile acid modulation** has also been studied in the treatment of IBS. In a double-blind placebo-controlled trial [77], administration of oral sodium chenodeoxycholate, a primary bile acid, in women with IBS-C resulted in accelerated colonic transit and increased stool frequency. Moreover, treatment with chenodeoxycholic acid was more effective in accelerating transit in subjects with lower bile acid synthesis rates, as suggested by lower levels of 7 α C4 (7 α -hydroxy-4-cholesten-3-one), a surrogate marker for bile acid synthesis.

Alternative forms of therapy for IBS have been suggested, such as herbs, mind-body therapies [78], acupuncture [79], transcutaneous interferential electrical stimulation [80], tripolar spinal cord stimulation [81]. However, their role is uncertain.

Conclusion

IBS is now being recognized as a multifactorial disease in which both central and peripheral pathophysiological mechanisms are involved. The ideal situation would be the development of simple and non-invasive diagnostic tests for this condition. Moreover, a better understanding of the mechanisms that lead to IBS could help develop pathogenesis-oriented therapy and improve patient approach. Further research is required to shed more light on this complex disorder.

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