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REVIEW

Need for a comprehensive medical approach to the neuro-immuno-gastroenterology of irritable bowel syndrome

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Abstract

Irritable bowel syndrome (IBS) is defined by the Rome Ⅲ criteria as symptoms of recurrent abdominal pain or discomfort with the onset of a marked change in bowel habits with no evidence of an inflammatory, anatomic, metabolic, or neoplastic process. As such, many clinicians regard IBS as a central nervous system problem of altered pain perception. Here, we review the recent literature and discuss the evidence that supports an organic based model, which views IBS as a complex, heterogeneous, inter-dependent, and multi-variable inflammatory process along the neuronal-gut axis. We delineate the organic pathophysiology of IBS, demonstrate the role of inflammation in IBS, review the possible differences between adult and pediatric IBS, discuss the merits of a comprehensive treatment model as taught by the Institute of Functional Medicine, and describe the potential for future research for this syndrome.

Key words: Irritable bowel syndrome; Abdominal pain; Inflammation; Probiotics; Stress

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INTRODUCTION

Functional abdominal pain (FAP) and irritable bowel syndrome (IBS) are debilitating and common conditions. IBS is defined by the Rome Ⅲ criteria as, "symptoms of recurrent abdominal pain or discomfort and a marked change in bowel habit for at least six months, with symptoms experienced on at least three days of at least three months, with two of the three following findings: (1) Pain is relieved by a bowel movement; (2) Onset of pain is related to a change in frequency of stool; and (3) Onset of pain is related to a change in the appearance of stool"[1].

FAP occurs in 10%-15% of school-aged children, of which 17%-24% have pain significant enough to disrupt their activity, and 13%-15% awaken from their sleep due to the pain[2,3]. Up to 53% of children with abdominal pain continue to have abdominal pain as adults, and 18% are ultimately diagnosed with IBS[4].

Chronic abdominal pain is associated with significant morbidity, including depression[2], decreased quality of life measures[5], and disability leading to inability to work[6]. Adults with abdominal pain have higher rates of potentially unnecessary surgeries[7-10]. Patients with IBS and FAP are costly to the medical system[11,12]. Both children and adults with IBS frequently visit the offices of primary care physicians and gastroenterologists[13]. Adults with IBS

have significantly more hospitalizations, outpatient visits, diagnostic testing, and overall medication use than well patients[14]. A large percentage of the medical costs associated with IBS are related to hospitalizations and inpatient diagnostic testing, such as endoscopies[15]. Antidepressants and other neuropharmacological agents help the symptoms of IBS[16], but these treatments have their own limitations and potential adverse effects.

IBS is thought to be just a functional problem that is "without demonstrable evidence of a pathological condition such as an anatomic, metabolic, infectious, inflammatory, or neoplastic disorder"[17]. IBS is seen as a non-organic syndrome, primarily involving altered perception and processing of pain. As a result, the majority of current therapies for IBS revolve around stress reduction, alteration of pain pathways, and alleviation of symptoms[16].

In this literature review, we delineate the gastrointestinal-neuro-immune pathophysiology of IBS and discuss the link between inflammation and pain. We believe that more effective treatment models are possible through a patient-centered approach that simultaneously treats the multiple variables that lead to IBS, as addressed in this review. The integration of this IBS treatment model may improve patient outcomes while reducing the medical cost burden of IBS. This paper will also discuss the possible differences between adult and pediatric IBS and present potential areas of future research.

STRESS AND THE GASTROINTESTINAL-NEURO-IMMUNE AXIS

Stress in various forms predisposes individuals to developing IBS[18-20] and increases IBS symptoms in children[21]. Abuse or other significant stressors change the neurobiology of stress and alters the levels of corticotropin-releasing factor (CRF) or hormone (CRH)[22], a hypothalamic stress hormone. CRF activates the pituitary-adrenal axis and mediates behavioral, autonomic, immune, and visceral responses to stress[23]. Patients with IBS have enhanced stress responses and release higher amounts of CRF in response to stress[24].

Stress changes the physiology of the gastrointestinal tract. Maternal separation of rat pups causes CRF-mediated mucosal barrier dysfunction with macromolecular permeability and increased bacterial adherence/penetration of the gastrointestinal mucosa with translocation to the spleen[25]. These animals also have mitochondrial swelling of the gut epithelial cells, immune cell infiltration, mucus depletion, and mast cell degranulation[23,26-29]. Stressed human beings show similar findings[30].

Stress compromises the integrity of the gut and induces inflammation through numerous pathways, as demonstrated by several published papers[22,28,31]. CRF released from the hypothalamus can directly influence human colonic mast cells[32,33], which then induce intestinal epithelial pathophysiology and mucosal barrier defects[34-37]. Substance P (SP) and calcitonin gene-related peptide (CGRP)-containing gastrointestinal efferent neurons can also influence mast cells[38-41] and result in degranulation[42] and release of TNF-α[43]. These compounds, in turn, result in gut inflammation and intestinal permeability[44].

These stress-induced changes in the gastrointestinal tract "persist after the stressor is removed from the animal"[37]. This is likely to be due to the ability of mast cells to influence their environment. In rats, inflammation results in increased mast cell-neuronal contacts and mucosal nerve cell density that last well beyond the initial insult[43,45]. Gastrointestinal inflammation in humans also results in neuron proliferation[46-48]. Stress and inflammation modulate nerve growth factor (NGF), which then affects mucosal nerve remodeling[49,50], sprouting, and synaptogenesis[51]. Mast cells, in close contact with neurons, synthesize and release NGF, and thus, can alter neuronal density and synaptogenesis[49,52].

Furthermore, inflammation preceding a psychological stress can alter the epithelial response to stress signals and make the gut more susceptible to stress[53]. In addition, inflammation can change the morphology of mast cells and their intracellular contents, further changing the susceptibility of the gut to various future stressors[54-57].

Inflammation can play an important part in the manifestation of IBS symptoms[58]. Once the inflammatory cascade is activated, this immune response can create a vicious cycle of self-perpetuating inflammation. Activated mast cells can directly release CRF[59]. Patients with inflammatory bowel disease (IBD) and IBS have CRF-immunoreactive macrophages, enterochromaffin cells, lymphocytes, neutrophils, and eosinophils, which are present in higher concentrations than in healthy controls[60-63]. CRF induces lymphocyte proliferation[64] and macrophage release of pro-inflammatory cytokines (TNF-α, IL-1, and IL-6)[65]. These activated immune cells, in turn, locally release CRF and other immune peptides[61,66], which then activate mast cells[22]. Mast cell-derived tryptase is another compound that recruits lymphocytes, eosinophils, and macrophages[67], and can further perpetuate inflammation.

INFLAMMATION INDUCED NEUROLOGICAL TONE

Patients with IBS have central processing abnormalities associated with the perception of pain[68-71]. Colonic irritation can lead to visceral hypersensitivity[72]. Patients with IBS have inflammatory changes in their gut mucosa, which can only be identified by quantitative histopathology, immunohistochemistry, and electron microscopy[73]. These patients have increased numbers of mast cells in the mucosa of the colon[30,74,75]. Mast cell concentrations and their distance from mucosal nerve cells are positively associated with various IBS symptoms[75]. The tryptase released from these mast cells can directly activate gastrointestinal neurons in animals and humans, and can cause visceral hypersensitivity[76-78]. Tryptase cleaves and activates transmembrane proteins called proteinase-activated receptor-2 (PAR2), which are found on the primary afferent neurons of the gastrointestinal tract[79]. Activation of PAR2 receptors leads to neuronal activation, which then creates the experience of

chronic pain.

In addition to central nervous system activation, patients with IBS also have sensitization and upregulation of the dorsal horn[70,71,80], which explains the cutaneous hyperalgesia found in the lower extremities, rather than upper extremities, due to viscerosomatic convergence of nociceptive afferent neurons from the colon/rectum and lower extremities[81]. Seybold *et al*[82] review the mechanisms by which gastrointestinal inflammation leads to gastrointestinal primary afferent neuronal activation and spinal cord activation/sensitization and inflammation.

If true, the perpetual mild mast-cell mediated inflammation can trigger the "excessive or prolonged stimulation of extrinsic afferents (that) may also result in the development of neuronal sensitization, at peripheral, spinal, or higher CNS levels, such that perception of sensations from the bowel is heightened, resulting in symptoms of urgency, bloating, and pain"[83]. This subclinical inflammation may also influence gastrointestinal serotonin pathways.

IBS, NEUROLOGICAL TONE, AND SEROTONIN

Serotonin (5-HT) can influence the motor function and sensitivity of the gastrointestinal tract[84-89]. Serotonin exerts a range of effects *via* its seven receptor subtypes (5-HT_1 to 5HT-7). Serotonin receptor $5HT_5$, $5HT_6$, $5HT_7$ are found in the brain, whereas $5HT_1$, $5HT_2$, $5HT_3$, $5HT_4$, and $5HT_7$ are the gastrointestinal serotonin receptors[90]. A large majority of the body's serotonin is stored in gastrointestinal enterochromaffin cells (EC)[85]. Patients with diarrhea predominant IBS have increased EC cells[91-93], which are activated by inflammation to release serotonin and may result in the elevated serotonin levels found in patients with IBS[94,95]. Tegaserod, a partial $5HT_4$ agonist has been used for constipation dominant IBS and Alosetron, a $5HT_3$ antagonist, in diarrhea dominant IBS.

Serotonin reuptake transporters (SERT) in the gut epithelial cells terminate the effects of serotonin[96,97] and influence serotonin concentrations and symptoms of IBS[85]. Patients with IBS have genetic polymorphisms that lead to lower expression of transport proteins and less serotonin reuptake[83,98,99]. The noted inflammation may also alter SERT expression and decrease its function in patients with IBS[100]. Further studies on the modulation of the gastrointestinal tract serotonin pathways may help further define and treat IBS.

INTESTINAL PERMEABILITY, CHRONIC INFLAMMATION, AND ANTIGENS

The presence and activity of mast cells, along with other inflammatory cells, alone are not likely result in chronic inflammation. Other intestinal antigens, such as food, bacteria, and fungi, are likely to be needed to perpetuate the inflammation in the presence of an impaired gastrointestinal epithelial barrier.

Healthy individuals have tight junctions that help to form the gastrointestinal epithelial barrier along with mucous, SIgA, and other peptides. This epithelial barrier controls the interaction between luminal bacteria and antigens and the mucosal immune system[22,101]. It also allows immune tolerance of food antigens and bacteria. Activation of PAR2 not only leads to neuronal activation, but also to epithelial barrier defects in patients with IBS[102,103].

Low level PAR2 activation of the myosin light chain kinase (MLCK), causes phosphorylation of the myosin light chain, which then leads to contraction of the actin-myosin ring. Tight junction protein zona occludens-1 (ZO-1) relocalizes into the cytoplasm and disrupts the tight junctions, which increases paracellular permeability. High level PAR2 activation in the rat colon results in localized inflammation and increased production of TNF-α and IFN-γ. INF-γ decreases ZO-1 expression and alters the actin cytoskeleton organization[104]. TNF-α activates MLCK and results in tight junction protein relocation[105,106]. A more detailed discussion of these pathways can be found in articles by Gareau *et al*[23] and Cenac *et al*[103].

Children and adults with IBS have increased intestinal permeability[107,108]. Increased intestinal permeability results in "mucosal barrier defects (that) allow the passage of an increased load of luminal antigens of dietary and bacterial origin which, in turn, elicit the activation of mucosal immune responses"[109].

Various triggers can activate mast cells. Bacteria are powerful antigens for the gastrointestinal immune system[110-115]. Stress can result in increased bacterial adherence and penetration into the gastrointestinal mucosa[23,25-27], which may increase the interaction between the luminal bacteria and local immune response. This may explain why patients with IBS have higher antibody titers to specific bacterial flagella than healthy controls[116]. The DNA of these bacteria can interact with toll-like receptors[117], which then influence the immune system through regulation of tumor necrosis factor alpha and interferon gamma[118].

Escherichia coli, Campylobacter, and other bacteria can negatively influence the GI immune system and result in gastrointestinal inflammation and intestinal permeability[46,91,119-121]. Conversely, commercially available beneficial bacteria, in the form of probiotics, can reduce gastrointestinal inflammation[122-125], reverse or prevent intestinal permeability[120], and stop bacterial adhesion[126] and translocation[27,127]. Probiotics can also reverse visceral hypersensitivity from various causes[128,129], including stress[130]. Probiotics attenuate the upregulation of pain pathways at the spinal and supraspinal levels[131], and induce epithelial cells to express micro-opiate receptor 1 (MOR1) and cannabinoid 2 (CB2) opioid receptors[132]. Probiotics can reduce the symptoms of IBS[133,134].

Adults with IBS have gastrointestinal microflora that are significantly different than those of healthy populations[135]. Children with IBS are also likely to have significant alterations in their gastrointestinal microflora. We speculate that there may be a subset of children who are predisposed to developing IBS through repeated or

prolonged exposure to antibiotics for various reasons (recurrent otitis media, sepsis, meningitis, osteomyelitis, vesicoureteral reflux, acne, *etc*). Various antibiotics, including Augmentin, the macrolides, and amoxicillin significantly alter the composition of the bacteria in the GI tract[136-138]. Antibiotic use has been related to increased rates of IBS and functional abdominal pain[139,140].

Gastrointestinal bacteria are also influenced by the diet. Dietary soluble fiber encourages the growth of beneficial species like lactobacilli and bifidobacteria[141-143]. In mice, a white bread diet significantly prolonged antibiotic induced bacterial perturbations[136]. It is common knowledge that the standard American diet lacks fiber, and thus may predispose human beings to have prolonged antibiotic induced bacterial perturbations.

Prebiotics are short chain carbohydrates that help some of the beneficial bacteria or probiotics in the intestines to grow more effectively[142,143]. Prebiotics may decrease IBS symptoms[144-146]. Prebiotics are fermented by probiotics and metabolized into short chain fatty acids (SCFA). SCFAs can decrease inflammation and are used in maintaining the intestinal epithelial lining[147]. While breast milk naturally contains prebiotics[148], up until a few years ago, most infant formulas did not contain prebiotics. Thus, there may be a population of children who were formula fed and required several courses of antibiotics that now have perturbed gastrointestinal flora, as well as intestinal epithelial barriers. We believe that these children may be at risk of developing IBS.

Food proteins are other significant antigens for the gut immune system. Food antigens induce mast cell activation[149] and degranulation, which can lead to visceral hypersensitivity. In children, certain foods may exacerbate intestinal permeability and the elimination of the foods help resolve the IBS symptoms[150]. Elimination of certain foods may decrease immune activation by removing the allergic antigenic load to the local immune system. In patients with IBS, sodium cromoglycate can eliminate IBS symptoms[151-153] by preventing the degranulation of mast cells and inhibiting the release of inflammatory mediators, following contact with an allergen[154].

Over 60% of patients believe that certain foods worsen their IBS symptoms and that elimination of these foods can reduce their symptoms[155-157]. Some believe that these food reactions are psychological in origin[158-160]. Blinded food challenges have raised many questions about the validity of elimination diets for IBS treatment[161-163]. There is also a growing body of evidence to support the use of elimination diets as part of a treatment protocol for IBS[164-167]. Milk, wheat, and eggs are the most commonly identified food triggers[163].

Another potential antigen for the gastrointestinal immune system is *Candida albicans*. Adult studies have shown that *Candida* does not play a significant role in patients with IBS[168,169]. To our knowledge, the role of candida in pediatric IBS has not been determined. Some children who have received numerous courses of antibiotics, such as amoxicillin, can have disruption of the bacterial balance and have overgrowth of the commensal *Candida*[137,170-173]. *Candida* induces inflammation. It produces alcohols and glycoproteins that stimulate mast cells to produce histamine and prostaglandins[174,175]. *Candida* also produces inflammatory prostaglandins that affect mammalian cells[176], as well as proteases that degrade the gastrointestinal IgA and, thus, allow candida to overcome the local immune defense mechanisms[177]. Candidal proteases can induce a B-cell response and result in increased inflammation[174]. In animals and humans, *Candida* perpetuates intestinal inflammation[169].

SMALL INTESTINAL BACTERIAL OVERGROWTH

Another possible contributing factor to IBS signs and symptoms is small intestinal bacterial overgrowth (SIBO), defined as bacterial counts greater than 10^5 cf/mL from small intestinal aspirates[178]. Controversy exists over the ideal method of assessing SIBO[178-181]. A significant number of patients with IBS complain of bloating and pain. SIBO may explain this bloating and pain, as well as other IBS-like-symptoms[182-184]. Several studies have shown antibiotics to be helpful in reducing the symptoms of IBS[185-188].

Patients with IBS who have delayed gastric emptying have a higher risk of developing SIBO[189-192]. Stress is one cause of delayed gastric emptying[193-196]. Once SIBO is present, it can trigger an inflammatory response. SIBO, through abnormal gastrointestinal flora fermentation, may be another cause of IBS symptoms and must be considered in the evaluation of the patient. Furthermore, proton pump inhibitors can also increase the risk of SIBO by decreasing gastric acidity and further perturbations of the gastrointestinal flora species[170,197-200]. We speculate that SIBO may play a larger role in adults with IBS than in children. Further studies are required to elucidate the various differences between adult and pediatric IBS.

CONCLUSION

The evidence presented in our review suggests that IBS is an organic disease with a complex pathophysiology (Figure 1) that is difficult to identify by standard diagnostic tools. The pathophysiology of IBS varies from person to person and from children to adults. The underlying mast cell mediated inflammation of IBS, along with serotonin signaling, can drive the chronic nociceptive input from the periphery to dynamically maintain the altered central processing defects and perception of pain[70,80,201].

In addition to the pathophysiology, clinicians must focus more attention on the well known and less well characterized risk factors that may predispose individuals to developing IBS (Table 1). It is our belief that clinicians should further use the field of neurogastroenterology to better understand the effects of stress on the gastrointestinal tract. Clinicians and researchers must work to develop and adopt models to help us better predict and prevent this condition in susceptible individuals. For chil-

Table 1 Risk factors for irritable bowel syndrome

Genetics/family history
Stress/high academic performance/parental psychiatric disorders
Recurrent or chronic antibiotic use
Bacterial or viral enteritis
Unrecognized food sensitivities
Low fiber diet/diet high in simple carbohydrates
Formula feeding
Chronic acid suppression

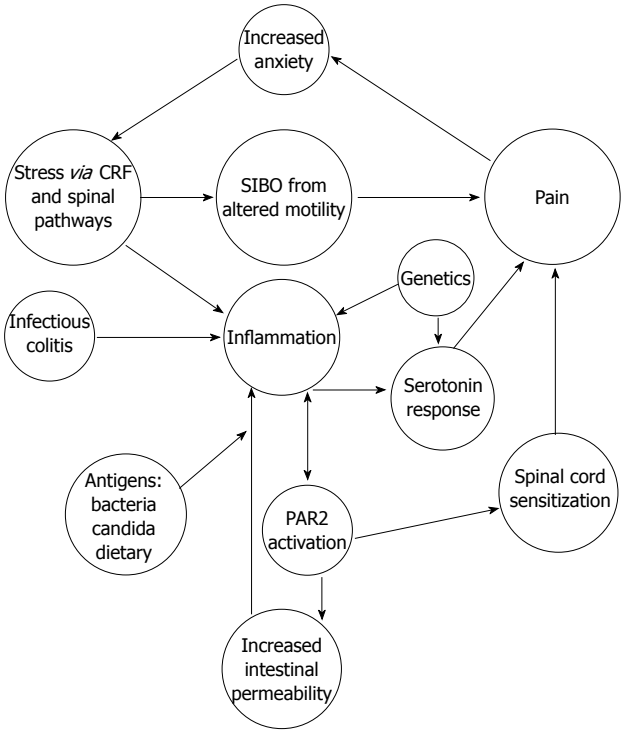

Figure 1 Proposed pathophysiology of irritable bowel syndrome. CRF: Corticotropin-releasing factor; SIBO: Small intestinal bacterial overgrowth; PAR2: Proteinase-activated receptor-2.

dren, these models will require additional studies to evaluate the impact of recurrent antibiotic use and resultant overgrowth of candida on the development of IBS.

Effective treatment models for IBS must reflect the complex physiology of IBS and simultaneously address multiple pathophysiological factors to break the vicious cycle of inflammation and ultimately allow for cessation of symptoms. The Institute of Functional Medicine (IFM)[202] has created such a model of care for IBS. The IFM model has the potential to provide significant improvement in patient care, while reducing healthcare costs and deserves further consideration and evaluation. Please refer to the IFM website and various publications for a more detailed discussion on treatment options.

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