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REVIEW

Low-grade inflammation plays a pivotal role in gastrointestinal dysfunction in irritable bowel syndrome

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Abstract

The pathogenesis of irritable bowel syndrome (IBS) is considered to be multifactorial and includes psychosocial factors, visceral hypersensitivity, infection, microbiota and immune activation. It is becoming increasingly clear that low-grade inflammation is present in IBS patients and a number of biomarkers have emerged. This review describes the evidence for low-grade inflammation in IBS and explores its mechanism with particular focus on gastrointestinal motor dysfunction. Understanding of the immunological basis of the altered gastrointestinal motor function in IBS may lead to new therapeutic strategies for IBS.

Key words: Irritable bowel syndrome; Serotonin; Enteritis; Gastrointestinal motility

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INTRODUCTION

Irritable bowel syndrome (IBS) is seen throughout the world with estimated prevalences ranging from 9%-23%[1], representing an important clinical problem. It is accompanied by a poor quality of life[2]. Its symptoms include abdominal pain or discomfort associated with changes in bowel habits for which no obvious cause can be found on routine investigations[3] and its diagnosis is commonly dependent on the symptom-based Rome criteria (Table 1).

The pathogenesis is considered to be multifactorial and includes psychosocial factors, gastrointestinal (GI) dysmotility, enhanced perception of sensory stimuli conveyed from the gut wall to the central nervous system, stress, corticotrophin-releasing factor, infection, microbiota, genetics and gut wall immune activation[4]. It is now well recognized that an episode of gastroenteritis can trigger IBS symptoms, known as post-infective IBS (PI-IBS). Low-grade inflammation and immune activation are evident in biopsies both from patients with IBS[5] and PI-IBS[6]. It is becoming clear that low grade inflammation in the mucosal compartment of the gut could alter function in the underlying neuromuscular tissues from animal studies. IBS represents a clinical entity largely diffused which may heavily affect the patient's quality of life and a strong need of oriented therapeutic interventions could be avaible.

This review describes the evidence for low-grade inflammation in patients with IBS, explores its mechanism

with particular focus on the inflammation-induced GI motility and highlights its implications for understanding the pathophysiology of IBS.

EVIDENCE OF INFLAMMATION IN IRRITABLE BOWEL SYNDROME

Cytokines and immune cells

Several reports have described increased numbers of T cells in various lymphoid compartments of the small or large intestine in IBS patients[5,7,8]. Proinflammatory cytokines such as interleukin (IL)-1β, IL-6 and tumor necrosis factor (TNF)-α in peripheral blood mononuclear cells[9] and IL-6 and IL-8 in serum[10] were reported to be increased in IBS patients. Thus, low-grade inflammation can be detected through biopsies from intestine and blood.

Subsequent studies in IBS patients revealed increased numbers of mast cells in the lamina propria of the terminal ileum[11] and mucosa of the colon[12,13]. It is becoming clear that mast cells may affect the sensorimotor function and contribute to IBS symptoms[13,14]. Barbara *et al*[13] showed that activated mast cells released significant amounts of various mediators including tryptase and histamine. It has been reported that mast cell tryptase elicits neuronal hyperexcitability[15,16] while histamine activates visceral sensory nerves *via* histamine-1 and -2 receptors[17], indicating that tryptase and histamine are candidate mediators for the gut sensorimotor dysfunction in IBS[18].

PI-IRRITABLE BOWEL SYNDROME

Epidemiological studies have indicated that 6%-17% of patients with acute gastroenteritis develop IBS[19]. Low-grade inflammation and immune activation are evident in biopsies from patients with PI-IBS and there is also evidence of increased intestinal permeability[4].

Neurotransmitters

Serotonin: Serotonin (5-hydroxytryptamine, 5-HT) is found in the GI tract and central nervous system and functions as a neurotransmitter[20]. 5-HT is the most studied neurotransmitter in IBS. About 95% of the body's 5-HT is localized in the GI tract and 5% is present in the brain. In the GI tract, 5-HT is synthesized in serotonergic neurons in the enteric nervous system as well as in enterochromaffin (EC) cells of the GI mucosa.

EC cells produce and secrete far more 5-HT than central or peripheral serotonergic neurons and it reaches the GI lumen and blood[21]. Overflowing 5-HT from EC cells, taken up and concentrated in platelets, is virtually the sole source of blood 5-HT. 5-HT exerts its actions by binding to its receptors (5-HT_{1-7}) which are present on intrinsic and extrinsic primary afferent neurons. The large range of effects of 5-HT mainly results from the presence of the multiple receptor subtypes on enteric neurons, EC cells, gastrointestinal smooth muscle cells, enterocytes and immune tissues. Seven families and multiple subtypes of 5-HT receptors have now been identified[22]. The 5-HT_{1-4} and 5-HT_7 receptors are known to affect gut motor functions[23-26]. 5-HT is well known to increase in various GI disorders such as carcinoid syndrome, celiac disease, acute bacterial enteritis and inflammatory bowel disease (IBD)[27,28]. Several studies have shown that plasma 5-HT is increased in patients with IBS[29,30]. Furthermore, the effectiveness of 5-HT_3 antagonists for IBS with diarrhea (IBS-D) has been demonstrated[31] and 5-HT_3 antagonists are currently widely used for IBS-D in various countries. PI-IBS has been associated with increased numbers of EC cells[32]. Although IBS with constipation (IBS-C) patients showed decreased plasma 5-HT[29], colonic[29,33] and duodenal[34] mucosal 5-HT appeared to be increased. Opiate-induced constipation does not alter the 5-HT content or mucosal serotonin transporter (SERT) level in humans, suggesting that the changes in 5-HT metabolism in IBS-C are primary[27].

Table 1 Rome Ⅲ criteria

Recurrent abdominal pain or discomfort for at least 3 d per month in the last 3 mo associated with two or more of the following
Improvement with defecation
Onset associated with a change in frequency of stool
Onset associated with a change in form (appearance) of stool

5-HT released in the mucosa is rapidly taken up by serotonin transporters in nerve terminals or mucosal enterocytes and vascular endothelial cells[35]. The associations between SERT transcription levels, polymorphisms and IBS phenotypes have been investigated. The SERT gene-linked polymorphic region (SERT-LPR), an area 12 kb upstream of the SERT exon that has short (s) and long (l) alleles, is thought to influence the level of transcription[36].

Emerging biomarkers

The identification of reliable biomarkers represents a major step forward in the management of disease. The physiological changes accompanying IBS have been shown to be reflected in changes in the expression levels of biomarkers[37-39]. The reliable serum biomarker for IBS is expected to reduce an unnecessary colonoscopy caused by symptom-based criteria.

Lembo *et al*[40] investigated blood-based diagnostic tests to differentiate IBS from non-IBS using the Smart Diagnostic Algorithm and complex patterns of the serum concentrations among 10 biomarkers, including IL-1β, growth-related oncogene-α, brain-derived neurotrophic factor, anti-*Saccharomyces cerevisiae* IgA antibodies, anti-CBir1 antibodies, anti-human tissue transglutaminase antibodies, TNF-like weak inducer of apoptosis (TWEAK), anti-neutrophil cytoplasmic antibodies, tissue inhibitor of metalloproteinase-1 and neutrophil gelatinase-associated lipocalin. They demonstrated that the positive predictive value was 81% and the negative predictive value was 64% at 50% IBS prevalence in the validation cohort[40]. Therefore, the pathophysiology of IBS is heterogeneous and the identification of multiple biomarkers is more reliable than detection of a single biomarker for IBS.

Microbiota

The intestinal microbiota influences a broad array of host

organs including the gut and brain and is an important determinant of normal function in these systems. Disruption of the delicate balance between the host and the intestinal microbiota (termed dysbiosis) results in changes in the mucosal immune system that range from overt inflammation, as seen in Crohn's disease, to low-grade inflammation without tissue injury, as seen in a subset of IBS patients. The dysbiosis induced by infection, diet or antibiotics can produce the low-grade inflammation seen in IBS[41]. Malinen *et al*[42] showed that *Lactobacillus* species were decreased in IBS-D patients while *Veionella* species were increased in IBS-C patients.

Other groups have obtained evidence that the intestinal microflora of patients with IBS differs from that of healthy subjects[43,44]. It has recently been suggested that IBS symptoms are partly caused by a process designated small intestinal bacterial overgrowth (SIBO)[45,46]. Further therapeutic manipulation of the gut flora with antibiotics[47] or probiotics[48] improves the symptoms of IBS but is controversial. We consider SIBO as a subtype of IBS more than a distinct entity.

These lines of evidence provide proof of the concept that the intestinal microbiota can induce the persistent gut dysfunction seen in IBS.

GI MOTILITY AND SMOOTH MUSCLE

GI motility is defined by the movements of the digestive system including two fundamental patterns of motility, propulsion and mixing. Several players including central nerves, enteric nerves, interstitial cells of Cajal (ICC) and smooth muscles contribute to a coordinated regulation of GI motility. Among them, smooth muscle probably plays the most important role in GI motility since the patterns of motility observed in gut are characteristic of smooth muscle which has different properties from skeletal muscle. The contractile properties of smooth muscle are mainly regulated by the phosphorylation of regulatory light chains of myosin Ⅱ (LC_{20})[49] which is driven by the balance between myosin light chain kinase (MLCK) and myosin light chain phosphatase (MLCP) activities. MLCK depends on Ca^{2+}-calmodulin. Intracellular Ca^{2+} [$(Ca^{2+})_i$] is the primary determinant of smooth muscle contraction. However, MLCP functions independently of Ca^{2+}-calmodulin and is regulated by G protein-coupled signaling pathways. Inhibition of MLCP leads to an increase in both MLC phosphorylation and contractile force development in smooth muscle without any changes in $(Ca^{2+})_i$[50]. This enhancement of the contractile response to Ca^{2+} is commonly referred to as 'calcium sensitization'[49]. In general, several protein kinases including Rho-associated kinase (ROK)[51], protein kinase C (PKC)[52], integrin-linked kinase (ILK)[53,54] and zipper-interacting protein kinase (ZIPK)[55] have been shown to contribute to regulation of MLCP and Ca^{2+} sensitization, mediated directly by phosphorylation of the myosin phosphatase targeting subunit of MLCP (MYPT1) and/or indirectly by phosphorylation of a PKC-potentiated phosphatase inhibitor protein-17 kDa (CPI-17). In addition, it has been shown that mitogen activated protein kinase pathways also contribute to intestinal smooth muscle contraction and Ca^{2+} sensitization possibly *via* regulation of MLCP[56].

GUT MOTOR FUNCTION IN INFLAMMATION

Conditions ranging from infective acute enteritis or colitis to IBD and functional disorders such as IBS are accompanied by altered GI motility[57] which can be a reflection of altered function of enteric nerves, ICC or smooth muscles. Alterations in GI motility with resultant changes in transit contribute to the abdominal pain, intestinal cramping and diarrhea. Colonic mast cell infiltration and mediator release in the proximity of mucosal innervations may contribute to abdominal pain perception in patients with IBS[13]. Patients with IBD in remission often generate IBS symptoms, termed IBD/IBS, and have pain and diarrhea similar to those in IBS patients in association with minimal or no evident intestinal inflammation[58].

These findings suggest that low-grade inflammation contributes to the GI motor dysfunction and abdominal symptoms in patients with functional GI disorders and IBD in remission. Since we cannot use whole human intestinal tissue to investigate the pathophysiology of IBS, we have tried to establish bench-to-bedside animal models for the development of new therapies.

Which immune cells and mediators and how do they affect gastrointestinal motility?

Macrophages: Macrophages perform a key role in innate defense against foreign invaders and produce a number of cytokines such as IL-1β, IL-6 and TNF-α. In animal experiments, macrophages infiltrate the gut wall including the neuromuscular layers during nematode infection in mice. It was reported that macrophages were not critical for the change in muscle contraction in *Trichinella spiralis*-infected mice[59] although another group reported that alternative activated macrophages affected the muscle hypercontractility in *Nippostrongylus brasiliensis*-infected mice[60].

T lymphocytes (Th1/Th2): T lymphocytes are crucial for many immune responses, including those associated with animal models such as dextran sulfate sodium colitis[61], 2,4,6-trinitrobenzenesulfonic acid colitis[62], nematode infection[57,63] and anti-CD3 antibody-induced enteropathy[64]. Antigen-presenting cells present antigens to $CD4^+$ T helper (Th) cells. Th cell-dependent immune responses are generally divided into two major subsets, Th1 and Th2[65]. Th1 cells predominantly produce interferon (IFN) γ and IL-2 while Th2 cells produce IL-4, IL-5, IL-9 and IL-13. Th1 and Th2 cells cross-regulate one another. While few generalizations can be made, it appears that contractile dysfunction depends on the specific inflammatory environment. Recent accumulated data from animal

models have shown that Th1 and Th2 immune response was associated with hypocontractility or hypercontractility of inflamed intestinal smooth muscle respectively.

Schwartz *et al*[66] showed that surgical manipulation suppressed jejunal contractions with upregulation of IL-6, TNF-α, cyclooxygenase-2 and inducible nitric oxide synthase. It has been shown that both TNF-α[67] and IL-1β[68] were associated with hypocontractility of inflamed intestinal smooth muscle. Furthermore, we have shown that incubation of IFNγ with intestinal smooth muscle decreased carbachol-induced smooth muscle cell contraction[69]. It has also been shown how these Th1-related cytokines cause hypocontractility of inflamed intestinal smooth muscle. TNF-α and IL-1β inhibited carbachol-induced contraction *via* down-regulation of CPI-17[62] and L-type Ca^{2+} channels[70] respectively.

On the other hand, the Th2 cytokines IL-4 and IL-13 acting *via* Stat6 mediate the development of nematode infection-induced intestinal muscle hypercontractility which contributes to worm expulsion[71-73]. Other studies supported our finding that Th2 responses mediated muscle contractility in nematode *N. brasiliensis*-infected mice[60,74]. Although it remains to be investigated how these Th2-related cytokines mediate hypercontractility of inflamed intestinal smooth muscle, Ihara *et al*[61] showed that mitogen-activated protein kinase pathways played crucial roles in the Th2 cytokine-mediated Ca^{2+} sensitization and hypercontractility observed in inflamed colonic circular smooth muscle from dextran sulfate sodium-treated mice.

Th1/Th2 balance: To evaluate the role of Th1/Th2 in infection-induced alterations of enteric muscle function, Khan *et al*[75] investigated the effects of IL-12 overexpression on intestinal muscle contractility and worm expulsion in *T. spiralis*-infected mice. IL-12 gene transfer *via* a single injection of a recombinant adenovirus vector expressing IL-12 (Ad5IL-12) in *T. spiralis*-infected mice effectively inhibited the development of infection-induced intestinal muscle hypercontractility and prolonged worm survival in the gut. A shift to a Th1 response after overexpression of IL-12 significantly altered the intestinal muscle hypercontractility in this Th2-based enteric infection.

Furthermore, we evaluated the association of 5-HT with Th1/Th2 responses. 5-HT influences intestinal homeostasis by altering the gut physiology and has been implicated in the pathophysiology of various GI disorders such as IBD, IBS and GI infection[35,36,76,77]. In a colonic parasitic infection with *Trichuris muris*, resistant strains (BALB/c, C57BL/6 and NIH Swiss) expelled the parasites through the generation of a Th2 response whereas susceptible strains (AKR and B10.BR) developed a chronic infection with activation of a Th1 response[78].

We used *Trichuris muris*-infected AKR (susceptible to infection with generation of a Th1 response), BALB/c (resistant to infection with generation of a Th2 response), Stat4-deficient (impaired in Th1 responses) and Stat6-deficient (impaired in Th2 responses) mice to explore the mechanism of the EC cell and 5-HT responses in Th1/Th2-dominant environments[79]. We found that the EC cell and 5-HT responses to the same infectious agent were influenced by Th1 or Th2 cytokine predominance, suggesting that the immunological profile of the inflammatory response is important in the regulation of EC cell biology in the gut. Furthermore, we evaluated the 5-HT response and intestinal motility in an IBD/IBS model using T cell-induced enteropathy in Th1/Th2-dominant environments[80]. In BALB/c mice, carbachol-induced intestinal smooth muscle cell contraction was significantly increased at d 7 after anti-CD3 antibody injection when the tissue damage returned to the normal histological appearance. We also observed that 5-HT protein in the intestine was significantly increased at d 7. On the other hand, in AKR mice, carbachol-induced muscle cell contraction was significantly decreased at d 7. 5-HT protein in the intestine was also decreased at d 7. We showed that Th1 and Th2 cytokines had opposing effects on intestinal muscle contraction *via* 5-HT signaling in the post-inflammation phase in this model.

Th17: Previous concepts regarding the roles of Th cells in chronic inflammatory and autoimmune diseases have been challenged by the description of a novel T-cell subset characterized by the production of IL-17[81]. Several disorders originally considered to be Th1-mediated have been reclassified as Th17-mediated inflammation[82,83]. Th17 cells produce IL-17, IL-17F and IL-22, thereby inducing a massive tissue reaction owing to the broad distribution of the IL-17 and IL-22 receptors. Th17 cells also secrete IL-21 to communicate with the cells of the immune system. The differentiation factors (TGF-beta plus IL-6 or IL-21), the growth and stabilization factor (IL-23) and the transcription factors (STAT3, RORgammat and RORalpha) involved in the development of Th17 cells have just been identified[84]. IL-17 is a proinflammatory cytokine that activates T cells and other immune cells to produce a variety of cytokines, chemokines and cell adhesion molecules. This cytokine is augmented in the sera and/or tissues of patients with contact dermatitis, asthma and rheumatoid arthritis[82]. Although the Th1/Th17 balance in human IBD remains unclear, IBD seems to have a relationship with Th17 cells[85]. Low-grade inflammation in the mucosa is considered to be a factor involved in the pathophysiology of IBS and further investigations of Th17 cells in the intestinal mucosa of patients with IBS should therefore be carried out[86]. A recent study showed that Th17 cells were increased during acute infection with *T. spiralis* and that jejunal smooth muscle strips cultured with IL-17 showed enhanced contractions elicited by acetylcholine in a concentration-dependent manner[87]. We found that IL-17 protein in the small intestine was upregulated in mice injected with an anti-CD3 antibody[88] and that IL-17 incubation with smooth muscle cells enhanced carbachol-induced smooth muscle cell contraction (unpublished observation). Further investigations are required using IL-17-/- mouse and IL-17 antagonist to confirm the role of IL-17-induced muscle hypercontractility (Table 2).

VISCERAL HYPERSENSITIVITY

Abdominal pain is an essential symptom of IBS and visceral hypersensitivity is the most widely accepted mechanism[89]. Visceral sensitivity is regulated at the level of the peripheral (mucosa/submucosa), spinal cord and central nervous system. Non-inflammatory mediators such as stress, glycerol and glutamate, as well as inflammatory mediators have the potential to trigger visceral pain[89].

Inflammatory mediators such as prostaglandin E_2 from inflammatory cells, or chemical mediators such as ATP, bradykinin, 5-HT, substance P and calcitonin gene-related peptide, directly activate nerve endings and trigger the release of algesic mediators, histamine, 5-HT, nerve growth factor and prostanoids from other cells and afferent nerves, resulting in an increasing response of pain[89]. Nerve fibers expressing the capsaicin receptor, transient receptor potential vanilloid type-1, were increased in colonic mucosa from IBS patients and may contribute to the visceral hypersensitivity and pain in IBS[90]. Recent animal studies showed that 5-HT_{2B} receptor antagonists[91,92], melatonin[93], corticotrophin-releasing hormone receptor 1 antagonists[94] and protease-activated receptor-4[95] inhibited visceral hypersensitivity. These molecules are candidates for novel therapies against the visceral hypersensitivity in IBS.

SECRETOMOTOR DYSREGULATION

Secretomotor neurons

Disordered defecation in IBS is directly related to the physiology of the enteric secretomotor neurons. Secretomotor neurons are excitatory motor neurons in the submucosal plexus of the enteric nervous system which innervate and stimulate secretion from the intestinal crypts of Lieberkuhn, Brunner's glands and goblet cells.

Secretomotor neurons have receptors that receive excitatory and inhibitory synaptic inputs from other neurons in the integrative circuitry of the enteric nervous system and from sympathetic postganglionic neurons. They are also influenced by paracrine chemical messages from non-neural cell types in the mucosa and submucosa such as EC cells and immune/inflammatory cells[17,90].

Activation of the excitatory receptors on secretomotor neurons stimulates the neurons to fire and release their transmitters at neuroepithelial junctions in the crypts. Secretomotor neurons express excitatory receptors for acetylcholine, 5-HT and histamine. The overall result of the activation of the excitatory receptors and associated increase in secretomotor neuronal firing is stimulation of the secretion of H_2O, electrolytes and mucus from the crypts into the intestinal lumen[90].

Knowledge of the cellular neurobiology of submucosal secretomotor neurons is key to understanding the pathophysiology of secretory diarrhea and constipation. Suppression of secretomotor firing by antidiarrheal agents such as opiates is manifested as harder-drier stools. On the contrary, stimulation by chemical mediators such as acetylcholine, 5-HT and histamine is manifested as more liquid stools[90]. The proinflammatory cytokines IL-1β and TNF-α increased epithelial tight junction permeability *in vitro* in Caco-2 cells in a dose- and time-dependent manner[96,97]. This effect was mediated by an increase in myosin L chain kinase expression and activity. IFNγ is well known to increase tight junction permeability in the T84 cell line accompanied by activation of the PI3-kinase pathway[98]. Green tea[99] and probiotics[100] are candidates for reducing the mucosal hyperpermeability seen in IBS.

Table 2 Immune cells and mediators affecting gastrointestinal motility

Immune cells/mediators	Muscle contractility	Ref.
Macrophage	↑→	[59,60]
T cell	↑↓	[57,61-64,72]
Proinflammatory cytokines; IL-1β, IL-6, TNFα	↓	[62,66-68,70]
Th1 cytokines; IFNγ, IL-12	↓	[69,75]
Th2 cytokines; IL-4, IL-9, IL-13	↑	[60,71-74]
Th17 cytokines; IL-17	↑	[87,88]
Th1/Th2 balance; Th1 > Th2	↓	[79,80]
Th1 < Th2	↑	[61,79,80]
5-HT	↑	[17,20,27,28,80]
MAP kinase	↑	[56,61]
CPI-17	↓	[62]
L type Ca^{2+} channel	↓	[70]

TNF: tumor necrosis factor; IFN: interferon; IL: interleukin; 5-HT: serotonin; MAP: mitogen activated protein; CPI-17: protein kinase C-potentiated phosphatase inhibitor protein-17 kDa; Ref: references.

EFFECTIVE TREATMENTS

Several emerging clinical trials for IBS are ongoing that target visceral hypersensitivity, motility, neurotransmitters, microbiota and immune systems acting peripherally and/or centrally. Thus far, 5-HT agents have been the most effective for IBS[101,102]. A systematic review showed that the 5-HT_3 antagonist alosetron and 5-HT_4 agonist tegaserod were more effective than placebos and that serious adverse events were rare, although the Food and Drug Administration announced discontinued marketing of tegaserod due to a potential risk of adverse events, cerebrovascular and cardiovascular ischemic events in 2007[101,102].

One of the excitatory receptors on secretomotor neurons belongs to the 5-HT_3 serotonergic receptor subtype[20,103,104]. The observed efficacy of blockade of 5-HT_3 receptors by a 5-HT_3 antagonist in the treatment of diarrhea in diarrhea-predominant IBS suggests that overstimulation of secretomotor neurons by 5-HT is a significant pathophysiological factor in this form of IBS[105,106]. On the contrary, 5-HT_4 agonists stimulate GI motility and intestinal secretion and have demonstrated efficacy in improving bowel habits for constipation-predominant IBS[107].

Three 5-HT_4 agonists, prucalopride, ATI-7505 and TD-5108, in development are reported to have greater selectivity for 5-HT_4 over other receptors and have advanced to human trial. 5-HT_3 receptor antagonist, ramose-

tron, was effective and well tolerated in the treatment of abdominal pain, discomfort and bowel habits in IBS-D patients[108].

IMPLICATIONS

It is becoming increasingly clear that inflammation of the intestinal mucosa and nerves causes the altered GI dysfunction seen in IBS. Several studies have been performed to detect robust and reliable biomarkers for IBS[40,109,110]. However, IBS contains many different conditions with different underlying causes and different responses to therapy and different mechanisms, biomarkers and therapies therefore need to be identified in each IBS subgroup. Understanding the underlying immunological basis of the altered GI motor dysfunction in IBS by considering the role of the Th1/Th2 balance or Th17 cytokines may ultimately lead to new therapeutic strategies for IBS.

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