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Review

Inflammation and Overlap of Irritable Bowel Syndrome and Functional Dyspepsia

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Irritable bowel syndrome (IBS) and functional dyspepsia (FD) are common functional gastrointestinal disorders (FGIDs) and account for a large proportion of consulting patients. These 2 disorders overlap with each other frequently. The pathogenesis of IBS or FD is complicated and multi-factors related, in which infectious or non-infectious inflammation and local or systemic immune response play significant roles. There are few studies focusing on the mechanism of inflammation in patients with overlap syndrome of irritable bowel syndrome and functional dyspepsia (IBS-FD). This review focuses on current advances about the role of inflammation in the pathogenesis of IBS and FD and the possible mechanism of inflammation in IBS-FD.
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Key Words
Dyspepsia; Inflammation; Irritable bowel syndrome

Introduction

As common functional gastrointestinal disorders (FGIDs), irritable bowel syndrome (IBS) and functional dyspepsia (FD) are lack of gastrointestinal organic lesions, but seriously affect the quality of life of patients. According to the epidemiological data, 26.7-48.7% of IBS patients and 20.0-42.1% of FD patients have overlapping symptoms.[1-3] Overlap syndrome leads to more serious clinical manifestations, worse quality of life and more difficult therapy.[4] The symptoms of IBS or FD are usually induced by diet, gastrointestinal infection, gut microbiota alteration, stress, psychological disorders and other unknown factors. The chronic inflammation after infection or non-infectious inflammation related to the above factors and immune response lead to visceral hypersensitivity, dysfunction of brain-gut axis and intestinal mucosal barrier, which may be the causes of IBS or FD symptoms. However, whether inflammation has the same mechanism in overlap syndrome of IBS and FD (IBS-FD) remains unclear and there are few related studies to confirm this. In this article, we reviewed recent advances about mechanism of inflammation in IBS-FD and provided references about the possible mechanism of inflammation in IBS-FD.

The Role of Gastrointestinal Infection in the Pathogenesis of Irritable Bowel Syndrome, Functional Dyspepsia, and Overlap Syndrome of Irritable Bowel Syndrome and Functional Dyspepsia

In the population suffering acute gastrointestinal infection, the

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prevalence of post-infectious IBS (PI-IBS) and post-infectious FD (PI-FD) is 12.7% and 9.5%, while the odds ratio of IBS and FD following gastrointestinal infection is 3.5 and 2.5, respectively.[5] According to previous studies, pathogens including *Shigella*, *Salmonella*, *Escherichia coli*, *Campylobacter jejuni*, *Vibrio cholerae*, *Clostridium difficile*, norovirus, *Giardia lamblia*, and *Trichinella spiralis* were considered to be associated with IBS, while pathogens that correlated with FD included *Helicobacter pylori*, *Shigella*, *Salmonella*, *E. coli*, *C. jejuni*, *C. difficile*, norovirus, and *G. lamblia*.[5-7] A recent prospective cohort study found that the incidence of PI-IBS, PI-FD, and IBS-FD was 16.5%, 7.4%, and 4.7%, respectively, 1 year after acute gastrointestinal infection, and the difference was statistically significant compared with healthy controls without infection history.[8] Mearin et al[9] found that 36.0% of all the patients with PI-FD or PI-IBS 1 year after the outbreak of acute *Salmonella* gastroenteritis had overlapping symptoms. The incidence of IBS-FD 3 years after acute *G. lamblia* infection was higher than the control group without infection (44.0% vs 29.0%).[10] Spiller[11] put forward the hypothesis that the site of acute infection may be related to the outcome of symptoms in post-infectious FGIDs. If the infection is limited to the proximal intestine, patients are more likely to develop symptoms of FD; if the distal intestine or colon is involved, symptoms of IBS may occur. When the proximal and distal intestine are both involved, patients are more likely to develop overlap syndrome of IBS and FD.[11] However, there is a lack of prospective studies to confirm the above hypothesis.

In the months to years after acute gastrointestinal infection, mild chronic inflammation remained in the gastrointestinal tract and mainly reflected by increase and activation of inflammatory cells such as mast cells (MCs), eosinophils, and macrophages in the mucosa, which was difficult to be found by routine blood tests and endoscopy.[12-16] It has been proved that the local and systemic immune response concomitant or secondary to inflammation can lead to damage of the intestinal mucosal barrier, dysfunction of enteric nervous system and brain-gut axis, as well as abnormal sensory and motor functions of the gastrointestinal tract. These changes are related to epigastric pain syndrome (EPS) and postprandial distress syndrome of FD in the upper gastrointestinal tract, abdominal pain, and altered bowel habits of IBS in the lower gastrointestinal tract. We will detail evidence of literatures in the following paragraphs.

The Chronic Inflammation and Immune Response Secondary to Gastrointestinal Infection

After the pathogen of acute infection is removed, although acute mucosal injury is repaired, the ability of the immune system to terminate inflammation is impaired so that mild chronic inflammation is left in the gastrointestinal mucosa of PI-IBS or PI-FD patients. The persistent immune response to mild inflammation of the gastrointestinal mucosa may be involve in the pathogenesis of PI-IBS or PI-FD. A previous study has found that the number of MCs in the terminal ileum mucosa in patients with PI-IBS was significantly increased compared with that in control subjects.[12] The intraepithelial T lymphocyte counts in rectum of patients with PI-IBS were still in a high level 1 year after *Campylobacter* enteritis.[13] The increase of MCs located within 5 μm of intestinal nerve fibers was significantly correlated with severity and frequency of abdominal pain/discomfort in patients with IBS.[17] Compared with nonspecific FD patients or healthy controls, the histological score of chronic gastric inflammation and the number of activated MCs within 5 μm of nerve fibers in the gastric antrum were significantly greater in patients with PI-FD.[14] It has been confirmed that there existed persisting focal CD8+ T lymphocyte and eosinophil aggregates, decreased CD4+ T lymphocytes and increased macrophage counts surrounding the crypts in the duodenum of patients with PI-FD.[15,16] Duodenal eosinophilia was proved to be associated with early satiety,[18] and there was a significant correlation between epigastric burning and the degree of duodenitis in patients with PI-FD.[16] Inflammatory cells release pro-inflammatory cytokines such as TNF-α, IFN-γ, IL-6, IL-8, IL-18, and anti-inflammatory factors such as IL-10 and IL-13, which are important markers of immune response and play important roles in the regulation of inflammatory cascade. The expression levels of IL-6, IL-18, and IFN-γ in colonic and rectal mucosa of patients with PI-IBS were higher than those in controls and patients with non-PI-IBS while the expression level of IL-10 was lower.[19,20] Compared with the control group, plasma IL-6, IL-18, and TNF-α levels in PI-IBS were significantly higher while the plasma IL-10 level was lower.[21,22] There is no comparative study about the levels of multiple inflammatory factors between PI-FD and non-PI-FD or healthy subjects.

Damage of Intestinal Mucosal Barrier Associated With Gastrointestinal Infection

The intestinal mucosal barrier consists of symbiotic bacteria and mucus layer, epithelial cells and cell-cell junctions, as well as the lamina propria containing connective tissue.[23] In patients with post-infectious FGIDs, pathogens are usually eliminated and the damage of intestinal mucosal barrier is mainly caused by the post-infectious intestinal flora dysbiosis and chronic inflammation. Toll-like receptor (TLR) is a type of receptor protein which is mainly involved in recognizing microbial products, mediating infection-

related immune response and inflammatory signal transduction. Homologous ligands such as some bacterial components such as flagellin and lipopolysaccharide bind to TLR and mediate the activation of mature MCs and release of inflammatory mediators such as histamine, tryptase and prostaglandin E2, which may be the main mechanism of intestinal flora dysbiosis causing low-grade inflammation.[24]

Flora dysbiosis affect the normal amino acid metabolism, leading to composition changes of the mucus layer and the damage of the mucus barrier. Most of threonine in diet is used to synthesize secretory mucin, and restriction of threonine from diet can reduce the synthesis of secretory mucin in intestinal epithelial cells.[25] The metabolism of glycine, serine, and threonine is closely related to the abundance of some species of *Bacteroides* (eg, *Bacteroides thetaiotaomicron*) and *Firmicutes* (eg, *Faecalibacterium prausnitzii*).[26,27] The abundance of *Faecalibacterium* including *F. prausnitzii* in patients with IBS was significantly lower than that of healthy controls.[28] The microbial diversity of colonic mucosa and feces in PI-IBS patients was reduced,[29] and the Index of Microbial Dysbiosis was correlated with the degree of abdominal pain and increased bowel movements.[27] In patients with FD, the abundance of anaerobic bacterium including *Prevotella*, *Veillonella*, and *Actinomyces* in duodenal mucosa was lower than that of healthy controls,[30] but a lack of study was found in the exploration of the abundance change of *Bacteroides* and *Firmicutes* in patients with FD.

Tight junctions and adherens junctions are important components of the intestinal mucosal barrier. Tight junctions are protein complexes composed of transmembrane proteins including claudins and occludin, junction adhesion molecules, and intracellular protein zonula occludens (ZO). The intracellular domains of protein complexes are anchored on the cytoskeleton by ZO.[31,32] Adherens junctions are mainly composed of E-cadherin, catenin and actin. It has been confirmed that the activation of MCs in jejunum mucosa of patients with IBS was correlated with the decreased expression of ZO-1 as well as the degree of diarrhea symptoms.[33] Activated MCs release tryptase, which binds to the protease activated receptor (PAR) on the basolateral side of intestinal epithelial cells. The combination of PAR with PAR1 leads to the increase of intestinal epithelial cell permeability through mechanisms such as apoptosis and the activation of myosin light chain kinase (MLCK) resulting in the redistribution of tight junction proteins.[34] The pro-inflammatory cytokines such as TNF-α released by inflammatory cells can induce the contraction of actin-myosin ring at the top of intestinal epithelial cells, the redistribution of ZO-1 and occludin, the decrease of transepithelial electrical resistance, and the increase of intestinal epithelial cell permeability to macromolecules by inducing the phosphorylation of MLCK.[35] In patients with FD with infiltration of eosinophils and MCs, the transepithelial electrical resistance of duodenal mucosa decreased, suggesting that the integrity of mucosal barrier was damaged.[36] Further study found that the expression of ZO-1 and occludin was abnormal and the phosphorylation of serine/threonine residues of occludin reduced, which may be the cause of duodenal mucosal integrity damage.[36]

The Influence of Gastrointestinal Infection on Enteric Nervous System

There are a large number of MCs adjacent to neuron-specific enolase, substance P (SP), and 5-hydroxytryptamine (5-HT) positive nerve fibers in the digestive tract mucosa.[37] The contact between cell membrane of MCs and the axons of adjacent nerve fibers lays a structural foundation for the neuroimmune interactions of the intestinal mucosa. Compared with healthy controls, the number of MCs around the nerve fibers of colon and terminal ileum in patients with PI-IBS increased significantly.[12] Animal experiments and studies on patients with IBS-D suggested that nerve growth factor (NGF) released by MCs bound with tyrosine kinase A receptor on sensory nerve endings, which promoted the proliferation of nerve fibers expressing transient receptor potential vanilloid type-1 (TRPV1), calcitonin gene related peptide (CGRP), and SP, and improved the activation of TRPV1 in the meantime. Under these circumstances, sensory nerve fibers released more pain-related neuropeptides (such as CGRP and SP) when stimulated, leading to increased visceral sensitivity.[38,39] Activated MCs also released adenosine triphosphate, prostaglandin, and other inflammatory mediators, which bind to P2X receptor of purinergic neurons, prostaglandin receptor, and TRPV1 to excite cholinergic motor neurons and promote intestinal movement.[40]

Inflammatory cells such as MCs and eosinophils produce and release NGF and neurotrophin resulting in local tissue hyperinnervation (neural sprouting, and neural and ganglionic hypertrophy), and a switch in the neurochemical code toward preferential expression of neuropeptides (eg, SP and CGRP) which are frequently present in nociceptive neurons.[41,42] These changes may be related to visceral hypersensitivity of patients with FD. In addition, the aggregation and degranulation of duodenal eosinophils in patients with FD were correlated with the increase of the density and sprouting of fine nerve fibers in duodenal mucosa, which was more significant in patients with EPS.[43] Another study pointed out that the infiltration of eosinophils in duodenal mucosa of patients with FD was correlated with the structural change (abnormal ganglion structure

and gliosis) and functional impairment (decreased calcium responses to depolarization and electrical stimulation) of the submucosal nerve plexus,[44] which may affect neuronal and muscular functions and lead to clinical symptoms in patients with FD.

Enterochromaffin cells (ECs) concentrate around the afferent nerve endings of gastrointestinal mucosa and synthesize and release 5-HT to the lamina propria. 5-HT regulates gastrointestinal motility through binding to 5-HT_2 receptor and 5-HT_4 receptor, meanwhile influencing visceral sensation through binding to the 5-HT_3 receptor.[45,46] Increased ECs was one of the acute changes following *Campylobacter* enteritis which could persist for more than a year[47] and was proved to be an important independent predictor of developing PI-IBS.[48] The number of ECs as well as 5-HT released by them in gastric mucosa of patients with PI-FD were significantly higher than those of patients with non-PI-FD and healthy controls, correlating with the degree of mucosal inflammation.[14] TNF-α and IFN-γ in inflammatory response downregulated the expression of selective 5-HT reuptake transporter, resulting in the decrease of 5-HT reuptake and sustained effect of 5-HT.[49]

The Influence of Gastrointestinal Infection on Brain-gut Axis

Enteric nervous system interacts with the central nervous system manifesting as emotional and physiological stress that can affect mucosal function of secretion and barrier, increase visceral sensitivity, and change gastric emptying and intestinal transit. Conversely, change of gastrointestinal motility, visceral inflammation and injury amplify the signals of the ascending visceral afferent pathway and affect brain activity, causing more intense pain and emotional/mental disorders including anxiety and depression.[50,51] There are 2 main neural regulatory pathways involved in visceral sensation. One is the excitatory pain-regulatory pathway, which consists of sensory nerves of the brain (anterior cingulate cortex, insula, hippocampus, amygdala, etc) and spinal cord. The function of this pathway is in facilitation (sensitization) in the visceral hypersensitivity state.[52] The other is the inhibitory pain-regulatory pathway, which mainly includes the vagal afferent pathway and is in a low state while visceral hypersensitivity occurs.[53] During a chronic inflammatory period, increased MCs release nociceptive molecules such as protease, histamine, platelet activating factor, leukotriene, cytokines (eg, TNF-α, IFN-γ, IL-1β, and IL-6). These inflammatory mediators acted on the adjacent nociceptive dorsal root ganglion neurons to influence the excitability and sensory threshold of neurons and cause visceral hypersensitivity through enhancing the calcium influx of neurons and increasing the firing rates of submucosal neurons.[54,55] 5-HT, released by ECs, is a type of important mucosal signaling molecule, while those secreted by enteric neurons is an important neurotransmitter, and constitutes significant effects on functions of brain-gut axis.[56,57] Chronic high concentration of 5-HT binding to the 5-HT_3 receptors on the nociceptive neurons of the vagus in the colorectal mucosa could enhance the pain perception induced by colorectal distention, which could be blocked by vagotomy or 5-HT_3 receptor antagonists.[46]

Corticotropin releasing factor (CRF) is mainly produced by the paraventricular nucleus of the hypothalamus, cerebral cortex, hippocampus, amygdala, and locus coeruleus. Its mRNA is highly expressed in the adrenal gland, gastrointestinal tract, thymus, skin, placenta, and inflammatory cells. CRF plays a significant role in the regulation of brain-gut axis. Animal experiments showed that CRF inhibited gastric contraction and gastric emptying through binding to CRF_2 receptors and stimulated colon transit and defecation by activating CRF_1 receptors.[58] Cytokines such as IL-1β and bacterial endotoxins induced by gastrointestinal infection could cross the blood-brain barrier, affect CRF neurons in the hypothalamus and activate the hypothalamic-pituitary-adrenal (HPA) axis.[59] Overman et al[60] found that exposure of pig ileum to specific concentrations of CRF increased the permeability of intestinal epithelial cells by promoting the release of TNF-α and protease from MCs. As a type of neuromodulator, TNF-α excites the neurons of nucleus tractus solitarii and inhibits gastric motility through the vago-vagal reflex pathway, which may be related to delayed gastric emptying in FD or overlap syndrome. Hussain et al[61] established the animal model of IBS-D overlapping with FD by intraperitoneal injection of CRF. They also found that trimebutine significantly regulated gastrointestinal motility by promoting gastric emptying as well as reducing bowel movements.[62] It is reasonable to speculate the mechanism of trimebutine may be relevant to inhibition of CRF.

Taken together, we summarize the possible inflammatory and immune activation in gastrointestinal mucosa of patients with post-infectious IBS-FD in Figure.

Possible Mechanism of *Helicobacter pylori* Infection in Overlap Syndrome of Irritable Bowel Syndrome and Functional Dyspepsia

Although meta-analysis does not support a specific association between IBS and *H. pylori* infection,[63] some evidences suggest that *H. pylori* infection may be involved in the pathogenesis of IBS. The positive rate of *H. pylori*, cytotoxin-associated gene A, and vacuolating cytotoxin A alleles (eg, s1 and s2) in patients with IBS-D were significantly higher than those in healthy controls.[64] Both

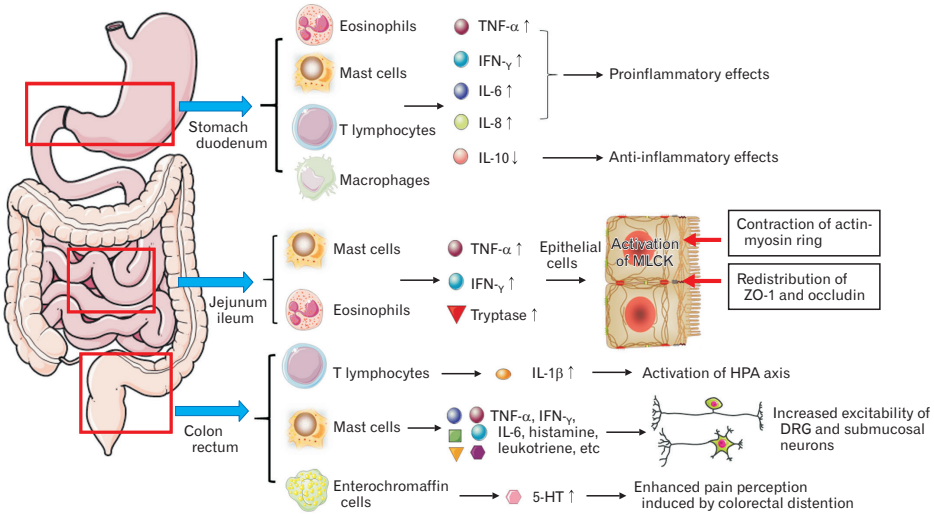

Figure. The possible inflammatory and immune activation in gastrointestinal mucosa of patients with post-infectious irritable bowel syndrome–functional dyspepsia. Inflammatory cells (mast cells, eosinophils, T lymphocytes, and chromaffin cells) are activated and release inflammatory factors (TNF-α, IFN-γ, IL-1/6/8, etc) and inflammatory mediators (histamine, leukotriene, platelet activating factor, 5-hydroxytryptamine [5-HT], etc) to participate in the inflammatory response, resulting in the increase of epithelial permeability through the redistribution of tight junction proteins and visceral hypersensitivity through 5-HT and increased excitability of afferent neurons. MLCK, myosin light chain kinase; HPA, hypothalamic-pituitary-adrenal; ZO-1, zonula occludens-1; DRG, dorsal root ganglion.

vacuolating cytotoxin A and neutrophil-activating protein significantly activated MCs and increased the release of pro-inflammatory cytokines such as IL-6.[65,66] A cross-sectional, observational study has concluded that duodenal lymphocytosis was significantly associated with bloating of FD patients, and the simultaneous presence of duodenal lymphocytosis and *H. pylori* infection was significantly more prevalent in FD patients than in control subjects.[67] In multivariate analysis, the odds of experiencing severe symptoms in patients with severe microscopic duodenitis was 2.2 times greater than in individuals with very mild, mild, or moderate duodenitis.[68] A large population study showed that *H. pylori* infection and any dyspepsia-related consultation significantly increased the likelihood of an IBS related consultation.[69] Duodenal inflammation caused by *H. pylori* infection may play a role in the pathogenesis of patients with IBS-FD, which needs to be confirmed in the future with more studies in patients with IBS-FD.

Mönnikes et al[70] showed that discomfort and pain thresholds on gastric distension were lower in *H. pylori*-positive FD patients. These patients also showed higher antral mucosal levels of CGRP and SP, which negatively paralleled the levels of discomfort and pain thresholds, demonstrating the involvement of SP and CGRP in the sensitization of afferent neuronal pathways.[70] Choi et al[71] found that *H. pylori* infection upregulated the expression of TRPV1 and NGF genes in human gastric cell lines. The expression of TRPV1 and NGF genes in gastric mucosa of successful *H. pylori* eradication patients was significantly reduced compared with patients without *H. pylori* eradication after 1 year follow-up, and the decreased expression of TRPV1 and NGF genes was correlated with the improvement of symptoms of FD patients.[71] It was also demonstrated that NGF, TRPV1, SP, and CGRP were involved in the development of visceral hypersensitivity in patients with IBS. For example, the high level of NGF in the mucosa of rectum and sigmoid was related to the increase of sensory nerve fibers expressing TRPV1 and CGRP in patients with IBS-FD.[39] The expression of microRNA-199 was significantly reduced in patients with IBS-D and was related to visceral pain since the upregulation of microRNA-199 decreased visceral pain via inhibition of TRPV1 signals.[72] However, no study have attempted to confirm the relationship between the above mechanism and *H. pylori* infection in patients with IBS.

Eradication of *H. pylori* improved the symptoms of some patients with FD. Therefore, the Rome IV Committee recommended eradication treatment for *H. pylori*-positive patients with FD.[73] A number of studies have explored the efficacy of *H. pylori* eradication in patients with IBS but no consistent conclusions have been reached.[74,75] The positive rate of *H. pylori* in patients with IBS-FD and whether *H. pylori* eradication benefits patients with IBS-FD have not been reported.

The Role of Non-infectious Inflammation in the Pathogenesis of Irritable Bowel Syndrome, Functional Dyspepsia, and Overlap Syndrome of Irritable Bowel Syndrome and Functional Dyspepsia

Small Intestinal Bacterial Overgrowth

In IBS and FD patients without gastrointestinal infection history, the continuous low-grade inflammation of the intestinal mucosa may also be related to small intestinal bacterial overgrowth (SIBO), since intestinal flora makes significant difference in mucosal and systemic immune response. In recent years, SIBO is considered to be significantly associated with functional bowel disease, especially IBS.[76] A recent meta-analysis showed that the incidence of SIBO in IBS patients is 36.7% (95% CI, 24.2-44.6) and it was closely associated with intestinal inflammation.[77,78] There are few studies focusing on the relationship between SIBO and FD. In a small sample Brazilian study, SIBO was found in 56.5% (13/23) of patients with FD but not observed in healthy controls ($P = 0.005$).[79] Japanese scholars tested SIBO in 38 patients with refractory functional gastrointestinal diseases (11 FD, 10 IBS, and 17 IBS-FD) and 2 patients were positive (1 FD and 1 IBS-FD). The symptoms of 2 patients with positive SIBO were significantly improved and their breath hydrogen levels decreased to normal following levofloxacin administration for 7 days, suggesting that the occurrence of FD symptoms may be related to SIBO.[80]

SIBO occurs because bacterium in the distal intestine move into the small intestine for various reasons, which may cause malnutrition, abnormal intestinal motility, diarrhea, abdominal distention, and other symptoms. The overgrowth of harmful bacterium produces a variety of toxic substances including ammonia, D-lactate, endogenous bacterial peptidoglycan, etc. These substances as well as bacterium themselves stimulate intestinal immune cells to produce pro-inflammatory cytokines resulting in sustained mild intestinal inflammation and immune activation, impaired intestinal mucosal barrier function, and increased intestinal sensitivity. It was confirmed that the increase of IL-1α and IL-1β in the upper gastrointestinal mucosa of patients with IBS was associated with SIBO.[81] Bacterial translocation was associated with enhanced local immune response in the small intestinal mucosa including the increase of plasma cells secreting IgA and IgM in the lamina propria.[82] Clinical research demonstrated that rifaximin effectively relieved the abdominal distention symptoms of IBS or FD patients and the incidence of adverse reactions was low in long-term follow-up.[83,84] Rifaximin is a type of oral broad-spectrum antibiotic acting on the local gastrointestinal tract. According to the study on a stress-induced rat model, rifaximin reduced the total load of intestinal flora, increased the relative abundance of lactobacilli, regulated the imbalance between the levels of pro-inflammatory factors and anti-inflammatory factors caused by stress (that is, downregulated the levels of pro-inflammatory factors such as IL-17, IL-6, and TNF-α and upregulated the level of anti-inflammatory factor IL-10) so as to inhibit stress-induced chronic inflammation of mucosa, reduce intestinal permeability, and improve visceral hypersensitivity.[85] At present, lack of large-scale studies, research focuses on the incidence of SIBO in patients with IBS-FD, the correlation between SIBO and overlapping symptoms, and the benefits from rifaximin treatment in patients with IBS-FD.

Food Allergy

Dietary factors play a more and more vital role in the etiology and pathophysiology of IBS and FD. In addition to their direct effects on sensitive mucosal receptors, food components also participate in the pathogenesis of IBS and FD by inducing mucosal immune response. In IBS patients with food allergy, acute eosinophil degranulation in duodenum and permeability of intestinal mucosa increased, and these patients had a 4-fold increase in prevalence of atopic disorders compared with controls.[86] In the study of Fritscher-Ravens et al,[87] diluted food antigens were administered directly to the duodenal mucosa, which immediately caused duodenal mucosal breaks, increased intervillous spaces, and increased intraepithelial lymphocytes in IBS patients with a suspected food intolerance to candidate food antigens. Duodenal eosinophilia of patients with FD was associated with history of allergy (including food allergy) and early satiety.[88] In addition, α-amylase/trypsin inhibitors in wheat act as strong activators of innate immune responses in monocytes, macrophages, and dendritic cells induced gastrointestinal inflammation by activating TLR-4.[89]

In type I anaphylaxis, the combination of IgE antibody and food allergens induces degranulation of MCs and the recruitment of eosinophils, basophils, and T-lymphocytes in the intestinal mucosa. These cells proliferate and secrete T helper 2 cytokines including IL-4, IL-5, and IL-13. Tryptase released by MCs activates eosinophils through PAR-2 receptor expressed on eosinophils. Activated eosinophils degranulate and release major basic proteins, a type of endogenous allosteric inhibitor of agonist binding to the M2 muscarinic receptor, resulting in the enhancement of smooth muscle contraction.[90] Early studies have found that IBS patients produced

higher IgG4 titers for certain dietary antigens (such as wheat, beef, pork, and lamb) compared to controls, and a food elimination diet based on serum IgG/IgG4 antibodies was able to improve overall symptoms in IBS patients.[91] Similarly, FD patients had significantly higher titers of IgG antibody to egg and soybean than controls.[92] It is suggested that IBS or FD symptoms caused by dietary antigens may be involved in the IgG mediated MCs sensitization.

Psychological or Mental Stress

Studies on animals and humans both have demonstrated that stress was closely correlated with the activation of inflammation and immune response, and its possible mechanism includes the efferent cholinergic pathway of the vagus and the pro-inflammatory and anti-inflammatory effects of central and peripheral CRF signaling pathways.[93] Compared with control group rats, the number of CD4+ T lymphocytes and the expression of occludin and ZO-1 in the duodenum mucosa of the stressed rats decreased significantly.[94] The results showed that acute stress could lead to abnormal immune function of the duodenal mucosa, damage of mucosal barrier, and increased permeability of intestine. In addition, the secretion of CRF by the hypothalamus increased under stress, which promoted the degranulation of MCs in ileum of pigs and the release of TNF-α and tryptase, leading to the increase of intestinal permeability.[95] The activation of HPA axis and the increase of intestinal mucosal permeability induced by public speech and intravenous injection of CRF could be inhibited by the MC stabilizer sodium cromoglycate, indicating that MCs participated in the inflammatory response mediated by CRF.[96] Moreover, the activation of HPA axis was associated with the increase of IL-6 in peripheral blood.[97] Other studies confirmed the relationship between inflammation and mental disorders showed that patients with anxiety and depression had immune dysfunction manifesting as the increase of serum C-reactive protein, IL-6, IL-5, IL-13, TNF-α, and other inflammatory mediators.[98,99] Patients with IBS-FD had more serious psychological problems than those without overlapping symptoms.[100] Multivariate logistic regression analysis showed that anxiety was an independent factor affecting the overlap of IBS and FD symptoms.[101] Antidepressants are used in practice for FGIDs patients with epigastric or lower abdominal pain. Current evidence for the use of antidepressants is much stronger in IBS than in FD.

In summary, SIBO, food allergy and mental or psychological stress all participate in the local or systemic chronic inflammatory response and immune dysfunction of IBS/FD as stimulants. The changes of MCs, eosinophils and lymphocytes, and the increase of pro-inflammatory cytokines released by them lead to the increase of intestinal permeability and the disorder of the brain-gut axis, resulting in gastrointestinal symptoms through mechanisms similar to that of PI-IBS/PI-FD.

The Guiding Significance of Inflammatory Mechanism in the Treatment of Patients With Overlap Syndrome of Irritable Bowel Syndrome and Functional Dyspepsia

Therapies such as food elimination diet, *H. pylori* eradication, antibiotics and antidepressants/anti-anxiety agents (currently called neuromodulators)[102] can remove the initiating factors of inflammatory response and become potential therapeutic options for IBS-FD patients. However, there is no case-control study to confirm the efficacy of the above treatments in patients with IBS-FD. Other treatments that may be effective for patients with IBS-FD include drugs that inhibit the intermediate links of inflammation besides rifaximin as mentioned above.

Mesalazine plays an anti-inflammatory role through affecting various mediators and signaling pathways that mediate leukocyte chemotaxis and epithelial defense function (such as free radical-scavenging, promoting proliferation of intestinal epithelial cells, and inhibiting their apoptosis). In recent years, the therapeutic effect of mesalazine on intestinal inflammation of IBS patients has been widely reported. It was confirmed that mesalazine reduced the number of MCs in the colonic mucosa of IBS patients and down-regulated the function of MCs.[103] A randomized controlled study of Barbara et al[104] showed that mesalazine treatment was not superior to placebo at the primary end point of the study (significant improvement of abdominal pain/discomfort), but some IBS patients (11.6%) showed sustained response (relief of abdominal pain and improvement of overall symptoms) to the treatment. As altered mucosal immune activity is a pivotal pathogenic factor in PI-IBS, Andresen et al[105] observed that mesalazine reduced the risk of PI-IBS after infection with Shiga-like toxin-producing *E. coli* O104:H4 through its modulatory action on mucosal immunity. Since mesalazine mainly acts on the lower gastrointestinal tract to exert its anti-inflammatory effect and considering the potential side effects of its long-term treatment, we can speculate that mesalazine benefits little to patients with FD or IBS-FD, but there are no relevant studies.

MCs are the main inflammatory cells involved in the inflammatory response of IBS and FD. Pretreatment with disodium cromoglycate, a MC stabilizer, could prevent the increased intestinal permeability due to acute stress and CRF injection.[96] Oral disodium cromoglycate treatment could also significantly improve

gastrointestinal symptoms of patients with IBS by reducing activation of MCs.[106] Meta-analysis showed that the improvement rate of FD symptoms after being treated by 5-HT_4 receptor partial agonist (tegaserod) was significantly higher than placebo.[107] Tegaserod was also significantly better than placebo in relieving IBS symptoms such as abdominal distention and constipation.[108] 5-HT_3 receptor antagonists (alosetron and cilansetron) were more effective than the comparators in achieving global improvement in IBS symptoms and relief of abdominal pain and discomfort.[109] Through inhibiting the synthesis of 5-HT, tryptophan hydroxylase inhibitor could down-regulate the release of 5-HT in inflammatory response, which is beneficial to the improvement of symptoms in patients with IBS-D.[110] In the future, more studies will be needed to explore the efficacy of these drugs in patients with IBS-FD.

Summary and Outlook

The subtype analyses regarding IBS-FD showed that IBS with constipation was the most prevalent subtype in the postprandial distress syndrome-IBS overlap group, while IBS with diarrhea was the most frequently reported type in the EPS-IBS group,[111] which suggest that visceral hypersensitivity or dyskinesia of the whole gastrointestinal tract and abnormal perception and processing to pain in the central nervous system may be the pathophysiological characteristics of IBS-FD. As mentioned earlier, comorbidity with psychological or mental illness also increases the risk of overlap syndrome.

Table. The Known and Possible Mechanism of Inflammation in Patients With Overlap Syndrome of Irritable Bowel Syndrome and Functional Dyspepsia

Pathogenic factors	Disease	Pathophysiological changes	Pathophysiological outcomes	Related symptoms
Gastrointestinal infection	IBS	Intestinal MCs, T lymphocytes ↑[12,13] and inflammatory cytokines ↑[19,20] Downregulation and redistribution of ZO-1 and occludin[33] TRPV1, SP, and CGRP positive nerve fibers ↑[38,39] Increased excitability of DRG and submucous plexus neurons[54,55] Persistent activation of CRF neurons,[59] CRF binding to CRF_1 receptor[61,62]	Increased intestinal permeability Visceral hypersensitivity Colon transit and defecation ↑[61,62]	Diarrhea[33] Abdominal pain
	FD	Gastric and duodenal MCs, eosinophils ↑[14-16] Abdominal expression of ZO-1 and occludin[36] SP, CGRP positive nerve fibers ↑[41,42] CRF binding to CRF_2 receptor[61,62]	Damage of duodenal mucosal barrier Visceral hypersensitivity Gastric contraction and gastric emptying ↓[61,62]	Epigastric burning[16] Early satiety[18]
SIBO	IBS	IL-1α and IL-1β ↑[81]	Mild inflammation and immune activation of intestinal mucosa	Abdominal distention relieved by rifaximin[83]
	FD	NA	NA	Abdominal distention relieved by rifaximin[84]
Food allergy	IBS	Increased intervillous spaces and intraepithelial lymphocytes in intestinal mucosa[87] Titers of IgG antibody to specific food antigen ↑[91]	Increased intestinal permeability	
	FD	Duodenal eosinophilia[88] Titers of IgG antibody to specific food antigen ↑[92]	NA	Early satiety[88]
Psychological or mental stress		CD4+ T lymphocytes ↓[94] CRF secreted by hypothalamus ↑, degranulation of MCs and released TNF-α and tryptase ↑[95] Expression of occludin and ZO-1 in duodenal mucosal ↓[94]	Increased intestinal permeability Visceral hypersensitivity	

SIBO, small intestinal bacterial overgrowth; IBS, irritable bowel syndrome; FD, functional dyspepsia; MC, mast cell; ZO, zonula occludens; TRPV1, transient receptor potential vanilloid type-1; SP, substance P; CGRP, calcitonin gene related peptide; DRG, dorsal root ganglion; CRF, corticotropin releasing factor; NA, not applicable.

It is worth mentioning that inflammation may be involved in any level of the above pathogenesis. The mechanism of inflammation in IBS-FD can be summarized as various factors such as acute gastrointestinal infection, intestinal flora disorder, food allergy, or stress lead to the damage of intestinal epithelial barrier and antigen presentation, causing the activation of inflammatory cells and release of pro-inflammatory cytokines and chemokines of inflammatory cells. Then, the activation and degranulation of inflammatory cells leads to different degree of gastrointestinal mucosal inflammation and immune response resulting in visceral hypersensitivity, dysfunction of gastrointestinal motility, sensory and secretion. Compared with IBS or FD patients, the systemic or local inflammation of the gastrointestinal tract induced by infection, diet, microbiota alteration, mental or psychological factors may be more obvious in patients with IBS-FD. In addition, the distribution of inflammation is probably more extensive in the digestive tract, which involves the upper and lower digestive tract at the same time or subsequently, resulting in overlapping symptoms.

The known and possible mechanisms of inflammation in patients with IBS-FD is summarized in Table. Although there are few studies regarding IBS-FD, this article specifically reveals many commonalities of inflammation in the mechanism of IBS and FD and provides references for future research direction. In the future, we need more researches focusing on confirming the pathogenesis of IBS-FD, and more high-quality, multi-center, and large-sample studies on efficacy to guide clinicians to optimize the treatment of patients with IBS-FD.

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