

# The significance of infectious disease and microbiota in functional gastrointestinal disorders

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## Abstract

The definition of functional gastrointestinal disorders (FGID) used to be disorders that do not manifest into organic abnormalities. However, it was pointed out that chronic GI dysfunctions can develop following bacterial infections such as acute gastroenteritis and viral infections, and some organic changes in the epithelial cell structure, immunocompetent cells, and inflammatory cytokines were revealed. Recent advancements in analytical methods helped discover changes in the intestinal microbiota of patients with FGID. Correlations between the effects on the enteric environment and clinical conditions and symptoms of FGID are drawing increasing attention.

## KEYWORDS

intestinal bacteria, post infectious irritable bowel syndrome, small intestinal bacterial overgrowth

## 1 | INTRODUCTION

Functional gastrointestinal disorders (FGID) was used to be considered as disorders that do not have organic or biochemical etiology, as abnormalities were not observed endoscopically or in the blood test results. Recently, advancements have been made in the gross examination as well as the microscopic examination of the GI tract, and the findings of patients with FGID that are different from those of healthy people, including pathological changes, immune response, and other findings, began to come to light. In addition, it has been shown that infectious diseases are often the cause of FGID, and this has gathered much attention. Attempts have also been made to learn about organic abnormalities in order to understand the clinical manifestations of FGID. FGID is classified into functional dyspepsia (FD) and irritable bowel syndrome (IBS), whose symptoms are mainly associated with the upper GI tract and the lower GI tract, respectively. In this report, correlations between infectious diseases, intestinal bacteria, and immune response and FD and IBS are summarized.

### 1.1 | Infectious diseases and FGID

#### 1.1.1 | *Helicobacter pylori* infection and FD

Ever since *Helicobacter pylori* (*H. pylori*) was discovered in 1983, its correlations with diseases such as ulcers, hyperplastic polyps, MALT lymphoma, and gastric cancer have been revealed. Correlations between FD and *H. pylori* infection are also being examined by many researchers. Firstly, in a comparison of FD symptoms in *H. pylori*-positive FD patients vs *H. pylori*-negative FD patients, severity of symptoms was equivalent. For the evaluation of *H. pylori*'s role in FD, symptoms were compared before and after eliminating *H. pylori* in *H. pylori*-positive FD patients. Many reports were made for both patients whose symptoms improved and did not improve, but the meta-analysis of 17 reports in 3566 *H. pylori*-positive FD patients suggested that *H. pylori* eradication therapy is more effective in symptom improvement compared to other treatment.<sup>1</sup> In 2008, a randomized double-blind comparison study was performed, and it was shown that *H. pylori* eradication therapy improves FD symptoms just as good as other medical treatment.

Although it has not been demonstrated that *H. pylori* infection is an underlying cause of FD, there is a growing consensus that *H. pylori* infection is associated with FD symptoms. The Asia-Pacific Consensus Guidelines therefore recommend proactive use of *H. pylori* eradication therapy for *H. pylori*-positive FD patients.<sup>2</sup>

Is there any organic difference in *H. pylori*-positive and *H. pylori*-negative FD patients? Many reported no clear correlation between the endoscopically diagnosed severity of gastritis and FD symptoms. However, Gargala et al.<sup>3</sup> reported a significantly higher number of intraepithelial lymphocytes (IELs) in the duodenal mucosa of *H. pylori*-positive FD patients compared to that of *H. pylori*-negative FD patients and healthy people. As it has also been reported that the number of CD8+ IELs in the duodenal mucosa as well as stimulation-induced cytokine production is high in patients with FD, further analysis of correlations between IELs and FD is desired.

### 1.1.2 | Postinfectious FD (PI-FD)

Development of IBS symptoms following acute GI infection has been noted historically, and this condition was recently established as postinfectious irritable bowel syndrome (PI-IBS) (details are discussed below): Thus, correlations between FD and acute infectious diseases have also been studied. In 2002, Tack et al. performed a study in 400 FD patients and found out that 25% (n=98) of patients developed acute conditions that started with fever, vomiting, diarrhea, or other symptoms suggestive of acute inflammation. Additionally, the gastric retention of FD patients who developed acute conditions decreased more than that of other patients with FD.<sup>4</sup> A prospective study was performed in people of a village in Spain who were victims of the group infection outbreak on June 23, 2002, which involved a *Salmonella*-contaminated cake, when a total of 1243 people developed acute GI symptoms. A follow-up study was performed in 677 of the 1243 people, and 13.4% reported FD symptoms a year after the outbreak. As only 2% of healthy people in the control group reported FD symptoms, it was demonstrated for the first time that FD is triggered by infectious diseases.<sup>5</sup> Interestingly, this study investigated about PI-IBS at the same time. Development of PI-IBS significantly increased a year after the outbreak (10%), but in people who developed both FD and IBS, FD symptoms were observed more frequently than IBS symptoms (patients who developed FD symptoms only, 43%; both FD and IBS symptoms, 36%, IBS symptoms only, 21%). The concept of PI-IBS was introduced earlier than PI-FD, but the actual number of patients with PI-FD is likely to be higher. Studies on PI-FD also focused attention on the duodenal mucosa, and its biopsy specimens have been pathologically analyzed. As a result, a high number of aggregated CD8+ T cells and CD68+ macrophages were observed in PI-FD patients, but the number of CD4+ T cells was low.<sup>6</sup> PI-IBS has been associated with an increased number of neuroendocrine cells in the epithelial cells and IELs, but this was not the case with PI-FD. This means that GI infections possibly manifest differently in the upper and lower GI tract. It would be useful to examine organ-specific postinflammation changes in the future PI-FD studies.

### 1.1.3 | Postinfectious IBS (PI-IBS)

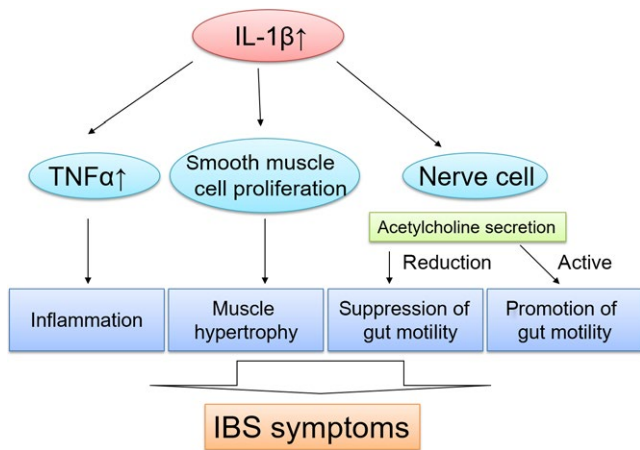
Postinfectious development of IBS, whose symptoms are mostly associated with the lower GI tract, has been known for quite some time. The first report was made during WWII when a British soldier who was infected with amebic dysentery in North Africa developed IBS symptoms after returning home. This case is considered the beginning of the PI-IBS concept. A prospective study of patients who were involved in a group GI infection showed that 31% of patients had IBS symptoms, and the symptoms were still present a year after the infection. Another study reported that 25% of acute enteritis patients had IBS symptoms 6 months after getting injected.

Subsequently, a large-scale prospective study was performed in the UK in 584 308 healthy people. A total of 318 people developed bacterial enteritis during the observational period, and 12 of whom were diagnosed with IBS a year after the onset of bacterial enteritis. The risk of developing IBS was 4%/y, whereas in people who did not develop bacterial enteritis, the risk was 0.35%/y. It was thus demonstrated that acute enteritis is associated with a significantly higher risk of IBS development. As patients who had intestinal infection continually develop PI-IBS symptoms for some reason, they have become a useful model for understanding the clinical manifestation of IBS.

PI-IBS has thus been reported in association with bacterial enteritis, and more recently, PI-IBS associated with viral gastroenteritis has also been observed. A follow-up study was performed for the major norovirus gastroenteritis epidemic that took place in Italy in 2009. A year after the epidemic, PI-IBS was diagnosed at a very high rate (n=40/103), indicating that not only bacterial but viral acute gastrointestinal conditions can also induce PI-IBS.

Pathological changes in PI-IBS have much been studied. This type of study involves a postinfection sequential rectal biopsy followed by a microscopic examination of the changes in the histological findings. Evaluation criteria include, mainly, cellular infiltration of epithelial cells, IELs, lamina propria lymphocytes (LPLs), macrophages, and mast cells. It was previously thought that pathological characteristics of epithelial cells including that of hematoxylin and eosin stained epithelial cells were not significantly different from that of normal epithelial cells. However, an increased number of endocrine cells were observed immediately after acute infection, and a year afterward, the total number of endocrine cells did not decrease and was higher than that of healthy people. Furthermore, analysis of the proteins produced by the endocrine cells of PI-IBS patients revealed an increased number of enterochromaffin cells that produce serotonin, although normally, peptide YY is what is mainly produced.<sup>7</sup>

Regarding the lymphocyte component, the number of both IELs and LPLs significantly increases at the time of infection and normally, a high number of IELs and LPLs gradually decline as the infection ameliorates. However, it was not normalized a year later and was still increasing at a certain rate. As for mast cells, an increase in the number of cells has been reported by more than one article. The number of mast cells observed in the large intestinal biopsy specimens was not increased but that of mast cells observed in the ileocecal region and the ileum terminal biopsy specimens of both PI-IBS



**FIGURE 1** Functional regulation of IL-1 $\beta$  in PI-IBS

and IBS patients was increased compared to normal people. Mast cells are rich in intracellular chemicals whose activities are known to trigger inflammation and enhance mucosal permeability. The increased number of mast cells in the ileocecal biopsy specimens of IBS patients strongly suggested occurrence of focal and faint inflammatory response.

Cytokine production was analyzed in an attempt to understand the severity of the said inflammation associated with the minimal cell increase. In patients with PI-IBS, the number of macrophages was not increased but interleukin-1 beta (IL-1 $\beta$ ) produced by macrophages was increased. Additionally, increased blood IL-6 and interferon concentrations, that is, increased level of inflammatory cytokines, have also been reported. The lactose and mannitol tolerance test that assessed permeability of intestinal mucosa revealed increased permeability in PI-IBS patients compared to normal people, and the possible involvement of inflammatory cytokines including TNF- $\alpha$  and interferon was suggested. Moreover, IL-1 $\beta$  directly affects the smooth muscle cells of the GI tract, and it thickens the GI muscle layers by promoting cellular proliferation. Biopsy of the all intestinal layers of patients with PI-IBS also revealed thickened intestinal muscle layers, strong inflammatory cell infiltration in the nerve plexus between muscle layers, reduced number of nerve cells, and a reduced number of interstitial cells of Cajal, suggestive of close association with a regulation dysfunction of GI motility. IL-1 $\beta$  is also known to have impact on nerve cells of the intestine. It has been reported that IL-1 $\beta$  regulates or promotes the regulation of acetylcholine and nor-epinephrine secretions, and it causes a regulation dysfunction of GI motility although the active site may be different depending on the local environment (Figure 1). In summary, histological and immunological evaluation has so far helped us to understand that acute infectious diseases trigger mild mucosal changes in the lower GI tract and is closely related to IBS symptoms.

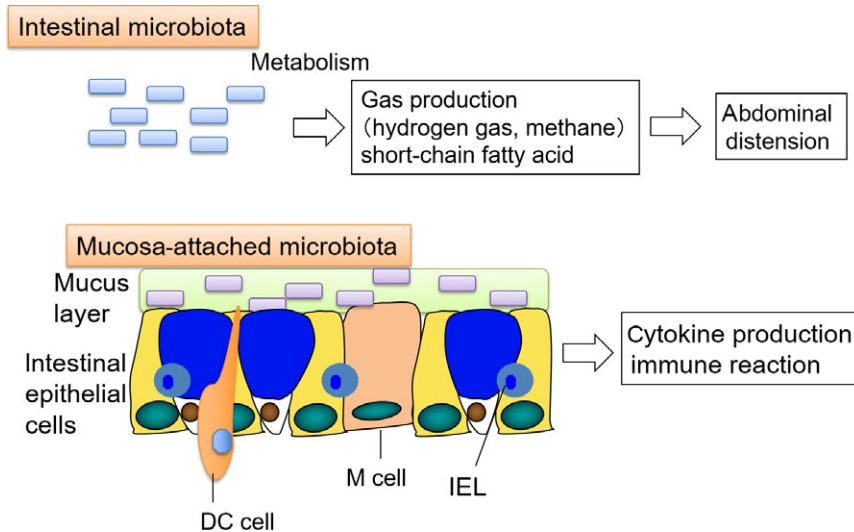
## 1.2 | Intestinal bacteria and FGID

Correlations between intestinal bacteria and FGID have been pointed out, as the intestinal microbiota of patients with FGID is different

from that of healthy people and symptoms often improve with an anti-flatulent or an antibiotic agent. The number of intestinal bacteria is low in the stomach and jejunum ( $10^1$ - $10^3$  cfu/mL), but it increases in the ileum ( $10^4$ - $10^7$  cfu/mL) and the colon ( $10^{10}$ - $10^{13}$  cfu/mL). In terms of bacterial species, more aerobic bacteria are observed on the jejunum side and more anaerobic bacteria on the ileum side. As thus, the intraluminal environment differs greatly depending on the part of the intestinal tract.<sup>8</sup>

### 1.2.1 | Small intestinal bacterial overgrowth (SIBO) and IBS

A high concentration of hydrogen ions in the intestinal tract of patients with FGID has been reported, and they were thought to be produced as a result of intestinal bacteria's metabolic response. Subsequently, clinical conditions associated with increased bacterial proliferation in the jejunum were confirmed, and SIBO was then defined as abnormal bacterial proliferation of  $\geq 10^5$  cfu/mL in the duodenum and jejunum. It has been suggested that SIBO-related bacterial proliferation results in increased metabolism in the lumen, which triggers compromised motility and gas retention that can lead to symptoms such as abdominal distention and flatulence. Possible involvement of SIBO in the etiology of IBS is controversial, and concrete evidence has not yet been discovered. The problem lies in the breath tests that are used for measuring the bacterial volume in the jejunum. Lactulose hydrogen breath test (LHBT) and glucose hydrogen breath test (GHBT) are breath tests that evaluate metabolism of intestinal bacteria based on the hydrogen gas and methane gas in breath. All studies that employed these breath tests for confirming the presence of SIBO in IBS patients reported a high SIBO positive rate, from 11% to 78%.<sup>9</sup> However, it has recently been pointed out that the transit time of lactulose used in these breath tests is faster in IBS patients compared to that of healthy people, and by the time IBS patients' breath is collected, lactulose has already reached the large intestine. This means that the results of the breath tests IBS patients undergo may reflect the bacterial volume in the large intestine, not in the jejunum. It is an ideal to measure the bacterial volume directly, but it is relatively difficult to approach the jejunum and a large-scale evaluation has not yet been performed. Comparatively large-scale studies performed to date include an analysis of jejunal fluids that have been aspirated from 162 IBS patients. The presence of  $\geq 10^5$  cfu/mL of bacterial volume, namely, SIBO was observed in 4% of the patients, but it was not statistically significant, as the equivalent proportion of healthy people also had SIBO. In another study, duodenal fluids were endoscopically collected from 148 IBS patients. Bacterial volume of  $\geq 10^5$  cfu/mL was detected in only 2% of IBS patients, whereas in the control group (n=542), over 10% of people had SIBO.<sup>10</sup> Tests that measure actual bacterial volume do not help demonstrate the relationship between IBS and SIBO. However, advancements have been made in small bowel endoscopy such as balloon endoscopy, and the small intestine can now be approached relatively easily. Detailed examination in a form of a large-scale study is desirable for the future.



**FIGURE 2** The effect of intestinal microbiota on gut

### 1.2.2 | Intestinal bacterial species and IBS

In addition to the volume, the species of intestinal bacteria have also been the focus of attention. Majority of reports involved examination of the intestinal bacteria excreted in stools, which were then cultured for species identification in early days. However, as many intestinal bacterial species cannot be cultured, examination and comparison were limited to the species that could be cultured. Subsequently, it was discovered that bacterial genomes contain a bacteria-specific common sequence named 16S rDNA which can be a target for PCR, and direct identification and quantification of the species of the bacteria in stool became possible without culturing. There seems to be no report from a large-scale study, but some studies have been performed in about 10-20 IBS patients. These studies examined IBS by the types of symptoms, that is, the diarrhea type and the constipation type, and evaluated volume variability of specific intestinal bacterial species. Thus far, advancement has been made for the bacterial species that cannot be cultured, but the PCR species identification is not possible unless the genetic sequence is known and a certain level of bacterial volume is available. In recent years, the next generation sequencer is used to directly identify the genetic sequence of bacteria in stools. Jeffery et al. analyzed intestinal bacteria in the stools of 37 IBS patients and 20 healthy people. As a result, they succeeded in sequencing 30 000 genes per person and bacterial species were then identified and classified. A cluster analysis was then performed based on the bacterial species being identified, and the bacterial structural patterns were analyzed. Twenty-two out of 37 IBS patients had clusters that are different from those of healthy people, and 11/37 IBS patients had the same clusters as those of healthy people. IBS patients' clusters were characterized as having a high number of *Firmicutes* spp. and a lower number of *Bacteroidetes* spp. compared to healthy people.

Another interesting finding was obtained when clinical symptoms were compared between the IBS patients who have IBS-specific intestinal bacteria clusters and the IBS patients who have the same intestinal bacteria clusters as healthy people: Prevalence of depression was significantly higher in the IBS patients who have the same intestinal

bacterial clusters as healthy people. This result suggested that IBS patients can be classified into two types, that is, patients who develop IBS due to psychological reasons in spite of having normal intestinal microbiota and patients who develop IBS due to infection or other diseases that result in altered intestinal microbiota.<sup>11</sup>

Regarding the correlations with PI-IBS, there are no reports that showed a significantly different intestinal microbiota in PI-IBS patients and non-PI-IBS patients. It is very difficult to compare changes in the intestinal bacteria before and after infection, as the onset of acute infection cannot be predicted. However, once how infectious diseases affect the intestinal microbiota is demonstrated, the understanding of the clinical manifestations is likely to be enhanced greatly.

Most of the intestinal microbiota examinations use enteric fluids or stools, and they are useful in studying the effects on the intraluminal metabolism. However, it is also important to study about bacteria attached to mucus. It has been noted that the intraluminal microbiota and mucosal microbiota are completely different. As bacteria attached to mucus have a direct impact on the epithelial cells in the intestinal tract and the immunocompetent cells in mucus, evaluation of the bacteria attached to the mucus of IBS patients is anticipated (Figure 2).

## 2 | CONCLUSION

In this article, I have summarized infectious diseases and a role of intestinal bacteria in patients with FGID. Immunological changes observed in PI-FGID patients and changes in the intestinal microbiota of IBS patients are being reported as new findings, as advancements are being made to the analytical methods. Slight changes can now be detected as the sensitivity of analytical methods increased, but there is no research that aimed to discover how such changes manifest into clinical conditions. It is important to comprehensively understand immunology of the GI tract, microbiota, GI motility regulation, and psychological impact in order to find out how FGID manifest in clinical conditions. I believe a study approach that correlates the

immunological changes and changes in the intestinal bacteria of IBS patients with the clinical conditions of inflammatory intestinal diseases is effective. I am hopeful that understanding the clinical conditions of IBS which so many patients are affected with will contribute to deeper understanding of the clinical conditions of inflammatory intestinal diseases.

#### CONFLICT OF INTEREST

The authors have stated explicitly that there are no conflicts of interest in connection with this article.

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