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Mini Review

Bacteriotherapy reintroduces "old friends" in IBD

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Various strains of microorganisms inhabit the human gut and greatly impact human health. Recent advances in next-generation sequencing techniques revealed a correlation between alterations to the composition of gastrointestinal microbiota: called dysbiosis, and inflammatory bowel disease (IBD), a chronic inflammatory intestinal disorder comprising ulcerative colitis (UC) and Crohn's disease (CD). These alterations are suspected to be the causes of IBD. Significant lifestyle and environmental changes in modern developed countries may be responsible for the altered gastrointestinal microbiota, and may have greatly contributed to the rapid rise of IBD in the modern era. To date, many trials attempted to treat IBD by restoring altered microbiota using such methods as probiotics and fecal microbiota transplantation (FMT). Currently, more sophisticated and convenient methods of FMT are being devised. The focus is now on FMT, which is expected to be the new direction of IBD therapy.

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Introduction

Inflammatory bowel disease (IBD), which comprises ulcerative colitis (UC) and Crohn's disease (CD), is characterized by chronic inflammation of the gastrointestinal tract. IBD patients experience remission and relapse cycles of inflammation, and present with diarrhea, bloody stools, and weight loss. While the cause of IBD remains unknown, more than 160 IBD-associated susceptibility genes have

been identified in multiple studies[1], suggesting that genetic factors probably contribute to IBD. As the most widely used IBD therapy is immunosuppressive therapy, inappropriate immune response is strongly implicated in the pathogenesis of IBD. Environmental factors are considered to mediate genetic factors and inappropriate immune responses. To date, the most consistent pathogenetic mechanism proposed for IBD is an inappropriate immune response against gastrointestinal commensal microbiota triggered by environmental factors[2].

The number of IBD patients has shown tremendous increases, especially in developed countries in the modern era. The involvement of alterations to gastrointestinal microbiota in IBD patients has been highlighted as a potential cause. Sellon et al. reported that genetically engineered IBD mice do not develop colitis in germ-free conditions[3], suggesting that gastrointestinal microbiota play an important role in triggering IBD inflammations. The human gastrointestinal tract contains 10^{14} bacteria composed of over 1,000 species that build gastrointestinal microbiota[4]. The recent advance of next-generation sequencing techniques has made it possible to exhaustively analyze gastrointestinal microbiota. Using this technique, it was discovered that a disruption of the balance of microbiota known as "dysbiosis" likely contributes to IBD exacerbation[5]. Modern people have developed methods of preservation, transportation, and sewage disposal, promoted hygienic environments, and dramatically changed their diets to include high sugar/low dietary fiber. As a result, modern people have lost the balance of their gastrointestinal microbiota, in a manner, their "old friends". Given this change, it has become apparent that the number of IBD patients has been increasing gradually with the modernization and westernization of our lifestyles. In this review, we will discuss the correlation between IBD and alterations of gastrointestinal microbiota, as well as bacteriotherapies that reconnect us with our "old friends".

Lifestyle changes and microbiota

When no freezing or refrigeration technology was available, fermentation was the essential method of food preservation. People of this era frequently ate fermented food, including probiotics (e.g., Lactobacillus), and naturally inoculated many types of beneficial bacteria into their gastrointestinal tracts. Along with modernization and industrialization, the development of freezing and refrigeration technology has allowed for long-term food preservation independent of fermentation. In addition, the development of transportation technology has enabled the year-round availability of fresh meat, fish, and vegetables while the development of sewage disposal systems made our environment more sanitary, allowing us to preserve food without the risk of rottenness. Thus, people have come to prefer fresh food to fermented food, leading to a decline in the consumption of fermented food. Furthermore, the improvement of hygiene in our environments decreases our exposure to numerous bacteria[6]. The components of our diet have also changed dramatically in the modern era. Modern individuals prefer foods that are low in dietary fiber to those that are rich in fiber because of the ease of digestion. Significant reductions in the intake of dietary fibers, which feed microbiota in the human gut, greatly affect the microbiotic environment and reduce the diversity of gastrointestinal microbiota. Additionally, the changes in amount of consumption of fat and other nutrients also lead to dysbiotic state of gut microbiota[7].

The mode of child delivery and infant feeding method also affect gut microbiota[8, 9]. Pre-birth, children are in germ-free conditions. They are then exposed to many types of microorganisms at delivery for the first time, and are colonized by commensal microorganisms within 2-3 days after birth. Several beneficial bacteria such as Lactobacillus species are present in the birth canal. These beneficial bacteria colonize by ingestion when the baby is delivered vaginally. However, a baby delivered by Caesarean section is not exposed to these bacteria. While human breast milk was traditionally thought to be sterile, it in fact contains many bacteria and is one of the main sources by which bacteria are ingested[10]. Bifidobacterium, a known probiotic, constitutes a substantial portion of the microbiota in the feces of breast-fed infants[11]. Human milk may form healthier gut microbiota in infants[12].

This is the manner in which gastrointestinal microbiota of modern people, especially those in westernized and industrialized countries, has been dramatically altered along with the loss of beneficial bacteria.

Dysbiosis may induce intestinal inflammation in IBD

To date, no specific bacterium that induces IBD has been identified. In the pathophysiology of IBD, the condition is thought to result from the alteration of microbiota (i.e., dysbiosis) rather than from a single specific pathogen[13]. Indeed, several studies reported reduced diversity and total

amounts of gastrointestinal microbiota in IBD patients[5, 14-16]. The alterations of gastrointestinal microbiota in IBD include reduced numbers of *Firmicutes* and *Bacteroidetes* and increased numbers of *Proteobacteria* and *Actinobacteria*. These changes are more obvious in inflamed sites than in non-inflamed sites, suggesting the possible involvement of dysbiosis in intestinal inflammation. However, it remains uncertain whether these alterations of microbiota in IBD patients are a cause or a consequence of the inflammation. Interestingly, wild-type mice co-housed with genetically engineered mice that developed spontaneous UC-like colitis also developed a similar colitis[17]. This suggests that a state of dysbiosis in the gut can affect normal gastrointestinal microbiota and induce a dysbiotic state. Altered microbiota can also promote intestinal inflammation.

Recently, several studies showed correlations between the intestinal immune systems of IBD patients and specific bacterial strains. For example, segmented filamentous bacteria (SFB) are sufficient to induce Th17 cells in the intestine[18]. Th17 cells secrete IL-17 and IL-22 and play a crucial role in IBD inflammation. Clostridia ferment dietary fibers and produce short-chain fatty acids (SCFAs) such as acetate, butyrate, and propionate. Butyrate is one of the most significant sources of nutrients for colonocytes, and contributes not only to the proliferation of intestinal epithelium but also to the suppression of intestinal inflammation. Several studies have revealed that butyrate induces the differentiation of regulatory T cells that produce IL-10 and suppress excessive immune responses[19-21]. Furthermore, Atarashi et al. reported that a mixture of 46 mouse-derived *Clostridia* strains promoted transforming growth factor (TGF)-β and induced IL-10-producing regulatory T cells in inoculated germ-free mice[22]. These results suggest that the decrease of Clostridia observed in IBD patients causes aberrant functioning of regulatory T cells and reduces their numbers, consequently contributing to the development and maintenance of intestinal inflammation. Recently, our group demonstrated that *clostridium butyricum (CB)* prevents acute experimental colitis in mice through induction of IL-10, an anti-inflammatory cytokine[23]. CB directly triggered IL-10 production by intestinal macrophages in inflamed mucosa via the TLR2/MyD88 pathway. The colitis-preventing effect of *CB* was negated in macrophage-specific IL-10-deficient mice, suggesting that induction of IL-10 by intestinal macrophages is crucial for the probiotic action of *CB*. These results suggested that *CB* may be useful for IBD patients to prevent colitis as probiotics.

Restored dysbiosis as a treatment for IBD

As discussed above, the abnormal composition of gastrointestinal microbiota is likely to be deeply involved in the pathophysiology of IBD, and communicated dysbiotic gastrointestinal microbiota can cause the development of intestinal inflammation. Against the background of these findings, several trials have attempted restoring the abnormal microbial composition for the treatment of IBD (Fig. 1).

1)Probiotics

Probiotics are live bacteria that benefit human health. Several clinical trials have demonstrated the efficacy of probiotics for IBD. For example, *Escherichia coli* Nissle 1917 was found to be safe and effective in maintaining remission in UC equivalent to that of mesalazine[24]. VSL#3, a mixture of 8 strains of bacteria, showed efficacy in the prevention of recurrent pouchitis[25]. VSL#3 has also been shown to be effective in inducing remission in active UC patients[26]. There is little evidence for the efficacy of probiotics in CD patients. One systematic review of randomized control trials of probiotics in IBD patients concluded that there is insufficient evidence to recommend probiotic use in CD patients[27].

However, two Cochrane reviews evaluated the efficacy of probiotics for the induction or maintenance of remission in UC patients[28, 29], finding limited benefits of probiotics in

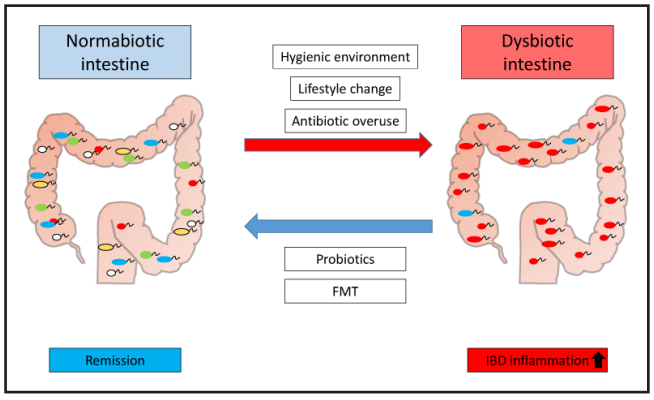

Fig. 1 Treatment restoring altered microbiota in IBD
The overuse of antibiotics, lifestyle changes, and the development of a hygienic environment resulted in the dysbiosis of normal gut microbiota. Dysbiosis may cause intestinal inflammation, and it is thus expected that treating dysbiosis with probiotics or fecal microbiota transplantation will dramatically improve these inflammations.

this population. Therefore, probiotics are not recommended as first-line therapy in IBD. There are three considerable reasons for the limited efficacy of probiotics in IBD.

First, probiotics contain a lower bacterial load compared to the gastrointestinal microbiota. There are only 10^6-10^9 bacteria in probiotics, 100,000-fold less bacteria than the amount found in human beings. Thus, the impact of probiotics on gastrointestinal microbiota may be very small.

Second, there is a lack of bacterial diversity in probiotics. Probiotics are composed of only a single strain or a few strains of bacteria. By contrast, the gastrointestinal microbiota is composed of over 1,000 species of bacteria that build a complex web of interactions. A few types of bacterial strains may exert only a small effect against these complex interactions.

Finally, the mechanism of "colonization resistance", whereby ingested pathogens and foreign substances are excluded to maintain health and homeostasis may inhibit probiotic colonization. Healthy hosts with normal immune function are consistently exposed to pathogenic microorganisms, but they rarely develop diseases because of colonization failure due to colonization resistance. Commensal bacteria that build gastrointestinal microbiota have evolved and adapted to fulfill the nutritional niche, and compete with colonizing pathogens[30]. Indirect mechanisms of colonization resistance that are mediated by the immune system have also been suggested. Commensal bacteria have acquired tolerance to both the immune response and to antimicrobial peptide (AMP)[31]. When pathogenic bacteria invade a host intestine, commensal bacteria induce the host's immune response as well as the production of AMP. Consequently, pathogenic bacteria are eliminated from the intestine before they can adapt to the environment.

Thus, probiotics may have a limited effect on aberrant gastrointestinal microbiota. Probiotics can only ameliorate the symptoms of IBD, but are considered insufficient to strongly induce remission in IBD patients. For maintenance treatment, probiotics may be considered for patients who are intolerant to 5-ASA preparations; however, the underlying evidence is weak to recommend its use in all UC patients, and the specific probiotic is not available in many countries[32].

2)Fecal microbiota transplantation

Fecal microbiota transplantation (FMT) is a underexplored treatment geared at restoring aberrant microbial compositions by delivering fecal microbiota derived from healthy donors into the recipient's gastrointestinal tract. *Clostridium difficile* infection (CDI) is an intestinal disorder that is often associated with a course of antibiotics that causes dysbiosis in the gut. It is difficult to cure patients with recurrent CDI by repeatedly administering vancomycin or metronidazole. A randomized controlled trial comparing FMT with standard vancomycin therapy for recurrent CDI was conducted in the Netherlands[33]. Surprisingly, FMT had a much higher cure rate (81%) than standard antibiotic treatment (31%) in these recurrent CDI patients. The fecal microbiota used in FMT are obtained from healthy donors and contain numerous strains of bacteria that are thought to easily adapt to the intestinal environment because they previously colonized the donor intestine.

FMT has been highlighted as a treatment for restoring dysbiosis in IBD. In what was the first implementation of FMT in UC, in 1989, Bennet et al. reported one UC patient with a continuously active intestinal inflammation who received FMT and experienced remission[34]. Borody et al. reported using consecutive FMT to induce remission in 6 active UC patients, all of whom achieved clinical remission without severe adverse events[35].

However, there are several reports of UC patients who received FMT and did not achieve clinical remission[36-38]. Kump et al. reported that patients whose microbiota dramatically changed to resemble the composition of the donor fecal microbiota did not achieve clinical remission. By contrast, Angelberger et al. reported that the successful inoculation of fecal microbiota from donor feces into the recipient intestine correlated with the successful achievement of clinical remission.

A systematic review published in 2014 included 18 studies of FMT for IBD and a total of 122 patients. According to the results, 54 of 119 patients (45%) achieved clinical remission without severe adverse events[39]. However, publication bias should be considered because of the inclusion of 8 case studies. Supported by these backgrounds, we conducted a clinical trial treating IBD with using FMT in Japan[40].

There are several obstacles to FMT treatment. FMT transfers not only beneficial bacteria, but also pathogenic microorganisms such as hepatitis B virus, hepatitis C virus, human immunodeficiency virus, *Helicobacter pylori*, and pathogenic amoebae. Therefore, donors must be screened carefully. Despite careful screening, the risk of transmitting unknown pathogens remains[41]. FMT may also transfer extra-intestinal disorders that have significant correlations with alterations of intestinal microbiota such

as type 2 diabetes mellitus, multiple sclerosis, chronic fatigue syndrome, and idiopathic thrombocytic purpura[42]. In addition, such donor screenings are time consuming, making FMT unsuitable for the patient with severe disease activity requiring immediate intervention.

To resolve these issues, the feces bank named "Open Biome" was established in the US in 2012. A recent report showed that frozen FMT inocula have equivalent efficacy for treating CDI patients[43]. The "Open Biome" pools frozen feces obtained from donors proven safe via screening. Owing to the "Open Biome", the application of FMT to the treatment of dysbiosis is easier, quicker, and safer.

Future perspectives

The routes of infusion of fecal material vary across trials and include administration by nasogastric tube, nasojejunal tube, esophagogastroduodenoscopy, colonoscopy, or retention enema. Because FMT is a developing treatment, the optimal administration route is uncertain. FMT protocols are still complicated, and a more convenient method is needed. Youngster et al. conducted an open-label, single-group trial of a less invasive and easier to use FMT treatment in which they orally administered frozen encapsulated fecal material from unrelated donors to relapsing CDI patients. They reported no serious adverse events and a 90% rate of clinical resolution[44]. In Europe and the United States, an ongoing trial is investigating the use of "Artificial Feces" that contain large amounts of various probiotics instead of FMT[45]. "Artificial Feces" excel in terms of cost, stability of effectiveness, and ethicality. These advantages facilitate the treatment's commercialization for restoring dysbiosis.

Recent reports and our study found that specific strains of bacteria suppress intestinal inflammation in a gnotobiotic murine model[23, 46]. In this manner, microorganisms that can potentially resolve inflammation are being identified. The future progress of research on bacteria and bacterial interactions that correlate with intestinal inflammation may identify optimal combinations of probiotics that will enable the formulation of ideal "Artificial Feces" that can restore dysbiosis much more easily, safely, and effectively.

Conclusion

As discussed above, the numbers of IBD patients in developed countries are rapidly increasing along with alterations of the composition of gastrointestinal microbiota. FMT may have great potential for restoring dysbiosis and treating IBD. However, there is limited evidence regarding FMT for IBD, and better optimized and more sophisticated methods of FMT are needed.

Feces are generally viewed as "dirty" materials, however, the feces of healthy individuals are actually a "clean drug" that contains an enormous number of beneficial bacteria and can restore the disrupted balance of microbiota. It is important to reacquaint the gastrointestinal tract with these "old friends", and to maintain a "clean" gastrointestinal tract in the management of IBD. Therefore, further insights into probiotics should be obtained through cooperation not only between clinicians and drug companies, but also with the food industry.

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Conflict of interests

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