Review

Role of gut microbiota in inflammatory bowel disease pathogenesis

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The role of the gut microbiota, especially bacterial flora, in the pathogenesis of inflammatory bowel disease (IBD) is becoming clearer. Advances in gut microbiota analysis and the use of gnotobiotics models have underscored the importance of gut bacteria and their metabolites in the progression of IBD. Fecal microbiota transplantation has shown promise in clinical trials for ulcerative colitis started as Advanced Medical Care B in Japan, raising expectations for its outcomes. This review explores the gut microbiota's role in IBD, encompassing both current knowledge and future prospects.

***Key Words*: fecal microbiota transplantation, gut microbiota, inflammatory bowel disease, ulcerative colitis**

Inflammatory bowel diseases (IBD), primarily comprising Crohn's disease (CD) and ulcerative colitis (UC), are chronic conditions characterized by persistent inflammation of the gastrointestinal tract. These diseases are known not only for causing chronic intestinal inflammation but also for their relapsing and remitting nature, which leads to complications and surgical interventions, ultimately diminishing patients' quality of life (QOL). The specific etiology of IBD remains unclear; however, it is believed to result from a complex interplay of genetic, environmental, and immunological factors. Among environmental factors, IBD patients have been reported to exhibit reduced diversity in gut microbiota, with an increase in specific harmful bacteria. This dysbiosis may excessively stimulate the immune response in the gut, potentially leading to chronic inflammation. The relationship between IBD and gut microbiota is highly complex. A healthy gut microbiota plays a crucial role in maintaining the intestinal barrier function and modulating the immune system. However, in IBD patients, this balance is disrupted, potentially leading to the dominance of pathogenic bacteria, exacerbating inflammatory responses in the gut. This review will discuss the dysbiosis in IBD, the current state of fecal microbiota transplantation (FMT) therapy, the prospects of microbiome-based drug development using live biotherapeutic products (LBPs), regulatory T cells (Tregs) induction therapies, and prevention strategies for IBD. It will cover both the findings from gut microbiota research to date and the future directions in this field.

Dysbiosis in IBD

Interest in the gut microbiota was piqued in 2013 with the discovery that 17 Clostridium species in Japanese individuals induce Tregs.[1] This finding significantly influenced subsequent research on IBD and the gut microbiota. Gnotobiotic mice transplanted with healthy human feces exhibited suppressed experimental colitis, implicating Tregs in this anti-inflammatory effect.[2,3] Further analyses revealed that short-chain fatty acids (SCFAs), particularly butyrate, produced by gut bacteria utilizing dietary fiber, induce Tregs from naive T cells.

In clinical studies, Andoh *et al.*[4] analyzed the gut microbiota of IBD patients using terminal restriction fragment length polymorphism (T-RFLP) and found decreased levels of Clostridium clusters IV and XIVa in CD compared to healthy individuals. These bacteria, which were identified by Atarashi *et al.*,[1] are butyrate-producing species. Additionally, mucosa-associated microbiota analysis revealed a reduced prevalence of butyrate-producing bacteria such as *Faecalibacterium*, *Coprococcus*, *Prevotella*, and *Roseburia* in CD.[5,6] Notably, patients achieving remission with anti-TNFα antibody therapy showed recovery of these bacteria and increased SCFA concentrations.[7]

These findings suggest that butyrate-induced Tregs from specific gut bacteria, particularly those targeting the gastrointestinal tract, may lead to new therapeutic strategies for IBD.

Current Status of FMT in IBD

In 2013, the effectiveness of FMT from healthy donors for recurrent Clostridium difficile infections (CDI) was reported, generating significant global interest.[8] At that time, CDI was rising in North America and Europe, especially severe cases and fatalities linked to hypervirulent strains, such as 027 and 078. According to the Centers for Disease Control and Prevention and the Food and Drug Administration (FDA), nearly 500,000 people in the U.S. contract CDI annually, with 15,000–30,000 deaths. FMT proved high effectiveness, with a relapse-free rate exceeding 90%, normalizing gut microbiota dysbiosis.

Although clinical trials for CDI have not advanced in Japan, BiomeBank in Adelaide, Australia, has received approval from the Therapeutic Goods Administration in 2022 for FMT to treat recurrent CDI symptoms. The FDA also approved "Rebyota®," a microbiota-based product derived from human feces, for recurrent CDI in individuals over 18 years old. This marked the first approval of a fecal-based pharmaceutical product in developed countries, such as the U.S., Europe, and Japan.

Since then, FMT has been used to treat various diseases associated with gut microbiota dysbiosis, including UC and CD, and numerous clinical studies have been conducted worldwide. In Japan, the efficacy of FMT for UC has gained attention with pilot trials conducted by a team led by Professor Ishikawa at Juntendo University. The research group proposed "antibiotic FMT" (A-FMT) therapy, which involves the administration of three antibiotics (amoxicillin, fosfomycin, and metronidazole) before FMT.[9] Starting in July 2014, this clinical research demonstrated that effective bacterial species (Bacteroides) from donors could be efficiently transplanted to restore gut microbiota

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diversity. Long-term follow-up studies revealed that patients and fecal donors (siblings or the same generation within a 10-year age difference) significantly improved long-term therapeutic effects.[10]

In January 2023, A-FMT therapy for left-sided and pancolitis UC was approved as Advanced Medical Care B. Juntendo University Hospital, Kanazawa University Hospital, and Shiga University of Medical Science Hospital are participating in this trial, supported by Metagen Therapeutics, Inc. (Tsuruoka, Japan), which manages donor recruitment, sample management, microbiota solution preparation, and quality control. The trial is progressing smoothly, and registration is expected to be completed by 2024.

Numerous clinical trials worldwide have explored FMT's efficacy for diabetes, irritable bowel syndrome, and other conditions.[11] However, a cautious approach is essential, particularly in Japan, where the safety and efficacy results from Ishikawa's group should guide future developments.

Live Biotherapeutic Products (LBPs) for Microbiome Drug Development

Global efforts are underway to develop microbiome-based drugs using live biotherapeutic products (LBPs). Although a mixture of 17 Clostridium species has not reached commercialization, VE303, a combination of eight gut bacteria, has shown efficacy against CDI.[12] Clinical trials for vancomycin plus oral LBP (SER-287) for UC reported clinical remission rates of 40%.[13] Various trials have explored combinations of live bacteria to correct gut microbiota dysbiosis and achieve clinical benefits (Table 1).

Microbiome drug development also includes administering low-molecular-weight substances and peptides produced by the gut bacteria, using phages for bacterial modification, and next-generation LBPs involving gene-editing technologies. Despite the fierce competition, a collaborative microbiome drug development project targeting Japanese patients with IBD is anticipated.

Preventive Strategies and Reducing the Incidence of IBD

The incidence of IBD is rising, with Japan experiencing an annual increase of 3.7%. Although several risk factors have been identified, preventive measures have not yet been implemented. Gut microbiota and environmental changes are critical risk factors, and dietary approaches can effectively reduce this risk.[14] Integrated analyses of lifestyle and gut microbiota revealed a strong negative correlation between butyrate-producing bacteria, such as *Eubacterium eligens*, *Roseburia hominis*, *Faecalibacterium prausnitzii*, *Butyrivibrio crossotus*, and both rural living environment and lifeline diet scores (LLDS). The LLDS calculates scores based on nine heath-beneficial food groups and three harmful groups. AGA guidelines recommend a Mediterranean diet during IBD remission to improve the gut microbiota.[15] Higher occupancy of butyrate-producing bacteria correlates with higher Mediterranean diet scores.[16] Additionally, a healthy lifestyle, including not smoking, maintaining normal weight, physical activity, a Mediterranean diet, and alcohol restriction, reduces mortality in patients with IBD.

Genetic risk factors for UC and CD have been analyzed, and a polygenic risk score has been proposed. A prospective cohort study of 500,000 individuals over 12 years in the UK demonstrated that lifestyle improvements significantly reduce UC and CD risk.[17] Targeting the gut microbiota and environment is a promising approach for reducing IBD.

Table 1. Development of live biotherapeutic products (LBPs) for intestinal inflammation

Donor-derived enema
REBYOTA (RBX2660)
Donor-derived oral capsules
VOWST (SER-109)
RBX7455
CP-101
Designed oral capsule
VE-303
NTCD-M3
MET-2
ART24 (ADS024)
DSM33864
SER-262/287

Unresolved Issues in Treg Therapy

The gut microbiota→butyrate→Treg pathway is critical for improving IBD pathology. However, ten years after the discovery of the 17 Clostridium cocktails, drug development did not progress, indicating unresolved issues in this pathway. Although gut bacteria and butyrate are essential for Treg induction, additional factors are required for Treg maintenance and longevity. One such factor is the central regulatory mechanism involving the vagus nerve. Teratani *et al.*[18] identified a liver-brain-gut neural arc that maintains peripherally-induced Tregs (pTregs) in the gut, emphasizing the importance of multi-organ communication.[19] The solitary tract nucleus of the medulla detects gut inflammation and sends anti-inflammatory signals to the vagus nerve.

Numerous new treatments for IBD have been developed, including the use of anti-IL-23 antibodies, which have demonstrated remarkable efficacy. Despite limited IL-23 receptor (IL-23R) analysis, recent findings reveal its expression in both innate and adaptive immune cells, identifying IL-23R as a susceptibility gene for CD. Uchiyama *et al.*[20] found high IL-23 mRNA expression in healing mucosa, predicting relapse risk. Higashimura *et al.*[21] demonstrated that zinc deficiency in M1 macrophages enhanced IL-23 secretion, suggesting that zinc acts as a signaling molecule. Hashimoto *et al.*[22] identified D-alanine, a gut bacterial metabolite that inhibits IL-23 signaling and suppresses experimental colitis. IL-23R is also expressed in Tregs, where it sends negative signals. Clinical studies have explored the use of IL23R-CAR-Tregs for cellular therapy in CD.

Conclusion

This review focuses on the role of the gut microbiota in IBD over the past decade, emphasizing the gut microbiota→butyrate→Treg pathway. Despite the challenges in drug development, significant progress has been made in FMT, microbiome drugs, and CAR-T cell therapy. Addressing the rising number of patients with IBD requires comprehensive information and further research, especially in Japan, where nutritional studies focusing on Mediterranean diets lag behind those in Western countries.

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Conflict of Interest

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