TOPIC HIGHLIGHT

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Enteric microbiota leads to new therapeutic strategies for ulcerative colitis

Wei-Xu Chen, Li-Hua Ren, Rui-Hua Shi

Wei-Xu Chen, Li-Hua Ren, Rui-Hua Shi, Department of Gastroenterology, The First Affiliated Hospital of Nanjing Medical University, Nanjing 210029, Jiangsu Province, China

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Correspondence to: Rui-Hua Shi, MD, PhD, Department of Gastroenterology, The First Affiliated Hospital of Nanjing Medical University, 300 Guangzhou Road, Nanjing 210029, Jiangsu Province, China. ruihuashi@126.com

Telephone: +86-25-83674636 **Fax:** +86-25-83674636

Abstract

Ulcerative colitis (UC) is a leading form of inflammatory bowel disease that involves chronic relapsing or progressive inflammation. As a significant proportion of UC patients treated with conventional therapies do not achieve remission, there is a pressing need for the development of more effective therapies. The human gut contains a large, diverse, and dynamic population of microorganisms, collectively referred to as the enteric microbiota. There is a symbiotic relationship between the human host and the enteric microbiota, which provides nutrition, protection against pathogenic organisms, and promotes immune homeostasis. An imbalance of the normal enteric microbiota composition (termed dysbiosis) underlies the pathogenesis of UC. A reduction of enteric microbiota diversity has been observed in UC patients, mainly affecting the butyrate-producing bacteria, such as *Faecalibacterium prausnitzii*, which can repress pro-inflammatory cytokines. Many studies have shown that enteric microbiota plays an important role in anti-inflammatory and immuno-regulatory activities, which can benefit UC patients. Therefore, manipulation of the dysbiosis is an attractive approach for UC therapy. Various therapies targeting a restoration of the enteric microbiota have shown efficacy in treating patients with active and chronic forms of UC. Such therapies include fecal microbiota transplantation, probiotics, prebiotics, antibiotics, helminth therapy, and dietary polyphenols, all of which can alter the abundance and composition of the enteric microbiota. Although there have been many large, randomized controlled clinical trials assessing these treatments, the effectiveness and safety of these bacteria-driven therapies need further evaluation. This review focuses on the important role that the enteric microbiota plays in maintaining intestinal homeostasis and discusses new therapeutic strategies targeting the enteric microbiota for UC.

Key words: Ulcerative colitis; Enteric microbiota; Dysbiosis; Probiotic; Fecal microbiota transplantation; Polyphenol

Core tip: The human gut is comprised of a large, diverse, and dynamic microbiota. The enteric microbiota plays an important role in regulating anti-inflammatory and immunoregulatory activities. An imbalance of the normal enteric microbiota composition, termed dysbiosis, underlies the pathogenesis of ulcerative colitis (UC). Therefore, manipulation of the dysbiosis is an attractive strategy for UC therapy. This review discusses new therapies associated with the regulation of enteric microbiota for UC patients.

INTRODUCTION

Inflammatory bowel disease (IBD) refers to chronic relapsing or progressive inflammatory conditions that may affect the entire gastrointestinal tract. Ulcerative colitis (UC) and Crohn's disease (CD) are two main clinically defined forms of IBD[1]. In UC, inflammation is limited to the lining of the colon, whereas transmural inflammation occurs along any part of the gastrointestinal tract in CD[2]. The incidence and prevalence of IBD have continued to increase over the past few decades throughout various regions around the world[3]. Although the precise pathogenesis is unknown, it is thought that the etiology of IBD involves dysfunction of the mucosal immune system, which develops from complex interactions between the host immune system and genetic and environmental factors[4].

The gastrointestinal tract is possibly the most complicated immune organ of the entire human body. The intestinal mucosa is continuously exposed to a variety of commensal microbiota and food antigens. The gut must suppress excessive immune responses to antigen stimulation, and thus uses both acquired and innate immune systems to maintain intestinal homeostasis. Evidence suggests that the development and function of the intestinal immune system depend on its specific enteric microbiota, which appears to have co-evolved[5]. However, a disruption in this system can lead to aberrant immune responses to the enteric microbiota and cause chronic inflammation within the gut[5,6]. This review focuses on the important role the enteric microbiota plays in maintaining intestinal homeostasis and discusses new therapeutic strategies for UC that are based on the enteric microbiota.

HUMAN ENTERIC MICROBIOTA

The human gut is a complex anaerobic environment with a large, diverse, and dynamic enteric microbiota composed of more than 100 trillion microorganisms - which is ten times greater than the total estimated number of human cells[7]. Until the 1990s, the characterization of the enteric microbiota was limited to the use of bacteriological culture. However, less than 30% of fecal microorganisms observed under the microscope can be cultured. More recent advances in culture-independent techniques, such as 16S rRNA gene probing-based strategies and metagenomics, have broadened our knowledge of the complexity of this ecosystem[8,9]. The enteric microbiota is comprised of more than 1000 different bacterial species[10]. The majority of these organisms are from seven phyla: *Firmicutes*, *Bacteroidetes*, *Proteobacteria*, *Fusobacteria*, *Verrucomicrobia*, *Cyanobacteria* and *Actinobacteria*. Over 90% of the human enteric microbiota are from the *Firmicutes* and *Bacteroidetes* phyla, though *Proteobacteria* are also common[11,12].

There is a beneficial, symbiotic relationship between the host and the enteric microbiota. The host provides a nutrient-rich habitat to support the microbiota, which in return confers a huge diversity of genes and metabolic functions. First, the enteric microbiota provides nutrition to the host by fermenting non-digestible substrates and producing short chain fatty acids, and aiding in the absorption of ions, amino acids and vitamins. Second, the microbiota provides a barrier against pathogenic bacteria. Furthermore, the microbiota plays an important role in regulating immune and gut homeostasis[13]. Importantly, an imbalance of this symbiotic state, termed dysbiosis, underlies the pathogenesis of several inflammatory diseases, including allergic skin and respiratory disorders, rheumatoid arthritis, type I diabetes and IBD[14].

Enteric microbiota in IBD

Quantitative and qualitative changes in the composition of the enteric microbiota have been observed in IBD, through a decrease in the diversity and an increase in the concentration of bacterial species[13,15]. Dysbiosis in patients with CD has been well characterized, with a loss of bacteria from the *Firmicutes* phylum, including *Faecalibacterium prausnitzii* (*F. prausnitzii*), the major butyrate-producing bacterium in cluster IV of the *Clostridium leptum* phylogenetic group in the gut[16]. Butyrate, the key energy source for colonic epithelial cells, represses the production of pro-inflammatory cytokines in the intestinal mucosa. The reduction of *F. prausnitzii* in mucosal and fecal samples represents the most replicated species-specific finding so far in CD[17-19].

The decrease in enteric microbiota diversity has also been described in UC[20-22]. Researchers found significantly fewer *Bacteroides* and *Clostridium* (*C. coccoides* and *C. leptum*) in fecal samples of UC patients[23], and a higher amount of *Enterococcus* and *Gammaproteobacteria*[21,22,24]. Additionally, Machiels *et al*[25] found that two well-known butyrate-producing *Firmicutes* bacteria, *Roseburia hominis* and *F. prausnitzii*, were reduced in UC patients. Moreover, the presence of butyrate-producing members of the clostridial cluster was found to vary with disease activity[26]. Varela *et al*[27] found that counts of *F. prausnitzii* were associated with relapse and maintenance of clinical remission, with low counts corresponding to short-term remission, and counts increasing during remission. In another study that analyzed both fecal and biopsy UC specimens, *F. prausnitzii* was sharply decreased in both, whereas *Bifidobacterium* was significantly increased in the biopsy specimens of active UC[28]. However, the composition of fecal and mucosal-associated microbiota changes throughout the progression of UC[26,29].

It is still not clear whether the observed dysbiosis contributes to the development or is a consequence of UC. Although studies documenting the association between microbial changes and UC do not necessarily predict cause-effect relationships, they at least demonstrate that the enteric microbiota plays an important role in anti-inflammatory and immunoregulatory activities, which can benefit UC patients[30].

THERAPIES ASSOCIATED WITH ENTERIC MICROBIOTA

Current therapeutic strategies for treating UC typically follow a step-up strategy, including mesalazine, steroids, immunosuppressants and biologics[31]. Although a considerable proportion of patients maintain remission when using this approach, a significant number of them experience persistent disease activity and ultimately require colectomy[32]. Therefore, the development of new and effective therapies is needed. Manipulation of the dysbiosis is an attractive therapeutic approach, particularly because the interaction between enteric microbiota and the host immune system perpetuates the inflammation in UC.

Fecal microbiota transplantation

Fecal microbiota transplantation (FMT) involves the administration of fecal material from a healthy donor into the intestinal tract of a recipient. This approach attempts to recover the composition and function of the enteric microbiota in patients with chronic gastrointestinal infections and IBD[33]. FMT was first used in 1985 when standard treatments for *Clostridium difficile* infection (CDI) failed. The efficacy achieved by this application[34] has since brought greater attention to FMT, which is increasingly being used for treatment of IBD, and UC in particular.

A case report published in 2003 documented six patients with UC who were treated with FMT[35]. Some symptoms of UC improved within one week, and a complete reversal of symptoms was achieved in all patients by four months after FMT. In addition, there was no clinical, colonoscopic, or histologic evidence of UC in any patient after 1 to 13 years, even without any UC medication. Another study observed ten children with mild to moderate UC for four weeks after receiving FMT[36]. Seven of these subjects showed a clinical response within one week, and six maintained a clinical response after one month. Among these, three subjects achieved clinical remission after one week, which was maintained throughout the four-week observation period. Furthermore, no serious adverse events were noted.

In contrast, some studies report only temporary clinical improvement from FMT rather than remission. Kump *et al*[37] reported short-term clinical improvement with colonoscopic FMT in six patients that were non-responsive to standard medical therapy. Although there were significant changes in the composition of the intestinal microbiota that resulted in a partial improvement of UC-associated dysbiosis, none of the patients achieved clinical remission. Similar results were obtained in a study of five patients with moderate to severe active UC by Angelberger *et al*[38]. Only one patient was observed to have some clinical improvement, and none of the five patients achieved remission during the 12-wk study. A transient increase in the similarity to donor microbiota and an increase in phylotype richness were detected in two patients less than four weeks after FMT. Notably, the microbiota of the sole clinical responder was similar to the donor's, even 12 wk after FMT, which was characterized by successive colonization by the anti-inflammatory and short-chain fatty acid-producing *F. prausnitzii*, *Rosebura faecis* and *Bacteroides ovatus*.

There are several possible explanations for the failure of FMT in some UC patients[39]. First, enteric microbiota in non-responsive patients may be affected by other factors, including dietary intake or exposure to cigarette smoke. Second, the dysbiosis may be a result of UC, rather than the cause. Finally, the type of patients used in these studies may have affected the results, as those with more severe disease and for whom current medical therapies had failed were chosen.

Because of the uncertain effectiveness and safety issues, the study of FMT in UC is complicated. Further studies should identify the best candidates for FMT, such as patients with mild to moderate UC, those who already have a medically induced remission, or those who are newly diagnosed. In addition, more attention should be paid regarding the side effects accompanying FMT. A recent case report of a patient with UC that had been in remission for more than 20 years experienced a UC flare-up after FMT for treatment of CDI[40]. Thus, additional careful studies are needed to determine the effectiveness and safety of FMT, and to facilitate the development of FMT as potential therapy for UC patients.

Probiotics and prebiotics

Probiotics are living non-pathogenic microbes, including *Lactobacilli*, *Bifidobacteria*, *Enterococci* and some yeast species[41], which can benefit the host and affect the structure and function of the enteric microbiota through diverse mechanisms[42]. Prebiotics, which also confer benefits to the host, are defined as non-digestible, selectively fermented, short-chain carbohydrates that allow specific changes in the composition and/or activity of the enteric microbiota[43]. Probiotics and prebiotics have been widely used in the prevention and treatment of important gastroenterological conditions, such as irritable bowel syndrome (IBS) and IBD, and infectious diarrhea.

A probiotic mixture named VSL3, consisting of four strains of *Lactobacilli*, three strains of *Bifidobacteria* and one strain of *Streptococcus thermophiles*, was effectively used as maintenance therapy in patients with recurrent or chronic pouchitis[44-46]. Further evidence supporting the use of probiotics was provided by a one-year, placebo-controlled, double-blind study involving a total of 29 children with active UC who received either VSL3 or a placebo in addition to steroid induction and mesalamine maintenance treatment[47]. A greater number of patients receiving the placebo relapsed within one year, suggesting that VSL3 is effective in maintaining remission. In two randomized, double dummy trials, Nissle 1917, a non-pathogenic strain of *Escherichia coli* (*E. coli*), was as effective as standard mesalamine in maintaining remission in UC[48,49]. In both studies, there were no differences in the percentage of patients who relapsed or the safety profile

of one-year treatments with *E. coli* Nissle 1917 or mesalamine. However, an open-label, randomized trial by Zocco *et al*[50] demonstrated that treatment with *Lactobacillus* GG was more effective than mesalamine in prolonging the relapse-free time in UC patients.

Probiotics also have the capability to induce remission. In a multicenter, randomized, double-blind, placebo-controlled trial including 147 adult patients with mild to moderate UC, a greater percentage of patients receiving VSL3 for 12 wk achieved remission or had greater activity reduction [reduced UC disease activity index (UCDAI) score] than those receiving a placebo[51]. In a similarly designed clinical trial, 144 patients who received VSL3 for 8 wk in addition to their standard pharmaceutical therapy had greater improvement in UCDAI scores, and a significant reduction in rectal bleeding[52]. A study by Oliva *et al*[53] demonstrated that local administration of *Lactobacillus reuteri* ATCC 55730 with standard oral mesalazine reduced the inflammation of rectal mucosa in pediatric UC patients.

The prebiotic germinated barley foodstuff has also been shown to reduce the clinical activity of UC together with the baseline treatment, and to maintain remission[54-56]. The combined use of probiotics and prebiotics, such as *Bifidobacterium* and galacto-oligosaccharide, has also been shown to improve the clinical status of UC patients[57]. However, other studies using combinations of probiotics and prebiotics have been less successful[58,59]. Future probiotic and prebiotic treatments for UC patients should be more personalized, taking into consideration the age of patient, the phase of disease, and the molecular pattern of dysbiosis[60]. Moreover, therapeutic outcomes may be improved by careful selection of agents, and protective commensal enteric species may be more suitable.

Antibiotics

There have been conflicting results over the past few decades concerning the efficacy of antibacterial therapies for the treatment of UC[31]. However, a combination of broad-spectrum antibiotics completely inhibited spontaneous colitis in animal models, suggesting a possible rationale for antibiotic combination therapy for the treatment of UC[61,62]. A two-week antibiotic combination therapy consisting of amoxicillin, tetracycline and metronidazole (ATM) was shown to be effective against *Fusobacterium varium* in UC patients[63,64]. *F. varium* has been detected in the colonic mucosa of a large number of UC patients[65] and can induce UC lesions in experimental animals[66]. ATM treatment was also shown to provide more efficient improvement, remission and steroid withdrawal than a placebo in a double-blind, multicenter trial including 105 patients that was conducted in patients with chronic mild to severe relapsing UC[67]. This treatment has also shown efficacy in the treatment of refractory or steroid-dependent UC[68].

Helminth therapy

Modern hygienic practices prevent exposure to helminth parasites, though epidemiological data suggest that people who carry helminths have fewer immune-mediated diseases[69,70]. Helminth infections may alter the abundance and composition of enteric microbiota, resulting in a greater proportion of anti-inflammatory strains[71]. The biocomplexity of the gut, including the interactions between parasites and the enteric microbiota, can influence inflammatory processes[72]. Studies in rodent models revealed that intestinal parasites and parasite products regulate host immunity and alleviate IBD-like inflammation[73]. In a randomized, double-blind, placebo-controlled trial involving 54 patients with active UC, a greater number of patients who received 12 wk of treatment with *Trichuris suis* ova showed improvement with no side effects[74]. Similarly, three patients with active UC in an open trial attained remission with 12-wk *T. suis* ova treatment with no side effects[75].

Dietary polyphenols

Diet can modulate enteric microbiota composition, with a strong effect on human health[76]. Polyphenols are members of a large family of plant-derived compounds that have drawn attention due to their antioxidant and anti-inflammatory properties[77]. Some *in vitro* and *in vivo* studies indicate that dietary polyphenols and their metabolites can interact with the enteric microbiota, and enhance the growth of probiotic bacteria[78-84]. Due to the prebiotic potential and anti-inflammatory effects, polyphenol supplementation could potentially serve as a complementary medicinal approach to UC. However, there have been no clinical trials evaluating the use of dietary polyphenols for the treatment of UC.

CONCLUSION

As a form of IBD, UC has its own genetic, pathogenic and therapeutic identity. Despite recent advances in UC therapeutic resources, a considerable proportion of UC patients are still refractory to conventional treatment. Dysbiosis is an important immunologic and pathologic process in UC. Accumulating knowledge of dysbiosis has promoted the use of enteric microbiotal regulation as a novel promising adjuvant in UC therapy. Therapeutic approaches, such as FMT, probiotics, antibiotics, dietary polyphenols, and helminth therapy, can alter the abundance and composition of enteric microbiota, and improve patient outcomes. However, the safety and effectiveness of bacteria-driven therapies need further evaluation.

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