Review Article

INFECTIOUS Microbes & Diseases

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Probiotics and Their Metabolites Ameliorate Inflammatory Bowel Disease: A Critical Review

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Abstract

Crohn disease and ulcerative colitis are the two main manifestations of inflammatory bowel disease (IBD), and both are highly morbid. However, the precise etiology of IBD is still unknown and effective therapeutics are yet to be discovered. It is becoming increasingly clear that a combination of factors, including genetic background, host immune response, and microbial reduced diversity status are related to IBD. The cardinal symptom of IBD patients is the imbalance of the intestinal microflora. According to previous studies, both probiotics and symbiotic microbiota can play a protective role through intestinal micro-ecosystem regulation, epithelial barrier integrity enhancement, and inflammation reduction. Therefore, probiotics can provide an alternative or auxiliary method to traditional IBD treatment. Here, we reviewed the possible pathogenesis of IBD, summarized the possible mechanisms of probiotics modulation of IBD, and emphasized the prevention and treatment targets of probiotics-mediated IBD, with the aim to provide theoretical support for the treatment of IBD patients by probiotics in clinical trials.

Keywords: inflammatory bowel disease; metabolites; probiotics; the regulatory mechanism

Introduction

Inflammatory bowel disease (IBD) is an idiopathic and chronic disease that involves the ileum, rectum, and colon.[1] According to the incidence, severity, progression, and remission at different stages, IBD is mainly divided into ulcerative colitis (UC) and Crohn disease (CD)[2,3] and unfortunately, there is no cure.[4,5] Currently, researches on IBD mainly focus on the changes in intestinal microflora composition and function. However, with the development of molecular biology instruments and techniques, researchers have found that intestinal microorganisms, microbial metabolites, and the interaction between intestinal microflora and the intestinal epithelial mucosal immune system play an important role in IBD.[6,7] With the deepening of studies on IBD, researchers found that patients have an intestinal microflora disorder phenomenon. In addition, the metabolic spectrum of patients has also changed in the IBD period, such as short-chain fatty acids (SCFAs), bile acids (BAs), and tryptophan (Trp) metabolites, which may be related to the pathogenesis of IBD.

Probiotics are considered to be promising agents. Probiotics and symbiotic microflora can play a protective role by intestinal micro-ecosystem regulation,[8] epithelial barrier integrity enhancement, and immune and inflammatory response reduction.[9] Therefore, they have great potential in the prevention and treatment of mild or severe intestinal mucositis. Nevertheless, there are few clinical studies on the effect of probiotics on IBD. It should be noted that some results suggested caution of these drugs in the relapse on IBD,[10] because the effectiveness of probiotics is affected by species, dose, and disease types. The duration of treatment also depended on the clinical indications.

Here, we reviewed the known possible causes of IBD, focusing on probiotics administration that works to restore the balance of the intestinal microflora and mediate the production of intestinal metabolites to relieve or ameliorate IBD symptoms. The aim of this review is to provide theoretical support for IBD patients' treatment with probiotics in clinical trials and also provide new ideas and directions for the prevention and treatment of IBD.

Pathogenesis of IBD

The incidence of IBD is increasing globally, but a function therapy of IBD has not yet been found. In IBD patients, the integrity of the mucosal barrier and immune regulation are damaged. Through the damaged epithelial barrier or microfold cell (M cell) transport, the pathogen stimulates dendritic cells and macrophage to release proinflammatory cytokines (eg, IL-1, IL-6, IL-8, IL-12, IL-23, TNF-α) that can induce T helper cells Th1 and Th17 to secrete the pro-inflammatory cytokines IL-1, IL-17, IL-22, IFN-γ,

Editor: Stijn van der Veen

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Author contributions: PL and QG conceived and designed the review paper; YL, ML, JP, ZZ, ZG, PL, and QG drafted the manuscript; QZ revised the manuscript; all authors read and approved the final manuscript.

Funding: This work was supported by the Key Research and Development Program of Zhejiang Province (No. 2020C04002), the National Natural Science Foundation of China (No. 31972974, No. 31871775), and the Chinese Academy of Engineering Academy-Locality Cooperation Project (2019-ZJ-JS-02). The funders had no role in study design, data collection and interpretation, writing of the manuscript, or the decision to submit the manuscript for publication.

Conflicts of interest: The authors reported no conflicts of interest.

Infectious Microbes & Diseases (2021) 3:1

Received: 15 August 2020 / Received in final form: 30 November 2020 / Accepted: 2 December 2020

https://doi.org/10.1097/IM9.0000000000000046

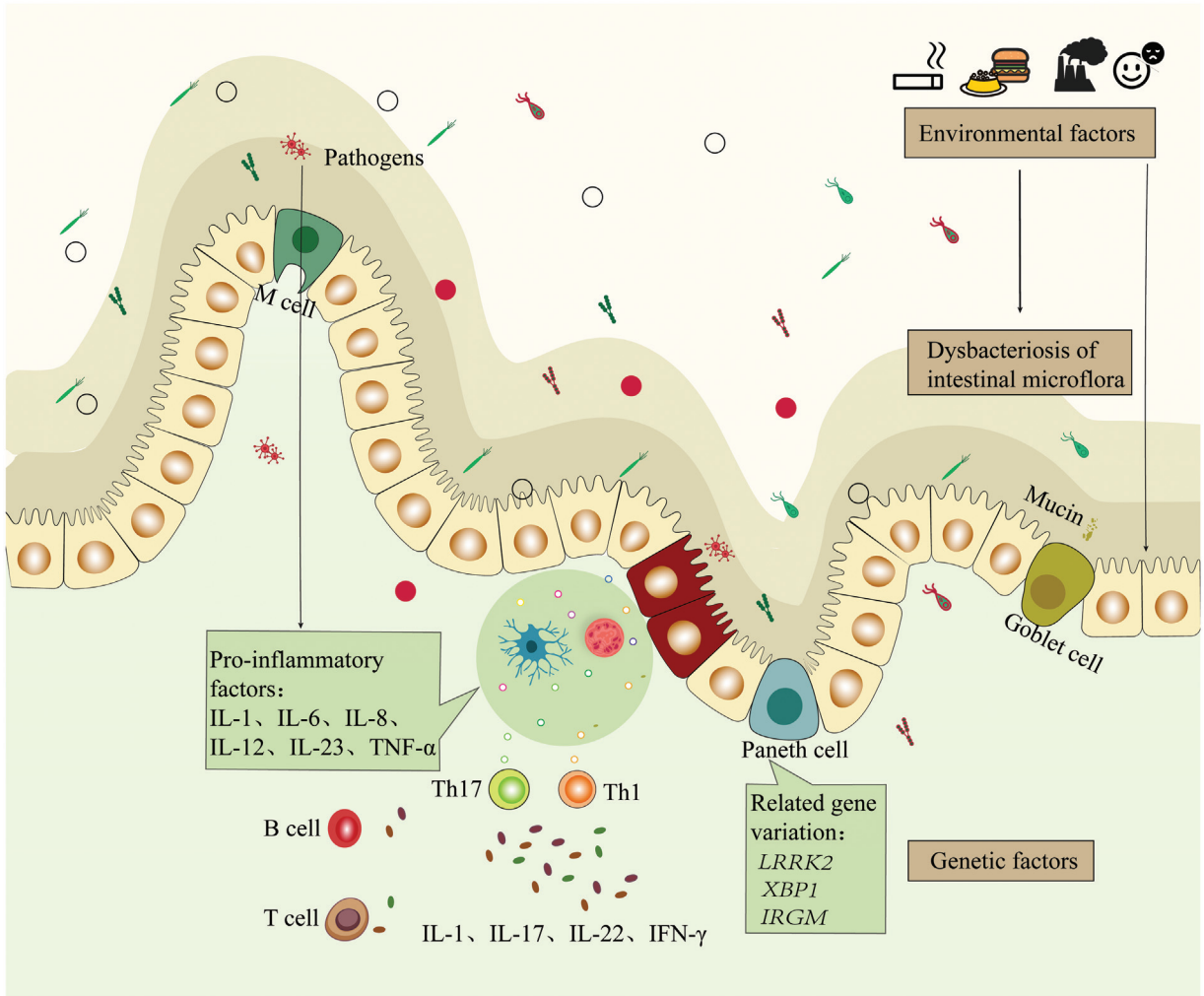

Figure 1. The possible pathogenesis of inflammatory bowel disease (IBD). The pathogenesis of IBD was not limited to one aspect, but was more likely to be affected by the complex interactions between environmental, genetic and microbial factors. The intestinal epithelium is at the crossroad of IBD pathogenesis by coordinating the link amongst the factors implicated in the disease onset. Flares of diseases have been associated with environmental factors, such as smoking, stress and diet. Host genetics can itself influence the gut microbial composition or immune response to affect the disease pathogenesis. Microbial dysbiosis, which is defined as an increase in pathogenic bacteria concomitant with decreases in beneficial bacterial species, might promote the occurrence of IBD.

and TNF, leading to persistent enteritis.[11] According to the cytokine environment, effector B cells and effector T cells differentiate by producing immunoglobulin and further form chronic inflammation. Understanding the factors that increase the risk of disease facilitates the design of specific interventions in the early stages. This can prevent disease development and improve outcomes in patients with IBD.[11] Besides, the cause of IBD has not been limited to one aspect, but is more likely to be affected by the complex interactions between the environmental, genetic, and microbial factors (Figure 1).

Environmental factors

On the etiology of IBD, research has shown that the environment is the main cause of the disease.[12] Roda et al. deemed that changes in lifestyle and behavior, exposure to environmental pollution, and changes in diet make IBD more common in urban centers.[11] In Western countries, smoking is the most studied environmental factor in IBD, and it has been associated with an increasing risk of CD.[13] In Japan, passive smoking has also been confirmed as an increased risk of IBD.[14] Moreover, Rengarajan et al. found that stress can also cause an outburst of IBD.[15]

There are also other factors. Ng et al. supported the importance of dietary factors in the development of IBD.[16] Dietary factors might influence the risk of developing IBD. For example, through the data analysis of mice models, Khan et al. found that monosaccharide intake can easily induce colitis by regulating the intestinal microflora of mice.[17] In addition, regular consumption of alcohol or meat also can increase the incidence rate of IBD.[18]

Genetic factors

Compared with environmental factors, genomic information that determines the risk of IBD has made greater progress. IBD genetics research has identified more than 200 loci, which can explain 8% to 13% of disease susceptibility risk differences.[19] For example, Ouellette found that mutations in the *LRRK2*, *XBP1*, and *IRGM* genes cause changes in the survival and function of the special intraepithelial lymphocyte Paneth cells, which are located at the bottom of the Lieberkühn crypts in the small intestine.[20] These changes are the characteristics of

secondary inflammatory changes in CD. In addition, nuclear factor kappa light chain enhancer of the activated B cell (NF-κB) signals in intestinal epithelial cells are equally important. It has many activating factors, when these activating factors mutate, they will cause the misregulation of NF-κB and induce chronic inflammation.[21]

Currently, there are many research reports on IBD-related genes, but the genetic variation itself cannot fully explain the variation and phenotype of IBD due to the age, location, and complications at the time of diagnosis. Therefore, genetic factors are still not able to explain the specific pathogenesis of IBD.

Microbial factors

A healthy host is tolerant to the microbiota and maintains homeostasis. However, the number and composition of the intestinal microflora of IBD patients are changed, and the diversity and stability generally show a decline,[22] which has led to an increase in pathogenic bacteria. Microbial factors have been shown to be essential for the pathogenesis of IBD.[23]

Mycobacterium avium was designated as a cause of CD, and it was the first organism to be identified as the pathogen associated with IBD. Adhesive invasive *Escherichia coli* is seemingly specific to ileal CD and can induce the release of TNF-α (a key cytokine in IBD). Besides, some researchers speculated that *Helicobacter pylori*, which has been found in primate colitis, may also be a pathogen in IBD.[12] Zhang et al. found that *Proteus* is more common and abundant in stool and colon biopsy samples of CD patients.[24]

Particularly, there are other microbial factors such as BAs and Trp metabolites that may induce IBD. For example, BAs can participate in the defense of the intestinal mucosa through their antibacterial properties. The metabolism of BAs is affected by the intestinal microbiota, which in turn affects signal transduction through BA receptors. Metabolic disorders of BAs may greatly affect intestinal homeostasis and the course and/or phenotype of IBD, then induce the occurrence of IBD.[25] Nikolaus et al. found that the Trp metabolites produced by the microbiota can be used as biomarkers of ecological disorders and play a role in the mucosal immune response. Therefore, Trp deficiency may lead to the occurrence or exacerbation of chronic inflammation.[26]

Although there is a close relationship between the host and the microbiome, it is still challenging to determine a credible association between the microbiome and IBD.

The effect of probiotics on IBD

When probiotics are administered in appropriate proportions, they have a positive effect on the host body (mice or/and volunteers). As a potential therapeutic agent for IBD, probiotic intervention strategies may be proven to be a feasible option for IBD patients in the future.[27] The most studied probiotics are *Lactobacillus* and *Bifidobacterium* species and therapeutic effects on UC have been reported, such as induction or maintenance of the remission period and symptom alleviation, but research data on their role in CD recurrence is very limited. For example, *Lactobacillus plantarum*, *Lactobacillus paracasei*, *Saccharomyces boulardii*, and *Bifidobacterium infantis* have been proven to be able to alleviate the symptoms in mice and humans with enteritis.[28]

Researchers have demonstrated that a variety of probiotics can regulate inflammation and prevent or treat IBD through a mice enteritis model (Table 1). For example, Rodríguez-Nogales et al. found that *S. boulardii* could significantly reduce the up-regulated expression of miR-155 and miR-223 in inflammatory mice. The up-regulation of miR-223 is related to the regulation of the inflammatory body complex and the production of IL-1β, in which miR-155 and miR-223 are significantly increased in inflammatory mice, while miR-143 and miR-375 are significantly reduced by about 2-fold compared with the non-inflammation group. In addition, *S. boulardii* increased some proteins involved in maintaining epithelial integrity, such as MUC-2, MUC-3, and tight junction proteins.[27]*Lactobacillus delbrueckii* from dairy products regulates the NF-κB pathway and reduces the inflammatory state of the dextran sulfate sodium (DSS) colitis mice model. Similarly, Javed et al. found *B. infantis* played an early role in intestinal diseases, reducing colitis, enteritis, and the early incidence rate of intestinal diseases. Compared with the

Table 1

Probiotic effectiveness in murine model of colitis

Induction type	Administration strategy	Probiotic effect	References
TNBS	30 mice; 7–9 weeks old; 26 days; *Saccharomyces boulardii*, 5.0×109 CFU/day	The expression of miR-155 and miR-223 was decreased to maintain the integrity of epithelial protein	29
DNBS	60 mice; 6–8 weeks old; 25 days; *Lactobacillus fermentans* CECT5716, *Lactobacillus salivarius* CECT5113, *E. coli* Nissle1917, *Saccharomyces boulardii* CNCMI745, 5.0×10^8 CFU/day	All probiotics showed intestinal anti-inflammatory effect, but slightly different, especially in miRNA expression. Similarly, probiotics improved malnutrition associated with colitis	84
DSS	32 mice; 8 weeks old; 14 days; *Lactobacillus casei* LH23, DSS group (oral PBS); LH23 treatment group (1.0×10^8 CFU/day); heat kill LH23 treatment group (1.0×10^8 CFU/day)	*L. casei* LH23 can significantly improve DSS-induced colitis in mice by reducing the number of macrophages and inflammatory cytokines secreted	85
DSS	32 mice; 6 weeks old; 2 weeks; *Lactobacillus rhamnosus* LDTM7511, 1.0×10^9 CFU/day	LDTM 7511 significantly inhibited the production of nitric oxide in RAW264.7 macrophages stimulated by lipopolysaccharide	86
DSS	30 mice; 5 weeks old; 22 days; *Propionibacterium freundii*, 1.0×10^8 CFU/day	*Propionibacterium* ameliorated acute colitis by restoring the number of goblet cells and stimulating MUC2 expression in intestinal goblet cells	87
DSS	24 mice; 6–7 weeks old; 14 days; 3.0×10^9 CFU/day; mice in Akk group (*Akkermansia muciniphila*), DP group (DSS + PBS) and CP Group (water + PBS) were given the same amount of anaerobic sterile PBS	In Akk treated mice, histological trauma was improved, mucosal integrity was stabilized, resulting in smooth and continuous localized closure protein and ZO-1, and colitis was relieved.	88

DNBS: dinitrobenzene sulfonic acid; DSS: dextran sulfate sodium; TNBS: 2,4,6-trinitrobenzene sulfonic acid.

control group of 2,4,6-trinitrobenzene sulfonic acid (TNBS)-induced enteritis mice, most samples of rats fed with *B. infantis* showed almost complete preservation of the epithelial cell layer, reduced presence of crypt abscesses, and no significant decrease in mucus depleted goblet cells.[29] *Bifidobacterium adolescentis* IM38 has also been demonstrated to improve colitis induced by a high-fat diet in mice by inhibiting the activation of intestinal microflora NF-κB and the production of lipopolysaccharide. Sokol et al. showed that *Faecalibacterium prausnitzii* was able to induce a strong anti-inflammatory reaction in mice with TNBS-induced colitis. *F. prausnitzii* and its supernatant were able to significantly reduce colitis, reduce body weight, normalize part of the colon length and improve Wallace and Ameho scores. Moreover, treatment with *F. prausnitzii* or its supernatant tended to counteract all bacterial malnutrition observed in colitis control mice.[30] However, not all probiotic strains are effective against IBD enteritis. In a hapten model of colitis, Kennedy and his colleagues found that the beneficial effects of *L. plantarum* 299 on intestinal permeability, body weight change, colonic microscopic score, and blood albumin level in rats were not shown,[31] contrary to some other available reports. This suggested that the immunomodulatory characteristics of probiotics may be different, and these differences may be the result of experimental conditions, such as animal models (rats, mice), selected colitis inducers (DSS or TNBS), the severity of colitis (dose-dependent), and differences among bacterial strains.[32]

Further, similar results were obtained from IBD volunteers based on the effects of probiotics on mice with enteritis (Table 2). In patients with chronic enteritis, *S. boulardii* also had a palliative effect, and administration of *S. boulardii* to IBD volunteers helped maintain remission and intestinal sealing.[32] In terms of remission of IBD, *Lactobacillus rhamnosus* may be able to alleviate IBD for 1 year like the conventional drug mesalazine, which is not inferior to the established standard mesalazine and can provide significantly better efficacy in delaying the recurrence of UC.[33] However, the combination of the two did not have a great advantage and did not improve the condition of volunteers. Perhaps mesalazine interfered with *L. rhamnosus* GG (LGG) adhesion and colonization of the intestinal mucosa.[28] *Bifidobacterium* can not only improve enteritis in mice, but also improve IBD in human. Tamaki et al. found that after administration of *Bifidobacterium longum* 536 to mild to moderate UC patients in Japan, these patients had good supplemental tolerance to *B. longum* 536 after 8 weeks, and the ulcerative colitis disease activity index scores, EI scores and Mayo scores decreased.[34] In addition, compared with the non-probiotic supplement group, *B. infantis* BB02 reduced the clinical symptoms of IBD, protected colon structure, and reduced edema. However, Matsuoka et al. found that the *Bifidobacterium brevis* strain was ineffective in maintaining remission in patients with UC. Even in IBD volunteers treated with a mixture of *B. brevis* strain Yakult and *Lactobacillus acidophilus* for 48 weeks, no significant difference was observed.[35] VSL#3, a high concentration probiotic preparation, was used in IBD volunteers as eight living freeze-dried bacterial species. The results showed that treatment with VSL#3 combined with balasalazide was more effective than treatment with mesalazine or balasalapeptide only against effective remission.[36] The effectiveness of VSL#3 has also been demonstrated in children with mild to moderate UC (mean age 12 years old). *E. coli* is usually regarded as a harmful bacterium, but *E. coli* Nissle 1917 (EcN) belongs to the category of probiotics. The adhesion of EcN to intestinal epithelial cells-407 of IBD patients was dose and time-dependent. EcN formed a biofilm of non-pathogenic bacteria in vivo, which prevented pathogenic microorganisms from entering the cell surface.[37] By analyzing the bacterial composition, biochemical indicators, inflammatory markers, and activity scores of IBD patients, it was found that pentosan combined with probiotics could improve the

Table 2

Probiotic effectiveness in human model of IBD

Type of IBD	Administration strategy	Probiotic effect	References
UC	82 participants; 22–70 years old; 2 months; Bifid Triple Viable 2 capsules; 3 times/day	Improvement of symptoms and in inflammation, and reduction of IL-1β expression and increase in IL-10 and IgA expression in the colonic mucosa	89
UC	90 participants; 19–69 years old; 8 weeks; VSL#3; 3 g/day	Improved all parameters (symptoms, remission time and others)	90
UC	30 participants; 47 ± 1.59 years old; 8 weeks; *Lactobacillus fermentum*, *Lactobacillus delbruekii*; 2400 mg/day	Maintaining remission and preventing recurrence of UC	91
UC	32 participants; 18–65 years old; 6 weeks; VSL#3; 4 sachets	Induction of remission period	92
UC	22 participants; 18–65 years old; 8 weeks; *Bifidobacterium infantis* 35624; 1.0×10^{10} CFU/day	Biomarkers of systemic inflammation in reducing gastrointestinal and extraintestinal inflammatory diseases	93
UC	187 participants; 33 years old; 12 months; LGG; 1.8×10^{10} CFU/day	Increase in remission maintenance time	33
UC	60 participants; 44.5 years old; 2 years; *Lactobacillus salivarius, Lactobacillus acidophilus* and *Bifidobacterium bifidus* strain BGN4. It was not mentioned the dose	Disease activity indexed improvement and reduced in the recovery time	94
UC	30 participants; 47 years old; 8 weeks; *Lactobacillus delbruekii* and *Lactobacillus fermentum*; 1.0×10^{10} CFU for sachets	Improvement of mucosal inflammation and lesion	91
CD	119 participants; >16 years old; VSL#3; 9.0×10^{11} bacteria^2/day	Lower concentration of pro-inflammatory cytokines in the intestinal mucosa and lower relapse rate	95
CD	Four participants; 10–18 years old; 6 months; LGG 1.0×10^{10} CFU, 2 times/day	Reduction of disease activity index and intestinal permeability	96
CD	98 participants; >18 years old; 6 months; *Lactobacillus johnsonii* LA1 4.0×10^9 CFU/day	No therapeutic effect	82
CD	Ten participants; 19–42 years old; 13.0 ± 4.5 months; *Bifidobacterium breve* (3.0×10^{10} CFU/day), *Bifidobacterium longum* (1.5×10^{10} CFU/day) and *Lactobacillus casei* (3.0×10^{10} CFU/day), Psyllium 9.9 g/day	Improvement of symptoms, such as diarrhoea and nabdominal pain	97

CD: Crohn disease; IBD: inflammatory bowel disease; UC: ulcerative colitis.

bacterial composition of IBD patients and reduce the level of inflammatory cytokines.[38] However, the clinical results are unknown, which is worthy of further verification.

Regulation mechanism of probiotics on IBD

Probiotics, microorganisms with beneficial effects on the host, can maintain intestinal microflora health, reduce pathogen growth and colonization, and enhance the mucosal barrier integrity and immune modulation through competition or occupation.[39,40] Moreover, probiotics secrete substances (such as defensins or vitamins) that also stimulate specific mucosal immune function.[41] With in-depth studies, knowledge on the regulatory mechanisms involving the effects of probiotics on IBD has been largely improved (Figure 2). Probiotics not only help to restore the normal intestinal microbial community, but also maintain the barrier function of intestinal epithelial cells by mediating the metabolites of intestinal microflora (especially SCFAs, BA derivatives, and Trp metabolites). That will enhance and regulate the innate and adaptive host immune responses, thereby inhibiting the production of inflammatory cytokines through different signaling pathways,[42] and achieve the purpose of IBD prevention and treatment.[43]

Probiotics and their metabolites

Probiotics can inhibit inflammation through a variety of mechanisms that affect intestinal microflora. Yan et al. confirmed that LGG derived soluble protein p40 could prevent and treat experimental colitis by relying on epidermal growth factor receptors.[44] Probiotics can also produce cytokines by affecting the epithelial cells and have anti-inflammatory effects. Zhang et al. found that LGG reduced TNF-α induced IL-8 production by affecting the NF-κB pathway in Caco-2 cells.[45] Moreover, culturing epithelial cell monolayers with probiotics can prevent changes in epithelial permeability caused by the pro-inflammatory cytokines TNF-α, and IFN-γ.[46]

Probiotics and their metabolites may also enhance and regulate innate and adaptive immune responses of the host through other mechanisms. Probiotics may stimulate the production of a

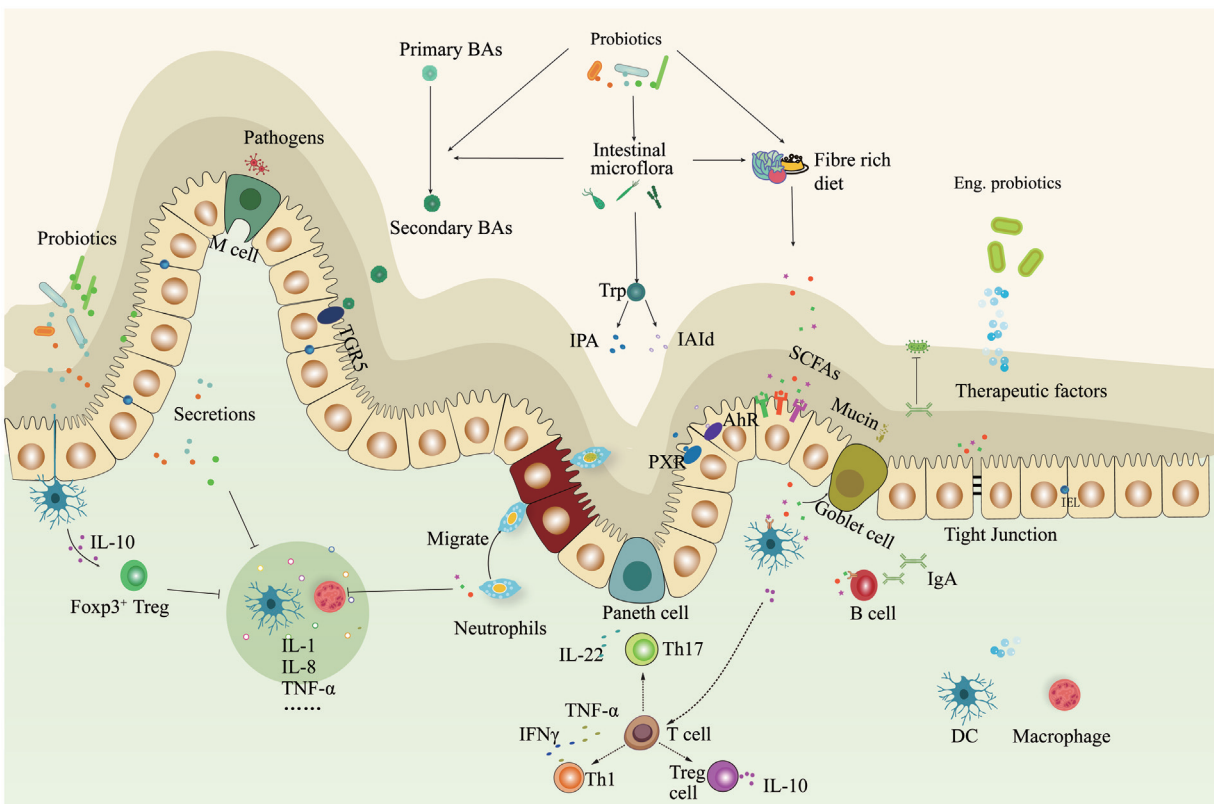

Figure 2. The regulation mechanisms of probiotics on inflammatory bowel disease (IBD). Probiotics can cause immune regulation through direct interaction with intestinal epithelial cells, stimulate specific mucosal immune functions by secreting substances such as defensins or vitamins, or maintain the barrier function of intestinal epithelial cells by mediating the metabolites of intestinal microflora. For example, molecules produced by probiotics (eg, bacteriocin) trigger dendritic cells (DCs) to release IL-10, thus maintaining Treg cells. Short-chain fatty acids (SCFAs) are mainly produced by the fermentation of indigestible sugars through the beneficial bacteria in the colon. Some probiotics can directly produce SCFAs like acetic acid and butyric acid. SCFAs regulate intestinal barrier integrity by inducing secretion of IL-18 by intestinal epithelial cells and upregulating the expression of tight junction. SCFAs induce neutrophil migration to inflammatory sites and enhance their phagocytosis. The differentiation of T cells is mediated both by SCFA regulation of DCs and by the direct action of SCFAs on T cells. SCFAs regulate the generation of Th1, Th17, and Treg in different cytokine milieus. SCFAs also inhibit intestinal macrophage production of pro-inflammatory cytokines, and possibly induce intestinal IgA production of B cells. Bile acids (BAs) and microbiota interact in a bidirectional manner, and the BAs that reach the colon are converted into secondary BAs by bacteria. BAs can exert many metabolic and immune effects, through binding to transmembrane G protein-coupled receptor 5 (TGR5). Tryptophan (Trp) can be metabolized into a series of indole metabolites by the intestinal microbiota, some of which can be used as aromatic hydrocarbon receptor (AhR) ligands. Indole propionate (IPA) helps to maintain barrier function and inhibits mucosal TNF-α production through binding to the pregnane X receptor (PXR). Engineered probiotics release therapeutic factors (eg, IL-10) within the gut, clear the toxins produced by pathogens, and modulate the immune system. M cell: microfold cell.

defensin from intestinal crypts,[47] thereby controlling the proliferation of normal microflora in the crypts and influencing the morbidity of IBD in these areas. For example, bacteriocin can be used as antibacterial peptides, which can promote the dominant position of its producing bacteria in its niche, to improve the competitiveness of probiotics. Similarly, Bassaganya-Riera et al. found that conjugated linoleic acid produced by probiotics could maintain intestinal homeostasis by inducing and activating PPARγ and δ, thus preventing and improving inflammatory enteritis.[48] Von Schilde et al. found that lactococcin (a protease secreted by *L. paracasei*) could degrade some pro-inflammatory chemokines, including CXC chemokine ligand 10, to inhibit the recruitment of inflammatory cells to mucosal tissues, and prevent colitis in mice.[49] Segawa et al. confirmed that polyphosphate secreted by *Lactobacillus brevis* had a protective effect on epithelial cells by activating mitogen-activated protein kinase p38.[50] Levit et al. simultaneously compared a riboflavin-producing lactic acid bacterium strain with commercially available vitamin supplements in a TNBS-induced mouse colitis model. It was confirmed that soybean milk fermented by *L. plantarum* CRL2130 could produce riboflavin, which had an anti-inflammatory effect.[51] Therefore, microorganism-produced riboflavin and other vitamins may be used as a new tool for probiotics to treat IBD. Some researchers have found that other unidentified compounds secreted by *Enterococcus faecalis* and *L. paracasei* could inhibit the activation of NF-κB and protect the tissues of patients with IBD from experimental colitis or ongoing inflammation.[52] A recent study showed that long-chain fatty acids produced by EcN could bind to and activate PPARγ to exert anti-inflammatory effects, thereby inhibiting DSS-induced colitis in mice.[53]

With the development of genetic engineering technology, genetically modified lactic acid bacteria have also been classified as microbial therapy and the next generation of probiotics. Probiotics can be given certain functional characteristics through techniques like gene editing, such as the release of direct-acting anti-inflammatory factors in the intestine, or the targeted treatment of inflammation-related metabolic changes that can control inflammation-induced biological disorders, thereby preventing and improving IBD.[54] Some recombinant probiotics have been used in experiments with mouse enteritis models. Steidler et al. confirmed that genetically engineered *Lactococcus lactis* had a therapeutic effect by secreting IL-10, which significantly reduced the symptoms of DSS-induced colitis in mice.[55] Carmen et al. also found that *L. lactis* containing an IL-10 expression vector had a protective effect on mice with TNBS-induced inflammation.[56] In addition, a new study showed that engineered lactic acid bacteria could deliver IL-1Ra to the colon, thus inhibiting IL-1 signaling. IL-1Ra-expressing *L. lactis* provided by oral administration in acute colitis mice was also able to reduce the symptoms of colitis.[57] Moreover, the potential of recombinant probiotics as a credible treatment was further demonstrated in phase I clinical trials of CD patients.[58]

Short-chain fatty acids

When a sufficient number of probiotics are colonized in the intestine, the diversity of the intestinal microbiota is improved, promoting the growth of the number of SCFAs-producing bacteria, and thereby increasing the levels of SCFAs in the intestine. SCFAs have a probiotic effect on the intestine by strengthening the intestinal barrier and inhibiting inflammation.[59,60] SCFAs are mainly produced by the fermentation of indigestible sugars through the beneficial bacteria in the colon. Besides, some probiotics can directly produce SCFAs like acetic acid and butyric acid. Acetate contributes to the defensive function of the host intestinal epithelial cells, and butyrate can upregulate tight junctions and regulate epithelial permeability.[59] Researchers have found that SCFAs levels might play an important role in the pathophysiology and/or progression of IBD. Compared with healthy people, acetic acid, butyric acid, and propionic acid levels in the feces of IBD patients are significantly reduced.[61]

Huda-Faujan et al. found that the typical intestinal microecological disorder in IBD patients was associated with the decrease of bacterial species producing SCFAs, such as *F. prausnitzii* and *Roseburia hominis*, consistent with the metabolomics study of human IBD patients.[61] This characteristic may have a great effect on the treatment of IBD patients. The intervention of probiotics can directly or indirectly mediate the intestinal microbiota through its metabolites, affect the secretion of SCFAs, and play a probiotic effect. In some animal experiments, Fukuda et al. found that the reason why *Bifidobacteria* could regulate the host defense response and prevent infectious diseases was partly due to the increase of acetate in the intestine.[62] They showed that acetate improved intestinal defense mediated by epithelial cells, thereby protecting the host from fatal infections.[62] Bian et al. found that *Pediococcus pentosaceus* LI05 was able to reduce intestinal inflammation by maintaining intestinal epithelial integrity and regulating immune status, intestinal microbiome, and metabolite composition (such as increased production of SCFAs), providing a potential treatment for colitis.[63]

Moreover, probiotics can also indirectly mediate intestinal microflora through the secretion of vitamins and other metabolites, thereby promoting the synthesis of SCFAs, and ultimately alleviating or improving IBD patients. For example, most of the growth of the intestinal microflora depends on folate, which is produced by microbial fermentation in the intestine. When folate-producing lactic acid bacteria are colonized in the intestine, the folate produced in the intestine is used by other non-folate-producing strains,[64] thereby maintaining the balance of intestinal commensal bacteria and increasing the level of SCFAs.

Although SCFAs have been shown to exert multiple beneficial effects on the host, several animal and human trials have found that SCFAs either lack therapeutic efficacy or exacerbate inflammation.[65,66] Conflicting results suggest the function of SCFAs depends on context and disease severity.

Bile acids

BAs, like complex metabolic integration factors and signal factors, are mainly small molecules synthesized by the liver from cholesterol in a multi-enzyme process. However, the primary BAs in the colon are mainly derived from bacterial biological modification.[67] BAs and microbiota interact in a bidirectional manner, and the BAs that reach the colon are converted into secondary BAs by bacteria. In addition to regulating their synthesis, BAs also exert many metabolic and immune functions by binding to various receptors such as FXR receptor and transmembrane G protein-coupled receptor 5.

In IBD patients, the metabolism of BAs will be abnormal, with the primary BAs increased in serum and feces, and the secondary BAs decreased.[6,68] Therefore, dysregulation of intestinal BAs is considered a mediator of the pathogenesis of human IBD and colorectal cancer.[25,69] Many signaling pathways activated by

BAs have become attractive therapeutic targets for metabolic disorders.[70] Degirolamo et al. found that VSL#3 probiotic was able to promote ileal BAs deconjugation with subsequent fecal BA excretion and induce hepatic BA biosynthesis via down-regulation of the gut-liver FXR-FGF15 axis.[71] In a DSS-induced colitis mice model, VSL#3 administration significantly reduced the disease activity index score and colitis morbidity.[72] Therefore, regulating BA metabolism may be a potential target for the treatment of IBD.

Tryptophan metabolites

Trp is an essential aromatic amino acid that must be obtained from the human diet. It can be metabolized into a series of indole metabolites by intestinal microbiota, some of which can be used as aromatic hydrocarbon receptor (AhR) ligands (a receptor related to the pathogenesis of IBD), and some indole derivatives can be used as agonists of AhR, thereby affecting the stability of the intestinal environment. Therefore, probiotic intervention combined with Trp metabolism can be used as a treatment strategy, by changing the intestinal microflora, increasing the production of AhR ligand, and ultimately protecting the host from intestinal inflammation. Takamura et al. found that *Lactobacillus bulgaricus* could activate the AhR pathway in the mouse colon, thereby improving the symptoms of DSS-induced colitis.[73]*Clostridium sporogenes* can convert Trp into indole propionate (IPA) to protect mice from colitis induced by DSS.[74] Similarly, Zelante et al. found that *Lactobacillus reuteri* bacteria produced indole-3-aldehyde in the presence of luminal Trp. indole-3-aldehyde can activate ILC3 cells through AhR to produce IL-22 to protect against mucosal inflammation and show antifungal activity, which is important to resist colonization by *Candida albicans* and protect the intestinal mucosa.[75] Overall, Trp and its metabolites can not only be used as effective biomarkers of IBD but also become therapeutic targets of IBD.

Probiotics administration as a treatment for IBD

In the clinical treatment of IBD, probiotics have shown some positive effects.[76] The principal probiotics used in humans are *Bifidobacteria*, LGG, and synbiotics. Currently, no probiotics has been proven to successfully treat CD, but probiotic interventions have been proven to be promising for UC patients.

For adult patients, several probiotics have been used in clinical trials. A randomized study conducted by Ishikawa et al. reported that after treating UC patients with *L. brevis* strain Yakult powder and galactooligosaccharides (synbiotic group) for 1 year, their clinical status was improved after evaluation by colonoscopy.[77] Tamaki *et al* determined the efficacy of *B. longum* 536 in inducing remission of mildly to moderately active UC in Japan.[34] A recent short-term, double-blind, randomized placebo-controlled clinical trial conducted by Bjarnason et al. showed that multi-strain probiotics were associated with decreased intestinal inflammation in patients with UC, but not with CD.[76] At the same time, clinical studies in children with IBD showed similar results. For example, Miele et al. conducted the first pediatric, 1-year, placebo-controlled double-blind trial. Their results showed that the endoscopic and histological scores of the VSL#3 group were significantly lower than those of the placebo group, and proved its role in maintaining remission of UC.[78] A prospective, randomized, placebo-controlled study included 31 children with mild to moderate ulcerative proctitis/UC. Based on oral mesalazine treatment, they received *L. reuteri* ATCC 55730 within 8 weeks enema or placebo. The clinical, endoscopic, histological, and immune evaluations (IL-10, IL-1β, TNF-α, IL-8) were performed. Clinical and endoscopic improvements of the probiotic group were better, and the histological score of the *L. reuteri* group was significantly lower. Only in the *L. reuteri* group, IL-10 was significantly increased, while IL-1β, TNF-α, and IL-8 were significantly decreased.[79] Furthermore, *L. lactis* displayed a therapeutic effect on IBD and is currently undergoing a phase I clinical trial.[80]

More than 70% of CD patients undergo surgery throughout their lives. New lesions usually appear shortly after ileocolectomy, followed by clinical recurrence, and eventually new complications and further surgery. In a randomized placebo-controlled trial by Prantera et al. among patients undergoing CD surgery and from whom all diseased intestines were removed, patients were randomly assigned to receive 12 billion cfu's of lactic acid bacteria or the same dose of a placebo for 1 year. Colonoscopy was performed at the end of the trial or at the onset of symptoms. The aim was to determine whether oral probiotic LGG was able to prevent the recurrence of CD after surgery or reduce its severity. As a result, LGG did not seem to prevent endoscopic recurrence within one year, nor did it reduce the severity of recurrent lesions.[81] At the same time, Marteau et al. found through a randomized placebo-controlled trial that *Lactobacillus johnsonii* LA1 did not have enough effect to prevent endoscopic recurrence of CD.[82]

Despite the strength of the conceptual basis for the beneficial use of probiotics, the existing literature and in particularly randomized clinical trials on the use of probiotics in IBD are extremely limited. Further research is required to establish whether probiotics are able to reduce the incidence of clinical relapses in asymptomatic IBD patients.

Conclusions and prospects

With the continuous investigation of immunology and molecular biology analysis, we have a clearer and more intuitive understanding of the etiology of IBD on the microscopic level. For example, T cell differentiation, the NF-κB pathway and autophagy have been identified to participate in the pathogenesis of IBD. According to the literatures, oral probiotics can restore balance of the intestinal microflora, mucosal barrier function, and immune tolerance. Probiotics' metabolites also can maintain intestinal health and prevent and treat disease. Probiotic interventions mainly use *Lactobacillus* and *Bifidobacteria*. However, not all probiotics are harmless to healthy individuals. *L. plantarum* MF1298 and *L. plantarum* v299 have been shown to have harmful side effects on healthy human intestinal tissues, such as crypt destruction and inflammatory cell recruitment.[52,83] Therefore, probiotic therapies with novel microbiota members need to be developed. Recently, engineered probiotics have also been used in the prevention and treatment of IBD. It shows great potential in changing the host immune response in experimental mice models and could be an effective microbial therapy. Although a large number of in vitro studies and many experimental results based on animal models of IBD have shown that various probiotics may have some therapeutic potential, researchers have not yet translated these findings into human studies. And there is insufficient data to recommend routine use of probiotics to induce or maintain remission of UC or CD. As a result, the effectiveness of probiotics must be demonstrated in different research models, especially in clinical trials. Future studies need to consider the heterogeneity of existing data,

especially the dose, duration of treatment, and the strains used, to provide more wise recommendations for the clinical efficacy of probiotics in IBD.

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How to cite this article: Liang Y, Liu M, Pu J, Zhu Z, Gao Z, Zhou Q, Gu Q, Li P. Probiotics and their metabolites ameliorate inflammatory bowel disease: a critical review. *Infect Microb Dis* 2021;3(1):4–13. doi: 10.1097/IM9.0000000000000046