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Review Article

The potential therapeutic role of *Lactobacillus reuteri* for treatment of inflammatory bowel disease

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Abstract: Inflammatory bowel disease (IBD) is a chronic intestinal disease of unknown etiology. However, recent studies have established a pathological role of disordered intestinal microbiota and immune dysregulation. Clinical studies have suggested that the reconstruction of the normal intestinal flora in patients with IBD can reverse the dysbiosis caused by genetic, environmental, dietary, or antibiotic factors to ameliorate the symptoms of IBD. *Lactobacillus reuteri* is widely present in the intestines of healthy individuals and regulates the intestinal immune system, reducing inflammation through multiple mechanisms. This review summarizes the current knowledge of the role of *L. reuteri* in maintaining intestinal homeostasis and considers its possible value as a new therapeutic agent for patients with IBD.

Keywords: Inflammatory bowel disease, *Lactobacillus reuteri*, intestinal microbiota, immunoregulation, fecal microbiota transplantation, double-positive intraepithelial T lymphocytes

Introduction

Inflammatory bowel disease (IBD), which includes both ulcerative colitis (UC) and Crohn's disease (CD), is characterized by chronic gut inflammation of unknown etiology. The main clinical symptoms of IBD are abdominal pain, diarrhea, and hematochezia, which seriously affect the quality of life of patients. Although IBD has long been associated with a Western lifestyle, its incidence has been on the rise in Asia in recent years. Current estimates speculate that the prevalence of IBD in China will increase to 0.1% within decades, with the number of patients exceeding 1.5 million in 2025, on par with the burden of disease in Western countries [1]. Factors influencing the occurrence and development of IBD include aberrant immune responses, genetic susceptibility, intestinal dysbiosis, persistent intestinal infections, chronic intestinal mucosal barrier injury, poor diet, and others. Fecal microbiota transplantation (FMT) is a novel therapeutic strategy that has shown encouraging benefits in patients with IBD, refractory *Clostridium difficile* infection (rCDI), diarrhea-type and constipation-type irritable bowel syndrome, insulin resistant diabetes, obesity, Parkinson's disease, idiopathic thrombocytopenic purpura, and other related conditions [2]. FMT was first recommended for treating rCDI in the United States in 2013 [3] and was subsequently used in China to treat severe CD-complicated intestinal fistula infections [4]. *Lactobacillus reuteri* is a normal resident species of the healthy gut microflora that can prevent IBD by altering the intestinal micro-environment and the immune system [5, 6]. Recent studies have shown that *L. reuteri* promotes the clonal expansion of $CD4^+CD8\alpha\alpha^+$ double-positive intraepithelial T lymphocytes (DPIELs), a unique subset derived from $CD4^+$ T cells, in the intestinal mucosa. DPIELs are immunotolerant cells that reduce inflammation due to active immune responses, and therefore can decrease intestinal inflammation in IBD patients [7].

The intestinal microbiota and IBD

Characteristics of the intestinal microbiota

The human gut has been estimated to harbor a complex community of approximately 100 tril-

lion microbial organisms, including bacteria, viruses, fungi, and protozoa, which collectively constitute the microbiota (also referred to as the microbial fora). Although microbes outnumber the host cells 10 to 1 in the human gut, the total number of microbial genes is actually 200-fold higher than human gene copies [8, 9]. Microbial colonization of the intestine begins at birth when infants acquire microbes from the birth canal, skin, feces, and breast milk [10, 11]. More than 1,000 species of bacteria are estimated to reside in the human intestinal tract, predominantly consisting of obligate anaerobes from the *Firmicutes*, *Bacteroides*, *Proteobacteria,* and *Actinomycetes* phyla [12]. The Gram-positive *Firmicutes* and Gram-negative *Bacteroides* have been shown to account for more than 90% of the intestinal flora [13]. The number and composition of the gut bacteria differ markedly from the esophagus to the rectum, with the density increasing from 10/g in the esophagus and stomach to 10^{12}/g in the colon and distal gut. Correspondingly the microbial diversity is lower in the upper segment of the stomach and small intestine compared to the lower gastrointestinal tract.

The gut microbiota exists as a complex multicellular community that, in health, exists synergistically with its host. This microbial community plays an important role in influencing host physiology in health and disease [14]. The benefits that a healthy gut microbiota provides for the human host can be grouped into three categories: nutrition, immune development, and host defense [15]. Bacteria produce short chain fatty acids (SCFAs) via anaerobic fermentation of complex carbohydrates, regulate fat metabolism, metabolize xenobiotics such as drugs, pesticides, and carcinogens, and synthesize vitamin K, group B vitamins, and amino acids that are essential for human nutrition [16, 17]. In addition, the gut microbiota helps maintain the structural integrity of the intestinal mucous barrier by preventing colonization by pathogenic bacteria through the production of antibacterial compounds [18]. Finally, the proper functioning of the intestinal innate immune system strongly depends on the resident microflora; the gut microbiota modulates T-cell repertoires and regulates the T helper (Th) cell profile. Regulatory T cells (Tregs) are $CD4^+$ T cells that have a role in regulating or suppressing other cells in the immune system [15].

Associations between the intestinal microbiota and IBD

IBD is a multifactorial disease that is influenced by environmental, genetic, immunological, and microbial factors [19]. Several independent lines of evidence support the strong link between the composition of the intestinal microbiota and incidence of IBD, including the absence of colitis in germ-free animal models [20], decreased biodiversity and altered composition of the fecal and intestinal microbiota of IBD patients [21], clinical benefits from treatment of patients with IBD with probiotics such as VSL#3 (a mixture of four lactic acid bacteria, three bifidobacterial, and one streptococcus) [22], and the therapeutic impacts of treatment with different antibiotics (metronidazole, amoxicillin, doxycycline, and vancomycin) in patients with severe refractory UC and IBD [23]. Furthermore, the global spread of IBD appears to be associated with the increasing westernization of dietary patterns and the overuse of antibiotics, two factors that have been shown to affect the intestinal microbiome and to increase the risk of IBD in genetically susceptible individuals [24, 25].

A sequencing-based comparison of the intestinal microbiota of patients with IBD and healthy individuals revealed significantly less diversity among individuals with IBD. Importantly, the proportion of harmful bacteria such as *Bacteroides* and *Enterobacteria* (including *Escherichia coli*) increased, however the relative abundance of beneficial *Firmicutes* decreased [17, 26]. In recent decades, *E. coli* and, in particular, adherent-invasive *E. coli* (AIEC) pathotype, has been implicated in the pathogenesis of IBD [27]. Reduced abundance of microbes that produce SCFAs have been observed in patients with IBD [28]. A systematic review of 73 controlled studies describing the fecal and intestinal microbiota of patients with CD found a significant decrease in the microbial richness of the lumen and mucous membranes, mainly due to a decrease in *Firmicutes* species. On the other hand, the numbers of *Bacteroides* and *Enterobacteriaceae* species were significantly increased, especially *E. coli* [29]. In this review, we have summarized recent research on the differences in microbial composition between patients with IBD (either UC or CD) and healthy controls, as summarized in **Table 1** [30-36].

Table 1. Microbial alterations in inflammatory bowel disease

Object of study	Cohort description	Sample type	Outcomes	Reference
Adult	121 CD, 75 UC, 27 control	Mucosal biopsies and Fecal samples	CD • Roseburia, Phascolarctobacterium, Ruminococcaceae, Faecalibacteria prausnitzii (ileal disease) ↓ • Enterobacteriaceae ↑ UC • Roseburia, Phascolarctobacterium, Leuconostocaceae, Odoribacteriaceae ↓	Morgan et al. [30]
Adult	15 UC, 15 control	Feces	UC • Ruminococcus bromii, Eubacterium rectale, Roseburia sp. and Akkermansia sp ↓ • Fusobacterium sp., Peptostreptococcus sp., Helicobacter sp., Campylobacter sp. and Clostridium difficile ↑	Rajilic-Stojanovic et al. [31]
Pediatric	243 CD, 73 UC, 43 control	Mucosal ileal biopsies	CD • Neisseriaceae, Gemellaceae, Fusobacteriaceae, Veillonellaceae, Pasturellaceae, Enterobacteriaceae and Epsilonproteobacteria ↑ • Bifidobacteriaceae and Firmicutes including Lachnospiraceae, Clostridiales and Erysipelotrichaceae ↓ UC • Limited changes noted	Haberman et al. [32]
Pediatric	13 CD, 10 UC, 12 control	Mucosal ileal biopsies	CD • Limited changes noted UC • Microbial richness ↓	Alipour et al. [33]
Adult	28 CD, 30 UC, 30 control	Mucosal colonic biopsies	CD • Faecalibacterium prausnitzii, Bacteroides, Blautia, Ruminococcus, Roseburia, Coprococcus, Lachnospiraceae ↓ UC • Limited changes noted	Rehman et al. [34]
Adult	35 CD, 15 control	Mucosal colonic biopsies and subgroup of fecal samples	CD • Enterobacteriaceae, Fusobacteriaceae in mucosal colonic biopsies ↑ • Enterobacteriaceae, Pseudomonadaceae, Streptococcaceae and Erysipelotrichaceae in subgroup of fecal samples ↑ • Bacteroidaceae, Prevotellaceae, Lachnospiraceae and Ruminococcaceae and Veillonellaceae in mucosal colonic biopsies and in fecal samples ↓ • Microbial richness in subgroup of fecal samples ↓	Eun et al. [35]
Pediatric	13 CD, 12 UC, 12 control	Mucosal biopsies	CD • Microbial richness ↓ • Faecalibacteria prausnitzii ↑ UC • Limited changes noted	Hansen et al. [36]

Abbreviations: CD, Crohn's disease; UC, ulcerative colitis.

Fundamentally, an altered intestinal microecology forms the pathological basis of IBD, and although the specific etiological species have not been confirmed, probiotics have provided clinical benefits for IBD patients. Together, this work suggests that the dysbiosis that contributes to the development of disease will become increasingly treatable as our microbiological understanding of IBD continues to improve.

Application of FMT in treating IBD

The underlying motivation for FMT is a need to restore the balance in intestinal flora that has been disrupted by antibiotics, most strongly exemplified by its role in treating rCDI. FMT can improve the disordered intestinal microecology of IBD patients, compensate for the reduced symbiosis, restore barrier function and permeability, and maintain immune function of the intestinal mucosa. This technique was first used by Eiseman in 1958 to successfully treat four patients with fulminant pseudomembranous colitis and was administered via enema [37]. In the past few decades, FMT has been used to treat rCDI and, more recently, has emerged as a potential treatment option for IBD. The first case report of using FMT for IBD was published in 1989 by Bennet and Brinkman, who used it to treat a patient with chronic UC that was refractory to sulfasalazine and steroids. Colonoscopy after 3 months of retention enema transplants of stool from a healthy donor showed that the acute inflammation had subsided, and the patient remained free of symptoms through 6 months [38]. However, because IBD alternates between periods of active disease and remission, the single 6-month follow-up did not necessarily confirm the long-term efficacy of FMT. A retrospective analysis conducted in 2003 showed that six patients with refractory UC achieved complete, medication-free remission after FMT with no recurrence after 1-13 years of follow-up, indicating the potential long-term efficacy of FMT [39]. **Table 2** summarizes the main published case series and reports of FMT in IBD [40-48]. Collectively, these studies were typified by small sample sizes and inconsistent outcomes. Therefore, two recent randomized trials were designed to rigorously evaluate the clinical efficacy of FMT treatment for UC. Moayyedi et al. studied the benefits and risks of administration of fecal enema or placebo to patients with UC once a week for 6 weeks and found that 9 out of 38 (24%) patients in the FMT group and 2 out of 37 (5%) patients in the placebo group were in remission. However, the improvements in symptoms and quality of life were similar for patients in both groups. Notably, those patients with UC with a history of less than 1 year of disease were more likely to enter remission. Following FMT treatment, all subjects showed a greater diversity of intestinal microorganisms that was similar to the diversity of the donor samples [49]. In another randomized controlled study conducted at the Amsterdam Academic Medical Center, secondary FMT was performed within 3 weeks of the first round of treatment in IBD patients by placing nasal intestinal tubes. While 30% of the patients receiving donor FMT were in remission at week 12, only 20% of patients receiving placebo (autologous feces) were in remission. There was no statistically significant difference in clinical and endoscopic remission between the two groups, which may be due to limited numbers. Patients who exhibited a clinical improvement in disease in both groups were found to have increased diversity of fecal microorganisms at week 12, in contrast with the non-responders in both groups [50].

A review of 12 reports on a total of 111 adult IBD patients that received FMT reported an overall therapeutic success rate of 77.8%. In addition, FMT has been shown to have a 90% therapeutic success rate for patients with UC, as defined by the cessation of symptoms or reduction in ulcerative colitis activity index (UCAI) [51]. A meta-analysis of the four randomized controlled trials that have been conducted to date demonstrated that clinical remission was achieved in 39 of 140 (28%) UC patients in the donor FMT groups compared with 13 of 137 (9%) patients in the placebo groups ($P< 0.01$). However, there was significant variability in the designs of these four clinical trials, ranging from differences in the route of administration of FMT, methods for FMT preparation, the total number of FMTs administered, and differences in definition of primary outcomes [52]. Some of this variability reflects the real-world difficulties associated with standardizing a newly emerging therapy that is dependent on inherently variable donor samples. Data on the benefits of FMT for patients with CD are somewhat more limited than UC. Case reports have

Table 2. Main case series and reports of fecal microbiota transplantation in inflammatory bowel disease

IBD type	Patients	Route	Infusion volume	Number of infusions	Outcome characteristics	Reference
UC	1	Enema	NR	1	Documented remission for 6 mo and ceased medications	Bennet et al. [38]
UC	1	Enema	NR	NR	Documented remission for 6 mo and ceased medications	Borody et al. [40]
CD	1	Enema	NR	NR	Symptoms-free and receiving no medications 4 mo after FMT	
UC	6	Enema	200-300 g/200-300 mL	6	Documented remission from 1 to 13 yr and ceased medications	Borody et al. [39]
CD	1	Colonoscopy + Enema	200-400 mL	1 + 9	CD related improvement was not reported	Grehan et al. [41]
UC combined with CDI	4	Colonoscopy	220-240 mL	1	Colitis activity was improved, and CDI was cured	Hamilton et al. [42]
CD combined with CDI	6	Colonoscopy + Enema	220-240 mL	1 or 2	Two cases underwent a second FMT due to CDI recurrence, but the efficacy of FMT on CD was not reported	
UC combined with CDI	1	Colonoscopy	300 mL	1	Documented symptom-free for 8 mo without CDI recurrence	Zainah et al. [43]
UC	3	Repeated rectal infusions	NR	Daily infusion for 2 to 6.5 mo	Documented improvement from 1 to 36 mo	Borody et al. [44]
CD combined with CDI	3	Colonoscopy	18-397 g/180-600 mL	1	Symptoms such as diarrhea improved or resolved 3 mo after FMT	Patel et al. [45]
CD combined with CDI	2	Colonoscopy Upper endoscopy	18-397 g/180-600 mL	2	CDI recurred in one case after the first FMT by colonoscopy, and a second FMT was performed by upper endoscopy; but the efficacy of FMT on CD was not reported	
UC	6	Colonoscopy	300-500 mL	1	Documented improvement, but no remission within 2 wk after FMT	Kump et al. [46]
UC	10	Enema	165 mL	5	78% and 67% subjects achieved clinical response within 1 wk and 1 mo after FMT, respectively	Kunde et al. [47]
CD	1	NR	NR	NR	Response to FMT for 6 mo followed by relapse	Gordon et al. [48]
CD	1	Gastroscope	150 mL	1	Documented clinical remission for more than 9 mo	Zhang et al. [4]

Abbreviations: FMT, fecal microbiota transplantation; UC, ulcerative colitis; CDI, *Clostridium difficile* infection; CD, Crohn's disease; NR, not reported.

shown mixed results, with some suggesting clinical and endoscopic remission and others demonstrating no effect [53]. A study of a cohort of 30 patients with refractory mid-gut CD found a 77% rate of clinical remission at one month following a single FMT administered via the nasoduodenal route [54]. Taken together, FMT may be a valuable treatment for refractory IBD compared to traditional therapies such as anti-inflammatory steroids and immunosuppressive agents; however, its definitive clinical benefits are currently difficult to estimate given the significant heterogeneity of clinical study designs and methods used for therapeutic preparation and administration.

L. reuteri and IBD

Characteristics and clinical efficacy of treatment with L. reuteri

L. reuteri is a Gram-positive facultative anaerobe of the genus *Lactobacillus*. It is a slightly irregular campylobacter with rounded ends and is widely present in the intestines of vertebrates wherein it ferments sugar to lactic acid, acetic acid, and ethanol [55]. It is one of the few lactic acid bacteria that have adapted to survive in the human stomach and can grow in the presence of gastric acids and bile salts. In addition, *L. reuteri* has been detected in the upper regions of the small intestine, where it colonizes the mucosal layer.

L. reuteri, when administered as a probiotic, helps to restore the balance of intestinal flora and to inhibit diarrhea through multiple mechanisms. It produces metabolites such as organic acids, hydrogen peroxide, bacteriocins, and other antagonistic substances that inhibit the growth and reproduction of harmful bacteria and prevent antibiotic-induced diarrhea [6]. In addition, *L. reuteri* colonies in the digestive tract form a biological barrier that blocks the adhesion of pathogenic bacteria to the gastrointestinal mucosa, inhibits pathogenic growth by competing for nutrients, and neutralizes bacterial toxins. *L. reuteri* metabolizes glycerin to produce reuterin and 3-hydroxypropionaldehyde (3-HPA), which is a low molecular weight, neutral, and soluble bacteriocin that exists as a mixture of its hydrate and dimeric forms. Low doses of reuterin have been shown to inhibit the growth of various pathogens, such as *E. coli*, *Salmonella typhimurium*, *Candida albicans*, *Bacillus subtilis*, *Aspergillus flavus*, *Campylobacter jejuni,* and *Clostridium sporogenes* [56]. Importantly, *L. reuteri* also modulates the host immune response by decreasing the production of proinflammatory cytokines and promoting the development of Tregs [6]. Recent mechanistic studies have suggested that *L. reuteri* CCM 3625 produces tyramine under certain culture conditions and that *L. reuteri* E and *L. reuteri* KO5 produce biogenic amines, including histamine and tyramine, which may reduce the inflammatory response in the gastrointestinal tract [55]. Consistent with this finding, *L. reuteri* can prevent intestinal anaphylaxis and regulate the intestinal immune response [57]. It has been shown that feeding newborn rats with *L. reuteri* DSM 17938 can prevent necrotizing enterocolitis (NEC) and inhibit Treg-deficiency-associated autoimmunity in the newborn rats. Feeding *L. reuteri* did not affect clinical phenotype or inflammatory biomarkers in plasma and stool, but *L. reuteri* increased the proportion of $Foxp3^+$ Tregs in the intestine. *L. reuteri* also exerts a major influence on the plasma metabolome, upregulating amino acid metabolites formed via the urea, tricarboxylic acid, and methionine cycles and increasing tryptophan metabolism [58].

The anti-inflammatory effects of L. reuteri in IBD

IBD is a chronic gastrointestinal disease that results from a dysregulated immune response to specific environmental triggers in a genetically predisposed individual. Increasing evidence has suggested a central role for dysbiosis of the gut microbiota in contributing to this immune-mediated intestinal inflammation [26]. Although the relationship between *L. reuteri* and IBD has gained considerable attention in recent years, the results of studies to date are not conclusive. The intestinal microbiome of patients with IBD and healthy individuals show qualitative and quantitative differences. Typically, the relative abundance of *Escherichia*, *Fusobacterium,* and *Proteobacteria* genera are increased, and *Bacteroides*, *Bifidobacterium,* and *Clostridium* groups IV and XIVA are decreased in patients with IBD and in mouse models of colitis [59]. Of note, these microbiome changes are correlated with inflammation of the intestinal mucous membrane [59]. It has been reported that treatment with *L. reu-*

teri R2LC or 4659 significantly reduced inflammation of the intestinal mucosa in a mouse model of dextran sodium sulfate (DSS)-induced UC, acting by decreasing the expression of proinflammatory markers like MPO, IL-1B, IL-6, and mKC (mouse keratinocyte chemokines). In addition, probiotic treatment with *L. reuteri* reverses the DSS-induced thinning of the intestinal mucus and promotes the synthesis of connective tissue tight junction proteins at the bottom of the colon recess [60]. Petrella et al. have demonstrated therapeutic benefits following administration of *L. reuteri* ATCC PTA 4659 (of human origin) and *L. reuteri* R2lc (of rodent origin) in a rat model of UC [61]. Impressively, continuous supplementation with *L. reuteri* almost completely prevented the occurrence of DSS-induced UC in rats, likely due to sustained colonization of the intestinal tract by the probiotics. A recent study demonstrated that the prophylactic administration of *L. reuteri* F-9-35 had anti-inflammatory effects in the DSS-induced mouse model of colitis [5]. This preventive effect was attributed to the reduced transcription of mRNA for *COX-2*, *TNF-α*, and *IL-6* as well as the restoration of the intestinal balance between *Firmicutes* and *Bacteroidetes* [5]. Taken together, the data from these studies suggest that probiotic treatment with *L. reuteri* has an anti-inflammatory effect on the gut that may provide therapeutic benefits in IBD.

The immune-regulatory effects of L. reuteri in IBD

The vast intestinal microbial community secretes effector molecules and physically interacts with host pattern receptor proteins to initiate a complex, two-way regulation of the local immune system and the dynamics of the microbial population. Innate immune responses are elicited by the recognition of bacterial pathogen-associated molecular patterns (PAMPs) by the host pattern recognition receptors (PRRs) present on leukocytes, including Toll-like receptors (TLRs), NOD-like receptors (NLR), and C-type lectin receptors (CLRs), canonically forming the proinflammatory response that is thought to be the pathogenic basis of IBD [62]. Studies of animal models with decreased expression/activation of NOD-like receptor or TLR signaling have revealed a complex and context-dependent role of innate immunity in colitis, ranging from protective to proinflammatory [63]. A recent study showed that *L. reuteri* DSM 17938 attenuates experimental NEC by inducing tolerogenic intestinal dendritic cells (DCs) and Tregs, which in turn reduce the proliferation of proinflammatory lymphocytes and production of inflammatory cytokines via a mechanism that is dependent on TLR2 [64]. In the healthy gut, these immune responses are kept in check through various regulatory pathways, and any disruption to this tightly controlled system can trigger an inflammatory response. Indeed, reports have suggested that mucosal immune system dysfunction plays an important role in IBD pathogenesis [6, 65]. Intestinal immunomodulation is mainly regulated by intraepithelial T lymphocyte subsets [66]. Because DPIELs are common to mice and humans, preclinical studies of the effects of treatment *L. reuteri* on this immune cell type may provide new insights into IBD treatment.

Indeed, in 2017, a study showed that *L. reuteri* is an intestinal microbe that can regulate DPIELs. In that work, rats were randomly divided into two groups: a high-DPIEL group and a group without DPIELs. The intestinal flora of the DPIEL group was transferred to the other group of mice. Strikingly, the recipient mice then generated a considerable amount of DPIELs, which then disappeared after treatment with antibiotics, indicating that the intestinal flora plays an important role in the regulation of intestinal immunity. In addition, the researchers transplanted Gram-positive, neomycin-resistant bacteria into rats and found that none of the other five types of *Bacteroidetes* had this effect [7], whereas *L. reuteri* influenced whether $CD4^+$ T cells could differentiate into DPIELs. Disordered intestinal flora can further aggravate a pre-existing inflammatory condition in order to induce IBD [18].

Tryptophan (Trp) is an anti-inflammatory essential amino acid that supports the intestinal flora. A recent study found that Trp supplementation in a mouse model of colitis reduced the levels of threonine, methionine, and proline, which in turn decreased the colonic concentration of IL-22 and altered the intestinal microbiome [67]. Interestingly, Trp concentration in the intestinal lumen may be related to *Lactobacillus*-mediated regulation of intestinal immunity. Marco Colonna et al. were the first to show that

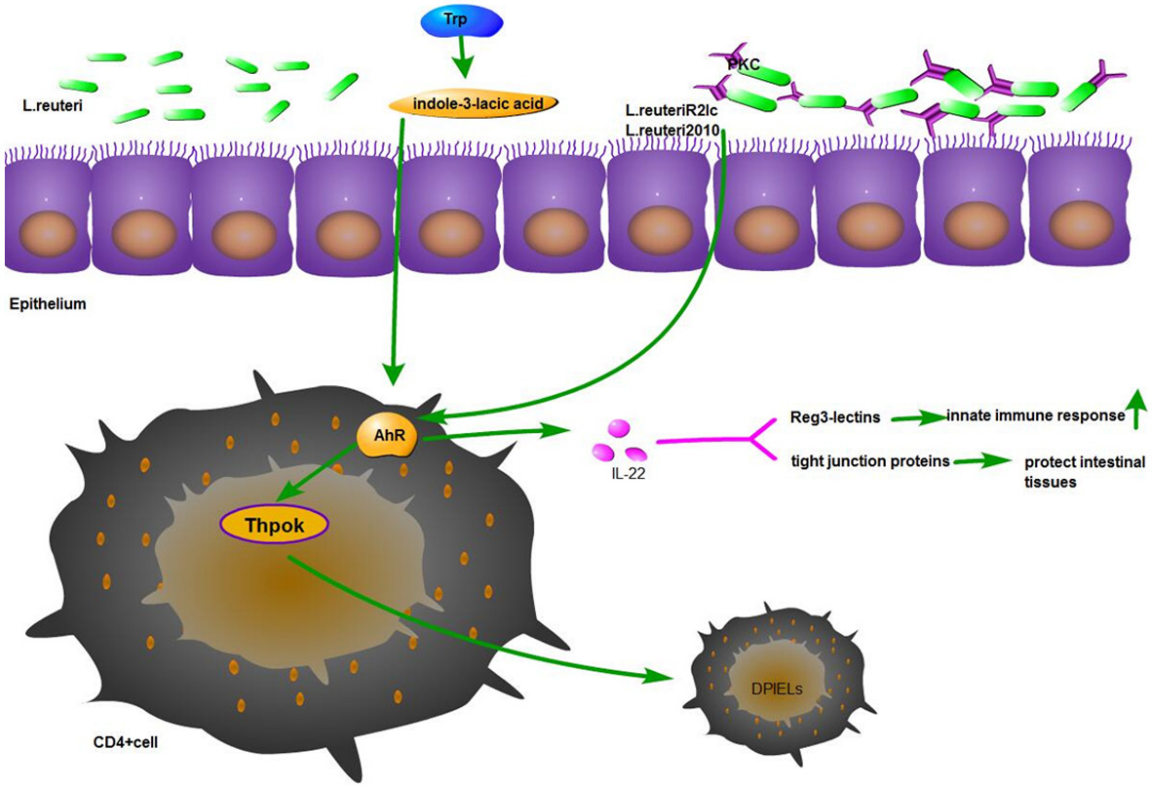

Figure 1. The immune-regulatory role of *L. reuteri* in IBD. *L. reuteri* provides indole derivatives of dietary Trp, such as indole-3-lactic acid, which activate AhR and lead to the down-regulation of Thpok and subsequent reprogramming of $CD4^+$ IELs into DPIELs. The R2lc and 2010 strains of *L. reuteri* activated AhR through the cluster of polyketone synthase (PKS), a pathway that is unrelated to Trp metabolism. Activation of AhR promotes the production of interleukin-22 (IL-22), which enhances the innate immune response by inducing production of antimicrobial peptides (Reg3-lectins) to fight off intestinal pathogens and to protect intestinal tissues from damage due to inflammation by increasing the expression of tight junction proteins.

L. reuteri promotes the differentiation of T cells into DPIELs by metabolizing Trp to indole-3-lactic acid, which then activates the aryl hydrocarbon receptor (AhR) on $CD4^+$ T cells to downregulate the transcription factor Thpok and ultimately induce their differentiation into DPIELs [7] (**Figure 1**). This finding is consistent with prior reports of the molecular implications of the down-regulation of Thpok [68]. To further study the relationship between *L. reuteri* and Trp in the generation of DPIELs, the researchers fed normal and gnotobiotic mice lacking intestinal bacteria with high, medium, or low doses of Trp for 4 weeks. Although even high-dose Trp failed to induce DPEIL production in the aseptic mice, it significantly increased the amount of DPIELs measured in the normal mice in a dose-dependent manner. Taken together, these studies suggest that the beneficial bacteria residing in the healthy intestines require Trp to carry out their immunomodulatory functions. This is further corroborated by the higher incidence of intestinal inflammation in individuals with genetic defects in Trp-metabolizing enzymes [7].

A recent study showed that the R2lc and 2010 strains of *L. reuteri* activated AhR through the cluster of polyketone synthase (PKS), a pathway that is unrelated to Trp metabolism. Activation of AhR is important for the production of interleukin-22 (IL-22), which can enhance the innate immune response by inducing production of antimicrobial peptides (Reg3-lectins) to fight off intestinal pathogens and to protect intestinal tissues from inflammation damage by inducing tight junction proteins [69] (**Figure 1**). Researchers also demonstrated that a deficiency in $Foxp3^+$ Treg cells results in dysbiosis of the gut microbiome and a dramatically increased likelihood of developing autoimmunity in scurfy (SF) mice. However, remodeling the

microbiota with additional *L. reuteri* prolonged survival and reduced multiorgan inflammation in these SF mice. *L. reuteri* appears to help improve the metabolomic profile that is disrupted by Treg cell deficiency, for example by restoring levels of the purine metabolite inosine [70]. In an *in vitro* experiment, treatment with *L. reuteri* DSM 17938 cell-free supernatant (*L. reuteri*-CFS) was shown to induce an immunotolerant phenotype in retinoic acid (RA)-driven mucosal-like dendritic cells, which had subsequent effects on Tregs. Indeed, treatment with *L. reuteri*-CFS further influenced the tolerogenic phenotype of RA-DC by downregulating many genes involved in antigen uptake, antigen presentation, and signal transduction, as well as several chemokine receptors, while upregulating the production of IL-10, a tolerogenic cytokine [71]. Other studies have indicated that *L. reuteri* 5289 causes DCs to release IL-10 and inhibits the production of IL-12 by DCs in response to co-culture with other bacteria that typically induce production of IL-12; remarkably, they observed that the *L. reuteri*-mediated inhibition of IL-12 production was associated with prolonged ERK1/2 MAP kinase phosphorylation [72]. The role of *L. reuteri* in intestinal immunity is not yet fully understood. Significant detailed mechanistic studies are needed to better understand how *L. reuteri* contributes to a healthy intestinal environment.

The anti-osteoporosis effects of L. reuteri in IBD

Approximately 10% to 40% of IBD patients may suffer from at least one extraintestinal manifestation, sometimes including metabolic bone diseases such as osteopenia and osteoporosis [73]. A study showed that both osteopenia and osteoporosis are strongly associated with IBD, ranging from 32% to 36% for osteopenia and from 7% to 15% for osteoporosis [74]. A Swiss IBD cohort study of 877 patients found a prevalence of bone density alteration in 20% of IBD patients and identified, by multivariate logistic regression analysis, that an extended history of disease, perianal disease, and corticosteroid use were independent risk factors of bone density loss [75]. Bone alterations in patients with IBD appear to have a staggeringly complex multifactorial etiology: disruption of gut-bone immune signaling interactions, inflammation-related bone resorption loss, genetic factors, interactions between microbiota and pathogenic microorganisms, multiple intestinal resections, steroid treatments, reduced absorption of minerals, and vitamin D deficiency are all possible factors which may, together or individually, contribute to the alteration of bone mineral density [76]. Indeed, it is not clear whether inflammation directly causes the loss of bone mineral density or if other intermediary factors contribute to the decline of bone mineral density in patients with IBD.

Probiotic bacteria supplementation has been demonstrated to be beneficial for bone health [77, 78]. A study found that treating healthy mice with *L. reuteri* enhances bone density in male mice, but not in females. This work showed that probiotics increased mineral density in the distal femur metaphyseal region as well as in the lumbar vertebrae and increased osteoblast serum markers in male mice [79]. However, the host and bacterial mechanisms responsible for mediating these effects however are not well understood.

A recent study found that *L. reuteri* secretes factors that regulate T lymphocytes, which play an important role in mediating positive bone density outcomes. In that work, researchers administered *L. reuteri* via drinking water for 4 weeks to male wild-type or RAG knockout (which lack mature T and B lymphocytes) mice. Although *L. reuteri* treatment increased bone density in wild type mice, no significant increases were seen in RAG knockout mice, suggesting that lymphocytes are indeed critical for the *L. reuteri*-mediated beneficial effects on bone density. *Ex vivo* studies using whole mesenteric lymph nodes (MLN) as well as $CD3^+$ T cells, demonstrated that the administration of live *L. reuteri* and its secreted factors have concentration-dependent effects on the expression of cytokines, including the anti-inflammatory cytokine IL-10. Further, they found that the effects of *L. reuteri* on lymphocytes are negatively regulated by a RIP2 inhibitor, suggesting a role for NOD signaling in this regulatory network. Finally, this study showed that T cells from MLNs treated with *L. reuteri* supernatants secrete factors that enhance the expression of osterix, a transcription factor involved in osteoblast differentiation, in MC3T3-E1 osteoblasts [80]. Despite these informative

findings, the exact mechanisms by which *L. reuteri* in the intestinal tract exerts a systemic effect to promote bone health remains to be fully elucidated. Although these findings highlight just several potential mechanisms by which *L. reuteri* is able to improve bone health, they pave the way to potential targets for future therapeutic research on disease outcomes related to IBD.

The antifungal effects of L. reuteri in IBD

An increased relative abundance of intestinal fungi has long been suspected to play a role in the pathogenesis of IBD [81]. Gastrointestinal fungi may be beneficial or detrimental to the host [82, 83], but relevant data are currently scarce. Several IBD-associated genes, such as *CARD9*, are involved in immune responses to fungi [84]. Moreover, mice lacking major genes responsible for fungi sensing, such as *CARD9* or *DECTIN1*, have an increased fungal microbiota load and are more susceptible to colitis [85, 86]. These data suggest a link between fungal microbiota and IBD pathogenesis.

Several studies have shown an increased level of *Candida albicans* in patients with IBD [81, 87]. It has been shown that the fungal microbiota is skewed in patients with IBD, with an increased *Basidiomycota/Ascomycota* ratio, a decreased proportion of *Saccharomyces cerevisiae,* and an increased proportion of *Candida albicans*, relative to healthy subjects [81]. A recent study found an elevation of (1→3)-β-D-glucan (BG, a component of the fungal cell-wall) in the serum of patients with IBD and endoscopic moderate colitis in clinical remission, supporting a possible role for gut fungi in IBD. In mice, the administration of *Candida* by oral gavage was found to worsen the increase mortality, was associated with more severe colon histology findings, and increased gut leakage. Treatment of mice with DSS + *Candida* induced higher proinflammatory cytokines both in intestinal tissue and in blood. However, treatment with *Lactobacillus rhamnosus* L34 attenuated the effects of both DSS + *Candida* and DSS alone through the attenuation of gut local inflammation, reversal of gut-leakage, correction of fecal dysbiosis, and a reduction in systemic inflammation [87]. A study found that probiotic treatment with *L. rhamnosus* GR-1 and *L. reuteri* RC-14 strains led to potent protection against all of the tested *Candida glabrata* strains. Treatment with these lactobacilli reduced fungal aggregation, inhibited fungal growth, and eventually led to death of *Candida glabrata* [88]. Based on the above results, we speculate that *L. reuteri* may play an additional therapeutic role in IBD through its effects on fungi. However, specific studies aimed to assess that hypothesis are required.

Therapeutic potential of L. reuteri in IBD

Treatment via FMT can reconstruct and restore the healthy intestinal microbial flora, maintain intestinal homeostasis, decrease the secretion of inflammatory factors, and regulate intestinal mucosal immunity, each of which can ameliorate the symptoms of IBD. As discussed in this review, *L. reuteri* not only inhibits the growth of harmful bacteria and fungi as well as reduces intestinal inflammation, but it also up-regulates the differentiation of DPIELs in the small intestines, which in turn maintain the intestinal microecology and dampen immune responses [5, 7, 57]. Impressively, significant research has shown that *L. reuteri* has anti-osteoporotic and antifungal effects in IBD. Together, these findings suggest that *L. reuteri* has considerable potential as a targeted therapeutic intervention for patients with IBD.

Conclusion and outlook

L. reuteri prevents intestinal disturbances such as diarrhea by restoring the intestinal microbial flora and regulating intestinal immune function. Although the mechanisms by which *L. reuteri* influences these outcomes have become increasingly clear with recent research, further biochemical and genetic analyses are required to fully understand its potential as a treatment for IBD.

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Disclosure of conflict of interest

None.

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