

Probiotics and prebiotics in chronic inflammatory bowel diseases

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Abstract

The prokaryotic and eukaryotic cells of the colon exist in a highly complex, but harmonious relationship. Disturbances in this remarkable symbiosis can result in the development of inflammatory bowel diseases (IBD). Although the etiology of IBD is not entirely understood, it is known that the chronic inflammation of Crohn's disease, ulcerative colitis and chronic pouchitis are a result of an overly aggressive immune response to the commensal intestinal flora in genetically susceptible hosts. Recent studies have enhanced our ability to understand the interaction between the host and its intestinal microflora and the role the microflora plays in maintaining intestinal homeostasis. As we begin to understand the benefits conferred to the intestine by the microflora, the notion of modifying the composition of the bacterial load to improve human health has arisen. A significant body of research now exists investigating the role of probiotics and prebiotics in ameliorating chronic intestinal inflammation. This article will begin with an overview of the role of the commensal microflora in maintaining mucosal immune homeostasis, and how a dysregulated immune response to the intestinal microflora results in IBD. This will be followed by a summary of the use of probiotics and prebiotics in experimental and human IBD.

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Key words: Colitis; Crohn's disease; Microflora; Immunity; Probiotics; Prebiotics

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INTRODUCTION

At birth, the gastrointestinal tract is a sterile environment. Initial exposure of the gut to microbes occurs during the birthing process from the maternal fecal and vaginal flora. Within a few months after birth, a relatively stable microbial population is established^[1]. This abundant, diverse and dynamic intestinal microflora normally lives in a complex, symbiotic relationship with the eukaryotic cells of the mucosa. About 100 trillion bacterial cells benefit from the constant nutrient flow, stable temperature and niches for various metabolic requirements provided by the intestinal environment. Likewise, the host benefits from the ability of the intestinal microflora to synthesize vitamin K, exert trophic effects on intestinal epithelial cells, salvage energy from unabsorbed food by producing short-chain fatty acids, inhibit the growth of pathogens, sustain intestinal barrier integrity and maintain mucosal immune homeostasis. Studies from germ-free animals reveal that the absence of resident intestinal microflora results in significant alterations in intestinal structure and function, including slender villi, shallow crypts, low leukocyte count^[2,3], a decrease in the number and density of Peyer's patches^[4] and decreased stimulation of migrating motor complexes^[5].

In their co-evolution with bacteria, vertebrates develop pattern-recognition receptors, which are activated by specific molecular patterns unique to bacteria, fungi and viruses that are absent in eukaryotes (lipopolysaccharides, peptidoglycan, ssRNA, muramyl dipeptide, flagellins, *etc*). These include the Toll-like receptors (TLRs) and nucleotide oligomerization domains (NODs). TLRs and NODs are critical for the initiation of innate immune defense responses. Activation of their signaling cascades usually results in the production of pro-inflammatory cytokines. TLR signaling also provides a link between innate and adaptive immunity, as TLR signaling results in the maturation of dendritic cells, which activate adaptive immune responses^[6]. Although stimulation of these receptors in most parts of the immune system results in production of inflammatory cytokines, these ligands are not only tolerated by the gut mucosal immune system, but also essential for adaptation to intestinal bacteria and maintenance of homeostasis^[7]. The tolerance to the intestinal microflora is not completely understood, but several aspects of commensal physiology have been defined which contribute to their inability to activate the immune system. Some commensal bacteria can modify

TLR ligands, resulting in a hypoactive immune response. For example, the endotoxic portion of LPS is pentacyclated in many *Bacteroides* species, and has minimal toxicity^[8]. An important feature of commensal bacteria is their inability to penetrate the intestinal epithelial barrier. If some of these organisms do penetrate, they are usually rapidly phagocytosed by the innate mucosal immune system. Indeed, in a healthy host, the systemic immune system appears to be ignorant of the intestinal microflora^[9]. Maintaining tolerance to these intestinal bacteria is a remarkable accomplishment achieved by the mucosal immune system, and disturbances in this bacterial-epithelial homeostasis result in considerable deleterious effects on the host.

Role of the commensal flora in IBD

Although many studies have investigated the possibility of a single infectious agent causing Crohn's disease and ulcerative colitis, also called chronic inflammatory bowel disease (IBD), none has yet been discovered. The intestinal bacteria are now believed to be involved in the initiation and perpetuation of IBD. The prevailing theory explaining the development of IBD is that the adaptive immune system is hyper-responsive to the commensal intestinal microflora in genetically susceptible individuals^[10]. This hypothesis is supported by several observations: most inflammation occurs in areas with the highest density of intestinal bacteria, broad spectrum antibiotics improve chronic intestinal inflammation, and surgical diversion of the fecal stream can prevent recurrence of Crohn's disease. Most importantly, despite differences in the pathogenesis of chronic intestinal inflammation, a consistent feature of many animal models of IBD (such as IL-10 knockout mice and HLA-B27 transgenic rats) is the failure to develop chronic intestinal inflammation when these animals are raised in germ-free conditions^[11,12]. Dysbiosis is also observed in IBD patients. Adherent and intramucosal bacteria, particularly *Bacteroides spp*, *Escherichia coli* (*E. coli*) and *Enterobacterium spp* are more abundant in patients with Crohn's disease (CD) than in controls^[13,14] (Figure 1). Bacterial overgrowth and dysbiosis are also associated with the development of chronic pouchitis, the inflammation of the ileal reservoir created after ileo-anal anastomosis following colectomy in ulcerative colitis (UC) patients^[15]. In addition, several selected commensal bacterial species can induce and perpetuate colitis in genetically susceptible rodent models of chronic intestinal inflammation^[16,17] (Figure 1).

Other immune dysfunctions in IBD include aberrant secretion of pro-inflammatory cytokines and chemokines by intestinal epithelial cells and mucosal immune cells^[18-20], altered TLR4 signaling^[21], defective antigen presenting cell function^[22] and lack of T-cell apoptosis^[23]. It is now clear that ignorance of the systemic immune system to commensal intestinal microflora is lost in IBD patients, as shown by enhanced and persistent cell-mediated and humoral immune responses to these bacteria^[24].

At least 7 genetic loci conferring susceptibility to CD, ulcerative colitis (UC) or both, have been identified^[25]. Interestingly, such susceptibility genes associated with CD

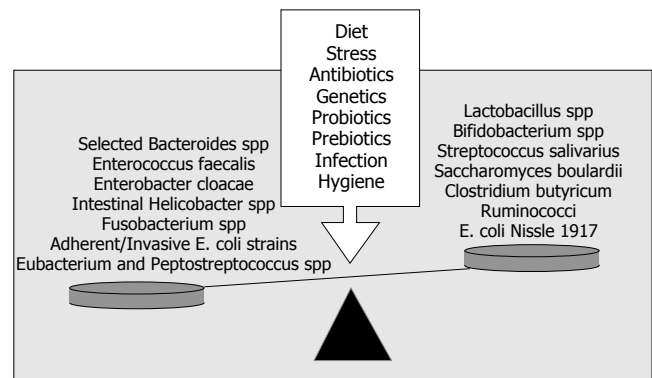


Figure 1 Microbial balance and dysbiosis. The pathogenic immune responses present in IBD are triggered by the presence of luminal bacteria. The balance of beneficial vs aggressive intestinal microbes is responsible for either mucosal homeostasis or chronic inflammation. A number of environmental and genetic factors influence the balance of beneficial vs aggressive microbes. Adapted from^[63].

involve polymorphisms of the NOD2 gene^[26] (the pattern recognition receptor for muramyl dipeptide) which can result in hampered innate immune functions by impairment of TLR function^[27], defective clearance of invasive bacteria by macrophages^[28], and decreased production of defensins^[29].

The recognition of the compelling association between intestinal microflora and the development of IBD has led to an abundance of studies investigating the therapeutic potential of altering luminal bacteria using probiotics and/or prebiotics.

Probiotics, prebiotics and synbiotics

Probiotics are defined as living organisms in food and dietary supplements which, upon ingestion, improve the health of the host beyond their inherent basic nutrition^[30]. Probiotics are typically lactic acid bacteria selected from the gut flora, and are able to survive stomach acid and bile, maintain viability throughout extended periods of storage and are safe for human consumption. Other species have also shown some beneficial effects, such as *E. coli* Nissle 1917 and *Saccharomyces boulardii* (Figure 1). Probiotic bacteria have verifiable beneficial properties, including the ability to improve epithelial barrier function, modulate the mucosal immune system, and alter the intestinal flora.

Prebiotics are non-digestible dietary carbohydrates, e.g., lactosucrose, fructo- and galacto-oligosaccharides, inulin, psyllium, bran, germinated barley (Figure 2), which stimulate the growth and metabolism of endogenous enteric protective bacteria upon consumption. Beneficial effects of prebiotics are also associated with changes in colonic short-chain fatty acids (SCFA) due to fermentation by colonic bacteria^[31]. Synbiotics are combinations of probiotics and prebiotics, and are also an emerging therapeutic modality. Restoration of normal microflora using probiotics, prebiotics or synbiotics has been investigated in numerous gastrointestinal and other disease states, including infectious diarrhea, *H. pylori* infection, irritable bowel syndrome, colorectal cancer, lactase deficiency, pancreatitis, atopy, and IBD.

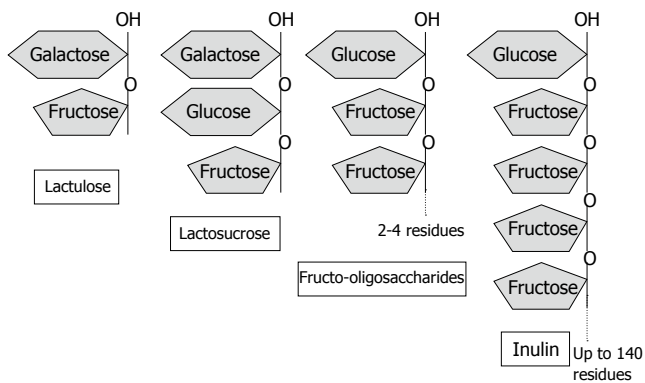


Figure 2 Basic structures of various prebiotic substances. Structurally, prebiotics are a mixture of polymers and oligomers comprising branching chains of fructose units.

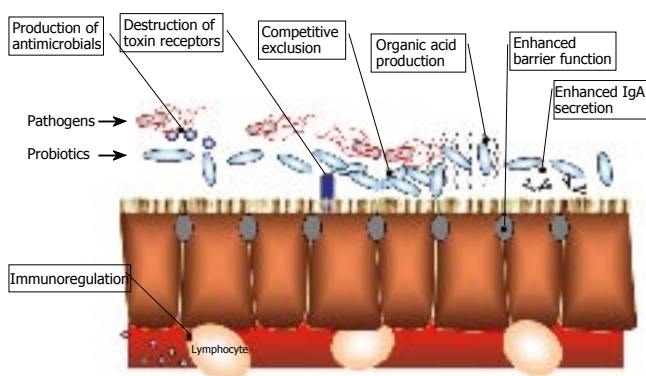


Figure 3 Mechanisms of probiotic activity.

Protective mechanisms of probiotics by ameliorating chronic intestinal inflammation

Probiotic bacteria have beneficial effects on the intestinal epithelia both directly and indirectly, including enhanced barrier function, modulation of the mucosal immune system, production of antimicrobials, and alteration of the intestinal microflora (Figure 3).

Alteration of the mucosal immune system. The presence of probiotics has been shown to result in several modifications in the mucosal immune response, including augmented antibody production^[32,33], increased phagocyte^[34] and natural killer cell activity^[35-38], modulation of the nuclear factor kappa-B (NFκB) pathway^[39-41], and induction of T cell apoptosis^[42]. Generally, probiotics increase the production of intestinal anti-inflammatory cytokines (such as IL-10 and TGF-β), while reducing the production of pro-inflammatory cytokines (e.g., TNF-α, interferon-γ, IL-8)^[43-46]. Several probiotic bacteria, including *B. breve*, *Streptococcus thermophilus*, *B. bifidum* and *Ruminococcus gnavus* have been shown to secrete metabolites that reduce LPS-induced TNF-α secretion^[47]. *L. reuteri* reduces TNF-α and *Salmonella typhimurium* induces IL-8 secretion *in vitro*, by inhibiting nuclear translocation of NFκB and preventing the degradation of IκB^[48]. Administration of the probiotic cocktail VSL#3 (consisting of *L. acidophilus*, *L. bulgaricus*, *L. casei*, *L. plantarum*, *B. breve*, *B. infantis*, *B. longum*, *S. thermophilus*) to IL-10 deficient mice results in

colitis reduction and a concomitant reduction in mucosal secretion of TNF-α and interferon-γ^[49]. *E. coli* Nissle 1917 is able to down-regulate the expansion of newly recruited T-cells into the mucosa and limit chronic intestinal inflammation^[50]. In SAMP1/Yit mice, *Lactobacillus casei* strain Shirota inhibits IL-6 production in LPS-stimulated large intestinal lamina propria mononuclear cells and down-regulates nuclear translocation of NFκB^[51]. Patients with a recent ileo-anal pouch anastomosis who responded to probiotic therapy have reduced mRNA levels of IL-1β, IL-8 and IFN-γ, and fewer polymorphonuclear cells compared with patients who receive placebo^[52]. Probiotic treatment has also been shown to reduce IFN-γ and IL1-α expression and decrease inducible-nitric oxide synthase and gelatinase activities in pouch biopsy samples from patients with pouchitis^[53]. In mucosal explants of ileal specimens from patients with Crohn's disease, probiotics reduced TNF-α release and the number of CD4 cells^[54]. In addition to live probiotics, components of probiotic bacteria can also exert effects on the mucosal immune system. For example, genomic DNA isolated from VSL#3 inhibits TNF-α-induced IL-8 secretion, mitogen-activated protein kinase activation and NFκB activation^[41] in HT-29 cells.

Improved barrier function. Various probiotic bacteria can enhance intestinal epithelial barrier function. For example, oral administration of VSL#3 results in normalization of impaired colonic barrier function and restoration of intestinal epithelial integrity in IL-10 deficient mice and enhancement of epithelial resistance in T-84 cells^[49]. Barrier function was enhanced not only by live bacteria, but also by a proteinaceous secreted product of VSL#3^[49]. Several strains of lactobacilli are also capable of up-regulating intestinal MUC3 mRNA expression, thereby improving barrier function by increasing the mucus layer^[55,56]. *Lactobacillus* GG (*L. GG*) improves barrier function by inhibiting apoptosis of intestinal epithelial cells^[57]. *S. thermophilus* and *L. acidophilus* have been shown to enhance phosphorylation of actinin and occludin in the tight junction, thereby preventing the invasion of enteroinvasive *E. coli* into human intestinal epithelial cells^[58].

Alteration of the intestinal flora. Probiotics suppress the growth and invasion of pathogens in several ways. They competitively exclude pathogenic bacteria by occupying the limited physical space in the mucus layer and on epithelial cells. They also engage pattern-recognition receptors and consume substrate otherwise available to other (pathogenic) microbes. In addition, probiotics render their microenvironment inauspicious for pathogens by secreting antimicrobial substances such as hydrogen peroxide, organic acids, and bacteriocins. For example, both *in vitro* and *in vivo* experiments demonstrate that *B. infantis* suppresses the growth of *Bacteroides vulgatus*^[59]. VSL#3 has been shown to inhibit *Salmonella dublin* invasion into T-84 cells^[49]. Patients with pouchitis treated with VSL#3 have been demonstrated to have increased bacterial diversity in the pouch, and decreased fungal diversity^[60].

Probiotics may also alter the intestinal microflora by changing the fatty acid profile in the colon. VSL#3

Table 1 Clinical studies of probiotics in IBD

Author	Design	Group (Dose/d) (n)		Results
		Probiotic	Comparator	
Induction of remission of ulcerative colitis				
Kato 2004 ^[87]	DB, R, C	Bifidobacterium fermented milk (100 mL) (10)	Placebo (10)	Reduced UCDAI ($P < 0.05$)
Rembacken 1999 ^[85]	DB, R, C	<i>E. coli</i> Nissle 1917 (1×10^{11} cfu) (57)	Mesalamine (59)	As effective as mesalamine at attaining remission
Bibiloni 2005 ^[88]	Open-label	VSL#3 (3.6×10^9 cfu) (32)	None	77% remission or response rate
Ishikawa 2003 ^[86]	R, C	Lactobacillus and Bifidobacterium-fermented milk (100 mL) (11)	Placebo (10)	Reduced exacerbation of symptoms ($P < 0.01$)
Borody 2003 ^[90]	Case reports	Fecal enema (6)	None	100% remission
Maintenance of remission of ulcerative colitis				
Kruis 2004 ^[82]	DB, R, C	<i>E. coli</i> Nissle 1917 ($2.5\text{-}25 \times 10^9$ cfu) (162)	Mesalamine (165)	As effective as mesalamine at maintaining remission ($P = 0.003$)
Zocco 2006 ^[83]	Open-label	Lactobacillus GG (1.8×10^{10} cfu) (65)	Mesalamine (60) Mesalamine + LGG (62)	No difference in relapse rates at 12 mo. LGG more effective than mesalamine at prolonging relapse-free time ($P < 0.05$)
Shanahan 2006 ^[117]	DB, R, C	Lactobacillus salivarius or Bifidobacterium infantus (1×10^9 cfu) (52/group)	Placebo (53)	No improvement of time to relapse
Venturi 1999 ^[84]	Open-label	VSL#3 (1×10^{12} cfu) (20)	None	75% maintained clinical and endoscopic remission
Induction of remission of Crohn's disease				
Schultz 2004 ^[97]	DB, R, C	Lactobacillus GG (2×10^9 cfu) (5)	Placebo (6)	No difference in remission rates
McCarthy 2001 ^[118]	Open-label	Lactobacillus salivarius (1×10^{10} cfu) (25)	None	Reduced disease activity compared with baseline
Gupta 2000 ^[119]	Open-label	Lactobacillus GG (2×10^{10} cfu) (4)	None	Improvement in CDAI scores compared with baseline ($P < 0.05$)
Maintenance of remission of Crohn's disease				
Prantera 2002 ^[95]	DB, R, C	Lactobacillus GG (1.2×10^{10} cfu) (23)	Placebo (22)	No significant difference in remission
Campieri 2000 ^[120]	R, C	VSL#3 (3×10^{11} cfu) (20)	Mesalamine (20)	Equivalent to mesalamine in preventing recurrence
Marteau 2006 ^[94]	DB, R, C	Lactobacillus johnsonii LA1 (2×10^9 cfu) (48)	Placebo (50)	No difference in endoscopic recurrence
Malchow 1997 ^[93]	DB, R, C	<i>E. coli</i> Nissle 1917 (5×10^{10} cfu) (16)	Placebo (12)	No difference in remission rates
Bousvaros 2005 ^[96]	DB, R, C	Lactobacillus GG (2×10^{10} cfu) (39)	Placebo (36)	No difference in time to relapse
Guslandi 2000 ^[91]	R, C 6 mo	Saccharomyces boulardii (1 g/d) + mesalamine (2g) (16)	Mesalamine (16)	Significant prolongation of remission ($P < 0.05$)
Induction of remission of pouchitis				
Kuisma 2003 ^[80]	DB, R, C	Lactobacillus GG (1×10^{10} cfu) (10)	Placebo (10)	No difference in PDAI
Laake 2004 ^[121]	Open-label	Lactobacillus acidophilus and Bifidobacterium lactis-fermented milk (500 mL) (51)	None	Improved PDAI, no difference in histology
Gionchetti 2000 ^[78]	DB, R, C	VSL#3 (6 g) (20)	Placebo (20)	Increased remission time ($P < 0.001$)
Mimura 2004 ^[122]	DB, R, C	VSL#3 (6 g) (20)	Placebo (16)	Increased remission time ($P < 0.0001$)
Gionchetti 2003 ^[79]	DB, R, C	VSL#3 (1×10^{11}) (20)	Placebo (20)	Increased remission time ($P < 0.05$)

DB: Double-blind; R: Randomized; C: Controlled; UCDAI: Ulcerative colitis disease activity index; CDAI: Crohn's disease activity index; LGG: Lactobacillus GG; PDAI: Pouchitis disease activity index.

probiotic strains are also capable of converting linoleic acid to conjugated linoleic acid, a fatty acid with anti-inflammatory and anti-carcinogenic properties^[61].

Use of probiotics in inflammatory bowel disease treatment

Results from various animal studies and clinical trials using probiotics to treat intestinal inflammation have generated considerable excitement. Data are now emerging which suggest that probiotics are capable of preventing relapse of chronic intestinal inflammation. Some probiotics can even treat mild to moderately active IBD^[62,63]. However,

at present there is a relative lack of rigorously designed, randomized, placebo-controlled trials. Level 1 evidence is only available for the use of probiotics in post-operative chronic pouchitis while level 2 and 3 evidence supports the use of probiotics in treatment of CD and UC^[62] (Table 1).

Experimental colitis

More than 20 animal models of IBD are available^[64] and have been widely used to study the efficacy and mechanisms of probiotics in ameliorating inflammation in order to provide support for human clinical trials. In

IL-10 knockout mice, *L. plantarum* 299v^[65], *L. reuteri*^[46], *L. salivarius* subspecies *salivarius* 433118, *B. infantis* 35624^[66], *L. salivarius* subspecies *salivarius* UCC118^[67] and VSL#3^[49] have all been shown to successfully attenuate intestinal inflammation. *L. GG* prevents recurrent colitis in HLA-B27 transgenic rats after antibiotic treatment, whereas *L. plantarum* has no effect^[68]. Both VSL#3 and *L. GG* significantly ameliorate sulfhydryl-blocker iodoacetamide-induced colitis in rats, whereas they have no effect on dinitrobenzene sulfonic acid-induced colitis^[69]. Improved inflammation in 2,4,6-trinitrobenzene sulfonic acid (TNBS)-induced colitis has also been demonstrated after oral administration of *L. salivarius* ssp. *salivarius* CECT5713 and *L. plantarum* NCIMB8826^[70,71]. Dextran sulphate sodium (DSS)-induced colitis in mice is ameliorated by soluble bacterial antigens extracted from *E. coli* (strain *Laves*) or by *Bifidobacterium* strains *breve*, *catenulatum*, and *longum*^[72,73]. Daily administration of live but not heat-killed auto-aggregating *L. crispatus* reduces the severity of DSS-colitis in mice^[74]. *L. reuteri* significantly reduces the colonic inflammation caused by both acetic acid and methotrexate in rats^[75,76]. Interestingly, DNA from VSL#3 has been reported to reduce colonic inflammation, thus improving intestinal barrier function in IL-10 KO mice and DSS-induced colitis^[41,77].

Chronic pouchitis

Probiotics can maintain antibiotic-induced remission in patients with chronic pouchitis after colectomy for refractory UC. Gionchetti *et al.*^[78] have completed placebo-controlled trials using the probiotic cocktail VSL#3 in patients with chronic relapsing pouchitis, showing that the remission rate after 1 year is 90% in the group treated with VSL#3 versus 60% in the placebo group. Another study by the same group also showed that VSL#3 is also capable of preventing the development of chronic pouchitis during the first year after pouch surgery for UC^[79]. Ten percent of patients who took VSL#3 developed pouchitis, compared with 40% of the placebo group. A double-blinded, prospective, randomized placebo-controlled trial was carried out in 20 patients treated with *L. GG* versus placebo for 3-mo^[80]. In contrast to the study with the probiotic cocktail VSL#3, no significant differences were observed in chronic pouchitis disease activity in *L. GG*-treated patients despite an increased total fecal lactobacilli: anaerobe ratio, illustrating the inefficacy of *L. GG* for this condition.

Ulcerative colitis

Numerous studies have investigated the use of probiotics for maintenance of remission of UC in humans. A small study investigating the use of non-pathogenic *E. coli* Nissle 1917 versus low-dose mesalamine showed that it can maintain remission of quiescent UC, with a relapse rate of 16%-67% in those treated with *E. coli* versus 11%-73% in the mesalamine group^[81]. In a double-blind, randomized trial involving 327 patients, Kruis *et al.*^[82] compared the effectiveness of an oral preparation of *E. coli* Nissle 1917 with mesalamine for maintaining remission of UC, and found that at the end of the 12-mo study, there is no significant difference between the two study groups, with

relapses occurring in 36.4% of the *E. coli* Nissle 1917 group and 33.9% of the mesalamine group (significant equivalence, $P = 0.003$). Recently, Zocco *et al.*^[83] investigated the efficacy of *L. GG* in maintaining remission of UC and found that there is no difference in relapse rate between the 3 groups after 6 and 12 mo. However, *L. GG* is more effective than standard mesalamine treatment in prolonging relapse-free time ($P < 0.05$). An open-label study of VSL#3 showed that 15/20 UC patients remain in remission after 1 year^[84].

Several studies have addressed treatment of established UC with probiotic therapy. A study using *E. coli* Nissle 1917 has demonstrated its equivalence to mesalamine for inducing remission of UC^[85]. Ishikawa *et al.*^[86] evaluated *Bifidobacterium*-fermented milk in the treatment of UC, and symptoms of exacerbation were observed in 3 of 11 patients in the treated group versus 9 of 10 patients in the untreated group after 1 year ($P = 0.01$). However, no difference was observed in endoscopic disease activity. A placebo-controlled trial with bifidobacteria-fermented milk for 12 wk in UC patients with active disease showed that endoscopic disease activity index and histological score are significantly reduced in the treatment group compared with those in placebo group^[87]. Uncontrolled administration of daily VSL#3 for 3 mo induces remission in 19 of 30 patients (63%), with a response rate of 87%^[88]. As in all of these reported studies, the increased luminal probiotic bacteria return to baseline levels within one month after stopping probiotic treatment, indicating only transient colonization by these probiotic bacteria. In another small study administration of a combination of 3 bifidobacterium species for 2 mo was superior (20% remission) to placebo (93% remission) in maintaining remission of UC induced by sulfasalazine and glucocorticoids^[89]. This effect correlates with decreased mucosal TNF- α , IL-1 β and increased mucosal IL-10 levels^[89]. An interesting study by Borody *et al.*^[90] showed that altering the gut microflora in UC patients achieved dramatic outcomes by administration of a freshly prepared enema from a healthy donor to six patients with relapsing refractory UC after broad spectrum antibiotics. This results in a remarkable reversal of all symptoms after 4 mo and sustained remission after 1-3 year of treatment.

Crohn's disease

Thus far, the use of probiotics for the prevention and treatment of CD is less substantiated than for the prevention and treatment of UC, although some studies certainly show promise. The effect of probiotics in maintaining remission of CD has been reported in an open-label study in patients receiving mesalamine alone versus mesalamine and *S. boulardii*^[91]. At 6 mo, 37.5% of patients had a clinical relapse in the former group versus 6.3% of patients in the probiotic group. In an open-labeled study, McCarthy *et al.*^[92] reported that oral administration of *L. salivarius* UCC118 significantly reduces disease activity in patients with mild to moderate CD. In a randomized, placebo-controlled pilot study, patients with CD were treated with steroids and randomized to non-

Table 2 Clinical studies of prebiotics in IBD

Author	Design	Group (Dose/d) (n)		Results
		Prebiotic	Comparator	
Induction of remission of ulcerative colitis				
Kanauchi 2003 ^[112]	Open label	Conventional therapy + Germinated barley foodstuff (20-30 g) (21)	Conventional therapy	Improved UCDAI
Furrie 2005 ^[114]	DB, R, C	<i>B. longum</i> (2×10^{11}) + inulin/oligofructose (6 g) (9)	Placebo (9)	Sigmoidoscopy scores reduced ($P = 0.06$) β -defensins, TNF α , IL-1 α levels decreased ($P < 0.05$)
Maintenance of remission of ulcerative colitis				
Hanai 2004 ^[113]	Open label	Conventional therapy + Germinated barley foodstuff (20 g) (22)	Conventional therapy (37)	Improved UCDAI and endoscopic scores
Induction of remission of Crohn's disease				
Lindsay 2006 ^[115]	Open label	Fructo-oligosaccharides (15 g) (10)	None	Increase in IL-10 expressing intestinal dendritic cells

pathogenic *E. coli* Nissle 1917 as probiotic therapy or placebo^[93]. After 1 year, there were fewer relapses in the probiotic group, but this was not statistically significant. Despite these promising studies, there are numerous reports on the inefficacy of some probiotics in CD. A randomized, placebo-controlled study of 98 patients showed that *L. johnsonii* LA1 is ineffective in preventing postoperative recurrence of CD^[94]. A placebo-controlled trial with *L. GG* is also ineffective in preventing postoperative recurrence of CD in patients undergoing bowel resection^[95]. Other studies have failed to detect benefits of *L. GG* in maintaining remission of CD in children^[96] and adults^[97,98]. Although *L. GG* has demonstrated its efficacy in treating rotaviral^[99] and antibiotic-associated^[100] diarrhea, results in IBD patients have been particularly underwhelming for this bacterial species, highlighting the species-specificity of colitis protection by probiotics.

PREBIOTICS IN INFLAMMATORY BOWEL DISEASE

Experimental colitis

Studies using prebiotics have been performed mostly in animal models. Lactulose and inulin have been shown to attenuate inflammation in IL-10 knockout mice and DSS-induced colitis respectively^[46,101]. The combination of inulin and oligofructose (mixture 1:1) is also effective in preventing the development of colitis in HLA-B27 transgenic rats^[102]. This beneficial effect is observed in conjunction with an increase of intestinal bifidobacteria and lactobacilli. Another study in HLA-B27 transgenic rats showed that the effects of the synbiotic "SIM", a combination of lactobacilli, bifidobacteria and the prebiotic inulin, are attributed to the inulin rather than the probiotics. The ingested probiotic bacteria are not detectable in the cecal content, yet the microflora profile of their cecal contents is altered^[103]. In that study, inulin was also shown to specifically stimulate the growth of *Bifidobacterium animalis*. DSS-induced colitis rats fed with goat's milk oligosaccharides maintain their body weight, have reduced colonic myeloperoxidase activity and clinical

symptoms and increased MUC-3 expression compared with control rats^[104]. Goat's milk oligosaccharides also causes decreased anorexia, weight loss, bowel wall thickening and necrotic lesions in TNBS-induced colitis in rats, compared with untreated controls^[105]. In another study of TNBS-induced colitis in rats, a 2-wk feeding of lactulose prior to the induction of colitis reduces myeloperoxidase activity, colonic TNF α and leukotriene B production, in conjunction with an increase of lactobacilli and bifidobacteria species in feces^[106]. Lactulose has also demonstrated a dose-dependent beneficial effect on DSS-induced colitis in rats, including improvements of colonic ulceration areas, body weight changes, diarrhea, bloody stools and a reduction of myeloperoxidase activity and microscopic colitis^[107].

However, not all studies using prebiotics have resulted in positive outcomes. Moreau *et al*^[108] found that fructo-oligosaccharides are ineffective in improving DSS-induced colitis in rats. Holma *et al*^[109] have reported a similar inefficacy of galacto-oligosaccharides in TNBS-colitis rats.

Several studies have investigated the use of an insoluble mixture of glutamine-rich protein and hemicellulose-rich dietary fiber termed germinated barley foodstuff (GBF). Fukuda *et al*^[110] found that feeding GBF to rats with DSS-induced colitis results in significantly reduced colonic inflammation scores, and increased butyrate concentrations in cecal contents. Kanauchi *et al*^[111] have observed similar results in DSS-colitis rats, and further determined that the dietary fiber component rather than the protein component of the GBF is responsible for the beneficial effects of GBF.

Ulcerative colitis

Table 2 lists the clinical trials using prebiotics to treat IBD. Although there is a paucity of human studies using prebiotics, the few emerging studies showed that there is potential for this treatment modality. A multi-centered open-label trial reported that oral administration of GBF to patients with mild to moderately active UC for 24 wk results in a significant decrease in clinical activity index, compared to controls^[112]. An open-label study of 22 UC

patients in remission showed that a daily oral intake of 20 g GBF results in a significantly improved clinical activity index and endoscopic score at 3, 6 and 12 mo, and a reduced relapse rate, compared with controls^[113]. A recent randomized, double-blinded controlled trial by Furrir *et al.*^[114] examined the use of synbiotics in 18 patients with active UC, using a combination therapy of *B. longum*, inulin and oligofructose, and found that sigmoidoscopy inflammation scores are reduced in the synbiotic-treated population when compared to placebo. Intestinal levels of TNF- α and IL-1 β are also reduced. Additionally, rectal biopsies have demonstrated reduced inflammation and greater epithelial regeneration in the synbiotic-treatment group.

Crohn's disease

A small, uncontrolled study of 15 active CD patients reported that 21 d of fructo-oligosaccharide (15 g) intake results in a significant decrease of disease activity, an increase of intestinal bifidobacteria and modifications of Toll-like receptors and IL-10 expression in mucosal dendritic cells^[115].

CONCLUSION

The link between intestinal microflora and IBD is now well established, and altering the composition of the microflora using probiotics and prebiotics holds promise as a therapeutic strategy for ameliorating chronic intestinal inflammation. Future developments in this field must include rigorous double-blind, placebo-controlled trials, using probiotics and prebiotics along with a further understanding of their protective mechanisms. Due to their excellent safety profile and lack of serious side effects, there are few contraindications to the consumption of prebiotics, probiotics and synbiotics by IBD patients. Further understanding of the interactions between microbes and gastrointestinal tract will help identify which strains of bacteria and/or which prebiotics may be effective in the treatment of different types of chronic inflammatory disease.

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