



# Probiotic Effects on Inflammatory Bowel Disease<sup>1,2</sup>

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## Abstract

Components of the commensal flora, including Bifidobacteria and Lactobacilli, have been associated with beneficial effects on the host. These beneficial effects include maintenance of intestinal homeostasis, competitive exclusion of pathogens, production of antimicrobial compounds, promotion of gut barrier function, and immune modulation. Probiotics currently can be administered in dairy yogurts and drinks and also in the form of sachets or capsules. Although preliminary studies are clearly promising, placebo-controlled, randomized, double-blind clinical trials are required to clarify the role of probiotic bacteria in the treatment of inflammatory bowel disease. The choice of probiotic bacteria, the optimal dose, mode of administration, and duration of therapy still need to be established. Detailed strain characterization is also required for all potential probiotic strains. As evidence accumulates to suggest a breakdown in tolerance toward ubiquitous intestinal bacteria, it appears logical to intervene by modulating the enteric flora. Increasingly, research suggests that probiotics may offer an alternative or adjuvant approach to conventional therapy by altering the intestinal microflora and modulating the host immune system. *J. Nutr.* 137: 819S–824S, 2007.

Despite the passage of a century since Metchnikoff observed that consumption of certain bacteria improves intestinal health, it is only in recent years that the scientific basis of these claims has been investigated. This renewed interest in the enteric microflora and in host-microbe interactions has resulted from a number of factors, including the need to accurately identify the composition of the microflora in health and disease, to understand the impact of their metabolic activity on the host, and to study the detrimental effect of a breakdown in immune system tolerance to enteric flora in the pathogenesis of inflammatory diseases such as inflammatory bowel disease (IBD).<sup>3</sup> In addition, the mechanisms underlying the interaction of the flora and the epithelium

and their role in the development and function of the gastrointestinal tract require further investigation.

Examination of the interactions between bacteria and their hosts has focused primarily on pathogens because of their clinical significance. However, the mammalian intestine contains a diverse array (>400 different species) of nonpathogenic bacteria numbering up to  $10^{11}$  cells per gram of intestinal content (1). The intestinal microbiota is an active metabolic entity that produces products for the host, such as vitamins K and B-12. The presence of this complex ecosystem also provides protection from pathogen invasion through competition for nutrients and epithelial binding sites (termed colonization resistance). In addition, colonization with bacteria is critical for the normal structural and functional development and optimal function of the mucosal immune system (2). Studies in mice have shown that the composition of intestinal intraepithelial lymphocytes may be markedly altered by gut microbial colonization. The B-cell follicles of Peyer's patches are quiescent in germ-free adult mice. Germinal centers, including sIgA<sup>+</sup> blasts, appear in the B follicles of formerly germ-free adult mice ~10–14 d after monoassociation with various gut commensal bacteria. Moreover, induction and/or maintenance of oral tolerance to ingested antigens also requires microbial colonization of the gastrointestinal tract in early life. Therefore, mucosal immune responses to the indigenous flora require precise control and an immunosensory capacity for distinguishing commensals from pathogens. However, unrestrained mucosal immune activation in response to bacterial signals from the lumen is a risk factor for development of IBD. This was clearly demonstrated in spontaneous murine models of colitis, such as the IL-10 knockout model, which remain disease-free when housed under germ-free conditions (3–6).

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<sup>3</sup> Abbreviations used: CD, Crohn's disease; DGGE, denaturing gradient gel electrophoresis; IBD, inflammatory bowel disease; UC, ulcerative colitis.

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Not all members of the gastrointestinal flora should be considered proinflammatory in nature. On the contrary, deliberate administration of certain commensal organisms (e.g., *Lactobacillus* and *Bifidobacterium* species) to murine models attenuates the development of colitis (7–11). In addition, human clinical trials have demonstrated efficacy in the maintenance of remission of chronic pouchitis (12–14). The mechanisms underpinning such effects are complex and not well understood. Proposed mechanisms include the production of lactic acid, bacteriocins, and other antimicrobials, competitive exclusion of pathogens from epithelial surfaces, alteration of mucosal permeability, and induction of various host immune responses.

### Probiotics

Probiotics are defined as living food supplements or components of bacteria that have been shown to have beneficial effects on human health (15). A probiotic bacterium is required to fulfill certain criteria to be of benefit. These include being of human origin and having generally regarded as safe (GRAS) status, acid and bile stability, adherence to intestinal cells, persistence for some time in the gut, ability to produce antimicrobial substances, antagonism against pathogenic bacteria, and ability to modulate the immune response (16). Probiotic activity has been associated with Lactobacilli, Bifidobacteria, *Streptococcus*, Enterococcus, nonpathogenic *E. coli*, and *Saccharomyces boulardii* (17).

A number of beneficial effects have been attributed to the consumption of probiotic products. These include alleviation of lactose intolerance, protection from or decreased duration of gastrointestinal infections, suppression of cancer, reduction in plasma cholesterol concentrations and consequently in coronary heart disease, and improved digestion and nutritional value of foods. Multiple mechanisms have been proposed to account for probiotic action in different clinical conditions. In the context of host defense against infection, probiotic mechanisms may include competitive metabolic interactions, the production of antimicrobials, and inhibition of adherence or translocation of pathogens. In the context of IBD, antiinflammatory bacteria may signal with the gastrointestinal epithelium and perhaps mucosal regulatory T cells or dendritic cells (18). The probiotic cocktail VSL#3 has been demonstrated to induce dendritic cell secretion of IL-10 while attenuating T-cell production of IFN- $\gamma$  (19). Probiotic effects on epithelial cell function have been demonstrated *in vitro* and *in vivo* (20). Stabilization of the gut barrier by probiotics may be important for therapeutic efficacy in IBD. Similarly, the exchange of regulatory signals between the commensal flora and the epithelial and subepithelial components of the mucosa may also be involved. Indeed, certain nonpathogenic organisms have been shown to counterbalance epithelial responses to invasive bacteria via the regulation of cytokine transcription factors (21).

### Significance of the enteric flora in IBD

Crohn's disease (CD) and ulcerative colitis (UC), collectively referred to as IBD, are chronic aggressive disorders with a prevalence of 0.1–0.5%. The 2 disorders have distinct features. UC is characterized by inflammation with superficial ulcerations limited to the mucosa of the colon. Inflammation normally starts in the rectum and continuously spreads throughout the large intestine. CD, however, is characterized by a discontinuous pattern, potentially affecting the entire gastrointestinal tract. In contrast to UC, inflammation in CD patients is transmural with large ulcerations, and occasionally granulomas are observed.

Genetic factors, immune system responsiveness, and environmental factors (such as the composition and metabolic ac-

tivity of the gut flora) are all believed to play a role in the progression of these inflammatory states. Clinical observations suggest that certain intestinal and extraintestinal bacterial infections sometimes precede or reactivate chronic intestinal inflammation. A number of microbial agents are implicated as initiating factors in the pathogenesis of IBD. These include *Mycobacterium paratuberculosis*, measles virus, *Listeria monocytogenes*, and adherent *E. coli*. However, results implicating any single microorganism in the etiology of IBD are controversial. Pathogens may drive intestinal inflammation in susceptible individuals via disruption of the mucosal barrier (allowing increased uptake of luminal antigens), mimicry of self-antigens, and activation of the mucosal immune system via modulation of transcription factors such as NF- $\kappa$ B.

Enhanced mucosal permeability is thought to play a pivotal role in the maintenance of a chronic inflammatory state. This may be a primary defect (i.e., genetically predisposed) or a secondary event either as a result of direct contact with pathogenic bacteria or as a consequence of intestinal inflammation. A defective epithelial barrier could result in a loss of tolerance to non-pathogenic resident enteric bacteria. Once these bacterial products have gained access to the submucosa, they can drive a variety of proinflammatory signaling pathways, further perpetuating the inflammatory state.

In recent decades abundant data have been generated from *in vitro* studies, experiments using animal models of intestinal inflammation, and clinical trials suggesting a critical role for normal luminal bacteria in the pathogenesis of IBD (18). The distribution of the lesions in these conditions is greatest in areas with the highest numbers of luminal bacteria. The continuity of the fecal stream has also been linked with disease activity; interruption of the fecal stream has been associated with clinical improvement, but relapse is predictable following surgical restoration. Lesions of CD may be induced by direct instillation of fecal contents into apparently unaffected loops of bowel in susceptible individuals (22,23). There is also persuasive evidence for loss of immunological tolerance to components of the commensal flora in patients with IBD, and this is reflected in their serology and cellular immune reactivity to enteric flora antigens (24,25).

The most compelling evidence for the interactive role of genes, bacteria, and immunity has been derived from experimental animal models of both Crohn's-like and colitis-like disease (26–28). There are >20 different spontaneously occurring or genetically engineered (KO or transgenic) animal models of IBD (28–30). Colonization with an enteric flora is required for full expression of disease. Thus, the normal flora is a common factor driving the inflammatory process irrespective of the genetic underlying predisposition and immunological effector mechanism. Although the composition of the flora may be important in IBD, the functional activity of the bacteria may also be of critical importance and should not be overlooked.

The manner in which the enteric immune system recognizes bacterial antigens also plays a role in disease susceptibility. Polymorphisms in the *NOD2* gene are associated with susceptibility to CD (31–33). Although the exact role of this mutation in CD remains unclear, *NOD2* mutations may lead to ineffective immune response to bacterial components and ineffective clearance of intracellular bacteria in human intestinal epithelia (34,35).

Additional susceptibility genes are currently being characterized (36,37).

Because there is abundant evidence for the role of luminal flora in the pathogenesis of IBD, the alteration of the microflora

through the introduction of probiotic bacteria could theoretically result in some clinical improvement. The modulation of cytokine expression and stabilization of the mucosal barrier by probiotics could promote disease resolution. Conventional drug therapy for IBD involves suppression of the immune system or modulation of the inflammatory response. Chronic antibiotic use is associated with negative side effects and the risk of bacterial resistance. Probiotics offer an alternative by altering the intestinal flora and modulating the immune response without the risk of side effects associated with conventional therapy.

### Probiotic therapy in animal models of IBD

A number of reports have been published that describe the influence of probiotic consumption on colitis in animal trials (Table 1). In particular, the IL-10 knockout mouse has been extensively studied.

IL-10 knockout mice develop colitis when colonized with a conventional flora but remain disease-free when maintained under germ-free conditions. Schultz et al. colonized IL-10<sup>-/-</sup> mice with *L. plantarum* 299v 2 wk before transfer from a germ-free environment to a specific pathogen-free environment (10). This resulted in significant attenuation of disease and a significant reduction in mesenteric lymph node IL-12 and IFN- $\gamma$  production. Madsen et al. demonstrated a role for *Lactobacillus reuteri* in prevention of colitis in IL-10<sup>-/-</sup> mice. Neonatal mice were shown to have a decreased concentration of colonic *Lactobacillus* species and an increased concentration of mucosal adherent bacteria. Oral administration of the prebiotic lactulose (shown to increase the levels of *Lactobacillus* species) and rectal swabbing with *L. reuteri* restored *Lactobacillus* levels to normal and reduced the number of adherent bacteria within the colon. These effects were associated with the attenuation of colitis (7). In a placebo-controlled trial, orally administered *Lactobacillus salivarius* UCC118 was shown to reduce the incidence of colon cancer and the severity of mucosal inflammation in IL-10<sup>-/-</sup>

mice. *L. salivarius* was also shown to modify the intestinal microflora in these animals as *C. perfringens*, coliforms, and enterococcus levels were significantly reduced in the probiotic-fed group (9). In another placebo-controlled trial the efficacy of *L. salivarius* UCC118 and *Bifidobacterium infantis* 35624 in attenuation of colitis in the IL-10<sup>-/-</sup> mouse model was demonstrated. Attenuation of disease activity was associated with modulation of the gut microflora as investigated by culture-independent 16S RNA denaturing gradient gel electrophoresis (DGGE). In addition, mucosal proinflammatory cytokine levels were significantly reduced (8). Further studies examined the effect of *B. infantis* 35624 on early inflammation in IL-10<sup>-/-</sup> mice and wild-type mice of the same genetic background. Pronounced changes occurred in the Peyer's patch following probiotic consumption, with IFN- $\gamma$  reduced in both wild-type and IL-10<sup>-/-</sup> mice. Interestingly, following in vitro stimulation with a pathogen, IFN- $\gamma$  was enhanced in wild-type mice but reduced in IL-10<sup>-/-</sup> mice (38).

The oral route of administration may not be required for certain probiotic effects. Reduced inflammatory scores and reduced production of proinflammatory cytokines have been observed in IL-10<sup>-/-</sup> mice that had been injected subcutaneously with *L. salivarius* UCC118 (39).

In order to enhance the probiotic effect in these murine models, investigators have combined probiotic treatment with prebiotics or antibiotics, or they have genetically engineered the probiotic strain to secrete antiinflammatory mediators. The prebiotic inulin and a combination of the probiotic organisms *L. acidophilus* La-5, *L. delbrückii* subsp. *bulgaricus*, *Bifidobacterium* Bb-12, and *Streptococcus thermophilus* significantly reduced inflammation in HLA-B27. The effect was enhanced by combination with metronidazole, suggesting a synergistic effect of the combination of anti- and probiotics in the treatment of experimental colitis (40). Genetically modified probiotics have been tested for their ability to attenuate colitis in the IL-10 knockout model and the DSS model. (41) *Lactococcus lactis* was

**TABLE 1** Probiotic efficacy in animal models

Probiotic microorganism	Type of study	Trial outcome	Reference
<i>Lactobacillus reuteri</i>	IL-10 <sup>-/-</sup> mice. N = 4–8 per group. Placebo-controlled trial	Probiotic lactulose and probiotic <i>L. reuteri</i> attenuated colitis and improved mucosal barrier function.	Madsen et al. (7)
<i>Lactobacillus salivarius</i> UCC118	IL-10 <sup>-/-</sup> mice. N = 10 per group. Placebo controlled	Reduced incidence of colon cancer and mucosal inflammation. Modulation of fecal flora.	O'Mahony et al. (9)
<i>Lactobacillus salivarius</i> UCC118 and <i>Bifidobacterium infantis</i> 35624	IL-10 <sup>-/-</sup> mice. N = 10 per group. Placebo controlled	Attenuation of disease. Modulation of gut microflora. Reduction in in vitro production of IFN- $\gamma$ , TNF- $\alpha$ , and IL-12. TGF- $\beta$ levels maintained.	McCarthy et al. (10)
<i>Lactobacillus salivarius</i> UCC118	IL-10 <sup>-/-</sup> mice. CIA model N = 10 per group. Placebo controlled	Attenuation of colitis and arthritis following subcutaneous administration of probiotic. Associated with reduction in proinflammatory cytokines	Sheil et al. (39)
<i>Bifidobacterium infantis</i> 35624	IL-10 <sup>-/-</sup> and wild-type N = 25 per group. Placebo controlled	Modulation of inflammatory cytokine response in the Peyer's patch of all mice. Differential response following in vitro stimulation.	Sheil et al. (38)
<i>Lactobacillus plantarum</i> 299v	IL-10 <sup>-/-</sup> mice. Placebo controlled	Attenuation of colitis. Reduction in IL-12 and IFN- $\gamma$ produced by stimulated mesenteric lymph node cells.	Schultz et al. (10)
<i>Lactobacillus rhamnosus</i> GG	HLA-B27 transgenic rats.	Prevented recurrence of colitis.	Dieleman et al. (11)
Combination of <i>L. acidophilus</i> La-5, <i>L. delbrückii</i> subsp. <i>bulgaricus</i> , <i>Bifidobacterium</i> Bb-12, and <i>Streptococcus thermophilus</i> .	HLA-B27 transgenic rats.	Attenuated colitis following treatment with the prebiotic inulin and a combination of probiotic organisms.	Schultz et al. (40)
<i>E. coli</i> strain Nissle 1917	IL-10 <sup>-/-</sup> and DSS colitis	Attenuation of colitis. Heat-inactivated and DNA had antiinflammatory effect in DSS colitis.	Kamada et al. (45)

engineered to secrete biologically active IL-10. A significant reduction in inflammation was observed in both murine models. The investigators concluded that genetically engineered bacteria for local administration of a therapeutic agent, such as IL-10, are successful in the treatment and prevention of colitis and have potential for human use and application to other forms of therapy.

Isolated components of the probiotic cell may also have some therapeutic benefit. Bacterial DNA has been shown to have potent immunostimulatory effects. In 1 trial by Rachmilewitz et al. (42), bacterial DNA was used to attenuate colitis in a number of murine models, both experimental and spontaneous (42). In addition, they demonstrated that attenuation of DSS colitis was caused by VSL#3 DNA mediated through Toll-like receptor 9 signaling, and nonviable bacteria were equally effective in reducing inflammation in this model (43). Heat-inactivated *E. coli* strain Nissle 1917 and its isolated DNA were also administered in the DSS model, and an antiinflammatory effect was demonstrated (44).

Interestingly, specific immunostimulatory DNA sequences have also been shown to attenuate the production of proinflammatory cytokines which are elevated in the mucosa of UC patients, suggesting that the animal model data may be applicable to human disease states such as IBD (45).

### Probiotic trials in IBD patients

Although it is unclear whether the abnormal composition of the enteric flora contribute to the pathogenesis of IBD, evidence from animal models and clinical observations (e.g., antibiotics are effective in certain patients) have prompted the examination of a wide variety of probiotic strains in the treatment of IBD (Table 2).

A number of published studies have examined the efficacy of *Lactobacillus casei* strain GG in the treatment of IBD. Malin et al. reported that in pediatric CD, consumption of *Lactobacillus* GG was associated with increased gut IgA levels, which could promote the gut immunological barrier (46). Gupta et al.

reported improved clinical scores and improved intestinal permeability in an open-labeled pilot study with a small number of pediatric CD patients (47). In an open-labeled study patients with pouchitis were treated with *Lactobacillus* GG and fructooligosaccharide. Patients reported a beneficial effect when the probiotic-prebiotic mix was administered as an adjuvant to antibiotic therapy. Remission was induced as documented by suppression of symptom scores (48).

Kruis et al. reported in a randomized, double-blind clinical trial with 120 UC patients that oral administration of *E. coli* strain Nissle 1917 as a maintenance treatment of remission showed no difference in relapse rates compared with patients on mesalazine. Relapse rates were 11.3% for the mesalazine-treated group and 16.0% for the *E. coli* group. Life table analysis showed a relapse free time of  $103 \pm 4$  d for mesalazine and  $106 \pm 5$  d for *E. coli*. From the results of this preliminary study, probiotic treatment appears to offer another option for maintenance therapy of UC (49). These results were confirmed by Rembacken et al. (50). A total of 116 patients with active UC were recruited into this study, and 75% and 68% of the mesalazine and *E. coli* groups achieved remission, respectively. The relapse rate in both groups was markedly higher than the investigators anticipated, 73% for the mesalazine group and 67% for the *E. coli* group. The time to relapse was not significantly different between the groups. In a large (327 patients) multicenter, randomized, double-blind, remission maintenance study, *E. coli* was shown to be as effective as mesalazine in maintaining remission and therefore offers an alternative to mesalazine in maintenance of remission in UC patients (51).

VSL#3, a mixture of 4 lactobacilli strains (*Lactobacillus plantarum*, *Lactobacillus casei*, *Lactobacillus acidophilus*, *Lactobacillus delbrueckii* ssp. *bulgaricus*), 3 bifidobacteria strains (*Bifidobacterium infantis*, *Bifidobacterium breve*, *Bifidobacterium longum*), and 1 strain of *Streptococcus salivarius* ssp. *thermophilus*, has been examined in UC, CD, and pouchitis patients. Gionchetti et al. (12) demonstrated the efficacy of this probiotic mix in maintenance of remission in patients with

**TABLE 2** Probiotic therapy in IBD

Probiotic microorganism	Type of study	Trial outcome	Reference
<i>Lactobacillus</i> GG	Human trial. Open study. <i>N</i> = 14.	Increase in gut IgA response.	Malin et al. (46)
	Human trial. <i>N</i> = 4. Open trial.	Improved intestinal permeability and CDAL.	Gupta et al. (47)
	Human trial. Open-labeled study. <i>N</i> = 10 per group.	Probiotic and prebiotic fructooligosaccharide induced remission in pouchitis trial when administered as adjuvant to antibiotic.	Friedman et al. (48)
<i>E. coli</i> strain Nissle 1917	Randomized, double-blind human trial. <i>N</i> = 120	Patients with active UC demonstrated similar relapse rates compared with patients on mesalazine.	Kruis et al. (49)
	Randomized, double-blind human trial. <i>N</i> = 116	Confirmed result from Kruis et al. (49)	Rembacken et al. (50)
	Randomized, double-blind human trial. <i>N</i> = 327	Remission maintained in patients receiving probiotic.	Kruis et al. (51)
	Randomized, double-blind human trial. <i>N</i> = 28	Remission maintained in patients receiving probiotic with steroids compared with steroids and placebo.	Malchow et al. (55)
VSL#3	Randomized double-blind placebo-controlled human trial. <i>N</i> = 40	Maintenance of remission in chronic pouchitis.	Gionchetti et al. (12)
	Open trial. <i>N</i> = 20.	15% relapse compared with 100% in control group.	
	Randomized double-blind placebo-controlled human trial. <i>N</i> = 40	Maintenance of remission in UC patients.	Venturi et al. (13)
<i>Saccharomyces boulardii</i>	Human trial. Double-blind study. <i>N</i> = 20	Patients with UC had 20% remission when given rifaximin and VSL#3 compared with 40% in mesalazine treated group.	Campieri et al. (14)
		Reduced frequency of bowel movements in UC patients.	Pein and Holz (56)
		Relapse observed in 6.25% UC patients receiving probiotic plus mesalazine compared with 37.5% on mesalazine alone.	Guslandi et al. (54)

chronic pouchitis. In a randomized, double-blind, placebo-controlled trial, 40 patients in clinical and endoscopic remission received VSL#3 or placebo for 9 mo. All patients received 1 mo of antibiotic treatment before the trial. At the end of the study 3 patients (15%) had relapsed in the VSL#3 group compared with 20 (100%) in the placebo group. The effect of VSL#3 on maintenance of remission in UC patients was evaluated using an open-label design. Twenty patients in remission were treated for 12 mo. At the end of the trial 15 of 20 patients (75%) remained in remission (13). In a double-blind, randomized study the efficacy of VSL#3 combined with antibiotic treatment on the postoperative recurrence of CD was compared with treatment with mesalamine alone. Forty patients were randomized to receive rifaximin for 3 mo followed by VSL#3 for 9 mo or mesalamine for 12 mo. At the end of the trial 20% of the patients had recurrent CD in the probiotic/antibiotic group, whereas 40% of patients in the mesalamine group relapsed (14).

The yeast *Saccharomyces boulardii* has been successfully used in the prevention of antibiotic-associated diarrhea (52,53) and in the treatment of other types of diarrhea. In a randomized double-blind trial with 32 CD patients in clinical remission receiving either mesalamine or mesalamine plus *S. boulardii*, clinical relapse was observed in 6.25% of patients receiving mesalamine plus *S. boulardii* vs. 37.5% in the mesalamine alone group (54).

Although the trials summarized above are promising, the current consensus is that a number of larger controlled trials are necessary before the use of probiotics as a routine medical treatment is warranted.

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