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Evidence for the use of probiotics and prebiotics in inflammatory bowel disease: a review of clinical trials

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Human subjects and their enteric microbiota have evolved together to reach a state of mutual tolerance. Mounting evidence from both animal models and human studies suggests that inflammatory bowel disease (IBD) represents a malfunction of this relationship. The enteric microecology therefore represents an attractive therapeutic target with few side effects. Probiotics and prebiotics have been investigated in clinical trials as treatments for IBD, with conflicting results. The evidence for the use of probiotics in the management of pouchitis is persuasive and several studies indicate their effectiveness in ulcerative colitis. Trials of probiotics and prebiotics in Crohn's disease are less convincing. However, methodologies vary widely and a range of probiotic, prebiotic and combination (synbiotic) treatments have been tested in a variety of patient groups with an assortment of end points. Conclusions about any one treatment in a specific patient group can therefore only be drawn on evidence from relatively small numbers of patients. The present article reviews the role of the intestinal microbiota in the pathogenesis of IBD and addresses the clinical evidence for the therapeutic manipulation of bowel microbiota using probiotics, prebiotics and synbiotics in IBD.

Prebiotic: Probiotic: Ulcerative colitis: Crohn's disease: Pouchitis

The ability to define self from non-self and thereby maintain the integrity of the individual in the face of interference from other organisms is the defining property of an immune system. In the gut, however, this vital role must be executed in the setting of conflicting demands of constant exposure to ingested elements of the host's environment and antigens from commensal organisms at a mucosa adapted to permit uptake and absorption of nutrients. The operation of gastrointestinal immunity must therefore be particularly subtle in order to discriminate between pathogens to be excluded and symbiotic commensals to be tolerated. The gut immune system is unique in being able to contain organisms that if encountered in another bodily compartment would evoke an aggressive and damaging response. There is mounting evidence that the inflammatory bowel diseases (IBD) represent a malfunction of this singular attribute of tolerance to the commensal microbiota, which focuses attention on this relationship as a potential therapeutic target.

IBD comprises a spectrum of disorders characterised by inflammation, ulceration and stricturing of the gastrointestinal tract that results in symptoms of abdominal pain, diarrhoea and gastrointestinal bleeding. The two major phenotypes are ulcerative colitis (UC) and Crohn's disease (CD), both of which are chronic, relapsing and remitting diseases. UC causes continuous mucosal inflammation from the anal verge and is confined to the colon, whereas CD presents with discontinuous patchy transmural inflammation throughout the intestinal tract, with a predilection for the terminal ileum.

Conventional drug therapy is associated with a number of disadvantages, including a considerable burden of side effects and less than comprehensive efficacy, with a marked percentage of patients still requiring surgery to resect the affected gut. In UC, for example, a colectomy and ileal pouch anal anastamosis may be required either for refractory or fulminant disease or to reduce the risk of colon cancer in patients with dysplasia. Unfortunately, symptoms of inflammation can recur within the pouch resulting in the syndrome of pouchitis.

Inflammatory bowel disease and the gastrointestinal microbiota

The causes of IBD have not been fully elucidated, but are thought to involve both genetic and environmental factors.

Abbreviations: CD, Crohn's disease; IBD, inflammatory bowel disease; LGG, *Lactobacillus rhamnosus GG*; UC, ulcerative colitis.

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More recently, a number of observations have suggested that the intestinal microbiota may drive intestinal inflammation. First, experimental animal models of IBD remain healthy when reared in a germ-free environment and develop inflammation only after colonisation with commensal bacteria (Onderdonk *et al*. 1981; Sellon *et al*. 1998). Second, higher concentrations of bacteroides and enterobacteria are found in regions of inflamed mucosa in CD (Swidsinski *et al*. 2002). Third, in both CD (Harper *et al*. 1985; D'Haens *et al*. 1998) and pouchitis (de Silva *et al*. 1991) disease activity deteriorates when the faecal stream is surgically restored to previously-defunctioned intestine, implying that the lumen contents are necessary for the development of inflammation. Finally, the association between ileal CD and mutations in the bacterial-sensing caspase-activating recruitment domain 15 gene implicate bacterial recognition as a central pathogenic process (Ogura *et al*. 2001).

In contrast, there is also evidence that the commensal microbiota protect the mucosa from inflammation by decreasing intestinal permeability, increasing epithelial defence mechanisms and promoting an immunoregulatory acquired immune response (Madsen *et al*. 1999; Macpherson & Uhr, 2004; Rakoff-Nahoum *et al*. 2004). The mechanisms by which commensal microbiota interact with the gut immune system have been the focus of recent interest. There is evidence that metabolites produced by bacteria may interact directly with gut epithelial cells to enhance mucosal integrity (Venkatraman *et al*. 2000; Stempelj *et al*. 2007). In addition, commensal bacteria may compete with pro-inflammatory species to decrease access to the mucosa (Lee *et al*. 2003). Finally, microbial signature molecules, termed pathogen-associated molecular patterns, can interact with pattern recognition receptors such as toll-like receptors expressed on many host cells, including both epithelial cells and lamina propria dendritic cells (Stagg *et al*. 2003).

Despite this evidence for a microbial influence in pathogenesis, drugs conventionally used to induce and maintain remission in IBD, such as aminosalicylates (5-aminosalicylic acid drugs), corticosteroids, immunosuppressants (azathioprine and methotrexate) and infliximab (a chimeric monoclonal antibody), primarily act to suppress the enhanced immune response and do not affect the microbiota. Antibiotic-mediated microbial manipulation has some efficacy, especially in active CD and pouchitis, but cannot be used to maintain remission because of lack of long-term efficacy and side effects (Sutherland *et al*. 1991; Borgaonkar *et al*. 2000). None of these treatments is effective in all patients and all treatments are associated with major side effects. Therapies such as prebiotics and probiotics that selectively manipulate the gastrointestinal microbiota present a novel treatment option with a low side-effect burden.

Probiotics are living microbes that when introduced to the gut are capable of benefiting the host, and include organisms such as lactobacilli, bifidobacteria, Gram-positive cocci, enterococci and yeast species such as *Saccharomyces boulardii* (Fuller, 1989). Prebiotics are selectively fermented short-chain carbohydrates that allow specific changes both in the composition and/or activity of the gastrointestinal microbiota that confers health benefit, and include fructo-oligosaccharides and galacto-oligosaccharides (Gibson & Roberfroid, 1995). Combinations of probiotics and prebiotics are termed synbiotics. Evidence from animal models of IBD indicates that probiotics can alter the intestinal microbiota and ameliorate disease (Sartor, 2004). Similarly, prebiotics have been shown to enhance lumenal immunoregulatory bacteria, reduce the activity of pro-inflammatory transcription factors (e.g. NF-κB) and attenuate inflammation (Holma *et al*. 2002; Cavin *et al*. 2005). In addition, prebiotics produce SCFA, such as acetate and butyrate (Probert *et al*. 2004), that inhibit mucosal inflammation, impacting on both epithelial and dendritic cell function (Kinoshita *et al*. 2002; Millard *et al*. 2002).

The present review aims to assess the evidence that these observations from *in vitro* experiments and animal models can be put into clinical practice. The evidence presented relates to trials that have assessed these therapies in terms of clinical outcomes in IBD, and the data extracted and presented are the clinical end points in each study.

Probiotics and prebiotics in pouchitis

The most compelling evidence for the use of probiotics in IBD comes from randomised double-blind placebo-controlled trials of VSL#3 (a mixture of four species of lactobacilli, three species of bifidobacteria and *Streptococcus thermophilus*; Vsl Pharmaceuticals Inc., Gaithersburg, MD, USA) in patients with pouchitis (Table 1). Gionchetti *et al*. (2000) have assessed the efficacy of VSL#3 as a maintenance treatment in forty patients with chronic relapsing pouchitis after antibiotic-induced remission. After 4 months fewer relapses were found to occur in the intervention group than in the control group. Moreover, all patients were subsequently found to relapse 3 months after cessation of VSL#3. The same group (Gionchetti *et al*. 2003) have assessed VSL#3 in the primary prevention of pouchitis in forty patients following surgery. The incidence of pouchitis was found to be reduced and the quality of life improved in the VSL#3-treated group compared with the placebo group at the end of the intervention period; these beneficial effects were associated with faecal colonisation with the probiotics. Finally, a further study (Mimura *et al*. 2004) has confirmed the effectiveness of VSL#3 as maintenance therapy in patients with recurrent or chronic pouchitis. Importantly, these studies demonstrate high relapse rates of pouchitis in the placebo group, perhaps reflecting intensive patient follow-up resulting in the detection of relapse that may have otherwise remained subclinical.

In contrast, Shen *et al*. (2005) have reported no significant benefit of VSL#3 in maintaining antibiotic-induced remission in thirty-one patients. After 8 months twenty-three patients had discontinued VSL#3 because of symptom recurrence and two had discontinued because of side effects. In the six patients who remained on VSL#3 endoscopic evidence of mild to moderate pouchitis was found to persist even though symptom scores were not significantly different from baseline. Although the fact that

Table 1. Clinical outcomes of published studies investigating the efficacy of probiotics and prebiotics in the treatment of pouchitis

Reference	*n*	Trial design	Study groups		Duration (months)	Relapse rate (%)		*P*
			Intervention (daily dose)	Control		Intervention	Control	
Gionchetti *et al.* (2000)	40	R, DB	VSL#3 (6 g)	Pl	9	15	100	<0·001
Welters *et al.* (2002)	20	R, DB, CO	Inulin (24 g)	Pl	2·2	NR	NR	NR
Friedman & George (2000)	10	OL	LGG (one tablet) plus FOS (one tablet)	–			–	
Gionchetti *et al.* (2003)	40	R, DB	VSL#3 (3 g)	Pl	12	10	40	<0·05
Kuisma *et al.* (2003)	20	R, DB	LGG (>10^{10} CFU)	Pl	3	NR	NR	NR
Gosselink *et al.* (2004)	117	OL	LGG (300 mg)	HC	36	8	35	0·01
Mimura *et al.* (2004)	36	R, DB	VSL#3 (6 g)	Pl	12	15	94	<0·01
Laake *et al.* (2005)	51	OL	Fermented milk (500 ml)	–	1	NR	–	–
Shen *et al.* (2005)	31	OL	VSL#3 (6 g)	–	8	81	–	–

R, randomised; DB, double blind; CO, cross-over study; OL, open label; VSL#3, a mixture of four species of lactobacilli, three species of bifidobacteria and *Streptococcus thermophilus*; LGG, *Lactobacillus rhamnosus* GG; FOS, fructo-oligosaccharide; Pl, placebo; HC, historical controls; CFU, colony-forming units; NR, not reported.

patients purchased, stored and administered the VSL#3 themselves may have resulted in lower compliance, this methodology may more faithfully reproduce patients' use of probiotics outside clinical trials.

Trials of other probiotics in the management of pouchitis have yielded mixed results. One observational study of patients receiving *Lactobacillus rhamnosus GG* (LGG) after pouch formation (Gosselink *et al*. 2004) has reported a lower rate of pouchitis than in historical controls. However, Kuisma *et al*. (2003) have found no difference in mean pouchitis scores between placebo and LGG-treated groups at the end of a 3-month study period, despite an increase in faecal lactobacilli : anaerobes. Finally, a reduction in endoscopic and clinical disease activity associated with an increase in faecal probiotic species has been demonstrated (Laake *et al*. 2005) in fifty-one patients with pouchitis after surgery for ulcerative colitis who consumed a fermented milk containing lactobacilli and bifidobacteria. These studies suggest that therapeutic benefit requires a combination of probiotic species (as with VSL#3) or that the component(s) responsible for the anti-inflammatory effect in combination preparations have specific properties that monotherapy probiotics do not. This concept supports the use of prebiotics that increase concentrations of several commensal immunoregulatory bacteria.

Two studies have examined the use of prebiotics or synbotics in pouchitis. Ten patients with antibiotic-refractory or antibiotic-dependent pouchitis treated with a combination of LGG and fructo-oligosaccharide in an open-label study were reported to experience complete clinical and endoscopic remission (Friedman & George, 2000). Welters *et al*. (2002) have reported that intake of inulin is associated with a decrease in histological and endoscopy scores in twenty patients in a cross-over study with a 4-week wash-out period between 3-week study phases. No patient had overt clinical pouchitis at enrolment and no change in clinical activity scores was detected, although overall pouchitis disease activity index was found to be significantly reduced during the inulin period ($P = 0{\cdot}01$). Prebiotic use was shown to be associated with a reduction in the faecal concentration of *Bacteroides fragilis* ($P = 0{\cdot}02$), but had no effect on lactobacilli or bifidobacteria. In addition, inulin was found to increase faecal butyrate concentrations ($P = 0{\cdot}01$) and reduce pH, with no effect on secondary bile acid concentration.

Probiotics and prebiotics as maintenance therapy in ulcerative colitis

Many trials have examined the use of probiotics to maintain remission in patients with UC. Three controlled trials (Kruis *et al*. 1997, 2004; Rembacken *et al*. 1999) have demonstrated that *Escherichia coli* Nissle 1917 has similar efficacy to conventional 5-aminosalicylic acid (mesalazine) treatment, but the evidence to support the use of other probiotic species is less persuasive (Table 2). Kruis *et al*. (1997) have demonstrated equivalence of mesalazine and *E. coli* Nissle 1917 in patients with UC in remission over 12 weeks. However, studying the maintenance of remission over a short follow-up period is of limited clinical applicability. Rembacken *et al*. (1999) have treated patients for a 12-month period and have also demonstrated equivalence of mesalazine *v*. *E. coli* Nissle 1917. Remission was induced using standard medical treatment (including topical and systemic corticosteroids) alongside the study intervention. No significant difference was found in the time to achieve remission or mean duration of remission, although the lack of a placebo group may make it difficult to distinguish a small difference in effectiveness from the effect of potent doses of steroid. Finally, no significant difference was found in the rate of relapse in a study comparing *E. coli* Nissle 1917 with mesalazine in 327 patients using no concomitant medication (Kruis *et al*. 2004). Probiotic therapies such as *E. coli* Nissle 1917 that have equivalent efficacy but fewer side effects than conventional drug therapies present an appealing alternative for patients and physicians.

Several studies examining the use of lactobacilli or bifidobacteria as maintenance treatment in UC have demonstrated conflicting results. In patients randomised to three open-label arms (LGG *v*. mesalazine *v*. LGG plus mesalazine) it was demonstrated (Zocco *et al*. 2006) that both the lactobacillus arms experience a prolonged

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Table 2. Clinical outcomes of published studies investigating the efficacy of probiotics in maintenance of remission in ulcerative colitis

Reference	*n*	Trial design	Study groups: Intervention (dose/d)	Study groups: Comparator (dose/d)	Duration (months)	Relapse rate (%): Intervention	Relapse rate (%): Comparator	*P*
Kruis *et al.* (1997)	120	R, DB, DD	*E. coli* Nissle (200 mg)	Mesalazine (1·5 g)	3	16	11	NS
Rembacken *et al.* (1999)	116	R, DB, DD	*E. coli* Nissle (>10^{10} CFU)	Mesalazine (2·4 g)	12	32	25	0·05
Venturi *et al.* (1999)	20	OL	VSL#3 (6 g)	–	12	20	–	–
Ishikawa *et al.* (2003)	21	R	Fermented milk (100 ml)	Pl	12	27	90	<0·01
Cui *et al.* (2004)	30	R, DB	Bifidobacteria (1·26 g)	Pl	8	20	93	<0·01
Kruis *et al.* (2004)	327	R, DB, DD	*E. coli* Nissle (200 mg)	Mesalazine (500 mg)	12	36	34	SE 0·03
Zocco *et al.* (2006)	180	R, OL	LGG (>10^{10} CFU) LGG (>10^{10} CFU) + mesalazine (2·4 g)	Mesalazine (2·4 g)	12	15 17	20	0·77
Shanahan *et al.* (2006)	157	R, DB	*L. salivarius* (10^9 CFU) *B. infantis* (10^9 CFU)	Pl	12	NR	NR	NR

R, randomised; DB, double blind; DD, double dummy; OL, open label; *E. coli* N, *Escherichia coli* Nissle 1917; CFU, colony-forming units; VSL#3, a mixture of four species of lactobacilli, three species of bifidobacteria and *Streptococcus thermophilus*; LGG, *Lactobacillus rhamnosus* GG; *L. salivarius*, *Lactobacillus salivarius*; *B. infantis*, *Bifidobacterium infantis*; Pl, placebo; NR, not reported; SE, significant equivalence.

relapse-free time compared with 5-aminosalicylic acid treatment alone, despite no significant differences in overall relapse rates and clinical, histological or endoscopy scores. In a study of thirty patients with UC in medically-induced remission (Cui *et al*. 2004) those receiving supplements of bifidobacteria were found to have fewer relapses during the 2-month follow-up period than those receiving placebo (20% *v*. 93% respectively). In addition, those receiving the probiotic were found to have increases in the concentration of faecal lactobacilli and bifidobacteria, a reduction in the expression of the pro-inflammatory transcription factor NF-κB and enhancement of anti-inflammatory cytokine release. Similarly, Ishikawa *et al*. (2003) have demonstrated a reduction in the number of disease exacerbations in a group of Japanese patients receiving fermented milk containing *Bifidobacterium breve*, *Bifidobacterium bifidum* and *Lactobacillus acidophilus* compared with placebo. However, this clinical benefit was not found to be associated with an increase in steroid-free remission or endoscopic improvement in disease activity. In contrast, a recently presented study (Shanahan *et al*. 2006) comparing *Lactobacillus salivarius* with *Bifidobacterium infantis* or placebo in 157 patients with UC in medically-induced remission has demonstrated no significant benefit of either of the probiotics when compared with placebo. Finally, treatment with VSL#3 to maintain remission in UC (Venturi *et al*. 1999) was found to result in an increase in faecal concentrations of VSL#3 bacteria and a reduction in faecal pH. At the end of the study only four of twenty patients had experienced relapse.

There are few data on the efficacy of prebiotics or synbiotics in the maintenance of remission in UC.

Probiotics and prebiotics to treat active ulcerative colitis

Four diverse studies have examined the use of probiotics to treat active UC (Table 3). Tursi *et al*. (2004) have compared low-dose balsalazide + VSL#3 *v*. medium-dose balsalazide alone *v*. mesalazine alone in patients with mild- to moderately-active UC. Remission rates and time to remission were found to be shortest in the combination group. These results suggest that probiotics may augment the effect of 5-aminosalicylic acid drugs, although the balsalazide doses used in this study were lower than those generally used in clinical practice. In a second study in which clinical benefit of VSL#3 was also demonstrated (Bibiloni *et al*. 2005) VSL#3-specific organisms were detected in only three of eleven patients after microbiological analysis of mucosal biopsies. This finding suggests that the clinical effect observed was not the result of a direct influence of VSL#3-derived organisms at the mucosa.

Efficacy of direct delivery of the probiotic to the colon with *E. coli* Nissle 1917 enemas in left-sided UC has been demonstrated (Matthes *et al*. 2006). A significant dose-dependent response was found and the time to remission was also reported to be shortest in the group receiving the highest dose of enema. A fourth probiotic was studied by Kato *et al*. (2004), who treated active UC with fermented milk (*B. breve* strain Yakult, *B. bifidum* strain Yakult and a *L. acidophillus* strain). Although no difference was found in either remission rates or histological and endoscopy scores between the groups, the clinical activity scores were found to be lower in the treatment group than in the placebo group at the end of the study period.

A series of small open-label studies have evaluated a prebiotic germinated barley foodstuff (a glutamine-rich protein and dietary fibre, mainly hemicellulose) and have found evidence for therapeutic efficacy. For example, when the germinated barley foodstuff was administered to ten patients with mild- to moderately-active UC (Mitsuyama *et al*. 1998) a significant decrease was found in mean clinical activity score after 4 weeks of treatment ($P<0·05$) associated with increases in faecal SCFA concentration.

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Table 3. Clinical outcomes of published studies investigating the efficacy of probiotics in induction of remission in patients with active ulcerative colitis

Reference	*n*	Trial design	Study groups: Intervention (dose/d)	Study groups: Comparator (dose/d)	Duration (weeks)	Remission rate (%): Intervention	Remission rate (%): Comparator	*P*
Mitsuyama *et al.* (1998)	10	OL	Germinated barley foodstuff (30 g)	–	4	NR	–	NR
Tursi *et al.* (2004)	90	R, OL	VSL#3 plus balsalazide (2·25 g)	Balsalazide (4·5 g) Mesalazine (2·4 g)	8	80	77 53	0·02
Kato *et al.* (2004)	20	R, DB	Fermented milk (100 ml)	Pl	12	40	30	NS
Biblioni *et al.* (2005)	34	OL	VSL#3 ($>10^{10}$ CFU)	–	6	53	–	–
Furrie *et al.* (2005)	18	R, DB	Synergy 1 (6 g) and *B. longum* ($>10^{11}$ CFU)	Pl	4	NR	NR	NR
Matthes *et al.* (2006)	90	R, DB	*E. coli* Nissle enema (ml): 40 20 10	Pl	4	53 44 27	18	0·04

OL, open label; R, randomised; DB, double blind; VSL#3, a mixture of four species of lactobacilli, three species of bifidobacteria and *Streptococcus thermophilus*; CFU, colony-forming units; *B. longum*, *Bifidobacterium longum*; *E. coli* N, *Escherichia coli* Nissle 1917; DB, double blind; Pl, placebo; NR, not reported.

Table 4. Clinical outcomes of published studies investigating the efficacy of probiotics and prebiotics in maintenance of remission in patients with Crohn's disease

Reference	*n*	Trial design	Study groups: Intervention (dose/d)	Study groups: Comparator (dose/d)	Duration (months)	Relapse rate (%): Intervention	Relapse rate (%): Comparator	*P*
Guslandi *et al.* (2000)	32	R, OL	*S. boulardii* (1 g) plus mesalazine (2 g)	Mesalazine (3 g)	6	6	38	0·04
Campieri *et al.* (2000)	40	R, OL	VSL#3 (6 g)	Mesalamine (4 g)	12	20	40	NR
Prantera *et al.* (2002)	45	R, DB	LGG (4·92 g)	Pl	12	17	11	0·30
Schultz *et al.* (2004)	11	R, DB	LGG ($>10^9$ CFU)	Pl	6	60	67	NS
Bousvaros *et al.* (2005)	75	R, DB	LGG ($>10^9$ CFU)	Pl	24	31	17	0·18
Marteau *et al.* (2006)	98	R, DB	*L. johnsonii* ($>10^9$ CFU)	Pl	6	49	64	0·15
Van Gossum *et al.* (2007)	70	R, DB	*L. johnsonii* ($>10^9$ CFU)	Pl	3	15	14	0·91
Chermesh *et al.* (2007)	30	R, DB	Synbiotic 2000*	Pl	24	25	20	NS

R, randomised; OL, open label; DB, double blind; *S. boulardii*, *Saccharomyces boulardii*; VSL#3, a mixture of four species of lactobacilli, three species of bifidobacteria and *Streptococcus thermophilus*; LGG, *Lactobacillus rhamnosus* GG; CFU, colony-forming units; *L. johnsonii*, *Lactobacillus johnsonii*; Pl, placebo; NR, not reported.
**Pediacoccus pentoseceus*, *Lactobacillus raffinolactis*, *Lactobacillus paracasei susp paracasei* 19, *Lactobacillus plantarum*, 2·5 g β-glucans, 2·5 g inulin, 2·5 g pectin, 2·5 g resistant starch.

A pilot study of eighteen patients in a randomised double-blind double-dummy study over 1 month (Furrie *et al.* 2005) has suggested that synbiotics may have a role in reducing inflammation in UC. A non-significant trend in favour of the combination of oligofructose and inulin and a probiotic (*Bifidobacterium longum*) was observed on histological and sigmoidoscopic assessment but clinical measures were not found to be different. However, mucosal-associated bifidobacteria concentrations were found to increase in association with a decrease in both pro-inflammatory cytokines such as TNFα and IL-1α and antimicrobial human β-defensin peptides. It is disappointing that the observed alterations in mucosal cytokine balance did not translate into more clinically-important changes in symptoms or endoscopic appearance. It may be that this rapid change in cytokine expression would give rise to a more clinically-relevant change if treatment were sustained for a longer period.

Probiotics and prebiotics to maintain remission in Crohn's disease

Evidence for the use of probiotics as maintenance therapy in CD is not persuasive, with only two studies reporting positive results (Table 4). A study comparing *S. boulardii* + the antibiotic mesalazine with mesalazine alone (Guslandi *et al.* 2000) has shown fewer relapses in the former group in patients with medically-induced remission of CD. Campieri *et al.* (2000) have shown benefit of VSL#3 in preventing post-operative recurrence in forty patients randomised to 3 months of rifaximin followed by 9 months of VSL#3 or to 12 months of mesalazine.

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Table 5. Clinical outcomes of published studies investigating the efficacy of probiotics and prebiotics in induction of remission in patients with active Crohn's disease

Reference	*n*	Trial design	Study groups: Intervention (dose/d)	Study groups: Comparator (dose/d)	Duration (weeks)	Remission rate (%): Intervention	Remission rate (%): Comparator	*P*
Malchow (1997)	28	R, DB	*E. coli* N (100 mg)	Pl	12	75	92	NS
Gupta *et al.* (2000)	4	OL	LGG ($>10^{10}$ CFU)	–	24	100	–	–
Lindsay *et al.* (2006)	10	OL	FOS (15 g)	–	3	40	–	–

R, randomised; DB, double blind; OL, open label; *E. coli* N, *Escherichia coli* Nissle 1917; LGG, *Lactobacillus rhamnosus* GG; CFU, colony-forming units; FOS, fructo-oligosaccharide; Pl, placebo.

Conversely, three studies using LGG (Prantera *et al.* 2002; Schultz *et al.* 2004; Bousvaros *et al.* 2005) have not confirmed the effectiveness of this probiotic as a maintenance strategy after surgically- or medically-induced remission. No significant differences in clinical or endoscopic recurrence rates were found between patients receiving LGG or placebo to maintain surgical remission (Prantera *et al.* 2002). Similarly, Schultz *et al.* (2004) have shown no significant effect of LGG in maintaining remission induced with antibiotics and steroids. Finally, no significant difference in time to relapse was found between probiotics or placebo (inulin) in a paediatric population with medically-induced remission (Bousvaros *et al.* 2005). In this study few patients were found to have high concentrations of faecal lactobacilli (7% and 20% in the intervention and placebo groups respectively). The study was terminated early because of lack of effect and poor recruitment. It is important to note that the placebo (inulin) is a prebiotic that could therefore mask any difference in efficacy between the groups. It could even be argued that the non-significant trend towards fewer relapses in the placebo group could relate to the higher dose of inulin administered, although this explanation is unlikely at the low doses employed.

Finally, two randomised double-blind placebo-controlled studies (Marteau *et al.* 2006; Van Gossum *et al.* 2007) have reported no effect of *Lactobacillus johnsonii* LA1 in preventing recurrence in patients with CD in surgically-induced remission. Marteau *et al.* (2006) have found no significant difference in endoscopic recurrence in comparison with placebo 6 months after surgery. Likewise, Van Gossum *et al.* (2007) have reported no significant difference from placebo in endoscopy scores or clinical relapse rates after 3 months of treatment.

The use of prebiotic therapies in the maintenance of remission in CD has not been studied extensively. In patients with CD who have undergone ileal resection treatment with Synbiotic 2000 (*Pediacoccus pentoseceus, Lactobacillus raffinolactis, Lactobacillus paracasei susp paracasei* 19, *Lactobacillus plantarum*, 2·5 g β-glucans, 2·5 g inulin, 2·5 g pectin, 2·5 g resistant starch) was reported to have no impact on maintaining remission when compared with placebo (Chermesh *et al.* 2007). It is notable that the investigators had intended to enrol twice as many patients, but were unable to do so because of reluctance of patients and physicians to risk exposure to the placebo arm.

Probiotics and prebiotics to treat active Crohn's disease

Two studies have evaluated probiotics in patients with active CD (Malchow, 1997; Gupta *et al.* 2000), but neither study has demonstrated convincing efficacy, in part because of concomitant drug prescription and small numbers of patients (Table 5). Malchow (1997) has compared *E. coli* Nissle 1917 with placebo in patients with active CD who also received the steroid prednisolone, 60 mg daily for 11 weeks, to induce remission and then continued with probiotics or placebo during a 12-month maintenance phase. The proportion of patients achieving remission and time to remission was found to be similar in the two groups, suggesting that the remission was mostly attributable to steroid use. In the maintenance phase there was a non-significant trend towards decreased relapse rates in the *E. coli* group *v.* the placebo group. Gupta *et al.* (2000) have examined the effects of LGG in four children with steroid refractory CD, two of whom were taking concomitant metronidazole. Colonisation with LGG and a sustained reduction in disease activity associated with improved intestinal permeability was demonstrated in all patients, suggesting a mechanism for the therapeutic effect.

Prebiotics have also been investigated in the treatment of CD. Lindsay *et al.* (2006) have found that fructo-oligosaccharide reduces disease activity in active CD. As in healthy controls (Langlands *et al.* 2004), fructo-oligosaccharide was shown to increase faecal bifidobacteria concentrations, although no corresponding change in mucosal-associated bifidobacteria was found. However, a significant increase was found in mucosal bifidobacteria in those patients entering remission. Evidence was found of an immunoregulatory shift in dendritic cell activity, with an increase in the proportion of IL-10-positive dendritic cells ($P = 0{\cdot}06$), and enhanced toll-like receptor 2 and toll-like receptor 4 expression ($P = 0{\cdot}086$ and $P < 0{\cdot}001$ respectively). A placebo-controlled trial to confirm the benefit of fructo-oligosaccharide in active CD is underway.

Limitations to research on probiotics and probiotics in inflammatory bowel disease

Interpretation of the current literature on probiotics and prebiotics in IBD is undermined by marked heterogeneity between trials frequently precluding meta-analysis. Variables include the choice of probiotic or prebiotic studied

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and their dose, the trial design and the outcome measures reported. The populations studied differ, with some trials enrolling patients with active disease and others studying maintenance of remission induced by antibiotics, conventional drug treatment or surgery. Few studies stratify for disease distribution, and none have reported the prevalence of genotypes that may impact on the response to therapies manipulating the microbiota. Most studies have enrolled small numbers of patients, which limits statistical power and is of particular relevance given the high placebo response rates seen in clinical trials of IBD (Su *et al*. 2004, 2007). Finally, no study has included details of the patients' diet, which could exert a marked influence on the efficacy of microbial therapies.

The precise mechanisms of action of probiotics and prebiotics remain to be elucidated. For example, it is not clear whether intestinal colonisation with probiotic species is necessary, as some studies demonstrating clinical efficacy have not demonstrated such colonisation (Bibiloni *et al*. 2005). Further research is required to determine whether prebiotics exert their effect via increased numbers of bacteria that are recognised by intestinal immune cells or via effects of SCFA on the mucosa. *In vitro* studies examining the effect of prebiotics in enterocyte models may reveal direct effects on the mucosa. Investigation of changes in immune cell phenotype on exposure to prebiotics and/or bacteria may elucidate such relationships.

Conclusions

There is a well established link between the intestinal microbiota and the inflammation associated with IBD, with evidence for both pro-inflammatory and regulatory effects. Thus, selective manipulation of the microbiota is an attractive therapeutic strategy for the treatment of disease and maintenance of remission.

The evidence for the use of probiotics in IBD is strongest in the case of pouchitis, particularly for the use of VSL#3. In addition, *E. coli* Nissle 1917 appears to be at least equivalent to 5-aminosalicylic acid treatment in UC and may be useful in the form of enemas for distal disease. However, studies of probiotics in CD have been disappointing, and a recent Cochrane systematic review (Rolfe *et al*. 2006) has concluded that their use could not be recommended on the available evidence. Prebiotics are frequently evaluated as part of a synbiotic combination, making it difficult to isolate their individual effects. However, few adverse events have been reported for either probiotics or prebiotics in any of these studies, confirming the safety of these treatments.

At present, there is some evidence to support the use of probiotics and prebiotics in IBD, although large trials using standardised methodology are required to confirm this evidence. However, the investigation of the therapeutic application of these treatments increases understanding of the role of the gastrointestinal microecology in the pathogenesis of IBD. With improved knowledge of the mechanisms by which the gastrointestinal mirobiota determine gut immune responses, clinical research will be better focused to select appropriate investigational probiotic treatments and patient groups, and trial outcomes can be more meaningfully translated into clinical practice.

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