Antibiotics and probiotics in treatment of inflammatory bowel disease

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INTRODUCTION

The rationale for using antibiotics and probiotics in the treatment of inflammatory bowel disease (IBD) is based on convincing evidence implicating intestinal bacteria in the pathogenesis of the disease[1]. The distal ileum and the colon are the areas with the highest bacterial concentrations and represent the sites of inflammation in IBD. In addition, pouchitis, the non-specific inflammation of the ileal reservoir after ileo-anal anastomosis, appears to be associated with bacterial overgrowth and dysbiosis. Furthermore, pouchitis does not occur prior to closure of the ileostomy.

Patients with Crohn's disease (CD) consistently respond to diversion of faecal stream, with immediate recurrence of inflammation after restoration of intestinal continuity or infusion of luminal content into the bypassed ileum[2,3]. Moreover, the composition of the enteric flora is altered in patients with IBD, and enteric bacteria or their products have been found within the inflamed mucosa of patients with CD[4]. Increased number of aggressive bacteria such as Bacteroides, adherent/invasive Escherichia coli and enterococci, and decreased number of protective lactobacilli and bifidobacteria have been observed in IBD[5].

However, the most compelling evidence that intestinal bacteria play a role in IBD has been derived from animal models. Although there is a great diversity in genetic defects and immunopathology, a consistent feature of many transgenic and knockout mutant murine models of colitis is that the presence of normal enteric flora is required for full expression of inflammation[6]. Indeed, there is evidence that immunological tolerance to commensal bacteria is lost in patients with IBD[7,8]. These findings have led to the proposal that manipulation of intestinal microbiota flora, either with antibiotics or probiotics, may be therapeutic in IBD. Some suggested mechanisms of action of antibiotics and probiotics are shown in Table 1.

There is a growing body of evidence from animal studies and clinical trials that antibiotics and probiotics have therapeutic effects in ulcerative colitis (UC), CD and pouchitis.

ANTIBIOTICS

Animal model studies

In several rodent models the use of broad-spectrum an-
Antibiotics can both prevent and treat experimental colitis, whereas metronidazole and ciprofloxacin can only prevent experimental colitis but cannot reverse the established disease. Broad-spectrum antibiotics are effective in almost all models of acute and chronic colitis, but they have only a transient efficacy in HLA-B27 transgenic rats. Interestingly, ciprofloxacin and metronidazole have selective efficacy in different colonic regions of interleukin-10 (IL-10) knockout mice, suggesting that different bacteria cause inflammation in different colonic segments. These studies suggest that most clinical forms of IBD may respond to a specific combination of broad-spectrum antibiotics.

**Ulcerative colitis**

Only a few trials on the use of antibacterial agents have been carried out in UC and the results are controversial. Most clinicians have used antibiotics as an adjuvant therapy for severe UC. Dickinson et al. carried out a double-blind controlled trial on the use of oral vancomycin as an adjunct for acute exacerbations of idiopathic colitis and found that there is no significant difference between the two treatment groups, with only a trend towards a reduction in the need for surgery in patients treated with vancomycin.

Intravenous metronidazole used in conjunction with corticosteroids, is as effective as placebo in inducing remission in patients with severe UC. In a double-blind, placebo-controlled trial in patients with acute relapse of UC, Burke et al. randomized 84 patients to receive corticosteroids plus oral tobramycin or placebo and found that after 1 wk of treatment, 74% of patients in the tobramycin treatment group and 43% in the placebo group achieve complete symptomatic remission. However, the combination of tobramycin and metronidazole does not have any beneficial effect when compared with a standard steroid treatment in severely acute UC.

Mantzaris et al. investigated ciprofloxacin in a randomised, placebo-controlled study and randomized 70 patients with mild to moderate active UC to receive either 250 mg ciprofloxacin twice a day or placebo for 14 d and found that 70.5% of patients in the ciprofloxacin group and 72% in the placebo group achieve remission. Moreover, a short course of intravenous ciprofloxacin is not effective as an adjunctive treatment to corticosteroids in severe UC. In contrast, some efficacy of ciprofloxacin has been observed in a more recent randomised placebo-controlled trial when ciprofloxacin is administered for 6 mo to patients with active UC poorly responding to conventional therapy with steroids and mesalazine. At the end of the study, the treatment-failure rate was 21% in the ciprofloxacin-treated group and 44% in the placebo group ($P < 0.002$). This difference was detected using clinical criteria; while endoscopic and histological findings showed differences only at 3 mo but not at 6 mo.

The non-absorbable broad-spectrum antibiotic, rifaximin, was investigated in a small controlled study to evaluate its efficacy and systemic absorption in patients with moderate to severe active UC refractory to steroid treatment. Twenty-eight patients were randomised to receive either rifaximin 400 mg twice daily or placebo for 10 d as an adjunct to standard steroid treatment. Although there is no significant difference in the clinical efficacy score between the two treatments, only rifaximin determines a significant improvement in stool frequency, rectal bleeding and sigmoidoscopic score.

Whilst rifaximin does not permanently alter the colonic microbiota, resistant *Bifidobacterium* species have been found after 3 intermittent courses in patients with UC.

**Crohn’s disease**

Broad-spectrum antibiotics are widely used to treat CD, but large controlled trials have not yet been performed (Table 2).

Metronidazole has been the most investigated agent. In 1978, Blchfeldt et al. found that there is no difference between metronidazole and placebo-treated patients in a placebo-controlled, double-blind, crossover trial. However, a positive trend in favour of metronidazole is observed when only the colon is involved. In the National Cooperative Swedish study, metronidazole has been compared with sulphasalazine as a primary treatment for Crohn’s disease. Although no significant difference is found between the two groups, metronidazole is effective in patients who fail to respond to sulphasalazine. In

| Antibiotics | Probiotics |
|-------------|------------|
| Eradication of bacterial antigenic triggers | Inhibition of pathogenic enteric bacteria by: |
| Elimination of bacterial overgrowth | decreasing luminal pH |
| Reduction of pro-inflammatory bacterial toxins | secretion of bacteriocidal proteins |
| Potential immunosuppressive properties of antibiotics | resisting colonization |

Increasing IgA production

Increasing interleukin-10 and TGF-β, and decreasing TNF levels

**Table 1** Suggested mechanisms of action of antibiotics and probiotics

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The use of antibiotics in the prevention of post-
operative disease recurrence has also been investigated. Rutgeerts *et al* [43] have assessed the efficacy of metronidazole at 20 mg/kg per day in a placebo-controlled double-blind study. In their study, sixty patients were randomised to receive either metronidazole or placebo for 12 wk and endoscopic relapse was evaluated by Rutgeerts score at the end of the treatment. They found that metronidazole significantly decreases the incidence of severe endoscopic relapse (grade 3 or 4) and the clinical recurrence rate. More recently, ornidazole used continuously for 1 year, has been shown to be significantly more effective than placebo in the prevention of clinical and endoscopic recurrence in the neoterminal ileum [46].

**Pouchitis**

The awareness of the crucial importance that faecal stasis and bacterial overgrowth may play a role in the pathogenesis of acute pouchitis has led clinicians to treat patients with antibiotics. Antibiotics have become the mainstay of treatment for pouchitis, although controlled trials are not available. Metronidazole is the first-line treatment, and most patients with acute pouchitis respond quickly to its administration of 1-1.5 g/d [45,46]. A double-blind, randomised, placebo-controlled, crossover trial was carried out by Madden *et al* [47] to assess the efficacy of 400 mg of metronidazole three times daily *per os* for two weeks in 13 patients (11 completed both arms of the study) with chronic, unremitting pouchitis. The found that metronidazole is significantly more effective than placebo in reducing stool frequency (73% and 9%), even without improvement in endoscopic appearance and histological grade of activity. However, a significant proportion of patients (55%) may experience side-effects while using metronidazole, including nausea, vomiting, abdominal discomfort, headache, skin rash and metallic taste. Recently Shen *et al* [48] compared the efficacy and side-effects of ciprofloxacin and metronidazole in treating acute pouchitis in a randomised clinical trial. Seven patients received ciprofloxacin (1 g/d) and nine patients received metronidazole (20 mg/kg per day) for 2 wk. The results of this study have shown that both ciprofloxacin and metronidazole are efficacious in the treatment of acute pouchitis. Both reduce the total pouchitis disease activity index (PDAI) scores and lead to a significant improvement in symptoms as well as endoscopic and histological scores. However, ciprofloxacin leads to a greater reduction in PDAI scores as well as improvement in symptoms and endoscopic scores. Furthermore ciprofloxacin is better tolerated than metronidazole (33% of metronidazole-treated patients reported adverse effects, compared with none in the ciprofloxacin group).

Given the management difficulties posed by chronic refractory pouchitis, the use of combined antibiotic treatment has been explored. In an open trial, 18 patients with active pouchitis not responding to standard therapy (metronidazole or ciprofloxacin) for 4 wk, were treated orally with rifaximin (2 g/d) plus ciprofloxacin (1 g/d) for 15 d. Symptom assessment, endoscopic and histological evaluations were performed at screening and after 15 d using PDAI scores. The results indicate that 16 out of 18 patients (88.8%) improve (*n* = 10) or go into remission (*n* = 6) with the median PDAI score before and after therapy being 11 and 4 respectively (*P* < 0.002) [49].

More recently, 44 patients with refractory pouchitis received metronidazole (800 mg to 1 g/d) and ciprofloxacin (1 g/d) for 28 d. The results reveal that 66 patients (82%) go into remission with the median PDAI score before and after therapy being 12 and 3 respectively (*P* < 0.0001), and the patients’ quality of life is significantly improved after the treatment (median IBD Questionnaire score increased from 96.5 to 175) [50].

### PROBIOTICS

The use of probiotics for the purpose of health maintenance and disease prevention is first proposed by Elie Metchnikoff, the Russian Nobel prize winner [51], who at the turn of the last century suggested that a high concentration of lactobacilli in the intestinal flora is important for the health and longevity of humans. Probiotics are defined as “living organisms, which upon ingestion in a certain number exert health benefits beyond inherent basic nutrition” [52].

A number of bacteria are associated with probiotic activity (Table 3). For clinical application, probiotic strains need to be resistant to acid and bile and the ability to be metabolically active within the luminal flora where they should ideally survive but not persist in the long term. They should be antagonistic to pathogenic bacteria and safe for human use while maintain their viability and beneficial properties during the manufacturing processes [53].

### Animal model studies

Encouraging results of probiotic therapy have been obtained in experimental colitis. Administration of *Lactobacillus reuteri* can significantly reduce inflammation in acetic acid-and methotrexate-induced colitis in rats [54-56]. More recently *Lactobacillus* *sp.* has been shown to be able to prevent the development of spontaneous colitis in IL-10 deficient mice [57], and continuous feeding with *Lactobacillus plantarum* improves an established colitis in the same knockout model [58]. A strain of *Lactobacillus salivarius* (subsp. *salivarius*) reduces the rate of progression from inflammation to dysplasia and colonic cancer in IL-10 deficient mice [59], and *Bifidobacterium infantis* and of *Lactobacillus salivarius* are able to attenuate inflammation and reduce the ability to produce Th1-type cytokines in the IL-10 knockout model [60].

VSL#3 is characterised by a very high bacterial concentration (each packet containing 450 billion viable bacteria) and the presence of a cocktail of eight different bacterial species. This product contains viable lyophilised bacteria of four strains of lactobacilli (*L. casei, L. plantarum, L. acidophilus, L. delbrueckii* subsp. *bulgaricus*), three strains of

| Table 3 Organisms associated with probiotic activity |
|-----------------------------------------------|
| **Most commonly** | **Other bacterial strains** | **Yeast** |
| Lactobacilli | Enterococci | Saccharomyces boulardii |
| Bifidobacteria | Non-pathogenic E. coli | |
Ulcerative colitis

Promising results of probiotics have been found in the treatment of UC. In 3 recent trials involving the non-pathogenic strain of Escherichia coli Nissle 1917, similar efficacy has been observed to that of mesalazine in the maintenance treatment of UC.[62-64]

We carried out a pilot study using the probiotic cocktail, VSL#3, as maintenance treatment for patients with UC in remission, allergic or intolerant to sulphasalazine and mesalazine, to assess its impact on the faecal flora. Twenty patients received 6 g a day of VSL#3 (1800 billion bacteria) for 12 mo and were assessed clinically and endoscopically at baseline, at 6 and 12 mo, and in the event of a relapse. Stool culture and determination of faecal pH were also performed at different intervals.[65] Microbiological determination showed a significant increase in concentration of lactobacilli, bifidobacteria and Streptococcus thermophilus, evident after just 20 d, which persisted throughout the treatment period, and returned to basal levels within 15 d after treatment. Faecal concentration of Bacteroides, enterococci, coliforms, Clostridia and total anaerobes and aerobes was not affected, but faecal pH was significantly reduced by the treatment. Fifteen of the twenty patients (75%) remained in remission throughout the treatment period.[66]

Furthermore, VSL#3 at very high dosage (3600 billion bacteria/d) can induce remission in 63%, with a positive response in a further 23% of patients with active mild to moderate disease.[67]

In addition, an open uncontrolled 4-wk study found that the yeast Saccharomyces boulardii could induce remission in 71% of patients with mild to moderate UC.[68] These studies highlight the wide range of organisms that may be beneficial as probiotic therapy for UC.

Crohn’s disease

Campieri et al.[69] performed a randomised trial to evaluate the efficacy of a combination of rifaximin and the probiotic preparation, VSL#3, in the prevention of post-operative recurrence of CD. Rifaximin (1.8 g/d) for 3 mo, followed by VSL#3 (6 g daily) for 9 mo, was compared with mesalazine (4 g/d) for 12 mo in 40 patients after curative resection for CD. After 3 mo of treatment, the antibiotic-probiotic combination resulted in a significantly lower incidence of severe endoscopic recurrence compared to mesalazine [2/20 (10%) vs 8/20 (40%)]. This difference was maintained throughout the study period [4/20 (20%) vs 8/20 (40%)].[69]

No such clinical effect was seen in a study by Prantera et al.[70] who reported that the probiotic Lactobacillus GG could not prevent post-operative disease recurrence in an 1-year double-blind, placebo-controlled trial. Similar negative results have been recently reported by the GETAID French group. A randomised double-blind, placebo-controlled study showed that Lactobacillus johnsonii LA1 (4x10^9 cfu/d) is not superior to placebo in preventing endoscopic recurrence of CD.[70]

In a small pilot study,[71] treatment with capsules containing E.coli Nissle 1917 was compared to placebo in the maintenance of steroid-induced remission of colonic CD. Twelve patients were treated with E.coli Nissle and 11 with placebo. The results showed that at the end of the 12-wk treatment period, the relapse rate is 33% in the E.coli group and 63% in the placebo group. Unfortunately, because of the small number of patients treated, this difference did not reach statistical significance.

However, a small comparative open study[72] showed that the combination of Saccharomyces boulardii (1 g/d) and mesalazine (2 g/d) is significantly superior to mesalazine (3 g/d) in maintenance of remission, suggesting that probiotic treatment in CD may be beneficial. More recently, a double-blind trial showed that Lactobacillus GG is not superior to placebo in prolonging remission in children with CD when given as an adjunct to standard therapy[73].

Pouchitis

Although probiotics are less widely used in clinical practice than antibiotics, they may be efficacious in the prevention and treatment of pouchitis. We have compared the efficacy of VSL#3 with placebo in the maintenance and treatment of chronic pouchitis[74]. Forty patients who obtained clinical and endoscopic remission after 1 mo of combined antibiotic treatment (rifaximin 2 g/d + ciprofloxacin 1 g/d) were randomised to receive VSL#3, 6 g daily (1800 billion bacteria/d) or a placebo of identical appearance for 9 mo. Clinical assessment was carried out every month, endoscopic and histological assessments were performed at entry and subsequently every two months. Stool samples were cultured before and after antibiotic treatment and subsequently every month during maintenance treatment. Relapse was defined as an increase of at least 2 points in the clinical portion of the PDAI and confirmed endoscopically and histologically. Whilst all 20 patients treated with placebo had a relapse during the 9 mo follow-up period, 17 of the 20 (85%) patients treated with VSL#3 remained in remission at this point. Interestingly, all these 17 patients had a relapse within 4 mo after the active treatment. Faecal concentrations of lactobacilli, bifidobacteria and Streptococcus salivarius subsp. thermophilus were significantly increased within 1 mo after VSL#3 treatment, and remained stable throughout the study. However, this increase did not affect the concentration of the other bacterial groups, suggesting that the beneficial effect of treatment is not mediated by suppression of endogenous luminal bacteria.

A recent study examining the maintenance of remission in patients with refractory or recurrent pouchitis showed that remission is achieved in 85% of patients treated with VSL#3, 6 g/d (1800 billion bacteria/d) and 6% in
the placebo group after 1 year of treatment[73]. In addition, continuous administration of VSL#3 results in a significant increase in IL-10 tissue levels, a significant decrease in tissue levels of the pro-inflammatory cytokines (TNF alpha, IL-1 and IFN gamma) and a decrease in matrix metalloprotease activity[76]. This may aid our understanding of the mechanisms of action by which VSL#3 maintains remission in pouchitis. In contrast, Lactobacillus GG is ineffective in preventing relapse in patients with chronic pouchitis[77].

We have also carried out a double-blind, placebo-controlled trial to evaluate the efficacy of VSL#3 in preventing pouchitis onset following ileal-anal anastomosis for UC[78]. Forty patients were randomised to receive VSL#3, 3 g per day (900 billion bacteria/d) or an identical placebo for 12 mo. Patients were assessed clinically, endoscopically and histologically at 1, 3, 6, 9 and 12 mo according to the PDAI. The results indicate that patients treated with VSL#3 have a significantly lower incidence of acute pouchitis compared with those treated with placebo during the first year of ileostomy closure (10% vs 40%; P < 0.05). Moreover, the IBD Questionnaire score is significantly improved in the group treated with VSL#3, and the median stool frequency in patients not developing pouchitis, is significantly less in the VSL#3 group compared with the placebo group[79].

CONCLUSION

There is strong evidence that enteric commensal bacteria are involved in the pathogenesis of IBD. Therefore, modification of the gut bacterial flora by antibiotics and probiotics may be effective in treating UC, CD and pouchitis.

Antibiotics are a well established, efficacious treatment option for various manifestations of IBD. Antibiotics play an essential role in treating the septic complications of CD, including intra-abdominal and perianal abscesses and perianal fistulae, although their use in CD as a primary therapy is poorly documented. There is good evidence that ciprofloxacin, metronidazole or their combination is effective in Crohn’s colitis and ileocolitis, though not in isolated ileal disease. Large controlled trials are needed to define optimal antibiotic regimens. In addition, their use in UC is not supported by the available studies and large trials with broad-spectrum agents are required. Although proper controlled trials have not yet been conducted, the use of antibiotics in pouchitis is largely justified.

Probiotics provide an attractive alternative to antibiotics in the treatment of IBD as trials to date have shown that they safe and have no side-effects. Promising results have been obtained from studies using probiotics, in both the prevention of relapse and the treatment of mild to moderate attacks of UC. Studies using probiotics in the treatment of CD are less clear due to conflicting and limited data. There is also considerable evidence that the highly concentrated cocktail of probiotics, VSL#3 is efficacious in preventing pouchitis onset and relapse.

Studies have highlighted the importance of selecting a well characterised probiotic preparation for treatment. In fact, viability and survival of bacteria in many available preparations are unproven. It should be remembered that the beneficial effect of one probiotic preparation does not imply efficacy of other preparations containing different bacterial strains, because each individual probiotic strain has its unique biological properties.

There is a need to improve our understanding of the composition of the enteric flora and the relationship between intestinal physiology and the luminal ecosystem. Only then can we truly optimise the bacteria-modifying treatments now available to effectively treat IBD.

REFERENCES

1. Sartor RB. Review article: Role of the enteric microflora in the pathogenesis of intestinal inflammation and arthritis. Aliment Pharmacol Ther 1997; 11 Suppl 3: 17-22, discussion 22-23
2. Rutgeerts P, Goboes K, Peeters M, Hiele M, Penninckx F, Aerts R, Kerremans R, Vantrappen G. Effect of faecal sample diversion on recurrence of Crohn’s disease in the neoterminal ileum. Lancet 1991; 338: 771-774
3. D’Haens GR, Geboes K, Peeters M, Baert F, Penninckx F, Rutgeerts P. Early lesions of recurrent Crohn’s disease caused by infusion of intestinal contents in excluded ileum. Gastroenterology 1998; 114: 262-267
4. Guarner F, Casellas F, Borruel N, Antolin M, Videla S, Vilaseca J, Malagelada JR. Role of microecology in chronic inflammatory bowel diseases. Eur J Clin Nutr 2002; 56 Suppl 4: S34-S38
5. Neut C, Bulois P, Desrouaux P, Membre JM, Lederman E, Gambiez L, Cortot A, Quandalle P, van Kruiningen H, Colombel JF. Changes in the bacterial flora of the neoterminal ileum after ileocolonic resection for Crohn’s disease. Am J Gastroenterol 2002; 97: 939-946
6. Sartor RB. Targeting enteric bacteria in treatment of inflammatory bowel diseases: why, how, and when. Curr Opin Gastroenterol 2003; 19: 351-358
7. Dohmann R, Kaiser I, Herrmann E, Mayet W, Ewe K, Meyer zum Büschenfelde KH. Tolerance exists towards resident intestinal flora but is broken in active inflammatory bowel disease (IBD) Clin Exp Immunol 1995; 102: 448-455
8. Dohmann R, May E, Heike M, Knolle P, Neurath M, Meyer zum Büschenfelde KH. T cell specificity and cross reactivity towards enterobacteria, bacteroides, bifidobacterium, and antigens from resident intestinal flora in humans. Gut 1999; 44: 812-818
9. Rath HC, Schultz M, Freitag R, Dieleman LA, Li F, Lindel HJ, Schölmerich J, Sartor RB. Different subsets of enteric bacteria induce and perpetuate experimental colitis in rats and mice. Infect Immun 2001; 69: 2277-2285
10. Madsen KL, Doyle JS, Tavernini MM, Jewell LD, Rennie RP, Federak RN. Antibiotic therapy attenuates colitis in interleukin 10 gene-deficient mice. Gastroenterology 2000; 118: 1094-1105
11. Hoentjen F, Harmens HJ, Braat H, Torrice CD, Mann BA, Sartor RB, Dieleman LA. Antibiotics with a selective aerobic or anaerobic spectrum have different therapeutic activities in various regions of the colon in interleukin 10 gene deficient mice. Gut 2003; 52: 1721-1727
12. Fiorucci S, Distruiti E, Moncarelli A, Barbanti M, Palazzini E, Morelli A. Inhibition of intestinal bacterial translocation with rifaximin modulates lamina propria mononuclear cells reactivity and protects against inflammation in a rodent model of colitis. Digestion 2002; 66: 246-256
13. Baniias G, Marin M, Moskaluk CA, Odashima M, Ross WG, Rivera-Suves J, Cominelli F. Down-regulation of intestinal lymphocyte activation and Thi cytokine production by antibiotic therapy in a murine model of Crohn’s disease. J Immunol 2002; 169: 5308-5314
14. Yamada T, Deitch E, Specian RD, Perry MA, Sartor RB, Grisham MB. Mechanisms of acute and chronic intestinal inflammation induced by indomethacin. Inflammation 1993; 17: 641-662
15. Onderdonk AB, Hermos JA, Dzink JL, Bartlett JG. Protective

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tion of Lactobacillus reuteri R2LC and oat fiber on acetic acid-induced colitis in the rat. *Scand J Gastroenterol* 1993; 28: 155-162

Mao Y, Nobaek S, Kasravi B, Adawi D, Stenram U, Molin G, Jeppsson B. The effects of Lactobacillus strains and oat fiber on methotrexate-induced enterocolitis in rats. *Gastroenterology* 1996; 111: 334-344

Madsen KL, Doyle JS, Jewell LD, Tavernini MM, Fedorak RN. Lactobacillus species prevents colitis in interleukin 10 gene-deﬁcient mice. *Gastroenterology* 1999; 116: 1107-1114

Schultz M, Veldkamp C, Dieleman LA, Grether WB, Wyrick PB, Tonkonogy SL, Sartor RB. Lactobacillus plantarum 299V in the treatment and prevention of spontaneous colitis in interleukin-10-deﬁcient mice. *Inflamm Bowel Dis* 2002; 8: 71-80

O’Mahony L, Feehely M, O’Halloran S, Murphy L, Kiely B, Fitzgibbon J, Lee G, O’Sullivan G, Shanahan F, Collins JK. Probiotic impact on microbial ﬂora, inﬂammation and tumour development in IL-10 knockout mice. *Aliment Pharmacol Ther* 2001; 15: 1219-1225

McCarthy J, O’Mahony L, O’Callaghan L, Shell B, Vaughan EE, Fitzsimons N, Fitzgibbon J, O’Sullivan GC, Kiely B, Collins JK, Shanahan F. Double blind, placebo controlled trial of two probiotic strains in interleukin 10 knockout mice and mechnistic link with cytokine balance. *Gut* 2003; 52: 975-980

Shiboleth O, Karmeli F, Eliakim R, Swennen E, Brüggen P, Gionchetti P, Campieri M, Morgenstern S, Rachmilewitz D. Variable response to probiotics in two models of experimental colitis in rats. *Inflamm Bowel Dis* 2002; 8: 399-406

Madsen K, Cornish A, Soper P, McKaigney C, Jijon H, Yachimec C, Doyle J, Jewell L, De Simone C. Probiotic bacteria enhance murine and human intestinal epithelial barrier function. *Gastroenterology* 2001; 121: 580-591

Kruis W, Schütz E, Fric P, Fiss G, Judmaier G, Stolte M. Double-blind comparison of an oral Escherichia coli preparation and mesalazine in maintaining remission of ulcerative colitis. *Aliment Pharmacol Ther* 1997; 11: 853-858

Rembacken BJ, Snellings AM, Hawkey PM, Chalmers DM, Axon AT. Non-pathogenic Escherichia coli versus mesalazine for the treatment of ulcerative colitis: a randomised trial. *Lancet* 1999; 354: 635-639

Kruis W, Fric P, Pokrotnieks J, Lukás M, Fiss G, Kacskák M, Kamm MA, Weismueller J, Beglinger C, Stolte M, Wolff C, Schulze J. Maintaining remission of ulcerative colitis with the probiotic Escherichia coli Nissle 1917 is as effective as with standard mesalazine. *Gastroenterology* 2000; 119: 305-309

Venturi A, Gionchetti P, Rizzello F, Johansson R, Zucconi E, Brüggen P, Mezzetti D, Campieri M. Impact on the composition of the faecal flora by a new probiotic preparation: preliminary data on maintenance treatment of patients with ulcerative colitis. *Aliment Pharmacol Ther* 1999; 13: 1103-1108

Bibiloni R, Fedorak RN, Tannock GW, Madsen KL, Gionchetti P, Campieri M, De Simone C, Sartor RB. VSL#3 probiotic-mixture induces remission in patients with active ulcerative colitis. *Am J Gastroenterol* 2005; 100: 1539-1546

Guslandi M, Giollo P, Testoni PA. A pilot trial of Saccharomyces boulardii in ulcerative colitis. *Eur J Gastroenterol Hepatol* 2003; 15: 697-698

Campioni M, Rizzello F, Venturi A, Poggioli G, Ugolini F, Helwig U, Amadini C, Romboli E, Gionchetti P. Combination of Antibiotic and Probiotic Treatment is efficacious in prophylaxis of post-operative recurrence of Crohn’s Disease: A randomised controlled Study vs. Mesalazime. *Gastroenterology* 2000; 118: A781

Prantera C, Scribano ML, Falasco G, Andreoli A, Luzi C. Ineffectiveness of probiotics in preventing recurrence after curative resection for Crohn’s disease: a randomised controlled trial with Lactobacillus GG. *Gut* 2002; 51: 405-409

Marteau P, Lemann M, Seksik P, Laharie D, Colombel JF, Bouchnik Y, Cadot G, Soule JC, Bourreille A, Metan E, Lerembouers E, Carbonnel F, Dupas JL, Veyrac M, Coffin B, Moreau J, Abitbol V, Blum-Sperisen S, Mary JY. Ineffectiveness of Lactobacillus johnsonii L1 in prophylaxis of postoperative recurrence in Crohn’s disease: a randomised, double-blind, placebo-controlled GETAID trial. *Gut* 2006; 55: 842-847

Malchow HA. Crohn’s disease and Escherichia coli. A new approach in therapy to maintain remission of colonic Crohn’s disease? *J Clin Gastroenterol* 1997; 25: 653-658

Guslandi M, Mezzi G, Sorgi M, Testoni PA. Saccharomyces boulardii in maintenance treatment of Crohn’s disease. *Dig Dis Sci* 2000; 45: 1462-1464

Bousvaros A, Guandalini S, Baldassano RN, Botelho C, Evans J, Ferry GD, Golden B, Hartigan L, Kugathasan S, Levy J, MurrayKF, Oliva-Hemker M, Rosh JR, Tolia V, Zholudev A, Vandevoort JA, Hibberd PL. A randomized, double-blind trial of Lactobacillus GG versus placebo in addition to standard maintenance therapy for children with Crohn’s disease. *Inflamm Bowel Dis* 2005; 11: 833-839

Gionchetti P, Rizzello F, Venturi A, Brüggen P, Matteuzzi D, Bazzocchi G, Poggioli G, Miglioli M, Campieri M. Oral bacteriotherapy as maintenance treatment in patients with chronic pouchitis: a double-blind, placebo-controlled trial. *Gastroenterology* 2002; 10: 305-309

Mimura T, Rizzello F, Helwig U, Poggioli G, Schreiber S, Talbot JC, Nicholls RJ, Gionchetti P, Campieri M, Kamm MA. Once daily high dose probiotic therapy (VSL#3) for maintaining remission in recurrent or refractory pouchitis. *Gut* 2004; 53: 108-114

Ulisse S, Gionchetti P, D’Alò S, Russo FP, Pesce I, Ricci G, Rizzello F, Helwig U, Cifone MG, Campieri M, De Simone C. Expression of cytokines, inducible nitric oxide synthase, and matrix metalloproteinases in pouchitis: effects of probiotic treatment. *Am J Gastroenterol* 2001; 96: 2691-2699

Kuisma J, Mentula S, Jarvinen H, Kahri A, Saxelin M, Farkkila M. Effect of Lactobacillus rhamnosus GG on ileal pouch inﬂammation and microbial flora. *Aliment Pharmacol Ther* 2003; 17: 509-515

Gionchetti P, Rizzello F, Helwig U, Venturi A, Lammers KM, Brüggen P, Vitali B, Poggioli G, Miglioli M, Campieri M. Prophylaxis of pouchitis onset with probiotic therapy: a double-blind, placebo-controlled trial. *Gastroenterology* 2003; 124: 1202-1209