Probiotics and inflammatory bowel disease

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Probiotics are defined as 'mono- or mixed cultures of live micro-organisms which, when applied to animal or man, beneficially affect the host by improving the properties of the indigenous microflora'.[1] Both *Lactobacilli* spp. and *Bifidobacterium* spp. are frequently applied as probiotics. Probiotic bacteria for humans are preferably of human origin: they have to be safe for the host, genetically stable, and capable of surviving passage through the gastrointestinal tract.[2] Among the effects claimed for probiotics are beneficial immunomodulation, reduction of serum cholesterol, improved lactose digestion and protection against colon cancer.[2,3] Probiotics have also been studied in infectious diarrhoea, inflammatory bowel disease and pouchitis.[3,4] In this review we focus on the possible value of probiotics in inflammatory bowel disease (IBD).

BACTERIAL FLORA IN INFLAMMATORY BOWEL DISEASE

In ulcerative colitis (UC) the inflammatory response is confined to the mucosa and submucosa of the colon with clear demarcations. In Crohn's disease (CD), the entire gastrointestinal tract can be involved and the inflammation can extend through the intestinal wall from mucosa to serosa. Areas of inflammation may be interspersed with relatively normal mucosa. In CD, the predominant symptoms are diarrhoea, abdominal pain and weight loss whereas in UC diarrhoea is the main symptom, often accompanied by rectal bleeding. Both diseases are common in the industrialized world, with highest incidences in North America and Northern Europe.[5] In a European study,[6] the average annual incidence was 5.9/100 000 for CD and 11.2/100 000 for UC. The peak age of onset for both diseases is between 15 and 30 years with a second minor peak between 55 and 80 years. CD shows a higher incidence in females than in males. UC seems more equally distributed between the sexes, with a tendency to a male preponderance.[5]

The aetiology of IBD is unknown but both genetic and environmental factors are thought to contribute. Dietary habits, oral contraceptives and breastfeeding have come under suspicion as conditioning factors. Smoking is positively associated with CD, non-smoking with UC. The intestinal bacterial flora is thought to be an important factor in the development and recurrence of IBD, but exactly how has not yet been elucidated. Current treatment of IBD relies mainly on anti-inflammatory drugs, immunomodulators, nutritional supplements and surgery.

The human gastrointestinal tract contains about 10^{14} bacteria, with small numbers in the stomach ($<10^3$/mL) rising with descent of the tract to 10^{11}–10^{12}/mL in the colon. Here the anaerobes outnumber the aerobes 100–1000-fold.[4,7] Among other beneficial effects, the intestinal bacterial flora contributes to digestion of nutrients and metabolism of (pro)carcinogens and has an important barrier function against pathogens. For example, short-chain fatty acids are produced by anaerobic bacterial fermentation of luminal carbohydrates and proteins; the intestinal epithelial cells depend on short-chain fatty acids (especially butyrate) as energy source. Furthermore, products of intestinal bacterial flora such as peptidoglycan and lipopolysaccharides are immunostimulants.

The triggering of chronic intestinal inflammation seems to depend somehow on the flora. In laboratory animals such as interleukin (IL)-10 deficient mice or chemically treated rats, IBD-like intestinal inflammation will not occur without the presence of an intestinal bacterial flora.[8,9] In health, the intestinal immune response to the resident bacteria will normally be limited by three factors—homoeostasis in the bacterial flora, relative impermeability of the mucosal barrier, and a suppressive immune response. The genetically susceptible person in whom the intestinal bacterial flora may induce chronic intestinal inflammation is likely to be characterized by a low suppressive immune response or a deficient ability to heal injured intestinal mucosa.[10]

A defective suppressive immune response has been reported in patients with IBD.[11,12] Normally, intestinal antigens will be taken up by macrophages or antigen-presenting cells and will subsequently be recognized by CD4+ T cells. The CD4+ T cells can be divided into subsets based on the profile of cytokine production: Th1 lymphocytes produce IL-2 and interferon-gamma and are associated with cellular immune responses and increased

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IgG2 production. The Th2 lymphocytes produce II-4, IL-5, IL-6 and IL-10 and are associated with hypersensitivity reactions and increased IgG1 production. A balance between these cytokine profiles is important for an effective suppressive immune response.

The pattern of cytokine production indicates that CD is a Th1-condition.[11,12] In CD there is overproduction of IgG with relative deficiency of mucosal IgA. In UC there is preferential expression of Th2 cytokines—IL-4 and IL-5—and an autoimmune response to epithelial cells.[11,12]

The hypothesis that the intestinal bacterial flora contributes to the pathogenesis of IBD is supported by several experimental and clinical observations in man. The parts of the gut with highest bacterial counts are the sites most affected by IBD—i.e. the terminal ileum and colon[4]—and antibiotic treatment has lessened disease activity in both UC and CD.[7] Enteric bacteria and their products have been detected within inflamed mucosa of patients with Crohn's disease.[4] Crohn's disease is improved by diversion of the faecal stream from the affected segment[13] and also by washing-out of the luminal contents.[14] Several groups have investigated the composition of the intestinal bacterial flora in CD patients. Faecal numbers of anaerobic bacteria, especially *Bacteroides* spp., were reported greater than in healthy controls.[15–17] Giaffer *et al.*[18] found no difference in total anaerobes between active CD patients, inactive CD patients and healthy controls; but they did find more aerobes and enterobacteria in active CD, and fewer lactobacilli in CD patients, than in healthy controls. A decrease in bifidobacteria but not lactobacilli has also been reported.[19] Other research groups have focused on the role of specific pathogens in CD such as *Mycobacterium paratuberculosis*, *Listeria monocytogenes* and paramyxovirus but overall the results are hard to interpret.[20]

Recently, mutations in the NOD2 gene (renamed as CARD15) on chromosome 16 (IBD1 locus) were reported to be associated with CD but not UC. NOD2 proteins are cytosolic proteins that are involved in the intracellular recognition of bacterial components and activate nuclear factor κB (NF-κB), a transcriptional factor that contributes to innate immunity. Innate immunity is an important host defence mechanism expressed in monocytes, granulocytes and dendritic cells.[21] Mutations in the NOD2 gene are found in about 20% of CD patients. The exact role of NOD2 is not yet clear but the finding is consistent with the hypothesis that the bacterial flora is relevant to the pathogenesis of CD.

In UC patients, enteric infection is seldom detected but a change in the intestinal bacterial flora has been observed. Increased numbers of aerobes were found in colonic mucosal biopsies and in faecal samples.[15,17,22] A decrease in numbers of obligate anaerobic bacteria and lactobacilli in active but not in inactive UC was reported by Fabia *et al.*[23] Increased numbers of anaerobes have also been reported,[17,22] although less than in CD patients.[17] In this context a possible role was found for *Bacteroides vulgatus*: numbers were high and the antibody response to this bacterium was enhanced.[22] The mucosal barrier of UC patients has been characterized by a thin mucus layer and subnormal epithelial cell metabolism of butyrate.[15,20] Epithelial butyrate metabolism can be blocked by deficient production of short-chain fatty acids[15] and by the hydrogen sulphide released by excess numbers of sulphate-reducing bacteria.[24]

The intestinal flora has been investigated not only in UC and CD but also in pouchitis. Pouchitis develops in 7–45% of patients with an ileal pouch and is most frequently encountered in patients with UC.[25] It is characterized by an increase in aerobes, a decrease in anaerobes, an increase in bile acids and a decrease in short-chain fatty acids with a subsequent increase in the faecal pH.[25–27] Some workers have reported an increase in anaerobes.[4,28] Work in this whole area is plagued by inconsistent findings. Studies are difficult to compare because culture methodologies differ, active and inactive disease are not always analysed separately, and drug use and disease localization are often not taken into account. Although all the above-mentioned findings support the hypothesis that the intestinal bacterial flora contributes to the pathogenesis of IBD, more work is needed to clarify the mechanisms.

PROBIOTICS AND INFLAMMATORY BOWEL DISEASE

Because of the evidence implicating the intestinal bacterial flora in IBD, various attempts have been made to modify the flora with probiotics. In animals with experimental colitis orally or rectally administered lactobacilli have yielded improvements. In IL-10 deficient mice, *Lactobacillus plantarum* 299v prevented onset of disease and reduced established colitis.[29] In methotrexate-treated rats the onset and severity of colitis was reduced when *L. plantarum* 299v was given orally[30] but this lactobacillus had no effect on established colitis induced in rats by another method (TNBS/E).[31]

L. reuteri (R2LC) attenuated the development of colitis in IL-10 deficient mice,[32] in acetic-acid treated rats[23] and in methotrexate treated rats;[30] *L. salivarius* UCC118 decreased mucosal inflammatory activity in IL-10 deficient mice;[33] finally, a multispecies probiotic (VSL#3) given to IL-10 deficient mice with established colitis normalized gut barrier function, reduced proinflammatory cytokines and lessened histological disease.[34]

Table 1 gives an overview of intervention studies with probiotics in patients with UC or CD. In an open study, oral administration of *L. casei* GG (*L. GG*) was judged to

have increased mucosal IgA in children with either active or inactive CD.[35] When *L. GG* was given for twelve months to 4 children with moderately active CD receiving stable doses of corticosteroids, all improved clinically and 3 were able to have their steroids reduced.[36] *L. GG* was recovered from faecal samples in amounts ranging from 10^7 to 10^9 colony-forming units/g. 2 of the 4 patients received metronidazole as well as the probiotic, and this may have influenced the clinical outcome. Guslandi[37] studied 10 patients with inactive CD of ileum, colon or both, free from treatment with steroids for one month or other immunosuppressive agents for three months. He prescribed mesalazine 1 g twice daily in combination with *Saccharomyces boulardii* for six months; only one patient relapsed.

Subsequently, Guslandi *et al.*[38] compared two regimens in 32 patients with either mesalazine 1 g three times daily or mesalazine 1 g twice daily in combination with *S. boulardii* (number unreported) for six months. The relapse rate was significantly lower in patients treated with mesalazine plus *S. boulardii* (6%) than in those treated with mesalazine alone (38%). Evaluation of this result is hindered by the difference in doses of mesalazine. In an overview, Mattila-Sandholm *et al.*[39] reported on an open study in which *L. salivarius* UCC118 was been given for six weeks to 20 patients with relapsed CD. Clinical remission could not be induced in all patients but an overall increase in quality of life was recorded; detailed information on this study is not available. Only two studies in CD patients were placebo controlled. Malchow[40] compared the non-pathogenic *Escherichia coli* Nissle 1917 with placebo for one year in 32 patients with active colonic CD who were also treated with a standardized prednisolone schedule. Remission rates were similar in the two groups, but subsequently a lower relapse rate was seen in the *E. coli* treated group (33% versus 64%); statistical analyses were lacking. Prantera *et al.*[41] studied 37 well-defined patients after a 'curative' resection for CD who were randomized to receive *L. GG* or placebo for one year. Clinical recurrence was observed in about the same proportions (17% versus 11%, respectively).

In 1989, Bennet *et al.*[42] reported the case of an adult with active UC, refractory to standard treatment regimens, who was symptom-free for six months after treatment with a short course of antibiotics followed by an enema of 'faecal bacteria' from a healthy donor.[42] *E. coli* Nissle 1917 has been compared with mesalazine in three studies of UC patients. Kruis *et al.*[43] included 103 patients in remission who were randomized to receive mesalazine 500 mg three times daily or *E. coli* for 12 weeks and, using a double-dummy design, found similar relapse rates for the two regimens. 23 patients were also treated with corticosteroids but this was not further discussed. Although the per-protocol analyses and intention-to-treat analyses gave similar results, it was not clear why 33 patients did not complete the total protocol. Furthermore, the study period was short for a maintenance study. Comparable results were later reported (in abstract) for 327 patients treated for twelve months in a similar design.[44] In another double-dummy study[45] clinically active UC patients (n=116) were treated for one week with gentamicin and then randomized to receive *E. coli* Nissle 1917 or mesalazine 800 mg three

Table 1 **Probiotics in patients with inflammatory bowel disease**

Reference (No.)	n	*Disease activity*	*Treatment*	*Duration*	*Effect*
Crohn's disease					
Malin 1996 (35)	14 children	Active/inactive	*L. GG*	10 days	↑ Mucosal IgA
Malchow 1997 (40)	28 adults	Active	Prednisolone+'*E. coli* vs placebo'	12 months	Reduced relapse rate (64% – 33%)
Guslandi 1999 (37)	10 adults	Inactive	*S. boulardii*+mesalazine	6 months	Only 1 relapse
Mattila-Sandholm 1999 (39)	20 adults	Active	*L. salivarius* UCC118	10 days	No remission, ↑ quality of life
Gupta 2000 (36)	4 children	Moder. active	*L. GG*	6 months	↓ Intestinal permeability, clinical disease activity
Guslandi 2000 (38)	32 adults	Inactive	'*S. boulardii*+5ASA' vs 5-ASA	6 months	Reduced relapse rate (38% – 6%)
Prantera 2002 (41)	37 adults	After 'curative' resection	*L. GG* vs placebo	12 months	Similar endoscopic recurrence
Ulcerative colitis					
Bennet 1989 (42)	1 adult	Active	Antibiotic+faecal enema	Once	Induced remission
Kruis 1997 (43)	108 adults	Inactive	*E. coli* vs mesalazine	12 weeks	Similar relapse rate
Kordecki 1998 (46)	19 adults	Active	*L. plantarum* (9 active/10 inactive)	?	6 of 9 patients treated with active form: in remission
Rembacka 1999 (45)	116 adults	Active	*E. coli* vs mesalazine	12 months	Similar remission and relapse rates
Venturi 1999 (47)	20 adults	Inactive	Multispecies (VSL#3)	12 months	75% still in remission and changed faecal flora
Kruis 2001 (44)	327 adults	Inactive	*E. coli* vs mesalazine	12 months	Similar relapse rate

times daily for twelve months; in addition, all patients were treated with corticosteroids. Remission rates and subsequent relapse rates were the same in the two groups. A small open study has been performed in active UC patients.[46] 6 of 9 patients given viable *L. plantarum* 299v reached remission, compared with none of 10 patients treated with inactivated bacteria. The abstract does not give information on concomitant use of medication or how disease activity was scored. Finally, in an uncontrolled study, Venturi *et al.*[47] examined the effect of a multispecies probiotic (three strains of bifidobacteria, four of lactobacilli and one of *Streptococcus salivarius* sp. *thermophilus* (i.e. VSL#3)) for twelve months in 20 patients with inactive UC who were intolerant to mesalazine. 15 of 20 patients remained in remission. Venturi's is the only research group who also investigated the effect of the probiotic product on the faecal flora, finding an increase in bifidobacteria, lactobacilli and *S. salivarius*, but no change of other bacterial species.

Gionchetti *et al.*[48] performed a double-blind placebo-controlled study with VSL#3 in 40 pouchitis patients with clinical and endoscopic remission. The multispecies probiotic significantly reduced the number of patients with relapses in the nine-month treatment period—15% versus 100%.

CONCLUSION

Studies on probiotics in animal models of colitis are promising. Although clinical results in IBD patients are also encouraging, the data are limited and few studies are placebo-controlled. Additional placebo-controlled double-blind studies in CD and UC, active and inactive, taking into account other medical therapy, are required before recommendations can be offered on routine use of probiotics in IBD. More data are also needed on the properties of the various bacterial strains for different clinical indications, as well as information on dose and duration of treatment. If probiotics do prove to have beneficial effects in IBD, investigation of the mechanisms may well lead to further advances in treatment.

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