REVIEW ARTICLE

Probiotics for the young and the not so young

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Although interest in the effects of the intestinal flora on health dates from the beginning of the 20th century, controlled clinical trials did not begin until its end. Oral administration of probiotic lactobacilli has been shown to alleviate and prevent atopic eczema. Similar effects were accomplished by administering fructose and glucose oligosaccharides (prebiotics) which encouraged the proliferation of endogenous lactobacilli and bifidobacteria. Nonpathogenic Escherichia coli has been shown to protect premature infants from infection. Twenty years later, infants given this E. coli were still found to be less allergic. Meta-analyses have confirmed that Lactobacillus rhamnosus strain GG prevents and treats rotavirus diarrhoea. By reversing the changes in bowel flora which occur with age comes the prospect of rejuvenating ageing immune systems, preventing Clostridium difficile infections and bowel cancer, thereby fulfilling the early promise of prolongation of life.

Keywords Bifidobacterium, Elderly, Gut microflora, Infants, *Lactobacillus*, Probiotics.

INTRODUCTION

Interest in the potential health benefits of bowel flora began at the start of the 20th century with Elie Metchnikoff, a Russian expatriate working at the Pasteur Institute, who hypothesized that the presence of lactose-fermenting bacteria in the colon could prolong life (Metchnikoff 1907). The theme was taken up by the Japanese scientist Dr Minoru Shirota, who isolated a promising probiotic strain subsequently named *Lactobacillus casei* Shirota, and established the company, Yakult Ltd. By the end of the 20th century, interest revived when Erica Isolauri's group showed that atopic dermatitis could be prevented by oral administration of lactobacilli (Isolauri *et al*. 2000). It is fitting that studies into the potential health benefits of probiotic bacteria have now come full circle and there is increasing evidence for positive influences on health in the elderly (Tuohy *et al*. 2000; O'May and Macfarlane 2005).

As was shown back in 1921 by Rettger and Chepplin (1921), the gut flora can be modified favourably by the administration of exogenous probiotic microorganisms (Goldin and Gorbach 2008) or by the administration of poorly absorbed oligosaccharides (prebiotics) which favour the growth of endogenous lactose-fermenting bacteria. When the probiotics and prebiotics are combined, the resulting product is a synbiotic.

MODIFICATION OF INTESTINAL FLORA IN INFANCY

Atopic dermatitis and the bowel flora

The first inkling that the nature of the intestinal flora could protect against the development of atopy arose from epidemiological studies from Sweden (Alm *et al*. 1999). The European Philosopher Rudolph Steiner (1861–1925) founded the Anthroposophic Movement. Devotees eschew many aspects of western medicine and favour spontaneously fermented vegetables, rich in lactobacilli. Their children were shown to have fewer positive skin prick tests and less atopy than their peers in the same village whose parents did not espouse the anthroposophic lifestyle. The role of lactobacilli in allergy prevention was confirmed by studies in Swedish and Estonian children. Allergy was more common in the more affluent Sweden, but non-allergic children in both countries were colonized by lactobacilli (and bacteroides), whereas in the allergic

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children, *Staphylococcus aureus* predominated in Sweden and *Escherichia coli* in Estonia.

The association was demonstrated in a groundbreaking clinical trial from Finland, in which infants and children with eczema recovered quicker if they were given *Lactobacillus rhamnosus* strain GG, in addition to a milk-free diet. Of great interest was the subgroup of breast-fed infants with eczema, who improved when their mothers were given *L. rhamnosus* strain GG. Measurement of inflammatory markers in the stool showed that the lactobacilli were exerting an anti-inflammatory effect on the gut. The same group showed that *L. rhamnosus* strain GG could decrease the risk of atopic dermatitis from 46% to 23% in the infants of mothers given the lactobacilli during pregnancy and continued during lactation; unfortunately, the prevention did not extend to asthma or rhinitis (Kalliomaki *et al*. 2001).

Bifidobacteria predominate in the intestinal flora of breast-fed babies. Breast milk contains a complex mixture of oligosaccharides which favours the growth of bifidobacteria. When a mixture of oligosaccharides (fructo- and galacto-oligosaccharides) is added to infant formulae, bifidobacteria predominate in these infants' stools (Veereman 2007). Their stools resemble the unformed stools of breast-fed babies, as does the stool pH and their short-chain fatty acid content. Formula-fed babies have a more diverse microbiota, with more clostridia and Gram-negative anaerobes (Edwards 2006). Recent studies have investigated the possibility that the bifidobacteria flora convey protection against infection and allergy. As in the trials with *L.* rhamnosus strain GG, the above prebiotics reduced the risk of atopic dermatitis by from 23% to 10% when administered to infants (Moro *et al*. 2006).

Interesting work conducted in the Czech Republic followed the progress of a group of children for 20 years (Lodinova-Zadnikova *et al*. 1991, 2003) after they were administered a nonpathogenic *E. coli* O83:K24:H31 as infants. This resulted in the strain remaining dominant in the intestine over several months, stimulating both serum and local antibody. In high-risk infants, the probiotic appeared to decrease the number of infections and reduce fatalities and antibiotic use. Questionnaires completed after 10 and 20 years showed a significant reduction in the occurrence of allergies in the probiotic-fed group compared to a control group. The occurrence of repeated infections was lower in the probiotic group compared to controls after 10 years, but not 20 years.

Probiotics and acute diarrhoea

A metareview concluded that the efficacy of probiotics in the treatment of paediatric diarrhoeal disease could be considered to be well established (Szajewska *et al*. 2006), but more trials are needed, in particular, to prove efficacy for each strain (Canani *et al*. 2007). The main benefit appears to be for reducing the diarrhoea duration, and was most helpful for watery diarrhoea and viral gastroenteritis. A similar review by Sazawal *et al*. (2006) concluded that probiotics reduced the associated risk of diarrhoea among children by 57% (35–71%), based on analysis of 34 randomized, blinded placebo-controlled trials. McFarland *et al*. (2006) examined 39 studies and concluded that probiotics were a safe and effective method to prevent and treat acute paediatric diarrhoea. Most studies on diarrhoea in children have been with *L. rhamnosus* strain GG, but efficacy has been shown for other strains, including *E. coli* Nissle 1917, *L. casei* DN-114 001, *L. casei* Shirota, *Lactobacillus reuteri* DSM 12246, *L. paracasei* ST11, *L. rhamnosus* 19070-2 and strains of *L. acidophilus*, *Bifidobacterium bifidum* and *Saccharomyces boulardii* (Examples: Sugita and Togawa 1994; Guandalini *et al*. 2000; Pedone *et al*. 2000; Rosenfeldt *et al*. 2002a,b; Sarker *et al*. 2005; Henker *et al*. 2007; Szajewska *et al*. 2007). In randomized trials involving almost 1000 children, the duration of acute diarrhoea was decreased by 1 day (2 days in the case of rotavirus diarrhoea).

Probiotics and the short gut

Our own experience with the therapeutic effect of lactobacilli began in 1998. Faced with an infant who had no remaining gut beyond the proximal jejunum, and whose daily stool output averaged 10% of his body weight, we administered *L. casei* Shirota via a gastrostomy feeding tube. His jejunal effluent changed from a mixed faecal flora to lactobacilli while his diarrhoea reduced dramatically and his sodium absorption markedly increased (Candy *et al*. 2002). We became convinced that probiotic bacteria had genuine therapeutic applications.

Other published reports contribute to the evidence for probiotic and synbiotic benefit in children with short bowel syndrome (Vanderhoof *et al*. 1998; Kanamori *et al*. 2001, 2003, 2004).

Probiotics and necrotizing enterocolitis

The infant described above lost the majority of his intestine from necrotizing enterocolitis, in which varying lengths of gut develop a pathological condition resembling gas gangrene. Premature babies are predisposed, perhaps because the sterile environment in which they are nursed delays the acquisition of a protective gut flora. Evidence for probiotic benefit with necrotizing enterocolitis is still limited to a few published studies, but these have shown positive results (Cooke *et al*. 2008). Administration of a probiotic containing *L. acidophilus* and *Bifidobacterium infantis* reduced the incidence of necrotizing enterocolitis in preterm infants when compared with historical controls

(Hoyos 1999). The same combination was shown to be effective in a study conducted in China (Lin *et al*. 2005), where the probiotics were fed enterally with breast milk. In a study in Israel (Bin-Nun *et al*. 2005), low birth-weight babies were randomized to receive either a probiotic supplement (a combination of *B. bifidus*, *B. infantis* and *Streptococcus thermophilus*) or no supplement at all. It was concluded that probiotic supplement reduced both the incidence and the severity of necrotizing enterocolitis in the premature neonatal population.

MODIFICATION OF BOWEL FLORA IN OLDER PEOPLE

Changes in bowel flora with age

The predominant species of bowel flora changes throughout life. Bifidobacteria, which are dominant in the neonatal period and early infancy are joined by bacteroides, *E. coli* and enterococci, lactobacilli and clostridia. These genera replace bifidobacteria in later life. In old age, an adverse change occurs in the relative numbers of different populations and the nature of species in the gut (Tuohy *et al*. 2000; Hopkins and Macfarlane 2002; Hopkins *et al*. 2002; Hebuterne 2003). Levels of bifidobacteria, for example, start to drop after the age of 60 and can fall by 1000-fold. One study has examined the link between the gut microbiota and frailty in the elderly (Van Tongeren *et al*. 2005). The faecal flora of elderly people with a high frailty score showed a significant reduction in the number of lactobacilli (26-fold), and reduced numbers of other predominant bacterial groups (*Bacteriodes/ Prevotella* and *Faecalibacterium prausnitzii*). In very frail elderly people, the number of Enterobacteriaceae was significantly higher. Thus, the possibility arises that modification of bowel flora in later life may have continuing health benefits.

Pseudomembranous colitis

Serious, life-threatening diarrhoea may follow administration of antibiotics. Endoscopic examination of the colon shows multiple yellow plaques at sites of mucosal necrosis. The disease is caused by *C. difficile*. In severe cases, colectomy may be required. Mortality in the UK from *C. difficile* exceeds methicillin-resistant *S. aureus*. Older people are at risk; infants can harbour *C. difficile* without symptoms. Possibly, the changes in bowel flora with age are a factor conferring susceptibility.

There have been several metareviews on the potential benefits of probiotics in prevention and/or treatment of antibiotic-associated diarrhoea (Barbut and Meynard 2002; D'Souza *et al*. 2002; Dendukuri *et al*. 2005; McFarland 2006; Sazawal *et al*. 2006). The general conclusion from such reviews was that probiotics may be of use, particularly lactobacilli and yeast, and particularly for prevention rather than treatment of the problem. Studies on pseudomembranous colitis have used the yeast *S. boulardii* which has been shown to prevent recurrence after treatment of *C. difficile* diarrhoea with vancomycin or metronidazole. A formulation of *L. acidophilus* and *B. bifidus* decreased the risk of *C. difficile* diarrhoea from 7.3 to 2.9% following a course of antibiotics. We are currently investigating the possibility that the administration of a *Lactobacillus* probiotic to older patients in a rehabilitation ward will decrease the risk of acquiring *C. difficile* diarrhoea.

Nutritional benefits

It has been suggested that probiotics (and prebiotics) enhance intestinal calcium absorption, which could help prevent osteoporosis. The mechanisms for this have been variously suggested as the following: increased solubility of minerals due to an increase in bacterial production of short-chain fatty acids; enlargement of the absorption surface by promoting proliferation of enterocytes mediated by the bacterial metabolites, notably lactate and butyrate; and increased expression of calcium-binding proteins. Probiotics are thought to degrade mineral-complexing phytic acid, stimulate calcium uptake by enterocytes and have an antiarthritic effect (Scholz-Ahrens *et al*. 2007).

Constipation

As many as 14 million people in the UK may be affected by constipation, and this problem is particularly likely to affect elderly people because of the increased likelihood of reduced mobility and reduced intake of fibre. There is relatively good evidence for certain probiotic strains for the relief and/or prevention of constipation. Probably more than one mechanism of activity involved. Production of short-chain fatty acids by species of lactobacillus and bifidobacterium is thought to help reduce the pH of the gut lumen, thereby stimulating the intestinal musculature and peristalsis. It has also been suggested that probiotics may improve transit time by increasing faecal bacterial mass and bacterial metabolism of bile acids in the colon. Probiotics may also affect gas production in the bowel, stimulating production of the hormone cholecystokinin and increasing the stimulatory response of the smooth muscle of the gut.

Several studies have been reported describing benefit with regard to constipation symptoms for certain probiotic strains such as *Bifidobacterium animalis* ssp. *lactis* DN-173010, *L. casei* Shirota, and a combination of *L. rhamnosus* and *Propionibacterium freudenreichii* strains (Koebnick *et al*. 2003; Marteau *et al*. 2002; Ouwehand *et al*. 2002; Hongisto *et al*. 2006; Matsumoto *et al*. 2006). A

double-blind placebo-controlled trial of *L. casei* Shirota, for example, in adults with abdominal pain and constipation showed improvement in symptoms in those randomized to receive the lactobacilli.

Overgrowth of oral yeast

The elderly are vulnerable to candida infection, partly because they may have poor oral hygiene and reduced salivary flow. The impairment of the immune system associated with old age is a further contributory effect. One study in Finland estimated that as many as 75% of elderly people in the country had oral colonization with yeast (Nahri *et al*. 1999). A recent double-blind placebo-controlled study has reported the efficacy of probiotics (*L. rhamnosus* GG, *L. rhamnosus* LC705, *P. freudenreichii* ssp. *shermanii* JS) delivered in a cheese product, in controlling oral candida and hyposalivation in people aged over 70 years (Hatakka *et al*. 2007).

Immune stimulation

The majority of lymph nodes in the body are located in the gut; one estimate puts this at 85% (MacDonald and Bateman 2006). The importance of the gut microbiota in the development and education of the immune system was first demonstrated with germ-free animals. Now the interaction of certain probiotics with the innate and acquired arms of the immune system is being elucidated at a molecular level. For example, probiotics have been reported to restore natural killer cell activity in individuals with low activity (Nagao *et al*. 2000; Gill *et al*. 2001; Morimoto *et al*. 2005). In a controlled study, 60 elderly subjects received an additional daily supplement to their normal diet, which consisted of a probiotic *L. paracasei* strain, a prebiotic fructo-oligosaccharide and vitamins and other nutrients (Bunout *et al*. 2004). After the study period, the subjects were given an influenza and pneumococcus vaccine. Various immune parameters were measured. This trial also showed increase in natural killer (NK) cell activity in the test group compared to the control group. During a 12-month follow-up period, the test group reported significantly fewer infections.

A controlled pilot study has been conducted on elderly people, with yogurt containing the probiotic *L. casei* DN-114 001. There was evidence of a potential 20% reduction in the duration of winter infections (gastrointestinal and respiratory), although no difference in incidence (Turchet *et al*. 2003). Other studies have been reported with adults and children, showing similar benefits (Hatakka *et al*. 2001; De Vrese *et al*. 2005; Tubelius *et al*. 2005; Winkler *et al*. 2005). These results suggest that probiotics may offer benefit to the elderly, people who are known to suffer immunosenescence, which is associated with increased morbidity and mortality rates with infectious diseases. The activity of NK cells and phagocytes is reduced in old age, as well as negative changes in T-cell effectiveness.

Cancer prevention

There is increasing evidence to show that the intestinal microbiota may be involved in the aetiology of colorectal cancer, and that certain probiotics and synbiotics may offer benefit, through a range of mechanisms of activity (Commane *et al*. 2005). Manipulation of the gut flora in favour of the beneficial bacteria genera may be one strategy that could help reduce risk of colon cancer. Clinical trials are difficult with cancer, and it is realized that evidence for probiotic benefit will involve the investigation of biomarkers that indicate either the likelihood of cancer occurrence or the rate of cancer development. Positive results from *in vitro* studies, using cell lines and animals are now beginning to be confirmed in clinical trials (Ishikawa *et al*. 2005; Rafter *et al*. 2007). Probiotic organisms are thought to bind and degrade carcinogens of dietary origin and displace bacteria which produce these metabolites (Goldin *et al*. 1980; Ling *et al*. 1994; Spanhaak *et al*. 1998; De Preter *et al*. 2004, De Preter *et al*. 2007). Such carcinogens, such as nitrosamines, are absorbed and excreted in the urine. Hence probiotics may also help prevent bladder cancer, and there is some clinical trial evidence for this (Aso and Akaza 1992; Aso *et al*. 1995; Ohashi *et al*. 2002).

Chronic liver disease

Neutrophil function can also be boosted by *L. casei* Shirota in adults with chronic alcoholic liver disease (Stadlbauer *et al*. 2008). The presumed mechanism of action is by suppressing intestinal coliform bacteria, thereby normalizing gut permeability and its barrier function and decreasing endotoxaemia. Evidence from this and previous studies indicated that it was the high level of endotoxin in the blood that caused suppression of neutrophil function.

CONCLUSIONS

The health benefits of probiotic bacteria were first mooted hundred years ago, when Metchnikoff hypothesized that a balanced bowel flora would confer a long and healthy life. Early research focused on the prevention of infection in low birth-weight babies and infants, prevention of atopic dermatitis and treatment of acute diarrhoea. Similar effects can be achieved by encouraging the growth of lactobacilli and bifidobacteria by oral administration of fructose and galactose oligosaccharides. Interest has returned to health of the elderly, with positive results in immune stimulation,

cancer prevention pseudomembranous colitis and constipation. The next challenge is to investigate the benefits of probiotic administration at a public health level.

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