**Immunologic effects of probiotics and human health**

**Introduction**

The human gastrointestinal tract (GIT) harbours an extremely complex and diverse microbial ecosystem representing over 500 different species. While a majority of indigenous bacteria are benign or beneficial, some possess the potential to cause disease; in healthy individuals, a balance exists between these populations.

In addition to nutritional and barrier functions, the intestinal microflora plays an important role in guiding the development of a balanced immune system and maintaining gut homeostasis. Perturbations in the microbial homeostasis due to factors such as antibiotic therapy, stress and infection enhances predisposition to increased risk of infectious diseases, cancers and immunoinflammatory disorders.

Recent studies have shown that modulation of the intestinal flora with probiotics can be used to restore gut microbial balance and promote health; probiotics are defined as live microbial food/feed supplements with health-promoting attributes.

**Probiotics and immune function**

There is strong evidence that specific strains of lactic acid bacteria (LAB) are able to stimulate as well as regulate several aspects of natural and acquired immune responses. It has also been demonstrated that significant differences exist in the ability of bifidobacteria and lactobacilli strains to influence the functioning of the immune system and this may account for some of the inconsistencies in data on immunomodulatory efficacy of probiotics.

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**Effect on innate immune function**

The innate or non-specific immune responses constitute the first line of host defence against invading pathogens. The major cellular effectors of non-specific immunity include phagocytic cells (monocytes, macrophages and polymorphonuclear [PMN] cells), and natural killer cells (NK cells). Phagocytic cells play a central role in host defence against microbial infections, whereas NK cells are pivotal for combating viral infections and cancers. In addition, innate immune responses play an important role in shaping the development of acquired immune responses orchestrated by CD4 T cells.

The ability of probiotics to stimulate innate immune function in healthy human subjects is well documented. Enhanced phagocytic activity of peripheral blood leucocytes in healthy volunteers has been reported after consumption of *Lactobacillus johnsonnii* La1 or *Bifidobacterium lactis* Bb12 and similar effects have been obtained with *Lactobacillus acidophilus* and *Lactobacillus GG*.

In elderly subjects, probiotic intake has been reported to be effective in restoring the age-related decline in phagocyte function. Augmentation of NK cell activity (Figure 1), and increases in the percentage of NK cells in the peripheral blood of human volunteers following regular consumption of yogurt or milk containing probiotics have also been reported. Both phagocytic function and augmentation of NK cell function in the elderly following administration of LAB was significantly correlated with age (Figure 2).

However, several other studies have found no effect of probiotic consumption on natural immune function. Whether this has been due to the poor immunostimulatory capacity of the probiotic strains used or some other reason is not known. Differences in the ability of LAB to influence immune function are well documented.

**Humoral immune responses**

Administration of specific strains of LAB to human subjects has also been shown to enhance humoral immune responses to natural infections and systemic or oral immunisations. In a randomised, placebo-controlled study, Kaila et al. observed significantly higher numbers of IgG-, IgA- and IgM-secreting cells in children administered *Lactobacillus* GG fermented milk during acute phase of rotavirus infection (diarrhoea) compared with children receiving a placebo (90% of the *Lactobacillus S GG* group compared with 46% of the placebo group). Viable cells were found to be more efficient at stimulating rotavirus-specific immune response than heat inactivated cells.

Link-Amster and colleagues reported significantly higher serum IgA antibody response to *Salmonella typhi*, following immunisation, in subjects fed yoghurt containing *Bifidobacterium bifidum* and *L. acidophilus* La1 than in the control group. Improved immunogenicity of a live rotavirus vaccine in subjects receiving *Lactobacillus* GG, compared with the control group, has also been demonstrated.

Recent studies by de Vrese et al. have shown that supplementation with specific strains of probiotics is also able to improve protection against polioviruses by...
stimulating the production of virus-neutralising antibodies. Feeding of a formula supplemented with bifidobacteria to infants, immunised against poliovirus several months prior to enrolment in the study, has also been shown to enhance the levels of total faecal IgA and anti-poliovirus faecal IgA. Together these observations suggest that specific strains of LAB are endowed with potent adjuvant properties and could be used for improving efficacy of oral vaccines and as immunostimulants to promote recovery from infectious illnesses.

**Cytokine production**

Cytokines represent the largest and most pleotropic group of immune response mediators. Initiation, maintenance and resolution of both innate and acquired immune responses depend upon cell-to-cell communication via cytokines. Intake of probiotics and fermented milk products has been shown to influence cytokine production (IFN-α, IFN-γ, TNF-α, IL-2, IL-6, IL-10 etc) by cells of the immune system and thus modulate a range of host immune responses.

**Infectious diseases**

Several well-controlled studies have demonstrated that oral administration of specific strains of LAB could reduce the duration and severity of diarrhoea in infants and children hospitalised for acute rotavirus diarrhoea. The effectiveness of probiotics in the prevention of diarrhoeal disease in randomised, placebo-controlled trials, has also been demonstrated. Abundant evidence also exists for the efficacy of probiotic therapy in the prevention and treatment of antibiotic-associated and *Clostridium difficile*-induced diarrhoea (see article by T Riley, this issue). Recent studies by Hatakka and colleagues have shown that oral consumption of probiotics could also be effective in enhancing protection against respiratory tract infections.

The precise mechanisms by which probiotics mediate protection are not known. However, the ability of probiotics to mediate protection against viral infections and at extra-intestinal sites (e.g. the respiratory tract) suggests that stimulation of the immune system may be responsible, at least in part, for these protective effects.

Reduction in the duration of diarrhoea in children hospitalised for acute viral diarrhoea, following administration of probiotics, was associated with enhanced specific immune responses (rotavirus-specific IgA secreting cells and serum IgA). Augmentation of immune responses following administration of probiotics in children with acute respiratory tract infections (numbers of T helper cells, IFNα and IFNγ production and NK cell activity) and oral immunisation with live microorganisms (specific antibody responses) has also been reported.

Other mechanisms by which probiotics contribute to protection against intestinal infections include competition for nutrients and adhesion sites, production of anti-microbial substances (e.g. acids, bacteriocins) and upregulation of mucus production.

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**In Focus**

**Figure 1.** Dietary consumption of *Bifidobacterium lactis* HN019 (DR10™) enhances natural cellular immune function in healthy middle-aged and elderly subjects.

**Figure 2.** Relative increases in NK-cell tumoricidal activity following consumption of *Lactobacillus rhamnosus* HN001 (DR20™) supplemented milk in healthy elderly subjects.
**Cancers**

Numerous animal studies have shown that administration of LAB could prevent the establishment, growth and metastasis of transplantable and chemically induced tumors and that probiotic-mediated stimulation of the immune system may be responsible for these effects.

An inverse relationship between the consumption of yoghurt and fermented milk containing lactobacilli or bifidobacteria and the incidence of colon and breast cancer has also been reported in epidemiological and population-based case-control studies.

However, there is little direct experimental evidence regarding the anti-cancer efficacy (tumour suppression) of probiotics in humans. Aso et al. reported the protective effect of *Lactobacillus casei* strain Shirota on the recurrence of superficial bladder cancer in a randomised, controlled, multi-centre study. Although associated immune responses were not assessed in these studies, enhanced NK cell activity in adult colon-cancer patients given *L. casei* Shirota suggests that probiotics may suppress tumour development through activation of the immune system.

**Immunoregulation**

T helper cells can be classified into distinct subpopulations, Th1 and Th2, according to their cytokine secretion and function. Recently, the presence of a further subtype of T cells, with immunosuppressive function and cytokine profile, termed T regulatory cells (Tr cells) has been described.

A balance between Th1 and Th2 cells is essential for immune system homeostasis. Immune-mediated diseases such as allergies, autoimmune and immunoinflammatory disorders represent disruption of Th1/Th2 balance. Several studies have shown that specific LAB could be effective in down-regulating over expressed Th1/Th2 immune responses.

The mechanisms by which probiotics modulate Th1/Th2 responses are not fully understood. Recent observations suggest that LAB-induced Tr cells may play a pivotal role in the regulation of polarised Th1/Th2 immune responses (Figure 3).

**Allergic disorders**

Atopic disorders (e.g. atopic dermatitis, allergic rhinitis and asthma) represent an aberrant immune response to environmental or food allergens. It involves over-activation of Th2 cells producing IL-4 and IL-5 and is characterised by an increased synthesis of IgE and activation and recruitment of eosinophils. Elimination diets or elemental formulations are used to control atopy.

However, recent studies have shown that the rise in the prevalence of atopy in western societies is associated with lack of exposure to bacteria early in life. Differences in the neonatal gut microflora precede the development of allergy and children with high prevalence of atopy have lower amounts of lactic acid bacteria in their bowel flora than non-atopic children. Studies by Isolauri and colleagues have provided evidence that supplementation with probiotics could be used to prevent and manage allergic disorders.

Weaning of breast-fed infants with atopic eczema to a hydrolysed formula supplemented with *Lactobacillus GG* resulted in significant improvements in symptoms and down-regulation of inflammatory responses as indicated by a reduction in the soluble CD4 in serum and eosinophilic protein X in urine, compared with subjects given formula without lactobacilli. *Lactobacillus GG* supplementation to pregnant mothers with a family history of atopy and to their infants postnatally for 6 months, has also been reported to significantly reduce the incidence of atopic eczema at 2 years of age, compared with the placebo group.

Beneficial effects of probiotics against atopy were associated with increased concentration of TGF-β in the milk of mothers.

However, little is known about the efficacy of probiotics in preventing allergic disorders in adults. *Lactobacillus GG* administration was found to have no beneficial effect in young adults or teenagers with birch-pollen or apple allergy.

Several mechanisms by which probiotics mediate their anti-allergy effects have been suggested. These include: induction of T cells, activation of Th1 cells, reduced immunogenicity of potential allergens through modification of their structure and stabilisation of gut mucosal barrier.
Immuno-inflammatory bowel disease

The inflammatory bowel diseases (IBDs), Crohn’s disease, ulcerative colitis and pouchitis, are disorders of unknown aetiology. Genetic factors as well as an abnormal host immune response to some members of indigenous flora are suggested to play a role in the initiation and perpetuation of IBD. Differences in the composition of intestinal microflora between patients with IBD and healthy individuals have also been observed. Recent studies have shown that probiotic therapy may be effective in the management of IBD. For example, treatment with a cocktail of LAB (8 strains) significantly reduced the relapse rate and the severity of clinical symptoms in patients with pouchitis (inflammation of the ileo-anal pouch formed after colectomy), compared with the placebo. The beneficial effects of *Saccharomyces boulardii* in maintaining remission in patients with Crohn’s disease have also been demonstrated; the relapse rate in subjects receiving mesalazine alone in combination with *S. boulardii* (6.2%) was significantly lower than in subjects receiving mesalazine alone (37.5%).

**Future perspectives**

There is strong evidence that specific strains of probiotics are able to influence functioning of the immune system. This includes both stimulation and regulation of the immune function depending upon the immunological status of the host. There is also evidence that probiotic intake is effective in the prevention and/or management of acute gastroenteritis and rotavirus diarrhoea, antibiotic-associated diarrhoea and intestinal inflammatory disorders such as Crohn’s disease and pouchitis, and paediatric atopic disorders. The efficacy of probiotics against bacterial infections and immunological disorders such as adult asthma, cancers, diabetes, arthritis in humans remains to be proven.

In addition, major gaps exist in our knowledge regarding the mechanisms by which probiotics modulate immune function, and the optimum dose, frequency and duration of treatment for different probiotic strains. Different LAB strains vary in their ability to modulate immune function and therefore efficacy of each strain needs to be carefully demonstrated through rigorously designed (randomised, double-blind, placebo-controlled) studies. These studies should also include assessment of relevant immunological biomarkers that could help elucidate mechanisms by which probiotics mediate their beneficial effects.

**References**