Probiotics, Immunomodulation, and Health Benefits

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Abstract Probiotics are defined as live microorganisms that, when administered in adequate amount, confer a health benefit on the host. Amongst the many benefits associated with the consumption of probiotics, modulation of the immune system has received the most attention. Several animal and human studies have provided unequivocal evidence that specific strains of probiotics are able to stimulate as well as regulate several aspects of natural and acquired immune responses. There is also evidence that intake of probiotics 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, and arthritis in humans remains to be proven. Also, major gaps exist in our knowledge about the mechanisms by which probiotics modulate immune function. Optimum dose, frequency and duration of treatment required for different conditions in different population groups also remains to be determined. Different probiotic strains vary in their ability to modulate the immune system and therefore efficacy of each strain needs to be carefully demonstrated through rigorously designed (randomised, double-blind, placebo-controlled) studies. This chapter provides an over view of the immunomodulatory effects of probiotics in health and disease, and discusses possible mechanisms through which probiotics mediate their disparate effects.

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Introduction

The human gastrointestinal tract harbors a diverse microflora representing several hundred different species. The colonization of the gastrointestinal tract begins immediately after birth. The colonization pattern is affected by factors such as mode of delivery, initial diet, and geographical location (Fanaro et al., 2003). In breastfed infants, between days 4 and 7, bifidobacteria become predominant, accumulating to 10^{10}–10^{11} CFU/g. Thus, nearly 100% of all bacteria cultured from stools of breastfed infants are bifidobacteria (Mitsuoka, 1996). During weaning, when an adult diet is consumed, the stools of infants shift to the Gram-negative bacillary flora of adults; bifidobacteria decrease by 1 log, the number of bacteroidaceae, eubacteria, peptostreptococcaceae, and usually Gram-positive clostridia outnumber bifidobacteria, which constitute 5–10% of the total flora. Lactobacilli, megaspherae, and veillonellae are often found in adult feces, but the counts are usually less than 10^7 CFU/g. In elderly persons, bifidobacteria decrease, clostridia significantly increase, as do lactobacilli, streptococci, and enterobacteriaceae (Woodmancey et al., 2004).

It is estimated that the gastrointestinal tract of an adult human contains 10^{13} bacteria, 10 times the number of eukaryotic cells in the body. The density of bacterial colonization increases progressively from the stomach ($10^{3–4}$ CFU/g) to the colon ($10^{10–11}$ CFU/g). Based on their effect on the intestinal environment, these bacteria can be grouped into three categories: beneficial bacteria, harmful bacteria, and bacteria exhibiting an intermediate property. Harmful bacteria are those that possess pathogenicity or transform food components into harmful substances (ammonia, amines, hydrogen sulfide, and indole from proteins) and include *Clostridium, Veillonella*, *Proteus,* and the Enterobacteriaceae family. Beneficial bacteria represented by *Bifidobacterium* and *Lactobacillus* suppress the harmful bacteria and exert many beneficial physiological effects. They have no harmful effect on the host. *Bacteroides*, *Eubacterium,* and anaerobic streptococci belong to the intermediate group. These bacteria do not show any virulence under normal conditions, but they may cause opportunistic infections when the host immunity or resistance is lowered (Ishibashi et al., 1997). Normally, a delicate balance exists among various communities of the intestinal flora and the harmful bacteria remain under check, leading to a healthy state. However, this balance can be altered as a result of many endogenous (nutrient availability, diet, diarrhea, etc.) and exogenous (antibiotic therapy, excessive hygiene, stress, aging, etc.) factors (Suskovic et al., 2001). Disturbances in the intestinal ecosystem are generally characterized by a remarkable increase in bacterial counts in the small intestine, by an increase in the numbers of aerobes, mostly enterobacteriaceae and streptococci, by the reduction or disappearance of bifidobacteria, and/or often by the presence of *Clostridium perfringens* (Mitsuoka, 1992). Recent studies have provided overwhelming evidence that the administration of probiotics could be effective in restoring intestinal microbial balance and gut homeostasis.

Probiotics are defined as live microorganisms that, when administered in adequate amounts, confer a health benefit on the host (FAO/WHO, 2001).

Intestinal Microflora and the Development of the Immune System

The immune system of a newborn is immunologically naïve and functionally immature (Kelly & Coutts, 2000). Exposure to antigens during early life is essential to drive the development of the gut mucosal immune system and to maintain immune homeostasis. Microbial antigens derived from the resident flora and the environment play a pivotal role in the maturation of gut-associated lymphoid tissue (Glaister, 1973; Moreau et al., 1978) and normal resistance to disease (Yamazaki et al., 1982). This has been clearly demonstrated in studies on germ-free mice. Germ-free animals have a poorly developed immune system; they have fewer IgA plasma cells and intraepithelial lymphocytes in the intestinal mucosa, and lower levels of immunoglobulins, compared to their conventionally reared counterparts (Gordon & Bruckner-Kardoss, 1961; Crabbe, 1968; Crabbe et al., 1970; Gordon & Pesti, 1971; Glaister 1973), and exhibit increased susceptibility to disease (Roach & Tannock, 1980; Yamazaki et al., 1982). However, the normal development of the immune system is restored when the germ-free animals are reared in a conventional environment or given normal intestinal microflora (Bauer et al., 1965; Crabbe et al., 1999). The role of the microbial flora in the development and regulation of host immunity is also highlighted by differences in the intestinal microflora and humoral immune responses of vaginally born versus Caesarean-delivered infants whose mothers received prophylactic antibiotics (Gronlund et al., 2000); microbial colonization in vaginally born infants is associated with the maturation of mucosal immune responses, especially circulating IgA- and IgM-secreting cells.

Another important role of intestinal microflora in the induction and maintenance of oral tolerance has also been demonstrated. GF mice fail to develop oral tolerance, whereas reconstitution of the gut microflora in GF mice at the neonatal stage, but not later, leads to the development of normal tolerance (Sudo et al., 1997). A reduced microbial exposure in Western societies has also been associated with an increased incidence of atopic and autoimmune disorders (Rook & Stanford, 1998).

Probiotics and Human Health

Consumption of probiotics is associated with a range of health benefits including stimulation of the immune system, protection against diarrheal disease and nosocomial and respiratory tract infections, lowering of cholesterol, attenuation of overt immunoinflammatory disorders (such as inflammatory bowel

disease, allergies), and anticancer effects. The scope of this chapter is limited to the immunomodulatory effects of probiotics only. Readers are advised to see reviews by Guarner and Malagelada (2002), Gill and Guarner (2004), Sullivan and Nord (2006), and Quigley and Flourie (2007) for information on other health benefits of probiotics.

Probiotics and Modulation of Intestinal Microflora

Metchnikoff (1907) first proposed the hypothesis that colon bacteria adversely affect human health by "autointoxication." Further, he proposed that the longevity of Bulgarian peasants was due to the consumption of large quantities of fermented milk containing live beneficial microorganisms. As delineated in earlier sections, the intestinal microbiota impacts markedly on the immunology, biochemistry, physiology, and nonspecific disease resistance of the host (Gordon & Pesti, 1971). These observations have prompted the view that modification of the composition of the intestinal microbiota by means of dietary supplements might promote health (Goldin & Gorbach, 1992; Roberfroid, 1998). Most commonly used probiotic bacteria belong to the genera *Lactobacillus* and *Bifidobacterium* (Prasad et al., 1999). Lactobacilli are Gram-positive, nonspore-forming rods, catalase-negative, and usually nonmotile and do not reduce nitrate. The most frequently used lactobacilli species include *Lb. acidophilus*, *Lb. salivarius, Lb. casei, Lb. plantarum, Lb. fermentum,* and *Lb. brevis* (Mikelsaar et al., 1998). On the other hand, bifidobacteria are Gram-positive, nonspore-forming rods with distinct cellular bifurcating or club-shaped morphologies. The most commonly used species include *B. animalis, B. longum, B. bifidum,* and *B. infantis.*

In terms of the probiotic dose, it is generally thought that at least 10^9 CFU/day need to be ingested (Ouwehand et al., 2002). In a study aimed at determining the impact of consumption of *Bifidobacterium longum* (10^9 CFU/day) on the fecal flora of healthy adult human subjects, it was found that the fecal levels of lecithinase-negative clostridia were significantly reduced (Benno & Mitsuoka, 1992). In another study, intake of yogurt enriched with *B. longum* was found to significantly increase bifidobacteria counts in the feces of treated subjects compared with subjects given control yogurt (Bartram et al., 1994). Langhendries and co-workers (1995) reported the impact of consuming fermented infant formula containing viable bifidobacteria (10^6 CFU/g of *B. bifidum*) in full-term infants, wherein significant increases in resident bifidobacteria were observed. Fermented milk containing *Lb acidophilus* LA2 was consumed by human adult volunteers for seven days; the resident lactobacilli as well as bifidobacteria increased significantly in the feces (Hosoda et al., 1996). After investigating the impact of consumption of follow-up formula (NAN BF) containing *B. bifidum* strain Bb12 on fecal flora, the authors reported that the resident bifidobacteria significantly increased and the clostridia counts decreased (Fukushima et al., 1997). In a study involving adult human

subjects consuming yogurt containing *Lb. acidophilus* and *B. bifidum*, Chen et al. (1999) reported that, after 10-day consumption, the subject fecal analysis displayed significant increase in the resident bifidobacteria and, at the same time, a significant drop in the coliforms counts. In a relatively long-term study (six-month preintervention period, six-month intervention, and three-month postintervention) on the effects of consuming *Lactobacillus rhamnosus* DR20 on the microecology of healthy human subjects, Tannock et al. (2000) reported that the strain DR20 was detected at different levels in different test subjects during the intervention period. The presence of DR20 among numerically predominant strains was related to the presence or absence of a stable indigenous population of lactobacilli during the control period. In addition, it was concluded that consumption of the DR20-containing milk product (1.6×10^9 CFU/day) transiently altered the lactobacilli and enterococci population of the feces of the majority of consumers. In another study, human subjects consumed *B. lactis* HN019 containing (3×10^{10} CFU/day) reconstituted milk for four weeks. At the end of the four weeks, the resident bifidobacteria and lactobacilli content increased significantly, and hence the probiotic was able to transiently impact the gut flora toward a beneficial effect. The probiotic counts in feces reached as high as 12.5×10^8 CFU/g (Gopal et al., 2003).

The effective probiotic dose for desired efficacy has received much attention recently, which probably is a result of both commercial (cost) and scientific interest. In order to determine the effective dose of *B. animalis* subsp. *lactis* Bb12, four doses (10^8, 10^9, 10^{10}, 10^{11} CFU/day) were given to adult volunteers in different groups. The fecal recovery of Bb12 increased significantly with increasing dose; however, the fecal bacterial composition was unaffected (Larsen et al., 2006). In another study, the effective dose of *B. lactis* HN019 that could influence the fecal flora of elderly (mean age: 69.5 years) human subjects was investigated. Three doses (5×10^9; 1×10^9, and 6.5×10^7 CFU/day) were administered in reconstituted milk. The probiotic intervention increased the number of resident bifidobacteria and reduced the enterobacteria. In addition, the enterococci and lactobacilli counts were increased. Even the lowest dose administered influenced the fecal microflora composition in elderly subjects (Ahmed et al., 2007). Elderly gut microecology appears to be more amenable for manipulation with bifidobacteria as the natural levels drop in the elderly. All these studies conclusively provide evidence that probiotic administration, though at a smaller level (10^9 CFU/day into 10^{14} CFU/g gut content) when compared to the total number of microbiota, can influence the intestinal microecology and deliver the desired health benefits.

Probiotics and Stimulation of the Immune System

Several animal and human studies have provided evidence that specific strains of lactic acid bacteria are able to stimulate as well as regulate several aspects of natural and acquired immune responses. It has also been shown that significant

differences exist in the ability of bifidobacteria and lactobacilli strains to modulate the immune system and that the responses are dose-dependent. Several excellent reviews on the immunomodulatory effects of probiotics have been published in recent years; readers are advised to consult these for additional information (Gill, 1998, 2003; Erickson & Hubbard, 2000).

Immunological detection of probiotics and probiotic-derived products in the gut is performed by specialized membranous cells (M cells), overlying the Payer's patches and the epithelial cells. Dendritic cells, distributed throughout the subepithelium, have also been shown to have the ability to directly sample lumenal antigens. Antigens taken up by M cells are delivered to antigen-presenting cells (APCs) that process and present antigens to naïve T cells. APCs are able to discriminate between closely related microbes and their products through the expression of pattern-recognition receptors (e.g., TLRs and CD14) that recognize pathogen-associated molecular patterns (PRRs). The nature of cytokine secretion, phenotype, and state of activation of APCs determine whether T cells differentiate into T helper 1 (Th1), T helper 2 (Th2), or T regulatory (Treg) cells. Subsequent activation of Th1 cells leads to the production of IFN-γ, TNF-α, and IL-2 and is associated with the development of cell-mediated and cytoxic immunity; activated Th2 cells mainly secrete IL-4, IL-5, and IL-13, which promote antibody production and are associated with atopy; Treg cells secrete IL-10 and TGF-β, which downregulate activities of both Th1 and Th2 cells.

Innate (Nonspecific) Immunity

The innate responses constitute the first line of host defense and operate nonselectively against pathogens/abnormal antigens. The major cellular effectors of nonspecific immunity include epithelial cells, phagocytic cells (monocytes, macrophages, neutrophils), and natural killer cells (NK cells). Probiotics have been found to modulate the functions of all these cells.

Effect on Phagocytic and NK Cell Activity

Phagocytic cells are effective in eliminating microbial pathogens, whereas NK cells are crucial for defense against viral infections and cancers. The ability of probiotics to enhance the phagocytic activity of peripheral blood leucocytes (monocytes/macrophages and PMN) has been demonstrated in a number of human studies (Gill, 2003). Intake of *Lb. johnsonii* La1, *B. lactis* Bb12, *L. rhamnosus* HN001, or *B. lactis* HN019 resulted in the enhanced phagocytic capacity of peripheral blood leukocytes (PMN and monocytes) in healthy subjects (Schiffrin et al., 1995; Donnet-Hughes et al., 1999). The PMNs exhibited significantly greater improvement in phagocytic capacity compared with

monocytes. The increases in phagocytic activity were dose-dependent (Donnet-Hughes, 1999) and were maintained for several weeks after cessation of probiotic intake (Schiffrin et al., 1995; Gill et al., 2001a, b). In another study, *Lactobacillus* GG was found to induce activation of neutrophils (increased the expression of phagocytosis receptors CR1, CR3, FcγRI, and FcαR) in healthy subjects but to inhibit milk-induced activation of neutrophils in milk-hypersensitive subjects (Pelto et al., 1998). An enhanced oxidative burst or microbicidal capacity of PMN cells in subjects fed probiotics or yogurt has also been demonstrated (Arunachalam et al., 2000; Mikes et al., 1995; Parra et al., 2004).

It has also been reported that probiotic intake is able to restore the age-related decline in phagocytic cell function (Gill, 2002). Aged subjects fed milk containing *Lb. rhamnosus* (HN001) or *Bifidobacterium lactis* (HN019) for three to six weeks exhibited significantly higher phagocytic activity than subjects fed milk without probiotics (Arunachalam et al., 2000; Gill et al., 2001a, b; Gill & Rutherfurd, 2001; Sheih et al., 2001). Importantly, subjects with relatively poor preintervention immunity status consistently showed greater improvement in phagocytic cell function than subjects with adequate preintervention immune function (Gill et al., 2001c). Furthermore, enhancement in phagocytic capacity was also age-related, with subjects older than 70 years exhibiting significantly greater improvements in immune function than those under 70 years (Gill et al., 2001a, b; Gill & Rutherfurd, 2001).

The augmentation of NK cell activity (*ex vivo*) and increases in the percentage of NK cells in the peripheral blood in healthy subjects following regular consumption of yogurt or milk containing probiotics have also been demonstrated (Gill et al., 2001b; Chiang et al., 2000; Sheih et al., 2001; Olivares et al., 2006). As with phagocytic activity, improvements in NK cell function in the elderly subjects, following intake of probiotics, were significantly correlated with age (Gill et al., 2001c). Similar observations regarding enhancement of phagocytic and NK cell function have been made in animals fed probiotics (Gill, 1998; Cross, 2002). Differences in the ability of live versus dead bacteria have also been reported.

It is important to note, however, that several studies have found no effect of probiotic intake on natural immune function (Spanhaak et al., 1998). Whether this has been due to the poor immunostimulatory ability of the probiotic strains used, suboptimal dose, probiotic viability, or some other reason is not known. Strain- and dose-dependent differences in the ability of LAB to modulate immune function are well documented (Donnet-Hughes et al., 1999; Gill, 1998).

Acquired Immunity

The acquired immunity comprises antibody- and cell-mediated responses and is characterized by its specificity and memory.

Consumption of specific probiotics has been shown to enhance antibody responses to natural infections and to systemic and oral immunizations (Isolauri et al., 1995; Majamaa et al., 1995; Kaila et al., 1992, 1995; Fukushima et al., 1998; Link-Amster et al., 1994; de Vrese et al., 2001). In a randomized, placebo-controlled study, Kaila et al. (1992) found significantly higher levels of specific mucosal and serum antibody responses in children with rotavirus following administration with *Lactobacillus* GG fermented milk compared with children receiving a placebo. It has also been demonstrated that viable probiotics are more efficient at stimulating rotavirus-specific immune response than the nonviable bacteria; the proportion of subjects exhibiting rotavirus-specific response at the convalescent stage was higher in the live group (10 out of 12 children) compared with the group given dead bacteria (2 out of 13) (Majamaa et al., 1995; Kaila et al., 1995).

Significantly superior antibody responses and seroconversion rates following parenteral or oral immunization in subjects given probiotics have also been demonstrated. Following immunization with a *Salmonella* vaccine in subjects given probiotics (*B. bifidum*, *L. acidophilus* La1, *Lactobacillus*), significantly higher specific serum IgA antibody and IgA-secreting cell responses were reported (Link-Amster et al., 1994; He et al., 2000). Consistent with these observations, a trend toward increased anti-*Salmonella* IgA levels in subjects receiving LGG and oral *Salmonella* vaccine was reported by Fang et al. (2000). The enhanced immunogenicity of a live rotavirus vaccine in infants given probiotics has also been observed; infants given oral rotavirus vaccine and *Lactobacillus* GG had significantly more IgA- and IgM-secreting cells compared with infants given vaccine only (Isolauri et al., 1995). In another investigation, supplementation with specific strains of probiotics was shown to enhance the efficacy of poliovirus vaccine (de Vrese et al., 2001). In a randomized, double-blind, placebo-controlled study, subjects given yogurt containing *L. rhamnosus* and *L. paracasei* had significantly higher virus-neutralizing antibody responses (mainly IgA) following vaccination with live attenuated polioviruses compared with subjects given placebo (chemically acidified milk). The levels of polio-specific serum IgG and IgA in volunteers consuming yogurt were also significantly increased (de Vrese et al., 2001). In another study, administration of a formula containing bifidobacteria to infants who were immunized against poliovirus several months prior to enrollment in the study was found to enhance total fecal IgA and anti-poliovirus fecal IgA (Fukushima et al., 1998). Similar effects of probiotic administration on antibody responses to a range of antigens and bacterial pathogens have been reported in several animal studies (Gill, 1998).

Together these observations suggest that specific strains of LAB exhibit potent adjuvant properties. The adjuvant effects of probiotics appear to be mediated through improved antigen presentation function: increased transport of antigenic materials across the gut mucosa and upregulation of antigen-presenting molecules and co-stimulatory molecules on immune cells (Heyman, 2001) and/or an increased number of B cells (De Simone et al., 1991). Thus,

probiotics could be effective in improving the efficacy of oral and parenteral vaccines.

Cytokine Production

Cytokines comprise the largest and most pleiotropic group of immune response mediators. Initiation, maintenance, and resolution of both innate and acquired immune responses are regulated by cell-to-cell communication via cytokines.

The ability of probiotics to induce cytokine production by a range of immunocompetent cells may explain how they are able to influence both innate and acquired immune responses. Several studies have reported enhanced levels of IFN-γ, IFN-α, and IL-2 in healthy subjects given probiotics (de Simone et al., 1986; Solis-Pereyra & Lemonnier, 1991; Wheeler et al., 1997; Halpern et al., 1991; Aattouri & Lemonnier, 1997; Kishi et al., 1996; Arunachalam et al., 2000). Long-term consumption of yogurt has also been shown to increase the production of IL1β, IL-6, IL-10, IFN-γ, and TNF-α (Halpern et al., 1991; Aattouri & Lemonnier, 1997; Solis-Pereyra & Lemonnier, 1993; Miettinen et al., 1996). *In vitro*, LAB-induced production of IFN-γ, IL-1, TNF-α, IL-10, IL-12, IL-18, and TGF-β by mononuclear cells and DCs has also been demonstrated (Cross et al., 2002; Miettinen et al., 1998; Gill & Guarner, 2004; Lammers et al., 2003; Niers et al., 2005).

IL-12 and IL-18 induce IFN-γ production by T, B, and NK cells, while IFN-γ enhances the phagocyte capacity of phagocytic cells, induces MHC1 and MHCII expression on a variety of cells, potentiates antitumor cytotoxicity, stimulates helper T cell function, and improves the immunogenicity of vaccines (Nussler & Thomson, 1992). TNF-α, together with IFN-γ, increases the microbicidal capacity of macrophages and exerts cytotoxic effect against tumors. IFN-α plays an important role in early stages of host protection against viruses, bacteria, and cancers. IL-1 stimulates proliferation of T and B cells; IL-6 induces differentiation to antibody-secreting plasma cells; IL-2 stimulates proliferation and differentiation of B cells and NK cells and plays a role in the induction and regulation of T cell-mediated immune responses. IL-10 and TGF-β play an immunoregulatory role (Gill, 2003).

Probiotic-Induced Immunostimulation and Disease Resistance

Infectious Diseases

Infections with gastrointestinal and respiratory tract pathogens (bacteria and viruses) continue to be a major health problem worldwide. Several well-controlled studies have provided evidence that the administration of specific

strains of probiotics could be effective in the prevention and/or treatment of infectious diarrhea (Table 1). A meta-analysis of studies published between 1966 and 2000 revealed that the administration of probiotics, compared to a placebo, was effective in reducing the duration of acute rotavirus diarrhea by 0.7 days (95% confidence interval: 0.3–1.2 days) and the frequency of diarrhea by 1.6 stools on day 2 of treatment (95% confidence interval: 0.7–2.6 fewer stools).

The results of several recent studies Table 1 have further shown that oral intake of probiotics is also effective against respiratory tract infections (Hatakka et al., 2001; Habbermann et al., 2001; Turchet et al., 2003; de Vrese et al., 2006). Several mechanisms by which probiotics mediate their protective effects have been suggested. However, their relative contribution remains unknown. The ability of probiotics to mediate protection at extraintestinal sites and against viral infections strongly suggests that probiotic-induced immune stimulation may be a major contributor.

An association between enhanced specific and nonspecific antibody responses (IgA-secreting cells and serum IgA) and a reduction in the duration of diarrhea in children hospitalized for acute viral diarrhea following the administration of probiotics have been reported in a number of studies (Kaila et al., 1992, 1995; Majamaa et al., 1995; Guandalini et al., 2000). An augmentation of immune responses (number of T-helper cells, NK cell activity, secretion of IFN-α and IFN-γ) and a reduction in the symptom score, duration of common cold episodes, and days with fever in subjects given probiotics during the winter/spring period have also been observed (De Vrese et al., 2006). Similarly, several animal studies have reported a positive relationship between enhanced immune responses (serum and mucosal antibodies, phagocytic cell function, and NK cell activity) and resistance to infection (*Salmonella*, *E. coli,* etc.) following oral administration with probiotics (Gill et al., 2001d; Shu & Gill, 2002).

Cancer

Studies in experimental animals have shown that supplementation with specific probiotic strains is effective in preventing the establishment, growth, and metastasis of chemically induced and transferrable tumors (Rafter, 2002; Capurso et al., 2006). In humans, probiotic supplementation has been shown to reduce the risk of colon cancer by inhibiting the transformation of pro-carcinogens to carcinogens, inactivating mutagenic compounds, and suppressing the growth of pro-carcinogenic bacteria. A negative association between the reduced incidence of cancer and the consumption of fermented dairy products, containing lactobacilli and bifidobacteria, has also been reported from a number of epidemiological and population-based case-control studies. However, there is little direct evidence of the antitumor efficacy of probiotics in

Table 1 Efficacy of Probiotics in the Prevention and Treatment of Diarrhea and Respiratory Diseases in Children: Some Examples

Probiotic Used	Study Population	Design	Outcome	Immune Effect	Reference
L. casei S train GG vs. placebo	Infants with diarrhea (82% due to rotavirus)	Double-blind, placebo-controlled	Reduction in number of motions/day (1.4 vs. 2.4; $P < 0.001$).	Not recorded	Isolauri et al. (1991)
L. case strain GG vs. placebo	Children with rotavirus diarrhea	Randomized, controlled	Reduction in duration of diarrhea (1.5 vs. 2.3 days; $P = 0.002$).	Not recorded	Isolauri et al. (1994)
LGG, L. casei subsp rhamnosus (Lactophilus) *or S. thermophilus* + *L. delbruckii* (Yalacta)	Children with acute rotavirus diarrhea	Randomized	Reduction in duration of diarrhea in LGG group (1.8 vs. 2.8 days in Lactophilus and 2.6 days in Yalacta groups).	Enhancement of rotavirus-specific IgA and specific antibody secreting cells	Majamaa et al. (1995)
L. reuteri	Infants with acute diarrhea	Randomized, placebo-controlled	Reduction in duration of diarrhea in *L. reuteri* group (1.7 vs. 2.9 days; $P = 0.07$).	Not recorded	Shornikova et al. (1997)
L. case strain GG vs. placebo	Children with diarrhea (unknown etiology)	Randomized, placebo-controlled	Reduction in duration of diarrhea in LGG group (7199 vs. 58.3 hours), reduction in the number of watery stools.	Not recorded	Guandalini et al. (2000)
B. bifidum, S. thermophilus vs. placebo	Children—prevention of diarrhea	Double-blind, placebo-controlled	Reduction in the incidence of diarrhea (8/26 vs. 2/29). $P < 0.035$ for rotavirus diarrhea.	Not recorded	Saavadra et al. (1994)
L. GG vs. placebo	Undernourished children (6 to 24 months old)—prevention of diarrhea	Randomized, placebo-controlled	Significantly fewer episodes of diarrhea in LGG group.	Not recorded	Oberhelman et al. (1999)

Table 1 (continued)

Probiotic Used	Study Population	Design	Outcome	Immune Effect	Reference
L. casei, *S. thermorphilus, L bulgaricus* or *S. thermophilus, L bulgaricus* vs. placebo	Children (19 months old)—prevention of diarrhea	Randomized, blind, placebo-controlled	Reduction in the duration of diarrhea in *L. casei* group over the 6 months ($P = 0.009$).	Not recorded	Pedone et al. (1999)
L. rhamnosus strains 573L/1; 573L/2; 573L/3 or placebo	Children (2 months to 6 years old)—with infectious diarrhea	Randomized, blind, placebo-controlled	Reduction in the duration of rotavirus diarrhea (76 ± 35 hours vs. 115 ± 67 hours; $P = 0.03$).	Not recorded	Szymanski et al. (2006)
B. lactis Bb12 or *L. reuteri* ATCC 55730	Children (4–10 months old)—prevention of infections	Randomized, double-blind, placebo-controlled	Reduction in the number (0.31 in control group; 0.13 in *B. lactis* group and 0.02 in *L. reuteri* group) and duration of episodes of diarrhea.	Not recorded	Weizman et al. (2005)
B. lactis Bb12	Children (8 months or younger)	Multicenter, double-blind, controlled study	Reduction in the duration of episodes of diarrhea in probiotic group (5.1 ± 3.3 days vs. 7.0 ± 5.5 days).	Not recorded	Chouraqui et al. (2004)
Bifidobacterium Bb12 alone or with *S. thermophilus*	Children (6–36 months)—prevention of rotavirus diarrhea	Placebo-controlled	Prevention of symptomatic rotavirus infection.	No significant increase in antibody levels in treatment group, indicating no infection (30% of control group showed subclinical infection)	Phuapradit et al. (1999)

Table 1 (continued)

Probiotic Used	Study Population	Design	Outcome	Immune Effect	Reference
Lactobacillus GG	Children (1–6 years old)—prevention of diarrhea and respiratory infections	Randomized, double-blind, placebo-controlled	Reduction in the number of days of absence from day care center due to illness (4.9 vs. 5 days; $P < 0.03$). Reduction (17%) in the incidence of respiratory tract infections ($P = 0.05$).	Not recorded	Hatakka et al. (2001)
Verum (*Lactobacillus* and *Bifidobacterium* spp)	Prevention of common colds in healthy adults	Randomized, double-blind, controlled	Significant reduction in duration of episodes (7.0 vs. 8.9 in control, $P < 0.045$)	Significant increase in cytotoxic plus T suppressor cells ($CD8^+$) and T helper cells ($CD4^+$)	De Vrese et al. (2006)
Verum (*Lactobacillus* and *Bifidobacterium* spp)	Prevention of common cold in healthy adults	Randomized, double-blind, placebo-controlled	Reduction (13.6%) in incidence of virally induced infections ($P = 0.07$). Significant reduction (54%) in number of days with fever ($P = 0.03$).	Significant increase in T-lymphocytes including $CD4^+$ and $CD8^+$ cells as well as monocytes	Winkler et al. (2005)

human subjects. Rafter and colleagues (2007) reported a protective effect of synbiotic therapy in a randomized, double-blind, placebo-controlled study involving polypectomized patients and colon cancer patients. Synbiotic administration for 12 weeks resulted in a significant reduction in colorectal proliferation and the capacity of fecal water to induce toxicity of colonic cells, along with an improvement in epithelial barrier function in polypectomized patients. Furthermore, symbiotic therapy prevented an increase in IL-2 secretion by peripheral blood mononuclear cells in the polypectomized patients and enhanced the production of interferon-γ in cancer patients. The protective effects of probiotic supplementation against bladder cancer have also been demonstrated (Aso et al., 1995; Sawamura et al., 1994). It was also suggested that probiotic-induced stimulation of the immune system, as indicated by increases in the percentages of T-helper cell and NK cells, and augmentation of NK cell activity may play an important role in the suppression of tumor development. Several other mechanisms by which probiotics mediate anticancer effects have also been suggested (Rafter, 2002).

Probiotics and Attenuation of Immunoinflammatory Disorders

Inflammatory Bowel Disease (IBD)

Inflammatory bowel disease (IBD) consists of mainly two forms: Crohn's disease (CD) and ulcerative colitis (UC). Both diseases are chronic in nature and are characterized clinically by relapses and remissions. UC is characterized by inflammation with superficial ulcerations limited to the mucosa of the colon. Inflammation in CD patients is transmural with large ulcerations, and occasionally granulomas are observed. UC is generally confined to the large intestine, while CD shows a discontinuous pattern, potentially affecting the entire GI tract (Sheil et al., 2007). The etiology of the IBD is unknown. Results of several recent studies suggest that genetic factors and an abnormal host immune response to resident luminal bacteria are involved in the development and/or maintenance of IBD (Bonen & Cho, 2003; Mahida & Rolfe, 2004); CD is a Th1-mediated disease, whereas UC is a Th2-mediated disorder. Studies with animal models of IBD (genetically engineered and germ-free) have clearly demonstrated that the induction of intestinal inflammation is associated with the presence of enteric bacteria. The presence of enteric bacteria or their products in the inflamed tissue and alterations in patients with IBD have also been reported (Fedorak & Madsen, 2004). These observations have led to the evaluation of probiotic therapy as a means for modifying the luminal microbial environment and restoring immune homeostasis, for the management and treatment of IBD. The results of these interventions have been encouraging (Table 2). As per the criteria of evidence-based medicine, there is level 1 evidence to support the therapeutic use of probiotics for the treatment of

Table 2 Efficacy of Probiotics in the Prevention and Treatment of IBD: Some Examples

Probiotic Used	Study Population	Design	Outcome	Immune Effect	Reference
B. longum and synergy I	Patients with active UC	Double-blind, randomized controlled trial	Reduction in sigmoidoscopy scores (scale 0–6) in the probiotic group (3.1) compared with placebo (3.2).	Significant reduction in the mRNA levels for human β-defensins 2, 3, and 4 after treatment ($p = 0.016$,0.038, and 0.008, respectively). Also, reduction in TNF-α and IL1-α after treatment ($p = 0.018$ and 0.023, respectively)	Furrie et al. (2005)
VSL#3*	Ambulatory patients with active UC (treatment and preventing relapse of IBD)	Open-label experiment	Induction of remission/ response rate of 77% with no adverse events.	Not recorded	Bibiloni et al. (2005)
Fermented milk product containing *Lactobacillus* La-5 and *Bifidobacterium* Bb12	Patients with UC operated on with ileal-pouch-anal anastomosis and patients with ileorectal anastomosis	Open-label experiment	Reduction in the median endoscopic score of inflammation during intervention in the UC/IPAA patients.	Not recorded	Laake et al. (2005)
VSL#3	Patients with pouchitis (PADI score 7 or more)	Randomized and placebo-controlled	Remission was maintained for one year in 17 patients (85%) on VSL#3 and in one patient (6%) on placebo ($p < 0.0001$).	Not recorded	Mimura et al. (2004)

Table 2 (continued)

Probiotic Used	Study Population	Design	Outcome	Immune Effect	Reference
VSL#3	Patients with UC operated on with ileal-pouch-anal anastomosis.	Placebo-controlled	Effective in the prevention of the onset of acute pouchitis and improvement in the quality of life of patients with IPAA.	Significant reduction in mucosal mRNA expression levels of IL-1β, IL-8, and IFN-γ compared with placebo-treated patients. Increase in the number of polymorphonuclear cells	Gionchetti et al. (2003)
VSL#3	Patients with chronic pouchitis	A double-blind, placebo-controlled trial	Effective in the prevention of flare-ups of chronic pouchitis.	Not recorded	Gionchetti et al. (2000)
E. coli (Nissle, 1917)	Pateints with CD symptoms	Placebo-controlled trial with 24 patients	Reduction in relapse rate.	Not recorded	Malchow (1997)

CD = Crohn's disease; UC = ulcerative colitis.
*(Contains *Lactobacillus acidophilus, Lactobacillus plantarum, Lactobacillus casei, Lactobacillus delbrueckii, Bifidobacterium breve, Bifidobacterium longum, Bifidobacterium infantis, Streptococcus salivaris*).

postoperative pouchitis, and levels 2 and 3 evidence to support the use of probiotics for the treatment of UC and CD (Fedorak & Madsen, 2004). The modulation or regulation of dysregulated immune responses has been suggested to be the primary mechanism by which probiotics mediate their beneficial effects (described in the next section); the ability of different probiotic strains to induce distinct mucosal cytokine profiles and modulate polarized Th1 and/or Th2 responses is well documented (Ghosh et al., 2004).

Allergies

Allergies represent an exaggerated and imbalanced immune response to an environmental or food antigen. Classical allergy is a type I hypersensitivity reaction. It is driven by the preferential activation of Th2 cells producing IL-4 and IL-5 cytokines and is characterized by an increased synthesis of IgE and activation and recruitment of eosinophils. Depending upon the mode of allergen entry, allergic reactions are commonly manifested by urticaria, rhinitis, vomiting, and/or diarrhea.

Recent studies have shown that insufficient or aberrant exposure to microbes, early in life, may be responsible for the rise in the prevalence of allergies in the westernized countries over the past 40 years. It has also been reported that differences (qualitative and quantitative) in the composition of neonatal gut microflora precede the development of allergy; children who developed allergy had fewer bifidobacteria and enterococci and higher levels of clostridia and *Staphylocuccus aureus* in their intestinal flora than nonallergic children (Bjorksten et al., 2001); differences in the composition of GI microbiota in individuals from industrialized versus nonindustrialized countries have also been observed (Drasar, 1974). The increased risk of developing allergic rhinoconjunctivitis in infants born by Caesarean section (C-section), compared with those delivered vaginally, further demonstrates the crucial role of indigenous microflora in shaping the development of the immune system (Renz-Polster et al., 2005).

To date, many studies have examined the efficacy of probiotic supplementation in the prevention and treatment of allergic disorders. These studies have shown that probiotic supplementation could have a beneficial effect in infants at high risk of atopy and those presenting with cow's milk allergy and atopic eczema and dermatitis (Table 3). It has also been shown that the benefits of probiotic supplementation during infancy extend beyond infancy (Kalliomaki et al., 2003; Lodinova-Zodnikova et al., 2003). Interestingly, however, the administration of probiotics in young adults or teenagers with birch pollen or apple allergy, before, during, and after pollen birch season, was found to be ineffective in alleviating the symptoms of allergy or reducing the use of medicine. This suggests that probiotic intervention, before or immediately after

Table 3 Efficacy of Probiotics in the Prevention and Treatment of Allergic Diseases: Some Examples

Probiotic Used	Study Population	Design	Outcome	Immune Effect	Reference
Probiotics + galacto-oligosaccharide	Mother and infant pairs (up to 6 months). Prevention of food allergy, eczema, asthma, and allergic rhinitis	Randomized, placebo-controlled trial	Reduction in the incidence of atopic diseases ($p < 0.052$), eczema ($p < 0.035$), and atopic eczema ($p < 0.025$)	Not recorded	Kukkonen et al. (2007)
L. casei shirota	Patients with allergic rhinitis triggered by Japanese cedar pollen	Randomized, double-blind, placebo-controlled study	Reduction in nasal symptom-medication score in the probiotic group	Not recorded	Tamura et al. (2007)
Lb gasseri TMC0356	Subjects with perennial allergic rhinitis	Controlled trial with 15 subjects showing high serum IgE levels and allergic symptoms	Not reported	Reduction in serum total IgE levels ($p < 0.05$). Significant increase in the proportion of Th1 cells [on days 14 ($p < 0.01$) and after 28 ($p < 0.05$)]	Morita et al. (2006)
Bifidobacterium longum BB536	Subjects with history of Japanese cedar pollinosis (JCPsis)	Randomized, double-blind, placebo-controlled trial	Significant improvements in eye symptoms in the probiotic group ($p = 0.0057$). Also, reduction in rhinorrhea and nasal blockage	Decrease in JCP-specific IgE levels	Xiao et al. (2006)

Table 3 (continued)

Probiotic Used	Study Population	Design	Outcome	Immune Effect	Reference
Lactobacillus rhamnosus and *Bifidobacteria lactis*	Children with established atopic dermatitis	Placebo-controlled	Improvement in AD only in food-sensitized children	Not recorded	Sistek et al. (2006)
Lb. fermentum VRI-033	Children (6–18 months) with moderate to severe atopic dermatitis (AD)	Randomized, double-blind, placebo-controlled trial	Significant reduction in the SCORAD index over time ($p < 0.03$)	Reduction in IgE level [35.7 ($\pm$6.0) in placebo group versus 31.8 ($\pm$4.3) in probitoc group]	Weston et al (2005)
Lactobacillus fermentum PCC trademark	Young children with moderate-to-severe atopic dermatitis (AD)	Randomized, placebo-controlled trial	Improvement in AD severity	Significant increase in IFN-γ production following stimulation with PHA and SEB at the end of the supplementation period (week 8: $P = 0.004$ and 0.046) as well as 8 weeks after cessation of supplementation (week 16: $P = 0.005$ and 0.021)	Prescott et al (2005)
Enterogermania (containing *Bacillus clausii*)	Adult subjects (mean age 22.3 years) with allergic rhinitis	Controlled trial involving 10 subjects	Symptoms not reported	Significant decrease in IL4 levels ($p = 0.004$); significant increase in IFN-γ ($p = 0.038$), TGF-β ($p = 0.039$), and IL10 ($p = 0.009$) levels	Ciprandi et al. (2005)

Table 3 (continued)

Probiotic Used	Study Population	Design	Outcome	Immune Effect	Reference
Lactobacillus GG (LGG), a mixture of four probiotic strains (MIX)	Infants with atopic eczema/dermatitis syndrome (AEDS) and food allergy	Randomized, double-blind, placebo-controlled	Reduction in SCORAD in IgE-sensitized infants. Reduction in AT in the LGG group, but not in other treatment groups	Increase in IgA levels in the probiotic group compared with the placebo group (LGG vs. placebo, $p = 0.064$; MIX vs. placebo, $p = 0.064$), after challenge, in subjects with IgE-associated CMA infants, increase in fecal IgA ($p = 0.014$), and decrease in TNF-α compared to placebo	Viljanen et al. (2005)
Enterogermania (containing *Bacillus clausii*)	Allergic children (mean age: 4.4 years) with recurrent respiratory infections	Controlled trial involving 10 children attending nursery school	Symptoms not reported	*S*ignificant reduction in IL-4 levels ($p < 0.01$) and a significant increase in IFN-γ ($p < 0.05$), IL-12 ($p < 0.001$), TGF-β ($p < 0.05$), and IL-10 ($p < 0.05$) levels	Ciprandi et al. (2004)
Lactobacillus rhamnosus 19070-2 and *Lactobacillus reuteri* DSM 122460	Children (1–13 years old) with atopic dermatitis	Double-blind, placebo-controlled, crossover study	Improvement in SCORAD	Reduction in serum eosinophil cationic protein levels ($P = 0.03$) in the probiotic group	Rosenfeldt et al. (2003)

Table 3 (continued)

Probiotic Used	Study Population	Design	Outcome	Immune Effect	Reference
Probiotics	Mother-infant pairs with history of atopic diseases	Double-blind, placebo-controlled study	Significant reduction in the risk of developing atopic eczema in probiotic group compared to placebo (15% and 47%, respectively; $P = 0.0098$)	Significant increase in TGF-β2 level in human milk in probitotic group (2885 pg/mL) vs. placebo (1340 pg/mL) $P = 0.018$)	Rautava et al. (2002)
Lactobacillus GG	Mother-infant pairs with history of atopic eczema	Randomized, double-blind, placebo-controlled study	Significant reduction in the incidence of atopic eczema ($p < 0.008$)	No effect on IgE levels	Kalliomaki et al. (2001)
Bifidobacterium lactis Bb-12 or *Lactobacillus* strain GG (ATCC 53103)	Infants (mean age: 4.6 months) with history of atopic eczema	Randomized, double-blind, placebo-controlled study	Reduction in SCORAD in the *Bifidobacterium lactis* Bb-12 group to 0 (0–3.8), and in the *Lactobacillus* GG group to 1 (0.1–8.7) vs. unsupplemented 13.4 (4.5–18.2)	Reduction in the concentration of soluble CD4 in serum and eosinophilic protein X in urine	Isolauri et al. (2000)
Lactobacillus GG (ATCC 53103)	Milk hypersensitive and healthy adult subjects	Double-blind, crossover study	Downregulation of immunoinflammatory response in milk-hypersensitive subjects	Significant reduction in the expression of CR1, Fc-γRI, and Fc-αR in neutrophils and CR1, CR3 and Fc-αR in monocytes	Pelto et al. (1998)

CMA = cow's milk allergy; SEB = Staphylococcus aureus enterotoxin B

birth, is more effective in inducing immunological tolerance, as the immune system is immature, compared with older children with a fully mature immune system (Ouwehand, 2007). Several mechanisms by which probiotics exert preventive/therapeutic antiallergy effects have been suggested. These include reduced immunogenicity of potential allergens through modification of their structure (Rokka et al., 1997), stabilization of the gut mucosal barrier, and restoration of immune system homeostasis through induction of regulatory innate and adoptive immune responses (Guarner et al., 2006).

Mechanisms by Which Probiotics Correct Immunological Disorders

A balance between Th1-Th2 is considered important for immune system homeostasis. Allergic disorders that are mediated by Th2 cells and IBD together with autoimmune disorders (e.g., type 1 diabetes) driven by Th1 cells were therefore considered the result of an imbalance between Th1-Th2 responses (Rook & Brunet, 2005). However, a parallel rise in the incidence of allergies and autoimmunity and IBD (in industrialized countries) in the past few decades and the simultaneous occurrence of Th1- and Th2-mediated disorders suggest that this simple assumption is unable to explain the underlying mechanisms (Guarner et al., 2006). Recent studies have shown that a defective Treg cell activity may be the central cause; patients with type 1 diabetes and multiple sclerosis, and individuals with predisposition to allergy, exhibit deficient Treg cell activity (Guarner et al., 2006).

Evidence from *in vitro* and *in vivo* studies suggests that probiotics may mediate their beneficial effects through induction of regulatory T cells, rather than skewing of Th1 or Th2 responses (Fig. 2). Treg cells suppress both Th1-and Th2-type immune responses through production of IL-10 and TGF-β. Increased levels of TGF-β in breast milk (Rautava et al., 2002) and elevated levels of IL-10 and TGF-β in atopic children following administration of probiotics have also been observed (Pessi et al., 2000; Isolauri et al., 2000). The ability of probiotics to induce regulatory DCs (Hart et al., 2004; Drakes et al., 2004) that drive the polarization of T cells toward Treg cells has been demonstrated (Di Giancinto et al., 2005). An association between the increased expression of IL-10 and the prevention of flare-ups of chronic UC (Cui et al., 2004) and a reduction in pro-inflammatory cytokines in tissue obtained from subjects with pouchitis following treatment with probiotics have also been observed (Lammers et al., 2005). Probiotics have been demonstrated *in vitro* to increase IL-10 synthesis and secretion (in macrophages and T cells) without significantly modifying pro-inflammatory cytokines in inflamed mucosa of patients with active ulcerative colitis (Pathmakanthan et al., 2004).

A strong support for the role of Treg cells is also provided by the results of recent animal studies. Di Giacinto et al. (2005) reported an increased number of Treg cells bearing surface TGF-β, following administration of probiotics, in

an animal model of colitis. These cells were effective in conferring protection against colitis in a cell-transfer system. Importantly, the protective effect was dependent on TGF-β and IL-10 and was abolished by appropriate neutralizing antibodies. Furthermore, probiotics, whether delivered orally or subcutaneously, and bacterial DNA have been found to be effective in attenuating colitis and arthritis in mice (McCarthy et al., 2003; Sheil et al., 2004; Rachmilewitz et al., 2004). Chapat et al. (2004) showed that IFN-γ producing $CD8^+$ T cell- mediated ability of orally administered probiotic *L casei* to reduce skin inflammation due to contact sensitivity was Treg cell-dependent. In a recent study, probiotic administration was found to induce IL-10 production and prevent spontaneous autoimmune diabetes in the nonobese diabetic mouse (Calcinaro et al., 2005). This clearly suggests that the mechanisms by which probiotics mediate their effects are not restricted to the gut and are likely to be mediated by Treg cells. Once generated, Treg cells are able to move to other tissues (Rook & Brunet, 2005).

It has also been suggested that modulation of dendritic cell function by probiotics is a critical step that directs the polarization of naïve T cells to Treg cells (Braat et al., 2004). Different probiotics induce different DC activation patterns (expression of cytokines and maturation surface markers), with some strains exhibiting the ability to inhibit DC activation by other lactobacilli (Christensen et al., 2002); therefore, these are likely to exert different effects.

Thus, probiotics have been demonstrated to augment health benefits by influencing the gut flora composition and restoring the intestinal homeostasis. In addition, stimulation of the host immune system to enhance innate (macrophage and NK cell activity), humoral (pathogen-/vaccine-specific antibody and antibody-producing cells), and cell-based (Treg function) immunity has the potential to influence the general health of the world's population. Development of immunization procedures that avoid the use of needles and adjuvants is highly desirable, as it will reduce vaccine costs and make the large-scale implementation of immunization programs possible. Further research effort to understand the mechanisms by which the probiotics modulate the activity of macrophages and NK cells and enhance the immunogenecity of vaccines would help to identify potential candidate probiotics with superior properties.

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