23 Immunological Effects of Probiotics and their Significance to Human Health

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23.1 Introduction

Probiotics are defined as live microorganisms which when administered in adequate amounts confer a health benefit upon the host (FAO/WHO, 2001). Lactic acid bacteria, particularly *Lactobacillus* and *Bifidobacterium* species are commonly used as probiotics. Other less commonly used probiotics include the yeast *Sacchromyces cerevisiae* and some non-pathogenic *Escherichia coli* and *Bacillus* species. Studies over the past 20 years have demonstrated that probiotic intake is able to confer a range of health benefits including modulation of the immune system, protection against gastrointestinal and respiratory tract infections, lowering of blood cholesterol levels, attenuation of overt immuno-inflammatory disorders (such as inflammatory bowel disease, allergies) and anti-cancer effects. However, the strongest clinical evidence for probiotics relates to their effectiveness in improving gut health and modulating (via stimulation or regulation) the host immune system. This chapter provides an overview of the current status of our knowledge regarding the immunostimulatory and immunoregulatory effects of probiotics on the immune system and their significance to human health.

23.2 Gut Microbiota

The human gastrointestinal tract (GIT) is home to a complex and dynamic community of microbes representing over 500 species. Colonization of the GIT begins immediately after birth and evolves towards the normal adult flora over the first 24 months of life. In breast-fed babies, the microbiota is dominated by

bifidobacteria whereas *Bacteroides* species are found in abundance in formula-fed babies. It is estimated that there are ten times more bacteria than cells in the human body and that the combined genome of intestinal microbiota is 50 times greater than the human genome. Most of the microbes present in the gut are beneficial and confer a range of health benefits. Recent studies have shown that the human gut microbiome encodes a larger proportion of metabolic pathways that are important for human life than the human genome itself (Sekirov and Finlay, 2006). In a healthy state, a balance between different groups (health-enhancing and potential pathogens) of bacteria ensures intestinal homeostasis. Dearrangements in the intestinal microbial ecosystem perturb intestinal homeostasis and enhance susceptibility to disease. There is overwhelming evidence that probiotics could be used to restore intestinal microbial balance and optimize health.

23.3 The Immune System

The immune system is a highly adaptable defense system that has evolved to preserve the integrity of an organism by eliminating all elements perceived as foreign. The protective function is mediated by a complex network of cells and molecules that are capable of specifically recognizing and eliminating a large variety of pathogenic organisms. Following recognition of a pathogen/foreign agent, the immune system recruits appropriate effector cells and molecules to eliminate or neutralize the threat. This also leads to the induction and expansion of memory cells that ensure more prompt and augmented immune response (both humoral and cellular) to subsequent challenges by the same pathogen.

The immune system consists of innate and adaptive components. The innate (non-specific) immune responses constitute the first line of host defense and comprise a set of resistance mechanisms that are non-adaptive and non-specific to a given pathogen. The major effectors of innate immune system include phagocytic cells (neutrophils/polymorphonuclear cells (PMNs), monocytes/macrophages and dendritic cells) and natural killer cells (NK cells). Failure of the innate system to contain an infection, results in the activation of adaptive immune responses.

Unlike the innate immune system, the adaptive (specific) immunity exhibits a high degree of specificity and memory. It consists of both cellular and humoral components. The key cellular constituents of the adaptive system include thymus-derived T helper lymphocytes (Th) and cytotoxic T lymphocytes (Tc),

bone-marrow-derived B lymphocytes and accessory cells such as dendritic cells and macrophages. The Th cells can be further divided into two subtypes; Th1 and Th2, each carrying out distinct and opposing functions. A proper balance between Th1 and Th2 immune responses is critical for immune homeostasis. T cells influence the activities of other immunocompetent cells by producing a wide array of cytokines. The adaptive humoral immunity is mediated by antibodies produced by plasma cells (mature B lymphocytes). Central to the activation and regulation of immune responses is the production of cytokines (such as interferons, interleukins, colony-stimulating factors).

The innate and adaptive immune systems are highly integrated and interdependent. In addition to being a pre- requisite for adaptive immunity, the innate immune response is responsible for the detection and elimination of pathogens (Haddad et al., 2005).

23.4 Intestinal Microbiota and Immune Development

At birth, the immune system is immunologically naive and functionally immature (Kelly and Coutts, 2000). The commensal flora acquired during early life plays an important role in the development and maturation of the host immune system. This is best illustrated by studies with gnotobiotic animal models that exhibit enhanced susceptibility to infectious diseases (Roach and Tannock, 1980; Yamazaki et al., 1982). All aspects of the intestinal immune system (e.g., IgA plasma cells, CD4 T cells, dendritic cells, intraepithelial lymphocytes, levels of immunoglobulins) are underdeveloped in germfree mice (Crabbe et al., 1999; Crabbe, 1968; Gordon and Bruckner-Kardoss, 1961; Gordon and Pesti, 1971; Mazmanian et al., 2005; William et al., 2006) but are rapidly restored upon the introduction of even single bacterial species or when reared in a conventional environment (Bauer et al., 1965; Crabbe et al., 1999).

It has also been demonstrated that intestinal microbiota is pivotal for the induction and maintenance of oral tolerance. Germfree mice fail to develop oral tolerance. However, the reconstitution of gut microbiota at the neonatal stage, but not any later, results in the development of normal tolerance (Sudo et al., 1997). Defective immunoregulation resulting from reduced or aberrant exposure to microbes during early life has also been associated with an increased incidence of atopic and autoimmune disorders in western societies (Rook et al., 2006). The microbial species and their components that are critical for driving the development of the gut mucosal immune system during early life are not known.

However, differences in the intestinal microbiota and humoral immune responses of vaginally versus caesarean delivered infants whose mothers received prophylactic antibiotics (Gronlund et al., 2000), and enteric flora of atopic versus non-atopic infants (Bjorksten et al., 2001) suggests that the signals imparted by diverse species of microbes during early life are critical for educating and shaping the developing immune system.

23.5 Probiotics and Stimulation of the Immune System

Several *in vitro* and *in vivo* studies have shown that specific strains of probiotics are able to modulate the functioning of the immune system; stimulate the immune function to protect against infectious diseases and cancers (*Table 23.1*), and regulate over expressed immune responses associated with immunoinflammatory disorders such as allergy and IBD (Gill & Guarner, 2004). It has also been shown that probiotic strains exhibit large variation in their capacity to modulate the immune system and the degree of response is dose-dependent. The viability of

Table 23.1

Examples of immunostimulatory effects of probiotics observed in healthy human subjects

Function	Effects
Cellular immunity	↑ Phagocytic capacity of PMN and monocytes
	↑ Expression of phagocytosis receptors (CR1 and CR3 in PMN)
	↑ NK cell activity
	↑ $CD3^+$, $CD4^+$, $CD25^+$ and $CD56^+$ (NK cells) cells
	↑ Oxidative burst activity
Humoral immunity	↑ Serum and mucosal IgA levels
	↑ Serum and/or mucosal antibody responses (IgG, IgA or IgM) to oral/systemic immunizations (such as rotavirus, *S. typhi*, polio and Hib vaccine)
	↑ IgG, IgM, IgA immunoglobulin-secreting cells
Production of cytokines	↑ IFN-γ levels in blood
	↑ 2–5A-synthetase activity in blood mononuclear cells
	↑ IFN-α in serum
	↑ *In vitro* and *ex vivo* production of pro- and anti-inflammatory cytokines following stimulation with mitogens

probiotics has also been found to be critical for maximal effect (Gill & Rutherfurd, 2001a). The following sections are mainly focused on the immunomodulatory effects of probiotics in human subjects and readers can consult reviews by Gill (1998, 2003), Cross (2002), and Erickson and Hubbard (2000) for additional information on immunomodulatory effects in animal models.

23.6 Effect on Innate (Non-specific) Immune Responses

23.6.1 Phagocytic Cell Function

The effect of probiotic supplementation on phagocytic cell function and NK cell activity has been the subject of many human studies. Schiffrin et al. (1995) and Donnet-Hughes et al. (1999) reported an augmentation of phagocytic capacity of peripheral blood leucocytes (neutrophils and monocytes) in healthy adults given fermented milk containing *L. johnsonii* La1 or *B. lactis* Bb12. These improvements in phagocytic activity were found to be dose-dependent (Donnet-Hughes et al., 1999) and were maintained for several weeks after cessation of probiotic consumption (Gill et al., 2001a, b; Schiffrin et al., 1995). Furthermore, supplementation with probiotics or standard yoghurt has also been shown to counteract the decrease in phagocytic cell function caused by dietary deprivation of fermented foods in healthy adult human volunteers (Olivares et al., 2006a).

Neutrophils comprise about 60% of blood leukocytes and are the main phagocytic cells in blood. Monocytes constitute only 3–7% of blood leukocytes and therefore contribute relatively less to the overall phagocytic capacity of blood leukocytes. In studies with probiotics, PMNs have been found to exhibit significantly greater enhancement in phagocytic activity compared with monocytes (Schiffrin et al., 1995).

It has also been shown that probiotics mediate disparate effects on phagocytic function depending upon the health status of recipient subjects. *Lactobacillus rhamnosus* GG (*Lactobacillus* GG) supplementation increased the expression of phagocytosis receptors (CR1, CR3, FcγRI and FcαR) on neutrophils in healthy subjects but had an opposite effect in milk-hypersensitive subjects (Pelto et al., 1998). Similarly, observations in healthy volunteers and patients with atopic dermatitis have been made by Roessler et al. (2008). Probiotic supplementation led to a significant increase in phagocytic activity of monocytes and granulocytes in healthy volunteers, but had no effect in patients with atopic dermatitis.

In addition to enhancing phagocytic activity, probiotics have also been shown to enhance oxidative burst or microbicidal capacity of PMN cells in subjects fed probiotics (Arunachalam et al., 2000; Mikes et al., 1995; Parra et al., 2004; Roessler et al., 2008).

Ageing is associated with a decline in immune competence including phagocytic capacity of neutrophils and macrophages. It has been reported that probiotic administration is able to correct the age-related deficit in phagocytic cell function (Gill et al., 2001b, c). Healthy elderly subjects given milk containing *L. rhamnosus* (HN001) or *B. lactis* (HN019) for 3–6 weeks showed significantly higher phagocytic activity than subjects given milk without probiotics (Arunachalam et al., 2000; Gill et al., 2001a, b; Gill and Rutherfurd, 2001b; Sheih et al., 2001). Notably, subjects with relatively poor pre-intervention immunity status consistently showed greater improvement in phagocytic cell function compared with subjects with adequate pre-intervention immune status (Gill et al., 2001c). Furthermore, enhancement in phagocytic capacity was also age-related; subjects older than 70 years exhibited significantly greater improvements in phagocytic cell function than those under 70 years of age (Gill et al., 2001a, b; Gill and Rutherfurd, 2001a, b). A trend towards an increase in blood phagocytic activity in hospitalized, enterally-fed elderly whose initial level was low, following consumption of fermented milk containing *L. johnsonii* La1, has also been reported by Fukushima et al. (2007).

Neutrophil dysfunction is associated with increased susceptibility of patients with alcoholic liver disease to infectious diseases. Treatment with *L. casei* Shirota for 4 weeks was reported to be effective in significantly increasing the phagocytic capacity of neutrophils in patients with alcoholic cirrhosis (Stadlbauer et al., 2008).

However, contrary to these results, several studies have reported little or no effect of probiotic intake, even at high doses, on the phagocytic activity of blood leukocytes when compared to the placebo (Christensen et al., 2006). Failure of probiotics (*L. acidophilus*) to influence early innate immune responses in infants at high risk of developing allergic disease has also been reported by Taylor et al. (2006).

23.6.2 NK Cell Activity

Low NK cell activity is associated with enhanced susceptibility to infectious diseases and cancers. Elderly people with low NK cell activity exhibit a higher

mortality rate due to infection compared to their counterparts with adequate NK cell activity. Populations with low NK cell activity have also been found to exhibit a significantly higher risk of cancer than populations with intermediate or high NK cell activity.

Supplementation with probiotics has been shown to augment NK cell activity (*ex vivo*) in healthy adults and elderly subjects. For example, Nagao et al. (2000) and Takeda and Okumura (2007) reported significant enhancement of NK activity in middle-aged volunteers given fermented milk containing *L. casei* for 3 weeks. The effect was more pronounced in subjects with low NK activity. Similar observations have been made by others (Chiang et al., 2000; Gill et al., 2001a; Nagao et al., 2000; Olivares et al., 2006a, b; Sheih et al., 2001). The NK activity remained elevated for 3 weeks post cessation of probiotic intake (Nagao et al., 2000).

Ageing, smoking and viral infections (such as T-cell lymphotropic viruses) are all associated with decline in NK cell function (Matsuzaki et al., 2005; Morimoto et al., 2005; Ogata et al., 1997). Supplementation with specific strains of probiotics has been shown to be effective in correcting these deficits in NK cell function in all these population groups (Gill et al., 2001a; Matsuzaki et al., 2005; Morimoto et al., 2005). 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). It has been suggested that the enhancement of NK cell activity is mediated by probiotic-induced IL-12 (Takeda et al., 2006). Similar observations regarding enhancement of phagocytic and NK cell function have been made in animals fed probiotics (Cross, 2002; Gill, 1998). Differences in the efficacy of live versus dead probiotics have also been reported (Gill and Rutherfurd, 2001a, b).

Probiotic administration has also been shown to affect the relative proportions/numbers of NK cells. In a randomized, double-blind, placebo-controlled study, intake of milk fermented with *L. casei* DN114001 for 6 months during the puerperium stage was found to result in significant increase in NK cells (Ortiz-Andrellucchi et al., 2008). A significant increase in the absolute numbers/proportions of NK cells in healthy volunteers (Roessler et al., 2008), students under examination-related stress (Marcos et al., 2004) and adults immunized with influenza vaccine following probiotic administration have also been observed (Olivares et al., 2007).

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 capacity of the probiotic

strains used, sub-optimal dosage, probiotic viability or some other reason is not known. Strain- and dose-dependent differences in the ability of probiotics to modulate immune function are well documented (Donnet-Hughes et al., 1999; Gill, 1998).

Studies have also shown that commensal bacteria may reinforce innate immunity by stimulating mucosal IgA production, without the activation of T cells, and that this may be instrumental in reducing mucosal penetration by pathogenic microbes (McPherson and Uhr, 2004). Enhanced levels of fecal sIgA observed in infants fed probiotic-enriched formula suggests that, in addition to other mechanisms, probiotics may strengthen host defenses by stimulating production of mucosal IgA (Bakker-Zierikzee et al., 2006).

23.7 Effect on Adaptive (Specific) Immune Responses

As previously highlighted, the failure of the innate immune system to contain or eliminate a given pathogen results in the activation of adaptive immunity. The adaptive immunity is mediated by antibody and cell-mediated processes and is characterized by its specificity and memory. Antibodies produced by mature B lymphocytes (plasma cells) are effective at neutralizing or eliminating extracellular pathogens and antigens. Specific classes of antibodies perform distinct functions. For example, IgA is the predominant immunoglobulin produced at mucosal surfaces and is effective in preventing adherence of pathogens to the gastrointestinal mucosa. IgG and IgM are involved in systemic neutralization of bacterial toxins and promote phagocytosis by monocytes/macrophages. On the other hand, cell-mediated immunity plays a central role in protection against intracellular pathogens (including viral infections) and cancers.

Numerous studies using different probiotic strains have been conducted to determine the effect of probiotics on adaptive immune responses. These studies have provided evidence that specific strains of probiotics are effective in augmenting specific antibody responses to parenteral and oral vaccines and to some infectious agents. For example, administration of fermented milk containing *L acidophilus* La1 and bifidobacteria for 3 weeks was found to be effective in enhancing the efficacy of an orally delivered attenuated *Salmonella typhi* Ty21a vaccine in healthy human subjects; the increase in specific serum IgA titer to *S. typhi* Ty21a was more than fourfold and significantly higher ($P = 0.04$) in the probiotic group than the control group. A trend towards increased anti-*Salmonella* IgA levels in subjects receiving *Lactobacillus* GG and oral *Salmonella*

vaccine has also been reported (Fang et al., 2000). In a randomized, double-blind, placebo-controlled study, de Vrese et al. (2005) found a significantly higher virus-neutralizing antibody response (mainly IgA) following vaccination with live attenuated polioviruses in subjects given yoghurt containing *L. rhamnosus* and *L. paracasei* compared with subjects given placebo (chemically acidified milk). The levels of polio-specific serum IgG and IgA antibodies in subjects receiving yoghurt were also significantly increased. Enhancement of anti-influenza plasma IgA antibody responses following vaccination in adults given *L. fermentum* CECT5716 has also been reported (Olivares et al., 2007).

Similar observations on the beneficial effects of probiotics in children have also been made. Isolauri et al. (1995) reported enhanced efficacy of a live rotavirus vaccine in 2–5 years old children who received *Lactobacillus* GG concomitantly with rotavirus vaccination; children given *Lactobacillus* GG had significantly more IgA- and IgM-secreting cells compared with infants given vaccine only. In another study, infants given a formula containing bifidobacteria and immunized against poliovirus, several months prior to enrolment in the study, were found to exhibit higher total fecal IgA and anti-poliovirus fecal IgA responses (Fukushima et al., 1998). Furthermore, enhanced anti-poliovirus IgA antibody responses and a positive correlation between antibody titers and bifidobacteria counts in infants fed a fermented milk formula has also been reported (Mullie et al., 2004).

Probiotic supplementation has also been shown to improve responses to Hib immunization (higher frequency of protective Hib antibody titers and a trend towards higher anti-Hib IgG levels) in allergy-prone infants (Kukkonen et al., 2006). However, antibody responses to diphtheria and tetanus were not affected by probiotic intake.

Recently, West et al. (2008) determined the impact of *Lactobacillus* F19 (LF19) during weaning periods in infants on gastro-intestinal infections and IgG antibody responses to routine vaccines in a double-blind, placebo-controlled randomized intervention trial. It was concluded that feeding LF19 did not prevent infections, but increased the capacity to raise immune responses to protein antigens, with a pronounced effect in breast-fed infants (<6 months of age). The outcomes of these studies once again suggest that orally-ingested lactic acid bacteria have an adjuvant-like effect on the humoral responses.

The ability of probiotics to augment specific antibody responses following rotavirus infection has also been demonstrated. In a randomized, placebo-controlled study involving children with roravirus gastroenteritis, Kaila et al. (1992) found significantly higher levels of specific mucosal and serum antibody

responses in children given *Lactobacillus* GG fermented milk compared with children receiving a placebo. It has also been reported that viable probiotics are superior at stimulating rotavirus-specific immune responses than non-viable probiotics; the proportion of subjects exhibiting rotavirus-specific response at the convalescent stage was higher in subjects given live probiotics (10 out of 12 children) compared with the group given dead bacteria (2 out of 13) (Kaila et al., 1995; Majamaa et al., 1995).

To date, relatively few studies have examined the effect of probiotics on acquired cell-mediated immune responses in human subjects. However, there is some evidence that probiotic intake may influence the relative as well as absolute numbers or proportions of T and B lymphocytes. For example, Marcos et al. (2004) and Ortiz-Andrellucchi et al. (2008) reported increases in CD3, CD19 and CD8 T cell counts in subjects given probiotics.

The precise mechanisms by which probiotics mediate these adjuvant effects are still unclear. It appears likely, however, that probiotics mediate these effects by improving antigen presentation function (Heyman, 2001) and/or by stimulating an increase in the numbers of B cells (De Simone et al., 1991).

23.8 Cytokine Production

Cytokines are pleiotropic group of small signaling molecules that play a central role in the selection, initiation, maintenance and resolution of both innate and acquired immune responses.

Several studies have reported increased levels of IFN-γ (as well as 2′,5′-adnylate synthatase activity), IFN-α and IL-2 in healthy subjects following ingestion of probiotics (Aattouri and Lemonnier, 1997; Arunachalam et al., 2000; De Simone et al., 1986; Halpern et al., 1991; Kishi et al., 1996; Solis Pereyra and Lemonnier, 1991; Wheeler et al., 1997). Long-term consumption of conventional yogurt has also been shown to enhance production of IL1β, IL-6, IL-10, IFN-γ and TNF-α (Aattouri and Lemonnier, 1997; Halpern et al., 1991; Miettinen et al., 1996; Solis Pereyra and Lemonnier, 1993). Probiotic-induced secretion of cytokines such as IFN-γ, IL-1, TNF-α, IL-10, IL-12, IL-18 and TGF-β by mononuclear cells and DCs *in vitro* is also well documented (Lammers et al., 2003; Miettinen et al., 1998; Niers et al., 2005; Solis Pereyra et al., 1997). It has also been reported that the nature of cytokines induced by probiotic are strain-dependent.

The ability of probiotics to induce broad and disparate cytokine responses by immune cells may explain how they are able to exert diverse effects on immune

function. For example, IL-12 and IL-18 induce IFN-γ production by T, B and NK cells whilst IFN-γ enhances phagocyte-capacity of macrophages, induces MHC1 and MHCII expression on a variety of immunocompetent cells, augments anti-tumor cytotoxicity, promotes T helper cell function and enhances immunogenicity of vaccines (Nussler and Thomson, 1992). TNF-α, together with IFN-γ, potentiates the microbicidal capacity of macrophages and exerts cytotoxic effect against tumors. IFN-α plays an important role in early stages of host protection against viral infections 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 and NK cells and plays a role in the induction and regulation of T cell-mediated immune responses (Gill, 2003). IL-10 and TGF-β are pivotal for regulating polarized Th1 and Th2 responses.

23.9 Immunostimulation and Protection Against Infectious Diseases

An optimally functioning immune system is critical for protection against infections diseases. Specific populations groups with less than adequate immune competence, such as immunocompromised individuals, children and the elderly are known to exhibit increased susceptibility to gastrointestinal and respiratory tract infections. Current disease prevention and management strategies rely on the use of vaccines, antibiotics and other anti-microbials. However, the currently available vaccines are not fully effective and also there is a widespread occurrence of microbes that are resistant to commonly used antibiotics. Several well-designed studies have provided evidence that specific strains of probiotics may be useful in the prevention and control of gastrointestinal, respiratory and urogenital tract infections.

23.9.1 Gastrointestinal Infections

The effectiveness of probiotics in the management and treatment of diarrheal disease has been the subject of numerous randomized, double-blind, placebo-controlled studies and several meta-analyses (Johnston et al., 2006; McFarland, 2006, 2007; Sazawal et al., 2006; Szajewska and Mrukowicz, 2001; Van Neil et al., 2002). Despite significant heterogeneity between studies, these meta-analyses have concluded that probiotics are effective in reducing the duration of acute

diarrhea, incidence of antibiotic-associated diarrhea and the risk of traveler's diarrhea. For example, a meta-analysis of data from 34 masked, randomized, placebo-controlled trials showed that probiotics significantly reduced antibiotic-associated diarrhea by 52%, reduced the risk of travelers' diarrhea by 8% and that of acute diarrhea due to diverse causes by 34%. Furthermore, probiotic intervention was more effective in children (risk ratio: 0.43) compared with adults (risk ratio: 0.74). Similarly, the pooled results from 23 controlled trials showed that probiotics reduced the mean duration of diarrhea by 30 h, and were more effective in the treatment of acute diarrhea if given early during the course of illness (Allen et al., 2007).

Several mechanisms by which probiotics mediate their protective effects have been suggested (Gill, 2003). However, the relative significance of various mechanisms remains unclear. Results of several animal studies suggest that an augmentation of host immunity by probiotics may play an important role in host defense. For example, a positive relationship between resistance to challenge infection with *Salmonella* and *E. coli* and enhanced natural (phagocytic cell function, NK cell activity) and acquired immune responses (specific mucosal and serum antibody responses) in mice fed probiotics was reported by Gill et al. (2001d), Shu and Gill (2002), and Shu et al. (2000). Similar observations have also been made by others (reviewed by Cross, 2002).

In humans, however, only a few studies have reported concomitant measurement of immune responses (*Table 23.2*). It has been reported that a reduction in the duration of diarrhea in children with acute rotavirus gastroenteritis, following administration of probiotics, was associated with enhanced specific (frequency of cells producing rotavirus-specific IgA antibody and anti-rotavirus serum IgA levels) and non-specific immune responses (frequency of circulating IgG-, IgM- and IgA-secreting cells), (Majamaa et al.,1995; Kaila et al., 1995). Similarly, supplementation with *B. breve* in infants attending a residential institution was shown to reduce the frequency of rotavirus shedding and enhance titers of anti-rotavirus IgA in stool samples. Furthermore, Kaila et al. (1995) demonstrated that the protective effect of viable versus inactivated probiotics was due to their superior immunostimulatory capacity; infants receiving viable *Lactobacillus* GG exhibited higher anti-rotavirus serum IgA response and higher frequency of rotavirus-specific IgA-secreting cell responses compared with subjects given inactivated probiotics.

Since challenge infection studies are not possible in humans, several studies have examined immune responses to attenuated or non-virulent pathogens. These studies have indicated that probiotic supplementation is effective in

Table 23.2

Probiotic-induced immunostimulation and the prevention and/or treatment of diarrhea: examples

Author	Study design and population	Probiotic treatment/ Intervention	Health effect	Immune correlate
Kaila et al. (1992)	RDBPC Children with acute rotavirus diarrhea	*Lactobacillus* GG fermented milk or a placebo, following oral rehydration	↓ Duration of diarrhea (1.1 vs. 2.5 days)	↑ IgG, IgM and IgA-secreting cell numbers during the acute phase ↑ % of subjects with rotavirus-specific IgA-secreting cell response at convalescence stage (90% vs. 46%)
Majamaa et al. (1995)	RDBPC Children with rotavirus gastroenteritis	*Lactobacillus GG, L. casei* subsp. *rhamnosus* (Lactophilus) or a combination of *S. thermophilus* + *L. delbruckii* (Yalacta)	↓ Reduction in duration of diarrhea in LGG group (1.8 days vs. 2.8 days in Lactophilus and 2.6 days in Yalacta groups)	Enhancement of rotavirus specific IgA-secreting cell numbers and serum IgA antibody levels at convalescent stage in LGG group
Kaila et al. (1995)	Infants with acute rotavirus diarrhea	Viable or inactivated *Lactobacillus* GG, following oral rehydration	↓ Duration of diarrhea in subjects given viable compared with inactivated *Lactobacillus* GG	Higher anti-rotavirus serum IgA response and higher frequency of subjects with rotavirus-specific IgA-secreting cell response in subjects given viable vs. inactivated *Lactobacillus GG* (10/12 vs. 2/13)
Araki et al. (1999)	Infants attending a residential institution	*B. breve* (strain YIT 4064) for 28 days	↓ Rotavirus shedding	Tendency toward increased rotavirus-specific IgA in the stools
Phuapradit et al. (1999)	RDBPC Children (6–36 months age) Prevention of rotavirus diarrhea	*Bifidobacterium* Bb12 alone or with *S. thermophilus*	Prevention of symptomatic rotavirus infection	No significant increase in antibody levels in treatment group indicating no infection (30% of control group showed sub-clinical infection)

augmenting immune responses to live rotavirus (Isolauri et al., 2000), *Salmonella* (Link-Amster et al., 1994) and polio vaccines (de Vrese et al., 2005).

23.9.2 Extra-intestinal Infections

27.9.2.1 Respiratory Tract Infections

Perhaps the best evidence for a pivotal role of immunological defense mechanisms in host protection comes from studies demonstrating the effectiveness of probiotics against pathogenic organisms at extra-intestinal sites (Lenoir-Wijnkoop et al., 2008), particularly the respiratory tract (de Vrese et al., 2006; Habermann et al., 2001; Hatakka et al., 2001; Turchet et.al., 2003). Many of these studies have measured changes in immune function and health effects concomitantly and found that probiotic-mediated protection is accompanied by stimulation of host immunity (*Table 23.3*). For example, de Vrese et al. (2006) and Winkler et al. (2005) reported that significant reduction in total symptom score, the duration of common cold episodes and days with fever during an episode in healthy adults following probiotics supplementation during winter/spring period was accompanied by significantly higher cytotoxic/suppressor T cell and helper T cell numbers. In a randomized, double-blind, placebo-controlled study, a reduction in the duration of infections and the frequency of respiratory tract symptoms in probiotic-fed subjects was associated with enhanced phagocytic activity of blood leukocytes (Fukushima et al., 2007). Probiotic therapy also modulated inflammatory responses as indicated by a reduction in the levels of blood IFN-α. Normalization of perturbed intestinal flora in children with acute viral and bacterial respiratory tract infections and enhancement in aspects of T and B cell function, and NK cell activity in children with acute viral and bacterial respiratory tract infections following administration with probiotics, has also been reported (Lykova et al., 2000).

Furthermore, respiratory tract infections impair the host's capacity to produce interferons, a vital component of cell-mediated host defense mechanism. Supplementation with probiotics was found to restore the interferon production capacity of children suffering from acute respiratory tract infections (Lykova et al., 2001). As highlighted earlier, interferons (alpha and gamma) play an important role in host protection, especially against intracellular/viral infections.

Activation of Th1 type immune responses is pivotal for effective eradication of intracellular pathogens, including viruses. Infants that produce high levels of

Table 23.3

Efficacy of probiotics in the prevention of respiratory tract infections: some examples (*Cont'd* p. 916)

Author	Study design/population	Intervention	Health effect	Immune correlate
Fukushima et al. (2007)	RDBPC Enterally-fed in-patients (n = 24) aged over 70 years	Fermented milk containing *L. johnsonii* La1 or placebo	Significant reduction in the percentage of days with infection and lower frequency of respiratory symptoms in the probiotic group	↓TNF-α levels in blood ↑ Phagocytic activity of blood leukocytes in subjects whose initial levels were low in the La1 group
Cobo et al. (2006)	RDBPC Children (3–12 years old, n = 251)	Given *L. casei* (DN-114001) fermented milk (n = 142) or a placebo (n = 109) for 20 weeks to determine effect on infectious disorders	Lower incidence of lower respiratory tract infections in the probiotic group (32% vs. 49%, $P < 0.05$)	ND
de Vrese et al. (2006)	RDBPC Healthy adults (n = 479)	*L. gasseri* PA 16/8, *B. longum* SP 07/3 and *B. bifidum* MF 20/5 + vitamins and minerals or placebo	Reduction ($P < 0.06$) in total symptom score, duration of common cold ($P = 0.05$) and number of days with fever ($P = 0.02$) in the probiotic group	Significant increase in $CD8^+$ and $CD4^+$ cells in the probiotic treated group
Winkler et al. (2005)	RDBPC Health adults (n = 477)	Probiotics (*Lactobacillus* and *Bifidobacterium spp*) plus vitamins and minerals or placebo	Reduction ($P < 0.07$) in the incidence of respiratory tract infections, total symptom score $P = 0.12$), and number of days with fever ($P = 0.03$) in the probiotic group	Significant increase in T-lymphocytes (including $CD4^+$ and $CD8^+$ cells) and monocytes

Table 23.3

Author	Study design/population	Intervention	Health effect	Immune correlate
Tubelius et al. (2005)	RDBPC Healthy adults (n = 262)	*L. reuteri* or placebo	Fewer subjects in the *L. reuteri* group reported sick-leave compared with the placebo group (10.6% vs. 26.4%, $P < 0.01$; frequency of sick leave 0.4% vs. 0.9%, $P < 0.01$). Amongst the shift workers, 33% reported sick leave compared with 0% in the probiotic group, $P < 0.005$)	ND
Gluck and Gebbers (2003)	ORPC Healthy adults (n = 209)	Fermented milk drink containing *Lactobacillus* GG (ATCC 53103), *Bifidobacterium* sp B420, *L. acidophilus* 145, and *S. thermophilus*; or standard yogurt	Significant reduction ($P < 0.001$) in the number of potential pathogenic bacteria in nasal cavity in probiotic group	ND
Turchet et al. (2003)	ORC Free-living elderly subjects (n = 360)	*L. casei* (DN-114001) fermented milk or a placebo for 3 weeks	No effect on the incidence of infections Reduction (20%, $P < 0.05$) in the duration of infection in probiotic group	ND

Lykova et al. (2001)	Hospitalized children (n = 46); 33 with complicated forms of acute respiratory virus infection and 13 with vegetov – ascular dystonia (comparison group)	Bifidobacterin forte	Not reported	Normalization of an impaired interferon status of children with respiratory tract infections
Hatakka et al. (2001)	RDBPC Day-care children (1–6 years, n + 571) in 18 day care centers	Milk with or without *Lactobacillus* GG (n = 289) or standard milk	Children in LGG group had – 17% fewer respiratory tract infections, fewer days of absence from day care (16% ↓, $P < 0.05$) and longer time without respiratory symptoms (5 vs. 4 days, $p < 0.05$) and 19% less antibiotic usage ($P < 0.05$)	ND
Lykova et al. (2000)	Children with acute viral and bacterial infections of the respiratory tract (n = 129)	Bifidobacterin forte	Normalization of intestinal microbiota	Improvements in the indices of T and B cell immunity, NK cell activity and interferon producing capacity of blood leukocytes

ORC open label randomized controlled trial, *RDBPC* randomized double-blind placebo-controlled, ND not done, ORPC open, randomised, placebo-controlled

Th1 cytokines in response to respiratory viral infections (Sin et al., 2001) and mice that are genetically engineered to overproduce Th1 cytokines exhibit less severe disease following infection (Pinto et al., 2006). A negative relationship between PHA (phytohaemagglutinin)-induced secretion of IFN-γ by cord blood mononuclear cells and the incidence of viral illnesses during the first year of life has also been reported (Copenhaver et al., 2004).

23.9.2.2 Urogenital Infections

Vaginal flora in healthy women is dominated by *Lactobacillus* species. The depletion of lactobacilli is associated with increased occurrence of urinary and vaginal infections. As a result, the use of probiotics to maintain and/or restore vaginal microbiota and prevent and/or treat urogenital infections has been the focus of active investigation. Whilst a small number of well-designed studies have provided evidence for the therapeutic and prophylactic efficacy of specific probiotic strains, others have found little or no therapeutic benefit (Barrons and Tassone, 2008; Reid, 2008). Whether this has been simply due to differences in strains, dosages or frequency of treatment used by various studies or some other reason is unclear. The mechanisms by which lactobacilli/probiotics mediate their protective effects are also not fully known, but thought to include competitive exclusion, competition for nutrients, production of antimicrobial compounds (such as hydrogen peroxide, bacteriocins, organic acids) and biosurfactants and immune stimulation. However, direct evidence supporting a role for immune mechanisms is limited and requires further research. It is quite likely, however, that being a part of the common mucosal system, sensitized immune cells induced in the gastrointestinal tract may relocate to distant mucosal sites such as urogenital tract. This is supported by the effectiveness of orally-delivered probiotics against urogenital infections and the fact that most of these infections are caused by pathogens ascending from the rectal area.

23.10 Immunostimulation and Protection Against Cancer

Cancer, a major cause of morbidity and mortality, is one of the most serious health problems afflicting human population worldwide. In addition to genetic factors, many lifestyle factors (environmental and behavioral) have been implicated in the

development of cancer. Despite significant advances in science and technology, including sequencing of the human genome, progress in developing preventive and therapeutic strategies for cancer has remained slow. Emerging evidence from animal and human studies suggest that specific strains of probiotic may have a protective effect against colon and bladder cancers.

23.10.1 Colorectal Cancer

Colorectal cancer (CRC) is a major cause of death from cancer in the developed world (Saikali et al., 2004). It has been suggested that environmental factors (especially diet) and colonic microbiota play an important role in colorectal carcinogenesis. As a result, there has been an increasing interest in exploring the protective effects of fermented milks and probiotics against CRC. Results of animal studies have shown that administration of probiotics is effective in reducing the incidence of CRC/or of precursor lesions (Capurso et al., 2006). The protective effect was found to be more pronounced when probiotics were administered before but not after the carcinogenesis. *In vitro* studies have also yielded similar results. There is also evidence from epidemiological and case-control studies that consumption of fermented products may have a protective effect against colorectal cancer. However, there is relatively little direct evidence of the anti-cancer efficacy of probiotics or fermented products in human subjects. Rafter et al. (2007) reported reduction in several colorectal cancer biomarkers in a randomized, double-blind, placebo controlled trial involving polypectomized and colon cancer patients following synbiotic (a combination of prebiotic SYN1 and probiotics *Lactobacillus* GG and *Bifidobacterium lactis* BB12) intervention. Patients receiving synbiotics exhibited significant reduction in colorectal proliferation and the capacity of fecal water to induce necrosis in colonic cells, and an improved epithelial barrier function. Additionally, synbiotic consumption prevented an increased secretion of IL-2 by peripheral blood mononuclear cells in polypectomized patients and increased the production of IFN-γ in cancer patients. These favorable changes in CRC biomarkers were also accompanied by significant changes in fecal flora: populations of *Bifidobacterium* and *Lactobacillus* increased and *Clostridium perfringens* decreased. A reduced rate of tumors in cancer patients following probiotic intervention has also been observed in another large randomized clinical study (Ishikawa et al., 2005). The occurrence rate of tumors with a moderate or severe atypia and tumors larger than 4 mm was significantly lower in subjects receiving probiotics. The ability of specific strains

of probiotics to reduce the concentration of bacterial enzymes that convert procarcinogens to carcinogens has also been demonstrated in several human studies (Rafter, 2002).

The mechanisms by which probiotics mediate these protective effects are not clear, however, an association between enhanced immune function and tumor suppression suggest an important role of probiotic-induced immunostimulation in host protection. Perdigon et al. (1998) and Feghali et al. (1997) reported that an inhibition of carcinoma development in mice fed yoghurt was accompanied by a significant increase in the number of IgA secreting cells and $CD4^+$ T lymphocytes in the lamina propria of the large intestine together with a decrease in the number of IgG^+ and $CD8^+$ cells. Similarly, mice given cytoplasmic fractions of *L. casei* and *B. longum* were found to exhibit significant anti-Tumor immunity and enhanced counts of CD8 T cells, total T cells, NK cells and $MHCII^+$ cells (Lee et al., 2004). In another study, Takagi et al. (2001) showed that NK cells were pivotal for probiotic-mediated anti-cancer immunity. Delayed tumor onset and reduced tumor incidence in normal mice administered *L. casei* were associated with enhanced NK cell activity and numbers of NK cells. However, *L. casei* failed to exert any protective effect in beige mice that are genetically deficient in NK cells. In patients with Dukes A stage colorectal cancer, administration of *L.casei* Shirota was found to increase the percentage of T helper cells and NK cells and decrease the proportion of T suppressor cells (Sawamura et al., 1994). *In vitro*, probiotics have been shown to mediate anti-cancer effects by promoting apoptosis through enhancing mitogen activated protein kinase (MAPK) activities including C-Jun N-terminal Kinase and P38 MAPK, and down regulating Nuclear Factor-KB (NF-kB) dependent gene products that mediate cell proliferation (COX-2, cyclin D1) and survival (Bcl-2, Bcl-XL) (Iyer et al., 2008). The ability of probiotic-derived components to induce macrophage activation and significantly increase production of TNF-α and NO that exhibit cytotoxic effects against tumor cells (Caco-2, HT-29, and SW480) has also been observed by Lee et al. (2008a).

23.10.2 Bladder Cancer

Probiotic administration has also been shown to reduce the recurrence rate of superficial bladder cancer after transurethral resection (Aso et al., 1992, 1995). A similar protective effect of fermented milk containing *L. casei* Shirota against bladder cancer was reported by Ohashi et al. (2002). Stimulation of the immune

system, as indicated by increases in the percentage of T-helper cells and NK cells and enhancement of NK cell cytotoxic activity have been suggested to play an important role in the prevention of tumor development.

23.11 Probiotics and Attenuation of Immuno-Inflammatory Disorders

A balance between Th1 and Th2 responses is essential for immune homeostasis. Polarization of immune responses towards either the Th1-type or Th2-type results in an increased occurrence of immunoinflammatory disorders. For example, diabetes mellitus type-1 (DM) and rheumatoid arthritis (RA) are associated with an over expression of Th1 responses, whereas allergies are associated with Th2 cell predominance. Of the various factors suggested to be responsible for the increased incidence of immunoinflammatory disorders, a lack of or inappropriate exposure to microbes early in life (due to improved hygiene, vaccination and antimicrobial medication) that are essential for the development of a balanced immune system (establishment of regulatory networks) has received wide acceptance. Although, drug based therapy is available for controlling these disorders, it invariably suffers from adverse side effects and exorbitant cost beyond the reach of the low income population groups.

There is overwhelming evidence that specific strains of probiotics are endowed with unique immunoregulatory properties and therefore, may provide a safe and effective alternative to drug therapy for the prevention or management of immunoinflammatory disorders. The possible mechanisms by which probiotics attenuate various inflammatory disorders are discussed in the following section.

23.11.1 Allergies

Allergic disorders are the consequence of aberrant Th2 type immune responses to innocuous environmental antigens in genetically susceptible individuals and are characterized by increased IgE synthesis and recruitment and activation of eosinophils. At birth, the immune system of the newborn is biased towards Th2 profile. In healthy infants, the polarized Th2 profile is down-regulated by the activation of Th1 cells, whereas the Th2 profile remains augmented in atopic infants due to the absence of counter-regulatory processes. An alarming increase

in the incidence of allergic diseases in children and adults observed in recent years has been attributed to lack of exposure to microbial antigens during early life. Signals transmitted by microbes have been suggested to be pivotal for the maturation of the immune system and the development of regulatory networks. The role of indigenous flora in shaping the development of the immune system is also highlighted by qualitative and quantitative differences in the composition of microbiota of atopic versus non-atopic children (Bjorksten et al., 2001); children who develop allergy were found to have fewer bifidobacteria and enterococci than non-allergic children.

As specific probiotic strains exhibit potent immunoregulatory properties, several studies have examined their effectiveness in the treatment or prevention of allergic disorders, especially atopic dermatitis (*Table 23.4*). The results of these studies have been mix.

In an open label study, Majamaa and Isolauri (1997) reported improvement in SCORAD (SCOring Atopic Dermatitis) and reduction in inflammatory markers (fecal α1-antitrypsin and TNF-α) in children with AD following 1 month supplementation with probiotics. Similar observations on the therapeutic effects of *Lactobacillus* GG in children weaned onto a probiotic-supplemented hydrolyzed formula were made by Isolauri et al. (2000). However, in subsequent studies, improvements in eczema were found to be limited only to subjects with allergic sensitization (Rosenfeldt et al., 2003; Sistek et al., 2006; Viljanen et al., 2005). On the other hand, several studies have found no effect of LGG (Folster-Holst et al., 2006; Gruber et al., 2007) or other probiotics on treatment of atopic dermatitis in infancy (Betsi et al., 2008). Furthermore, a number of studies have also demonstrated the protective effect of probiotics in high-risk infants. Kalliomaki et al. (2001, 2003) demonstrated that probiotic supplementation prenataly and postnataly to mothers with a history of atopic disease and their newborns suppresses the development of atopic dermatitis in high-risk children in later life (at least up to the age of 4 years). Similar observations regarding the protective effects of *L. rhamnosus* HN001 given during pregnancy and lactation have also been reported (Wickens et al., 2008). It has been shown that probiotics may mediate their protective effect by modulating maternal and fetal immune responses and through the induction of immunoregulatory factors in breast milk (Prescott et al., 2008). Contrary to these observations, however, Kopp et al. (2008) reported that supplementation with *Lactobacillus* GG during pregnancy and early infancy neither reduced the incidence nor the severity of atopic dermatitis in affected children but was associated with an increased rate of recurrent episodes of wheezing bronchitis. Taylor et al. (2006) noted that supplementation with *L. acidophilus* was associated

Table 23.4

Efficacy of probiotics in the prevention and treatment of atopic dermatitis: some examples (*Cont'd* p. 924)

Reference	Study design and population	Probiotic used	Outcome	Immune effect
Wickens et al. (2008) and Prescott et al. (2008)	RDBPC	*L. rhamnosus* HN001, *Bifidobacterium lactis* H019 or placebo daily from 35 weeks gestation until 6 months if breast-feeding, and their infants (same treatment as mothers) from birth to 2 years	Infants receiving *L. rhamnosus* had a significantly lower risk of eczema	Mothers receiving *L. rhamnosus* had significantly higher levels of cord blood IFN-γ and higher proportion had detectable blood IFN-γ compared with placebo
	Mothers and infants with family history of allergic disease		No significant effect of probiotics on atopy	Higher levels of TGF-β1 and IgA in breast milk mother given probiotics Prescott et al., 2008)
Abrahamsson et al. (2008)	RDBPC	*L. reuteri* ATCC55730 daily to mothers for 4 weeks before delivery and mother and baby daily for 12 months after delivery	No effect on eczema	Significant reduction in skin test reactivity in probiotic group
	Mothers and infants with family history of allergic disease		Infants receiving probiotics had less IgE-associated eczema at 2 years of age	
Kopp et al. (2008)	RDBPC	*Lactobacillus* GG or placebo starting 4–6 weeks before expected delivery, followed by postnatal supplementation for 6 months	No effect on the incidence or severity of atopic dermatitis	No difference in total immunoglobulin concentrations or number of specific sensitization to inhaled allergens
	Mothers and infants with family history of atopic disease			
Hol et al. (2008)	RDBPC	*L. casei* CRL 431 + *B. lactis* Bb12 or placebo	No effect on tolerance to cows milk	Higher percentage of pan T cells and helper T cells in placebo group than seen in probiotic treated infants
	Infants with cows milk allergy			

Table 23.4 (*Cont'd* p. 926)

Reference	Study design and population	Probiotic used	Outcome	Immune effect
Taylor et al. (2006)	RDBPC Infants with family history of atopic disease	*L. acidophilus* LAVRI-A1 or placebo daily for the first 6 months of life	No effect on risk of atopic dermatitis Higher percentage of children with skin-prick test and atopic dermatitis in the probiotic group	Higher rate of sensitization in placebo group
Kukkonen et al. (2007)	RDBPC Mother and infant pairs (up to 6 months). Prevention of food allergy, eczema, asthma and allergic rhinitis	Probiotics + galacto-oligosaccharide	Reduction in the incidence of atopic diseases ($p < 0.052$), eczema ($p < 0.035$) and atopic eczema ($p < 0.025$)	Not recorded
Morita et al. (2006)	Controlled Trial Subjects (n=15) with perennial allergic rhinitis/showing high serum IgE levels and allergic symptoms	*L. gasseri* TMC0356	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 28 ($p < 0.05$)
Xiao et al. (2006)	RDBPC Subjects with history of Japanese cedar pollinosis (JCPsis)	*B. longum* BB536	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
Weston et al. (2005)	RDBPC Children (6–18 months) with moderate to severe Atopic dermatitis (AD)	*L. fermentum* VRI-033	Significant reduction in the SCORAD index over time ($p < 0.03$)	Reduction in IgE level (35.7 ($\pm$6.0) in placebo group vs. 31.8 ($\pm$4.3) in probiotic group

Prescott et al. (2005)	RDBPC Young children with moderate-to-severe atopic dermatitis (AD)	*L. fermentum* PCC trade mark	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)
Ciprandi et al. (2005)	Controlled trial Adult subjects (mean age 22.3 years, n=10) with allergic rhinitis	Enterogermania (containing *Bacillus clausii*)	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
Viljanen et al. (2005)	RDBPC Infants with atopic eczema/dermatitis syndrome (AEDS) and food allergy	*Lactobacillus* GG (LGG), a mixture of four probiotic strains (MIX)	Reduction in SCORAD in IgE sensitized infants. Reduction in AD 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
Ciprandi et al. (2004)	Controlled trial involving children attending the nursery school Allergic children (mean age 4.4 yr, n=10) with recurrent respiratory infections	Enterogermania (containing *Bacillus clausii*)	Symptoms not reported	Significant 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

Table 23.4

Reference	Study design and population	Probiotic used	Outcome	Immune effect
Rosenfeldt et al. (2003)	Double-blind, placebo-controlled, crossover study Children (1–13 year old) with atopic dermatitis	*L. rhamnosus* 19070–2 and *L. reuteri* DSM 122460)	Improvement in SCORAD	Reduction in serum eosinophil cationic protein levels (P = 0.03) in the probiotic group
Rautava et al. (2002)	RDBPC Mother-infant pairs with history of atopic diseases	Probiotics	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-β 2 level in human milk in probiotic group (2,885 pg/mL) vs. placebo (1,340 pg/mL) placebo; $P = 0.018$)
Kalliomaki et al. (2001) and Kalliomaki et al. (2003)	RDBPC Mother and infant pairs with history of atopic eczema	*Lactobacillus* GG prenataly to mothers and postnataly whilst breast feeding up to 6 months and to babies if not breast feeding until 6 months of age	Significant reduction in the incidence of atopic eczema up to 4 years of age	No effect on IgE levels/skin-prick test reactivity
Isolauri et al. (2000)	RDBPC Infants (mean age 4.6 months) with history of atopic eczema	*B. lactis* Bb-12 or *Lactobacillus* GG (ATCC 53103)	Reduction in SCORAD in the *B. lactis* Bb-12 group to 0 (0–3.8), and in the *Lactobacillus* GG group to 1 (0.1–8.7), vs. unsupplemented to 13.4 (4.5–18.2)	Reduction in the concentration of soluble CD4 in serum and eosinophilic protein X in urine
Pelto et al. (1998)	Double-blind, cross-over study Milk hyper sensitive and healthy adult subjects	*Lactobacillus* GG (ATCC 53103)	Down-regulation 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

RDBPC randomized double-blind placebo-controlled, *CMA* cows milk allergy Adapted from Gill and Prasad (2008)

with increased incidence of allergen sensitization in infants. The inability of probiotics to prevent the development of atopic dermatitis/eczema has been reported by others as well (Abrahamsson et al., 2008; Taylor et al., 2006). Whether this was simply due to difference in the strains used, probiotic dose, host or environmental factors is not clear. *Lactobacillus* GG, *B. lactis and L. rhamnosus* are known to possess potent immunomodulatory properties (reviewed earlier). Strain dependent differences in allergy-preventing efficacy of probiotics in humans have been demonstrated recently (Wickens et al., 2008).

It is important to note that probiotics administration has been found to be ineffective in influencing allergic manifestations in young adults and teenagers (Helin et al., 2002; Ishida et al., 2005). This suggests that the protective effects of probiotics are restricted to infancy when the immune system is still undergoing the learning/maturation process. Evidence from recent studies suggests that probiotics mediate their prophylactic or therapeutic anti-allergy effects through induction and activation of immunoregulatory and/or counter-regulatory immune responses. Ability of specific strains of probiotics to induce regulatory cells that produce IL-10 and TGF-β and inhibit proliferation and cytokine secretion by immune cells such as T cells has recently been demonstrated (Smits et al., 2005). Increased production of IL-10 and IFN-γ by PBMC *in vitro* and increased levels in breast-milk and serum following ingestion of probiotics has also been reported (Lammers et al., 2003; Pessi et al., 2000; Pohjavuori et al., 2004). Furthermore, induction of regulatory T cells in animal models (Di Giacinto et al., 2005) and increased *in vitro* production of regulatory cytokines (IL-10, TGF β after intake of probiotics) has also been demonstrated (Lammers et al., 2003). Involvement of other mechanisms such as reduction in the immunogenicity of potential allergens and strengthening of the mucosal barrier function have also been suggested to play a protective role.

Overall, the results of the clinical studies reported to date, although promising, are inconclusive. Further well-designed, large-scale, long-term studies are required to further evaluate the therapeutic and/or prophylactic effect of promising probiotic strains in defined population groups with specific endpoints. The precise mechanism involved in protection and the microbial factors responsible for inducing these responses also need to be elucidated.

23.11.2 Inflammatory Bowel Disease

Inflammatory Bowel disease (IBD) encompasses two distinct diseases; Crohn's disease (CD) and ulcerative colitis (UC). Both diseases are chronic in nature but

have quite distinct pathogeneses, underlying inflammatory profile, symptoms and treatment strategies (Geier et al., 2007). UC is largely restricted to the colon and/or rectum and is characterized by inflammation and superficial ulceration of the colonic mucosa. On the other hand, CD occurs as skip lesions in any region of the intestinal tract and is characterized by transmural granulomatous inflammation. Pouchitis is another related disorder that results from complicated ileal pouch-anal anastmosis (IPAA) surgery for UC. It is important to note that UC is a Th-2 immune response whereas CD is predominantly a Th-1 driven immune response.

The exact aetiology of IBD is unknown. Current evidence suggests that it results from a dysregulated immune response to certain enteric microbiota in genetically susceptible individuals. Although a range of microorganisms and their products have been identified in inflamed tissues of IBD patients, no specific microbe has been proven to cause IBD. There is overwhelming evidence, however, that the intestinal microbiota plays a central role in the initiation and perpetuation of the disease (Bengmark, 2007). This recently acquired knowledge has stimulated interest in exploring the effectiveness of new strategies aimed at manipulating intestinal microbiota and restoring immune system homeostasis.

Several studies have examined the potential of probiotic administration in the prevention and/or treatment of various inflammatory bowel disorders. There is a strong evidence for the effectiveness of specific probiotics (especially the commercial product VSL#3 containing a mixture of probiotic strains) in preventing the initial attack of pouchitis, and in maintaining antibiotic-induced remission in patients with recurrent or refractory pouchitis (Vanderpool et al., 2008). Promising results for the effectiveness of probiotics in UC have also been reported (Vanderpool et al., 2008). For example, *E. coli* Nissle has been found to be as effective as mesalazine in maintaining remission of UC. Protective effects of *Bifidobacterium*-fermented milk and VSL#3 in UC have also been demonstrated. Contrary to the above observations however, there is little evidence to support the effectiveness of probiotics in CD.

The primary mode of action of probiotics appears to be through restoration of gut microbial balance and mucosal barrier function, as well as up-regulation of immunoregulatory pathways. The ability of specific strains of probiotics to suppress production of pro-inflammatory cytokines and induce regulatory T cells is well documented (Lee et al., 2008b; Sheil et al., 2004; Vanderpool et al., 2008). For example, Cui et al. (2004) reported an association between increased expression of IL-10 and the prevention of flare-ups of chronic UC (Cui et al., 2004) and Lammers et al. (2005) found a reduction in pro-inflammatory cytokines in tissues obtained from subjects with pouchitis following treatment with probiotics. IL-10 and TGF-β have also been demonstrated to ameliorate inflammation

in *Helicobacter hepaticus*-induced IBD in an IL-10 deficient mouse model (Pena et al., 2005). A recent report has further shown that VSL#3 improves pouchitis disease activity index by increasing the number of mucosal regulatory T cells (Pronio et al., 2008).

Interestingly, in addition to live probiotics, some isolated components of probiotic bacteria have been demonstrated to exert immunomodulatory effects on the mucosal immune system. Even bacterial DNA has been demonstrated to have anti-inflammatory and immunomodulatory properties when administered subcutaneously in a number of animal models of colitis (Rachmilewitz et al., 2002). Genomic DNA isolated from VSL#3 inhibited TNF-α induced IL-8 secretion, mitogen-activated protein kinase activation and NFkB activation in HT-29 cells (Jijon et al., 2004). However, significant differences exist in the ability of various probiotics to induce anti-inflammatory versus pro-inflammatory cytokines and this may explain differences in the efficacy of various probiotic strains.

23.11.3 Diabetes Mellitus

Diabetes mellitus (DM) is a major cause of morbidity and mortality across the world. Type 1 Diabetes, which accounts for 1–10% of cases, results from autoimmune destruction of pancreatic β cells. Over-production of pro-inflammatory cytokines (such as IL-1β, TNFα and IFNγ) has been proposed as the possible mechanism for this (Rabinovitch and Suarez-Pinzon, 1998). Up-regulation of IL-10 has been shown to have a protective effect against this destruction of β cells (Goudy et al., 2003). The far more prevalent (90–95% of cases) Type 2 Diabetes involves a resistance of tissues to insulin, resulting in hyperglycaemia. An increased release of TNF-α, MCP-1 and additional products of macrophages and other cells that populate adipose tissue are thought to play a role in the development of this resistance (Wellen and Hotamisligil, 2005; Yang et al., 2002).

To date, only a small number of animal studies have explored the anti-diabetic effects of probiotics. Matsuzaki et al. (1997) reported that oral administration of *L. casei* in KK-A^y mice significantly decreased plasma glucose levels and inhibited the production of β cell specific $CD4^+$ T cells and cytokines (INFγ and IL-2) associated with the induction of autoimmune diabetes. They further reported that diets containing *L. casei* strongly inhibited destruction of β cells and nitric oxide production. Later, Calcinaro et al. (2005) reported that VSL#3 prevented autoimmune diabetes by reducing insulitis in non-obese diabetic (NOD) mice. This preventative effect was associated with an increased production of IL-10 by Peyer's patches and splenocytes and a decreased expression of IL-1-mRNA in the

pancreas. IL-10 is generally considered to be an anti-inflammatory cytokine acting primarily on antigen-presenting cells to inhibit antigen presentation and the production of inflammatory cytokines. The effectiveness of IL-10 in preventing the development of autoimmune diabetes in NOD mice has also been demonstrated previously (Goudy et al., 2001). In another study, Matsuzaki et al. (2007) investigated the effects of *L. casei* strain Shirota (LcS) in autoimmune disease models using NOD mice. From the age of 4 weeks, female NOD mice were fed a diet containing *L. casei* and the onset of diabetes was recorded thereafter. The incidence of diabetes in the control group was significantly higher than that of the *L. casei*-treated group, and pathological analysis of the *L. casei* -treated group revealed a strong inhibition of pancreatic β-cell destruction. Moreover, the proportion of $CD8^+$ T cells amongst splenocytes was decreased in the *L. casei*-treated group, suggesting an inhibition of autoreactive T cells.

Since the anti-diabetic effect of probiotics is highly strain specific, more extensive work with a large number of strains is required to establish the immunomodulating/immunoregulatory potential of probiotics as oral therapy against DM. A study examining the protective effects of probiotics against β-cell autoimmunity in children at genetic risk of type-1 diabetes (the PRODIA study) is currently being conducted in Finland (Ljungberg et al., 2006). In the near future, there is a strong possibility of developing a probiotics-based therapy for combating this massive public health burden.

23.11.4 Rheumatoid Arthritis

Rheumatoid arthritis (RA), another important autoimmune disease, is characterized by chronic synovitis and causes stiffness, pain, loss of mobility and progressive erosion (deterioration) of the joints. It usually affects multiple joints symmetrically; the hand and wrists most commonly, but also the elbows, neck, shoulders, hips, knee, and feet. Extra-articular manifestations of RA can include development of nodules under the skin (especially at the elbows), lymphadenopathy, vasculitis and even peripheral neuropathy. Although many questions concerning the aetiology of RA remain unanswered, cumulative evidence suggests that $CD4^+$ T cells, which exhibit a predominantly Th1 pattern of cytokine expression, play an important role in the pathogenesis of the disease. Blockade of IL-12 and/or TNF-α has been shown to reduce progression of collagen-induced arthritis in mice (Butler et al., 2008) and leads to significant clinical improvement in subjects with RA (van Oosterhout et al., 2005).

Studies in animal models have shown that probiotics possess the ability to regulate over-expressed Th1-type responses and exert a beneficial effect in experimentally-induced arthritis. Oral administration of *L. casei* to DBA/1 mice prevented the onset of type II collagen (CII)-induced arthritis (CIA), reduced anti-collagen type II IgG2a and IgG2b serum antibodies and suppressed the CII-induced secretion of IFN-γ from splenocytes (Kato et al., 1998). In another study involving IL-10 knockout mice, Sheil et al. (2004) reported that even subcutaneous administration of *L. salivarius* 118 was effective in attenuating the development of collagen-induced arthritis and that the probiotic effect was associated with reduced production of proinflammatory (Th 1) cytokines and maintained production of anti-TGF-β. Recently, So et al. (2008) reported that probiotics mediate this effect by down-regulating Th1 effector functions. *L. casei* administration reduced type II collagen (CII)-reactive proinflammatory molecules (IL-1 β, IL-2, IL-6, IL-12, IL-17, IFN-γ, TNF-α and Cox-2) by $CD4^+$ T cells. *L. casei* administration also reduced translocation of NF-kB into the nucleus and CII-reactive Th1-type IgG isotypes IgG2a and IgG2b, while up-regulating immunoregulatory IL-10 levels. Preventative and curative effects of both live and heat-killed *Lactobacillus* GG in experimentally induced arthritis (Baharav et al., 2004) and the ability of *Enterococcus faecium* to improve the anti-inflammatory and anti-arthritic effects of methotrexate in adjuvant-induced arthritis in rats have also been demonstrated (Rovensky et al., 2005).

However, there is relatively little evidence regarding the effectiveness of probiotics against arthritis from human studies. In a randomized, double-blind placebo-controlled study, Hatakka et al. (2003) reported a beneficial effect of probiotic administration in RA patients. Although there were no statistically significant differences in the activity of RA, more subjects given *Lactobacillus* GG over a 12 month period reported subjective wellbeing compared with the placebo.

23.12 Mechanisms by Which Probiotics Modulate Immune Function

23.12.1 Recognition of Probiotics by the Immune System

The gastrointestinal tract is the largest body surface area permanently exposed to the external environment. It is continuously bombarded with a wide array of antigens derived from food, resident microbiota and the environment. Thus, the

challenge for the gut is to allow absorption of nutrients and exhibit tolerance towards indigenous flora whilst at the same time mount an effective immune response against potential pathogens. To perform this function optimally, the gastrointestinal tract harbors the largest number of immunocompetent cells (known as gut-associated lymphoid tissue, GALT) in the body.

Probiotics and probiotic-derived products in the gut are recognized by M cells (specialized epithelial cells overlying Peyer's patches), epithelial cells and dendritic cells (DCs) distributed in the intraepithelial and submucosal layers. Epithelial cells respond to bacterial components by releasing a variety of chemokines and cytokines (Haller et al., 2000) that recruit immune cells such as DCs to the mucosa. Antigens (probiotics and their products) taken up by M cells are delivered to antigen presenting cells (APCs), mainly macrophages and (DCs) located in the dome region. Antigen-loaded DCs travel to mesenteric lymph nodes and present antigens to T and B cells. Similarly, antigens/microbes phagocytosed by macrophages are either destroyed or presented to T and B cells. Active transport of live commensals by DCs from the mouse gut lumen to intestinal mesenteric lymph nodes has recently been reported by McPherson and Uhr (2004). Sensitized T and B cells circulate through the lymph and bloodstream and traffic back to populate mucosa remote from the inductive sites. APCs recognize conserved microbial signature molecules called pathogen-associated molecular patterns (PAMPS) through pattern-recognition receptors (Toll-like receptors (TLRs), C-type lectin receptors and Nod-like receptors) that are secreted or expressed on the surface of immune cells. These receptors are specific for various microbial components such as lipopolysaccharides, peptidoglycan and bacterial DNA. On recognition of PAMPS, TLRs activate a cascade of signaling pathways that induce antimicrobial effector responses and inflammation. The ability of probiotics to influence the expression level of TLRs and other TLR-like receptors on mucosal and systemic cells (Vanderpool et al., 2008) and to induce cytokine secretion from DCs and macrophages through the TLR-mediated signaling pathways is well documented (Shida and Nanno, 2008). Furthermore, activation of TLRs expressed on APCs play a central role in the initiation of acquired immunity; antigen recognition by APCs is followed by the secretion of cytokines and expression of co-stimulatory molecules. The nature of cytokines in the milieu, type and dose of antigen, phenotype and state of activation of APCs determine whether naive T lymphocytes differentiate into T helper 1 (Th1), T-helper 2 (Th2) or T regulatory (Treg) cells. For example, Th1 differentiation is dependent on IFN-γ and IL-12, and the Th2 differentiation relies on the presence of IL-4. Subsequent activation of Th1 cells results in the production of

IFN-γ, IL-2 and TNF-α and is associated with the development of cell-mediated and cytotoxic immunity. Activated Th2 cells produce IL-4, IL-5 and IL-13 and these promote antibody production and are associated with atopy. Treg cells secrete IL-10 and TGF-β and down-regulate the activities of both Th1 and Th2 cells.

23.12.2 Effect on Epithelial Cells

The epithelium (enterocytes, IECs) lining the gastrointestinal tract constitutes the first line of host defense and plays an important role in maintaining intestinal homeostasis. IECs express several antigen-presenting and costimulatory molecules and function as immunoregulatory cells. IECs recognize (MAMPS through TLRs and NOD) and respond to different bacteria and bacterial products in a discriminatory manner. IECs secrete a wide array of proinflammatory cytokines and chemokines, including IL-8 and TNF-α, in response to stimulation by pathogenic bacteria (Kagnoff and Eckmann, 1997), but show little or no response to resident microbiota. Through the release of cytokines and chemokines, IECs alert the host immune system and direct the development and deployment of effector (innate and/or acquired) immune responses to sites where the mucosal barrier has been breached. It has been demonstrated that in contrast to pathogens, specific probiotics are able to attenuate inflammatory pathways in epithelial cells through a variety of mechanisms (Tlaskalova-Hogenova et al., 2005). The ability of probiotics to attenuate inflammation and down-regulate over expressed immune responses in animal models of colitis and in subjects with milk allergy and IBD is well documented.

23.12.3 Regulation of Skewed Th1 and Th2 Responses and Attenuation of Immunoinflammatory Disorders

As mentioned earlier, T cells can be classified as Th1, Th2 or Treg/Th3 cells based upon their cytokine profiles. Treg cells produce IL-10 and TGF-β and are able to down-regulate skewed Th1/Th2 immune responses. A balance between Th1-Th2 is pivotal for immunological homeostasis, and polarization of immune responses towards Th1 or Th2 underlies the development of various immunoinflammatory

disorders. For example, allergies are driven by over-activation of Th2 immune responses and IBD and autoimmune disorders such as Type 1 diabetes are predominantly driven by Th1 type immune responses. Evidence from recent studies suggests that defective Treg cell activity may be the central cause for the concurrent rise in Th1 and Th2-mediated diseases observed over the recent decades; patients with Type 1 Diabetes, Multiple Sclerosis and individuals with a predisposition to allergy development are known to exhibit deficient Treg cell function (Guarner et al., 2006).

Results of *in vitro* and *in vivo* studies also suggest that the primary mechanism of probiotic action in inflammatory diseases is likely to be through the induction of regulatory T cells. 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 been reported in several studies (Isolauri et al., 2000; Pessi et al., 2000). An association between increased IL-10 expression and the prevention of flare-ups of chronic UC (Cui et al., 2004) as well as a reduction in pro-inflammatory cytokines in tissue obtained from subjects with pouchitis following treatment with probiotics have also been demonstrated (Lammers et al., 2005).

Further support for the role of Treg cells in probiotic-mediated protection comes from studies that have shown an increased number of T reg cells following probiotic administration in both animals and humans. Di Giacinto et al. (2005) found an increased number of Treg cells bearing surface TGF-β, following administration of probiotics, in an animal model of colitis. These cells conferred protection against colitis in a cell-transfer system. Notably, the protective effect was dependent upon TGF-β and IL-10 and was abolished by appropriate neutralizing antibodies. An association between increased number of mucosal regulatory T cells and protection against puchitis has also been recently reported (Pronio et al., 2008). Chapat et al. (2004) showed that the ability of *L. casei* to reduce skin inflammation due to contact sensitivity was also Treg cell-dependent. In another 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).

It has also been suggested that an interaction between DCs and probiotics is a critical step determining the development of various forms of immunity or tolerance. Maturation of DCs towards functionally distinct DC1, DC2 or DCreg subsets selectively directs the polarization of naive T cells towards Th1, Th2 or Treg phenotypes (Kapsenberg, 2003). Different probiotic strains induce distinct and even opposing DC responses (expression of cytokines and

maturation surface markers) with respect to their Th1/Th2/Treg-driving capacity (Christensen et al., 2002). The ability of certain probiotics to induce regulatory DCs has been reported by Hart et al. (2004) and Drakes et al. (2004).

Cumulatively, these observations suggest that mechanisms by which probiotics mediate their protective effects are not limited to the gut and are most likely mediated by Treg cells. Once induced, Treg cells are able to travel to other tissues in the body (Rook and Brunet, 2005). The effectiveness of orally or subcutaneously administered probiotics and bacterial DNA in attenuating colitis and arthritis in mice further supports this view (McCarthy et al., 2003; Rachmilewitz et al., 2004; Sheil et al., 2004).

Thus, probiotics have been demonstrated to confer health benefits by influencing the composition of gut flora and restoring intestinal homeostasis.

23.13 Conclusion

Of the many health benefits associated with the intake of probiotics, modulation of the immune system has received most attention. It is well documented that specific strains of probiotics are endowed with unique immunomodulatory properties. In healthy individuals, they have been shown to enhance phagocytic and microbicidal capacity of PMNs and monocytes, the tumor cell-killing capacity of NK cells, the immunogenicity of bacterial and viral vaccines (oral and systemically-delivered) and specific antibody responses to enteric pathogens. Emerging evidence also suggests that potentiation of innate and acquired immune responsiveness by probiotics may play an important role in protection against infectious diseases (both intestinal and extra-intestinal infections) and cancers. However, further studies, with concomitant measurement of immune responses and health outcomes, are needed to confirm these findings.

In subjects with immunoinflammatory disorders (such as IBD and allergies), probiotic administration has been shown to reduce the incidence or relieve the symptoms of various disorders by down-regulating aberrant Th1 or Th2 responses. Although the exact underlying mechanisms have not been elucidated, the available data suggests that probiotics mediate their effect through the induction of regulatory T cells that produce IL-10 and TGF-beta. Recent studies, mainly in experimental animals, also highlight the potential for using probiotics for the prevention/management of other inflammatory disorders such as RA and DM (especially type 1 DM). However, these observations still remain to be confirmed in human subjects.

Despite these major advances, significant deficits still remain in our knowledge regarding the effectiveness of different probiotics against various conditions, as well as the mechanisms by which probiotics promote health and protect against disease. It is important to note in this context, that most of the health benefits ascribed to probiotics have been proven only for a limited number of probiotic strains - in many cases only for a single strain. Thus, large scale, well-designed trials in relevant population groups are needed to unequivocally prove the clinical efficacy of probiotics. Effective/optimum dose rate and frequency of treatment also remains to be established for various probiotics for different population groups and health conditions.

List of Abbreviations

AD	atopic dermatitis
AEDS	atopic eczema/dermatitis syndrome
APC	antigen presenting cells
CD	Crohn's disease
CRC	colorectal cancer
DCs	dendritic cells
DM	diabetes mellitus
GALT	gut-associated lymphoid tissue
GIT	gastrointestinal tract
IBD	inflammatory bowel disease
IEC	intraepithelial cells
ND	not done
NK cells	natural killer cells
ORC	open-label controlled trial
ORPC	open randomised placebo-controlled
PAMP	pathogen-associated molecular patterns
PMN	polymorphonuclear cells
RA	rheumatoid arthritis
RDBPC	randomised, double-blind, placebo-controlled
SCORAD	SCOring atopic dermatitis
Tc	T cytotoxic cells
Th	T helper cells
TLRs	Toll-like receptors
UC	ulcerative colitis

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