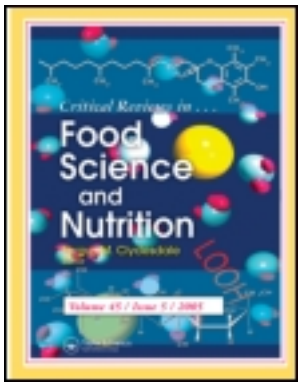

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Immune System Stimulation by Probiotic Microorganisms

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Review

Immune System Stimulation by Probiotic Microorganisms

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Probiotic organisms are claimed to offer several functional properties including stimulation of immune system. This review is presented to provide detailed informations about how probiotics stimulate our immune system. Lactobacillus rhamnosus GG, Lactobacillus casei Shirota, Bifidobacterium animalis Bb-12, Lactobacillus johnsonii La1, Bifidobacterium lactis DR10, and Saccharomyces cerevisiae boulardii are the most investigated probiotic cultures for their immunomodulation properties. Probiotics can enhance nonspecific cellular immune response characterized by activation of macrophages, natural killer (NK) cells, antigen-specific cytotoxic T-lymphocytes, and the release of various cytokines in strain-specific and dose-dependent manner. Mixture and type (gram-positive and gram-negative) of probiotic organisms may induce different cytokine responses. Supplementation of probiotic organisms in infancy could help prevent immune-mediated diseases in childhood, whereas their intervention in pregnancy could affect fetal immune parameters, such as cord blood interferon (IFN)-γ levels, transforming growth factor (TGF)-β1 levels, and breast milk immunoglobulin (Ig)A. Probiotics that can be delivered via fermented milk or yogurt could improve the gut mucosal immune system by increasing the number of IgA$^+$ cells and cytokine-producing cells in the effector site of the intestine.

Keywords Immunostimulation, cytokine, fermented milk, yogurt, infancy, pregnancy

INTRODUCTION

The word "probiotic" originated from Greek meaning "for life." Probiotics are "live microorganisms, which when administered in adequate amounts confer a health benefit on the host" (Shah, 2007). A number of genera of bacteria (and yeasts) are used as probiotics, including *Lactobacillus*, *Bifidobacterium*, *Leuconostoc*, *Pediococcus*, and *Enterococcus*, but the prime species believed to possess probiotic characteristics are *Lactobacillus acidophilus*, *Bifidobacterium* spp., and *Lactobacillus casei*. Species belonging to the genera *Lactobacillus* and *Bifidobacterium* have a long and safe history in the manufacture of dairy products and are also found as a part of the gastrointestinal microflora. Health benefits imparted by probiotic bacteria are strain specific; therefore, there is no universal strain that would provide all proposed benefits, not even strains of the same species. *Lactobacillus rhamnosus* GG (Valio, Culturelle), *S. cerevisiae* Boulardii (Biocodex), *L. casei* Shirota (Yakult), *L. acidophilus*, and *Bifidobacterium animalis* Bb-12 (Chr. Hansen) are certainly the most investigated probiotic cultures with established human health efficacy data (Shah, 2007). Health benefits derived from the consumption of foods containing probiotic bacteria such as *L. acidophilus*, *Bifidobacterium* spp., and *L. casei* are now well documented and have been reviewed (Lankaputhra and Shah, 1998; Wollowski et al., 2001; LeBlanc et al., 2002; Le Leu et al., 2005; Hlivak et al., 2005; Hsieh and Chou, 2006; Maldonado Galdeano and Perdigón, 2006; Shah, 2006; Shah, 2007; Vasiljevic and Shah, 2007; Amit-Romach et al., 2008; Paineau et al., 2008; Prescott et al., 2008; Pronio et al., 2008; Reid, 2008; Ryan et al., 2009; Van Hai et al., 2009). These health benefits include controlling gastrointestinal infections, improvement in lactose metabolism, anticarcinogenic and antimutagenic properties, reduction in serum cholesterol, improvement in inflammatory bowel disease, *Helicobacter pylori* infections, and immune system stimulation.

The mechanism of modulation of immune system by probiotic organisms is not entirely known, but it is believed to be due to competition for available nutrients in the colon, colonization

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site interference, and competition for binding sites on gut epithelial cells, production of bacteriocin, lowering of colonic pH, and nonspecific stimulation of the immune system (Shah, 2007). Probiotic organisms stimulate host's nonspecific resistance to microbial pathogens and thereby aid in their eradication. Probiotic bacteria may counteract the inflammatory process by stabilizing the gut microbial environment and the intestine's permeability barrier (Isolauri et al., 2001). Possible mechanisms of action of probiotic organisms include promotion of immunologic barrier via improvement in intestinal immunoglobulin A (IgA) and inflammatory responses and nonimmunologic gut defense barrier by controlling increased intestinal permeability and altered gut microecology (Isolauri et al., 2001).

IMMUNE SYSTEM STIMULATION

To efficiently handle microbial infections, the immune system needs to be in a state of "alert" that is maintained by the process of "immunostimulation." Immunity itself deals with highly complex mechanisms with multiple functions and encountering an antigen-elicited complex variety of immune responses that can be either cellular or humoral or both, based on mediating elements. In the humoral response, helper (CD4) T lymphocytes recognize the pathogen antigens complexed with class II major histocompatibility complex (MHC) proteins on the surface of antigen-presenting cell and produce cytokines that activate B cells expressing antibodies specifically matching the antigen. The B cells undergo clonal proliferation and differentiation to form plasma cells which then produce specific immunoglobulins (antibodies). Secreted antibodies contribute to host defense functions by binding to antigens on the surfaces of invading microorganisms and carrying out neutralization of toxins or viruses, which helps its uptake by phagocytic cells (Nairn, 1996).

In the cell-mediated response, the antigen-MHC class II complex is recognized by helper (CD4) T lymphocytes, while the antigen-MHC class I complex is recognized by cytotoxic (CD8) T lymphocytes. The numbers of active CD4 and CD8 cells are critical in maintaining cellular immune response. When an imbalance exists in the ratio of CD4 to CD8, cellular mechanisms are grossly impaired, leading to extreme susceptibility to development of many opportunistic infections, autoimmune diseases, and certain tumors (Kuby, 1992; Nairn, 1996). Each class of T cells produces cytokines, becomes activated, and expands by clonal proliferation. Cytokines are the soluble mediators of host defense responses, both specific and nonspecific, and have a critically important role in the effector mechanisms of eliminating foreign antigens (Nairn, 1996).

CD4 T cell population can be divided into three subsets based on specific cytokine secretion and effector functions such as Th1 cells (which secrete proinflammatory cytokines interleukin (IL)-2, interferon (IFN)-γ, and tumor necrosis factor (TNF)-β), Th2 cells (that produce the anti-inflammatory cytokines IL-4 and IL-5 and provide potent help for B cell activation, IgE and noncomplement-fixing subtypes of IgG), and Th2 cells (that mediate allergic immune responses) (Schulze-Koops and Kalden, 2001).

The immune system is composed of a complex array of immune responses that include two principal components: innate and adaptive immunity, which work together to protect us from external and internal injuries (Donkor et al., 2010). The innate immune system is hereditary, defending the host from infection by other organisms in a nonspecific manner. It is neither anticipatory nor clonal and does not respond to environmental changes. It does not confer long-lasting immunity to the host. It represents the first line of defense with natural killer (NK) cells as the primary cells involved in the identification and spontaneous lysis of offensive targets (virus-infected cells, tumor cells, bone marrow stem cells, and embryonic cells).

ROLE OF PROBIOTICS IN IMMUNE SYSTEM STIMULATION

Studies with animal models have demonstrated strong innate immune responses, developed after probiotic bacteria interventions. Probiotic organisms interact with the epithelial linings of the host recruit the immune cells to the site of infection and induce specific immune markers. *Lactobacillus paracasei* subsp. *paracasei* DC412 strain induced early innate immune responses after strong interaction with the cells forming the air pouch lining tissue of BALB/c (20–30 g) inbred mice or Fisher-344 inbred rats. These responses manifested as polymorphonuclear (PMN) cell recruitment, phagocytosis, and TNF-alpha (TNF-α) production, which paralleled the respective responses induced by probiotic *L. acidophilus* NCFB 1748 in the same model (Kourelis et al., 2010).

Oral administration of *L. casei* to BALB/c mice has been shown to activate immune cells of the innate immune response, with an increase in the specific markers of CD-206 and toll-like receptor (TLR)-2 cells (Maldonado Galdeano and Perdigón, 2006). The innate immune system via TLRs recognizes a large group of chemical structures in pathogens such as lipopolysaccharides (LPS) and lipoteichoic acids. This enables them to recognize foreign objects that trigger a cascade of immunological defense mechanisms, such as the production of pro- and anti-inflammatory cytokines (Anderson, 2000). TLRs are expressed mainly by macrophages and dendritic cells (DCs), and a variety of other cell types such as B cells and epithelial cells (Pasare and Medzhitov, 2005). The activation of TLRs results in the initiation of the response of the DCs, which leads to the production of cytokines and upregulation or downregulation of cell-surface molecules (Granucci and Ricciardi-Castagnoli, 2003). These signals critically influence further induction of both innate and adaptive immunity. Table 1 lists the main studies carried out on immune system stimulation by probiotic bacteria. It appears that *L. rhamnosus*, *L. casei*, and *L. acidophilus* are the most widely studied strains among *Lactobacillus* species and *Bifidobacterium lactis* among *Bifidobacterium* species.

Table 1 Studies reporting immune responses by probiotic bacteria

Probiotic studied	Administering protocol	Population tested	Immune response	References
B. lactis Bi-07, *B. lactis* Bl-04, *L. acidophilus* La-14, *L. acidophilus* NCFM, *L. plantarum* Lp-115, *L. paracasei* Lpc-37, *L. salivarius* Ls-33	Individually in the form of two capsules/day for 21 days containing 1×10^{10} cfu /capsule of bacteria	Humans (healthy volunteers aged 18–62 years)	During early response (day 0–21), serum IgG significantly increased in subjects consuming *Bifidobacterium lactis* Bl-04 and *L. acidophilus* La-14 ($P = 0.01$) compared with controls. During late response (day 21–28) serum IgA and IgM increased in subjects consuming *L. acidophilus* NCFMs. The overall vaccination titer was not influenced by the administration of the probiotics during the oral preparation of cholera vaccination protocol	(Paineau et al., 2008)
L. acidophilus, *B. infantis*, *B. bifidium*	In combination with yogurt starter, i.e., *L. bulgaricus* & *S. thermophilus*	Mice (female B6C3F1, eight weeks old)	Yoghurt supplemented with *L. acidophilus* and *Bifidobacterium* spp. stimulated enhanced mucosal and systemic anticholera toxin IgA	(Tejada-Simon et al., 1999; Sanders and Klaenhammer, 2001)
L. acidophilus, *L. reuteri*, *B. infantis*, *L. casei* GG	Individually via orally and anally with 1 mL of 10^7 cfu/mL probiotic and *Candida albicans*	Mice (C57BL/6 *bg*/*bg-nu*/*nu* and *bg*/*bg-nu*/+)	Increased the levels of IgG, IgA, and IgM in euthymic immunocompromised mice. Antibody and cell-mediated responses to *Candida albicans* in immunodeficient mice could decrease the incidence and severity of candidiasis	(Wagner et al., 1997)
L. acidophilus, *Bacillus subtilis*	Individually and in combination to get 1×10^7 cfu/g of bacteria supplemented to diet & fed 8% of body weight for eight weeks	Fish-Nile tilapia (*Oreochromis niloticus*)	Increase in hematocrit values and serum bactericidal activity in group given mixture of bacteria. A significant increase in the values of the nitroblue tetrazolium (NBT) assay, neutrophil adherence, and lysozyme activity in all probiotic-treated groups after one and two months of feeding compared with untreated control group	(Aly et al., 2008)
L. acidophilus (LAVRI/DSM)	Administered 1×10^9 bacteria in 0.2 m LPBS/day for two weeks to 5 mice/group and orally postchallenged with 1×10^8 *Candida albicans* blastoconidia	Mice (male BALB/c; H-2 d and DBA/2; H-2d, six–eight weeks old)	Stimulation of IL-4–nitric oxide paracrine loop. Enhancement of both IL-4 and IFN-γ production in the regional lymph nodes, and secretion of IFN-γ and nitric oxide in saliva	(Clancy, 2003; Elahi et al., 2003)
L. acidophilus LAVRI-A1	3×10^9 bacteria in maltodextrin (freeze dried powder in sachets reconstituted in 1–2 mL sterile water)/day or maltodextrin alone (control) for six months	Humans (infants-six months)	No significant difference to TLR-2 or TLR-4 mediated immune responses in probiotic group compared with control. No significant effects of probiotics on either Th1 or Th2 cell responses to allergens or other stimuli. Supplementation with *L. acidophilus* for the first six months of life did not affect early innate responses in infants at high risk of getting allergic diseases	(Clancy, 2003; Taylor et al., 2006)
L. acidophilus Lal, *B. bifidium* Bb12	Individually in the form of fermented milk supplemented with *B. bifidium* Bb12 (group 1, 1×10^{10} cfu/day) or *L. acidophilus* (group 2, 7×10^{10} cfu/day) for second three weeks	Humans (healthy volunteers, 12 females and 16 males, 23–62 years)	Enhanced phagocytosis after consumption of fermented products. Increased phagocytic capacity of granulocytes and monocytes	(Schiffrin et al., 1995)

Table 1 Studies reporting immune responses by probiotic bacteria *(Continued)*

Probiotic studied	Administering protocol	Population tested	Immune response	References
L. acidophilus Lal (*L. johnsonii* La1)	In combination with starter culture (*S. thermophilus*), 150-mL fermented milk for first three weeks (group 1, fermented milk base-control; group 2, milk fermented with 10^7 cfu/mL La1; group 3, milk fermented and stored for 21–28 days to get10^6 cfu/mL of La1)	Humans (healthy volunteers, 27 females and 15 males, 21–57 years)	Increased leukocyte oxidative burst activity and enhanced phagocytic capacity of PMN and mononuclear cells. Significant increase was observed with group receiving fermented product containing 10^7 cfu/mL of La1	(Donnet-Hughes et al., 1999; Isolauri et al., 2001; Gill, 2003)
L. acidophilus Lal	Milk (150 g) fermented with 10^8 cfu/g La1 or acidified milk without La1 (control) for 28 days	Humans (healthy volunteers)	Slight increase in serum IgA level in the group receiving La1 but did not affect on mucosal IgA level	(Marteau et al., 1997)
L. casei Shirota (TMC 0413), *L. rhamnosus* GG (ATCC 53103-TMC 0514)	Individually along with other LAB cultures, i.e., *L. acidophilus* (TMC 0313, 0356), *L. casei* (TMC 0402, 0409, 1001, 1002, 1003), *L. rhamnosus* (TMC 0503, 0510)	Murine macrophage-like cell line J774	All the tested bacteria increased the production of IL-6, IL-12, and TNF-α by J774.1 cells. Seven tested strains (TMC 0356, 0409, 0503, 0510, 1001, 1002, and 1003) induced the secretion of IL-10. *L. acidophilus* TMC 0356 produced the highest concentrations of IL-6, IL-10, IL-12, and TNF-α compared with other bacteria	(Morita et al., 2002; Shah, 2007)
L. acidophilus, La1, *B. bifidum* Bb12	In combination, full fat milk fermented with *S. thermophilus*, mesophilic streptococci, La1 and Bb12 to get 1×10^7–10^8 cfu/g of probiotic bacteria (3×125 g fermented milk /day) and orally challenged with *S. typhi* vaccine	Humans (healthy volunteers, 14 females and 16 males, 19–59 years)	Increased (more than fourfold) anti-*Salmonella typhi* antibody response in test group compared with control. Increase in serum total IgA level	(Link-Amster et al., 1994)
L. acidophilus (DDS-1 La1, NRRL 6934, NRRL B4527 and NRRL 0734), *B. bifidium*	Individually, alongwith *Escherichia coli* ATCC 25922	Murine macrophage cell line RAW264.7 of BALB/c origin	Induced the production of IL-1α and TNF-α. La1 induced higher secretion of IL-1α and TNF-α by macrophages than other lactobacilli, and bifidobacteria	(Rangavajhyala et al., 1997)
L. acidophilus, B. bifidum, Streptococcus faecalis	In combination via gavage on the day 1 of age, in two doses (1×10^5 & 1×10^6 cfu bacteria) and postinfected with (1×10^4 cfu) *Salmonella* serovar *typhimurium* at day 2 of age	Chickens (36 female)	No significant difference in IL-6 and IL-10 gene expression in cecal tonsils of chickens in different treatment groups but repression of IL-12 and IFN-γ expression in cecal tonsils of chickens infected with *Salmonella*	(Haghighi et al., 2008)
L. casei strain Shirota	Fermented milk (3×100 mL/day) containing 10^9 cfu *L. casei* Shirota/mL to the treatment group	Humans (20 healthy males, 40–65 years)	No significant effects were observed for any of the immune parameters measured including the percentages of T cells, CD4+ cells, CD8+ cells, NK cells and B cells, NK activity and production of IFN-γ, IL-1β and IL-2 between control and treatment group	(Spanhaak et al., 1998)
L. casei strain Shirota	Oral administration of suspension of Biolactis powder containing 2.6×10^{11} *L. casei* Shirota/g in distilled water	BALB/c mice (male, 7–10 weeks)	Increased the production of IFN-γ but not that of IL-4 or Il-5. Induced marked increase in the production of IL-12, probably by macrophages, which, in turn, stimulated the production of IFN-γ	(Matsuzaki, 1998; Kato et al., 1999; Gill, 2003)
L. casei, L. acidophilus	Oral administration of milk (20% suspension in water for eight days containing 2.4×10^9 cfu lactobacilli/day) fermented with *L. casei* (group 1), *L. acidophilus* (group 2), and mixture of both cultures in same proportion (group 3), and 10% skim milk powder to control group. Orally postchallenged with *Salmonella typhimurium*	Swiss albino mice (25–30 g weight, $n =$ 20–30/group)	Increased anti-*Salmonella* antibodies in serum and in intestinal fluid were found in the group of mice fed with the mixture (fivefold higher in serum and one- to twofold higher in intestinal fluid than control) and with *L. casei* fermented milk, respectively (twofold higher in serum and three- to fivefold higher in intestinal fluid than control)	(Perdigón et al., 1990)

(Continued on next page)

Table 1 Studies reporting immune responses by probiotic bacteria *(Continued)*

Probiotic studied	Administering protocol	Population tested	Immune response	References
L. casei	Oral administration of 1.2×10^9 cfu *L. casei* /day (for two, five, and seven consecutive days) suspended in sterile nonfat milk as a 20% (v/v) suspension in water (sterile milk for control group) and orally postchallenged with *Salmonella typhimurium* or *Escherichia coli* O_{111} K_{58}	Swiss albino mice (25–30 g weight, n = 20–30/group)	Increased levels of IgA anti-pathogen antibodies in intestinal secretions, whereas values of IgG anti-*Salmonella* and IgG anti-*Escherichia coli* were similar to control values in all groups	(Perdigón et al., 1991)
L. casei YIT 003	Intraperitoneal or intravenous administration (0.1 mg) of *L. casei* or other immunostimulant namely *Streptococcus pyogenes* (OK-432), Bacillus Calmette Guérin (BCG) or protein bound polysaccharide preparation isolated from *Coriolus vasicolor* (PSK) for 2, 7, or 13 days and intravenously postinfected with *Listeria monocytogenes*.	Mice (ddY, female, five weeks)	Increased phagocytosis in mice by activating the production of macrophages. Significantly prolonged survival against *Listeria* infection in *L. casei*-treated mice compared with groups of mice treated with other immunostimulants	(Tomioka et al., 1992)
L. casei strains (Danone strain 001 LAB-1, LAB-2 and Yakult)	Individually or in combination. Oral administration of milk fermented with one of three *L. casei* strains, yogurt, a mixture of LAB-1 and yogurt or milk. Oral postinfection with *S. typhimurium* (LD50 dosage).	Mice	Significant increase in circulating IgA levels, β-glucuronidase activity of peritoneal macrophages and phagocytosis index in mice fed with milk fermented with *L. casei* strains (LAB-1 and Yakult)	(Paubert-Braquet et al., 1995)
L. casei strain Shirota	Individually using *L. johnsonii* JCM 0212 as negative control	BALB/c mice (female, six–eight weeks) and offsprings of OVA23-3 mice (male, 15–20 weeks). Murine macrophage cell line, J774.1 and murine B lymphoma cell line	Induced IFN-γ, but inhibited IL-4 and IL-5 secretion, and markedly suppressed total and antigen-specific IgE secretion by OVA-stimulated splenocytes. Increased IL-12 production in the cell cultures containing *L. casei* by macrophages	(Shida et al., 1998; Cross, 2002)
L. casei ssp. *rhamnosus* GG	Individually as fermented milk product containing 10^{10}–10^{11} cfu *L. casei* GG, 125 g × 2/day (test group) or fermented then pasteurized yogurt containing $<10^3$ LAB, 125 g × 2/day (placebo)	Children (44 well-nourished comprised 39 rotavirus positive -22 in test group and 17 in placebo, 33.4% female, 7–35 months)	Increased IgA rotavirus-specific antibody-secreting cells in children who received *L. rhamnosus* GG during the acute phase of diarrhea	(Kaila et al., 1992; Marteau et al., 2001; Gill, 2003)
L. casei ssp. *rhamnosus* GG (ATCC 53103)	Individually containing 10^7 cfu/mL *L. casei* GG. Antiapoptotic response was compared with *L. acidophilus* (ATCC 393), *L. casei* (ATCC 4356), *Salmonella pullorum* and *Salmonella typhimurium*	Young adult mouse colon cell lines and human colonic epithelial carcinoma cell lines	LGG prevented cytokine-induced apoptosis in intestinal epithelial cell lines in culture by activating the antiapoptotic Akt/protein kinase B. It also inhibited the proapoptotic p38/mitogen-activated protein kinase by stimulating the production of TNF-α, IL-1β, IL-1α, or IFN-γ	(Yan and Polk, 2002; Shah, 2007)
L. casei CRL 431	Individually as *L. casei* cells suspended in sterile 10% (v/v) nonfat milk and administered at 1% (v/v) in drinking water for two, five, or seven consecutive days at 10^8 cfu/mL/mouse/day.	BALB/c mice (25–30 g weight, six weeks)	Increased IgA+ cells and IL-6 producing cells. The immune cells activated after oral *L. casei* administration to BALB/c mice were those of the innate immune response (with an increase in the specific markers of these cells, CD-206 and TLR-2), with no modification in the number of T cells	(Maldonado Galdeano and Perdigón, 2006; Shah, 2007)
L. casei, *L. acidophilus*	Mixture of both bacteria in the form of fermented milk and then challenged orally with *Shigella sonnei*	Mice	Increased survival against infection, decreased pathogen translocation to the spleen and liver; increased serum and gut mucosal anti-*Salmonella* antibody	(Nader De Macias et al., 1992; Cross, 2002)

Table 1 Studies reporting immune responses by probiotic bacteria *(Continued)*

Probiotic studied	Administering protocol	Population tested	Immune response	References
L. casei GG	Individually as reconstitution of two lyophilized preparations in water, one previously heat treated (85–100°C for 10 minutes) and the other as viable containing 1×10^{11} cfu/g *L. casei* GG	Humans (41 well-nourished children, 1.3–38.4 months comprised 26 rotavirus positive- 13 each in two groups)	Viable *L. casei* strain GG enhanced IgA cell response and serum IgA response to rotavirus. Clinical recovery was same in both groups. Rotavirus-specific IgM and IgG were not detected at convalescence in either groups	(Kaila et al., 1995)
L. casei GG, *L. casei* subsp. *rhamnosus* (Lactophilus), *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* (Yalacta)	Individually or in combination. Given one of three preparations, reconstituted in 5 mL water (×2/day/group for 5 days) including freeze-dried *L. casei* GG (group 1, $n = 16$) or Lactophilus (group2, $n = 14$), or Yalacta (group 3, $n = 19$)	Humans (49 rotavirus infected children, 6–35 months)	Increased number of rotavirus-specific IgA secreting cells in patients receiving *L. casei* GG compared with those receiving Lactophilus or Yalacta. Higher serum rotavirus IgA level at convalescent stage in *L. casei* GG and Lactophilus groups than in the Yalacta group	(Majamaa et al., 1995; Gill, 2003)
L. casei subsp. *rhamnosus GG* (ATCC 53103)	Individually as orally challenged with milk containing 2.6×10^8 cfu/day *L. casei* (200 mL × 2/day for one week, preceeded and followed by one-week period without milk proteins	Humans (milk-hypersensitive and healthy adults, 13 females, 4 males, 22–50 years)	In milk-hypersensitive subjects, milk with *L. casei* GG prevented the increase expression of receptors but in healthy individuals, it significantly increased the expression of phagocytosis receptors (CR1 and CR3), receptor for IgG (FcγRIII) and for IgA (FcαR) in neutrophils	(Pelto et al., 1998)
L. casei subsp. *rhamnosus G, L. lactis*	Individually as oral administration of lyophilized *L. casei* GG (4.0×10^{10} cfu/day), or *L. lactis* (3.4×10^{10} cfu/day) or ethyl cellulose (placebo) for seven days and challenged with *S. typhi* oral vaccine on day 1, 3, and 5 of the administration	Humans (30 healthy adult volunteers, 15 females, 15 males, 20–50 years)	Increase in specific IgA among the subjects receiving *L. casei* GG with the vaccine. Significantly higher CR3 receptor expression on neutrophils in *L. lactis* group than those receiving either the placebo or *L. casei* GG	(Fang et al., 2000)
L. casei subsp. *rhamnosus GG*	Oral administration of *L. casei* GG in conjunction with D × RRV rhesus-human reassortant live oral rotavirus vaccine	Humans (infants, two–five months)	Increased rotavirus specific IgM secreting cells suggesting enhanced immune response to oral rotavirus vaccine	(Isolauri et al., 1995; Dunne et al., 1999; Clancy, 2003)
L. rhamnosus (HN001), *L. acidophilus* (HN017), *B. lactis* (HN019)	Individually as oral administration of 10^9 cfu/day of HN001, HN017, HN019 in 50-μL skimmed milk (100 g/L) or 50-μL skimmed milk (control) without LAB for 10 days (set 1-oral challenged with cholera toxin on day 0 and 7) or for 28 days (set 2-oral challenged with tetanus vaccine on day 7 and 21)	BALB/c mice (male, 6–7 weeks)	Significant increase in the phagocytic activity of peripheral blood leukocytes and peritoneal macrophages in LAB groups compared with the control. Significant increase in IFN-γ response to stimulation with concanavalian A in spleen cells from LAB than cells from control mice. Serum antibody response, specific to both vaccines was significantly increased in the presence of LAB	(Gill et al., 2000)
L. rhamnosus HN001, *B. lactis* HN019	Individually as prenatal (two–five weeks) and postnatal (for the mother—six months if breast feeding and for the baby—two years) oral administration of 6×10^9 cfu/day *L. rhamnosus* ($n = 34$) or 9×10^9 cfu/day *B. lactis* ($n = 35$) or placebo ($n = 36$)	Humans (mother–baby pairs)	Supplementation with probiotic increased the cord blood IFN-γ levels in neonates, TGFβ1 levels in early breast milk—week 1 and breast milk IgA levels, compared with the placebo group	(Prescott et al., 2008)
L. rhamnosus HN001	Individually as skim milk powder -based diet alone (control, $n = 40$) or mixed with freeze dried culture to get 3×10^8 cfu/g *L. rhamnosus* for seven days ($n = 44$) and orally postchallenged with *E. coli* O157:H7 (0.1 mL/mouse-10^8 cfu)	BALB/c and C57BL/6 mice (six–eight weeks, male)	Significant increase in intestinal anti-*E. coli* IgA response and blood leucocyte phagocytic activity among probiotic fed mice compared with control group	(Shu and Gill, 2002)

(Continued on next page)

Table 1 Studies reporting immune responses by probiotic bacteria *(Continued)*

Probiotic studied	Administering protocol	Population tested	Immune response	References
L. rhamnosus HN001	Individually as reconstituted low-fat milk powder (25 g/200 mL, ×2/day) containing *L. rhamnosus* (group A, $n = 27$) or lactose hydrolyzed low-fat milk containing *L. rhamnosus* (group B, $n = 27$) from four–six weeks or without probiotic (one–three weeks in stage 1, seven–nine weeks in stage 3)	Humans (52 healthy elderly volunteers, 17 males, 35 females, 44–80 years)	Increased in relative levels of peripheral blood polymorphonuclear leukocytes cell response and natural killer cell tumor killing activity following consumption of *L. rhamnosus* HN001	(Sheih et al., 2001)
B. lactis HN019	Individually as oral administration of suspension of 10^9 cfu or 2×10^8 cfu *B. lactis* in reconstituted skim-milk and subsequently orally challenged with 8×10^5 cfu or 10^7 cfu *S. typhimurium* in two different experiments	BALB/c mice (six–eight weeks)	Increased in splenic lymphocyte proliferation response, blood and peritoneal cell phagocytic activity, and gut mucosal anti-*Salmonella* antibody titers in mice fed with *B. lactis*. It also increased survival, reduced cumulative morbidity index, and decreased pathogen translocation to the spleen and liver	(Shu et al., 2000)
L. rhamnosus strain GG (ATCC 53103), *L. paracasei* subsp. *paracasei* (CRL431)	Individually as low-fat milk supplemented with either bacteria (test) or without bacteria-placebo (control) followed by acidification. Administered 10^{10} cfu bacteria/serving (100 g) and orally postchallenged with live attenuated poliomyelitis virus vaccine	Humans (66 healthy male volunteers, 20–30 years)	Increased poliovirus neutralizing antibody titer, polio-specific serum IgG and IgA in subjects consuming probiotics following oral immunization with live attenuated polioviruses	(Gill, 2003; De Vrese et al., 2005)
B. animalis	Individually administered 200 μL/day (100 μL/day for week 1) of 1×10^9 cfu *B. animalis* or saline (control) followed by intraperitoneal sensitization and post challenge with ovalbumin by inhalation or saline aerosols	BALB/c mice (two weeks, equal male: female ratio)	Modestly reduced the number of infiltrating eosinophils and lymphocytes in the lungs but did not affect on allergen-specific serum immunoglobulin E levels. Skewed the Th1/Th2 balance toward Th1 in females, whereas in males significantly decreased ConA-induced IL-13 and a trend toward lower levels of ovalbumin-induced Th2 cytokines	(Ezendam et al., 2008)
B. breve M16V, *B. infantis* NumRes251, *B. animalis* NumRes252 and NumRes253, *L. plantarum* NumRes8, *L. rhamnosus* NumRes6	Individually as oral administration of 10^9 cfu probiotic /animal/day in 0.4 mL PBS or PBS alone (placebo) to 7–18 mice from day 28–42, followed by intraperitoneal presensitization and postchallenge with ovalbumin	BALB/c mice (male, five–eight weeks, 20–25 g)	Of the six strains, *B. breve* and *L. plantarum* inhibited response to methacholine, reduced the number of eosinophils in the bronchoalveolar lavage fluid, reduced both ovalbumin-specific IgE and IgG1. *B. breve* also reduced IL-4, IL-5, and IL-10 and reduced acute allergic skin reactions to ovalbumin	(Hougee et al., 2010)

Immunostimulation in Elderly and Adult Subjects

Probiotics as a dietary supplement are known to enhance some aspects of cellular immunity in humans, and more specifically in elderly subjects, characterized by the activation of macrophages, NK cells, antigen-specific cytotoxic T-lymphocytes, and the release of various cytokines. *B. lactis* (HN019) is the most extensively studied probiotic organism that has been reported to enhance T lymphocytes, PMN cell phagocytosis, and NK cell killing activity. In a study by Gill et al. (2001), milk supplemented with *B. lactis* (HN019) was consumed by 30 healthy elderly volunteers (age range: 63–84 years; median: 69 years) for nine weeks and an increase in total helper ($CD4^+$) and activated ($CD25^+$) T lymphocytes and NK cells were found while the proportions of cells staining positively for $CD8^+$ (MHC I-restricted T cells), $CD19^+$

(B lymphocytes), and human leukocyte antigen including HLA-DR$^+$ (MHC II-bearing antigen-presenting cells) remained unaltered throughout the trial (Gill et al., 2001).

In a randomized, double-blinded, placebo-controlled trial, 25 healthy elderly subjects (median age 69 years; range: 60 $\pm$ 83 years) consumed probiotic milk containing *B. lactis* HN019. The subjects exhibited enhanced levels of IFN-α upon stimulation of their peripheral blood mononuclear cells (PBMCs) in culture. Control group subjects ($n = 12$, 4 male, 8 female; median age: 67 years; range: 60 $\pm$ 75 years) consumed 180 mL reconstituted milk twice daily, whereas the test group subjects ($n = 13$, 3 male, 10 female; median age: 70 $\pm$ 83 years) consumed 180 mL milk containing 1.56×10^{11} cfu live *B. lactis* HN019. There was a significant increase in PMN cell phagocytic capacity among test group subjects after consumption of milk supplemented with *B. lactis*, whereas individuals who consumed *B. lactis*-supplemented milk or milk alone showed enhanced phagocyte-mediated bactericidal activity. "Even a relatively short-term dietary regime (six weeks) was sufficient to impart measurable improvements in natural immunity" (Arunachalam et al., 2000).

In another double-blind, three-stage before-and-after intervention trial, 50 healthy individuals (age range: 41–81 years; median age: 60 years) were randomly divided into two groups. In the first and last stage comprising 3 weeks, all the subjects consumed reconstituted low-fat milk, whereas in the middle stage of three months, one of two groups consumed low fat milk containing *B. lactis* and the other group consumed *B. lactis* in lactose hydrolyzed low-fat milk. An increase in PMN and NK cell activity was found to be greatest in subjects consuming *B. lactis* (HN019) in oligosaccharide-enriched low-fat milk as compared with the control group that consumed low-fat milk alone (Chiang et al., 2000).

Probiotics can modulate nonspecific cellular immune response indicated by an increased phagocytic activity. In a placebo-controlled, double-blinded, randomized crossover trial using 26 healthy volunteers (mean age: 25 years), yogurt supplement containing probiotic organisms (*L. acidophilus* 74-2 and *B. lactis* 420) given for three weeks significantly increased the phagocytic activity of granulocytes and monocytes from 92 to 95%. Other specific immune parameters and the oxidative burst activity of cells remained unaffected (Klein et al., 2008). Oligosaccharides aid in the colonization of transient flora in the human gastrointestinal tract and can help probiotics in rendering health benefits. Consumption of milk fermented with *L. acidophilus* strain La1 or *B. bifidum* by healthy adult subjects ($n = 28$, 16 male, 12 female; mean age: 36.9 years; age range: 23–62 years) for three weeks, also increased their overall phagocytic activity of blood leukocytes, particularly granulocytes (Schiffrin et al., 1995).

Immunostimulation in Infants

The establishment of normal bacterial populations during early infancy could prevent the colonization of potential pathogens in the gastrointestinal tract (Isolauri et al., 2001). Microbial exposure along with probiotic interventions in infancy could be helpful in developing immune system maturation for treatment and prevention of immune-mediated diseases in infancy and childhood. In a double-blinded, placebo-controlled, randomized intervention trial, one group of infants ($n = 89$) was given cereals supplemented with *L. paracasei* subsp. *paracasei* strain F19 (dose 10^8–10^{10} cfu/day) from 4–13 months of age and the control group ($n = 90$) was given cereals alone. These groups of infants were immunized with DTaP (diphtheria and tetanus toxoid, and acellular pertussis), polio and Hib-conjugate vaccines at 3, 5.5, and 12 months of age. *L. paracasei* subsp. *paracasei* strain F19 improved immune responses "by increasing antidiphtheria toxin concentrations when adjusting for breastfeeding duration and colonization with this strain ($p = .024$). There was an interaction of the intervention and colonization with the respective strain on antitetanus toxoid concentrations during the course of a vaccination ($p = .035$)," but the effect was revealed as statistically insignificant. The concentrations of anti-*Haemophilus influenzae* type B (Hib) capsular polysaccharide were increased after the first and second dose of Hib vaccine by breastfeeding duration such as in infants breastfed for $<$ six months compared with those breastfed $\geq$ six months ($p < .05$), with no effect by *L. paracasei* subsp. *paracasei* strain F19 (West et al., 2008). The mechanisms involving in such trials are still not known clearly.

Since the innate system is inborn, maternal supplementation with probiotic organisms may affect the fetal immune parameters and immunomodulatory factors in breast milk (Prescott et al., 2008). Prescott et al. (2008) evaluated the effects of maternal probiotic supplementation on immune markers in cord blood and breast milk. The authors demonstrated that supplementation with probiotics during pregnancy has a potential to influence fetal immune parameters as well as immunomodulatory factors in breast milk. Daily supplements of either *Lactobacillus rhamnosus* HN001 ($n = 34$; dose $= 6 \times 10^9$ cfu/day), *Bifidobacterium lactis* HN019 ($n = 35$; 9×10^9 cfu/day) or a placebo ($n = 36$) (containing dextran, salt, and a yeast extract) were given to pregnant women from two to five weeks before delivery and continuing for six months during lactation. Cord blood plasma and breast milk samples were collected at three to seven days, three months, and six months postpartum. The assays for cytokines (IL-13, IFN-γ, IL-6, TNF-α, IL-10, TGF-β1) and sCD14 in cord blood plasma and for total IgA in breast milk samples were performed. Neonates of mothers who received daily supplements of either *L. rhamnosus* HN001 or *B. lactis* HN019 had higher cord blood IFN-γ levels ($p = .026$) and statistically higher detectable (> 5 pg/mL) blood IFN-γ level ($p = .034$) compared with the placebo group, whose infants had undetectable IFN-γ levels (< 5 pg/mL). In probiotic groups, increased breast milk IgA levels [*L. rhamnosus* HN001 ($p = .011$) or *B. lactis* HN019 ($p = .008$)] and higher transforming growth factor (TGF) β1 levels were detected in early breast milk samples (week 1) [*L. rhamnosus* HN001 ($p = .075$) or *B. lactis* HN019 ($p = .041$)]. Higher breast milk IgA levels were detected in women collectively on probiotics at three months (79%, $p = .035$) with

increased and six months (65%, $p = .035$) compared with the placebo group (66% at three months and 44% at six months). Correlation was not found between neonatal- or breast milk sCD14 levels and subsequent allergic outcome in the studied population. However, possible implication of decreased neonatal plasma sCD14 levels in the *B. lactis* HN019 group compared with the placebo group ($p = .041$) is not clear (Prescott et al., 2008). The possible implication of maternal supplementation of probiotic organisms could have antenatal effects or potential postnatal influences via immunomodulators in breast milk, but their relationship with allergic outcome has yet to be resolved.

Controversial finding has also been reported in a double-blinded, placebo-controlled prospective trial, where pregnant women with at least one first-degree relative or a partner with an atopic disease were randomly given either the probiotic *Lactobacillus rhamnosus* GG (LGG) (ATCC 53103; 5×10^9 cfu/twice daily) or placebo (microcrystalline cellulose) for four to six weeks before expected delivery, followed by a postnatal period of six months. No difference was observed between the LGG-supplemented group and the placebo group in terms of the proliferative capacity of maternal or neonatal cord blood cells in response to IL-2, β-lactoglobulin, or LGG (Kopp et al., 2008a). LGG has in vitro effects on enhanced IL-10 and IFN-γ release of mononuclear cells. However, supplementation with LGG during pregnancy did not alter the proliferative capacity or cytokine pattern of neonate or breast milk (Kopp et al., 2008b). Significant differences in cytokine levels were found in early breast milk samples but in correspondence with the findings of previous author, the study of Prescott et al. (2008), also demonstrated no significant differences in breast milk cytokine levels detected at three and six months of lactation between the groups, even though breastfeeding women continued taking supplements of probiotic organisms (*L. rhamnosus* HN001, *B. lactis* HN019) for six months in the postnatal period.

Mucosal Immune System

The adaptive system is acquired through interactions with the environment. Humans as mammals have developed an extremely sophisticated adaptive immune system of both systemic and mucosal (local) type. Mucosal immunity can be viewed as a first line of defense that reduces the need for systemic immunity, which is primarily proinflammatory (Donkor et al., 2010). As the body's first-line-of-defense, the mucosal immune system is central to protection against invading pathogens. The mucosal immune system consists of physical (mucus), molecular (antimicrobial proteins), and cellular components that act synergistically to prevent microbes from invading the body. The digestive tract's immune system is often referred to as gut-associated lymphoid tissue (GALT), which is the example of mucosa-associated lymphoid tissue where intestinal DC populations are central to the immunomodulating effects of commensal or probiotic bacteria (Fig. 1). Circulating DCs can be activated to a varying extent by different species of *Lactobacillus* (Christensen et al., 2002). GALT represents the largest mass of lymphoid tissue and constitutes a vital part of the total immunological capacity of the host (Isolauri et al., 2001). Immune exclusion and immunosuppressive mechanisms, namely, "oral tolerance," work together for mucosal immunity. The role of maintaining the homeostatic balance between tolerance and immunity is crucially played by the intestinal epithelial cells (Artis, 2008).

The immune system regulates the colonization of the intestinal microflora by interfering with its ability to bind to the mucosa, whereas bacterial cells and metabolites modulate the immune system activity (Ouwehand et al., 2002). Mechanisms of immunomodulation include the induction of mucus production, macrophage activation by lactobacilli signaling, stimulation of secretory IgA and neutrophils, inhibition of release of inflammatory cytokines, and stimulation of elevated peripheral immunoglobulins (Senok et al., 2005). The secretory IgA is resistant to proteolysis and does not participate in any inflammatory response. Hence, it is most importantly involved in immune exclusion of foreign antigens by preventing binding to epithelial cells and penetration of microorganisms (Erickson and Hubbard, 2000; Ouwehand et al., 2002).

Intestinal epithelial cells are in direct contact with the intestinal microflora and play a significant role in immunological defense mechanisms. They express adhesion molecules that are important in residing T cells and other immune cells to perform regulatory functions. It has been suggested that the immune system might be beneficially affected by the presence of probiotic organisms through the action of recognition receptors expressed on the surface of epithelial cells (Isolauri et al., 2001). Probiotic organisms may directly or, by changing the composition (Reid et al., 2011) or activity of the intestinal microflora, indirectly influence the body's immune function (Marteau et al., 1997). They can improve the gut mucosal immune system by increasing the number of IgA+ cells and cytokine-producing cells in the effector site of the intestine (Galdeano et al., 2007; Dogi et al., 2010). Selected probiotic bacteria, including species of *Lactobacillus* and *Bifidobacterium*, are capable of enhancing the production of IgA (Yasui et al., 1992; Majamaa et al., 1995). Specific gut mucosal immunity may only be stimulated by viable probiotic bacteria. It was demonstrated in a study by Gill and Rutherfurd (2001b) that only live preparations of *L. rhamnosus* HN001 was able to enhance gut mucosal antibody response to cholera toxin vaccine in mice; nevertheless, live and heat-killed preparations of *L. rhamnosus* HN001 in mice have shown to enhance the phagocytic activity of blood and peritoneal leukocytes in a dose-dependent manner (Gill and Rutherfurd, 2001a).

Several reports have suggested that the consumption of probiotic fermented milk could modulate the intestinal microbial composition and metabolic activity. It stimulates the immune parameters and thus can be consumed as an immunopotentiator. *L. casei* strain Shirota is one of the most extensively used probiotics because of its health benefits and it has also been experimentally tested in animal models. Galdeano et al. (2009) analyzed the effect of probiotic fermented milk containing

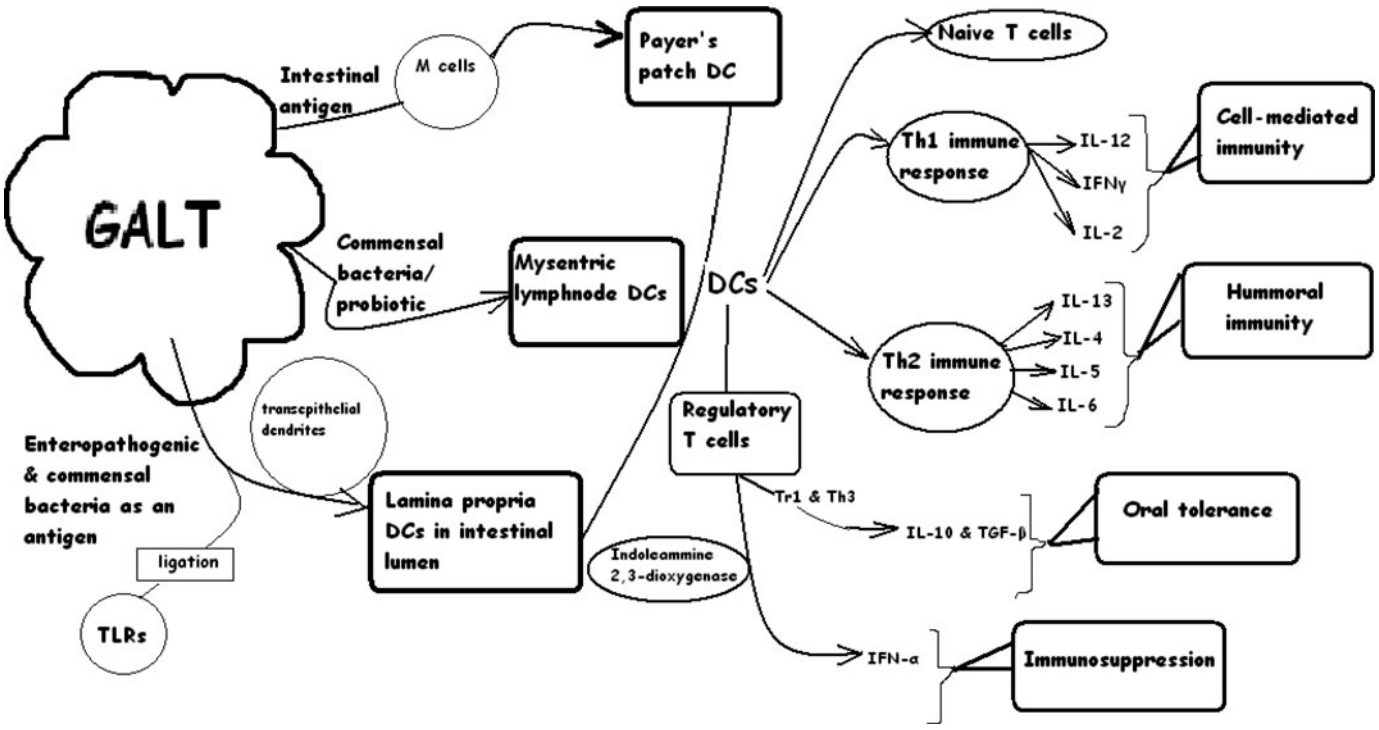

Figure 1 Role of gut-associated lymphoid tissues (GALT) in the immune system: Intestinal antigens are transported to Peyer's patch dendritic cells (DCs) via specialized enterocytes called M cells. Enteropathogenic and commensal bacteria are directly processed by lamina propria DCs in the intestinal lumen via transepithelial dendrites. This process can be induced by ligation of a variety of toll-like receptors (TLRs) expressed on epithelial cells. Commensal bacteria are encountered by macrophages and sequestered in mysentric lymph node DCs (MLN DCs) that prevent their entry into the systemic immune compartment. Intestinal DCs have the ability to activate naïve T cells and in directing helper T-cell responses toward Th1 or Th2 or regulatory patterns. Th1 immune responses critically depend on the ability of DCs to produce interleukin (IL)-12 and are characterized by the production of interferon (IFN)-γ and IL-2, which induce cell-mediated immunity. Th2 immune responses involve IL-4, IL-5, IL-6, and IL-13 and induce humoral immunity. DCs can lead T cells to take up regulatory functions and thereby induce oral tolerance. Induction of Tr1 and Th3 cells, and regulatory T cells, releases IL-10 or transforming growth factor (TGF)-β, respectively. The production of IFN-α or the induction of immunoregulatory enzyme, such as indoleamine 2, 3-dioxygenase (IDO), brings about interactions between DCs and regulatory T cells, which ultimately results in immunosuppression.

the probiotic bacterium *L. casei* DN 114001 on different parameters of gut mucosal immunity related to the nonspecific, innate, and adaptive response using BALB/c mice. The whole bacterium or fragments thereof interacted and stimulated the intestinal cells and gut-associated immune cells of BALB/c mice. There was an increase in the number of different immune cell populations: T and IgA^+ B lymphocytes, cells related with both innate and adaptive response (macrophages), and cells from the nonspecific barrier (goblet cells). Cytokine IL-6 that was released was associated with the IgA^+ B cell expansion and the enzyme calcineurine that was activated by *L. casei* in milk was responsible for the activation of the transcriptional factor NFAT (nuclear factor of activated T cells) (Galdeano et al., 2009). NFAT activation is known to regulate a variety of immune processes: apoptosis, anergy, T-cell development, and aging of immune system (Masuda et al., 1998). NFAT activation via calcineurin enzyme was reported in BALB/c mice (six–eight weeks old, weighing 25–30 g) when they were fed with 0.1 mL cell suspension (10^8 cfu/mL for gram-positive and 10^6 cfu/mL for gram-negative strains) of *L. acidophilus* (strains CRL 1462 and A9), *L. casei* (CRL 431) and *E. coli* (strains 129 and 137) for seven consecutive days by gavage. After the end of administration period, animals were sacrificed, and their small intestines were removed and processed for immunochemistry study. All the assayed strains increased the number of calcineurin(+) cells in lamina propria, whereas only gram-positive strains including *L. acidophilus* (strains CRL 1462 and A9) and *L. casei* (CRL 431) increased the TLR-9 expression (Dogi et al., 2010).

Feeding fermented milks containing *L. casei*, *L. acidophilus*, and a mixture of both to Swiss mice produced a remarkable effect on the activation of the immune system with an increase in both phagocytic and lymphocytic activity. No harmful effect such as hepatomegaly or splenomegaly was observed after prolonged administration of milk (Perdigón et al., 1988). However, contradictory findings were observed in a double-blind placebo-controlled study when human subjects ($n = 20$, healthy male, age 40–65 years) were used to observe the effect of consumption of milk fermented by *L. casei* strain Shirota (3×100 mL daily containing 10^9 cfu/mL) on the composition and metabolic activities of microflora and immune parameters. It was found that the immune parameters, including NK cell activity, phagocytosis, and cytokine production, remained unaffected after consumption of *L. casei* Shirota-fermented milk. However, composition (significant increase in *Bifidobacterium* count) and

metabolic activities (significant decrease in β-glucuronidase and β-glucosidase activities) of microflora were modulated following the probiotic intervention. The difference in findings was attributed variation in strain used, dose level, treatment period, and immune status of selected subjects (Spanhaak et al., 1998).

Interaction of probiotic microorganisms with intestinal epithelial cells and immune cells elicits changes in cell phenotype, the secretion of cytokines, and the activation/suppression of intracellular signaling pathways, all of which modulate host resistance (Ezendam et al., 2008). *L. paracasei* subsp. *paracasei* DC412 exhibited strong immunoregulatory activity and interacted strongly with GALT of BALB/c inbred mice or Fisher-344 inbred rats through stimulation of TLR2/TLR4-mediated signaling events leading to secretion of a certain profile of cytokines, namely, IFN-γ, TNF-α, IL-6, and IL-10 (Kourelis et al., 2010). In an ex vivo assay, the probiotic strain *L. casei* CRL 431 showed great activity on the immune cells and induced the significant release of IL-6 in a preparation enriched in intestinal epithelial cells, activating mainly cells of the innate immune system (Dogi et al., 2008).

Strain Specificity of Probiotic Organisms in Immunostimulation

The most fascinating feature and extensively studied effect of immune system modulation by probiotic bacteria is cytokine production. Probiotic bacteria induce the secretion of cytokines from intestinal epithelial cells in a strain-specific manner. Moreover, the same cytokine can be produced by multiple cell types, have multiple effects on the same cell, and act on many different cell types. In an ex vivo study, 2 of 5 *Lactobacillus plantarum* strains (BFE 5759 and BRE 1685), and 1 of 2 *L. johnsonii* strains (BFE 6128) stimulated HT29 intestinal epithelial cells to secrete IL-8 (West et al., 2009). Kourelis et al. (2010) found that *L. paracasei* subsp. *paracasei* DC412 strain produced IFN-γ, TNF-α, IL-6, and IL-10. The probiotic *L. acidophilus* NCFB 1748 induced the same cytokine profile in addition to IL-12β, and this response was potentially mediated by the synergy of TLR2 and TLR9. *L. plantarum* 299V, also known as "Lp299V" (also known as DSM 9843) induced the highest level of IL-12. *Streptococcus thermophilus* and *Leuconostoc* strains were found to be more potent inducers of Thl type cytokines IL-12 and IFN-γ than *Lactobacillus* strains (Kekkonen et al., 2008). He and others (He et al., 2002) found that *B. adolescentis* and *B. longum* induced significantly more pro-inflammatory cytokine secretion of IL-12 and TNF-α than did the other *Bifidobacterium*, namely, *B. bifidum*, *B. breve*, and *B. infantis*.

IL-10 and IL-12 are naturally produced by macrophages, B cells, and DCs. IL-12 induces cellular immunity that potently stimulates cytotoxic T cells and NK cells and enhances the production of Th1 cytokines (IL-2 and IFN-γ) and the proliferation of Th1 cells. In contrast, IL-10 negatively regulates cell-mediated immunity directly or via suppression of antigen-presenting cell functions including IL-12 production. Because of antitumor, antimetastatic, and antiangiogenic activity of IL-12, there has been an interest in testing IL-12 as a possible anticancer drug. In a study, inbred male BALB/c mice (aged 7–10 weeks) were orally administered with bacterial suspension of *L. casei* strain Shirota (2.6×10^{11} cfu/mL). The animals were sacrificed and spleen cells were isolated and cytokine (IFN-γ, IL-12, IL-4, and IL-5) assay was performed. X-ray-resistant splenocytes produced IL-12 after probiotic treatment but the presence of nylon wool column-passed splenocytes or Concanavalin A (Con A) did not affect IL-12 production. *L. casei* strain Shirota was found to stimulate the secretion of IL-12, which, in turn, induced the production of IFN-γ by x-ray- irradiated murine splenocytes in vitro (Kato et al., 1999). Moreover, *L. casei* has been found to inhibit antigen-induced IgE production through the induction of IL-12 secretion by macrophages in murine splenocyte cultures (Shida et al., 1998). In another study, *L. casei* strain Shirota was orally administered to mice that had been preinjected intraperitoneally with ovalbumin. Oral administration of *L. casei* strain Shirota to mice stimulated type 1 helper T (Th1) cell-associated-cytokines, activated the cellular immune system and inhibited the incidence of tumors and IgE production. In contrast, it lowered the production of type 2 helper T (Th2) cell-associated cytokines, such as IL-4, IL-5, IL-6, and IL-10, by splenocytes compared with the control group. More IL-12 production was increased by splenocytes in *L. casei* treated group than that of the control group (Yasui et al., 1999). The findings suggest a potential use of *L. casei* in preventing IgE-mediated allergy.

Lactobacilli can activate macrophage to secrete both inflammatory and anti-inflammatory cytokines. In a study carried out by Morita et al. (2002), 11 strains of *L. acidophilus*, *L. casei*, and *L. rhamnosus* induced the production of IL-6, IL-12, and TNF-α by murine macrophage cell line J774.1. Seven of these strains also induced the production of IL-10 by macrophage. IL-1β was not produced by either strain. However, *L. acidophilus* TMC 0356 significantly ($p < .0001$) induced the increased production of IL-6, IL-10, IL-12, and TNF-α compared with the other bacteria. *L. casei* strain Shirota induced IL-12 production in concentrations similar to be stimulated by *L. rhamnosus* GG.

In a study by Von der Weid et al. (2001), splenocytes from BALB/c mice or C57BL/6 were stimulated with LAB (*L. johnsonii* NCC533, *L. gasseri* NCC 2493, *L. paracasei* NCC2461, *L. casei* strain Shirota, and *L. casei* strain GG) and were subjected for cytokine analysis. *L. paracasei* strain NCC2461 and *L. casei* strain GG induced the largest amount of IL-10. The findings of previous studies revealing mutually antagonistic role of IL-10 and IL-12, and increased concentrations of IL-12 by *L. casei* strain Shirota, contradicted in this study, where *L. paracasei* (strain NCC2461) induced the highest levels of both IL-12 and IL-10 in splenocytes of BALB/c mice, whereas *L. casei* strain Shirota (a weak inducer of IL-10) was also found to be a poor IL-12 inducer, which is in contrast with the previous reports where the strain stimulated increased concentrations of IL-12.

Analysis of cytokine production in human PBMC in response to stimulation with 11 different potentially probiotic bacterial strains from *Streptococcus, Lactobacillus, Bifidobacterium, Lactococcus, Leuconostoc*, and *Propionibacterium* genera was carried out by Kekkonen et al. (2008). All tested

bacteria induced TNF-α production. The best inducers of Thl type cytokines IL-12 and IFN-γ were the strains of *Streptococcus thermophilus* and *Leuconostoc mesenteroides* subsp. *cremoris* (DSM18892). *Bifidobacterium* and *Propionibacterium* strains induced higher IL-10 production than all the other studied bacteria. In another study, *L. rhamnosus* E509, *L. rhamnosus* GG E522 (ATCC 53103), and *L. delbrueckii* subsp. *bulgaricus* E585 stimulated human PBMC and induced IL-1β, IL-6, and TNF-α mRNA expression and protein production. These strains have been shown to induce Th1 type cytokines IL-12, IL-18, and IFN-γ in human PBMC (Miettinen et al., 1998).

Cytokine Response of Probiotics in Combination Therapy

Bacterial combinations of probiotic organisms may induce different cytokine profiles compared with single bacterial preparation. Supplementation of a mixture of organisms and probiotics, namely, *S. thermophilus, L. acidophilus*, and *B. lactis* administered in a normal rat population led to some neutrophil infiltration in the mucosal layer and submucosa, which reflected stimulation of the immune system (Amit-Romach et al., 2010). Human intestinal lamina propria mononuclear cells, whole blood, or an enriched blood DC population were cultured with the mixture of eight different lactic acid bacteria (LAB) and probiotic strains in preparation/formula (VSL#3), including *L. acidophilus*, *L. delbrueckii* subsp. *bulgaricus*, *L. casei*, *L. plantarum*, *B. longum*, *B. infantis*, *B. breve*, and *S. thermophilus*. The bacterial combination upregulated production of IL-10 by DCs and downregulated production of IL-12 by DCs derived from human blood and lamina propria. Individual strains showed distinct immunomodulatory effect on DCs where increased anti-inflammatory effect was displayed by bifidobacterial strains, which upregulated IL-10 production by DCs, decreased expression of costimulatory CD80 molecule, and decreased interferon-γ production. In contrast, strains of lactobacilli decreased or had no significant effect on IL-10 production by DCs. Streptococcal strains also did not stimulated significantly IL-10 producing DCs. The proinflammatory effect of LPS was reduced by the suppression of IL-12 production in the presence of the probiotic combination while maintaining high production of IL-10 (Lammers et al., 2003; Hart et al., 2004).

Moreover, the cytokine response may vary greatly in the presence of different probiotic organisms or mixtures of probiotic organisms. In the study of Kekkonen et al. (2008), none of the bacterial combinations resulted in enhanced cytokine production after stimulation of the PBMC. The cytokine responses were also different when PBMCs were stimulated with combination of probiotic organisms (11 strains of *S. thermophilus*, *Lactobacillus*, *Bifidobacterium*, *Lactococcus*, *Leuconostoc*, or *Propionibacterium*) with gram-negative bacteria (*Escherichia coli*), with the purpose of testing the possibility of sharing a single TLR signal transduction pathway by these bacteria. Compared with the individual responses, there was an average production of TNF-α and reduction in IFN-γ production, with no increase of IL-10 production in PBMCs stimulated with combinations of probiotic bacteria and *E. coli*, although pure culture of *E. coli* induced TNF-α, IFN-γ, and IL-10 production. This suggests that different bacteria, whether gram-positive or gram-negative, compete with each other during host cell interactions (Kekkonen et al., 2008). It was found that in a combination therapy using mixtures of probiotic organisms, certain species may inhibit the stimulatory effect of others (Christensen et al., 2002).

Mucin Expression by Probiotic Organisms

Mucins are a large family of glycoproteins expressed by various epithelial cell types and malignant cells, bearing similarity in structural features but distinct in their tandem repeat peptides. They may be membrane-bound (MUC1, MUC3A, MUC3B, MUC4, MUC12, MUC13, and MUC17) or secreted (MUC2, MUC5AC, MUC5B, and MUC6). Abnormal expression of mucins has been reported to influence cellular growth, differentiation, transformation, adhesion, and invasion. It can be associated with cancer development and immune surveillance (Rakha et al., 2005). Some of the probiotics have been identified to actively participate in host epithelial barrier function via mucin production in which MUC2 and MUC3 have been extensively expressed by human intestinal epithelial cells. In vitro studies have shown that *L. plantarum 299v* and *L. rhamnosus GG* increased the expression of MUC2 and MUC3 mucin secretion from intestinal epithelial cells, which inhibit adherence of attaching pathogenic microorganisms to intestinal epithelial cells (Mack et al., 1999). Probiotic organisms (*L. plantarum* strain 299v and *L. rhamnosus* strain GG) induce MUC3 mucin transcription and translation with extracellular secretion of the MUC3 mucins. MUC3 mRNA expression was upregulated with HT29 cells coincubated with *L. plantarum* strain 299v. Selected probiotic *Lactobacillus* strains with the ability to adhere to intestinal epithelial cells such as *L. plantarum* strain 299v and *L. rhamnosus* strain GG induce upregulation of mucin (MUC3) gene expression, and subsequently extracellular secretion of mucin (MUC3) that leads to the inhibition of enteropathogenic adherence (*Escherichia coli* E2348/69). There was a direct correlation between upregulation of MUC3 mucin mRNA expression and extracellular secretion of MUC3 mucin. The same *Lactobacillus* strains that increased extracellular secretion of MUC3 mucin led to reduced adherence of enteropathogenic *Escherichia coli* E2348/69 during coincubation experiments (Mack et al., 2003).

In another study, human enterocyte Caco-2 cells were grown and incubated with control medium and *L. casei* GG (10^4 or 10^8 cfu) for 180 minutes, and monolayers were analyzed for MUC2 expression. Significant increase ($p < .05$) in protein densities (expressed as ratio to control group) for MUC2 was observed in probiotic treated monolayer as 8.6 ± 1.3 in case of low-dose group (10^4 cfu) and 15.6 ± 2.3 in the high-dose group (10^8 cfu). The stimulation of upregulation of MUC2 resulted in

an increased inhibition of bacterial translocation (Mattar et al. 2002).

Cytokine Expression by Probiotic Organisms or Their Cell Components

Many components of bacterial cell wall, such as lipopolysaccharides, peptidoglycans, and lipoteichoic acids, have shown to be involved in the cytokine induction. The cell wall components may activate macrophages, endothelial cells, smooth muscle cells, and neutrophils, and, in turn, these cells release several mediators. Several proteins may be produced by the activated macrophages, including cytokines such as TNF-α, IL-1, IL-6, IL-8, IL-12; metalloproteases, such as elastases and cathepsin; lipid mediators, such as prostaglandins; as well as reactive oxygen and nitrogen species. Stimulation of PBMC for the production of proinflammatory cytokines (TNF-α, IL-6, and IL-10) by LPS (from *E. coli*), peptidoglycan (from *S. aureus*), and lipoteichoic acids (from *S. aureus and E. faecalis*) was compared. In terms of concentration that elicited the cytokine response of PBMC, LPS was graded as 100–1000 times more efficient than peptidoglycan, and peptidoglycan was regarded 10–100 times more effective than lipoteichoic acids. LPS of gram-negative bacteria induced production of proinflammatory cytokines TNF-α and IL-6, as well as IL-10 (Erickson and Hubbard, 2000; Hessle et al., 2005).

Majority of identified probiotic organisms are from gram-positive bacterial group; therefore, there could be a possibility that probiotic bacteria or their cell components could stimulate monocyte or macrophage in the same fashion as do other gram-positive bacteria or their cell components. Purified lipoteichoic acid from gram-positive bacteria was found to have an immunostimulatory property on monocyte-macrophage U937 cell line. It has been shown to induce the release of TNF-α and IL-10 in a dose-dependent way. Lipoteichoic acid from a probiotic strain *L. acidophilus strain* A9 stimulated U937 cells to give enhanced levels of TNF-α and IL-10 compared with other gram-positive strains (nonprobiotic, *Lactobacillus acidophilus* CRL 1462; probiotic, *Lactobacillus casei* CRL 431), and gram-negative strains (*Escherichia coli* 129, *Escherichia coli* 137) (Dogi et al., 2010).

The suppression of the formation of proinflammatory cytokines and chemokines in the presence of probiotics has been reported in several in vitro studies. The response of the immune system to a probiotic organism was weaker than in the presence of a gram-positive pathogen. More importantly, it has been shown that human monocyte-derived DCs responded differently to different gram-positive bacteria. There was a strong expression of DC costimulatory molecules (CD80, CD83, and CD86) alongwith Th1-type response after stimulation of DCs with *Streptococcus pyogenes*. However, the expression of costimulatory molecules was moderate, and cytokine and chemokine concentrations were low in case of *L. rhamnosus*-stimulated DCs (Veckman et al., 2004). Cytokine responses of human PBMC to gram-positive cell walls of the normal intestinal microbiota, such as *Eubacterium aerofaciens* (ATCC 25986), *Eubacterium limosum* (ATCC 8486), *L. casei* (ATCC 11578), and *L. fermentum* (ATCC 14931), are similar in profile, level, and kinetics with those induced by LPS or cell wall derived from the pathogen *Streptococcus pyogenes* (ATCC 10389). Bacterial cell walls of these intestinal bacteria induced monocytes in PBMC to produce TNF-α, and IL-10, but not IL-4 or IFN-γ. The stimulation of IL-10 by cell wall or LPS was found to be serum-dependant (whereas TNF-α induction was partial serum dependant), involving both CD14-dependant and independent pathways. Moreover, the adherent cell population of monocyte in PBMC monolayer was responsible for the production of IL-10 and TFN-α (Chen et al., 1999).

Pure genomic DNA of probiotic strains has also been investigated for the expression of various cytokines. PBMC form healthy donors were incubated with pure DNA of probiotic strains of VSL # 3 formula (*B. infantis* B107, *B. breve* BBSF, *B. longum* BL04, *L. acidophilus* LA14, *L. delbrueckii* subsp. *bulgaricus* LB31, *L. casei* LC10, *L. plantarum* LPT, and *L. salivarius* subsp. *thermophilus* TA061), and with total bacterial DNA obtained from human feces collected before and after probiotic intake. DNA of *B. infantis*, *S. thermophilus*, *L. plantarum*, and *L. delbrueckii* subsp. *bulgaricus* stimulated a significant increase in IL-1β production, whereas DNAs of *B. longum*, *L. acidophilus*, and *L. casei* induced a significantly lower IL-1β production. DNAs of *B. breve*, *B. infantis*, and *S. thermophilus* stimulated increased levels of IL-6, whereas genomic DNAs of *B. longum* and *Lactobacillus* strains stimulated significantly lower concentration of IL-6. DNA isolated from *B. breve*, *B. infantis*, and *S. thermophilus* stimulated high secretion of IL-10, which was three times more than control (LPS; concentration of 20 ng/mL). Genomic DNA of *B. breve* was confirmed to be strong inducer of interleukins than *L. casei* DNA (Lammers et al., 2003).

It was found by Miettinen et al. (1996) that several live LAB were potent inducers of TNF-α, IL-6, and in some cases, IL-10 release from human PBMCs in quantities even higher than those induced with LPS. However, there were significant differences between LAB strains in the release of these cytokines in PBMC. *L. rhamnosus* E509, *L. rhamnosus* E522, *B. animalis* E508, and *L. acidophilus* E507 were the best inducer of TNF-α, and *L. rhamnosus* E509, *L. rhamnosus* E522, and *B. animalis* E508 were the best inducer of IL-6 among the tested LAB, including *B. longum* E505, *L. paracasei* subsp. *paracasei* E506, *L. acidophilus* E507, *B. animalis* E508, *L. rhamnosus* E509, *L. paracasei* subsp. *paracasei* E510, *L. rhamnosus* GG E522 (ATCC 53103), *Lc. lactis* subsp. *cremoris* E523, *Lc. lactis* subsp. *lactis* E414, and *L. plantarum* E98. Moreover, the stimulation of higher levels of IL-12 and TNF-α has been found by live *L. casei* Shirota than heat-killed preparations (Cross et al., 2004). In a different study, *L. salivarius* HA8 induced the production of proinflammatory cytokines IL-1β, IL-8, and TNF-α in the presence or absence of LPS, but the effect was more pronounced in the presence of LPS (Haza et al., 2005). Induction of these proinflammatory cytokines could indicate that LAB can

stimulate nonspecific immune responses. Although mechanisms of these immunological responses are still unclear, it can be comprehended that the cell wall composition and the involvement of different cell components may account for the differences in immunological responses.

Comparison of Cytokine Expression by Probiotic Organisms and Selected Gram-Negative Bacteria

To confirm that different types of bacteria elicit different immune responses and cytokine profile, various comparative studies have been carried out in which probiotic bacteria have been compared with gram-negative bacteria. *Klebsiella pneumoniae* (gram-negative) and *L. rhamnosus* were compared in a study where both cultures induced DC maturation but resulted in a different cytokine profile. Both bacteria induce the maturation of immature DCs (shown by expression of CD83 and CD86) but differentially matured DCs exhibited distinct activation patterns and functional properties. *K. pneumoniae* activated the expression of T-helper (Th) 1 type cells, whereas *L. rhamnosus* reduced the production of the proinflammatory cytokines (TNF-α) and interleukins (IL-6 and IL-8) by immature DCs. *K. pneumoniae* mature DCs produced significantly higher IL-12 and IL-18 than DCs matured in the presence of *L. rhamnosus* (Braat et al., 2004). In another study carried out by Cross et al. (2004), cytokine induction by *L. casei* Shirota (gram-positive probiotic) was compared against that of a novel gram-negative probiotic strain, *E. coli* Nissle 1917 using the murine monocyte/macrophage cell line J774A.1. Moreover, the differences in cytokine responses were compared against those induced by *E. coli*, an enteropathogenic coliform. Marked secretion of IL-12 and TNF-α by murine cells induced with all these strains were observed, whereas only coliforms induced the production of IL-10, with no or less induction of IL-18 or TGFβ. Secretion levels of IL-10, IL-12, and TNF-α were higher in live bacteria than in heat-killed preparations, whereas IFN-α was induced only by live coliforms. In contrast with the previous report, the patterns of cytokine response by probiotic bacteria were noticeably similar to the pathogenic coliform.

There is a common belief that gram-positive and gram-negative bacteria activate different immune receptors and induce a different cytokine profile. Comparisons of gram-positive (probiotic, nonprobiotic) and gram-negative strains were made by Dogi et al. (2008, 2010). They evaluated gut immune stimulation by *L. acidophilus* strains CRL 1462 and A9 (nonprobiotic), *L. acidophilus* CRL 924, *L. delbrueckii* subsp. *bulgaricus* CRL 423 (potential probiotic), *L. casei* CRL 431 (probiotic) as gram-positive strains, whereas *E. coli* 129 and *E. coli* 13-7 were the gram-negative strains. These were administered to BALB/c adult mice for two, five, or seven consecutive days by gavage. All the strains increased the number of IgA$^+$ cells. The gram-positive strains increased the number of IL-10$^+$ cells. In contrast, gram-negative strains increased the number of IL-12$^+$ cells. The probiotic strain increased mainly IFN-γ and TNF-α, as well as the number of CD-206$^+$ cells. All the gram-positive bacteria increased the number of TLR-2$^+$ cells and the gram-negative strains enhanced TLR-4$^+$ cells (Dogi et al., 2008). Oral administration of gram-positive (*L. acidophilus* strains CRL 1462, A9, *L. casei* CRL 431) and gram-negative (*E. coli* 129 and 13-7) bacteria to BALB/c mice stimulated the number of IFN-γ and TNF-α(+) cells (induced only by LPS stimulation) but not IL-10(+) cells in the total population of Peyer's patches. The main difference between gram-positive and gram-negative was in the expression of TLR-9, which was only increased by gram-positive organisms (Dogi et al., 2010). Since majority of probiotic bacteria that are used in commercial probiotic preparations comprise gram-positive group, the difference in cytokine expression and immune response by probiotic organisms and gram-negative bacteria could be attributed to their cell wall content and composition.

Modulation of GI Permeability and Th1:Th2 Balance

Probiotic organisms can modulate GI permeability and enhance epithelial barrier function in vitro and in animal models. Increased GI permeability permits the translocation of luminal antigens, including enteric microbial flora, into the systemic circulation. This translocation induces immune responses within the mucosa and may subsequently lead to chronic inflammation. A key function of the mucosal immune system is to control responses to luminal antigens to limit inflammation, which is one of the first responses of the immune system to infection with common symptoms of redness and swelling, caused by cellular infiltration and increased blood flow into a tissue. If enhanced, this response can damage the tissues (Perdigón et al., 1995). This state of control is achieved by regulating the balance of Th1:Th2 cytokines in favor of Th2 cytokines, including IL-2, TGF-β, IL-6, and IL-10, stimulated by probiotic organisms in a strain-specific manner and with dose-dependent effects (West et al., 2009). *B. animalis* was administered to rats to examine the Th1- and Th2-mediated immune responses. Cytokine profile assessed after culturing spleen cells with the mitogen concanavalin A (ConA) showed that *B. animalis* skewed the Th1:Th2 balance toward Th1 in female rats. However, in male rats a significant decrease in ConA-induced IL-13 and a trend toward lower levels of ovalbumin (OVA)-induced Th2 cytokines was observed. *B. animalis* reduced several immune parameters in the allergy as well as in the autoimmunity model (Ezendam et al., 2008). Probiotic bacteria, namely, *L. rhamnosus* GG, *L. gasseri* (PA16/8), *B. bifidum* (MP20/5), and *B. longum* (SP07/3) DNA modulated the Th1:Th2 response to some allergens dose dependently, where more than 50% of the effects appeared to be contributed by DNA. The tested live gram-positive probiotic bacteria and their genomic DNA inhibited stimulated secretion of Th2 cytokines (IL-4 & IL-5) by *Staphylococcus* enterotoxin A (SEA) and *Dermatophagoides pteronyssinus* (Dpt)- and enhanced the stimulation of IFN-γ. The magnitude of the probiotic bacterial effects differed between healthy and allergic patients (Ghadimi et al., 2008).

Cases of In Vivo Evidence in Controlling Infections

The immune system may be more efficient in controlling infections when preparations of probiotic organisms are consumed (West et al., 2008; Weichselbaum, 2009). Ingestion of *Lactobacillus* and *Bifidobacterium* species induced changes in the gut microbial flora, suppression of pathogen colonization in gastric mucosa, and thus the altered flora helped prevent antibiotic-associated diarrhea (Shah, 2004; Weichselbaum, 2009), *Clostridium difficile*-related colitis, rotavirus-associated diarrhea (Guandalini et al., 2000), inflammatory bowel disease (Amit-Romach et al., 2010), and urinary tract infections (Reid et al., 2001).

Cell-mediated immunity and intestinal microbial flora are involved in host resistance against bacterial infections like listeriosis. It was shown that orally administered viable *L. casei* Shirota strain YIT9029 was able to enhance host resistance against oral *L. monocytogenes* infection in Wistar rats; this was exhibited by the reduction in the number of *L. monocytogenes* in the gastrointestinal tract, as well as in the spleen and liver (De Waard et al., 2002).

The innate and adaptive systems are highly integrated and interdependent (Hoebe et al., 2004) in providing resistance to infection. Humoral immune responses involve induction, anticipation (immune memory), and clonal expansion and render specific immunity during the life of an individual. Several reports describing the effect of probiotic organisms on the production of IgA and IgG antibody response are summarized in Table 1. Oral administration of *B. breve* strain YIT4064 to mice activated the humoral immune system, augmented anti-rotavirus IgA production or anti-influenza virus (IFV) IgG production and protected against rotavirus infection or influenza infection, respectively (Yasui et al., 1999). *B. animalis* was found to be biotherapeutic against candidiasis in mice. It did not completely prevent but did reduce the incidence and severity of candida infection in mice. *B. animalis* apparently stimulated host resistance to candidiasis via thymus- and mucosa-associated lymphoid tissues. It stimulated T cell-dependent IgA and IgG antibody responses in athymic mice, possibly via extrathymic-matured T cells that are present in mucosal tissues and provided the best overall protection against mucosal and systemic candidiasis (Wagner et al., 1997).

The administration of yogurt supplemented with *L. acidophilus* and *Bifidobacterium* spp. enhanced mucosal and systemic IgA responses to cholera toxin immunogen. Significant increases in antibody responses of the IgA isotype were observed in mice fed with yogurts made with conventional starter bacteria (*L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus)* supplemented with *L. acidophilus*, *B. bifidum*, and *B. Infantis* as compared with mice fed with control yogurt without any probiotics (Tejada-Simon et al., 1999).

L. rhamnosus GG therapy has been associated with a significantly enhanced nonspecific humoral response during the acute phase of the rotavirus infection, reflected in the IgG, IgA, and IgM Ig-secreting cell numbers. *L. rhamnosus* GG enhanced recovery from rotavirus diarrhea via augmentation of the local immune defense. Furthermore, specific IgA response to rotavirus suggested a possible protection against reinfections (Kaila et al., 1992). In a randomized, controlled, and double-blind study, orally administered *L. rhamnosus* GG or *L. acidophilus* CRL431 to human subjects increased poliovirus neutralizing antibody titers (NT) and affected the formation of poliovirus-specific IgA and IgG in serum. This indicates that probiotic organisms can induce immunologic response to provide enhanced systemic protection of cells from virus infections by increasing production of virus neutralizing antibodies (De Vrese et al., 2005).

Milk fermented with *L. casei* and *L. acidophilus* could be used as a prophylactic against gastrointestinal infections by shigella. Higher levels of anti-shigella antibodies were found both in sera and in small intestinal fluid of mice fed with fermented milk containing a mixture of *L. casei* and *L. acidophilus*, suggesting that the protective immunity could be mediated by the mucosal tissue. Colonization of the liver and spleen with *Sh. sonnei* was markedly inhibited by pretreatment with fermented milk (Nader De Macias et al., 1992).

Role of Probiotic Organisms in Prevention of Atopic Dermatitis and Intestinal Tumor

Probiotic bacteria can alleviate atopic dermatitis (AD) and can differently modulate peripheral immune parameters in healthy subjects and patients with AD. A probiotic drink containing a combination of probiotic organisms, namely, *L. paracasei* Lpc-37, *L. acidophilus* 74-2, and *B. animalis* subsp. *lactis* DGCC 420, was administered to healthy volunteers and in patients with AD. Major lymphocyte subsets were not affected by the probiotic intervention. However, CD57(+) increased significantly in healthy subjects after probiotic intake and was not changed in patients, whereas CD4(+)CD54(+) decreased significantly in patients with AD and remained uninfluenced in healthy subjects. The expression of CD4(+)CD25(+) T cells was similar in healthy subjects and AD patients (Roessler et al., 2008). According to Prescott et al., 2005, the administration of the probiotic *L. fermentum* resulted in significant improvement in AD in very young children manifested by an increase in the capacity for Th1 IFN-γ responses and altered responses to skin and enteric flora. Supplementation with *L. rhamnosus*, but not *B. animalis* subsp *lactis*, in early life could also substantially reduce the cumulative prevalence of eczema, but not atopy by two years among high-risk children (Wickens et al., 2008). In a mouse model of OVA-allergic asthma, Hougee et al. (2010) found *B. breve* as the most potent antiallergic strain out of 6 strains tested. *B. breve* reduced IL-4, IL-5, IL-10, OVA-specific IgE, and IgG, and also acute allergic skin reactions (Table 1).

Probiotic organisms were shown to induce an immune response by inhibiting tumor development at extraintestinal sites (Commane et al., 2005). An inverse relationship existed between the rise and fall of NK cells and the incidence of tumor growth (Dussault and Miller, 1996). Oral administration of *L.*

casei strain Shirota inhibited incidence of tumors and IgE production (Yasui et al., 1999). Studies in animal models also suggested that raising NK activity by probiotic consumption may have potential effects on tumor development. An inflammatory immune response produces cytokine-activated monocytes and macrophages, which release cytotoxic molecules capable of lysing tumor cells in vitro (Commane et al., 2005). A decrease of inflammatory response was attributed to inhibit the tumor development at intestinal sites in mice when supplemented with yogurt containing probiotics. It resulted in the inhibition of the growth of intestinal carcinoma through increased activity of IgA, T cells, and macrophages (Perdigón et al., 1995). Probiotic organisms can also reduce the risk of colon cancer by inhibiting carcinogen-induced DNA damage in animals, but similar studies are lacking in human subjects.

CONCLUSIONS

Consumption of probiotic organisms in the form of fermented milk or yogurt has been shown to increase cellular immunity in elderly subjects and children. Moreover, maternal supplementation with probiotic organisms in pregnancy could have an effect on immune markers in cord blood and breast milk. This has the potential to influence fetal immune parameters. It is well established that the intestinal microflora affects the development and functioning of the immune sytem, so it is reasonable to conclude that intestinal microflora could be modulated by using probiotic organisms, which could provide means of improving the immune status of healthy and unhealthy individuals. Induction of cytokine secretion by probiotic bacteria exhibited strain specificity, and the response may also vary in the presence of different species of probiotic bacteria or a mixture of probiotic bacteria. Live probiotic bacteria are capable of inducing enhanced mucin expression, phagocytosis, and different profiles of cytokines, and the effect has also been observed in in vitro models by parts of probiotic cell including peptidoglycan, cell wall, LPS, and DNA. The immunostimulatory effect and cytokine expression may vary with gram-negative or gram-positive strains. Although there is strong evidence that probiotic organisms possess immunostimulatory properties, further research is needed to confirm that probiotic-mediated immunostimulation can control different infections or can promote increased resistance to different infections and diseases in animals and humans. In vitro evidence is rather well established, confirmation is also needed for in vivo models and in vivo study for an assessment of prolonged administration of probiotic organisms to different age groups including children and the elderly.

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