

# Symposium: Probiotic Bacteria: Implications for Human Health

## Probiotic Immunomodulation in Health and Disease<sup>1,2</sup>

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**ABSTRACT** Probiotics, microorganisms that have a favorable influence on physiologic and pathological processes of the host by their effect on the intestinal flora, may play a role in improving human health. One of the putative effects is the modulation of immune function. Thus, the mucosal immune system and methods to assess its function are reviewed briefly. Probiotic modulation of humoral, cellular and nonspecific immunity is reviewed, with emphasis placed on immune response in disease models. There are very few reports of human intervention studies with probiotics. However, some of the possible future directions for research with respect to probiotics, immunity, and human health are discussed. Although the application of probiotics has demonstrated trends with respect to altered aspects of immune response, the underlying mechanisms by which that occurs are unclear. *J. Nutr.* 130: 403S–409S, 2000.

**KEY WORDS:** • probiotics • lactic acid bacteria • immune response • functional foods • mucosal immunity

One of the most promising areas of development in the area of functional foods has been the use of prebiotics and probiotics and their role in human health and disease. Much of the research work in probiotics has focused on the gastrointestinal tract. There are a number of possible means by which probiotics may alter health; one of those putative effects is the alteration of immune function. Although the definition of probiotics continues to evolve, one possible definition is the following: microorganisms that have a favorable influence on the host by improving the indigenous microflora. Therefore, the purpose of this paper will be to focus on probiotics and their role in immunomodulation. First, we will review briefly the mucosal immune system and the mucosa-associated lymphoid tissue (MALT)<sup>4</sup>. Particular attention will be paid to the gut-associated lymphoid tissue (GALT) as a smaller subset of the MALT. Second, we will review some of the methods that have been used to study the mucosal immune system, focusing on some of the strengths and weaknesses. Third, we will discuss probiotic effects on immune response with an emphasis

on disease models. Finally, we will indicate some of the possible future directions for research with respect to probiotics, immunity and human health.

### The mucosal immune system

The immune system has evolved for the purpose of protecting us from pathogens. The site as well as the type of pathogen determines largely which type of immune response will be effective. The immune response involves recognition of the pathogen or foreign material and the mounting of a reaction to eliminate it. Immune responses fall broadly into two categories, innate immunity and adaptive immune response. The cells that mediate immunity include lymphocytes and accessory cells such as macrophages, antigen-presenting cells and, in some cases, epithelial cells. The first exposure to a foreign pathogen effects the innate immune response, one that is nonspecific. In that process, mediators such as cytokines may be released. Specific or adaptive immunity involves lymphocytes with receptors for a specific antigen and presentation of that antigen in the context of the major histocompatibility complex (MHC) by antigen-presenting cells. As a result, subsets of helper T cells (Th) may be activated. Cytokines secreted by the Th2 subset activate specific B cells for the antigen, whereas the Th1 subset is involved mainly in inflammation and the activation of cytotoxic T cells. These Th1 and Th2 cells may cross-inhibit function. Another subset, Th3, which is generated in the GALT, suppresses the function of effector cells by releasing an inhibitory cytokine, transforming growth factor- $\beta$ .

The lymphoid system contains those cells; it may be arranged into capsulated organs or may be represented as accu-

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<sup>4</sup> Abbreviations used: GALT, gut-associated lymphoid tissue; IFN, interferon; Ig, immunoglobulin; IL, interleukin; LPS, lipopolysaccharide; MALT, mucosa-associated lymphoid tissue; MHC, major histocompatibility complex; PG, peptidoglycan; sIgA, secretory immunoglobulin A; Th, T-helper cell; TNF $\alpha$ , tumor necrosis factor.

mulations of diffuse lymphoid tissue. The thymus and bone marrow are primary lymphoid organs and are the major site of lymphopoiesis. T cells mature in the thymus, whereas B cells mature in the fetal liver and bone marrow. Secondary lymphoid organs and tissues, which include the spleen, lymph nodes and MALT, are sites in which cellular and humoral immune responses occur. In this scheme, the spleen responds mainly to blood-borne antigens, whereas the lymph nodes respond to antigens circulating in the lymph. Those antigens may be absorbed through the skin or from the intestine. A group of lymphoid tissues, tonsils, Peyer's patches, bronchus-associated lymphoid tissue and urogenital lymphoid tissue respond to antigens, which have passed through the surface mucosal barriers. These aggregates of nonencapsulated lymphoid tissue, which may be found in the lamina propria and submucosal areas, make up the MALT.

**The MALT.** Diffuse accumulations of lymphoid tissue may be present in the lamina propria of the intestine wall. The epithelium over the Peyer's patches may be specialized to facilitate transport of antigen (see Heel et al. 1997 for review) due to the M cell, which is able to absorb and transport antigen (Keren 1992, Toy and Mayer 1996). That cell may also be able to process and present the antigen to lymphoid cells. Antigens, including pathogenic microorganisms, use M cells to cross the digestive epithelial barrier. The development of M cells appears to depend on the presence of lymphoid cells. Thus, the passage of antigens through M cells is an essential step in the development of the mucosal immune response as well as in the pathology of many infectious diseases.

In addition to the nonencapsulated lymphoid tissue of the MALT, lymphocytes are also found in the connective tissue of the lamina propria and within the epithelial layer. In fact, a majority of the T cells found in the intestine are present in the diffuse lymphoid tissue of the lamina propria. Lymphocytes within the lamina propria are mainly activated T cells; plasma cells may also be found in that location. Another group of T cells, intraepithelial lymphocytes, have phenotypic characteristics that differ from the lamina propria lymphocytes. Those lymphocytes are similar to the cells circulating in peripheral blood, many of which are T-cell receptor  $\gamma\delta^+$  and express CD8. Both populations of T cells have a subset of memory cells, CD45RO, a restricted lymphocyte common antigen. With activation of these lymphocytes, there is expression of a novel heterodimer. Intraepithelial lymphocytes can release cytokines such as interferon (IFN)- $\gamma$  and interleukin (IL)-5. Activation of a primary T response requires not only the antigen and MHC complex but also costimulating molecules on the surface of antigen-presenting cells. Those cells include bone marrow-derived B cells, macrophages and dendritic cells. The last-mentioned are potent initiators of a T-cell-dependent immune response.

**Humoral immune response.** Humoral immune responses at the mucosal level are mainly of the immunoglobulin (Ig)A isotype. Although IgG-, IgM- and IgE-secreting cells are also present, their levels of activity and number are much lower. In contrast to IgA in the serum, secretory IgA (sIgA) is present as a dimeric form in the gut. After synthesis, IgA binds to the membrane receptor on the abluminal surface of the epithelial cells. The polymeric IgA is transported to the mucosal surface while still bound to the membrane of the transport vesicle. After fusion with the cell membrane at the mucosal surface, IgA with the secretory component is released. Secretory IgA is resistant to proteolysis; it does not participate in an inflammatory response. Thus, a major function of sIgA is to mediate immune exclusion of foreign antigens by preventing binding to the epithelial cells and penetration of microorganisms.

**Recirculation of the mucosal immune system.** Lymphoid cells that are stimulated with antigen in the diffuse aggregates of lymphoid tissue migrate to the regional lymph nodes. Normally, lymphocytes leave the blood through regions of the postcapillary venule. Lymphocytes from the lymph node return to circulation via the efferent lymphatic pathways with ~2% of the lymphocyte pool recirculating each hour. The MALT may be considered a system distinct from the systemic lymphoid system because cells of the MALT recirculate mainly within the mucosal system. Lymphocytes activated in Peyer's patches pass through regional lymph nodes, such as the mesenteric group, through the thoracic duct and blood vascular system back to the intestinal lamina propria as well as other secretory tissues, including the respiratory tract, and the lachrymal, salivary and mammary glands. This specific recirculation is possible because the lymphoid cells recognize adhesion molecules that are specific for endothelial cells of the mucosal postcapillary venule. However, little is known about the immune regulation other than the strikingly regionalized disparity in class distribution of mucosal immunocytes (Brandtzaeg et al. 1999).

**Oral unresponsiveness or tolerance.** The MALT usually responds in two opposite fashions, i.e., in a positive manner for immunity to pathogenic organisms and in a negative manner to a large number of antigens of food as well as bacteria in the mucosal environment. This tolerance prevents the immune system from overresponding extensively to potential antigens. This unresponsiveness may be both T- and B-cell mediated. One potential mechanism is the induction of antigen-specific suppressor T cells found in Peyer's patches. Although the mechanism is unknown, antigen-nonspecific regulatory cells can also play an important role in down-regulating responses to specific antigens. Another possible mechanism would be a direct effect of antigen on mucosal lymphocytes resulting in the induction of clonal inhibition (Toy and Mayer 1996). Abnormalities or a reduction in oral unresponsiveness could result in hypersensitivity to oral antigens such as milk proteins in young children.

**Activational pathways of nonspecific immunity by bacterial cell wall products.** Both gram-positive and gram-negative bacteria are found in the human gut flora. Components of their cell wall may play an important role in a number of homeostatic mechanisms as well as nonspecific immunity. The bacterial cell wall consists of two major components. One of those, peptidoglycan (PG), is present in both gram-positive and gram-negative bacteria, whereas lipopolysaccharide (LPS) is expressed only by the gram-negative group. Small amounts of both PG and LPS are released continuously; during severe bacterial infection, large amounts of those compounds may be released. Small amounts of LPS or PG derived from the intestinal flora may be important for the development, maintenance and function of the immune system (Hamann et al. 1998). The action of LPS and PG on cell stimulation is a receptor-dependent process involving the cell surface CD14. The Toll-receptors, a conserved family associated with microbial pathogens, are coupled to signal transduction pathways that control expression of several inducible immune response genes (Kopp and Medzhitov 1999). Macrophages, endothelial cells, smooth muscle cells and neutrophils are activated by these cell wall components and in turn may release several mediators. A large group of proteins can be produced by LPS-activated macrophages, including cytokines, such as tumor necrosis factor- $\alpha$  (TNF $\alpha$ ), IL-1, IL-6, IL-8, IL-12; metalloproteases, such as elastase and cathepsin; lipid mediators such as prostaglandins; as well as reactive oxygen and nitrogen species. However, up to a 1000-fold higher concentration of

TABLE 1

*Methods used for study of intestinal humoral response<sup>1</sup>*

Measurement	Method	Advantage	Disadvantage
IgA or sIgA	Measured by: ELISA, RIA or immunofluorescence		
• Blood-sIgA or circulating IgA-producing cells		IgA easy and sIgA more difficult to measure in humans	Monomeric IgA is produced mainly in bone marrow; does not reflect intestinal response
• Feces		sIgA is produced mainly at mucosal sites, which reflects intestinal response	Proteolytic activity reflects only colonic response
• Saliva		Easy to measure; reflective of MALT	
• Gut lavage fluid		Most reflective of response in intestine	Invasive
IgM	Same as above	Replaces IgA for mucosal immunity in people with IgA deficiency	Levels usually low compared with IgA
IgG	Little detected in mucosal response		

<sup>1</sup> IgA, immunoglobulin A; sIgA, secretory IgA; MALT, mucosa-associated lymphoid tissue.

PG may be required to induce secretion of many of those compounds compared with LPS. It is not known, however, whether sufficient levels of PG are reached in vivo after severe bacterial infection to induce those macrophage functions in vitro. Thus, differential production of autocoids by probiotic bacteria vs. pathogenic bacteria in the intestinal microflora may have a pronounced influence on the induction of non-specific immunity.

#### Methods used for study of the mucosal immune system

When an antigen enters the body through an oral route, the first immune response that normally occurs is oral tolerance, through intraepithelial lymphocytes. When tolerance is abrogated, then an immune response occurs. The mucosal immune system contains precursors for IgA-synthesizing B lymphocytes and immunoregulatory T lymphocytes, which will determine whether oral tolerance or a strong immune response develops. Despite current knowledge of some of the components and events in mucosal immune response, the cellular and molecular events by which the digestive microflora influence the mucosal immune system are poorly understood (Salminen et al. 1998).

To study the effects of probiotics on the immune system, the oral route, the natural host route of the bacteria, should be the focus as should the MALT. One of the major responses in mucosal immunity is a humoral-immune response and the production of sIgA. Several different methods are available to assess the type and concentration of immunoglobulins (Table 1). The specificity of the immunoglobulin and the type are important considerations. For example, sIgA is produced mainly by MALT and may better reflect intestinal response than monomeric IgA, which may not specifically reflect intestinal response. The sIgA may be more difficult to measure than monomeric IgA; samples for assessment of sIgA are best obtained from gut lavage fluid or saliva. Obtaining samples for measurement of sIgA from those human fluids has the drawback of being quite invasive. Saliva may not be the optimal source; however, samples are easy to obtain from humans and saliva can be reflective of MALT activity. In the intestinal immune system, IgM levels are usually quite low compared

with IgA and little to no IgG can be detected in a mucosal response.

Measurement of cellular immune responses may be important in assessing mucosal immunity because of the extensive role played in many cellular interactions. This may be through direct cellular effector activity such as cytotoxic T cells, cellular interactions such as Th cells or the production of soluble mediators such as cytokines (Table 2). One of the most common methods used for assessment of immune function in humans is ex vivo assessment of lymphocyte proliferation. Lymphocyte proliferation in vitro may be induced specifically by a known antigen or nonspecifically by a mitogen. Although a commonly used method, the biological significance of mitogen stimulation is often questioned because of the broad non-specific nature of the stimulation. Moreover, stimulation of lymphocyte proliferation with mitogens by-passes many of the early events that occur in lymphocyte activation. Mitogens stimulate large numbers of lymphocytes, whereas antigens stimulate far fewer cells that are specifically sensitized to antigen. In addition, with antigen-specific stimulation, only T cells respond. Because stimulation of both specific and non-specific immunity may result in the production of cytokines, their assessment may be an important indicator of intestinal immune response. Although production of cytokines may be important for assessment, their constitutive levels are often difficult to detect. In contrast, assays of cytokine production after in vitro stimulation with agents such as LPS or PG are relatively straightforward. Measurement of cytokine production by lymphocytes and macrophages obtained from the MALT would be most reflective of probiotic local effects vs. a systemic response. Because cytokines often function at a focal or cell-to-cell level, the biological significance of assessing the influence of probiotics on systemic cytokine response remains to be established.

Other measurements have been used to determine the effects of probiotics on immune status. For example, number and type of immune cells may be assessed by the use of specific antibodies directed to cell surface markers. The methodology for that is relatively straightforward, and it is often used to determine the types of T and B cells present at a particular site. However, the counting of T and B cells in peripheral blood

TABLE 2

Methods used for study of intestinal cellular immune response

Measurement	Method	Advantages	Disadvantages
Lymphocyte proliferation assays Systemic or intestinal lymphoid cells	Stimulation with mitogen in vitro	<ul style="list-style-type: none"> <li>Stimulate large number of lymphocytes nonspecifically</li> <li>Easy for systemic assays</li> </ul>	<ul style="list-style-type: none"> <li>Biological significance?</li> <li>Biopsy needed for intestinal assays</li> <li>Stimulate few cells that are specifically sensitized to antigen</li> <li>Biopsy needed for intestinal assays</li> </ul>
	Stimulation with antigen in vitro	<ul style="list-style-type: none"> <li>Only T cells respond to antigen</li> <li>More sensitive than DTH</li> <li>Easy for systemic assays</li> </ul>	
Cytokine production	Stimulation of intestinal or systemic lymphoid cells in vitro	<ul style="list-style-type: none"> <li>Easy for systemic assays</li> </ul>	<ul style="list-style-type: none"> <li>Difficult for intestinal assays</li> <li>Biological significance of systemic response?</li> <li>Difficult to detect</li> </ul>
	Constitutive levels in sera, saliva or feces	<ul style="list-style-type: none"> <li>Biomarker?</li> </ul>	

DTH, delayed type hypersensitivity.

and tissue specimens has limited application in both the diagnosis and investigation of the pathophysiologic mechanism of many disease states. Another measure of nonspecific immune status is phagocytosis, mediated by peritoneal, circulating or splenic phagocytes. Although these assays are straightforward, the correlation with specific immune response, particularly at a distant site such as the intestine, is poorly understood.

### Probiotic modulation of the immune system

Probiotics such as *Lactobacillus acidophilus* and *Bifidobacterium bifidum* have been shown to influence select aspects of immune function. Such altered function can involve one or several components of an immune response, e.g., humoral, cellular or nonspecific immunity. Although several in vitro and in vivo studies on probiotic effects on immunity have been reported, the specific mechanisms of the observed changes remain unclear. Moreover, many probiotic preparations have been tested in several separate laboratories with diverse and sometimes contradictory results. In this section, we will review briefly the previous studies that have focused on probiotic effects on humoral response, cell-mediated responses and non-specific immunity. Reports of probiotic-induced alteration are not limited to the localized mucosal immune system; effects on systemic immune responses have also been reported.

**Humoral responses.** There have been several reports recently describing the effects of probiotics on sIgA in both rodents and humans (Table 3). Although the specific results varied, generally an enhanced sIgA production was observed

during probiotic treatment. For example, *L. casei*, *L. acidophilus* and yogurt enhanced the number of IgA-producing plasma cells in a dose-dependent manner (Perdigon et al. 1995). In another study, *L. casei* was shown to significantly increase the amount of sIgA in response to *Salmonella typhimurium* inoculation (Perdigon et al. 1991). This increased secretion of IgA was sufficient to prevent enteric infection. Similarly, the effect of feeding heat-killed *L. casei*, Shirota on IgE production in mice was evaluated after intraperitoneal preinjection with ovalbumin (Matsuzaki et al. 1998). *L. casei*, Shirota reduced serum IgE levels and IgE production in response to ovalbumin. In addition, in vitro production of IgE by spleen cells from mice fed *L. casei*, Shirota in response to restimulation with ovalbumin was inhibited in contrast to spleen cells from the control group (Matsuzaki et al. 1998). From these limited studies, it appears that *Lactobacillus* was able to enhance IgA production in experimental animal models.

**Immune status.** Few studies have been published assessing the effects of probiotics on the cellular aspect of the immune system. In one study, mice fed lactic acid bacteria had increased splenocyte proliferation in response to mitogens for T cells and T and B cells (De Simone et al. 1993). Other effects of probiotics on cellular responses have been observed in conjunction with specific diseases such as autoimmune diseases.

**Cytokine production.** Perhaps the most intriguing aspect of probiotic modulation of immune response is through its effects on cytokine production. Cytokines and their regulation of the immune system have been studied intensively in the last

TABLE 3

Probiotic modulation of humoral immunity

Probiotics	Species	Assessment	Effect
<i>Lactobacillus casei</i> Shirota, oral (heat-killed)	Rodent	Systemic antibody response to ovalbumin	Inhibited splenocyte immunoglobulin (Ig)E in vitro and serum IgE
<i>L. casei</i> , oral (live)	Rodent	Infection and antibody production in malnourished animals	Increased sIgA and reduced enteric infection
<i>L. acidophilus</i> + <i>Peptostreptococcus</i> , oral (live)	Rodent	Translocation of <i>Escherichia coli</i> and serum total anti- <i>E. coli</i> IgG, IgE and IgM	Decreased translocation and increased anti- <i>E. coli</i> IgM and IgE
<i>Bifidobacterium bifidus</i> , oral (live)	Human	Total IgA and response to polio virus	Increased sIgA

TABLE 4

Probiotic effects on cytokine production<sup>1</sup>

Probiotics	Species	Assessment	Effect
<i>Lactobacillus casei</i> , oral (dry)	Human	Serum IFN $\gamma$	Increased
<i>Lactobacillus</i> GG, oral (live)	Human	TNF $\alpha$ in patients with food allergy	Decreased fecal TNF $\alpha$
<i>Lactobacillus</i> , <i>Bifidobacterium</i> , and streptococcus (several strains), oral (live)	Rodent	Mitogen-induced IL-6, IL-12, IFN $\gamma$ , and TNF $\alpha$ production by intestinal lymphoid cells	Enhanced IL-6 and IL-12 ( <i>L. casei</i> and <i>acidophilus</i> ) Enhanced IFN $\gamma$ and NO ( <i>L. acidophilus</i> )

<sup>1</sup> IFN, interferon; TNF, tumor necrosis factor; IL, interleukin; NO, nitric oxide.

several years in cell lines and primary cells of both rodents and humans (Ha et al. 1999, Marin et al. 1998, Miettinen et al. 1998, Nicaise et al. 1993, Tejada-Simon et al. 1999a and 1999b). Several studies have shown that cytokine production by cells of the immune system can be altered by probiotic use (Table 4). For example, the effects of four commercial strains of *Streptococcus thermophilus* found in yogurt on cytokine production were evaluated with a macrophage cell line and a T-helper cell line and compared with active strains of *L. bulgaricus*, *Bifidobacterium adolescentis*, and *B. bifidum* (Marin et al. 1998). All cytokines studied, TNF $\alpha$ , IL-6, IL-2 and IL-5, were affected by heat-killed *S. thermophilus* in a strain- and dose-dependent fashion. All bacteria induced significant increases of IL-6 production in the macrophage cell line with *S. thermophilus*, 133 showing the greatest activity. The four *S. thermophilus* strains also strongly induced TNF $\alpha$  production. IL-6 and, to a lesser extent, TNF $\alpha$  production were also increased when the macrophages were costimulated with LPS and cells of the three groups of lactic acid bacteria. After concurrent stimulation of a T cell line with phorbol 12-myristate-13-acetate, seven of the eight strains enhanced IL-2 and IL-5 production significantly (Marin et al. 1998). In another study, the effect of bacterial flora on cytokine production from mouse resident peritoneal macrophages was investigated (Nicaise et al. 1993). The production of IL-1, IL-6 and TNF $\alpha$  was determined in germ-free mice and mice implanted with either *Escherichia coli* or *B. bifidum*. Macrophages from the implanted mice produced significantly more IL-1 and IL-6 in vitro than macrophages from germ-free mice (Nicaise et al. 1993).

More recent studies have assessed the effects of probiotics on cytokine gene transcription. For example, there was no effect of repeated oral exposure to viable or nonviable *L. acidophilus*, *L. bulgaricus*, *L. casei* or *S. thermophilus* on basal cytokine mRNA expression in Peyer's patches, spleen or lymph nodes of mice, after 14 d of exposure (Tejada-Simon et al. 1999b). In another study, human peripheral blood mononuclear cells were stimulated with three nonpathogenic *Lactobacillus* strains and with one pathogenic *Streptococcus pyo-*

*genes* strain. All bacteria strongly induced IL-1 $\beta$ , IL-6 and TNF $\alpha$  mRNA expression and secretion of the cytokine protein. *S. pyogenes* was the most potent inducer of secretion of IL-12 and IFN- $\gamma$ , and two of the *Lactobacillus* strains induced IL-12 and IFN- $\gamma$  production. All strains induced IL-18 protein secretion (Miettinen et al. 1998). Additional effects of probiotics have been to reverse the age-related decline in the production of cytokines (Famularo et al. 1997). For example, supplementing the diet of aging mice with several probiotic species restored IFN $\gamma$  and IFN $\alpha$  levels compared with control mice (Muscettola et al. 1994). The mechanism of this reversal is unknown but may involve the ability of lactic acid bacteria to adhere selectively to M cells of Peyer's patches.

**Nonspecific immunity.** Several studies have demonstrated the beneficial effects of lactic acid bacteria in boosting a nonspecific immune response. Probiotic bacteria have been shown to influence immune responses nonspecifically by enhancing phagocytosis of pathogens as well as modifying cytokine production (Table 5). Most studies that have reported the effects of probiotic treatment on phagocytosis have used macrophages isolated from treated animals. However, in one study, a strain of *L. acidophilus* isolated from a human newborn was inoculated into germ-free and conventional mice, and phagocytosis of *E. coli* was assessed in vivo (Neumann et al. 1998). The monoassociation of germ-free mice with this lactic acid bacteria for 7 d improved macrophage phagocytic capacity, as demonstrated by the clearance of *E. coli* inoculated intravenously. In another study, probiotic bacteria appeared to modulate the nonspecific immune response in normal, healthy subjects compared with hypersensitive subjects (Pelto et al. 1998). Milk-hypersensitive and healthy adults were challenged with milk with or without *Lactobacillus* GG. In the hypersensitive subjects, milk challenge significantly increased the expression of CR1, Fc $\gamma$ RI and Fc $\alpha$ R in neutrophils and CR1, CR3 and Fc $\alpha$ R in monocytes. In contrast, milk with *Lactobacillus* GG prevented the increase of the receptors expressed. In healthy control subjects, milk challenge did not influence receptor expression, whereas milk with *Lactobacillus* GG significantly increased the expression of CR1, CR3, Fc $\gamma$ RIII and Fc $\alpha$ R in neutrophils. From this work, the authors concluded that the response

TABLE 5

Effects of probiotics on nonspecific immunity

Probiotics	Species	Assessment	Effect
<i>Lactobacillus casei</i> Shirota, intravenous	Rodent	Peritoneal macrophages	Increased phagocytosis
<i>L. acidophilus</i> or <i>Bifidobacterium bifidum</i> , oral (live)	Rodent	Peritoneal or peripheral blood macrophages	Enhanced phagocytosis
<i>L. acidophilus</i> or <i>casei</i> , oral (live)	Rodent	Resident peritoneal macrophages	Enhanced phagocytosis
<i>L. casei</i> Shirota, oral (live)	Human	Peripheral blood	No effect on natural killer cell cytotoxicity in vitro

TABLE 6

Probiotic effects in rodent models of human disease<sup>1</sup>

Disease model	Probiotic	Assessment	Effect
Insulin-dependent diabetes mellitus	<i>Lactobacillus casei</i> , oral (live)	T-cell markers, splenic cytokines	Decreased CD4 <sup>+</sup> cells, IFN $\gamma$ and IL-2
Insulin-dependent diabetes mellitus	<i>L. casei</i> , oral (heat-killed)	Splenic B and T cell number and production of IFN $\gamma$ , IL-2,4,5,6,10	Decreased incidence of diabetes, increased CD45 <sup>+</sup> B-cells, decreased CD8 <sup>+</sup> T-cells. Decreased IFN $\gamma$ and increased IL-2
Collagen-induced arthritis (CIA)	<i>L. casei</i> Shirota, oral (live)	Joint swelling, DTH, collagen-stimulated IL-4 and IFN $\gamma$ production by spleen cells	Decreased CIA, anticollagen antibodies
Influenza immunization	<i>Bifidobacterium bifidus</i> , oral	Respiratory tract infection and anti-influenza virus IgG	Protection against lower resp. tract infections. Higher serum IgG levels

<sup>1</sup> IFN, interferon; IL, interleukin; DTH, delayed type hypersensitivity; Ig, immunoglobulin.

was immunostimulatory in healthy subjects, but down-regulatory in milk-hypersensitive subjects. Collectively, it appears that probiotic bacteria may have a selective influence on components of nonspecific immunity, but the mechanisms by which that occurs remain to be determined.

#### Possible future directions

Studies with probiotic bacteria indicate that select strains have the potential to be beneficial to human health. Studies that have used animal models of human diseases demonstrate that probiotic bacteria have the ability to alter select immunologic responses (Table 6). Although probiotic bacteria such as *L. casei* have been well documented to reduce some pathologic processes such as diarrhea, their ability to influence human immune function is not as clearly documented (Table 7). Although preliminary work is promising, an extensive number of well-controlled studies must be done to clarify specifically the influence that probiotic bacteria may have. Effects of these bacteria may be through alterations of non-specific immunity, potentially mediated through LPS, PG or both. One important issue will be to determine whether probiotics reduce inflammation either at the local or at the systemic level. Because probiotic bacteria cell wall products are likely to act at a local level, it will be important to know which processes are modified and what are the mechanisms for those modifications. Crohn's disease and ulcerative colitis are examples of potentially important models of intestinal non-specific immune dysfunction. Not only will it be important to determine whether probiotics can alter pathogenesis, but it

will also be important to determine the optimal use of probiotics for either prophylaxis or treatment.

With respect to effects on specific immunity, possible future directions include determining whether probiotics may be used as adjuvants for oral immunization (Pouwels et al. 1998). It is also possible that genetically engineered lactobacilli could be used as carriers for antigens to help induce oral tolerance (Maassen 1999). Some preliminary studies have indicated that probiotics could play a role in allergy, autoimmunity or gastrointestinal disease. It will be important to determine whether probiotic bacteria can influence those immunological processes in humans and which specific step may be altered.

Protein-energy malnutrition may be associated with immune suppression, particularly T-cell functions. Thus, it would be important to determine whether probiotics provided through food sources would enhance immune function. In all of these potential studies, it will be very important to identify the strains and determine the levels that are required to achieve the desired effects, whether it is prophylaxis or treatment for a general or specific health condition. It has already been demonstrated that not all strains of lactic acid bacteria exhibit probiotic effects. Extensive variation among species and strains belonging to the same species can be expected. It will also be important to assess whether the probiotics act to modulate the MALT or induce a generalized systemic response. To understand the mechanisms by which probiotics achieve their effects, the development of an in vitro model to mimic MALT would be very helpful.

Although the application of probiotics shows some prom-

TABLE 7

Effect of probiotics on select human disease<sup>1</sup>

Disease	Probiotic	Assessment	Effect
Asthma	<i>Lactobacillus acidophilus</i> , oral (live)	Serum IgE and IL-4	No alterations
Rotavirus infection	<i>L. GG</i> , oral (live)	Lymphocyte proliferation Diarrhea Serum total IgA and IgM and anti-rotavirus IgM and IgG	Duration of diarrhea decreased; Increased anti-rotavirus IgA
Crohn's disease and juvenile arthritis	<i>L. casei</i> , oral (live)	Anti-B $\beta$ -lactoglobulin IgA-secreting cells	Number increased

<sup>1</sup> Ig, immunoglobulin; IL, interleukin.

ising results and trends with respect to select aspects of immune modulation, the underlying mechanisms are unclear. Nevertheless, it will be important to understand the role of gut bacteria as immune modulators in health and disease.

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