Immunologic effects of yogurt

Simin Nikbin Meydani and Woel-Kyu Ha

ABSTRACT  Many investigators have studied the therapeutic and preventive effects of yogurt and lactic acid bacteria, which are commonly used in yogurt production, on diseases such as cancer, infection, gastrointestinal disorders, and asthma. Because the immune system is an important contributor to all of these diseases, an immunostimulatory effect of yogurt has been proposed and investigated by using mainly animal models and, occasionally, human subjects. Although the results of these studies, in general, support the notion that yogurt has immunostimulatory effects, problems with study design, lack of appropriate controls, inapposite route of administration, sole use of in vitro indicators of the immune response, and short duration of most of the studies limit the interpretation of the results and the conclusions drawn from them. Nevertheless, these studies in toto provide a strong rationale for the hypothesis that increased yogurt consumption, particularly in immunocompromised populations such as the elderly, may enhance the immune response, which would in turn increase resistance to immune-related diseases. This hypothesis, however, needs to be substantiated by well-designed randomized, double-blind, placebo-controlled human studies of an adequate duration in which several in vivo indexes of peripheral and gut-associated immune response are tested. Am J Clin Nutr 2000;71:861–72.

KEY WORDS Yogurt, lactic acid bacteria, Lactobacillus, Bifidobacterium, Enterococcus, Streptococcus, Salmonella, immunostimulatory effects, immune system, cancer, infection, gastrointestinal disorders, asthma, review

INTRODUCTION

Yogurt is defined by the Codex Alimentarius of 1992 as a coagulated milk product that results from fermentation of lactic acid in milk by Lactobacillus bulgaricus and Streptococcus thermophilus (1). Other lactic acid bacteria (LAB) species can be combined with L. bulgaricus and S. thermophilus. In the finished product, the LAB must be alive and in substantial amounts. LAB have been used for thousands of years to produce fermented food and milk products. Fermented products contain a variety of fermented microorganisms belonging to various genera and species, all of which produce lactic acid.

With few exceptions, milk and yogurt have similar vitamin and mineral compositions. During fermentation, vitamins B-12 and C are consumed and folic acid is produced. The differences in other vitamins between milk and yogurt are small and depend on the strain of bacteria used for fermentation. Although milk and yogurt have similar mineral compositions, some minerals, eg, calcium, are more bioavailable from yogurt than from milk. In general, yogurt also has less lactose and more lactic acid, galactose, peptides, free amino acids, and free fatty acid than does milk (2, 3).

After Metchnikoff (4) postulated that L. bulgaricus suppresses toxins produced by putrefactive bacteria in human intestines, many investigators studied the therapeutic effects of LAB. However, results were inconsistent. Varying reports of the therapeutic efficacy of LAB may be due to differences in the strains of LAB and experimental procedures used in the various studies. Although results obtained from studies in which LAB were administered parenterally might not be a good predictor of results of oral consumption of yogurt, both oral and parenteral administration of LAB, in general, were shown to strengthen nonspecific immune response or to act as adjuvants in antigen-specific immune response (5–8).

Most studies indicated that the potential therapeutic effects of LAB and yogurt, including their immunostimulatory effect, are due primarily to yogurt-induced changes in the gastrointestinal (GI) microecology. Increased amounts of LAB in the intestines can suppress the growth of pathogenic bacteria (9–12), which contributes in turn to reduced infection (13–15) and heightened anticarcinogenic effects (5, 16).

The immunostimulatory effect of LAB also depends on the degree of contact with lymphoid tissues while the bacteria are transiently colonizing the intestinal lumen (17, 18). Thus, the ability of LAB to survive in the GI tract can influence the bacteria’s immunogenicity (19–25). The survival rate of LAB in the GI tract varies with gastric pH (26). Within the Lactobacillus genus, L. acidophilus is more resistant to gastric juice than is conventional lactic culture, L. bulgaricus, and is more resistant...
than S. thermophilus (20). Of the 4 Bifidobacterium species studied (B. infantis, B. bifidum, B. adolescentis, and B. longum), B. longum was the most resistant to gastric acid (27). The LAB that survive the GI process adhere to epithelial cells in the wall of the GI tract (28–30) and can bind to the luminal surface of M cells (31). Animal studies showed that gut-associated lymphoid tissue is stimulated by these surviving LAB, resulting in enhanced production of cytokine and antibody (secretory immunoglobulin (sg) A) and increased mitogenic activity of Peyer’s patch (PP) cells and splenocytes.

In human studies, cytokine production (6, 32–36), phagocytic activity (37, 38), antibody production (39), T cell function (36, 40), and natural killer (NK) cell activity (32, 41) were shown to increase with yogurt consumption or when cells were exposed to LAB in vitro. There is some evidence that yogurt-induced immune enhancement is associated with a lowered incidence of conditions such as cancer, GI disorders, and allergic symptoms.

**Immune system functions**

The main functions of the immune system are to eliminate invading viruses and foreign microorganisms, to rid the body of damaged tissue, and to destroy neoplasms in the body. Healthy humans have 2 immune mechanisms: acquired (specific) immunity, which responds to specific stimuli (antigens) and is enhanced by repeated exposure; and innate (nonspecific) immunity, which does not require stimulation and is not enhanced by repeated exposure. Innate immune mechanisms consist of physical barriers, such as mucous membranes, and the phagocytic and cytotoxic function of neutrophils, monocytes, macrophages, and lymphatic cells (NK cells). Acquired immunity can be classified into 2 types on the basis of the components of the immune system that mediate the response, ie, humoral immunity and cell-mediated immunity. Humoral immunity is mediated by immunoglobulins produced by bone marrow–derived lymphocytes (B lymphocytes) and is responsible for specific recognition and elimination of extracellular antigens. Cell-mediated immunity is mediated by cells of the immune system, particularly thymus-derived lymphocytes (T lymphocytes) and is responsible for delayed-type hypersensitivity (DTH) reactions, foreign graft rejection, resistance to many pathogenic microorganisms, and tumor immunosurveillance. In addition to their involvement in nonspecific immunity, macrophages are important in cell-mediated immunity as antigen-presenting cells and through the production of regulatory mediators such as cytokines and eicosanoids. Several in vitro and in vivo tests were developed to assess the function of immune cells. Although the study of immune response in animals and humans is based on similar principles, the methods used to separate cells, the types of stimuli used in vitro, and the antigen used for in vivo challenge vary. In addition, the type of antibody used to measure different mediators or to determine cell-surface proteins is species specific.

**In vitro indexes of immune function**

To study immune function in vitro, immune cells are first separated from whole blood, lymphoid tissues, and gut-associated immune cells. The cells are then maintained and cultured with and without various immune cell stimuli. To measure the activity of isolated phagocytes, the cells are incubated with bacteria or other engulfable materials with or without opsonin for a limited time and then stained for uptake of foreign bodies. Lymphocytes are usually stimulated for varying lengths of time by a variety of stimuli (mitogens, antigens, and other stimulator or target cells) for measurement of their proliferative or cytotoxic activity or release of immunologically active molecules such as antibodies, cytokines, and eicosanoids.

**Phagocytic activity**

The ability to perform phagocytosis and kill microbes, including bacterial pathogens, is a major effector function of macrophages. These properties of macrophages are particularly important for host defense against facultative intracellular organisms, which can replicate within macrophages. The pathogenesis of facultative intracellular bacteria is determined by their ability to survive within macrophages. Several organisms were used previously as targets to determine macrophage killing. These include Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Salmonella typhimurium, Listeria monocytogenes, and Candida albicans.

Bacteria bind to complement components and the bacterium-complement complexes bind complement receptors on the surface of macrophages. Phagocytosis may also be mediated by specific antibodies that function as opsonins, which bind to particles, rendering them susceptible to phagocytosis. The bacterium-antibody complex then binds the macrophages via the Fc receptor and phagocytosis begins.

Measurement of phagocytic activity of macrophages was among the earliest techniques for evaluating the immunologic effects of LAB. This assay measures the ability of macrophages to bind, internalize, and phagocytose bacteria. Monocytes or macrophages isolated from human peripheral blood mononuclear cells (PBMCs) or from the peritoneal cavity of animals are mixed with bacteria in suspension and incubated at 37°C. Extracellular bacteria are then removed through washing and centrifugation or through washing only over sucrose. The degree of phagocytosis is determined by examining stained cells under oil-immersion microscopy and quantifying the number of internalized bacteria in each cell. This method takes into account not only the percentage of phagocytic cells but also the strength of the phagocytic ability of these cells, ie, how many bacteria are internalized by each cell (42).

**Lymphocyte proliferation assay**

Measurement of the proliferative response of lymphocytes is the most commonly used technique for evaluating cell-mediated immune response. Quantitative analysis of proliferative response involves measuring the number of cells in culture in the presence and absence of a stimulatory agent such as an antigen or a mitogen. The most common polyclonal mitogens used to test the proliferation of lymphocytes are concanavalin A (ConA), phytohemagglutinin, lipopolysaccharide (LPS), and pokeweed mitogen. T and B lymphocytes are stimulated by different polyclonal mitogens. ConA and phytohemagglutinin stimulate T cells, LPS stimulates B cells, and pokeweed mitogen stimulates both T and B cells. When mitogens are used, prior exposure of the host to the mitogens is not necessary. However, to measure antigen-specific proliferation, the host should be exposed to the antigen before the cells are stimulated with that antigen in vitro. Lymphocytes normally exist as resting cells in the G0 phase of the cell cycle.

When stimulated with polyclonal mitogens, lymphocytes rapidly enter the G1 phase and progress through the cell cycle. Measuring incorporation of [3H]thymidine into DNA is the most commonly used method for estimating changes in the number of cells. The proliferative assay is used to assess the overall...
immunologic competence of lymphocytes, as manifested by the ability of lymphocytes to respond to proliferation signals. Decreased proliferation, observed in chronic diseases such as cancer and HIV infection and in the aging process, may indicate impaired cell-mediated immune function.

Cytokine production

Cytokines, which are protein mediators produced by immune cells, are involved in the regulation of cell activation, growth and differentiation, inflammation, and immunity. Measurement of cytokine production, as determined by techniques such as bioassay, radioimmunoassay, and enzyme-linked immunosorbent assay, has been used to examine various immune functions. Details of cytokine measurement were published previously (42).

Interleukin 2 (IL-2) is a T cell growth factor produced by T helper (T\textsubscript{H}) 1 and NK cells. As an autocrine and paracrine growth factor, IL-2 induces proliferation and differentiation of T and B cells. IL-2 is responsible for the progress of T lymphocytes from the G\textsubscript{0} to the S phase in the cell cycle and also for stimulation of B cells for antibody synthesis. IL-2 stimulates the growth of NK cells and enhances the cytotytic function of these cells, producing lymphokine-activated killer (LAK) cells. IL-2 can also induce interferon (IFN-\gamma) secretion by NK cells. IFN-\gamma is an important macrophage-activating lymphokine. IL-2 secreted in culture media or biological fluids can be measured by immunoassay or bioassay, the most common of which uses the IL-2-dependent cytotoxic T lymphocyte line. Proliferation of cytotoxic T lymphocyte line cells reflects IL-2 activity. IL-2 activity in samples can be calculated according to a standard curve generated by adding varying concentrations of recombinant IL-2. Enzyme-linked immunosorbent assay is also used to measure IL-2. Although this assay is more specific than is the cytotoxic T lymphocyte activity assay, target cells can be lymphoblasts, tissue culture cells, or tumor cells that are labeled with \textsuperscript{3}H and then incubated with effector cells (stimulated effector cells in cytotoxic T lymphocyte assay or IL-2-stimulated effector cells in LAK assay) at different ratios. The percentage of \textsuperscript{3}H released represents the lysis of target cells, which reflects the cytotoxic activity of effector cells. Cytotoxic T lymphocytes, NK cells, and LAK cells are important in the host response to tumors and viral infections.

Flow cytometric analysis

Immune cells bear specific markers (antigens) on their surface that are used to identify various types of cells (eg, lymphocytes and macrophages) and cell subpopulations (eg, B and T lymphocytes). A population of cells can be classified into subsets according to the surface markers of the cells. Cell subsets play various roles in regulating immune response. For example, T cells can be classified as CD4\textsuperscript{+} or CD8\textsuperscript{+} cells, which are defined, according to their function, as T\textsubscript{H} cells and suppressor cells, respectively. Properly identifying cells with different surface markers may be a step toward understanding the cellular basis of immune response. For flow cytometry, a fluorescence-activated cell-sorter technology has been used widely to characterize and quantify viable subpopulations of immune cells. Flow cytometric analysis consists of 3 steps: 1) prepared cells are incubated with a specific antibody against a particular cell surface marker and labeled with fluorescent reagents, such as fluorescein isothiocyanate; 2) the stained cells are processed (identified and separated) by flow cytometry and appropriate data are collected; and 3) the collected data are analyzed to obtain quantitative information on cell subpopulations. Details of flow cytometry methods were published previously (42).

IN VIVO INDEXES OF IMMUNE FUNCTION

Although immune function is measured predominantly through in vitro methods, a limited number of in vivo methods are also available. In vivo methods may include factors not found under in vitro conditions and thus provide more accurate measurements.

Assessment of phagocytosis

In vivo activation of macrophages is important in suppressing tumor growth. The method depends mainly on measurement of the clearance from the circulation of intravenously injected materials (eg, colloids, bacteria, macromolecules, and opsonized red cells). Blood samples are collected at designated intervals after the intravenous inoculation of a colloidal carbon-particle suspension. After dissolution of the erythrocytes in blood samples, the contents of injected materials in blood are measured by optical density. Studies on the clearance of injected materials from the circulation provide information on the functional state of the macrophages in the liver (Kupffer cells), lung, and spleen. However, blood clearance of particulate matter is affected by factors such as the blood flow rate, the presence or absence of opsonized factors, the adhesiveness of particles to vessel walls, and changes in phagocytic cells in the liver.

Delayed-type hypersensitivity reaction

DTH reaction is used extensively as an in vivo assay to determine cell-mediated immune function and to assess immuno-
competence. Several investigators showed that a decrease in DTH is associated with increased mortality (50–53). DTH is based on an antigen-specific, T cell-dependent recall response manifested as an inflammatory reaction that reaches peak intensity ~24–48 h after antigenic challenge. In the DTH skin test, a small amount of soluble antigen is injected into the epidermis and superficial dermal tissue. Circulating T cells sensitized to the antigen from prior contact react with the antigen in the skin and induce a specific immune response, which includes mitosis (blastogenesis) and the release of soluble mediators. The reaction process involves antigen presentation by macrophages, release of IL-1 and tumor necrosis factor (TNF) from activated macrophages, release of IL-2 and IFN-γ from activated T cells, and interaction between these mediators. In humans and guinea pigs, the intensity of DTH is evaluated by measuring the redness and induration of an area of shaved skin exposed to the antigen (54). In humans, DTH to several recall antigens can be measured with multitest cell-mediated immunity (55), but this method does not work well in mice. An alternative method was developed in which antigens are injected subcutaneously into the footpad of a primed mouse (56). After 24 h, footpad swelling is measured with a caliper.

**Antibody production**

Immunization with appropriate antigens (viral or bacterial) can elicit serum antibodies. Particular antigens can produce an immune response at the mucosal level. Antibody production as a response to antigen challenge involves several cellular events, such as antigen processing and presentation, recognition of the presented antigen by T<sub>H</sub>1 cells, and T<sub>H</sub>1 cell activation and production of cytokines that augment the response of memory B cells. Therefore, the qualitative and quantitative assays for antibodies provide information about B cell responsiveness and T cell cooperation. Antibody response to a particular antigen, including vaccines and innocuous bacteria such as LAB, has been used as an index in evaluating the host resistance to infections.

**YOGURT COMPONENTS WITH POTENTIAL IMMUNOSTIMULATORY EFFECTS**

Although yogurt has long been known to bolster host-defense mechanisms against invading pathogens, the components responsible for these effects have not been fully defined (57, 58). The immunostimulatory effects of yogurt are believed to be due to yogurt’s bacterial components. However, the mechanism or mechanisms responsible for these effects have not been fully determined (57, 59). After entering the intestine, live or biologically active LAB particles may activate specific and nonspecific immune responses of gut-associated lymphoid tissue and the systemic immune response. The immunogenicity of intestinal bacteria depends on the degree of contact with lymphoid tissue in the intestinal lumen (17, 18). Therefore, dead bacteria are generally less efficient as antigens than are live bacteria because dead bacteria are rapidly dislodged from the mucosa (31, 60). Some studies, however, showed no difference in immunogenicity between viable and nonviable bacteria (61).

LAB are gram-positive bacteria with cell wall components such as peptidoglycan, polysaccharide, and teichoic acid, all of which have been shown to have immunostimulatory properties (62). In addition to cell wall components, immunostimulatory effects were observed with antigens originated from the cytoplasms of some strains of LAB.

Nonbacterial milk components and components produced from milk fermentation also may contribute to the immunostimulatory activity of yogurt. Peptides and free fatty acids generated by fermentation have been shown to enhance the immune response. Milk components such as whey protein, calcium, and certain vitamin and trace elements also can influence the immune system (62).

**Bacterial components**

The LAB most commonly used to ferment milk are _L. bulgaricus_ and _S. thermophilus_. To increase LAB’s survival rate and resistance to low pH and bile acid in the GI tract, LAB indigenous to the human intestine, including _L. acidophilus, Lactobacillus casei_, and _Bifidobacterium_ species, are now being used in yogurt production.

Yogurt consumption and oral administration of LAB were shown to stimulate the host immune system. It is believed that LAB are essential for yogurt to exert immunostimulatory effects and that the LAB cell walls contain the main immunomodulatory component (63). The LAB cell wall is composed mainly of peptidoglycan (30–70% of the total cell wall), polysaccharide, and teichoic acid. Peptidoglycans are glycopeptides released from the bacterial cell wall by bacteriolytic enzymes, such as lysozyme. Lysozyme, which is secreted into the intestine from paneth cells (64), can release peptidoglycan and muramyl dipeptide (MDP), a lower-molecular-weight product of peptidoglycan.

Peptidoglycans are known to have adjuvant effects on immune response (65–67). Binding sites for peptidoglycans were identified on lymphocytes and macrophages (68). LAB, which are very sensitive to lysozyme digestion, may liberate peptidoglycan in the intestine and induce adjuvant activity at the mucosal surface (39). Bogdanov et al (69) reported that the immunostimulatory activity in the host by cultured dairy products is mediated by glycopeptides in the bacterial cell wall.

MDP is a main constituent of peptidoglycan in the cell wall of pathogenic and nonpathogenic bacteria such as LAB. MDP stimulates macrophages to release IL-1, which is needed for activation of T lymphocytes (70–72) and induces IFN-γ production by lymphocytes (73). Tufano et al (73) showed that MDP stimulates the production of IL-1, IL-6, and TNF-α by monocytes as well as that of IL-4 and IFN-γ by lymphocytes. Aattouri and Lemoine (36) showed that MDP in vitro increased IFN-γ production, an effect that was diminished in the presence of anti-CD4 (which depletes CD4<sup>+</sup> T cells) or when monocytes were depleted. This indicates that MDP might stimulate PBMCs to produce IFN-γ through a CD4–T cell antigen receptor–human leukocyte antigen complex and that MDP might be a component of the bacterial cell wall, which is recognized by monocytes and presented to CD4<sup>+</sup> lymphocytes in the context of human leukocyte antigen. Also, enzymatic digestion of the LAB cell wall was shown to increase nonspecific host immunity to _L. monocytogenes_ in mice (74), to _Klebsiella_ infection (75), and to tumor cells (76) and to increase cytokine production by human PBMCs (36, 77), PP lymphocyte proliferative activity (62), and DTH and antibody titer to hepatitis B in guinea pigs (78). Sato et al (79) reported that the enhancement of host-defense activity by _L. casei_ against _L. monocytogenes_ infection in mice may be attributed to the cell wall components of _L. casei_, one of which is peptidoglycan. Other cell-wall components contribute to the immunostimulatory activity of LAB. Teichoic acids stimulate the production of IL-1, TNF-α, and IL-6 by monocytes in vitro (73, 80–83).
The cytoplasmic component of LAB was also shown to increase the proliferative response of PP cells in a strain-dependent manner. Rangavajhyala et al. (84) reported that the non-lipopolysaccharide (enterotoxin) component of L. acidophilus (strain DDS-1, L1) stimulates the production of IL-1α and TNF-α by mouse macrophages in vitro. Cell-free water-soluble extract of L. acidophilus and B. longum was found to stimulate phagocytic activity in an in vitro murine macrophage system (61). Thus, although many studies showed that peptidoglycan in the cell wall stimulates macrophages, antibody formation, and T lymphocyte activity, other bacterial components may exert some immuneenhancing effects.

The immunogenicity of LAB differs depending on the properties of a particular strain rather than on the common characteristics of the species (85). LAB’s exact structural attributes, however, have not been fully determined. The immunogenicity of LAB depends on the bacteria’s survival in the GI tract, resistance to gastric acid and bile acid, and ability to adhere to the mucosal surface (37).

Nonbacterial components

Although yogurt, like milk, is a rich source of protein, riboflavin, folic acid, and calcium, compositional changes occur as milk is converted into yogurt. These changes include a decrease in lactose and vitamins B-6 and B-12 and an increase in peptide, free amino acid, free fatty acid, folic acid, and choline contents. Yogurt contains calcium lactate whereas milk contains calcium caseinate. In addition to changes in nutrient and nonnutrient contents, other functional components are generated during fermentation.

Because proteases from microorganisms hydrolyze milk proteins more randomly than do intestinal proteases, bacterial proteases are not substrate specific. During fermentation of milk by LAB, the physicochemical state of milk proteins changes, causing significant amounts of free amino acids and peptides to be produced.

Proteolysis was shown to affect the phagocytic capabilities of macrophages (86). A more proteolytically hydrolyzed milk resulted in increased stimulation of phagocytosis from the pulmonary alveolar macrophages in mice (86). Therefore, peptides produced from the fermentation of milk may also contribute to the immunoenhancing effect of yogurt. Parker et al. (87) identified a hexapeptide, isolated from casein after enzymatic digestion, which, when intravenously injected into mice (5-wk-old females), improved resistance to K. pneumonia in vitro. In vitro, this hexapeptide stimulated the phagocytosis of sheep red blood cells by peritoneal macrophages of mice (87). In addition, these bioactive peptides might stimulate the proliferation and maturation of T cells and NK cells for the defense of the host against a wide range of bacteria, particularly enteric bacteria (88).

Denatured and native whey protein, both of which have remarkably higher cysteine contents than do other common edible proteins, may contribute to the immunostimulatory effects of yogurt. Cysteine is a rate-limiting component in the biosynthesis of glutathione (89). Glutathione is important for detoxification of endogenous and exogenous carcinogen and free radicals and in regulation of immune function. Depletion of cellular glutathione was reported to suppress mitogenic response of lymphocytes (90–93), to prevent lymphocytes from entering the S phase in the cell cycle (94), and to decrease antibody-dependent cellular cytotoxicity and spontaneous cell-mediated cytotoxicity (95, 96) and IL-2 induced LAK activity (97). McIntosh et al. (98) showed that rats fed whey protein had higher liver glutathione concentrations than did rats fed a control diet. Bounous et al. (99) showed that feeding whey protein concentrate to 3 HIV-positive persons for 3 mo significantly increased PBMC glutathione concentrations and body weights. Wong and Watson (100) showed that bovine whey protein increased antibody response to ovalbumin and DTH and proliferative response of splenocytes to T cell mitogen ConA in young BALB/c mice. Several studies showed that whey protein decreased the incidence of tumors (101) and neoplastic diseases (98, 102) and increased longevity (103).

Biochemical changes in milk fat may also occur during milk fermentation. Milk contains conjugated linoleic acid (CLA), a fatty acid with immunostimulatory and anticarcinogenic properties. CLA was discovered in meat products from ruminant animals, eg, cows and sheep, and in dairy products. Rumen bacteria can convert linoleic acid to CLA through biohydrogenation. It was speculated that biohydrogenation may occur during the fermentation of milk and that some new free fatty acids are formed after fermentation, depending on the origin of the milk and the bacterial strain (2, 104). Rao and Reddy (105) reported an increase in concentrations of free stearic and oleic acid after the fermentation of cow milk by L. bulgaricus or S. thermophilus, which they attributed mostly to partial saturation of the linoleic acid. Shantha et al. (106) reported that yogurt had a higher CLA content than did the milk from which it was processed. Fermented dahi (the Indian equivalent of yogurt) has a higher CLA content than does nonfermented dahi (107). Milk fermentation results in a complete solubilization of calcium, magnesium, and phosphorus and a partial solubilization of trace minerals (108). Therefore, milk fermentation may exert some effect on mineral bioavailability. Calcium and phosphorus were shown to be more bioavailable in yogurt than in milk (109). Long-term yogurt consumption was shown to be associated with a significant increase in serum ionized calcium (35). Calcium was shown to enhance immune function, including lectin binding by lymphocytes (110), IL-2 production (111), and lymphocyte tumor cytotoxicity (112).

Ayebo et al. (113) reported that the dialysate and the anion exchange fraction of yogurt showed significant inhibitory action against tumors in a mouse assay in vivo and suggested that increased antitumor or anticarcinogenic activity might be due to the enhancement of nonspecific immunity in the host. Biffi et al. (114) suggested that soluble compounds produced by LAB during milk fermentation can be used to prevent GI disorders and cancer. Perdigon et al. (115) reported that the supernate of fermented milk cultured with L. casei and L. acidophilus increased the immune response independent of the presence of lactobacilli. De Simone et al. (32) reported that filtered yogurt, which is free of microorganisms, increased IFN-γ production and NK activity of human peripheral blood lymphocytes.

These studies strengthen the notion that components of yogurt other than bacteria also may contribute to yogurt’s immunostimulatory effect. In addition, yogurt is a nutrient-dense food containing high-quality protein; vitamins, especially folic acid; and trace elements, all of which are necessary for maintaining optimal immune response.

**YOGURT AND IMMUNE FUNCTION**

Fermented milk containing viable LAB is known to be beneficial to health, acting as prophylaxis against intestinal...
infections (13, 15) and as an anticarcinogen (69, 116–120). In light of this, many investigators have evaluated the effect of yogurt on the immune responses of animals and humans.

**Animal studies**

Macrophages represent one of the first lines of nonspecific defense against bacterial invasion and tumors. Macrophages kill bacteria and tumor cells through several effector mechanisms, including the production of soluble factors such as nitric oxide, hydrogen peroxide, and superoxide (121–123). Macrophages can also use receptor-mediated attachments to kill tumor cells through direct cell-to-cell contact. The Fcγ receptor of immunoglobulin on macrophages enables these cells to attach to opsonized (IgG coated) tumor cells, thereby mediating tumor cell cytotoxicity (124). During activation, macrophages acquire the capacity to bind unopsonized tumor cells as well (125). Macrophage responses to bacteria and bacterial products are processed by a mechanism similar to that of tumoricidal activity (126). It was suggested that the antigen effect of LAB is due to enhancement of macrophage activity (115, 120, 127, 128).

A limited number of animal studies were conducted on the effect of yogurt on macrophages. Goulet et al (129) found that phagocytic activity of alveolar macrophages was significantly increased in mice fed milk fermented with *B. longum*, *L. acidophilus*, *L. casei rhamnous*, or *Lactobacillus helveticus* than in control mice fed ultrahigh-temperature-treated milk. However, no significant stimulation of phagocytic activity was observed with streptococci-fermented milk (129). Perdigon et al (130) showed that feeding milk (100 μg protein/d) fermented with *L. casei*, *L. acidophilus*, or both for 8 d increased the in vitro and in vivo phagocytic activity of peritoneal macrophages and antibody production against sheep red blood cells in Swiss mice. The activation of the immune system began on the third day, peaked on the fifth day, and decreased on the eighth day of feeding. However, a further increase in immune response was observed in mice given a dose of fermented milk (100 μg) on the 11th day of feeding.

Other studies in which reconstituted lyophilized LAB were administered orally or intraperitoneally showed enhancement of macrophage activity by LAB (74, 115, 120, 131). Kitazawa et al (132) reported that *L. acidophilus* induced production of IFN-α and IFN-β in murine peritoneal macrophage cell culture. If an antigen overcomes the nonspecific host-defense system, both the humoral and the cell-mediated immune responses are activated. Orally administered LAB may pass through the GI lumen to reach the local lymphatic organs in the gut. Subsequently, translocation of LAB can lead to the activation of the local immune system in the gut, which results, in turn, in mucosal antibody production, especially of slgA from PP cells (39, 133, 134). Generally, slgA is induced very poorly after intramuscular or subcutaneous immunization but can be induced vigorously by oral immunization (135). SlgA inhibits colonization of pathogenic microorganisms (enteric infection) and penetration of dangerous luminal antigens.

Perdigon et al (134) reported that orally administered LAB (*L. acidophilus* and *L. casei*) and yogurt feeding increased slgA production and the number of slgA-producing cells in the small intestine of mice in a dose-dependent manner. Puri et al (58) reported that serum IgA concentrations in yogurt-fed mice were significantly higher than concentrations in milk-fed mice after salmonellla challenge. These investigators proposed that the IgA secreted by the intestinal B cell enters the circulation and raises the serum IgA concentration. Takahashi et al (62) reported significantly (*P < 0.01*) greater specific IgG and IgA to LAB cytoplasm (*B. longum*) and cell wall (*L. acidophilus*) in mice fed LAB than in mice not fed LAB.

As with yogurt, orally fed *L. casei* was shown to increase slgA secretion into the intestinal lumen. Perdigon et al (133) proposed that the increase in slgA concentration is due to the stimulation of PP cells by LAB and a change in the ratios of CD4+ T lymphocytes (helper cells) to CD8+ T lymphocytes (suppressor cells).

De Simone et al (136) reported that mice fed live LAB (*L. bulgaricus* and *S. thermophilus*)-containing yogurt for 7 and 14 d had a higher percentage of B lymphocytes (*P < 0.01*) in the PP cells than did mice fed a control diet supplemented with cow milk. In addition, blastogenic response to phytohemagglutinin and LPS of PP cells from animals fed live LAB (*L. bulgaricus* and *S. thermophilus*)-containing yogurt for 14 or 21 d was significantly higher than that of the control group. In a similar experiment, Puri et al (58) showed that intestinal lymphocytes from mice fed live LAB-containing yogurt had a higher proliferative response to ConA and LPS than did mice fed milk after a challenge with *S. typhimurium*.

De Simone et al (137) studied the influence of a yogurt-supplemented diet on the immunocompetence and survival of animals subsequently infected with *S. typhimurium*. Their results suggested that feeding a diet supplemented with yogurt containing live LAB for 4 wk increases the rate of survival of young mice against *S. typhimurium* infection. These authors attributed the effect to the ability of live LAB to enhance local and systemic immune response. Interestingly, yogurt supplemented with heat-killed bacteria was not effective (137). Puri et al (59) studied the proliferative response of splenic lymphocytes to 3 mitogens (ConA, phytohemagglutinin, and LPS). These researchers reported that the mitogenic response to ConA and phytohemagglutinin was significantly higher in mice fed a yogurt diet than in mice fed a milk diet but that no significant difference was observed in response to LPS (59).

Muscellota et al (31) showed that in vitro production of IFN-γ from spleen cells of young (aged 7 wk) and old (aged 19 mo) mice fed live LAB (*L. bulgaricus* and *S. thermophilus*) was higher than that of control mice. They reported that a diet supplemented with lactobacilli for 7 and 15 d significantly (*P < 0.001*) increased IFN-α and IFN-γ production in young mice and reduced the cytokine levels in aged mice to less than those in the young mice.

It was suggested that the immunostimulatory function seen with oral administration of LAB is partially mediated by increased secretion of IFN-γ from PP cells in gut-associated lymphoid tissue. IFN-γ was shown to enhance expression of the secretory component, thus playing an important role in increasing external transport of dimeric IgA (49). Solis-Pereyra et al (49) showed that *L. bulgaricus* and *S. thermophilus* induced plasma IFN-α and IFN-β production in mice.

**Human studies**

Human studies examining the immunostimulatory effects of LAB focused primarily on the effect of yogurt consumption on ex vivo indicators of immune response, such as PBMC cytokine production (6, 32–36, 48, 138, 139), phagocytic activity (37, 38), specific humoral immune response (39, 140, 141), T lymphocyte (CD4+ and CD8+ function (36, 40), and NK cell activity (32, 41).

It was shown that phagocytic leukocyte activity of human blood cells, particularly granulocytes, was enhanced by the...
ingestion of fermented milk supplemented with \textit{L. acidophilus} La1 and \textit{B. bifidum} Bb12 for 3 wk (37, 38). Consumption by healthy humans of fermented milk containing \textit{L. bulgaricus} and \textit{S. thermophilus} was reported to stimulate cytokine production of PBMCs. De Simone et al (32) reported that lymphocytes cultured with \textit{L. bulgaricus} and \textit{S. thermophilus} produced more IFN-\gamma when stimulated with ConA than did control cultures. \textit{L. bulgaricus} was more effective than was \textit{S. thermophilus} in enhancing IFN-\gamma production. Increased production of IFN-\gamma by isolated T lymphocytes in young adults (aged 20–40 y) consuming yogurt containing live \textit{L. bulgaricus} and \textit{S. thermophilus} (450 g/d for 4 mo) was reported (35). Long-term consumption of yogurt containing viable LAB was shown to increase IL-1, IL-6, IL-10, IFN-\gamma, and TNF-\alpha production (6, 34–36, 49).

Contrary to the results of in vitro studies on cytokine release, yogurt consumption does not appear to affect plasma concentrations of IFN-\gamma. Trapp et al (48) reported that consuming 200 g yogurt/d for 1 y had no effect on plasma IFN-\gamma concentrations. IFN-\gamma has a very short half-life in plasma and is secreted locally in low amounts (47). Thus, using plasma IFN-\gamma concentrations to detect the effect of yogurt on IFN-\gamma production may not be appropriate. Solis-Pereyra and Lemmonnier (33) suggested that 2’-5’ A synthetase (an IFN-\gamma-inducible protein) be assayed instead of assaying for IFN-\gamma itself and showed that subjects consuming yogurt had higher plasma concentrations of 2’-5’ A synthetase than did subjects consuming milk. These authors also reported a transient increase in plasma IFN-\gamma concentrations. Link-Amster et al (39) showed that the \textit{S. typhimurium}-specific anti-IgA titer was 4 times higher in subjects fed fermented milk containing \textit{L. acidophilus} than in subjects fed diet without fermented milk (P < 0.04).

In summary, the results of animal and human studies indicate that yogurt consumption can stimulate certain in vitro indexes of immune response, such as cytokine production, macrophage activity, and lymphocyte mitogenic response. However, very few studies have investigated the effects of yogurt consumption on in vivo indexes of immune response. Furthermore, most of the studies lacked appropriate control groups and used short-term feeding protocols, which might induce a transient adjuvant effect rather than long-term stimulation of the immune response.

**YOGURT AND IMMUNE-RELATED DISEASE**

The health benefits of yogurt are due primarily to the ability of LAB to survive in the human GI tract. LAB commonly used for yogurt production were shown to survive in the stomach and were found in the feces (19, 20, 142), although survival rates are not known for all strains of LAB. Some strains of LAB show a higher fraction of sonicated cells of \textit{S. bulgarus} (37, 38). Consumption by healthy humans of fermented milk containing \textit{L. bulgaricus} and \textit{S. thermophilus} was reported to stimulate cytokine production of PBMCs. De Simone et al (32) reported that lymphocytes cultured with \textit{L. bulgaricus} and \textit{S. thermophilus} produced more IFN-\gamma when stimulated with ConA than did control cultures. \textit{L. bulgaricus} was more effective than was \textit{S. thermophilus} in enhancing IFN-\gamma production. Increased production of IFN-\gamma by isolated T lymphocytes in young adults (aged 20–40 y) consuming yogurt containing live \textit{L. bulgaricus} and \textit{S. thermophilus} (450 g/d for 4 mo) was reported (35). Long-term consumption of yogurt containing viable LAB was shown to increase IL-1, IL-6, IL-10, IFN-\gamma, and TNF-\alpha production (6, 34–36, 49).

Contrary to the results of in vitro studies on cytokine release, yogurt consumption does not appear to affect plasma concentrations of IFN-\gamma. Trapp et al (48) reported that consuming 200 g yogurt/d for 1 y had no effect on plasma IFN-\gamma concentrations. IFN-\gamma has a very short half-life in plasma and is secreted locally in low amounts (47). Thus, using plasma IFN-\gamma concentrations to detect the effect of yogurt on IFN-\gamma production may not be appropriate. Solis-Pereyra and Lemmonnier (33) suggested that 2’-5’ A synthetase (an IFN-\gamma-inducible protein) be assayed instead of assaying for IFN-\gamma itself and showed that subjects consuming yogurt had higher plasma concentrations of 2’-5’ A synthetase than did subjects consuming milk. These authors also reported a transient increase in plasma IFN-\gamma concentrations. Link-Amster et al (39) showed that the \textit{S. typhimurium}-specific anti-IgA titer was 4 times higher in subjects fed fermented milk containing \textit{L. acidophilus} than in subjects fed diet without fermented milk (P < 0.04).

In summary, the results of animal and human studies indicate that yogurt consumption can stimulate certain in vitro indexes of immune response, such as cytokine production, macrophage activity, and lymphocyte mitogenic response. However, very few studies have investigated the effects of yogurt consumption on in vivo indexes of immune response. Furthermore, most of the studies lacked appropriate control groups and used short-term feeding protocols, which might induce a transient adjuvant effect rather than long-term stimulation of the immune response.

Animal studies showed that LAB exerts anticarcinogenic effects (13, 148–152). Diet-induced microfloral alteration may retard the development of colon cancer (148). Some indigenous LAB, such as \textit{L. acidophilus} (153, 154), \textit{B. longum} (155), \textit{Lactobacillus} GG (156), and components of LAB (eg, insoluble fraction of sonicated cells of \textit{L. bulgaricus}) (117), were shown to exert tumor-suppressing effects.

Although the mechanisms by which LAB exert antitumor and anticarcinogenic effects are not fully understood, preliminary findings suggest that the potential mechanisms can be classified into 3 categories. One potential mechanism involves the changes in fecal enzymes thought to be involved in colon carcinogenesis. Nitrate was shown to be metabolized by nitrate reductase, an intestinal bacterial enzyme, to nitrite and may be metabolized further to nitrogen or ammonia. Nitrite may also be an important intermediary in the formation of N-nitroso compounds, which have been found to be highly carcinogenic in animals. Yogurt bacteria were shown to have nitrate reductase activity (157). Thus, these yogurt bacteria can reduce nitrite concentrations, thereby eliminating the substrate for the formation of carcinogenic compounds and nitrosamines (119, 153, 157).

A second possible mechanism involves LAB cellular uptake of mutagenic compounds, such as nitrite, in the gastrointestinal tract, thereby reducing the compounds’ potential conversion to carcinogenic compounds, nitrosamines (13). The third potential mechanism involves suppression of tumors by enhancement of immune response, as discussed previously (15, 154). Although animal studies showed that LAB may inhibit tumorigenesis, no evidence in this regard is available for humans. \textit{L. acidophilus}, however, was shown to reduce fecal enzyme activity of \(\beta\)-glucuronidase, nitroreductase, and azoreductase (119).

**Gastrointestinal disorders**

Yogurt’s microorganisms may prevent infections of the GI tract by influencing its microbial ecosystem. However, LAB that are colonized in the human intestine, \textit{L. acidophilus} and \textit{Bifidobacterium} species, are more resistant to gastric acid than are LAB conventionally used for yogurt fermentation (\textit{L. bulgaricus} and \textit{S. thermophilus}).

The inhibitory mechanisms of LAB against disease-causing bacteria are due primarily to 2 metabolites of lactic acid fermentation—organic acid (158, 159) and bacteriocin (160). It was also shown that prevention of and recovery from infection with pathogenic bacteria or viral infection in children with acute rotavirus-associated diarrhea can be enhanced through augmentation of the local immune defense, particularly by increasing the number of immunoglobulin-secreting cells (140, 141). In addition, oral microbial therapy with LAB can be effective in preventing antibiotic-induced GI disorders and in recovery from diarrhea. Colombel et al (161) reported that the simultaneous intake of \textit{B. longum}–containing yogurt with erythromycin reduced the frequency of GI disorders in human subjects who were taking erythromycin and a yogurt placebo. Thus, consumption of yogurt with LAB can reduce antibiotic-induced alterations of the intestinal microflora. Several animal studies also showed beneficial effects of yogurt consumption in building resistance to GI pathogens.

**Immunoglobulin E–mediated hypersensitivity**

LAB in yogurt are known to enhance concentrations of IFN-\gamma, which is produced mainly from \(T_h^1\) cells. IgE-mediated hypersensitivity (type 1 allergy) is triggered by antigens cross-linking...
with preformed IgE antibodies that are bound to antibody receptors (FceR1) on mast cell surfaces. The T\(_H2\) cytokine, IL-4, upregulates isotype switching of IgM to IgE but IFN-\(\gamma\) produced by T\(_H1\) cells inhibits isotope switching.

In human studies, it was shown that long-term consumption of large quantities of yogurt (450 g/d) can increase production of IFN-\(\gamma\) by lymphocytes (32), isolated T cells (35), and PBMCs (36). Shida et al (162) reported that _L. casei_ added in vitro to splenocytes from ovalbumin-primed BALB/c mice induced IFN-\(\gamma\) production but inhibited IL-4 and IL-5 secretion and markedly suppressed total and antigen-specific IgE secretion by ovalbumin-stimulated lymphocytes. Treatment of _L. casei_ with pepsin at low pH for 3 h had no effect on the ability of _L. casei_ to reduce IgE. This implies that oral consumption of _L. casei_ might also be effective in reducing IgE production. These results showed that yogurt might be effective in reducing IgE-mediated pathologies, such as asthma. Human studies, however, produced inconsistent results. Trapp et al (48) reported that consumption of yogurt (200 g/d) with live active cultures reduced allergic symptoms in young subjects but had no effect on IFN-\(\gamma\), total IgE, or specific IgE concentrations. Older subjects who consumed yogurt containing live bacteria, however, had lower IgE concentrations than did a control group. Wheeler et al (138) found no effect of yogurt consumption on asthma-related symptoms and pulmonary function in a group of patients with asthma.

**SUMMARY**

Many investigators have studied the therapeutic effects of yogurt and LAB commonly used in yogurt production on diseases such as cancer, infection, GI disorders, and asthma. Because the immune system is an important contributor to all of these diseases, the immunostimulatory effects of yogurt were studied by several investigators. Most of these studies used animal models; few human studies on the immunostimulatory effects of yogurt have been conducted.

Although the results of these studies mostly support the notion that yogurt has immunostimulatory effects, poor study design, lack of appropriate controls, and short duration of most of the studies limit the value of the conclusions that can be drawn from them. Most early animal and human studies included too few animals or subjects in each group and most did not include statistical analysis. Although more recent studies addressed these points, none provided the statistical basis for the selected number of subjects; that is, it seems that no power calculations were performed.

Most studies used short-term feeding protocols, which might induce a transient adjuvant effect rather than a long-term stimulation of the immune response. This was shown by several studies in which a maximum effect was seen with 2–5 d of yogurt consumption, after which the stimulatory effect of yogurt or yogurt bacteria diminished significantly. Furthermore, most studies investigated the effect of intravenous or intraperitoneal administration or in vitro application of yogurt bacteria on different variables of the immune response. Because yogurt is usually consumed orally and because bacterial and nonbacterial components of yogurt may be altered in the GI tract, the results of these studies may not reflect those that would be found if the yogurt had been consumed orally. Also, many studies lacked a placebo group or did not use a randomized, blinded design.

Most animal and human studies investigated the effects of yogurt on in vitro indexes of the immune response, whereas very few examined variables of the immune system in vivo. Because a quantitative correlation between in vitro tests of the immune system and resistance to diseases is not yet available, care should be taken in using the in vitro results as supporting evidence for health benefits of yogurt. Further, although most past studies focused on the peripheral immune response, the gut-associated immune system is increasingly being recognized as playing an important role in host defense. This aspect of the immune response is particularly relevant to determining the beneficial effects of yogurt because the systemic effects of yogurt may depend on the interaction of yogurt's bacterial components with the immune cells of the gut.

Despite the design problems of previous studies, these studies provide a strong rationale for the hypothesis that increased yogurt consumption, particularly in immunocompromised populations such as the elderly, may enhance immunity. This hypothesis, however, needs to be substantiated by well-designed randomized, double-blind, placebo-controlled human studies of adequate duration in which several in vitro and in vivo indexes of the immune response are tested. In particular, clinically relevant indexes such as response to vaccine and DTH should be included, as should a systematic evaluation of the gut-associated immune response. Future studies should use recent technical advances in fluorescent tagging of yogurt bacteria to enable an understanding of yogurt’s immunostimulatory effects. Information on the mechanisms by which yogurt protects is essential before the scientific community accepts claims regarding the health benefits of yogurt.

Although yogurt has long been believed to be beneficial for host-defense mechanisms, the components responsible for these effects or the way in which these components exert their immunologic modifications are not completely understood. The presence of LAB is thought to be essential for yogurt to exert immunostimulatory effects but components of nonbacterial yogurt, such as whey protein, short peptides, and CLA, are believed to contribute to yogurt’s beneficial effects as well. It is proposed that the LAB that survive through the GI tract, whether intact or modified, can bind to the luminal surface of M cells. LAB-bound M cells reaching to the dome region of PP cells stimulate local immune response, resulting in production of IFN-\(\gamma\) by \(\gamma\delta\) T cells. This may increase the M cell population with subsequent rapid amplification of bacterial translocation, which can further activate the local immune system, resulting in stimulation of the local and the systemic immune response. As mentioned previously, further studies are needed to substantiate this.

Finally, once the efficacy of yogurt in improving the immune response has been shown in humans, the benefits of these effects will need to be shown in large clinical trials in which the main outcomes are the incidence and severity of infectious disease. Infectious disease rather than other immune-related diseases are suggested because such studies can be conducted in a relatively short (eg, 1 y) time compared with studies of diseases such as cancer.

**REFERENCES**


