

Probiotics: effects on immunity¹⁻³

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ABSTRACT The gastrointestinal tract functions as a barrier against antigens from microorganisms and food. The generation of immunophysiologic regulation in the gut depends on the establishment of indigenous microflora. This has led to the introduction of novel therapeutic interventions based on the consumption of cultures of beneficial live microorganisms that act as probiotics. Among the possible mechanisms of probiotic therapy is promotion of a nonimmunologic gut defense barrier, which includes the normalization of increased intestinal permeability and altered gut microecology. Another possible mechanism of probiotic therapy is improvement of the intestine's immunologic barrier, particularly through intestinal immunoglobulin A responses and alleviation of intestinal inflammatory responses, which produce a gut-stabilizing effect. Many probiotic effects are mediated through immune regulation, particularly through balance control of proinflammatory and anti-inflammatory cytokines. These data show that probiotics can be used as innovative tools to alleviate intestinal inflammation, normalize gut mucosal dysfunction, and down-regulate hypersensitivity reactions. More recent data show that differences exist in the immunomodulatory effects of candidate probiotic bacteria. Moreover, distinct regulatory effects have been detected in healthy subjects and in patients with inflammatory diseases. These results suggest that specific immunomodulatory properties of probiotic bacteria should be characterized when developing clinical applications for extended target populations. *Am J Clin Nutr* 2001;73(suppl):444S–50S.

KEY WORDS Allergy, gastrointestinal tract, immune response, infants, inflammation, probiotics, antigen, lymphocytes, microflora

ROLE OF GUT MUCOSAL BARRIER IN HOST DEFENSE

The primary role of the gastrointestinal tract is to digest and absorb nutrients to meet the metabolic requirements and demands for normal human growth and development. In addition, the intestinal mucosa provides a protective host defense against the constant presence of antigens from food and microorganisms in the gut lumen. Protection against potentially harmful agents is ensured by many factors, including saliva, gastric acid, peristalsis, mucus, intestinal proteolysis, intestinal flora, and epithelial cell membranes with intercellular junctional complexes (1).

An abrupt change in gut barrier function occurs at birth when the gut switches from processing amniotic fluid to digesting milk. Food consumption initiates the release of trophic hormones

and the activation of secretion, motility, and absorption (2). During postnatal development, further maturational and adaptive events in the gut defense barrier include the appearance of mucosal proteins and digestive enzymes and the development of the intestinal flora. Gastric acidity is an important defense against microorganisms and secretion of hydrochloric acid by gastric mucosa develops during the first months of life (3). Goblet cell mucus that covers the epithelial surface of the gastrointestinal tract is an important physical barrier that interferes with intestinal attachment of luminal antigens. The establishment of normal bacterial populations can prevent overgrowth of potential pathogens in the gastrointestinal tract. Maturational changes also affect the epithelial cell membranes, a major mechanical interface between the internal environment of the host and the luminal contents. It was shown in experimental animals that postnatal maturation of small intestinal brush border membranes is associated with increased food-protein binding capacity (4). The capacity of antigens to bind to epithelial cells is related to the rate and route of antigen transfer and is shown to influence the intensity of mucosal immune responses (5).

GUT-ASSOCIATED LYMPHOID TISSUE

The surface of mucosal membranes is protected by a local adaptive immune system. The gut-associated lymphoid tissue represents the largest mass of lymphoid tissue in the human body. Consequently, it constitutes an important element of the total immunologic capacity of the host. The regulatory events of the intestinal immune response occur in different physiologic compartments: aggregated in follicles and Peyer's patches and distributed within the mucosa, the intestinal epithelium, and secretory sites (6). The intraepithelial T lymphocytes mainly exhibit a suppressor and cytotoxic phenotype, whereas the lamina propria cells exhibit a helper and inducer phenotype. The lamina propria is endowed with lymphocytes belonging to the B cell lineage. Immunoglobulin A (IgA) antibody production is abundant at mucosal surfaces. In contrast with IgA in serum, secretory IgA is present in dimeric or polymeric form. Secretory

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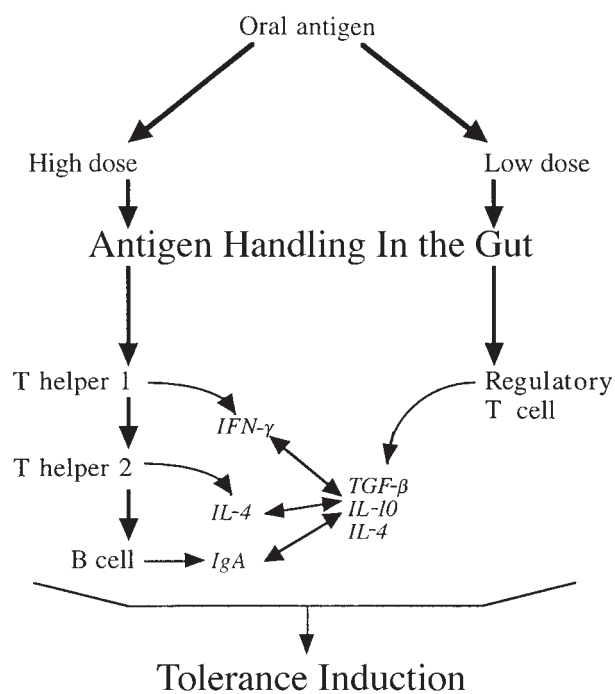


FIGURE 1. Mechanisms of oral tolerance in relation to the dose of antigen given. IFN, interferon; IG, immunoglobulin; IL, interleukin, TGF, transforming growth.

IgA is resistant to intraluminal proteolysis and does not activate complement or inflammatory responses, which makes secretory IgA ideal for protecting mucosal surfaces. There are differences between the upper and lower parts of the human gut-associated immune system in the isotype distribution of immunoglobulin-producing cells (7). IgA1 immunocytes predominate in the small intestine, whereas IgA2-producing cells are most frequent in the colon, the latter being more resistant to bacterial proteases.

The secretory IgA antibodies in the gut are part of the common mucosal immune system, which includes the respiratory tract, and lacrimal, salivary, and mammary glands. Consequently, an immune response initiated in the gut-associated lymphoid tissue can affect immune responses at other mucosal surfaces. The lymphocyte maturation cycle involves antigen transport across Peyer's patches and the presentation of antigens to T lymphocytes of a helper and inducer phenotype, which proliferate and induce B cell response. The specific antibody-secreting lymphocytes appear in peripheral blood 2–4 d after antigen exposure, reach a maximum concentration after 6–8 d, and persist in the blood for 2–3 wk. Studies show that these cells can reside in the gut. Homing receptors on lymphocytes, which interact with ligands on endothelial cells, target the migration of lymphocytes into tissues (8, 9). Antigen-specific systemic suppression after oral antigen introduction can be seen after 1–2 d and oral tolerance to systemic challenge becomes established within 5–7 d (10).

Data suggest that interactions of lymphocytes with the intestinal epithelium are perhaps more important than what was realized previously (11). Lymphocytes, particularly those of B cell lineage, can induce enterocytes into M cell like cells, a unique epithelium that comprises cuboidal epithelial cells, very few goblet cells, and specialized antigen sampling cells, which are typical to Peyer's patches. These cells effectively transfer parti-

cles and microbes from the gut lumen into underlying follicles. The induction of gut-seeking B cells, ie, by probiotics, may influence mucosal immunity beyond the secretion of IgA.

Intraepithelial lymphocytes, typically T cells with gamma, delta receptors, provide other unexplored mechanisms of mucosal immunity. These cells interact with the epithelial cells and protect the mucosa by killing infected cells and attracting other immune cells to combat infection. In mice, these cells act as exceptional T cells because they are generated neither in the thymus nor in the lymph nodes, but are instead generated locally in cryptopatches, which are cell clusters under the epithelial lining of the gut (12).

CONTROL OF ANTIGEN ABSORPTION IN THE GUT

The small intestine is challenged by a myriad of antigens encountered by way of the enteric route. Moreover, the small intestine is exposed to rapid and constant changes in the composition of the antigen load. Most antigens are excluded by a well-functioning mucosal barrier in the gut (1). In addition to the first line of gut defense, immune exclusion, specialized antigen transport mechanisms exist in the villous epithelium. Antigens are absorbed across the epithelial layer by transcytosis; here, the main degradative pathway entails lysosomal processing of the antigen. This second line of defense, immune elimination, is directed toward the removal of antigens that have penetrated the mucosa. A minor pathway allows for the transport of unprocessed antigens (13, 14). Peyer's patches, crucial in determining the subsequent immune responses to the presence of the antigen, are covered by the M cells. In general, antigen transport across this epithelium is characterized by rapid uptake and reduced degradation. Antigens are presented to subjacent T cells; these differentiate into various effector cells that mediate active immune suppression and promote the differentiation of IgA-secreting B cells (15). As a result of the absorption process across the intestinal mucosa, dietary antigens are altered into a tolerogenic form (Figure 1). Consequently, hyporesponsiveness to antigens, eg, food proteins, oral tolerance is a hallmark of the intestinal immune system.

ORAL TOLERANCE

It is recognized that the type of antigen, the route of antigen entry, and the dose of the antigen are crucial in determining the development of the T-cell response. The establishment of tolerance to orally administered antigen further depends on the age of the host and the timing of the encounter. Upon antigen exposure, immune cells respond with the release of a host of cytokines that then direct the subsequent immune responses. The demonstration that T helper 1- and T helper 2-like cells produce highly polarized patterns of cytokines has offered a conception of the distinct immune responsiveness to an antigen (16). Early events in the immunologic activation promote the generation of these cytokines. Interleukin 4 is obligatory for the development of T helper 2 phenotype, which may lead to enhanced IgE production, eosinophilia, and atopic disease. T-helper-1 cells are responsible for directing cell-mediated immune response to intracellular pathogens. In health, a critical balance is generated and maintained between protective mucosal immunity, including vigorous immune responses to pathogenic antigens, and systemic hyporesponsiveness specific to ubiquitous antigens, eg, food.

Oral tolerance is the immunologic hyporesponsiveness to antigens encountered through the enteric route (10, 15). Studies in experimental animals show that the dose and frequency of a fed antigen influence the course of tolerance acquisition (15). Feeding high doses of an antigen results in clonal deletion or anergy, whereas feeding low doses of an antigen results in active suppression subsequent to the induction of regulatory T cells in Peyer's patches (Figure 1). The regulatory T lymphocytes function through the production of suppressive cytokines, including interleukin 4, interleukin 10, and transforming growth factor β . Clonal deletion or anergy is preceded by the local production of interleukin 12, interferon γ (with consequent suppression of interleukin 4 and transforming growth factor β generation), and involves the apoptosis of T helper 1 cells. It is therefore suggested that one of the major mechanisms by which the gut-associated lymphoid tissue maintains homeostasis is via local cytokine regulation, particularly transforming growth factor β -associated low-dose tolerance.

Not all intraluminal antigens induce oral tolerance. Intraluminal bacterial antigens elicit specific responses in the gut-associated lymphoid tissue. This can be explained by the binding capacity of intraluminal bacterial antigens to epithelial cells, which allows antigen entry via enterocytes and escapes tolerance induction in Peyer's patches. Such tonic immune responses in the gut-associated lymphoid tissue may allow for control of the metabolic activity and balance of the gut microflora (15). Different adhesion capacities of antigens to epithelial cells have been reported and available probiotics have been classified according to this property (17). Strong adhesion of antigens to epithelial cells is associated with enhanced gut immune response. On the other hand, Duchmann et al (18) showed that healthy individuals are tolerant to their own microflora and that such tolerance is abrogated in patients with inflammatory bowel disease. Alteration of the properties of the indigenous microflora by probiotic therapy reversed some immunologic disturbances characteristic of inflammatory bowel disease (19). These data suggest that candidate probiotic bacteria play a paradoxical role in immune regulation: enhancement of gut-immune response and promotion of oral tolerance. Such paradoxical regulation of the immune response to enteral antigens seems to be a constant finding in the gut-associated lymphoid tissue, and oral tolerance is considered to be a concomitant effect of immune exclusion and suppression of systemic immune response, possibly attributed to the dual effect of the suppressor cytokine transforming growth factor β .

ORAL TOLERANCE OR ALLERGIC SENSITIZATION?

Many of the immunoregulatory aberrations favoring sensitization instead of tolerance induction prevail in early infancy. The intestine's antigen exclusion, elimination, and immune regulation mechanisms are incomplete during a variable period after birth, predisposing it to aberrant antigen uptake (1). The immature immunologic protection manifests itself in reduced capacity to generate IgA-producing cells. T-cell function is aberrant as well, and there are profound differences in immunoregulatory cytokine generation between the cells of infants and those of adults (20). In newborns, the cytokine profile is polarized away from cell-mediated immunity toward humoral immunity and the abundance of interleukin 4-generating cells during a critical period may divert the immunologic T-cell memory to T-helper-2 phenotype, which leads to enhanced IgE production and possibly to atopic sensitization (21).

The immature gut barrier may lead to aberrant antigen transfer and immune responses and thus explain the vulnerability of oral tolerance breakdown at an early age (22). It has been suggested that inadequate production of the antiinflammatory cytokine transforming growth factor β by neonatal lymphocytes predisposes a person to sensitization by low doses of enteric antigen (15). At an early age, such antigens are frequently derived from food and allergic reactions to foods are common (22).

In the context of inflammation, the altered rate, route, and mode of antigen presentation may lead to abrogation of oral tolerance. Intestinal permeability can be secondarily increased because of inflammation in the intestinal mucosa induced by viruses, bacteria, or dietary antigens (14, 23). A great amount of antigens may thus traverse the mucosal barrier, and the routes of transport may be altered. During the ensuing mucosal dysfunction caused by immaturity, infection, or hypersensitivity reaction, the normal pattern of antigen handling is impaired (1, 14), which may evoke aberrant immune responses and lead to sensitization (24). These data imply that allergic response to dietary antigens is caused by failure of the gut-associated lymphoid tissue to achieve or maintain oral tolerance to these antigens.

INTESTINAL FLORA: EFFECT ON THE GUT DEFENSE MECHANISMS

Microbial colonization begins after birth, but the development of the intestinal microflora and the gut barrier is a gradual process. The maternal intestinal flora is a source of bacteria colonizing the newborn's intestine. Colonization is also determined by contact with surroundings. Initially, facultative anaerobic strains dominate. Thereafter, differences exist in the composition of species, mainly because of the type of diet. Breast-feeding encourages the growth of bifidobacteria, whereas formula-fed infants have a more complex microflora made up of bifidobacteria, bacteroides, clostridia, and streptococci. After weaning, the composition of the microflora resembles that of the adult flora (7). Although bacteria are distributed throughout the intestine, the major concentration of microbes can be found in the large intestine.

The bacteria of the adult human gut include transient and indigenous types (25). The mouth harbors a complex microflora consisting of facultative and strict anaerobes, which includes streptococci, bacteroides, lactobacilli, and yeasts. The upper bowel (stomach, duodenum, and jejunum) has a sparse microflora with $\leq 1 \times 10^8$ colony-forming units/L contents. From the ileum and through the remainder of the digestive tract, bacterial concentrations gradually increase, reaching 1×10^{11} – 10^{12} colony-forming units/g in the colon. Up to 500 species of bacteria may be present in the adult human large intestine (7). Several reports indicated that 5 genera account for most of the viable forms of anaerobic bacteria in the large intestine: *Bacteroides*, *Eubacterium*, *Bifidobacterium*, *Peptostreptococcus*, and *Fusobacterium*. Various facultative and aerobic organisms are present in the colon. Cumulatively, it is estimated that bacteria account for 35–50% of the volume of the contents in the human colon.

The gut microflora is an important constituent in the intestine's defense barrier, as shown by increased antigen transport across the gut mucosa in the absence of an intestinal microflora. This notion is further supported by a demonstration that the gut microflora elicit specific immune responses at a local and a systemic level (14, 26, 27). Moreover, the gut flora is shown to induce and maintain oral tolerance in exper-



imental animal models (28). The intestinal flora allows for persistence of systemic hyporesponsiveness to an antigen and shortens the abrogation of hyporesponsiveness mediated by the *Escherichia coli* toxin.

In addition to participation in tolerance induction, intestinal colonization acts as an important antigenic stimulus for the maturation of the gut-associated lymphoid tissue (29, 30). The capacity to generate IgA-producing cells progressively increases in response to intestinal antigenic stimulation, particularly the establishment of the gut microflora (30, 31). Upon colonization, organisms have been shown to translocate to the mesenteric lymph node, but the number of translocating bacteria begins to decrease with the onset of specific IgA response, reflecting maturation of the intestine's immunologic defense mechanisms.

The role of the intestinal microflora in oral tolerance induction to the IgE response was investigated in germfree mice (32). In contrast with control mice, germfree animals maintained a T-helper-2-type immune response, eg, production of IgE antibodies, to orally administered ovalbumin. Abrogation of oral tolerance was due to the lack of intestinal flora. Aberrant IgE response by germfree mice could be corrected by the reconstitution of such flora at the neonatal stage, but not by any reconstitution exerted at a later age. These results suggest that in affecting the development of gut-associated lymphoid tissue at the neonatal stage, the intestinal bacterial flora plays crucial role in generating a T-helper-2 population that is susceptible to oral tolerance induction.

Our recent studies, in which we studied microflora development in vaginally born and cesarean delivery infants whose mothers received prophylactic antibiotics, indicate major differences in the culturable microflora (33). Differences were still observed at age 6 mo when a substantial proportion of children born by cesarean delivery were not colonized with *Bacteroides fragilis*. Colonization appeared to be associated with the maturation of humoral immune mechanisms. Interestingly, *B. fragilis* and, to a lesser extent, bifidobacteria, were important in this respect because infants harboring these organisms had more circulating IgA- and IgM-secreting cells. These results suggest that intestinal microflora is important in human individuals and that qualitative differences in the composition of the microflora might affect immunologic homeostasis. The effect of gut microflora on the maturation of the gut immune defense culminates in early infancy when the mode of immune responsiveness to antigens is consolidated (21, 22).

BACTERIOTHERAPY: PROBIOTICS

The demonstration that the gut microflora is an important constituent in the intestine's mucosal barrier has introduced the concept of probiotic therapy: therapeutic application of potentially beneficial microorganisms, which act as probiotics. A probiotic has been defined as a live microbial feed supplement that beneficially affects the host by improving its intestinal microbial balance (34). The definition is unsatisfactory for the purposes of human nutrition. Therefore, a European Commission concerted action program, coordinated by the International Life Sciences Institute, redefined probiotics as "A live microbial food ingredient that is beneficial to health" (7).

The criteria for a microorganism to be defined as probiotic include that the strain be of human origin, be safe for human use, be stable in acid and bile, and adhere to the intestinal mucosa

(35). The genera most frequently used as probiotics are *Lactobacillus* and *Bifidobacterium*.

PROBIOTICS: IMMUNOMODULATION OF LOCAL AND SYSTEMIC IMMUNE RESPONSE

Probiotic bacteria are shown to promote the endogenous host defense mechanisms. In addition to the effects of probiotics on nonimmunologic gut defense, which is characterized by stabilization of the gut microflora (7), probiotic bacteria have been shown to enhance humoral immune responses and thereby promote the intestine's immunologic barrier (14, 26). Moreover, probiotic bacteria have been shown to stimulate nonspecific host resistance to microbial pathogens (36, 37), and thereby aid in immune elimination, and to modulate the host's immune responses to potentially harmful antigens with a potential to down-regulate hypersensitivity reactions (27, 38).

Nonspecific immunomodulation

Oral introduction of lactobacilli can enhance nonspecific host resistance to microbial pathogens and thereby facilitate the exclusion of pathogens in the gut (36, 37). Several strains of live lactic acid bacteria have been shown to induce in vitro the release of the proinflammatory cytokines tumor necrosis factor α , and interleukin 6, reflecting stimulation of nonspecific immunity (39).

Oral introduction of *Lactobacillus casei* and *Lactobacillus bulgaricus* activates the production of macrophages (36) and administration of *L. casei* and *Lactobacillus acidophilus* activates phagocytosis in mice (37). Enhanced phagocytosis was also reported in humans by *L. acidophilus* Lal (40). Phagocytosis is responsible for early activation of the inflammatory response before antibody production. Phagocytes release toxic agents, eg, reactive oxygen intermediates and lytic enzymes, in various inflammatory reactions. Phagocytic activity results in the further recruitment of immunocompetent cells and the generation of inflammatory response. More recently, enhanced phagocytic activity was observed in atopic infants with food allergies compared with control infants, indicating that the capacity to generate and release functionally active products is increased in the phagocytes of patients with allergic inflammation (41). It is therefore interesting to note that probiotic bacteria were shown to modulate phagocytosis differently in healthy and allergic subjects: in healthy persons there was an immunostimulatory effect, whereas in allergic persons, down-regulation of the inflammatory response was detected (42).

In general, intestinal inflammation is accompanied with imbalance of the intestinal microflora (7). Rotavirus diarrhea is associated with an increased concentration of fecal urease (43), which is a proinflammatory mediator that predisposes gut mucosa to ammonia-induced destruction and thus to the overgrowth of urease-producing bacteria. A change of bacterial composition was also reported in patients with rheumatoid arthritis in comparison with healthy subjects, implying that the intestinal microflora constitutes an ecosystem that responds to inflammation beyond the gut. Indeed, fecal urease concentrations are shown to be elevated in patients with juvenile chronic arthritis (44). In such inflammatory states of infectious and noninfectious etiology, oral probiotic therapy proves to normalize fecal urease concentration (43, 44). Thus, probiotic therapy may help stabilize the gut microbial environment (7) and thereby prevent the generation of inflammatory mediators, which is a constant response of the gut-associated



lymphoid tissue to potentially harmful intraluminal antigens that have the potential to disrupt intestinal integrity (1, 6).

Proinflammatory cytokines, including interleukin 1, tumor necrosis factor α , and interferon γ , play a pivotal, yet paradoxical, role in inflammation. Experiments in cytokine transgenic knockout mice show that a harmless immune response to commensal gut microflora becomes a harmful inflammatory state in the absence of interleukin 10 and interleukin 2 (45). This indicates that inflammation is induced by an unbalanced local or systemic cytokine milieu. Oral bacteriotherapy with *Lactobacillus rhamnosus* GG (ATCC 53103) was shown to reduce elevated fecal concentrations of tumor necrosis factor α in patients with atopic dermatitis and cow milk allergy (46). Paradoxically, ingestion of lactobacilli in fermented milk products or as live attenuated bacteria was shown to potentiate the interferon γ production by peripheral blood mononuclear cells (47, 48). Interferon γ can promote the uptake of antigens in Peyer's patches (49), where specific IgA-committed cells are generated. An increase in systemic and mucosal IgA response to dietary antigens was shown after oral administration of lactobacilli (14, 26, 30). Therefore, ingestion of probiotic bacteria may stabilize the immunologic barrier of the gut mucosa by reducing the generation of local proinflammatory tumor necrosis factor α and by reinforcing the systemic production of interferon γ with physiologic protective effects in the gut. However, aberrant interferon γ production was shown to interfere with the induction of oral tolerance and to disrupt epithelial barrier integrity in the gut (15, 50, 51). Therefore, it is interesting to observe that specific strains of probiotic bacteria can normalize aberrant antigen-induced production of interferon γ in vitro (38). These data indicate that the immunomodulating effects of probiotic bacteria may depend on the immunologic state of the host. They further suggest that differences between specific strains of probiotic bacteria may exist.

Specific effects on immune response

Specific use of probiotics aims at modulation of the host's immune responses to potentially harmful antigens. Oral introduction of *Bifidobacterium bifidum* was shown to enhance antibody response to ovalbumin (52) and *Bifidobacterium breve* was shown to stimulate IgA response to cholera toxin in mice (53). In like manner, an increased humoral immune response, compared with that in control studies, including an increase in rotavirus-specific antibody-secreting cells in the IgA class, was detected in children with acute rotavirus diarrhea who received *L. rhamnosus* GG during the acute phase of diarrhea (26). The mean serum rotavirus IgA antibody concentration at the convalescent stage was also higher in those individuals receiving *L. rhamnosus* GG (54). In accordance with these observations, oral introduction of lactobacilli to suckling rats, who were sensitized with cow milk, increased the number of cells secreting antibodies to β -lactoglobulin (14). In human infants, cow milk allergy is associated with delayed-type hypersensitivity to cow milk proteins and a defective generation of local IgA responses, in addition to immediate-type IgE-mediated hypersensitivity (22). In atopic infants with challenge-proven cow milk allergy, a significant improvement in the clinical course of atopic dermatitis followed a probiotic-supplemented elimination diet (46).

The intestinal microflora contributes to the processing of food antigens in the gut. Certain bacterial species isolated from the gastrointestinal microflora can liberate low-molecular-weight pep-

tides, which trigger immune responses. Probiotic bacteria-derived proteases can degrade cow milk casein and thereby generate peptides with suppressive effects on the lymphocyte proliferation in healthy individuals (27). To further characterize the immunomodulatory effect of probiotics, a study was designed to investigate whether caseins degraded by probiotic bacteria-derived enzymes could modulate the cytokine production with anti-CD3 antibody-induced, peripheral blood mononuclear cells in atopic infants with cow milk allergy (38). Without hydrolyzation, casein increased the production of interleukin 4 in cultures from patients with atopic dermatitis, whereas *L. rhamnosus* GG-hydrolyzed casein reduced the production of interleukin 4. These results indicate that probiotics modify the structure of potentially harmful antigens and thereby alter the mode of their immunogenicity.

ANTIINFLAMMATORY PROPERTIES OF PROBIOTICS: COMPARISON OF SPECIFIC STRAINS OF PROBIOTIC BACTERIA

Whole bacterial cells are shown to enhance proliferation of immune cells (55) and induce production of proinflammatory cytokines, such as tumor necrosis factor α and interleukin 6 (39). In contrast, probiotic bacteria mediate suppression of lymphocyte proliferation and cytokine production by T cells (27, 38). Recently, we attempted to compare the antiproliferative effect of several probiotic bacterial strains in their nonviable forms (56; P Kankaanpää, Y Sütas, S Salminen, et al, unpublished observations, 2000). The probiotic strains were cultured separately and sonicated, the homogenates were filtered, and the enzymatic activity was found to be insignificant. The total protein concentration of the homogenates was estimated and three 10-fold dilutions were made accordingly. To determine the mitogen-induced proliferative responses of peripheral blood mononuclear cells to these homogenates, lymphocyte transformation tests were performed in healthy adults. A dose-dependent suppressive effect on mitogen-induced lymphocyte proliferation was observed in all experiments with probiotic homogenates. When the rate of proliferation was compared among cultures containing an identical protein concentration, a hierarchy of immunomodulation between probiotics was shown. The suppressive effect of 10 μ mol dexamethasone/L was comparable with that of successful probiotics, indicating that specific probiotic bacteria possess significant anti-inflammatory properties comparable to a therapeutic pharmaceutical agent. These findings further implicate the potential use of probiotic bacteria as immunomodulatory agents.

CONCLUSION: TARGETS OF PROBIOTIC THERAPY

Among the possible mechanisms of probiotic therapy is the promotion of the endogenous defense barrier of the gut. Promotion of nonimmunologic defense barrier in the gut includes normalization of increased intestinal permeability (23) and altered gut microecology (43). Another explanation for the gut-stabilizing effect could be improvement of the intestine's immunologic barrier, particularly intestinal IgA responses (26), and alleviation of intestinal inflammatory response (46). These data point to the conclusion that probiotics can be used as innovative tools for treating dysfunctions of the gut mucosal barrier, including acute gastroenteritis, food allergy, and inflammatory bowel disease (7) (Figure 2). Many of the probiotic effects are

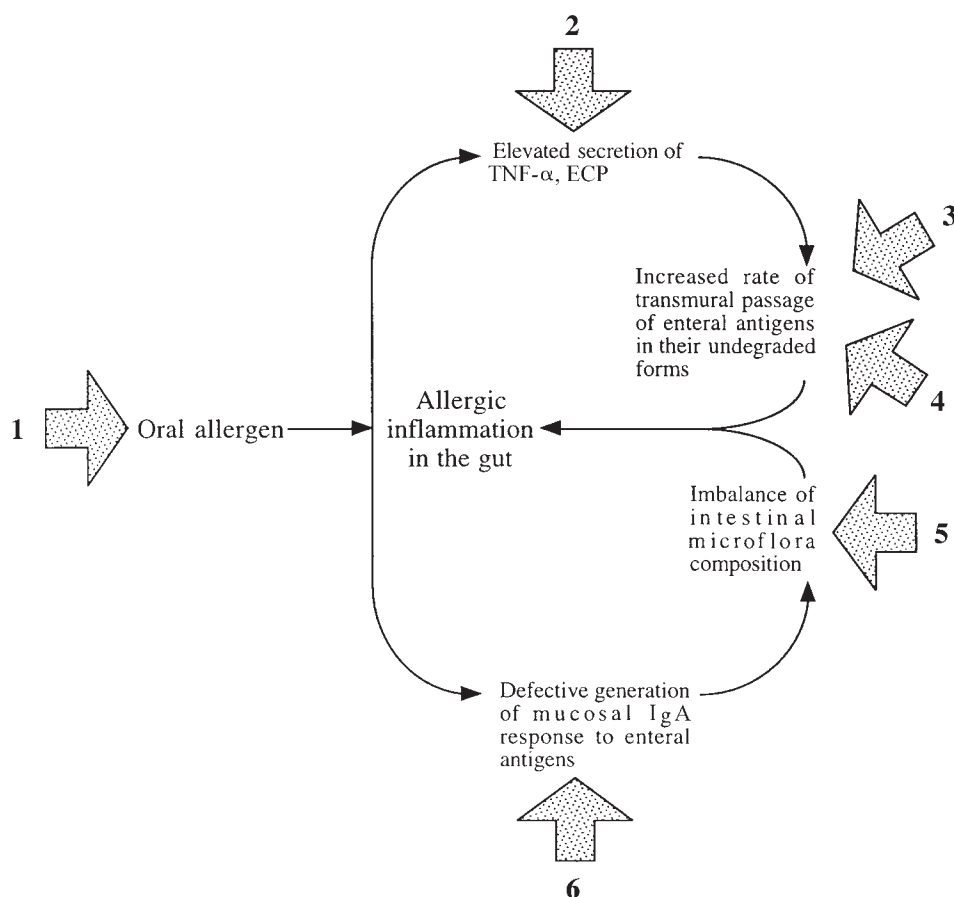



FIGURE 2. Suggested targets of probiotic therapy to modulate immune responses to dietary antigens during the course of allergic inflammation in the gut. Probiotic bacteria are shown to modulate allergic inflammation by 1) altering the immunogenicity of allergens via proteolytic activity, 2) reducing the secretion of inflammatory mediators in the gut, 3) reversing the increased intestinal permeability and enhancing the degradation of enteral antigens, 4) diverting the gut antigen uptake toward Peyer's patches, 5) normalizing the composition of intestinal microflora, and 6) enhancing mucosal immunoglobulin A (IgA) response to enteral antigens. TNF, tumor necrosis factor; ECP, eosinophil cationic protein.

mediated via immune regulation, in particular by control of the balance of proinflammatory and anti-inflammatory cytokines.

The results of the studies reviewed indicate that probiotic bacteria have several immunomodulatory effects: adjuvant-like properties and antiinflammatory properties. Moreover, both quantitative and qualitative differences in immune exclusion, immune elimination, and immune regulation exist among candidate probiotic bacteria. Distinct regulatory effects associated with probiotic consumption have been detected in healthy subjects and patients with inflammatory diseases. These observations reviewed together suggest that specific immunomodulatory properties of probiotic bacteria should be characterized during the development of clinical applications for extended target populations. 

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