Probiotics in human disease[1–3]

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ABSTRACT Western civilization is facing a progressive increase in immune-mediated, gut-related health problems, such as allergies and autoimmune and inflammatory diseases, and genetic factors are an unlikely explanation for these rapid increases in disease incidence. Two environmental factors that relate to the modern lifestyle in Western societies are hygiene and nutrition. There has been a decline in the incidence of microbial stimulation by infectious diseases as a result of improved hygiene, vaccination, and antimicrobial medication. In the past, methods of food preservation involved either the natural fermentation or drying of foods; thus, the human diet once contained several thousand times more bacteria than it does today. The development of probiotic, functional foods aims to "kill two birds with one stone," which is accomplished by providing a microbial stimulus to the host immune system by means of beneficial live microorganism cultures that are characteristic of the healthy, human gut microflora, ie, probiotics. Probiotic bacteria were shown to reinforce the different lines of gut defense, which are immune exclusion, immune elimination, and immune regulation. They were also shown to stimulate nonspecific host resistance to microbial pathogens, thereby aiding in pathogen eradication. Consequently, the best documented clinical application of probiotics is in the treatment of acute diarrhea. In humans, documented effects were reported for the alleviation of intestinal inflammation, normalization of gut mucosal dysfunction, and down-regulation of hypersensitivity reactions. These data show that probiotics promote endogenous host defense mechanisms. Thus, modification of gut microflora by probiotic therapy may offer a therapeutic potential in clinical conditions associated with gut-barrier dysfunction and inflammatory response. *Am J Clin Nutr* 2001;73(suppl):1142S–6S.

KEY WORDS Atopy, diarrhea, food allergy, gastrointestinal tract, infant, inflammation, probiotics

HEALTH BURDEN OF MODERN SOCIETY: FROM ALLERGIES TO INFLAMMATORY DISEASES

At the beginning of the third millenium, allergic diseases, atopic eczema, allergic rhinitis and asthma, together with chronic inflammatory bowel disease, Crohn disease, ulcerative colitis, diabetes, and arthritis, represent chronic diseases of rising importance in industrialized countries worldwide. Notwithstanding intensive research, the causes of these devastating inflammatory conditions remain unknown. In general, such outbreaks are thought to require genetic predisposition, immunologic disturbance, and the influence of intraluminal triggering agents, eg, allergens and antigens, bacteria, or viruses. Moreover, these diseases are associated with impairment of gut-barrier function (1, 2). These findings considered together would strongly suggest that the host defense mechanisms in the gut, primed to assimilate potentially harmful challenges, have decreased in Western societies during the past decades.

Given that significant immunologic and even inflammatory activation constantly prevails in the gut, deprivation of the stimuli priming for protective mechanisms directs the milieu in the gut toward a propensity to inflammatory disease (3). The earliest and most substantial driving forces for the development of the defense mechanisms in the gut are derived from dietary and microbial antigens. Specific strains of the healthy, normal gut microflora, ie, probiotics, promote gut-barrier functions, give maturational signals for the gut-associated lymphoid tissues, and balance the generation of pro- and antiinflammatory cytokines, thereby creating healthy interactions between the host and microbes in the gut that are needed to keep inflammatory responses regulated but concomitantly readily primed (4).

As a result, the diet and composition of the gut microflora may have an effect on the risk of inflammatory diseases (**Figure 1**). Conversely, these represent an exciting opportunity for the development of preventive and therapeutic dietary intervention strategies directed against the rising trend of inflammatory diseases in the modern world.

GUT-BARRIER FUNCTIONS: A TARGET OF PROBIOTIC THERAPY

The gastrointestinal tract provides a protective interface between the internal environment and the constant challenge from food-derived antigens and from microorganisms in the external environment (2). This first line of host defense is directed toward the exclusion of antigens, the elimination of foreign antigens that have penetrated the mucosa, and the regulation of ensuing antigen-specific immune responses (5). As a result, the gastrointestinal barrier controls antigen transport and the generation of immunologic phenomena in the gut. Even in physiologic conditions, a quantitatively insignificant but immunologically

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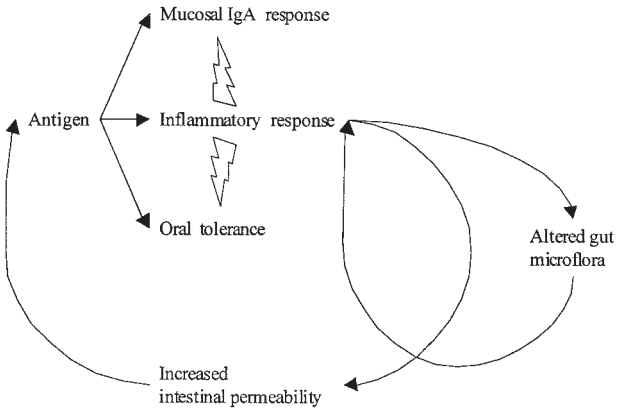

FIGURE 1. Gut microflora in inflammation. Inflammation is accompanied by an imbalance of the intestinal microflora, and a strong inflammatory response may be mounted to microfloral bacteria, leading to perpetuation of the inflammation and gut-barrier dysfunction. Ig, immunoglobulin.

important fraction of antigens bypass the defense barrier. Antigens are absorbed across the epithelial layer by transcytosis along the following 2 functional pathways: a degradative pathway that entails lysosomal processing of protein to smaller peptide fragments, thus reducing the immunogenicity of the protein and aiding host defense by diminishing the antigen load (>90% of internalized protein are absorbed this way), and a minor pathway that allows for the transport of intact proteins, which results in antigen-specific immune responses (6).

The regulatory events constituting the intestinal immune response take place in organized lymphoepithelial tissue and secretory sites. The organized lymphoid tissues are composed of Peyer's patches, which play an essential role in intestinal immune function, and lymphocytes and plasma cells that are distributed throughout the lamina propria. Intraepithelial lymphocytes are located above the basal lamina in the intestinal epithelium. These aggregations of lymphoid follicles are covered by a unique epithelium composed of cuboidal epithelial cells, very few goblet cells, and specialized antigen sampling cells, ie, M cells. Although blood-borne and tissue immunity has a predominance of immunoglobulin (Ig) G antibodies compared with IgA and IgM, IgA antibody production is abundant at mucosal surfaces and secretory IgA is present in dimeric or polymeric form (5). These secretory IgA antibodies in the gut form part of the common mucosal immune system, including the respiratory tract and lacrimal, salivary, and mammary glands. Consequently, an immune response initiated in the gut-associated lymphoid tissue can affect immune response at other mucosal surfaces.

Intestinal permeability is a reflection of the gut-barrier function (1). An immature gut barrier may lead to increased intestinal permeability and aberrant antigen transfer and immune responses, thus explaining vulnerability to infection, inflammation, and hypersensitivity at an early age. Intestinal permeability can be increased secondarily due to mucosal dysfunction that is induced by viruses, bacteria, or dietary antigens (7, 8). A great amount of antigens could thus traverse the mucosal barrier and the routes of transport could be altered.

Environmental factors, particularly those associated with intestinal inflammation, may flaw the normal immune regulation in the gut to the point of local and systemic inflammatory responsiveness (9). However, even in the absence of inflammatory stimuli from the environment, the healthy and mature intestine is in a proinflammatory state, provoking many differentiated and activated lymphocytes that generate proinflammatory cytokines, a state called *controlled inflammation* (10). The existence of active counterregulating processes primed to mount antiinflammatory responses may be mandatory for healthy interactions across the barrier.

NORMAL MICROFLORA AND GUT-BARRIER FUNCTIONS

Intestinal colonization is accompanied by an increase in the numbers of intestinal lymphocytes and maturation of mucosal immune function (11, 12). Intraluminal bacterial antigens elicit specific responses in gut-associated lymphoid tissue. It was shown in experimental animal models that the capacity to generate IgA-producing cells is initiated with the establishment of the gut microflora and with the onset of a specific IgA response to the number of translocating bacteria drops, reflecting maturation of the intestine's immunologic defense mechanisms (13). Moreover, there is a reduction in the number of lamina propria lymphocytes and the concentrations of serum immunoglobulin. It has been shown that the secondary lymphoid organs, ie, the spleen and lymph nodes, are poorly developed in germfree animals because of the lack of antigenic stimulation (11).

The role of the intestinal microflora in oral tolerance induction (ie, the unresponsiveness to nonpathogenic antigens encountered at the mucosal surface) to the IgE response was investigated in germfree mice (14). In contrast with control mice, germfree animals maintained their tendency to systemic immune response, eg, the production of IgE antibodies, after oral administration of ovalbumin. Abrogation of oral tolerance was due to a lack of intestinal flora. The aberrant IgE response in germfree mice could be corrected by reconstitution of the microflora at the neonatal stage but not later. These results suggest that the gut microflora direct the regulation of systemic and local immune responsiveness by affecting the development of gut-associated lymphoid tissue at an early age.

Parallel results were obtained in humans. Recent studies after microfloral development in vaginally born infants and in infants born by cesarean delivery showed major differences in culturable microflora (15). Colonization was associated with the maturation of humoral immune mechanisms, particularly of circulating IgA- and IgM-secreting cells (16).

The regulatory role of specific strains of the gut microflora was shown previously by a suppressive effect of immune responses to dietary antigens in allergic individuals (17), partly attributable to enhanced production of antiinflammatory cytokines, eg, interleukin 10 (18) and transforming growth factor β (19), whereas the capacity to stimulate nonspecific immune responses was retained (20, 21). Thus, as mucosal tolerance and immunization represent a continuum of immunologic competence in health (22), this pattern of immune response is not altered by the consumption of single and mixed cultures of probiotic microorganisms (23).

HEALTHY GUT MICROFLORA—THE SOURCE OF PROBIOTICS

Microbial colonization begins after birth, and initially, facultative anaerobic strains dominate. Thereafter, lactic acid bacteria

and coliforms become the predominant microorganisms of the gut microflora (11). After weaning, the type of diet determines the relative distribution of bacterial species. Breast-feeding encourages the growth of bifidobacteria in the gut, whereas formula-fed infants have a more complex microflora that contains bifidobacteria, bacteroides, clostridia, and streptococci. After weaning, the composition of the microflora resembles that of the adult flora (23). In the ileum, bacterial concentrations gradually increased to $\leq 10^{14}$ total bacterial cells of different culturable species. Several reports have indicated that 5 genera account for most of the viable forms of anaerobic bacteria: *Bacteroides*, *Eubacterium*, *Bifidobacterium*, *Peptostreptococcus,* and *Fusobacterium* (11, 23). Various facultative and aerobic organisms are also present in the colon. Most of these bacteria are hitherto uncharacterized because of the presence of nonculturable bacteria and the inaccuracy and insufficiency of the identification procedures available.

The complex ecosystem of the adult intestinal microflora is estimated to harbor ≈500 different bacterial species. Some of these species are considered potentially harmful because of their abilities of toxin production, mucosal invasion, or activation of carcinogens and inflammatory responses (23). The strains with health-promoting properties principally include bifidobacteria and lactobacilli. In infectious and inflammatory conditions the balance of the gut microecology is altered in such a way that the number of potentially pathogenic bacteria grows and the healthy interaction between the host and microbe is disturbed such that an immune response may be induced by resident bacteria.

Probiotics are beneficial bacteria that exist in the healthy gut microflora. The classification of a strain as *probiotic* requires that its beneficial physiologic effects be proven scientifically, that the strain be of human origin, be safe for human use, be stable in acid and bile, and that it adhere to the intestinal mucosa (23). The most frequently used genera fulfilling these criteria are *Lactobacillus* and *Bifidobacterium*.

PROBIOTIC FUNCTIONAL FOODS—AN OLD RECIPE FOR MODERN COOKING

The role of diet in health and well-being has changed as the science of nutrition has evolved. The principal role of the diet clearly lies in the provision of energy to meet the requirements of metabolism and growth. Currently, research is being directed toward improving our understanding of specific physiologic effects of the diet beyond its nutritional effect (24). The science of functional food evaluates the potential of the diet to promote health and well-being and to reduce the risk of diseases. A food can be defined as *functional* if it is shown to beneficially affect one or more target functions in the body beyond adequate nutritional effects in a way that is relevant to either the state of well-being and health, or to a reduction in disease incidence (23).

The Westernized diet includes few fresh nutritional components and among the nonnutritional components there are few microbes (25). It is characteristic of the diet in economically developed countries to include processed and sterile foods containing artificial sweeteners, preservatives, and in some extreme cases, even antibiotics. Such a diet may deprive the immune system of important tolerogenic signals from the environment. These include antiinflammatory processes promoted by specific microbes (17, 26, 27) and external antioxidants provided by fresh fruit and vegetables (28).

Inflammation is accompanied by an imbalance in the intestinal microflora (29–31; PV Kirjavainen, E Apostolou, T Arvola, SJ Salminen, GR Gibson, E Isolauri, unpublished observations, 2001), and a strong inflammatory response may be mounted to microfloral bacteria, leading to perpetuation of the inflammation (Figure 1). Oral introduction of probiotics may halt the vicious circle in normalizing the increased intestinal permeability and altered gut microecology, thus improving the intestine's immunologic barrier and alleviating the intestinal inflammatory response. The targets for probiotic therapy are thus identified as clinical conditions with impaired mucosal barrier function, particularly infectious and inflammatory diseases (4).

PROBIOTICS IN THE PREVENTION AND TREATMENT OF HUMAN DISEASE

Probiotic functional foods can improve specific physiologic functions in the human gastrointestinal tract, eg, the host immune defense, thereby reducing the risk of contracting illnesses. This conclusion is based on more recent in vitro and in vivo studies (4, 23).

Specific probiotic bacteria were shown to promote nonspecific host resistance to microbial pathogens (23). Several probiotic strains were shown to induce in vitro the release of proinflammatory cytokines, tumor necrosis factor α, and interlukin 6, which reflects the stimulation of nonspecific immunity (32). Enhanced phagocytosis was substantiated in humans by *Lactobacillus acidophilus* strain La1 (33) and *Lactobacillus rhamnosus* strain GG (20). These effects could be crucial in the exclusion and eradication of pathogens. The stimulation of the host's nonspecific and specific humoral immune responses to potentially harmful antigens has been documented for, among others, *Bifidobacterium bifidum*, *Bifidobacterium breve,* and *L. rhamnosus* GG (21, 34, 35). The specific IgA response could contribute to the preventive potential of probiotics. This was clinically documented in a reduction of diarrheal episodes in infants who were administered *Lactobacillus helveticus*– and *Streptococcus thermophilus*–fermented formula (36), *L. acidophilus*– and *Lactobacillus casei*–fermented milk (37), or a formula supplemented with *B. bifidum* and *S. thermophilus* (38).

The principal effect of probiotics is characterized by stabilization of the gut microflora (23). The clinical benefit of probiotics was shown when used to treat conditions in which the gut microecology is disturbed by changes in the environment (traveler's diarrhea) or by oral antimicrobial therapy (antibiotic-associated diarrhea). The value of probiotic preparations in prophylaxis for traveler's diarrhea has been assessed and more recent double-blind, placebo-controlled studies would indicate that some strains of lactic acid bacteria may protect against traveler's diarrhea (39). Similarly, evidence from recent well-controlled studies indicates that probiotics may be of value in the prevention of antibiotic-associated diarrhea (40). In balancing the gut microecology, the incidence of slower gastric emptying and partial hydrolysis of lactose during fermentation may be associated with the documented alleviation associated with symptoms of secondary lactose intolerance in adults (39).

The best-documented clinical application of probiotics is in the treatment of acute diarrhea and as adjunct therapy in gut-related inflammatory conditions (40). The beneficial, clinical effect of probiotics was explained by stabilization of the indigenous microflora (29), a reduction in the duration of rotavirus shedding (38), and a reduction in increased gut permeability caused by rotavirus infection (41) together with a significant

increase in IgA-secreting cells to rotavirus (21, 35). The multicenter study of the European Society of Pediatric Gastroenterology, Hepatology and Nutrition (42) extended this observation to preventing the evolution of rotavirus diarrhea toward a protracted course and thus confirmed the clinical benefit of probiotics in the treatment of rotavirus diarrhea in infants.

There is an increasing appreciation of the role of cytokines in regulating inflammatory responses at a local and systemic level. The ingestion of probiotic bacteria can potentially stabilize the immunologic barrier in the gut mucosa by reducing the generation of local proinflammatory cytokines (43, 44). Alteration of the properties of the indigenous microflora by probiotic therapy was shown to reverse some immunologic disturbances characteristic of Crohn disease (45), food allergy (44), and atopic eczema (19).

Recently, probiotics were shown to modulate the host's immune responses to foreign antigens with a potential to dampen hypersensitivity reactions (24). Unheated and heat-treated homogenates were prepared from probiotic strains, including *L. rhamnosus* strain GG, *Bifidobacterium lactis, L. acidophilus*, *Lactobacillus delbrückii* subsp. *bulgaricus,* and *S. thermophilus* (26). The phytohemagglutinin-induced proliferation of mononuclear cells was suppressed in these homogenates compared with controls with no homogenate, indicating that probiotic bacteria possess heat-stable, antiproliferative components, which could be therapeutically exploited in inflammatory conditions. Moreover, qualitative and quantitative differences between probiotic homogenates in these antiinflammatory properties were documented in vitro, even when adjusted for their protein concentrations or enzymatic activity (26, 27).

The intestinal microflora contribute to the processing of food antigens in the gut. To characterize the immunomodulatory effect of probiotics in allergic inflammation, a study was conducted to determine cytokine production by anti-CD3–induced peripheral blood mononuclear cells in atopic infants with cow milk allergy (46). Unhydrolyzed casein increased the production of interleukin 4, whereas *L. rhamnosus* strain GG-hydrolyzed casein reduced it. This indicates that probiotics modify the structure of potentially harmful antigens and reduce their immunogenicity. The clinical correlate of this effect is seen as a significant improvement in the clinical course of atopic dermatitis (eczema) in infants who were administered a probiotic-supplemented elimination diet, and in parallel, markers of intestinal (44) and systemic (19) allergic inflammation decreased significantly. Similar results were obtained in a study of milk-hypersensitive adults in whom a milk challenge in conjunction with a probiotic strain prevented the immunoinflammatory response characteristic of the challenge without probiotics (20). On the basis of these more recent studies of allergic inflammation, a novel target of probiotic therapy may be to control the excess formation of IgE and the development of T helper subset 2 cell–skewed immune responsiveness, both of which are key features of atopy. The T helper cells are divided on the basis of their cytokine profiles and IgE responses are under the control of cytokines that are produced by competing signals from the T helper cells. Patients with atopic disease manifest a high production of interleukin 4 (T helper subset 2 cells). Thus, the objective of the intervention is to redirect the immunologic memory away from the T helper subset 2 cell phenotype before such immune responsiveness to environmental antigens is consolidated.

CONCLUSION

Probiotic therapy is based on the concept of a healthy microflora. Probiotics can help stabilize the gut microbial environment and the intestine's permeability barrier and enhance systemic and mucosal IgA responses, thereby promoting the immunologic barrier of gut mucosa. The probiotic approach, ie, therapeutically consuming beneficial microorganism cultures of the healthy human microflora, holds great promise for the prevention and treatment of clinical conditions associated with impaired gut mucosal barrier functions and sustained inflammatory responses.

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