
Inventing probiotic functional foods for patients with allergic disease

Kirsi Laiho, PhD*; Arthur Ouwehand, PhD†; Seppo Salminen, PhD†; and Erika Isolauri, PhD*

Objective: The primary objective of this review is to discuss the rationale for and applicability of probiotics as part of the nutritional approach to the prevention and management of allergic disease.

Data Sources: This review represents a synthesis of recent literature published in the fields of probiotics, nutrition, and allergic disease.

Study Selection: The expert opinions of the authors were used to select the relevant data for the review.

Results: The hygiene hypothesis suggests that allergic disease may arise from a lack of counterbalancing microbial exposure at an early age. Thus, the initial compositional development of gut microbiota is considered a key determinant in the development of both the immune responder phenotype and the normal gut barrier functions. A wide range of data documents the specific actions of selected probiotics. The regulatory role of probiotics in allergic disease has been demonstrated as improving the clinical course or preventing the development of atopic eczema in infants given a probiotic-supplemented diet. In addition, certain types of fatty acids and antioxidants have been proposed for protective effects against the development of allergy and to diminish the inflammatory response in allergic disease.

Conclusions: The increase in the prevalence of allergic disease during the past decades is likely to be explained by changes in the environment, including reduced microbial exposure and altered food consumption. Scientifically composed functional foods containing probiotics and other functional components offer a nutritional strategy for both the prevention and the management of allergic disease. Further research is needed to characterize the gut microbiota and to clarify the mechanisms of action that control specific physiologic processes not only in the evolution of allergic disease in at-risk populations but also in the management of allergic diseases.

Ann Allergy Asthma Immunol 2002;89(Suppl):75–82.

INTRODUCTION

Approximately 20% of the population in western countries have some type of allergic disease, and no reversal of this increasing trend is expected. Atopic eczema, allergic rhinitis, and asthma constitute the most common chronic diseases of childhood. Despite the pronounced hereditary element in allergic disease, genetic factors are unlikely to explain the increased emergence of the atopic-type immune responsiveness to environmental antigens.

The rapid increase in allergic disease is likely to be related to the events in early life, when the immune responder phenotype is consolidated. As a result of improved hygiene, reduced family size, vaccination, and antimicrobial medication, the pressure of microbial stimulation through infectious diseases has declined. Indeed, the demonstration of an inverse association between infections early in life and atopy represents a substantial advance that has led to renewed scientific interest in the hygiene hypothesis concept introduced by Strachan¹ more than a decade ago.

An important source of microbial exposure is the establishment of the gut microbiota influencing the maturation of

the gut-associated lymphoid tissue.² Recently, investigators noted that differences in the neonatal gut microbiota precede the development of atopy.³ Indeed, the modification of the gut microbiota by means of probiotics induces beneficial effects that may reduce the risk of allergic disease.⁴

In addition to microbial exposure, nutrition of the infant may be an important determinant in the development of allergic disease. Evidence for programming by early nutrition has been suggested in animal studies as well as in epidemiologic studies linking markers of early nutrition to risk of chronic diseases (allergic disease, atherosclerosis, diabetes, hypertension, and obesity) in adulthood.^{5,6} Thus far, however, the dietary approach in attempts to prevent and treat allergy has focused on elimination diets as the first sensitizing antigens are derived from dietary proteins. A modern approach against allergic disease would be a nutritional management capable of preventing or depressing allergic inflammation. Such an approach could be based on the administration of probiotics. In addition, nutrients such as specific fatty acids and antioxidants could potentially be included in the dietary strategy for prevention and management of allergic disease.

Future research should clarify the mechanisms of probiotics and specific nutrients to control physiologic processes in the evolution of allergic disease in at-risk populations or in the management of allergic disease. Characterizing the mechanisms of actions of these dietary compounds will facilitate the development of functional foods with immunomodulatory properties.

* Department of Paediatrics, University of Turku, Turku, Finland.

† Department of Biochemistry and Food Chemistry, University of Turku, Turku, Finland.

Received for publication April 15, 2002.

Accepted for publication in revised form June 12, 2002.

ALLERGIC DISEASE

Allergic disease arises from an altered internal cytokine environment that directs immune T cells to differentiate into allergy-promoting T helper (TH)2 cells.⁷ TH2-skewed immunity to ubiquitous environmental antigens regulates the development of enhanced interleukin (IL)-4 and IL-5 synthesis, the continuity of which might lead to increased production of immunoglobulin (Ig)E, eosinophilia, and atopic disease.

During pregnancy, the internal milieu is polarized away from cell-mediated immunity (TH1-type) toward humoral immunity (TH2-type) to protect the developing fetus.⁸ Thus, the TH2 responder phenotype is universal at an early age, and a substantial overlap in the concentrations of IL-4 and IgE antibodies prevails at an early age between infants in whom atopy will subsequently develop and those in whom it will not.^{7,9} Rather than being a simple consequence of a TH2-polarized responder type, allergic disease could hence be ascribed to a lack of physiologic counterregulatory processes. Such signals are required for priming of the host-protective mechanisms and for redirecting of the polarized immunologic memory to a healthy balance. Transforming growth factor (TGF)- β -secreting immunomodulatory T cells (TH3 cells), are one good candidate for this objective because both TH1 and TH2 responses are downregulated by TGF- β .¹⁰

The host's major and primary microbial stimulation occurs along with the establishment of the gut microbiota.¹¹ Investigators have hypothesized that exposure to the commensal microbiota may be a key modulator of the immune system against atopy and atopic diseases. Thus, reduction of the risk of atopic diseases may be possible by modulation of the gut microbiota toward a normal balance.

PROBIOTICS—BASE FOR FUNCTIONAL FOODS

Healthy Gut Microbiota

The human intestine is a complex ecosystem, in which the body interacts with ingested food and the microbiota present. The normal intestinal microbiota have been estimated to consist of more than 400 different species of microbes.¹² The composition of gut microbiota changes along the gastrointestinal tract and with the health status and age of the host. The metabolic activity of the normal intestinal microbiota is equivalent to that of the liver, our body's metabolically most active organ. Therefore, the composition and activity of the intestinal microbiota clearly influence the health of the host.

The colon is the most heavily colonized part of the gastrointestinal tract, with 10^{10} to 10^{11} microorganisms present per gram of colonic contents and a considerable concentration on the mucosa. The major genera in the colon are *Bacillus*, *Bacteroides*, *Bifidobacterium*, *Clostridium*, *Eubacterium*, *Fusobacterium*, *Peptostreptococcus*, and *Ruminococcus*.¹³ A complex balance exists between the microbe populations and the host. Some genera or species of these genera may have a potentially detrimental effect on the host. Organisms with proteolytic activity, such as *Clostridium*, *Peptostreptococcus*, and *Bacteroides*, may produce ammonia, phenolic com-

pounds, and amines. In contrast, *Bifidobacterium*, *Eubacterium*, and *Lactobacillus* are mainly saccharolytic and produce short-chain fatty acids, which are an important energy source for the intestinal epithelium.¹⁴ Therefore, healthy gut microbiota would preferably consist of large numbers of saccharolytic and reduced numbers of proteolytic microbes.

Several studies have indicated that infants with allergic disorders have an aberrant composition of intestinal microbiota; the levels of clostridia are increased, and *Bifidobacterium* levels are reduced.^{3,15,16} In addition, the *Bifidobacterium* flora of infants with allergy consist mainly of *B. adolescentis*, which is characteristic of adults.¹⁷ Further, the properties of bifidobacteria isolated from the gastrointestinal tract of healthy infants differ from those isolated from infants with allergic disease.¹⁸ These data support the hypothesis that modification of the intestinal microbiota of infants with allergic conditions may aid in the management and prevention of allergy.

Origin and Desirable Properties of Probiotics

Two approaches can be used to increase the number of health-promoting microbes within the intestinal microbiota. Substrates that specifically stimulate the growth and activity of these microbes already present in the intestine, in particular, bifidobacteria, can be consumed (prebiotics), or selected strains of beneficial microbes can be consumed (probiotics). Currently, the health benefits of specific probiotics are better known than those of prebiotics.¹⁹

Because probiotics need to exert their effects in the intestine, most probiotic strains have been isolated from human intestinal contents. Some strains have also been isolated from raw milk, dairy products, or other sources. Because probiotics are generally required to be viable at the time of consumption and to remain viable and active in the intestine, specific properties are considered highly desirable for the strains used (Table 1). In addition to the desirable properties listed in Table 1, other characteristics of probiotics can be useful. These features usually relate to the mechanisms of action of the specific health effects ascribed to a probiotic.²⁰ Also of considerable importance, the characteristic properties of each probiotic should be safeguarded at the time of isolation. To guarantee scientifically proven health effects, rigorous quality control criteria must be established for evaluating the strain characteristics and keeping them unchanged or original during industrial processing.²¹

Mechanisms of Action of Probiotics

Many different mechanisms have been proposed for the action of probiotics. Modification of the composition and activity of the intestinal microbiota has always been one of the main aims of the use of probiotics. The beneficial effects of probiotics are likely to be related to their reinforcing the ability of microbiota to compete with and resist colonization by potential pathogenic microbes. We have demonstrated that Gram-positive species have been less frequent within predominant cultivable aerobic microbiota in the feces of infants

Table 1. Specific Desirable Properties for Probiotic Microorganisms

Property	Benefit
Resistance to pancreatic enzymes, acid, and bile	Survival of passage through the intestinal tract
Adhesion to the intestinal mucosa	Immune modulation
	Exclusion of pathogens
	Enhanced healing of damaged mucosa
	Prolonged transient colonization (?)
Human origin	Species-specific interactions with the host
Documented health effects	Proposed health effects are "true"
Safe	No health risk to consumer or environment
	No transfer of antibiotic resistance
Good technologic characteristics	Strain stability
	Neutral or positive contribution to flavor of product (when included in a fermented product)
	Viability in product; good shelf life
	Production in large scale
	Oxygen tolerance

with atopy than in healthy infants.¹⁶ Gram-positive bacteria have been reported as potent inducers of TH1 cytokines IL-12 and interferon- γ .²² These results warrant further studies on the role of Gram-positive species, among other probiotics, in reducing the risk of allergic disease in infants. Improved colonization resistance can be obtained through competition for mucus, mucosal binding sites and nutrients, and possibly through the production of antimicrobial substances.²³ At the same time, this enhanced colonization resistance of the normal microbiota will limit the permanent establishment of the probiotic in the intestine. Probiotics will usually disappear from the intestine 1 or 2 weeks after discontinuation of consumption.²⁴ Changes in the composition and activity of the intestinal microbiota may also modulate enzymatic activity in the lumen. For example, a reduction in urease activity will also decrease the exposure of the intestinal mucosa to ammonia.²⁵

Effects on the composition and activity of the intestinal microbiota are not the only known mechanisms of actions. Probiotics may also improve the processing of dietary antigens in the intestine. Enzymes derived from *Lactobacillus rhamnosus* strain GG have been observed to contribute to the degradation of antigens, a process that renders them less allergenic and downregulates the inflammatory response.²⁶ *L. rhamnosus* strain GG has also been noted to reverse the disturbed uptake of antigens during mucosal inflammation.²⁷

Specific probiotic strains have been found to improve intestinal mucosal barrier function. The production of short-chain fatty acids from the metabolism of the consumed probiotics may satisfy the energy requirements of the intestinal epithelium and thereby improve the functioning of the epithelial cells. Some lactobacilli and bifidobacteria have been shown to be able to enhance the production of IgA.^{28,29} This activity strengthens the gut mucosal barrier because IgA will complex with antigens and hence inhibit their penetration through the epithelium. In contrast to IgG, an IgA-antigen complex does not elicit an inflammatory response, and be-

cause IgA is relatively resistant to proteases, it is well adapted to its function in the intestine.³⁰ IgA-antigen complexes are also more readily taken up by Peyer patches, where they stimulate the production of IgA (and IgM) and thus further contribute to the immune exclusion of antigens. An increased uptake of antigens through Peyer patches may also lead to a reduced secretion of IgE, eosinophil activation, and, consequently, alleviation of the atopic inflammation. Indeed, *Lactobacillus casei* strain Shirota has been observed to inhibit the production of IgE in mice.³¹ However, Kalliomaki et al⁴ failed to note a reduction in IgE in infants at risk for allergy who were fed probiotics, although less atopic eczema developed than in the control subjects. This finding confirms the previous reports demonstrating that exposure to antigens (or sensitization) does not necessarily lead to the development of atopic disease.³² Therefore, different regulatory mechanisms are likely to determine whether sensitization will lead to atopy.

Different parts of the probiotic, generally Gram-positive, cell have been observed to be able to modulate the immune system. Cell wall material—in particular, peptidoglycan³³ and teichoic acids³⁴—but also cytoplasmic contents,^{35,36} have been suggested to elicit immune reactions. This finding could relate to the presence of nucleic acids in the bacteria. The feeding of relatively large amounts of probiotic bacteria also implicates the feeding of large amounts of nucleic acids, inasmuch as bacteria can consist of up to 50% nucleic acids by dry weight. Infants fed nucleotide-fortified cow's milk-based infant formula have been shown to have higher specific antibody titers to *Haemophilus influenzae* and diphtheria toxoids than infants fed unfortified formula.³⁷ In another study,³⁸ however, no differences were seen between antibody titers in infants fed soy-based formula versus nucleotide-supplemented soy formula. These studies did not mention specific oligonucleotides, although unmethylated CpG dinucleotides present in the bacterial DNA have been observed to affect the immune system.^{39,40} Currently, whether

certain strains of probiotics contain more CpG motifs, and hence would be more immunomodulatory, than others is not known. This possibility certainly warrants more attention in the future.

The precise mechanisms behind the immunomodulating activity of probiotics are still basically unknown. Adhesion to the intestinal mucosa is thought to be of major importance.³⁴ Close contact of the probiotics with the intestinal mucosa allows for benign translocation, which may lead to an enhanced interaction of the probiotics and the intestinal immune system. This interaction will stimulate naïve T cells to differentiate to TH1 cells under the influence of interferon- γ , IL-2, and IL-12, whereas the development of TH2 cells is downregulated under the influence of antagonistic activity toward IL-4. The results of the shift in T cell differentiation from TH2 to TH1 are a reduced production of IgE and an increased secretion of IgA, which in turn lead to a reduced allergic response.⁴¹ Species of the intestinal microbiota and probiotics may also influence the differentiation toward TH3 cells. To date, however, few investigative studies have focused on this process.

PROBIOTICS—REGULAR OR ALTERNATIVE MEDICINE?

Fermented foods have long been consumed by humans; in particular, fermented dairy products have had a persistent reputation for promoting health. Many of these proposed health benefits were based on anecdotal reports. With the increasing interest in functional foods during the past decade, the interest in probiotics has also increased.

Many probiotic preparations are sold in health stores as dietary supplements for self-medication. Unfortunately, a substantial number of these preparations do not contain the stated microbes or at least do not contain them in the declared amounts.⁴² Further, the health claims made for some of these products have not been substantiated by scientific evidence. As a consequence, the use of probiotics has often been considered in the realm of alternative medical practices.

Nevertheless, several companies, particularly in the dairy industry, have cooperated with research institutes to perform thorough scientific research on specific probiotic strains. The amount of documentation from well performed clinical, animal, and *in vitro* studies is increasing and suggests that selected probiotic strains indeed have specific beneficial health effects.²⁰ We believe that clinical trials of the same standards as those required for pharmaceutical agents will expand and increase the acceptance of probiotics for the treatment and the prevention of selected diseases. In the future, evidence-based studies may support the use of specific probiotics and confirm their health effects.

DIET—DAMAGE OR PROTECTION IN ALLERGIC DISEASE?

The environmental determinants underlying the increasing incidence of allergic diseases include changes in the western

diet composition during the past decades. Because dietary antigens may cause allergic inflammation, the role of the diet in allergic disease has been focused primarily on proteins. During the neonatal period, when the intestinal barrier functions are immature, antigens obtained from breast milk, infant formula, or solid foods may lead to an inflammatory response.⁴³ Thus, the dietary approach in attempts to prevent and treat allergy has centered on elimination diets.

Results of various elimination procedures, with dietary restrictions alone or in combination with some other environmental control measures, have been inconclusive regarding the prevention of allergic diseases, as reviewed by Zeiger.⁴⁴ Especially in long-term prevention of allergic disease, elimination diets during pregnancy and breast-feeding have proven unsuccessful. The advantage afforded by elimination diets seems to be in the silencing of specific allergic inflammation induced by an offending food, not in the primary prevention of the allergic disease.⁴⁵ Elimination diets may place affected infants at a constant risk for inadequate nutrition.⁴⁶ Further, dietary antigens may actually protect from allergic disease because they may induce tolerance to antigens rather than prevent sensitization.⁴⁷ Recent studies on the immunomodulatory properties of fatty acids and antioxidant properties of certain nutrients, including ascorbic acid, α -tocopherol, β -carotene, selenium, and zinc, have shown that food not only is a source of dietary antigens causing sensitization but also may contain protective factors.

Increasing available evidence suggests that dietary lipids, especially long-chain polyunsaturated fatty acids, regulate immune function and therefore contribute to the development and severity of symptoms of allergic disease. Mediators synthesized from dietary polyunsaturated fatty acids engage in immune regulation and may thus influence atopic sensitization.

Polyunsaturated n-6 series fatty acids derived from dietary linoleic acid (18:2, n-6) result in the production of eicosanoids, which are thought to have proinflammatory properties, whereas n-3 series fatty acids derived from dietary α -linolenic acid (18:3, n-3) seem to have less potent biologic functions, even anti-inflammatory properties.⁴⁸ The most notable of these mediators is arachidonic acid (20:4, n-6)-derived eicosanoid prostaglandin E₂ (PGE₂). PGE₂ results in increased IgE synthesis attributable to the induction of B-cell differentiation in the presence of IL-4.⁴⁹ The effects of PGE₂ may also be exerted through IgE-binding receptors. PGE₂ reduces the surface expression of these receptors, and the result is increased synthesis of IgE.⁵⁰ Indeed, the most frequently reported abnormality in cell fatty acid composition of patients with atopic disorders has been an imbalance between n-6 and n-3 series fatty acids,⁵¹⁻⁵³ which predisposes the patients to the adverse effects of PGE₂.

Despite their apparent proinflammatory role, n-6 fatty acids may also contribute to an anti-inflammatory intestinal environment because antigen stimulation upregulates PGE₂ production, which eventuates in suppression of antigen-specific T cell proliferation in gut-associated lymphoid tissue.⁵⁴

One could postulate that enhanced synthesis of prostaglandins contributes to an anti-inflammatory intestinal milieu through suppression of TH1-type cytokines, stimulation of anti-inflammatory IL-10 and TGF- β , and maintenance of the IgA environment.

In allergic disease, inflammatory processes result in increased endogenously generated oxidative stress and impaired homeostasis of oxygen radicals, which may be manifested by specific urinary markers.⁵⁵ Both cellular enzyme-based antioxidants and diet-derived antioxidants may counteract the oxidative stress and may diminish the inflammatory response in allergic disease.⁵⁶ Accordingly, antioxidants may be important in implementing the ability of the individual patient to restrain the inflammatory response and in avoiding injury to tissues. Low concentrations of plasma antioxidants (β -carotene, ascorbate, and α -tocopherol) have been measured in patients with wheezing illness,⁵⁷ and the low intake of antioxidants has been associated with bronchial reactivity and the risk of asthma.⁵⁸ In addition, higher dietary intake of vitamin E has been shown to be associated with lower serum IgE concentration.⁵⁹ These findings suggest that antioxidant deficiencies may be associated with symptoms of allergic disease. Although the potential role of antioxidants in the prevention of allergic disease is currently unknown, diet-derived antioxidants may be postulated to have protective effects.

Analyzing together the possible roles of fatty acids and antioxidants in the development of allergic disease, we recently demonstrated that the intake of saturated fatty acids in breast-feeding mothers was associated with atopic sensitization in the infant.⁶⁰ A cross-sectional study of adolescents in Taiwan⁶¹ recently found that the intake of saturated fatty acids was associated with an increased risk of asthma, as was vitamin C intake in the lowest quartile. The challenge in terms of prevention and management of allergic disease is to identify and clarify the mechanisms of action of the dietary factors that may be protective. Well controlled intervention studies must address the potential effects of different dietary modifications and supplementation to prevent and treat allergic disease. In the future, probiotics and specific nutrients may be incorporated into the same products to provide optimal functional foods for patients with allergic disease and those at risk for such disorders (Fig 1).

FUNCTIONAL FOODS FOR PREVENTION AND MANAGEMENT OF ALLERGIC DISEASE

Research in functional foods began in the early 1980s in Japan, and subsequently the concept spread to Europe and the United States. A food can be regarded as functional if it satisfactorily demonstrates beneficial effects on one or more target functions in the body, beyond adequate nutritional influences, in a way that is relevant to either the state of well-being and health or the reduction of the risk of a disease.⁶² In accordance with this definition and the common practice of avoidance of allergens, some hypoallergenic foods have been developed. In these foods, most allergens have been removed chemically or enzymatically⁶³ or by formulat-

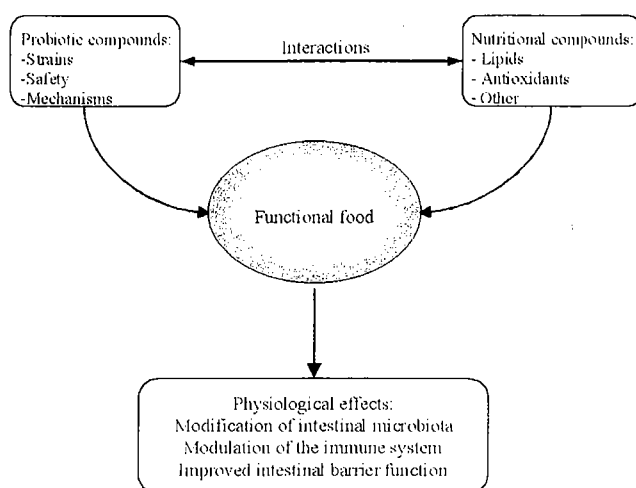


Figure 1. Diagram showing that development of an optimal functional food requires probiotic and nutritional compounds, which target specific physiologic functions in an effort to enhance health and reduce the risk of allergic disease.

ing the food product from different ingredients. A new approach has been used in the development of hypoallergenic rice. Rice was genetically modified to suppress the expression of a gene encoding for the 16-kDa allergen.⁶⁴

Instead of removing allergens from food and the environment, an alternative approach is to modulate the immune system and to provide it with sufficient stimulus, inasmuch as lack of microbial stimulation of the immune system during infancy has been suggested as a cause of allergic disease.¹ Indeed, supplementation of food with selected lactobacilli⁶⁵ and bifidobacteria⁶⁶ has been shown to aid in the management of atopic disease and to reduce the incidence of atopic disease in at-risk infants; thus, a prophylactic effect is provided.

Prebiotics might also have a similar immunomodulating effect by increasing the number of endogenous lactobacilli or bifidobacteria. This option, however, has as received little attention thus far¹⁹ as well as synbiotics, the combination of probiotics and prebiotics. The hypothesis is that the performance of probiotics may be improved by the addition of prebiotics because certain prebiotics have been shown to prolong the survival of probiotics.⁶⁷ This result has not been demonstrated for all synbiotics.⁶⁸

In general, the interactions between probiotics and other functional food ingredients are unknown. Antioxidants such as ascorbic acid were found not to affect the survival and performance of probiotics.⁶⁹ Interestingly, some strains of *Lactococcus*, *Lactobacillus*, and *Bifidobacterium* have been found to have antioxidant properties.^{70,71} Several strains of *Lactobacillus*, *Streptococcus*, *Lactococcus*, and *Propionibacterium* have also been observed to increase the level of conjugated linoleic acid,⁷² which has some proposed health effects. In contrast, a combination of probiotics and certain polyunsaturated fatty acids has been suggested to be detrimental to probiotics by altering their viability and adhesion proper-

ties.⁷³ These reports indicate that the properties of new functional food formulations must be carefully evaluated to ensure the efficacy of the ingredients in combination with other components as well.

FUTURE TRENDS

Probiotics and their health-related properties are modified by the food matrix. The matrix is important for survival in gastric conditions, but in early infancy, it may also provide the essence of normal gut colonization for the newborn. In particular, the fat composition and fatty acid content may affect the adherence properties of probiotics and facilitate changes in the early development of infant microbiota. This influence may already be transmitted by the fat components and carbohydrate composition present in breast milk. Clearly, more studies on the nutritional components and their interaction with specific probiotics are needed before functional foods for reducing the risk of atopic diseases can be developed. On the basis of our studies, the health benefits of probiotics can clearly be exerted through the mother. In the light of these observations, the nutrition recommendations for the pregnant or breast-feeding mother may have to be reconsidered. Thus, probiotics and prebiotics, under certain circumstances or for specific populations at risk for intestinal diseases, may have to be given to both the pregnant mother and the newborn. This situation establishes requirements for extremely careful planning of the food matrix and careful evaluation of nutritional composition of probiotic products designed for at-risk populations or patients with allergic disease.

Our studies have shown that infants at risk for allergy or already having allergic disease have highly specialized intestinal microbiota, differing especially in content and composition of bifidobacterial microbiota. During the first years of life, an adult-type pattern of stable indigenous gut microbiota is established concomitantly with the development of gut-associated lymphoid tissue in the most important effector organ of the adaptive immune system. The successful maturation of the gut mucosal immune system requires constant microbial stimulus from the developing gastrointestinal microbiota.

The characteristics of specific bifidobacteria or the stimulation by lactic acid bacteria of the right bifidobacteria in the intestine during the early life of healthy infants should form the basis for isolating new and novel strains for future foods for either treating atopic diseases in infants or reducing the risk of atopic diseases. Further research is clearly needed on intestinal colonization at birth and the influence of the mother's diet on breast milk composition and the infant's intestinal colonization pattern in connection with the protection of breast-feeding. In addition to new selection criteria for probiotic strains for at-risk atopic populations and nutritionally designed food matrices, nutrition education for pregnant and breast-feeding mothers must be planned. With these interventions, excellent possibilities exist for reversal of the increasing trend of occurrence of allergic diseases. The current

clinical practices, however, should not be modified until prospective clinical studies have repeatedly proven the beneficial effects of novel functional foods as a part of the recommended diet. Nevertheless, the use of some scientifically evaluated functional foods may be implemented in conjunction with current clinical practices.

REFERENCES

1. Strachan DP. Hay fever, hygiene, and household size. *BMJ* 1989;299:1259–1260.
2. Gronlund MM, Arvilommi H, Kero P, et al. Importance of intestinal colonisation in the maturation of humoral immunity in early infancy: a prospective follow up study of healthy infants aged 0–6 months. *Arch Dis Child Fetal Neonatal Ed* 2000;83:F186–F192.
3. Kalliomaki M, Kirjavainen P, Eerola E, et al. Distinct patterns of neonatal gut microflora in infants in whom atopy was and was not developing. *J Allergy Clin Immunol* 2001;107:129–134.
4. Kalliomaki M, Salminen S, Arvilommi H, et al. Probiotics in primary prevention of atopic disease: a randomised placebo-controlled trial. *Lancet* 2001;357:1076–1079.
5. Godfrey KM, Barker DJ. Fetal nutrition and adult disease. *Am J Clin Nutr* 2000;71(Suppl):1344S–1352S.
6. Jones CA, Holloway JA, Warner JO. Does atopic disease start in foetal life? *Allergy* 2000;55:2–10.
7. Prescott SL, Macaubas C, Smallacombe T, et al. Development of allergen-specific T-cell memory in atopic and normal children. *Lancet* 1999;353:196–200.
8. Piccinni MP, Beloni L, Livi C, et al. Defective production of both leukemia inhibitory factor and type 2 T-helper cytokines by decidual T cells in unexplained recurrent abortions. *Nat Med* 1998;4:1020–1024.
9. Kulig M, Tacke U, Forster J, et al. Serum IgE levels during the first 6 years of life. *J Pediatr* 1999;134:453–458.
10. Spiekermann GM, Walker WA. Oral tolerance and its role in clinical disease. *J Pediatr Gastroenterol Nutr* 2001;32:237–255.
11. Berg RD. The indigenous gastrointestinal microflora. *Trends Microbiol* 1996;4:430–435.
12. Moore WE, Holdeman LV. Human fecal flora: the normal flora of 20 Japanese-Hawaiians. *Appl Microbiol* 1974;27:961–979.
13. Tannock G. *Normal Microflora: An Introduction to Microbes Inhabiting the Human Body*. London: Chapman, Hall, 1995: 14–21.
14. Cummings JH, Macfarlane GT, Englyst HN. Prebiotic digestion and fermentation. *Am J Clin Nutr* 2001;73(Suppl):415S–420S.
15. Bjorksten B, Naaber P, Sepp E, Mikelsaar M. The intestinal microflora in allergic Estonian and Swedish 2-year-old children. *Clin Exp Allergy* 1999;29:342–346.
16. Kirjavainen PV, Apostolou E, Arvola T, et al. Characterizing the composition of intestinal microflora as a prospective treatment target in infant allergic disease. *FEMS Immunol Med Microbiol* 2001;32:1–7.
17. Ouwehand AC, Isolauri E, He F, et al. Differences in *Bifidobacterium* flora composition in allergic and healthy infants [letter]. *J Allergy Clin Immunol* 2001;108:144–145.
18. He F, Ouwehand AC, Isolauri E, et al. Comparison of mucosal adhesion and species identification of bifidobacteria isolated from healthy and allergic infants. *FEMS Immunol Med Microbiol* 2001;30:43–47.
19. Roberfroid MB. Prebiotics and probiotics: are they functional

- foods? *Am J Clin Nutr* 2000;71(Suppl):1682S–1687S.
20. Ouwehand AC, Kirjavainen PV, Shortt C, Salminen S. Probiotics: mechanisms and established effects. *Int Dairy J* 1999;9:43–52.
21. Tuomola E, Crittenden R, Playne M, et al. Quality assurance criteria for probiotic bacteria. *Am J Clin Nutr* 2001;73(Suppl):393S–398S.
22. Hessle C, Andersson B, Wold AE. Gram-positive bacteria are potent inducers of monocytic interleukin-12 (IL-12) while gram-negative bacteria preferentially stimulate IL-10 production. *Infect Immun* 2000;68:3581–3586.
23. Adlerberth I, Cerquetti M, Poilane I, et al. Mechanisms of colonisation and colonisation resistance of the digestive tract. *Microb Ecol Health Dis* 2000;11:223–239.
24. Goldin BR, Gorbach SL, Saxelin M, et al. Survival of *Lactobacillus* species (strain GG) in human gastrointestinal tract. *Dig Dis Sci* 1992;37:121–128.
25. Brigidi P, Vitali B, Swennen E, et al. Effects of probiotic administration upon the composition and enzymatic activity of human fecal microbiota in patients with irritable bowel syndrome or functional diarrhea. *Res Microbiol* 2001;152:735–741.
26. Sutas Y, Hurme M, Isolauri E. Down-regulation of anti-CD3 antibody-induced IL-4 production by bovine caseins hydrolysed with *Lactobacillus* GG-derived enzymes. *Scand J Immunol* 1996;43:687–689.
27. Pessi T, Sutas Y, Martinen A, Isolauri E. Probiotics reinforce mucosal degradation of antigens in rats: implications for therapeutic use of probiotics. *J Nutr* 1998;128:2313–2318.
28. Kaila M, Isolauri E, Soppi E, et al. Enhancement of the circulating antibody secreting cell response in human diarrhea by a human *Lactobacillus* strain. *Pediatr Res* 1992;32:141–144.
29. Link-Amster H, Rochat F, Saudan KY, et al. Modulation of a specific humoral immune response and changes in intestinal flora mediated through fermented milk intake. *FEMS Immunol Med Microbiol* 1994;10:55–63.
30. Helgeland L, Brandtzaeg P. Development and function of intestinal B and T cells. *Microb Ecol Health Dis* 2000;11:110–127.
31. Matsuzaki T, Yamazaki R, Hashimoto S, Yokokura T. The effect of oral feeding of *Lactobacillus casei* strain Shirota on immunoglobulin E production in mice. *J Dairy Sci* 1998;81:48–53.
32. Lau S, Illi S, Sommerfeld C, et al. Early exposure to house-dust mite and cat allergens and development of childhood asthma: a cohort study. Multicentre Allergy Study Group. *Lancet* 2000;356:1392–1397.
33. Stewart-Tull DE. The immunological activities of bacterial peptidoglycans. *Ann Rev Microbiol* 1980;34:311–340.
34. Morata de Ambrosini V, Gonzalez S, de Ruiz Holgado A, Oliver G. Study of the morphology of cell walls of some strains of the lactic acid bacteria and related species. *J Food Prot* 1998;61:557–562.
35. Pessi T, Sutas Y, Saxelin M, et al. Antiproliferative effects of homogenates derived from five strains of candidate probiotic bacteria. *Appl Environ Microbiol* 1999;65:4725–4728.
36. Tejada-Simon MV, Pestka JJ. Proinflammatory cytokine and nitric oxide induction in murine macrophages by cell wall and cytoplasmic extracts of lactic acid bacteria. *J Food Prot* 1999;62:1435–1444.
7. Pickering LK, Granoff DM, Erickson JR, et al. Modulation of the immune system by human milk and infant formula containing nucleotides. *Pediatrics* 1998;101:242–249.
38. Ostrom KM, Cordle CT, Schaller JP, et al. Immune status of infants fed soy-based formulas with or without added nucleotides for 1 year: part 1: vaccine responses, and morbidity. *J Pediatr Gastroenterol Nutr* 2002;34:137–144.
39. Klinman DM, Yi AK, Beaucage SL, et al. CpG motifs present in bacteria DNA rapidly induce lymphocytes to secrete interleukin 6, interleukin 12, and interferon γ . *Proc Natl Acad Sci USA* 1996;93:2879–2883.
40. McCluskie MJ, Weeratna RD, Payette PJ, Davis HL. The potential of CpG oligodeoxynucleotides as mucosal adjuvants. *Crit Rev Immunol* 2001;21:103–120.
41. Kirjavainen PV, Apostolou E, Salminen SJ, Isolauri E. New aspects of probiotics—a novel approach in the management of food allergy. *Allergy* 1999;54:909–915.
42. Hamilton-Miller JM, Shah S. Deficiencies in microbiological quality and labelling of probiotic supplements. *Int J Food Microbiol* 2002;72:175–176.
43. Isolauri E, Turjanmaa K. Combined skin prick and patch testing enhances identification of food allergy in infants with atopic dermatitis. *J Allergy Clin Immunol* 1995;97:9–15.
44. Zeiger RS. Dietary manipulations in infants and their mothers and the natural course of atopic disease. *Pediatr Allergy Immunol* 1994;5(Suppl):33–43.
45. Isolauri E, Tahvanainen A, Peltola T, Arvola T. Breast-feeding of allergic infants. *J Pediatr* 1999;134:27–32.
46. Arvola T, Holmberg-Marttila D. Benefits and risks of elimination diets. *Ann Med* 1999;31:293–298.
47. Kilburn SA, Pollard C, Bevin S, et al. Allergens in mother's milk: tolerization or sensitization? *Nutr Res* 1998;18:1351–1361.
48. Sellmayer A, Koletzko B. Long-chain polyunsaturated fatty acids and eicosanoids in infants—physiological and pathophysiological aspects and open questions. *Lipids* 1999;34:199–205.
49. Roper RL, Brown DM, Phipps RP. Prostaglandin E₂ promotes B lymphocyte Ig isotype switching to IgE. *J Immunol* 1995;154:162–170.
50. Roper RL, Phipps RP. Prostaglandin E₂ and cAMP inhibit B lymphocyte activation and simultaneously promote IgE and IgG₁ synthesis. *J Immunol* 1992;149:2984–2991.
51. Biagi PL, Hrelia S, Celadon M, et al. Erythrocyte membrane fatty acid composition in children with atopic dermatitis compared to age-matched controls. *Acta Paediatr* 1993;82:789–790.
52. Leichsenring M, Kochsiek U, Paul K. (n-6)-Fatty acids in plasma lipids of children with atopic bronchial asthma. *Pediatr Allergy Immunol* 1995;6:209–212.
53. Yu G, Duchén K, Björkstén B. Fatty acid composition in colostrum and mature milk from non-atopic and atopic mothers during the first 6 months of lactation. *Acta Paediatr* 1998;87:729–736.
54. Newberry RD, Stenson WF, Lorenz RG. Cyclooxygenase-2-dependent arachidonic acid metabolites are essential modulators of the intestinal immune response to dietary antigen. *Nat Med* 1999;5:900–906.
55. Omata N, Tsukahara H, Ito S, et al. Increased oxidative stress in childhood atopic dermatitis. *Life Sci* 2001;69:223–228.
56. Greene LS. Asthma, oxidant stress, and diet. *Nutrition* 1999;15:899–907.
57. Bodner C, Godden D, Brown K, et al. Antioxidant intake and adult-onset wheeze: a case-control study. Aberdeen WHEASE

-
- Study Group. *Eur Respir J* 1999;13:22–30.
58. Soutar A, Seaton A, Brown K. Bronchial reactivity and dietary antioxidants. *Thorax* 1997;52:166–170.
59. Fogarty A, Lewis S, Weiss S, Britton J. Dietary vitamin E, IgE concentration, and atopy. *Lancet* 2000;356:1573–1574.
60. Hoppu U, Kalliomaki M, Isolauri E. Maternal diet rich in saturated fat during breastfeeding is associated with atopic sensitization of the infant. *Eur J Clin Nutr* 2000;54:702–705.
61. Huang SL, Pan WH. Dietary fats and asthma in teenagers: analyses of the first Nutrition and Health Survey in Taiwan (NAHSIT). *Clin Exp Allergy* 2001;31:1875–1880.
62. Scientific concepts of functional foods in Europe. Consensus document. *Br J Nutr* 1999;81(Suppl):S1–S27.
63. Moriyama M, Tokue C, Ogiwara H, et al. Chemical and nutritional properties of hypoallergenic wheat flour. *Biosci Biotechnol Biochem* 2001;65:706–709.
64. Nakamura R, Matsuda T. Rice allergenic protein and molecular-genetic approach for hypoallergenic rice. *Biosci Biotechnol Biochem* 1996;60:1215–1221.
65. Majamaa H, Isolauri E. Probiotics: a novel approach in the management of food allergy. *J Allergy Clin Immunol* 1997;99:179–185.
66. Isolauri E, Arvola T, Sutas Y, et al. Probiotics in the management of atopic eczema. *Clin Exp Allergy* 2000;30:1604–1610.
67. Bezkorovainy A. Probiotics: determinants of survival and growth in the gut. *Am J Clin Nutr* 2001;73(Suppl):399S–405S.
68. Alander M, Matto J, Kneifel W, et al. Effect of galactooligosaccharide supplementation on human faecal microflora and on survival and persistence of *Bifidobacterium lactis* Bb-12 in the gastrointestinal tract. *Int Dairy J* 2001;11:817–825.
69. Dave RI, Shah NP. Effectiveness of ascorbic acid as an oxygen scavenger in improving viability of probiotic bacteria in yoghurts made with commercial cultures. *Int Dairy J* 1997;7:435–443.
70. Lin MY, Yen CL. Inhibition of lipid peroxidation by *Lactobacillus acidophilus* and *Bifidobacterium longum*. *J Agric Food Chem* 1999;47:3661–3664.
71. Stecchini ML, Del Torre M, Munari M. Determination of peroxy radical-scavenging of lactic acid bacteria. *Int J Food Microbiol* 2001;64:183–188.
72. Lin TY, Lin CW, Lee CH. Conjugated linoleic acid concentration as affected by lactic cultures and added linoleic acid. *Food Chem* 1999;67:1–5.
73. Kankaanpää PE, Salminen SJ, Isolauri E, Lee YK. The influence of polyunsaturated fatty acids on probiotic growth and adhesion. *FEMS Microbiol Lett* 2001;194:149–153.
- Requests for reprints should be addressed to:*
Erika Isolauri, PhD
Department of Paediatrics
University of Turku
20520 Turku, Finland
E-mail: erika.isolauri@utu.fi
-