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Probiotics: on-going research on atopic individuals

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The challenge for the modern health care system is to fight against the increasing prevalence of atopic disease. The introduction of scientifically composed probiotic functional foods for prophylactic or therapeutic purposes could be one solution. Probiotics are live microbial food supplements or components of bacteria which have beneficial effects on human health. Specific strains have been demonstrated to exert powerful anti-pathogenic, anti-inflammatory and anti-allergic effects. The hygiene hypothesis suggests that atopic disease may arise from a lack of counterbalancing microbial exposure at an early age. The initial compositional development of the gut microflora is considered a key determinant of the development of both the immune responder phenotype and normal gut barrier functions. The regulatory role of probiotics in human allergic disease was first emphasised in the demonstration of a suppressive effect on lymphocyte proliferation and interleukin-4 generation *in vitro*. Subsequently, a significant improvement in the clinical course of atopic eczema was reported in infants given a probiotic-supplemented diet. The potential of probiotics to reduce the risk of atopic disease has recently been demonstrated in a double-blind, placebo-controlled study: probiotics administered pre- and postnatally for 6 months to at-risk subjects reduced the prevalence of atopic eczema to half of that observed in infants receiving placebo. Ongoing research is directed towards the development of novel techniques to characterise the gut microflora. Future research will clarify the mechanisms to control specific physiological processes in the evolution of atopic disease in at-risk populations or in the management of allergic diseases.

Probiotics: Atopic disease: Functional foods

Atopic disease: a challenge for health care

The prevalence of atopic disease has been constantly increasing in Western societies. At present, about 20% of the population in Western countries suffer from atopic diseases, which are the most common chronic diseases of childhood. Genetic factors alone are unlikely to explain the emergence of the atopic eczema, allergic rhinitis and asthma.

Epidemiological studies have demonstrated an inverse association between atopy and sibling number (Strachan, 1989). On this basis, the hygiene hypothesis proposes that the rapid increase in atopy is related to reduced exposure to infections early in life, when the immune responder phenotype is consolidated. The hypothesis is supported by data showing that the immune response to microbial antigens is accompanied by preferential expression of T-helper (TH) 1-type cytokines possibly counterbalancing the TH2-polarised cytokine production of neonates (Romagnani *et al.* 1997). The continuity of TH2-type response might lead to enhanced immunoglobulin (Ig) E production, atopy, and atopic disease (Prescott *et al.* 1999).

The first condition to manifest itself is atopic eczema, and the first sensitising antigens are frequently derived from food (Isolauri & Turjanmaa, 1995). Most children become tolerant to food antigens by school age, when sensitisation to air-borne allergens takes over. Unlike food allergy, atopic respiratory diseases are not transient phenomena. The role of food allergy in the development of atopic disease remains obscure with many confounding factors. There are data to suggest that infants manifesting cow milk allergy in early infancy have a heightened risk of multiple food allergy (Isolauri & Turjanmaa, 1995) and asthma (Bergmann *et al.* 1998). Nevertheless, prevention of food allergy by elimination diets has not resulted in prevention of asthma (Zeiger & Heller, 1995), suggesting distinct immunoregulatory processes between these allergic diseases. Furthermore, it

Abbreviations: GALT, gut-associated lymphoid tissue; Ig, immunoglobulin; IL, interleukin; PG, prostaglandin; TH, T-helper.

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has been demonstrated that exposure to antigens does not necessarily lead to sensitisation or development of atopic disease (Platts-Mills *et al.* 2001).

The evolution of atopic immune responsiveness

The underlying factors in atopic sensitisation include genetic susceptibility, aberrant barrier functions of the skin epithelium and gut mucosa, and dysregulation of antigen-specific IgE production.

During pregnancy, the internal milieu is polarised away from cell-mediated immunity (TH1-type) towards humoral immunity (TH2-type) to protect the developing foetus (Piccinni *et al.* 1998). Consequently a significant overlap in the concentrations of interleukin (IL)-4, the key TH2 cytokine, and IgE antibodies prevails between atopics and non-atopics at an early age. Moreover, at birth, the gastrointestinal tract of the newborn is sterile and the maturation of gut-associated lymphoid tissue (GALT) is incomplete. During the first years of life an adult-type pattern of stable indigenous gut microflora is established concomitantly with the development of GALT, the most important organ of the adaptive immune system. The successful maturation of the gut mucosal immune system requires constant microbial stimulus from the developing gut microflora. Numerous experimental studies have shown that insufficient or aberrant stimulus result in defects in the immunological barrier of the intestine, particularly a defective mucosal IgA system (Moreau *et al.* 1978) and in abrogation of oral tolerance (Sudo *et al.* 1997).

The TH2 responder phenotype is associated with enhanced production of IgE antibodies against ubiquitous environmental antigens, eosinophilia, and consequently constitutes a hallmark of atopic diseases. The immune response for microbial antigens is accompanied by preferential expression of TH1-type cytokines and has been shown in *in vitro* studies to inversely relate to IgE response (Romagnani *et al.* 1997). However, categorisation of TH1 or TH2 immune responder phenotypes may not be justified.

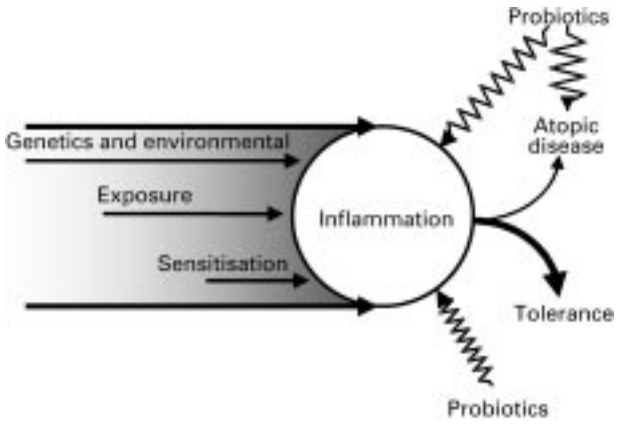

Fig. 1. Genetic factors together with environmental exposures and sensitisation lead to allergic inflammation which may favour the development of atopic disease. Probiotics may counteract early allergic inflammation as well as that in established atopic disease.

In support of such a concept, other T-cell subsets have been described, for example T-regulatory cells and TH3 cells, which have profound immunosuppressive properties, by producing mainly IL-10 and transforming growth factor β, respectively. These cytokines could counterbalance the TH2 response. Recent findings of Stene & Nafstad (2001) show a strong positive association between the occurrence of insulin dependent diabetes mellitus and symptoms of asthma at a population level. Similarly, our group recently demonstrated that the cumulative incidence of asthma in children with Crohn's disease or rheumatoid arthritis, both considered as TH1-type conditions, was significantly higher than in children without Crohn's disease or rheumatoid arthritis; $P<0{\cdot}001$ and $P=0{\cdot}016$, respectively (Kero *et al.* 2001). These results taken together demonstrate that the TH1 and TH2 diseases can coexist indicating a common environmental denominator behind these processes. The host's major and primary microbial stimulation occurs along with the establishment of the gut microflora (Berg, 1996). It has been hypothesised that exposure to commensal microflora may represent a key modulator of the immune system against atopy and atopic diseases. Thus, it may be possible to reduce the risk of atopic diseases by modulating the gut microflora towards a balanced normal microflora (Fig. 1).

Dietary factors and atopic disease

Several dietary factors may influence the inflammatory responses associated with atopic sensitisation. Equally, symptoms associated with an established atopic disease may be regulated by the diet. Food may be a source of dietary antigens causing sensitisation, but on the other hand, may also contain protective factors. Both macronutrients (protein, fat and carbohydrate) as well as micronutrients, especially antioxidants, may have a role in atopic disease.

Recently it has been suggested that the increased consumption of margarine and low intakes of fruits, vegetables and oily fish may be associated with the increased prevalence of atopic disease (Hodge *et al.* 1996; Black & Sharpe, 1997; Cook *et al.* 1997). These observations along with the experimental studies elucidating the immunomodulatory effects of individual nutrients have raised interest in the effects that diet may have upon the development and symptoms of atopic diseases. Furthermore, supplementation of the diet with non-nutrient compounds, probiotics, may induce specific health promoting effects.

Dietary antigens: sensitisation or tolerance?

Sensitisation to dietary antigens may occur prenatally, via breast milk or after weaning. Intra-uterine exposure to antigens has been suggested by demonstrating proliferative responses of cord blood lymphocytes to dietary antigens (Szepfalusi *et al.* 1997). However, the mechanisms and the causal association by which the intra-uterine exposure may result in atopic disease in later life are unclear (Jones *et al.* 2000). Food antigens, such as β-lactoglobulin, ovalbumin and gliadin, are also transferred to breast milk from the breast-feeding mother's diet. Concentrations of

antigens in breast milk, however, seem not to be directly associated with the incidence and symptoms of allergy in the infant (Cant *et al.* 1985). Antigens in breast milk may also be associated with tolerance induction rather than sensitisation (Kilburn *et al.* 1998). Induction of oral tolerance to dietary antigens may depend on several factors such as the dose and degradation of antigens (Barone *et al.* 2000). Degradation of antigens results in the formation of tolerogenic peptides that may promote the maturation of the neonatal immune system. However, early introduction of specific foods during weaning, especially those with structural similarity and cross-reactivity with inhalant allergens, may play a role in the development of atopic disease as it has been shown that early introduction of cereals may be a risk factor for grass pollen asthma (Armentia *et al.* 2001).

Despite the inconclusive association between antigen exposure and atopic disease, elimination of allergenic foods, e.g. cow's milk, egg, wheat and fish, from the pregnant or breast-feeding mother's or the infant's diet has previously been a common approach in attempts to prevent food allergy and atopic disease in high-risk infants. The benefits of elimination diets in prevention of atopic disease have been inconclusive (Zeiger, 1994). In a prospective randomised study of combined maternal and infant food allergen avoidance, a reduction in food allergy was observed before 2 years of age. However, at 7 years of age no difference in the prevalence of atopic disorders was observed between the prophylactic and control groups (Zeiger & Heller, 1995). The failure of elimination diets to prevent atopic disease may also be explained by the fact that besides allergenic proteins in the diet, other dietary factors, such as fatty acids might be associated with development of atopic disease.

Dietary fat: a link to atopic disease?

A possible link between dietary fat and the increased prevalence of atopic disease arises from the immunomodulatory properties of fatty acids. Mediators synthesised from dietary long-chain polyunsaturated fatty acids engage in immune regulation and thus may influence atopic sensitisation. The most notable of the mediators is arachidonic acid (20 : 4*n*-6)-derived eicosanoid prostaglandin (PG) E_2. PGE_2 results in elevated IgE synthesis due to the induction of B-cell differentiation in the presence of IL4 (Roper *et al.* 1995). The effects of PGE_2 may also be exerted via IgE-binding receptors (FcϵRII), as PGE_2 reduces the surface expression of the receptor, resulting in increased synthesis of IgE (Roper *et al.* 1992). Indeed, the most frequently reported abnormality in cell fatty acid composition of atopic patients has been an imbalance between series *n*-6 and *n*-3 fatty acids (Biagi *et al.* 1993; Leichsenring *et al.* 1995; Yu *et al.* 1998) predisposing patients to the adverse effects of PGE_2. Nevertheless, whether the observed alterations in the fatty acid composition of cells in patients with atopic disease result from a primary defect that contributes to the onset of atopic disease or is a consequence of atopic disease, is currently poorly understood.

The effects on dietary *n*-6 fatty acids may be counteracted by *n*-3 series fatty acids derived from dietary α-linolenic acid (18 : 3*n*-3) or directly from marine food sources (eicosapentaenoic acid; 20 : 5*n*-3 and docosahexaenoic acid; 22 : 6*n*-3). Eicosanoids derived from 18 : 3*n*-3 appear to have a less potent anti-inflammatory function compared to *n*-6 series fatty acids. For example 3-series thromboxanes derived from 20 : 5*n*-3 are less active in constricting blood vessels than 2-series thromboxanes derived from 20 : 4*n*-6 (Calder, 1997). The anti-inflammatory properties of *n*-3 fatty acids also arise from their capacity to inhibit the release of 20 : 4*n*-6 from membrane phospholipids, thereby reducing the production of eicosanoids derived from 20 : 4*n*-6 whilst the synthesis of *n*-3 fatty acid-derived eicosanoids increases (Whelan, 1996).

Considering the various immunomodulatory effects of different fatty acids, the repeatedly used method for the assessment of dietary fat intake by reporting the consumption of butter or margarine used as spread (Bolte *et al.* 2001; Haby *et al.* 2001) is inadequate for making conclusions on the associations between dietary intake of fatty acids and atopic disease. The quantitative assessment of intake of nutrients from the diet requires sophisticated methods, which aim to describe the usual intake of foods over a period of time (Willett & Buzzard, 1998). Besides, it is not possible to draw causal relations between diet and atopic disease from cross-sectional studies in atopic patients (Bolte *et al.* 2001; Haby *et al.* 2001). Early events in infancy may have regulated the disease progression rather than the situation at the time of the cross-sectional sampling. Similarly, as dietary habits are subjected to continuous change with the altering trends in food consumption and food supply, the outcomes of studies based on dietary intake data collected decades ago should be considered with reservation (Dunder *et al.* 2001). The estimation of dietary fatty acid intake by spread usage is especially misleading as the commercial margarines vary in the proportions of *n*-6 and *n*-3 fatty acids. This is especially intriguing as a recent study in mice shows that, despite their apparent proinflammatory role, *n*-6 fatty acids may also contribute to an anti-inflammatory intestinal environment as antigen stimulation up-regulates PGE_2 production from arachidonic acid with ensuing suppression of antigen-specific T-cell proliferation in GALT (Newberry *et al.* 1999). Due to potential interactions between nutrients, the relationship with respect to other nutrients of overall fatty acid composition and the quantity of fat within the diet may be crucial in the search for the optimal diet for prevention and management of atopic disease.

Dietary antioxidants to counteract inflammation

In atopic disease, inflammatory processes result in endogenously generated oxidative stress which dietary antioxidants, such as ascorbic acid, β-carotene, α-tocopherol, Se and Zn, may counteract (Greene, 1999). Low antioxidant intake may contribute to bronchial reactivity and the risk of asthma (Soutar *et al.* 1997). In addition, a higher dietary intake of vitamin E has been shown to be associated with lower serum IgE concentrations (Fogarty *et al.* 2000). However, the potential role of antioxidants in the prevention of atopic disease is currently not known.

Pre- and probiotic compounds in diet

Of the dietary carbohydrates, specific nondigestible oligosaccharides, particularly fructo-oligosaccharides, may prove to be beneficial in the management of atopic disease. Prebiotics are defined as nondigestible food ingredients that beneficially affect the host by selectively stimulating the growth and activity of one or a limited number of bacterial species in the colon. Many fructo-oligosaccharides have been shown to actively stimulate the growth of bifidobacteria, but the effects of intestinal flora modulation remain unclear (Gibson & Roberfroid, 1995).

Also, non-nutrient compounds in foods may have an important function in terms of atopic disease exerted via the modulation of gut microflora. The diet today contains several thousand times less bacteria than traditional food, as preservation methods such as natural fermentation have been replaced with modern methods including freezing, pasteurisation, ultra heat tested-treatment, vacuum storage and food additive use. Supplementation of the diet with probiotics may counteract this reduction in microbial exposure.

From nutrients to foods

Considering the possible role of fatty acids and antioxidants in the development of atopic disease, we recently demonstrated that in the diet of breast-feeding atopic mothers the intake of fat, especially saturated fatty acids, was relatively high and the intake of some antioxidants was relatively low compared to recommendations (Hoppu *et al.* 2000). These results, along with the failure of elimination diets to prevent atopic disease, suggest that atopic families should be encouraged to moderate their intake of fat and increase their intake of fruits and vegetables in the diet. No dietary advice on the supplementation of specific nutrients for atopic individuals can currently be given. More research is needed on the role of fatty acids and antioxidants in regulating inflammatory processes. Well-controlled intervention studies are also required to address potential effects of different dietary modifications and supplementation to prevent and treat atopic disease. In the future, probiotics and specific nutrients may be incorporated in the same products to provide optimal functional foods for at risk and atopic individuals. Unquestionably the interactions between probiotics and nutrients need to be studied. However, no single dietary supplement or functional food can resolve the challenge of atopic disease if the crucial role of the total composition of the diet is neglected.

Modification of gut microflora: benefits for the atopic individual?

The microflora composition of healthy infants is the basis for modifying the gut microflora for the benefit of atopic individuals. Allergic children are less often colonised with lactobacilli, but they have been reported to have higher numbers of coliform bacteria and *Staphylococcus aureus* (Björkstén *et al.* 1999). Bottcher *et al.* (2000) demonstrated that there are differences in the metabolic activity of gut microflora between allergic and non-allergic infants. *Clostridium difficile* and short-chain fatty acids, such as I-caproic acid, produced by clostridia are present at high levels in the microflora of allergic infants.

Kalliomäki *et al.* (2001*a*,*b*) were the first, using fluorescent *in situ* hybridisation technique, to demonstrate that distinct patterns of gut microflora exist in neonates who are likely to develop atopic diseases. The predisposing microflora include higher numbers of clostridia and lower numbers of *Bifidobacterium* species when compared to children remaining healthy. Later studies have shown that the differences were focused on the composition of bifidobacteria microflora. The *Bifidobacterium* species that prevail in healthy infants include *B. breve*, *B. infantis*, and *B. longum*. Allergic infants mostly harbour *B. adolescentis* resembling an adult microflora composition rather than that of healthy infants (Ouwehand *et al.* 2001). The properties of such a bifidobacterial microflora were demonstrated to be significantly different in terms of adhesion to the intestinal mucosa: bifidobacteria from healthy infants were more adhesive than the ones from allergic infants. The adhesive properties of bifidobacteria may relate to their enhanced colonisation ability within the intestinal mucosa (He *et al.* 2001). In the future, such a population of bifidobacteria may be a potential target for dietary interventions aimed at reducing the risk of atopic diseases. The fatty acid and carbohydrate composition of breast milk may influence the mucosal adhesion and intestinal colonisation properties of probiotic microbes. Thus the role of the infant diet on the development of the gut microflora has to be carefully considered (Fig. 2).

Probiotics and functional foods

The role of the diet has changed as the science of nutrition has evolved. Widespread interest in the possibility that certain food components may, by modulating specific target functions in the body, maintain or improve health as well

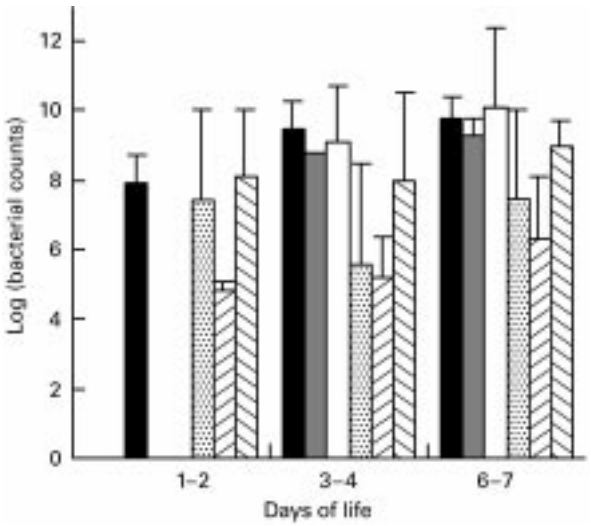

Fig. 2. Development of the faecal microflora during the first week of life. (■), *Bacteroides*; (■), *Eubacterium*; (□), *Bifidobacterium*; (▨), *Clostridium*; (▨), *Lactobacillus*; (▨), *Enterobacterium* species. (Modified after Benno & Mitsuoka 1986).

as reduce the risk of disease has given rise to the concept of functional foods. Functional foods have been defined by an ILSI Europe working group as foods that have specific proven health effects (Diplock *et al.* 1999). This requires functional foods to be scientifically studied and assessed for their efficacy and safety. Human studies are the main source of information to demonstrate efficacy.

Probiotics have been defined as live microbial food supplements that have been shown to benefit human health (Salminen *et al.* 1998). However, information is accumulating rapidly on the effects of non-viable probiotic cells, their cell wall and cytoplasmic components on human health. Thus, the definition may have to be revised in the future (Salminen *et al.* 1999). The rationale of the probiotic approach is based on the demonstration that the earliest and most substantial maturational signals for the gut barrier functions are derived from microbial antigens in the gut microflora. In particular the maturation of GALT evolves through bacterial colonisation (Moreau *et al.* 1978). Delayed maturation of humoral immune defence mechanisms, particularly of circulating IgA- and IgM-secreting cells, is a consequence of delayed compositional development of the gut microflora (Grönlund *et al.* 2000).

Until now, most probiotic products have been developed based on the function and importance of lactobacilli as the key organisms in the human gut. However, it has been documented that probiotic lactobacilli with proven efficacy also modify the gut *Bifidobacterium* microflora and the metabolic activity of clostridia (Benno *et al.* 1996; Kalliomäki *et al.* 2001*a*,*b*). The ratio of bifidobacteria to clostridia in the gut microflora has been suggested as important in reducing the risk of atopic diseases (Kalliomäki *et al.* 2001*a*). It is likely that particular *Bifidobacterium* species and strains may have an impact on the progress of the disease (He *et al.* 2001). Bifidogenic factors that promote the species and strains common in the healthy infant microflora should be selected as prebiotics for infants and small children. Thus, the adherence, immunomodulatory properties and substrate requirements of bifidobacteria deserve further clarification for the development of probiotic foods for the benefit of the atopic infant.

Probiotic functional foods have traditionally included fermented dairy products and more recently other fermented and non-fermented foods with added probiotic bacteria. In the future, targets should include more specific species and strains to be added to new types of food matrices to assist in dietary management and to reduce the risk of gastrointestinal tract related diseases. Probiotic functional food science will evaluate the potential of probiotics to alter several steps in atopic disease process and thereby aim to reduce the risk and symptoms of chronic atopic disease. Future probiotic foods should also fulfil nutritional requirements for subjects at risk for atopic diseases.

Mechanisms of probiotic action

The original mechanistic approach to probiotics established that many gastrointestinal related dysfunctions are based on disturbances or imbalances of the gut microflora. Thus, the main desired effect was to balance the gut microflora and thereby prevent or correct the microbial dysfunction. This still applies to probiotic studies and has been verified with many probiotic strains (Sanders, 2000). However, some probiotic effects, like immune modulation, may be achieved without changing the composition of the gut microflora measurably.

Allergic infants have been observed to have an aberrant gut microflora composition compared with healthy age-matched infants. *Bifidobacterium* levels are reduced and the gut microflora of allergic infants has an atypical composition, with mainly *B. adolescentis*, and increased levels of clostridia (Björkstén *et al.* 1999; He *et al.* 2001; Kalliomäki *et al.* 2001*a*). Normalising the microflora would therefore seem a feasible option. Although selected prebiotics have been shown to increase the levels of bifidobacteria (Roberfroid, 2001), they do not appear to be selective enough to stimulate the growth of specific *Bifidobacterium* species. Probiotics may therefore be a more promising option and selected probiotic bacteria have been used with success (Isolauri *et al.* 2000; Kalliomäki *et al.* 2001*b*).

By modulating the composition and/or activity of the gut microflora the exposure to dietary antigens can be changed. Enzymes derived from *Lactobacillus rhamnosus* GG have been observed to contribute to the degradation of antigens, rendering them non-allergenic (Sütas *et al.* 1996). In addition, certain fermentation products from the normal gut microflora and probiotics, such as butyrate, are an important energy source for the intestinal epithelium. They also improve the functioning of the epithelium and its role in the gut mucosal barrier (Wollowski *et al.* 2001). *L. rhamnosus* GG has also been observed to reverse the disturbed uptake of antigens during mucosal inflammation and to improve the mucosal degradation of antigens in rats (Pessi *et al.* 1998). The mechanisms behind these effects are not known, but may relate to better nutrition of the mucosal epithelium and the activity of microflora.

The effect of probiotics on the immune system is probably more important than modulation of the composition and/or activity of the gut microflora as far as atopic disease is concerned. For modulation of the activity of the immune system, changes in the composition of the gut microflora may not always be necessary, although it could be desirable for other proposed health effects of probiotics (Sanders, 2000). Selected lactobacilli and bifidobacteria have been shown to be able to enhance the production of IgA (Majamaa *et al.* 1995; Yasui *et al.* 1992). This strengthens the gut mucosal barrier, since IgA will complex with antigens thereby inhibiting their penetration through the epithelium. IgA–antigen complexes are also more readily taken up by the Peyer's patches where they stimulate the production of IgA (and IgM), thus further contributing to immune exclusion of the antigens. In contrast to IgG, an IgA–antigen complex does not elicit an inflammatory response and thus does not contribute to a worsening of the disease. In addition, IgA is relatively resistant to proteases, making it well adapted to its function in the intestine (Helgeland & Brandtzaeg, 2000).

An increased uptake of antigens through Peyer's patches may also lead to a reduced secretion of IgE and eosinophil activation, thereby alleviating the atopic inflammation. Indeed, *L. rhamnosus* GG has been shown to divert antigen uptake towards Peyer's patches (Isolauri *et al.* 1993), and *Lactobacillus casei* Shirota has been observed to inhibit the production of IgE in serum in response to intraperitoneal injections of ovalbumin in mice (Matsuzaki *et al.* 1998).

Different parts of the probiotic cell have been observed to be able to modulate the immune system. Cell wall material, peptidoglycan (Stewart-Tull, 1980) and teichoic acids (Morata de Ambrosini *et al.* 1998) and also cytoplasmic contents (Pessi *et al.* 1999; Tejada-Simon & Pestka, 1999) have been suggested to elicit immune reactions. This could relate to nucleic acids present in the bacteria. The feeding of relatively large amounts of probiotic bacteria also provides large amounts of nucleic acids as bacteria can consist of up to 50 % nucleic acids by dry weight. Nucleotide-fortified infant formula has been shown to result in higher specific antibody titres compared to unfortified formula (Pickering *et al.* 1998). This may be due to specific bacterial DNA sequences that have been observed to affect the immune system (Klinman *et al.* 1996).

The precise mechanism behind this immune modulation is still largely unknown, although adhesion to the intestinal mucosa is thought to be of importance (Morata de Ambrosini *et al.* 1998). Close contact of probiotics with the intestinal mucosa and possibly some benign translocation may lead to an enhanced interaction of probiotics and the intestinal immune system. This interaction will stimulate naive T-cells to differentiate to TH1 cells under the influence of interferon-γ, IL-2 and IL-12, while the development of TH2 cells is down-regulated under the influence of IL-4. The result of the generation of counter-regulatory TH1- and TH3-type immune responses is a reduced production of IgE and an increased secretion of IgA (Kirjavainen *et al.* 1999; Sanfilippo *et al.* 2000), which leads to a reduced allergic response.

The development of optimal probiotic preparations specifically designed to counteract atopic disease

Probiotic properties are strain-specific and probiotics have been shown to provide a wide range of health effects. The efficacy of probiotics varies in different disease states and intestinal dysfunctions. It has been recently reported that *L. rhamnosus* GG has no effect on colitis in an animal model whereas an animal-derived strain of *Lactobacillus reuteri* showed promising results (Holma *et al.* 2001). However, in our studies, no host specificity has been observed for human probiotics and a good adherence to intestinal mucus from other species including fish and dog has been shown for well-adhering human probiotic strains (Rinkinen *et al.* 2000; Nikoskelainen *et al.* 2001). We have shown that adherence to intestinal tissues is one of the key factors for selecting probiotics and that mathematical modelling can be successfully used for assessing competitive exclusion and adherence of probiotics in both human mucosal cell lines and human intestinal mucus model (Lee *et al.* 2000). Adherence should be assessed in actual competitive exclusion studies with the normal mucosal microflora to avoid false positive results in interpreting adherence results from traditional studies. New probiotics should therefore be characterised using adherence studies in humans or with human intestinal segments from the area where the target treatment is aimed. We have also recently shown that the composition of the *Bifidobacterium* flora in allergic infants is significantly different from healthy infants with *B. adolescentis* as the main *Bifidobacterium* species present (He *et al.* 2001). The target for functional foods and formula should be the modification of the gut microflora in such a way that it resembles a healthy bifidobacteria composition. Current prebiotics would very likely enhance the number of *B. adolescentis* further instead of other *Bifidobacterium* species that are typical for healthy children. Therefore, the potential of utilising specifically *B. breve*, *B. infantis* and *B. bifidum* or specifically selected probiotic strains enhancing the healthy *Bifidobacterium* flora should be explored as the method of choice for balancing the gut microflora composition. In

Table 1. Themes of on-going research in developing functional foods for allergic individuals

Problem	Suggestions for research
Aberrant microflora may be related to development of atopic disease	Development of methods for identification of gut microflora Identification of the differences in gut microflora between atopic and healthy subjects at different ages Detailed characterisation of bifidobacteria and bifidogenic components in balancing gut microflora
Development of functional foods for reducing the risk and management of atopic diseases	Characterisation of effective probiotic strains Characterisation of probiotic mechanisms in reducing the risk of atopic disease Identification of effective dietary components and their mechanisms of action Selection of prebiotic components to promote the healthy *Bifidobacterium* species and strains Investigation of detailed nutrient–strain interaction Selection of prebiotics, probiotics, nutrients and food matrices for functional foods

future, specific prebiotics to mimic breast-milk galacto-oligosaccharides should be isolated to promote the right type of *Bifidobacterium* flora in infants and children (Table 1).

Where should probiotic research be directed?

Understanding of the development of a healthy human gut microflora from infancy to senior years will be a key factor for developing more efficient probiotics. As the normal microflora alters with age, treatment targets may vary and may offer options for different populations for dietary modulation of the gut microflora. Detailed information on the composition and metabolic activity of the gut and mucosal microflora in health and disease will be required, including the impact of host age. Accurate, rapid and standardised microflora assessment methods are urgently needed to provide the required information.

Selecting scientifically characterised probiotics for different microflora or gut mucosal dysfunctions, such as colitis, or inflammatory bowel disease form important directions for probiotic research in the future. Such an approach will facilitate both alleviation and treatment of specific symptoms of dysfunction.

In infants, the microbial flora drives the maturation of the immune system and changes in its composition are likely to play a role in the prevalence of allergy. In early infancy the mode of birth, contact with mother's microflora via the breast and skin and the composition of breast milk influenced by the mother's diet may be the principal factors determining the development of atopic disease. These should be thoroughly understood for developing new dietary probiotics.

Clarifying the ways orally ingested probiotic microbes, the microflora and the gut's local and systemic immune system as well as dietary nutrients interact, is one of the key areas for future research. This would result in new criteria for selecting the types and activities of organisms aimed at normalising aberrant microflora, mucosa or metabolism. It also appears that bifidobacteria have a more important role than earlier understood. As *Bifidobacterium* strains have varying health effects, future probiotics may have to be isolated from the gastrointestinal tract of healthy infants, as aberrant microflora and the development of diseases may be influenced by specific species and even strains residing among the gut microflora.

The nutritional requirements of humans and the potential food matrices used for delivering probiotic organisms to improve health or to reduce the risk of disease have to be designed for each target population. For instance, to develop probiotic functional foods for preventing atopic diseases, in addition to scientifically selected and characterised probiotic microbes with proven efficacy, it is important to select components and food matrices which will enhance the nutritional impact of the food to counteract deficiencies and facilitate the delivery of the probiotic in the right and active form to both mothers and infants at risk of developing such diseases.

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