



# Antiallergic Effects of Probiotics<sup>1,2</sup>

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## Abstract

A considerable part of the Western population suffers from some form of allergy, and the incidence is still rising with no sign of an end to this trend. Reduced exposure to microbial allergens as a result of our hygienic lifestyle has been suggested as one of the possible causes. It has also been suggested that probiotics may provide safe alternative microbial stimulation needed for the developing immune system in infants. This idea is supported by the fact that allergic infants have been observed to have an aberrant intestinal microbiota. They were shown to have more clostridia and fewer bifidobacteria and, in addition, to have an adult-like *Bifidobacterium* microbiota. Clinical trials have shown that the standard treatment of infants with atopic eczema, extensively hydrolyzed infant formula, can be significantly improved through the addition of *Lactobacillus rhamnosus* GG or *Bifidobacterium lactis* Bb-12. It has also been shown possible to halve the incidence of allergy in at-risk infants through administration of *L. rhamnosus* GG to expecting mothers and subsequently to their infants during the first half-year of life. Many mechanisms have been proposed for these beneficial effects, ranging from improved mucosal barrier function to direct influences on the immune system. However, the exact mode(s) of action are not yet known. For the future, elucidation of these mechanisms will be an important target. Another important area will be the investigation of interactions between probiotics and other food components that influence allergies. This will enable optimization of probiotic use for the allergic subject. J. Nutr. 137: 794S–797S, 2007.

It is estimated that ~20% of the population in western countries suffers from some form of allergy. The incidence of allergy is still rising, and there are no indications that this trend will be reversed. A hereditary predisposition for allergy is thought to be involved: children who have family members with allergies have a higher risk of developing allergy as well (1). However, environmental factors appear to be required to trigger the disease.

The hygiene hypothesis suggests that insufficient or aberrant exposure to environmental microbes is one of the causes of the development of allergy. Reduced family size, improved hygiene, vaccination, the use of antimicrobial medication, and the consumption of almost sterile food have reduced and changed our exposure to microbes (2). Humans have evolved in an environ-

ment with a heavy bacterial load, and our immune system has been adapted to deal with that. With the advances in medicine and food processing, our contact with microbes has changed. The absence of such an appropriate microbial exposure may pose a problem for the development a child's immune system. In infants, the immune system is still developing; this provides an opportunity to direct development away from the allergic phenotype. Avoidance of allergens has been standard treatment in the past (3). This has met with limited success; allergen avoidance relieves the symptoms but does not treat the disease. Instead of avoidance, induction of tolerance by exposure to antigens may be the appropriate method. It is obvious that for public health reasons it is not desirable to abandon current medical and hygienic practices; therefore, safe alternatives have to be sought. Probiotics may be such safe alternatives for providing necessary microbial stimulation.

## The intestinal microbiota

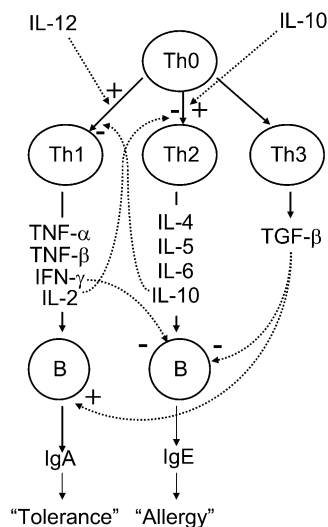
The normal intestinal microbiota has a diverse composition; a conservative estimate is that it consists of at least 400 species (4). This estimate was made on the basis of results from culture-based techniques. Because a large part of the intestinal microbiota can not be cultured with current techniques (5), it has been suggested that the number of microbial species in the human intestine may, in fact, exceed 1000.

This microbiota has a metabolic activity that equals that of the liver, our metabolically most active organ. The microbiota contributes to the digestion of exogenous and endogenous substrates, such as fibers and mucins. This provides the host with

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**Figure 1** Development of the "allergic" (Th2) or "tolerant" (Th1) phenotype. IL-10 stimulates the maintenance of the allergic phenotype, whereas IL-12 stimulates a shift toward the tolerant phenotype. Th3 cells, through the production of transforming growth factor- $\beta$ , further stimulate the shift toward tolerance. IgE may activate mast cells and cause allergic symptoms; IgA on the other hand may provide allergen exclusion.

additional energy in the form of fatty acids (6). It may, however, also expose the host to detrimental metabolic endproducts such as amines, sulfides, ammonia, etc.

Another important function of the intestinal microbiota is to provide a protective barrier against incoming bacteria, e.g., potential pathogens. This colonization resistance works through several different mechanisms: competition for nutrients and binding sites and production of antimicrobial substances (7).

The intestine is the body's largest immune organ; most of the antibody-producing cells reside in the intestine (8). A relatively recently recognized function of the intestinal microbiota is to provide stimulation of the immune system. Consumption of probiotics (and prebiotics) is, in most cases, aimed at modulating the composition and/or activity of the intestinal microbiota (9). This modulation can be expected to influence the immune system. Indeed, several probiotic strains have been observed to modulate some immune parameters after sufficient (time and amount) consumption (10–12). However, in many cases it is uncertain what the actual health benefit of this immune modulation is for the consumer, in particular the healthy consumer.

### The intestinal immune system

At birth, the immune system of an infant is not fully developed and tends to be directed toward a T-helper (Th)2 phenotype<sup>3</sup> (Fig. 1) to prevent rejection in utero. The Th2 phenotype leads, however, to the stimulated production of IgE by B cells and thus increases the risk for allergic reactions through activation of mast cells. Microbial stimulation early in life will reverse the Th2 bias and stimulate the development of a Th1 phenotype and stimulate the activity of Th3 cells (13). Their combined action will lead to the production of IgA by B cells. IgA contributes to allergen exclusion and will therefore reduce exposure of the immune system to antigens. Cytokines produced by the Th1 phenotype will also reduce inflammation and stimulate tolerance toward common antigens (14).

<sup>3</sup> Abbreviations used: Ig, immunoglobulin; SCORAD, clinical scoring of atopic dermatitis; Th, helper T cells.

### The intestinal microbiota of allergic infants

In the case of allergy, the rationale for modulating the intestinal microbiota is supported by observations that allergic children have a different microbiota composition than healthy infants. Children with allergy were found to have an aberrant microbiota even before the onset of allergy; they had higher levels of clostridia and lower levels of bifidobacteria (15,16). In addition to these quantitative differences in the *Bifidobacterium* microbiota, qualitative differences have also been observed. Infants with atopic dermatitis have been found to have a more adult type *Bifidobacterium* microbiota with high prevalence of *B. adolescentis*. Healthy infants, on the other hand, were found to be colonized mainly by *B. bifidum*, typical for breast-fed infants (17,18). However, children with respiratory allergy symptoms did not exhibit an aberrant microbiota composition (18). The bifidobacteria from infants with atopic dermatitis were found to induce a higher secretion of proinflammatory cytokines in vitro, whereas the bifidobacteria from healthy infants induced the secretion of more antiinflammatory cytokines (19). Also, bifidobacteria of dairy origin stimulated more antiinflammatory and less inflammatory cytokines than bifidobacteria from allergic infants. In addition to differing in their induction of cytokines, bifidobacteria from allergic and healthy infants also exhibited different in vitro adhesion to Caco-2 tissue culture cells (20) and intestinal mucus (21). This difference in adhesion to the intestinal mucosa may result in a different or reduced stimulation of the immune system through the gut-associated lymphoid tissue.

Not only the composition of the intestinal microbiota but also the metabolic activity of the microbiota may be different. Swedish children, who are at high risk to develop allergy, were found to have significantly higher levels of fecal butyrate, isovalerate, and caproate than Estonian children, who have a low risk for developing allergies (22).

### Treatment of atopic disease

A limited number of strains have been tested for their efficacy in the treatment and prevention of allergy in infants. Allergy may manifest in infants even when they are exclusively breast-fed. Standard treatment involves the feeding of extensively hydrolyzed formula (3). Supplementation of this type of formula with *Bifidobacterium lactis* Bb-12 or *Lactobacillus rhamnosus* GG has been found to lead to an earlier recovery than standard treatment alone, 2 mo vs. 6 mo (23). A combination of 2 *Lactobacillus* strains, *L. rhamnosus* 19070–2 and *L. reuteri* DSM 122460, was found to significantly reduce the clinical scoring of atopic dermatitis (SCORAD) in 1- to 13-y-old children with a positive skin prick test. But the SCORAD of children with no positive skin prick test remained unchanged. Interestingly, more than half of the subjects reported an improvement in their eczema, whereas only 15% in the placebo group reported improvement (24).

The 2 studies discussed used different probiotics preparations; this may explain the observed differences in outcome. But the differences may also relate to the differences in age of the patients studied. In young infants, the immune system is still developing. There is still a possibility to direct it toward tolerance. In older children, the allergic phenotype is already established, and here one may only be able to relieve the symptoms. Similarly, probiotics have not been very successful in alleviating symptoms of respiratory allergy. *L. rhamnosus* GG was not able to reduce the symptoms of birch pollen allergy in adults (25) despite its effectiveness in children. Similarly, *L. acidophilus* L-92 was reported only to relieve the subjective symptoms of cedar pollen allergy in adults (26).

## Prevention of allergic disease

In addition to treatment of allergy, it has been observed that selected probiotics can reduce the risk for the development of allergy. One of the earliest studies was performed with a non-pathogenic *Escherichia coli* administered to term and preterm infants. At 10 and 20 y of age, children treated with *E. coli* suffered significantly fewer allergic diseases than the subjects in the control group (27). In a recent study, the efficacy of *L. rhamnosus* GG on at-risk infants was studied; children of allergic mothers have ~50% risk of developing allergy. Pregnant allergic mothers were given *L. rhamnosus* GG or placebo from 2 to 4 wk before the calculated date of delivery in a randomized double-blind trial. After delivery, the children received *L. rhamnosus* GG for 6 mo. After 4 y, 46% of the children in the placebo group had developed atopic eczema, whereas in the probiotics group this was 26% (28). Surprisingly, the serum IgE levels did not differ between the 2 groups. This is in contrast to observations in mice, where *L. casei* Shirota was able to suppress the production of IgE (29).

## Mechanisms of antiallergic probiotic action

The precise mechanisms behind the favorable effects of probiotics on allergy are not entirely known. Several mechanisms have been observed in vitro and in animal studies (Fig. 2). In addition to modulation of the intestinal microbiota, probiotics have been observed to improve the barrier function of the intestinal mucosa (30), reducing leakage of antigens through the mucosa and thereby exposure to them. Direct modulation of the immune system may be through the induction of antiinflammatory cytokines or through increased production of secretory IgA (31). IgA will contribute to an exclusion of antigens from the intestinal mucosa. Further, enzymatic degradation of dietary antigens by enzymes from probiotics will reduce the load of and exposure to antigens (32). These and other mechanisms contribute to reduced exposure of the immune system to dietary antigens.

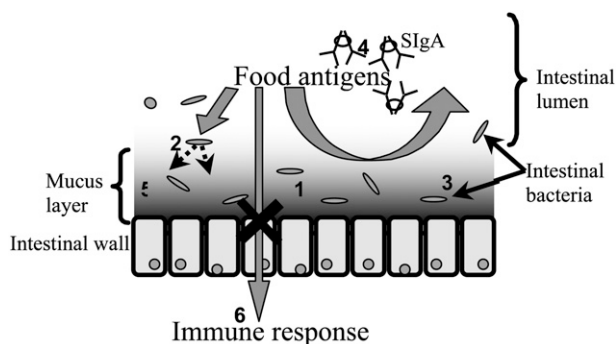
For the future, it will be important to determine the mechanisms behind the probiotic action on allergy. This will enable further improvement of the use of probiotics. A thorough knowledge of the intestinal microbiota of allergic and healthy infants presents an opportunity to select more effective strains or combinations of strains. Because probiotics modulate the composition and/or activity of the intestinal microbiota, it is important to obtain information on the intestinal microbiota, not only from fecal samples, as is common practice, but also

from the mucosa-associated microbiota. In addition to probiotics, (n-3) fatty acids (33) and antioxidants (34) have been suggested to contribute to a protection against allergy. Also, prebiotics may modulate the immune response through similar mechanisms as probiotics (35) and reduce inflammation (36). The influence on allergy of the combination of these dietary components and probiotics deserves further investigation.

Thus, although probiotic therapy appears to be a promising approach in the treatment and prevention of allergy, there are still a substantial number of questions that remain to be answered.

## Literature Cited

- Burke W, Fesinmeyer M, Reed K, Hampson L, Carlsten C. Family history as a predictor of asthma risk. *Am. J. Prev. Med.* 2003;24:160-9.
- Strachan DP. Hay fever, hygiene, and household size. *BMJ.* 1989;299:1259-60.
- Zeiger RS. Food allergen avoidance in the prevention of food allergy in infants and children. *Pediatrics.* 2003;111:1662-71.
- Moore WEC, Holdeman LV. Human fecal flora: The normal flora of 20 Japanese-Hawaiians. *Appl Microbiol.* 1974;27:961-79.
- Zoetendal EG, Akkermans ADL, de Vos WM. Temperature gradient gel electrophoresis analysis of 16S rRNA from human faecal samples reveals stable and host-specific communities of active bacteria. *Appl Environ Microbiol.* 1998;64:3854-9.
- Ouwehand AC, Derrien M, de Vos W, Tiihonen K, Rautonen N. Prebiotics and other microbial substrates for gut functionality. *Curr Opin Biotechnol.* 2005;16:212-7.
- Adlerberth I, Cerquetti M, Poilane I, Wold A, Collignon A. Mechanisms of colonisation and colonisation resistance of the digestive tract. *Microb Ecol Health Dis.* 2000;11(Suppl. 2):223-39.
- Brandtzaeg P. Current understanding of gastrointestinal immunoregulation and its relation to food allergy. *Ann N Y Acad Sci.* 2002;964:13-45.
- Saavedra JM, Tschernia A. Human studies with probiotics and prebiotics: clinical implications. *Br J Nutr.* 2002;87:S241-6.
- Pelto L, Isolauri E, Lilius E-M, Nuutila J, Salminen S. *Lactobacillus* GG modulates milk-induced immune inflammatory response in milk-hypersensitive adults. In *1st Workshop Fair CT96-1028, Selection and safety criteria of probiotics.* 1996. Helsinki, Finland.
- He F, Tuomola E, Arvilommi H, Salminen S. Modulation of humoral immune response through probiotic intake. *FEMS Immunol Med Microbiol.* 2000;29:47-52.
- Gill H, Rutherford KJ, Cross ML, Gopal PK. Enhancement of immunity in the elderly by dietary supplementation with the probiotic *Bifidobacterium lactis* HN019. *Am J Clin Nutr.* 2001;74:833-9.
- von der Weid T, Bulliard C, Schiffrin EJ. Induction by a lactic acid bacterium of a population of CD4<sup>+</sup> T cells with low proliferative capacity that produce transforming growth factor  $\beta$  and interleukin-10. *Clin. Diagn. Lab. Immunol.* 2001;8:695-701.
- Kirjavainen PV, Gibson GR. Healthy gut microflora and allergy: factors influencing development of the microbiota. *Ann Med.* 1999;31:288-92.
- Sepp E, Julge K, Mikelsaar M, Bjorksten B. Intestinal microbiota and immunoglobulin E responses in 5-year-old Estonian children. *Clin Exp Allergy.* 2005;35:1141-6.
- Kalliomäki M, Kirjavainen P, Eerola E, Kero P, Salminen S, Isolauri E. Distinct gut patterns of neonatal gut microflora in infants developing or not developing atopy. *J Allergy Clin Immunol.* 2001;107:129-34.
- Ouwehand AC, Isolauri E, He F, Hashimoto H, Benno Y, Salminen S. Differences in *Bifidobacterium* flora composition in allergic and healthy infants. *J Allergy Clin Immunol.* 2001;108:144-5.
- Murray CS, Tannock GW, Simon MA, Harmsen HJ, Welling GW, Custovic A, Woodcock A. Fecal microbiota in sensitized wheezy and non-sensitized non-wheezy children: a nested case-control study. *Clin Exp Allergy.* 2005;35:741-5.
- He F, Morita H, Hashimoto H, Kurisaki J-I, Ouwehand AC, Isolauri E, Benno Y, Salminen S. Intestinal *Bifidobacterium* species induce varying cytokine production. *J Allergy Clin Immunol.* 2002;109:1035-6.



**Figure 2** Mechanisms by which probiotics may influence food allergy. 1) Improved mucosal barrier function. 2) Degradation of food antigens. 3) Modulation of intestinal microbiota composition and activity. 4) Stimulated production of secretory IgA. 5) Change in mucus production. 6) Direct immune modulation.

20. Morita H, He F, Fuse T, Ouwehand AC, Hashimoto H, Hosoda M, Mizumachi K, Kurisaki J-I. Adhesion of lactic acid bacteria to Caco-2 cells and their effect on cytokine secretion. *Microbiol Immunol.* 2002;46:293-7.
21. He F, Ouwehand AC, Isolauri E, Hashimoto H, Benno Y, Salminen S. Comparison of mucosal adhesion and species identification of bifidobacteria isolated from healthy and allergic infants. *FEMS Immunol Med Microbiol.* 2001;30:43-7.
22. Norin E, Midtvedt T, Björkstén B. Development of faecal short-chain fatty acid pattern during the first year of life in Estonian and Swedish infants. *Microb Ecol Health Dis.* 2004;16:8-12.
23. Isolauri E, Arvola T, Sütas Y, Moilanen E, Salminen S. Probiotics in the management of atopic eczema. *Clin Exp Allergy.* 2000;30:1604-10.
24. Rosenfeldt V, Benfeldt E, Nielsen SD, Michaelsen KF, Jeppesen DL, Valerius NH, Paerregaard A. Effect of probiotic *Lactobacillus* strains in children with atopic dermatitis. *J Allergy Clin Immunol.* 2003;111:389-95.
25. Helin T, Haahtela S, Haahtela T. No effect of oral treatment with an intestinal bacterial strain, *Lactobacillus rhamnosus* (ATCC 53103), on birch-pollen allergy: a placebo-controlled double-blind study. *Allergy.* 2002;57:243-6.
26. Ishida Y, Nakamura F, Kanzato H, Sawada D, Yamamoto N, Kagata H, Oh-Ida M, Takeuchi H, Fujiwara S. Effect of milk fermented with *Lactobacillus acidophilus* strain L-92 on symptoms of Japanese cedar pollen allergy: a randomized placebo-controlled trial. *Biosci Biotechnol Biochem.* 2005;69:1652-60.
27. Lodinová-Zádníková R, Cukrowská B, Tlaskalová-Hogenová H. Oral administration of probiotic *Escherichia coli* after birth reduces frequency of allergies and repeated infections later in life (after 10 and 20 years). *Int Arch Allergy Immunol.* 2003;131:209-11.
28. Kalliomäki M, Salminen S, Pousa T, Arvilommi H, Isolauri E. Probiotics and prevention of atopic disease: 4-year follow-up of a randomised placebo-controlled trial. *Lancet.* 2003;361:1869-71.
29. 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.
30. Malin M, Verronen P, Korhonen H, Syväoja E-L, Salminen S, Mykkänen H, Arvilommi H, Eerola E, Isolauri E. Dietary therapy with *Lactobacillus* GG, bovine colostrum or bovine immune colostrum in patients with juvenile chronic arthritis: evaluation of effect on gut defence mechanisms. *Inflammopharmacology.* 1997;5:219-36.
31. Kaila M, Isolauri E, Soppi E, Virtanen E, Laine S, Arvilommi H. Enhancement of the circulating antibody secreting cell response in human diarrhea by a human *Lactobacillus* strain. *Pediatr Res.* 1992;32:141-4.
32. Pessi T, Isolauri E, Sütas Y, Kankaanranta H, Moilanen E, Hurme M. Suppression of T cell activation by *Lactobacillus rhamnosus* GG-degraded bovine casein. *Immunopharmacology.* 2001;1:211-8.
33. Mermer C, Mercola J. Omega-3s and childhood asthma. *Thorax.* 2002;57:281.
34. Shaheen SO, Sterne JAC, Thompson RL, Songhurst CE, Margetts BM, Burney PGJ. Dietary antioxidants and asthma in adults. *Am. J. Respir. Crit. Care Med.* 2001;164:1823-8.
35. Schley PD, Field CJ. The immune-enhancing effects of dietary fibres and prebiotics. *Br J Nutr.* 2002;87:S221-30.
36. Guigoz Y, Rochat F, Perruisseau-Carrier G, Rochat I, Schiffrin EJ. Effects of oligosaccharide on the faecal flora and non-specific immune system in elderly people. *Nutr Res.* 2002;22:13-25.