

*P.V. Kirjavainen
E. Apostolou
S.J. Salminen
E. Isolauri*

New aspects of probiotics – a novel approach in the management of food allergy

Authors' affiliations:

P.V. Kirjavainen, E. Apostolou, S.J. Salminen,
Department of Biochemistry and Food
Chemistry, University of Turku, Turku
E. Isolauri, Department of Paediatrics, Turku
University Hospital, Turku, Finland

Correspondence to:

Pirkka V. Kirjavainen
Department of Biochemistry and Food Chemistry
University of Turku
FIN-20014 Turku
Finland

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Introduction

Until now, the only treatment of food allergy has been the elimination of the offending food from the diet. However, recent studies indicate that probiotic bacteriotherapy has great potential in controlling the allergic inflammation associated with food allergy (1). Probiotics are microbial cell preparations or components of microbial cells which have a beneficial effect on the health and well-being of the host (2). Most of the strains in question belong to the genera of lactobacilli or bifidobacteria, and their general characteristics include human origin, assessed safety for human use, acid and bile stability, and ability to bind to the human intestinal mucosa (3). Conventionally, probiotics have been regarded as food supplements with a potential as nonspecific balancers of the gut microflora and potentiators of immunity.

Oral tolerance and allergic sensitization: the role of intestinal microflora

The gastrointestinal tract is constantly exposed to substantial amounts of luminal food and bacterial components. In the healthy gut, the immune system is able to create a balance between the protective mucosal immunity and systemic tolerance. In food allergy, this balance is impaired, and oral tolerance of dietary antigens is not achieved or maintained (4–6). The risk factors for the development of food allergy, immature gut barrier and type 2 T-helper (Th2) cell-skewed cytokine profile, are present in early infancy.

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This may lead to atopic sensitization, as the antigen uptake is aberrant and Th2 cells further produce interleukin (IL)-4, a cytokine essential for B-cell differentiation into immunoglobulin (Ig)E-producing cells, and IL-5, which is important for the activity of eosinophils.

Microbial colonization of the intestine begins after birth, but the development of the normal flora is a gradual process initially determined by contact with the maternal intestinal flora and surroundings and possibly by genetic factors (3, 7). The bacterial flora has been shown to counterbalance Th2 activity and promote oral tolerance (8, 9). It also affects the development of many other immune parameters, as indicated in studies on germ-free mice. For example, peritoneal macrophage function and lymphocyte proliferation have been demonstrated to be reduced in the absence of the intestinal flora (10–12).

The predilection of bacteria to promote the differentiation of Th1 cell lineage may, at least partly, be due to a specified CpG motif characteristic of bacterial DNA, which has been shown to induce polyclonal B-cell activation and secretion of Th1 cytokines IL-6, IL-12, and interferon (IFN)- γ (13). Our recent studies give some indication that lactic and propionic acid bacteria may influence, in a strain-dependent manner, the susceptibility of lymphocytes to mitogen-induced apoptosis (Kirjavainen et al., unpublished data). In theory, this, too, could affect the Th balance, as normally the Th1 lineage is more susceptible to apoptosis (14, 15). Lipopolysaccharide (LPS) of the Gram-negative flora may also play some role, as presentation of the polysaccharide portion of LPS appears to induce Th2 responses, while presentation involving the inflammatory lipid A may favour Th1 responses (16). Moreover, LPS may enhance the IgA response to dietary antigen (17), which otherwise could induce a Th2 response.

Clinical evidence for the potential use of probiotics in the management of food allergy

In the early 1980s, Russian scientists linked food allergy to abnormal intestinal microflora (18). Subsequently, Loskutova (19) reported that the administration of a mixture containing *Propionibacteria* and *Lactobacillus acidophilus* bacteria hastened the disappearance of food allergy manifestations. In a more recent study by Trapp et al. (20), volunteers given yoghurt had decreased concentrations of IgE in the serum and a lower frequency of allergies. Wheeler et al. (21) studied the effect of yoghurt on cellular, humoral,

and phagocytic function in adults with atopic allergy. Consumption of yoghurt, fermented with *L. bulgaricus* and *Streptococcus thermophilus*, induced no significant changes in any of the immune parameters investigated. However, more conspicuous results were observed when infants with atopic eczema and cow's milk allergy were given an extensively hydrolyzed whey formula supplemented with the probiotic *L. rhamnosus* strain GG [ATCC 53103] (1). In comparison to control subjects who received unsupplemented extensively hydrolyzed whey formula, probiotic supplementation resulted in a significant improvement of clinical symptoms and alleviation of intestinal inflammation associated with food allergy.

Targets for probiotic therapy in patients with food allergy

The possible mechanisms by which probiotics alleviate the inflammatory response in food allergy include their potentiating effect on the nonimmunologic and immunologic defence barrier of the gut and modified degradation of food allergens (Fig. 1) (1, 4, 22, 23). In theory, both of these actions can be either a direct response to the probiotic microorganisms or mediated by the normal intestinal flora.

In unbalanced intestinal flora, pathogens are abundantly present and the gut immune system may mount an inflammatory response to them (3). By producing antimicrobial substances and coaggregation with pathogens (2, 3), probiotics can normalize the intestinal flora and thereby alleviate inflammation, normalize permeability, and reduce the permeation of food antigens in hypersensitive subjects.

In order to influence the immune system, a probiotic microorganism must activate the lymphoid cells of the gastrointestinal lymphoid tissue, which populate the lamina propria and submucosa and are diffusely distributed among the epithelial cells (24). In theory, probiotics can affect these cells by various pathways, including direct contact by the intact probiotic microorganism, its fragment, or a metabolite; modification of the normal microflora and intestinal permeability to other antigens (e.g., bacterial peptides); and production of adjuvants, which modulate the processing of and immune responses to these other antigens (Fig. 2).

The effects of probiotics on nonspecific immunity are seen as downregulation of inflammatory responses. The anti-inflammatory properties of probiotics have been shown in a recent study of cell homogenates of *Lactobacillus* GG, *L. rhamnosus* LC705, *Bifidobacterium animalis* Bb12, *L. acidophilus* NCFB-L61748, *L. bulgaricus* ATCC 11842,

Streptococcus thermophilus T101, and *Propionibacterium freudenreichii* Shermanii strain JS (25). These homogenates were free of cell-wall components and enzymatic activity and were shown to be able to suppress effectively *in vitro* PHA-induced lymphocyte proliferation. These data would imply that probiotic bacteria possess significant, strain-dependent, antiproliferative properties.

Atopic infants have been shown to have overactive phagocytes (26), which may contribute to the allergic inflammation. Enhancement of phagocytic activity after administration of lactic acid bacteria has been observed in various experiments (27–34). Prominently, probiotics appear to modulate phagocytosis differently in healthy and allergic subjects (34). *Lactobacillus* GG has been demonstrated to be capable of alleviating the overactive phagocytic process induced by cow's milk challenge in milk-hypersensitive subjects, as shown by expression of phagocytosis receptors on neutrophils and monocytes. Conversely, healthy subjects evinced higher expression of receptors on these cells when they received *Lactobacillus* GG-supplemented milk than when they were given milk only. The difference in these responses could be due to a number of factors related to the immunologic differences between these groups. We suggest that one potential factor here is variability in the basal level of cytokines, as cytokines may have different immunomodulatory effects in different concentrations (35, 36). Another potential factor is the possible difference in antigen uptake and its effects on the immune response; i.e., while the antigen processing in healthy subjects is most likely to take place in the follicle-associated epithelium, milk-intolerant subjects may have morphologic changes in their gut epithelia allowing enhanced antigen translocation directly through the epithelial cells or through leaky tight

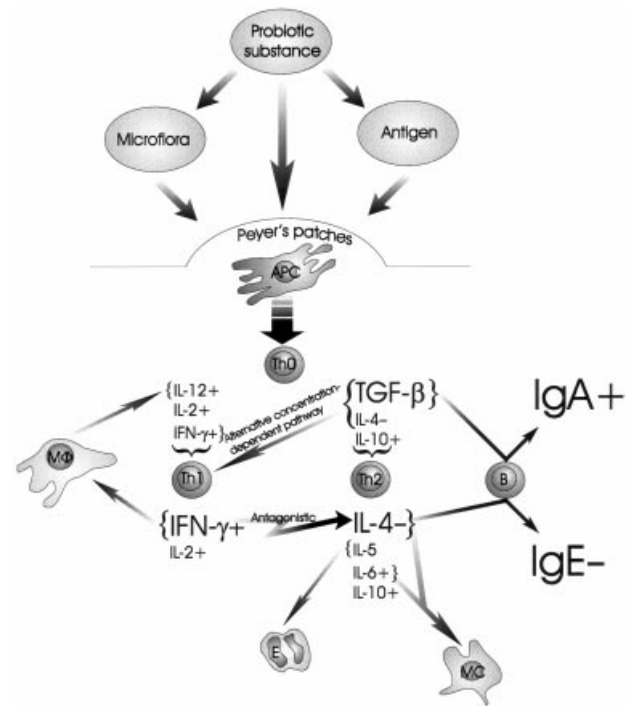


Figure 2. Model for Th1/Th2-mediated responses in alleviation of allergic inflammation by probiotics. Probiotic therapy can affect the immunocompetent cells of gut-associated lymphoid tissue directly due to a probiotic adjuvant or indirectly via modifications on microflora, or on uptake or degradation of food antigens. The probiotic adjuvant, or bacterial or dietary antigen is presented to naive T cells (Th0) by antigen-presenting cells (APC). The major cytokines (IFN- γ , IL-12, and IL-2) directing the differentiation of Th0 toward Th1 lineage appear to be enhanceable by probiotics (+) (43–46), while secretion of the most important Th2 cytokine IL-4 is downregulated (–) (45). Particularly, the ability to enhance the production of IFN- γ , a cytokine which is known to be antagonistic to many IL-4 activities, indicates that probiotics have the potential to alleviate atopic inflammation mediated by IgE, mast cells (MC), and eosinophils (E). The activation of IL-12 secretion by macrophages may have importance in reinforcing Th1 activity (46), and the concentration-dependent role of TGF- β in Th0 differentiation may largely enable the simultaneous enhancement of IgA secretion and downregulation of other characteristic Th2 responses.

junctions. Even small changes such as shortened villi may have an important role in that they may allow the probiotic antigen to adhere to receptors normally lying deeper in the glycocalyx or directly at the membrane surface, and thus facilitate its transepithelial uptake.

Bifidobacteria and lactobacilli have also been shown to enhance IgA production in Peyer's patches and potentiate IgA responses to potentially harmful antigens (37–41). An enhanced IgA response may prevent some potentially allergenic dietary antigens from invading the intestinal mucosa, and further alleviate the intestinal inflammation and downregulate hypersensitivity reactions. Secretory IgA may also downregulate the inflammation due to pathogenic bacteria by enhancing their exclusion. There is evidence of

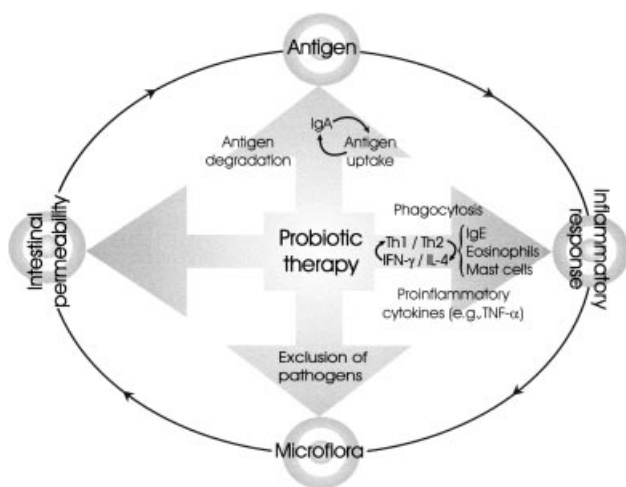


Figure 1. Targets for probiotic therapy.

distinctive B-cell populations secreting two different types of IgA (42). One of these is less specific and may be involved in the management of the composition of normal flora, whereas the other is mounted against pathogens entering via M cells of the Peyer's patches. Interestingly, antigen uptake through Peyer's patches may be enhanced by probiotics (4), and the IgA enhancement stimulated by a modified antigen uptake may have a self-regulatory nature, as IgA-complexed antigen is more readily taken up by Peyer's patches than free antigen.

Studies on the effects of probiotics on lymphokine secretion indicate that some of the immunologically mediated effects of probiotics could be due to direction of the differentiation of the CD4+ T-helper precursor Th0 cells toward Th1 lineages (Fig. 2). IL-4 is a potent inhibitor of the IFN- γ -directed differentiation of naive Th cells to Th1 cells; similarly, INF- γ is generally antagonistic to IL-4 action. However, Th1 cell differentiation has also been shown to be inducible by an alternative (IL-12-independent) pathway involving a combination of IL-4, transforming growth factor (TGF)- β , and endogenous production of IFN- γ (35). The cytokine stimulation appears to be concentration-dependent, as it has been shown in high IL-4 and fixed TGF- β concentrations that Th2 cell differentiation is promoted and Th1 development weak (35). In addition, the effect of TGF- β on Th1 development has been shown to be dependent on the concentration of IL-2: at low IL-2 concentrations, TGF- β inhibits and at high IL-2 concentrations stimulates Th1 development (36). Dependence on concentration is indicative of a possibility of different local effects.

Lactobacilli have been demonstrated to have the potential to induce the secretion of IL-2, IL-12, and INF- γ and to reduce the secretion of Th2 cytokines (43–46). Probiotics may also modify the immunomodulatory properties of native food proteins (22, 23). In contrast to purified casein, which upregulates IL-4 and INF- γ production in milk-hypersensitive atopic infants, *Lactobacillus* GG-degraded casein was shown to downregulate IL-4 production, with no effect on INF- γ release (22). These findings indicate that probiotics possess a potential as inhibitors of aberrant IgE secretion and eosinophil activation. Indeed, inhibition of IgE by lactic acid bacteria has been demonstrated in a murine model by Matsuzaki et al. (45). *Lactobacillus* Shirota significantly inhibited the IgE production induced by intraperitoneal ovalbumin injection, and *in vitro* IgE production by ovalbumin-restimulated splenocytes of *Lactobacillus* Shirota-treated mice was inhibited.

In addition to the modulation of secretion of cytokines such as IFN- γ , IL-12, and IL-4, which directly affect the differentiation of Th lineages, lactic acid bacteria appear to affect the secretion of several other cytokines. One of these is tumour necrosis factor (TNF)- α . *Lactobacillus* GG therapy might prevent its release by inducing IFN- γ production in T cells (1). On the other hand, with a mixture of *Bifidobacterium bifidum* and *L. acidophilus*, some elevation in the blood TNF- α concentration was noted in a different patient population (43). Similarly, *in vitro* studies have demonstrated that lactic acid bacteria can stimulate the secretion of TNF- α by murine macrophages and human peripheral lymphocytes (27, 44). Thus, it may be of interest that the inflammatory properties of TNF- α have been held to be dependent on the balance of Th1 and Th2 cytokines, so that in a "pure" Th1 response (without Th2) TNF- α acts as an additional macrophage-activating factor, whereas in mixed Th1 and Th2 (or Th0) responses, it may cause tissue damage (47). In our recent study, bifidobacteria- and *Lactobacillus* GG-treated infants evinced no changes in their TNF- α levels *in vivo* (Isolauri et al., unpublished), indicating a difference between *in vitro* and *in vivo* responses.

Safety of probiotics: treatment of allergy

Foods containing large numbers of lactobacilli and lactic acid bacteria have a long history of safe use. Epidemiologic data support the low risk of lactic acid bacteria (48, 49), and no adverse immunologic effects of probiotic bacteria have been reported in healthy persons (50). The total number of infections caused by or associated with lactic acid bacteria is extremely low considering that they are abundant inhabitants of the healthy microflora of all mucous membranes, and are widely used in fermented food products.

Although probiotics have been shown to have beneficial health effects on an immunocompetent host, there may be a risk of opportunistic infections. Wagner et al. (51) colonized immunodeficient mice with human isolates of *L. reuteri*, *Lactobacillus* GG, *Bifidobacterium animalis*, or *L. acidophilus*. These strains were innocuous to immunodeficient adult mice, but some deaths were observed in young mice with colonization by *L. reuteri* and *Lactobacillus* GG (51). Severely immunocompromised adult patients are also at some risk of infection when treated with live organisms. This was shown in a case report of a 73-year-old man with chronic lymphocytic leukaemia who had been treated with *Bacillus subtilis* spores for a month, and whose death may

have been associated with septicaemia caused by this strain (52).

Suggested virulence factors include strong adhesion, which may facilitate translocation and platelet aggregation associated with endocarditis (48, 53, 54). Comparing clinical *Lactobacillus* isolates from bacteraemia patients with normal intestinal strains and current probiotic strains, we found no indication that clinical bacteraemia strains have stronger adhesion than common probiotics. Clinical bacteraemia isolates also had no general property of platelet aggregation; rather, most of them were nonaggregating strains (55). Thus, adhesion and platelet aggregation may be related in some cases, but they do not appear to be strict virulence factors for lactic acid bacteria. When more specific probiotic microbes are selected or modified from the current strains, it may be important to assess both platelet aggregation properties and adhesion properties in more than one model (56, 57).

Subjects with food allergy may have a morphologically normal small-intestinal mucosa, characterized by increased intestinal permeability with or without intestinal inflammation. The administration of large numbers of live bacteria to such patients may give some cause for concern at the commencement of treatment by reason of decreased intestinal barrier function. Particular concern is focused on infants. Recent studies attest to the safety of specific probiotics such as *Lactobacillus* GG, which has been shown to promote gut barrier function and alleviate intestinal inflammation in infants with atopic dermatitis and food allergy, without adverse side-effects (1).

New probiotic strains are constantly being isolated. It cannot be assumed that these are as safe as the conventional or tested strains (50). Prior to incorporation of any novel bacterial strains into food products, each must be tested on a case-by-case basis for every disorder it is intended to treat, to ensure that it is as safe as the conventional food-grade organisms (58). Further studies on the safety of current

probiotics are called for in infants and young adults. Due to the concern over the safe use of probiotics, safety assessments have been given a top priority in the new European projects.

Conclusion and prospects for future research

In new approaches, functional foods are used like pharmaceuticals, directing well-defined physiological effects at specific clinical targets. With the use of probiotics, there is great scope in the management of food allergy, as they have been shown to potentiate the gut defence barrier by nonimmunologic and immunologic means, while the unwanted effects appear to be minimal. Probiotics normalize increased intestinal permeability and altered gut microecology, and potentiate the immunologic defence barrier of the host, at least by enhancing the IgA response and by alleviating the intestinal inflammatory response. The latter effect is due to modifications such as reduced activity of phagocytes, enhanced degradation of food antigens, and modulation of cytokine secretion toward the Th2 pattern, resulting in reduced IgE response.

Currently, the clinical data supporting the efficacy of probiotic therapy in the management of food allergy are limited. More information is needed to screen for optimal dosage regimens, to characterize the requisite properties of probiotics with an eye to strain selection, and to extend our knowledge of safety aspects. Due to the differences between systemic and gut immunity, efforts should also be focused on developing immunologic models which closely resemble the *in vivo* conditions in the gut and take full advantage of existing mucosal *in vitro* models such as the recently introduced M cell model (59).

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