

Pre and probiotics in the prevention and treatment of food allergy

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Purpose of the review

Recent studies on the pathogenesis of allergy in both man and experimental animals continue to show the importance of commensal bacteria in the gastrointestinal tract in stimulating and directing the immune system. The interest in modulating commensal bacterial flora with prebiotics and probiotics to prevent and treat food allergy has multiplied in recent years.

Recent findings

Studies on prevention suggest that probiotic supplementation reduces IgE-associated eczema. It seems that prenatal administration is of importance. Colonization seems to be readily achieved, so there is proof of concept. However, after cessation of the administration, a rapid decline in colonization is evident. IgE sensitization in atopic eczema is a risk factor for allergic airways disease, and the reduced IgE-associated atopic eczema demonstrated may be an indication of less respiratory allergic disease later.

Administration of probiotics to children with allergy or at risk of allergy seems to stimulate a low-grade inflammation by activating the innate immune system and further production of IL-10.

Summary

Modulation of commensal bacteria of the gut with probiotics has been shown to modulate the immune system and to have an effect on both the prevention and treatment of food allergy. The effects have been highly variable depending on the mode of treatment and the optimal treatment remains unsettled at present.

Keywords

atopic eczema, eczema, food allergy, prebiotics, prevention, probiotics, synbiotics

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Introduction

The contact between infants' gastrointestinal tract and foreign food proteins results in T-cell and B-cell responses which, not being associated with any symptoms, gradually fade out, and the great majority of infants become tolerant to food proteins. A minority, estimated at 2–10% fails to develop tolerance to the early introduction of food proteins. They show an aberrant immune response to food proteins, and eating of food eliciting immune response is associated with adverse symptoms, expressed in skin (atopic dermatitis or urticaria); in gastrointestinal tract (vomiting, diarrhoea, pains) and less frequently in respiratory tract (rhinitis or wheezing). This varying combination of symptoms and immune reaction is referred to as food allergy; it is usually the first sign of developing atopic diseases. Best known immune deviation causing food allergy is hyperactivity of Th2 cells resulting in production of food specific IgE antibodies; other mechanisms resulting particularly in a slow appearance of clinical symptoms after challenge must also operate. The hyperactivity of

Th2 arm most likely results from inappropriate control of these cells by regulatory T-cells operating by various mechanisms; secretion of immunomodulatory cytokines, as IL-10 and TGF- β , and by direct cell-to-cell contacts.

Experimental studies on gut flora and food allergy

Although the pathogenesis of allergy has a strong hereditary component, immunostimulation, particularly by the huge and active commensal bacterial flora in the intestine during early life, is important in directing the development of tolerance. The commensal bacterial flora of the host is probably acting through innate immune system. The importance of toll-like receptors (TLR) in the experimental food allergy was studied in mice with different genetic background (C3H and BALB/c). The deficiency of TLR4 influenced food sensitization and anaphylaxis to bovine β -lactoglobulin (BLG) and whole peanut antigen in a different way and intensity in the different strains. IgE and IgG responses in neither strain

were influenced by TLR4 status, whereas in C3H mice T-cell responses were Th2 skewed in TLR4^{-/-} mice, and this kind of effect not being observed in BALB/c mice. Anaphylaxis severity was increased by TLR4 deficiency in C3H mice, whereas in anaphylaxis resistant BALB/c mice its deficiency did not render the strain susceptible to anaphylaxis [1]. Other TLRs may also play a role as the stimulation of TLR9 by immune modulatory oligonucleotide (IMO) prevented the production of IL-5 and IL-13 and the development of peanut allergy. In the sensitized mice, it prevented the anaphylaxis and reduced inflammation in the gastrointestinal tract [2*].

In the murine model of food allergy, in which mice were orally sensitized with BLG in the presence of cholera toxin, food-induced anaphylaxis was diminished and the production of BLG-specific IgE and IgG1 almost completely abrogated by giving *Lactococcus lactis* transfected to secrete murine IL-10; part of the effect was attributable to the microorganism itself [3**]. In 5-week-old weaned mice, colonization by *Candida albicans* increased the permeability to ovalbumin at 30 min and resulted in increased levels of IgG1 antibodies in 3 weeks and IgE antibodies after 5 weeks of colonization. The increase of the permeability was dependent on the presence of mast cells. The authors discuss that the model fits the possibility of the association between food allergy and increased permeability of the gut [4]. Also in humans, the increased permeability of the gut has been associated with the severity of atopic dermatitis [5]. However, we have described an inverse correlation between the prematurity and development of allergies and lower levels of food antibodies in premature infants, suggesting that the high intestinal permeability of small premature infants, in fact, may result in tolerance to proteins in early foods and lower prevalence of allergies in later childhood [6,7].

Intestinal bacterial flora and allergy

Among 5-year-old Estonian children, those with allergic diseases had less commonly Bifidobacteria in their faeces than those without allergy, whereas Clostridia was more common in allergic children. The proportion of Clostridia was higher and that of Bifidobacteria lower in faeces from allergic children than in samples from nonallergic children. Clostridia counts correlated with the level of serum IgE in allergic children [8]. Japanese infants developing early allergy had different Bifidobacterium species compared with nonallergic infants; they particularly had adult type *Bifidobacterium (catenulatum)* as has been described earlier in another population [9]. Gore *et al.* [10*] studied faecal bacterial communities by culture-independent, nucleic acid-based analyses in infants aged 3–6 months. *Bifidobacterium pseudocatenulatum* was detected more commonly in faeces of infants with eczema (26%) than among control infants (4%); the same bacteria associated with

nonbreastfeeding (39%) compared with those on breastfeeding (9%).

Clinical studies on food allergy and probiotics

Clinical studies have focused on atopic eczema, the first manifestation of an atopic disease. The early small studies [11,12] on infants with atopic eczema showed dramatic alleviating effects of probiotics; however, in more recent studies on larger groups, the effects are weaker or absent. The largest cohort entails 230 infants in a randomized controlled trial (RCT), in which *Lactobacillus rhamnosus* GG (LGG), a mixture of four probiotics or placebo was given for 1 month to infants with atopic eczema. Half of them were diagnosed by double-blind placebo-controlled food challenge (DBPCFC) to have cow's milk allergy (CMA) [13]. Although probiotics had no additional therapeutic effect on atopic eczema in infants with or without CMA, in subgroup analysis LGG compared with placebo was superior in infants with IgE-associated eczema ($P = 0.027$) [13]. In a study on 102 infants [14*], treatment with LGG for 3 months resulted in the same rate of improvement of eczema as placebo in the whole study group and in those with sensitization. In two small RCTs, treatment with LGG for 2–3 months had no effect on the severity of atopic eczema compared with placebo in infants and small children [15,16]. Three months' treatment of young children (median age 4 years) with a combination of *L. rhamnosus* and *Bifidobacteria lactis* improved atopic eczema in the subgroup of food-sensitized children, whereas in the whole study group, there was no difference [17]. The effect did not last after the cessation of the treatment. Passeron *et al.* [18] evaluated the effect of a synbiotic (*L. rhamnosus* Lcr35 and a prebiotic preparation) compared with the prebiotic alone in 2–12-year-old children. Both treatments lasting 3 months reduced eczema severity with no difference between treatments. However, after stopping the treatment, deterioration of skin was observed in almost all patients suggesting that the cure was not due to spontaneous remission of disease [18]. The study, however, lacked a control group.

Four double-blind randomized controlled trials with probiotics to prevent allergy in high-risk families have been published. The first cohort has been followed for 7 years [19*]. Initially, 159 mothers from allergy-risk families were randomized to receive LGG 4 weeks before delivery and after delivery, the breastfeeding mothers continued LGG but bottle-fed infants received LGG until age 6 months. Seventy-three percent of the children completed the 7-year follow-up. The cumulative incidence of atopic eczema was significantly lower in the LGG group [43 vs. 66%; relative risk (RR) 0.64; confidence interval (CI) 0.45–0.92]. Positive skin prick tests (SPTs) were detected in 32% of the children with

no difference between the groups. The incidence of allergic airway diseases was low and similar in the study groups.

In the largest cohort studied [20^{••}], 1223 mothers carrying high-risk children were randomized to use a probiotic preparation containing LGG, *L. rhamnosus* LC705, *Bifidobacterium breve* Bb99 and *Propionibacter freudenreichii* ssp. *shermanii* JS or placebo 4 weeks before delivery. The newborn baby received the same probiotic added with a prebiotic galacto-oligosaccharide ($n=461$) or placebo ($n=464$) for 6 months. The follow-up at 2 years demonstrated significantly less atopic diseases in the probiotic group compared with the placebo group [odds ratio (OR) 0.65 (0.45–0.94)]. Atopic eczema represented 83% of atopic diseases at 2 years in the probiotic group and was reduced by 30% compared with placebo group. The cumulative incidences of atopic eczema were 12 and 18% in the probiotic and placebo groups, respectively [OR 0.61 (0.42–0.90)]. Eczema without sensitization was also reduced [OR 0.69 (0.52–0.93)]. Sensitization to food or airborne allergens was observed in 271 (30%) children with no significant differences between the groups. Studies of the faecal flora proved successful supplementation as Lactobacilli and Bifidobacteria more frequently were found in faeces of supplemented infants.

In another RCT, *Lactobacillus acidophilus* LAVRI-A1 or placebo was given only postnatally to 178 newborns of allergic women until 6 months [21[•]]. At 1 year, 77% completed the study and no differences in the rates of atopic eczema in the probiotic (43%) and placebo (39%) groups were found. Unexpectedly, increased frequency of sensitization was shown in the probiotic group 35/88 (40%) compared with the placebo 21/86 (24%) group. The proportion of children with atopic eczema and positive SPT was also greater in the probiotic group [23/88 (26%) vs. 12/86 (14%), $P=0.045$].

Lactobacillus reuteri was given to mothers from gestational week 36 and to infants during the first year of life. One hundred and eighty-eight of 232 families with allergic disease completed the study [22[•]]. The primary outcome was allergic disease with or without IgE sensitization. The cumulative incidence of eczema was similar, 36% in the probiotic and 34% in the control group. Significantly less IgE-associated eczema in the second year of life, however, was found in the probiotic group (8 vs. 20%; $P=0.02$). SPT reactivity was also less common in the probiotic group (14 vs. 31%; $P=0.02$).

Supplementing bottle-fed high-risk infants with prebiotic oligosaccharides up to 6 months of age resulted in less atopic eczema at 6 months [23]. All infants received a hypoallergenic formula.

Probiotics induce inflammation that supports tolerance

When either LGG or a combination of probiotics were given to the children with suspected CMA, levels of C-reactive protein (CRP) increased during the treatment; the highest and statistically significant increase was seen in the LGG group in which the serum IL-6 levels also increased [24]. In another study [25^{••}], the same combination of probiotics was given together with a prebiotic galacto-oligosaccharide to the infants with family history of allergy. Similarly, levels of CRP were higher in the synbiotic group than in the placebo group at the age of 6 months, the end of intervention period. These results show that probiotics stimulate the innate immune system and induce a low-grade inflammation that may further activate regulatory mechanisms beneficial for the down-regulation of allergic immune response. Increased levels of CRP at 6 months of age were negatively associated with the risk of allergy by the age of 2 years, not only in the synbiotic group but also in placebo group [25^{••}], suggesting that low-grade inflammation is an independent protective factor for allergy. It is possible that CRP, which is a factor limiting inflammation, downregulates the inflammation including allergic inflammation. In our opinion, it is more likely that the low-grade inflammation induced by probiotics activates regulatory mechanisms that protect from allergy.

Treatment of atopic eczema or food allergy with probiotics enhanced the IFN- γ response of peripheral blood mononuclear upon in-vitro stimulation [26,27] at the same time the symptoms were relieved. The children with allergy often show decreased IFN- γ response upon stimulation, which is likely related to their Th2 hyperreactivity or slow maturation of IFN- γ responsiveness. The enhanced IFN- γ response after treatment may be a sign of recovery of the Th1 response in the allergic children. Accordingly, the induction of IFN- γ or CRP by probiotics may be seen only in the group of individuals in whom a defect in these responses is present and the final immunomodulatory effect of probiotics depends not only on the bacterial strains used but also on the immunological status of the host. It should also be emphasized that the colonization of probiotics is transient.

The effects of probiotics are likely mediated through the pattern-recognition receptors of the dendritic cells that are in contact with luminal bacteria in the gut. Niers *et al.* [28] selected four strains of probiotic bacteria among the 13 strains they had studied earlier. The strains induced partial maturation of dendritic cells. In cocultures of *Bifidobacterium bifidum*-matured dendritic cells and T-cells, the proportion of IFN- γ producing T-cells increased and IL-4 producing T-cell decreased. Three of the strains activated TLR-2 [29[•]].

Probiotics may have a different potential to modulate the immune response *in vitro* vs. *ex vivo* [30[•]]. Oral administration of probiotics to children with food allergy resulted in a slightly decreased ex-vivo production of IL-10, TNF- α and IL-6, but the in-vitro stimulation of peripheral blood mononuclear cells in the presence of probiotics-indicated enhanced proliferation response and production of IFN- γ , IL-10 and TNF- α .

Probiotics decrease the antigenic load from intestinal lumen

In our treatment study [31], the children with IgE-mediated-CMA who received probiotics had higher levels of faecal IgA after CMA challenge than children who received placebo. The role of mucosal IgA is to neutralize the luminal antigens and it also participates in the transport of antigens to the gut lumen and decreases the penetrance of antigens from lumen. Together with an earlier study [5], these studies suggest that probiotics could modulate the antigenic load from intestine by their effect on the permeability of the intestinal epithelium and on the production of IgA. In the prevention study [25^{••}], we found that the children who received probiotics had higher levels of serum total IgA than those who received placebo, but the levels of IgA did not associate with the later risk of allergic diseases.

Probiotics and IgE

In clinical studies with beneficial effect [13,19[•],32], probiotics did not modulate IgE responses. In the Swedish prevention study [22[•]], the administration of *L. reuteri* decreased sensitization in a subgroup of the infants with allergic mother, but not in the whole group. Interestingly enough, we found that the infants who received the synbiotic preparation had higher total levels of IgE in serum than infants who received placebo [25^{••}]. This unexpected finding resembled the observations reported in association with helminth infections. Helminth infections are known to induce IgE, but paradoxically inhibit allergic reactions in animal models [33]. In populations with high prevalence of helminth infections, the risk of allergic diseases is low, although the serum levels of IgE in the population are high. The animal models suggest that helminth infections induce both Th2 and regulatory responses, inducing production of IL-10, TGF- β , the latter repressing the allergen-specific Th2 responses and development of allergic symptoms [33]. Although probiotics were associated with increased total IgE levels, IgE levels did not associate with the later development of allergic diseases in our prevention study [25^{••}]. It is reasonable that IgE levels do not correlate with the risk of allergy in this kind of intervention study as the IgE has a complex relation with both intervention, that is, pro-

biotics, and the outcome, that is, allergy. Our findings, however, indicate that the action of probiotics could resemble that of helminth infections; in both cases, the inflammation is associated with the induction of immune regulation that contributes to the survival of the parasite or colonizing bacteria, that is, probiotic, and benefits the host by supporting of oral tolerance.

Probiotics and regulatory T-cells

The role of the Foxp3 positive regulatory CD4CD25^{high} T-cells in allergy is unclear. In humans, naturally occurring thymus derived Foxp3 positive CD4CD25^{high} T-cells mediate suppression of effector T-cells and functional mutation in Foxp3 results in IPEX syndrome including the allergic phenotype [34]. Foxp3 positive CD4CD25^{high} T-cells are also induced in the periphery upon T-cell activation in humans, and these activation-induced Foxp3-expressing cells do not necessarily suppress effector cells [35]. In a prevention study [36[•]], children who later developed atopic eczema showed higher levels of Foxp3 expression after an in-vitro stimulation of peripheral blood lymphocytes (PBMC) with allergens at the age of 6 months than children who did not develop eczema. Probiotic supplementation and gut colonization did not modify these regulatory pathways, or the risk of developing atopic eczema. The activation of Foxp3 was here related to T-cell activation and did not show any clinically beneficial effect [36[•]].

IL-10 secretion by regulatory T-cells may be another mechanism of probiotics, not necessarily linked to activation of Foxp3 pathway. Studies in animal models suggest that probiotics induce IL-10 [33,37^{••}] supporting our findings in humans [24,25^{••}]. We found increased IL-10 levels in plasma in both treatment and prevention studies. Although IL-10 upregulation seems to be associated with probiotics, the source of IL-10 has not been identified in human studies. In animal models, the upregulation of IL-10 induced by probiotics is seen in mesenteric lymph nodes and Peyer's patches [37^{••}]. In an in-vitro study, two out of three strains of Lactobacilli primed human monocyte derived dendritic cells and promoted development of IL-10 secreting regulatory T-cells [38]. These regulatory T-cells inhibited proliferation of bystander T-cells depending on IL-10 production. The effect of these two probiotics on the induction of IL-10 secreting regulatory T-cells was mediated by their binding to C-type lectin dendritic cells-specific intercellular adhesion molecule 3-grabbin nonintegrin, DC-SIGN. Interestingly, the IL-10 responses have been reported to be decreased in atopic children [39] and recovery of IL-10 responses with ability to suppress the effector T-cells could be of importance.

Conclusion

Recent studies using probiotics in the prevention and treatment of food allergy and other allergic diseases show at best a modest effect. The effect is dependent on the strains used, length and mode of treatment. In prevention, the treatment should be given to the mother during late pregnancy and continued longer than practised up to date. Administration of probiotics to children with allergy or at risk of allergy seems to stimulate a low-grade inflammation by activating the innate immune system and production of IL-10.

A broader stimulus with different strains with documented effect given in an alternating fashion for long periods may result in better and more sustained effects both in a prevention and treatment.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 281).

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