

Probiotics prevent allergic diseases in high-risk children

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“...it has been shown that children who develop atopic eczema have a different fecal flora, with more clostridia and less bifidobacteria and lactobacilli than children who remain free of eczema.”

The increased prevalence of allergic diseases has been linked to improved hygienic conditions in Westernized societies. Children who have been in contact with stables with high levels of fecal bacteria and, independently, with farm milk, which also contains bacteria, have fewer allergic diseases at school age [1]. The effect is strongest early during the first year of life, the formative year for immune development. In a multicountry study evaluating children from both anthroposophic homes and farming families, it was shown that farm milk consumption is independently associated with protection from allergy and asthma [2].

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By measuring the bacterial content of feces, which reflects the bacteria present in the GI tract, it has been shown that children who develop atopic eczema have a different fecal flora, with more clostridia and less bifidobacteria and lactobacilli than children who remain free of eczema [3]. Furthermore, children from families that adhere to an anthroposophic lifestyle and those attending Rudolf Steiner schools consume more biodynamic food, which contains lactobacilli, and have less atopy than children from neighboring regular schools [4]. Lactobacilli are found naturally in the human intestine, as well as in dairy products and fermented vegetables, and were part of the normal human diet before refrigeration of food became common.

In clinical allergology, probiotics were first studied in the treatment of atopic eczema, with clear alleviating effects demonstrated [5]. The first allergy-prevention trial using probiotics in families at high risk for allergy was published in 2001, with very promising results [6]. Supplementing the mother with *Lactobacillus rhamnosus* GG (LGG) during the last month of pregnancy and later continuing supplementation during breastfeeding, or directly to the infants if they had not breastfed for 6 months, halved the incidence of atopic eczema. This effect was largely maintained at 4 and 7 years of follow-up [7,8]. However, no effect on respiratory allergies was observed. The set up of this study was replicated in a similarly sized study using the same dose of LGG bacteria, also to a cohort at high risk for allergy in Germany. LGG was given 1 month before delivery and 3 months postnatally to the breastfeeding mother, and from 3 to 6 months postnatally directly to the infant. Surprisingly, no effect on the incidences of atopic eczema could be reported. The reasons for this are unclear. Genetic differences are possible, but are unlikely to be the cause in two Westernized European countries [9].

“Lactobacilli ... were part of the normal human diet before refrigeration of food became common.”

We supplemented mothers in a large cohort in Finland at high risk for allergy with a mixture of four probiotics: LGG (5×10^9), *L. rhamnosus* LC705 (5×10^9), *Bifidobacterium breve* 99 (2×10^8) and

Propionibacter freudenreichii subspecies *shermanii* JS (2×10^9), or placebo, for 4 weeks before delivery, and we supplemented infants directly from birth to the age of 6 months. In the interim analysis at 2 years, the incidence of eczema was reduced from 32 to 26% (odds ratio [OR]: 0.74; 95% CI: 0.55–0.98) and the incidence of atopic eczema (i.e., eczema with positive specific IgE tests against common allergens), representing children with an increased risk for subsequent development of respiratory allergic diseases, was reduced from 18 to 12% (OR: 0.66; 95% CI: 0.46–0.95) [10].

We followed these children until the age of 5 years. Overall, 54% of the children had developed an allergic disease and 33% had developed an IgE-positive allergic (atopic) disease, with no differences in the probiotic- and placebo-treated groups. Eczema had developed in 39.3 and 43.3%, and atopic eczema in 24.0 and 25.1%, of the children in the probiotic and placebo groups, respectively, with no significant differences between groups. Allergic rhinitis was diagnosed in 20%, and asthma in 9%, of children, again with no differences between the two groups [11]. The favorable preventive effects, especially on rates of eczema demonstrated at 2 years, had disappeared by 5 years of age. Whether probiotics at best only postpone the development of eczema in small children or whether this waning effect is due to stopping the probiotic intervention at 6 months of age is not known. After the interrupted favorable immune stimulation offered by probiotics, the immune system may be redirected towards the genetically programed development of an allergic phenotype.

Nonetheless, parents would certainly be grateful if we are able to postpone the development of eczema for up to 2 years, since the treatment of eczema is a challenge in small children, with food allergies complicating the diagnostic evaluation of eczema and treatment implementation.

“Children born via Caesarean section develop more allergic rhinitis and asthma than children delivered vaginally.”

Children born via Caesarean section develop more allergic rhinitis and asthma than children delivered vaginally [12]. We were able to demonstrate a reduced incidence of atopic diseases in children born via Caesarean section who had received probiotic supplementation (24.3% vs 40.5%; OR: 0.47; 95% CI: 0.23–0.96). These children also showed considerably less atopic eczema (15.7% vs 30.4%; OR: 0.43; 95% CI: 0.19–0.95) [11].

We also evaluated the recovery of supplemented bacteria from collected fecal samples. The prevalence and the median counts of the supplemented probiotic bacteria were each significantly higher in the active group at 6 months. Furthermore, when assessing bifidobacteria in feces of children born by Caesarean section and vaginal delivery, the prevalence of bifidobacteria in the placebo group of Caesarean-born children was significantly lower, and this could be corrected by supplementation of the probiotic mix [11]. Cesarean section-delivered children are deprived of the bacterial load from contact with the mother's birth canal, and it is possible that these infants might especially benefit from the supplemented probiotic bacteria with regards to preventing the development of

allergy. The frequency of Caesarean sections in our cohort was 17%, whereas Caesarean sections accounted for up to 30% of all births in recent studies from the USA and Latin America [13,14], strengthening the impact of our observation.

Probiotics stimulate the mucosal immune system when they come into contact with it in the gut lumen. An immune response is raised against the bacteria, although what happens to this immune response if the probiotic bacteria settle in the gastrointestinal lumen is unknown. Probiotics, when passing through the GI tract, can stimulate the immune system all along the length of the intestine, whereas prebiotics specifically promote the growth of a selected species in the colon, thus they only stimulate the immune system in the colon. Therefore, giving bacteria directly to the child might be more effective, and this approach resembles the circumstances in less hygienic environments or mimics the situation that occurs in populations of children consuming farm milk or foods containing probiotics in their normal diet.

“Probiotics, when passing through the GI tract, can stimulate the immune system all along the length of the intestine...”

Whether or not it is possible to permanently change the microflora by early administration of probiotic bacteria remains unresolved. It appears that the microflora is very stable after forming; however, most studies on the development of the commensal flora have used culturing techniques and noncultivable bacteria that are not detected by these methods and that might possess strong immune-stimulating properties. Consequently, more studies evaluating the evolution of these microflora are needed. Recently, culture-independent techniques have become available, using 16S RNA techniques to evaluate the complex commensal flora. Studies evaluating the evolution of the variability of the commensal microflora would especially be of great value. Thereafter, studies evaluating the effect of interventional studies using probiotics on the commensal flora should be performed using large-scale microflora-assessment techniques.

A multicountry study assessing the commensal flora in children from Gothenburg (Sweden), London (UK) and Rome (Italy) was, however, unable to discern any particular bacterial group associated with the development of atopic eczema or food-specific IgE development by 1.5 years of age. Caesarean section-delivered children had a delayed maturation of the intestinal flora, with postponed colonization by *Escherichia coli*, *Bacteroides* spp. and *Bifidobacteria* spp., with other bacteria replacing these [15]. This study demonstrated that persistent colonization by lactobacilli was rare but also that the majority of infants were at least transiently colonized during their first year of life. The follow-up in this study is still short and, as the allergic phenotypes become more established later on, it will be interesting to see if the results persist.

Altering the microflora by administering probiotics appears to be a challenge. The gut is readily colonized by lactobacilli and bifidobacteria when they are administered, as has been shown in several studies [10,11,16]. However, after stopping the administration, they disappear within weeks or months [10]. This has

also been shown in studies where lactobacilli have been given to prevent allergic diseases; after stopping the intervention, the lactobacilli disappear. Giving four different probiotics (LGG, *L. rhamnosus* LC705, *Bifidobacterium animalis* subspecies *lactis* Bb-12 and *P. freudenreichii* subspecies *shermanii* JS) to healthy adults as capsules or in milk products, it was found that, following the intestinal survival of the lactobacilli after stopping the 2 weeks of intervention, LGG had the longest survival time, with 30% of subjects having LGG in their feces 3 weeks after the end of the intervention. Bacteria were detected by quantitative PCR from feces [17].

These results seem to indicate that when attempting allergy prevention by probiotics, the supplementation should be long-lasting – i.e., possibly years or even decades. The immune system can also be altered later in life, not just during early infancy, as has been shown in allergy-treatment studies using probiotics that showed increased levels of C-reactive protein and IL-6 [18,19].

The commensal gut microbiota play an important role in the development of the immune system and can be protective with regard to the development of a variety of immune-related diseases, including allergic diseases. Infants in Westernized countries are colonized more slowly and have a slower turnover of strains when compared with infants living in less hygienic environments. This could mean their immune systems are stimulated to a lesser degree.

Children growing up in Pakistan, an environment with a higher prevalence of germs, showed a very fast turnover of *E. coli* during the first 6 months of life, but strains originating from the mother were much more likely to persist in the infants' flora [20]. If this holds true for lactobacilli and other probiotic bacteria, it is in line with allergy-prevention studies, where positive results have been attained only if the supplementation was started with the mother, and continued to the newborn infant. One allergy-prevention trial using *L. acidophilus* failed to demonstrate an allergy-preventive effect when the intervention was started only after the infant was born [21].

“...mice reared in a germ-free environment have an underdeveloped immune system and do not develop oral tolerance.”

We also know from animal studies that mice reared in a germ-free environment have an underdeveloped immune system and do not develop oral tolerance. Administering bifidobacteria to these mice restores their ability to develop tolerance [22]. However, when bacteria enter the GI tract, they evoke an immune response in the gut immune system, including in the Peyer's patches and germinal centers, and result in the generation of a secretory IgA response. When these nonpathogenic bacteria persist in the gut, becoming commensal, the immune response gradually wanes as a consequence of a successful IgA response [23]. Many lactobacilli and bifidobacteria belong to the normal commensal flora.

The hygienic lifestyle of modern Westernized countries affects the incidence of childhood infections but has also influenced the colonization of the intestine [15,24]. However, the composition of

'healthy' microflora required to lead to sound development of the immune system, with normal oral tolerance and protection from allergies, is unknown at present.

Studies evaluating the gut microbiota from fecal samples have shown delayed colonization of *E. coli* in Caesarean section-delivered children compared with vaginally born children. Traditionally the first colonizers, *Enterobacteriaceae*, were detected late in both vaginally and Caesarean section-delivered children, having instead been replaced by the skin bacteria, staphylococci [24].

Studies on the mechanisms underlying the development of oral tolerance should help us elucidate more about allergic diseases so that we are able to better target immune stimulation with probiotics or other stimuli. Recent evidence indicates that food proteins in breast milk support the development of tolerance, since the administration of airborne ovalbumin to lactating mice led to efficient transfer of ovalbumin via breast milk to the offspring, which induced the development of oral tolerance and the absence of allergic disease in the progeny [25].

“Stronger stimuli, using for instance unpasteurized farm milk, might also lead to stronger immune stimulation and clinical allergy-preventive effects.”

It is possible that with more prolonged probiotic interventions, more sustained and possibly stronger effects could be seen. Stronger stimuli, using for instance unpasteurized farm milk, might also lead to stronger immune stimulation and clinical allergy-preventive effects. However, this entails risks of unwanted and potentially severe systemic infections with *Listeria* or other pathogens. A safer and more controlled approach would be the use of *in-vivo* studied probiotic bacteria, with documented immunologic effects and clinical preventive effects and a favorable safety profile. It is also possible that certain probiotics work better in mothers than they do in infants. Furthermore, a constant change in the given strain might lead to a stronger immune stimulus, directing the immune response away from the development of allergy.

During the course of evolution, humans have adapted to live in close and constant contact with our microflora and this has shaped our immune system. This lifelong symbiotic relationship between the normal flora and ourselves – the host – is important for our wellbeing. This interplay continuously tunes our immune system and can be associated with the absence of immunopathology, meaning no allergies or other immune-related diseases are present. The recent profound changes in our environment and lifestyle have led to less exposure to microbes and helminths. This 'microbial-deprivation' hypothesis, as put forward by Björkstén [26], states that these changes have also affected the development of our immune system, paving the way for the development of allergic and other immune-related diseases. However, there is no way of quickly returning to the unhygienic conditions prevailing 50 years ago in Europe, owing to the dangers of invasive infectious diseases. The addition of probiotics to our diet is one safe and investigated possibility of affecting this imbalance in the indigenous normal flora and of redirecting the immune deviation.

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