

Probiotics and prebiotic galacto-oligosaccharides in the prevention of allergic diseases: A randomized, double-blind, placebo-controlled trial

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Background: The increase in allergic diseases is attributed to a relative lack of microbial stimulation of the infantile gut immune system. Probiotics, live health-promoting microbes, might offer such stimulation.

Objective: We studied the effect of a mixture of 4 probiotic bacterial strains along with prebiotic galacto-oligosaccharides in preventing allergic diseases.

Methods: We randomized 1223 pregnant women carrying high-risk children to use a probiotic preparation or a placebo for 2 to 4 weeks before delivery. Their infants received the same probiotics plus galacto-oligosaccharides ($n = 461$) or a placebo ($n = 464$) for 6 months. At 2 years, we evaluated the cumulative incidence of allergic diseases (food allergy, eczema, asthma, and allergic rhinitis) and IgE sensitization (positive skin prick test response or serum antigen-specific IgE level >0.7 kU/L). Fecal bacteria were analyzed during treatment and at age 2 years. **Results:** Probiotic treatment compared with placebo showed no effect on the cumulative incidence of allergic diseases but tended to reduce IgE-associated (atopic) diseases (odds ratio [OR], 0.71; 95% CI, 0.50-1.00; $P = .052$). Probiotic treatment reduced eczema (OR, 0.74; 95% CI, 0.55-0.98; $P = .035$) and atopic eczema (OR, 0.66; 95% CI, 0.46-0.95; $P = .025$). Lactobacilli and bifidobacteria more frequently ($P < .001$) colonized the guts of supplemented infants.

Conclusion: Probiotic treatment showed no effect on the incidence of all allergic diseases by age 2 years but significantly prevented eczema and especially atopic eczema. The results suggest an inverse association between atopic diseases and colonization of the gut by probiotics.

Clinical implications: The prevention of atopic eczema in high-risk infants is possible by modulating the infant's gut microbiota with probiotics and prebiotics. (*J Allergy Clin Immunol* ■■■■;■■■:■■■-■■■.)

Key words: Allergy, prevention, probiotic, prebiotic, eczema, atopic eczema, high-risk infants, colonization

Improved hygiene and decreased exposure of the immature immune system to microbes in early life are implicated in the increase in allergic diseases in developed countries during recent decades.¹

Gut microbiota are the prerequisite for induction of oral tolerance,² and in infants features of the microbiota might relate to the establishment of allergies and IgE antibodies.³ The colonization of the gut that begins promptly after birth is affected by mode of delivery, maternal gut microbiota, and the early environment.⁴ After this initial colonization, diet affects the selection of bacterial strains. Breast milk contains plentiful indigestible oligosaccharides, which pass through the whole intestine and promote the growth and activity of commensal bacteria, mainly of bifidobacteria.^{5,6}

A promising approach in genetically high-risk infants seems to be prevention of allergic diseases by dietary supplementation of live commensal health-promoting bacteria, probiotics.⁷ Probiotic bacteria, which enhance mucosal barrier function, participate in degradation of protein antigens, and compete with pathogenic bacteria, promote early immune system maturation toward nonallergy and alleviate symptoms of eczema.⁸⁻¹¹ However, larger clinical trials are necessary to demonstrate probiotic effects in allergy prevention. Therefore in a large cohort of allergy-prone infants, we studied whether dietary supplementation of a combination of 4 probiotic strains with prebiotic galacto-oligosaccharides protects infants against allergic diseases.

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Abbreviations used

cfu: Colony-forming unit

OR: Odds ratio

METHODS**Participants**

Pregnant mothers carrying children at increased risk for allergy were recruited from antenatal clinics or through advertisements in the Helsinki suburban area, with its population of 900,000 and birth rate of 13,000 per year, to an allergy-prevention trial at the Skin and Allergy Hospital of Helsinki University. Mothers were eligible if at least one parent of the unborn child had a physician-diagnosed allergic disease, as evaluated in telephone interviews by trained personnel. Infants were born between November 2000 and March 2003. Infants born at less than 37 weeks of gestation, infants with major malformations, and B-twins were excluded.

Protocol

This randomized, double-blind, placebo-controlled study with 2 parallel groups used computer-generated block randomization to probiotic and placebo groups at 35 gestational weeks. In the probiotic group, during the 2 to 4 weeks before delivery, mothers twice daily took 1 capsule containing *Lactobacillus rhamnosus* GG(ATCC 53103), 5×10^9 colony-forming units (cfu); *L. rhamnosus* LC705 (DSM 7061), 5×10^9 cfu; *Bifidobacterium breve* Bb99(DSM 13692), 2×10^8 cfu; and *Propionibacterium freudenreichii* ssp. *shermanii* JS(DSM 7076), 2×10^9 cfu. Their newborn infants received 1 opened capsule containing the same probiotics mixed with 20 drops of sugar syrup containing 0.8 g of galacto-oligosaccharides once daily for 6 months after birth. In the placebo group mothers and their infants took capsules containing microcrystalline cellulose, and the infants received sugar syrup without galacto-oligosaccharides, all scheduled exactly as in the probiotic group. The products (supplied by Valio, Helsinki, Finland), which looked, smelled, and tasted identical, were delivered in numbered packages directly to the mothers for their use over a 4-week period and for the infants' use during the first 3 months. The rest was offered at the 3-month visit. The packages containing the capsules were stored frozen until opened and then refrigerated. The supplier regularly checked the viability of the bacteria in the capsules. The parents were instructed to bring the remaining products to the study visits and not to feed other probiotic preparations to the infants during the intervention.

At 3, 6, and 24 months, a pediatrician blinded to group assignment carefully recorded the infant's history of symptoms related to allergic diseases and clinically examined the infants. The parents of infants with any symptoms exhibited between these study visits were urged to contact the researchers. Any notification by parents during the follow-up period led to clinical evaluation.

At 3 months, the parents responded to questionnaires concerning pregnancy, birth, and parental education, and at 3, 6, 12, and 24 months responded to questionnaires concerning nutrition, symptoms of infantile colic, symptoms of allergic diseases, infections, antibiotic use, family size, day care, parental smoking, environment, and household pets.

Normal adapted cow's milk-based formula replaced breast milk when breast-feeding was insufficient. The use of hypoallergenic formula was restricted to infants allergic to cow's milk.

Compliance to the treatment was evaluated at 3 and 6 months by questioning the amount of doses not given and by counting the returned capsules.

The Ethics Committee of the Hospital for Children and Adolescents of Helsinki University approved the study protocol. Mothers signed a written informed consent form.

Outcome measures

Our main outcome measures were cumulative incidence of any allergic disease (food allergy, eczema, asthma, and allergic rhinitis) and IgE-associated (atopic) disease at age 2 years. Our secondary outcome measures were eczema and IgE sensitization.

Food allergy was diagnosed with an open food challenge performed in infants ($n = 124$) whose food-related symptoms (urticaria, eczema, excessive crying, vomiting, loose stools, poor weight gain, or abdominal pain) improved during a 2-week elimination diet.¹² Eczema was diagnosed according to the UK Working Party's criteria (an itchy skin condition plus ≥ 3 of the following: history of atopic disease in the family, dry skin during the last year, history of eczema, or visible eczema involving typical sites) and included both nonatopic and atopic eczema.¹³ Eczema was considered atopic only if IgE associated.¹⁴ Severity of eczema was evaluated by using the SCORAD score.¹⁵ Asthma was defined as 2 or more physician-diagnosed wheezing episodes accompanied by persistent cough or exercise-induced symptoms (including giggling and crying).¹⁶ The study pediatrician checked patient records when parents reported physician-diagnosed wheezing. Allergic rhinitis was defined as antigen-specific sensitization with a history of 2 or more symptoms (nasal discharge, blockage, and sneeze/itch) recurrently during antigen contact.¹⁷ Any allergic disease combined with a positive skin prick test response or serum antigen-specific IgE levels of greater than 0.7 kU/L at 2 years was considered IgE associated.

The skin prick tests were performed on the volar surface of the forearm with separate sterile lancets (ALK-Abellö, Hørsholm, Denmark). We used histamine dihydrochloride (10 mg/mL) as a positive control and the solvent (glycerin) as a negative control (ALK-Abellö). Skin prick tests were performed at 6 months ($n = 905$) with allergen extracts of egg white (1000 IC/mL; Stallergenes, Antony, France) and at 2 years ($n = 914$) with egg white, fish (1000 IC/mL; Stallergenes), cat, dog, birch, timothy (10 HEP; ALK-Abellö), cow's milk, and wheat grains (diluted in 0.9% sodium chloride). The wheal size was calculated as the mean of the longest diameter and its orthogonal diameter at 15 minutes. A wheal of 3 mm or more larger than the negative control was considered positive (the European Academy of Allergy and Clinical Immunology recommendation).¹⁸

At 2 years, a blood sample was drawn ($n = 687$) with parental permission. Anesthetic cream (AstraZeneca, Södertälje, Sweden) was applied to the puncture site before sampling. Centrifuged serum samples were stored at -50°C . Serum allergen-specific IgE levels against cow's milk, egg white, birch, timothy, dog, and cat were measured ($n = 684$) by means of immunoassay (ImmunoCAP System; Pharmacia Diagnostics, Uppsala, Sweden), with a detection limit of 0.01 kU/L. A concentration of greater than 0.7 kU/L was considered positive. Serum total IgE levels were measured by using the same immunoassay, with a detection limit of 2 kU/L.

In randomly selected infants we analyzed bacterial colonization of meconium ($n = 131$) and of fecal samples at 3 ($n = 98$), 6 ($n = 99$), and 24 ($n = 89$) months. Samples were frozen at -20°C within 15 minutes, brought frozen to the hospital, and stored at -40°C until analyzed for concentrations of lactic acid bacteria (MRS agar, LabM; International Diagnostics Group, Lancashire, United Kingdom) and bifidobacteria (Raffinose-bifidobacterium agar).¹⁹ Concentrations of the 2 *L. rhamnosus* strains were determined on MRS-vancomycin agar, and concentrations of *P. freudenreichii* JS were found on modified YEL agar.²⁰ The detection limit was 10^3 cfu/g feces. The 2 *Lactobacillus* strains and *P. freudenreichii* JS strain were identified

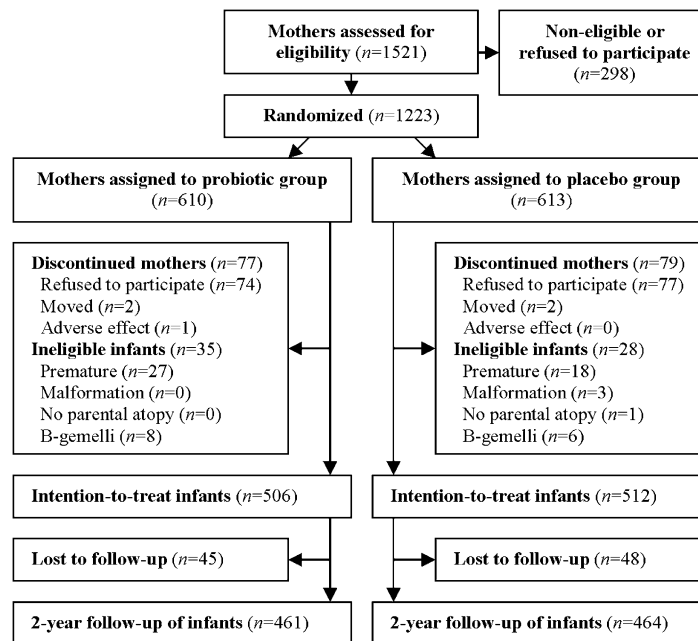


FIG 1. Diagram showing the flow of participants in the probiotic and placebo groups.

by means of random amplified polymorphic DNA. The PCR was performed with the Dynazyme polymerase kit (Finnzymes, Espoo, Finland), and random primers 5'AGTCAGCCAC3' and 5'ACGC-GCCCT3' were used for *L. rhamnosus* GG and LC705 and 5'CGAGCCGTC3' and 5'AGTCAGCCAC3' were used for *P. freudenreichii* JS. Primers were used at a concentration of 3 μ M and deoxynucleotides were used at 200 μ M. Initial denaturation was at 94°C for 2 minutes and a further 40 cycles at 94°C for 15 seconds, 37°C for 30 seconds (for *L. rhamnosus* GG and LC705), 30°C for 30 seconds (for strain JS), and 72°C for 2 minutes. The random amplified polymorphic DNA bands were separated in 1.5% agarose by means of gel electrophoresis.

Statistical analysis

Sample-size calculation was based on an expected 40% cumulative incidence of allergic diseases at age 5 years. To detect a 10% absolute reduction (odds ratio [OR], 0.64) by probiotics at a 5% significance level and with 90% power, the estimated size of each group was 597, which allowed a 20% dropout rate. The randomization code was kept by the database consultant and revealed only to the statistician (T.P.). Logistic regression analysis was performed to compare the cumulative incidences of allergic diseases at the end of the 2-year follow-up period. The results are given as ORs with 95% CIs. Potential confounders were first explored by using the Breslow-Day test, and stratified analyses were then performed. Multivariable logistic regression was used to adjust the outcomes for possible confounders: sex, delivery by means of cesarean section, breastfeeding, use of antibiotics during the intervention, and regular (daily or ≥ 2 days a week) use of probiotics after the intervention. An independent-samples *t* test was used for logarithmically transformed serum total IgE levels. Group comparisons are given as the ratio of probiotic to placebo. Intergroup fecal bacterial counts were analyzed by using the Mann-Whitney *U* test, and colonization differences were analyzed by using the χ^2 test, with results given as risk ratios and 95% CIs. Data were analyzed with SPSS, version 13.0 (SPSS Inc, Chicago, Ill).

RESULTS

Participants

Of the 1223 randomized mothers, 156 discontinued during pregnancy, and 63 of the newborn infants were ineligible (Fig 1). The intention-to-treat analysis comprised the 925 eligible infants who completed the 2-year follow-up period (Fig 1). Of them, sensitization was measured in 916 (99%). In the probiotic group 45 infants and in the placebo group 48 infants were lost to follow-up. Infants' baseline demographics and clinical characteristics were quite similar in the study groups (Table I). Infants were treated for a mean of 161 days in the probiotic group and 162 days in the placebo group. The 80% compliance level (≥ 144 treatment days) was 87% in the probiotic group and 88% in the placebo group. Some commercially available probiotic product was given during the intervention by error to 7 infants in the probiotic group and to 13 infants in the placebo group.

Allergic diseases and IgE sensitization

Probiotic treatment compared with placebo had no effect on the cumulative incidence of any allergic disease (OR, 0.85; 95% CI, 0.64-1.12). Of the study population, 29.2% had eczema, 7.8% had food allergy, 3.2% had asthma, and 1.4% had allergic rhinitis. IgE-associated (atopic) diseases tended to occur less frequently in the probiotic group (OR, 0.71; 95% CI, 0.50-1.00; Table II).

Eczema occurred less frequently in the probiotic group (OR, 0.74; 95% CI, 0.55-0.98). Relative risk reduction was 26%. Among infants with eczema, the mean highest SCORAD score at any visit was 20.1 (95% CI, 17.5-22.6) in the probiotic group and 20.5 (95% CI, 18.2-22.7) in the

placebo group. Probiotic treatment more pronouncedly reduced the incidence of atopic eczema (OR, 0.66; 95% CI, 0.46-0.95). Relative risk reduction was 34%. Eczema represented 88% of all allergic diseases and 92% of all IgE-associated diseases (Table II).

Probiotic treatment compared with placebo had no effect on sensitization at 6 months (12.7% vs 13.6%; OR, 0.92; 95% CI, 0.63-1.36) or at 2 years (Table II). The geometric mean serum total IgE level was 25.7 kU/L (95% CI, 22.2-29.8 kU/L) in the probiotic group and 27.2 kU/L (95% CI, 23.1-32.0 kU/L) in the placebo group (probiotic/placebo ratio, 0.95; 95% CI, 0.76-1.18).

When adjusted for possible confounders, the incidences of any IgE-associated disease, eczema, and atopic eczema were lower in the probiotic group compared with those in the placebo group (Table II).

Subgroup analysis

Boys were sensitized at a 32.4% (29.8% probiotic and 35.0% placebo) rate and girls at a 26.9% rate (26.2% probiotic and 27.5% placebo). Of boys, 28 (12.2%) of 229 in the probiotic group and 43 (19.0%) of 226 in the placebo group had atopic eczema (OR, 0.59; 95% CI, 0.35-0.99). In these respective groups, among girls, atopic eczema occurred in 29 (12.6%) of 230 and 39 (16.5%) of 237 (OR, 0.73; 95% CI, 0.44-1.23). The geometric mean serum total IgE level was higher in boys than in girls ($P < .001$).

Fecal colonization

Meconium samples contained bifidobacteria in 11 of 61 infants in the probiotic group and in 8 of 70 infants in the placebo group but contained no lactobacilli or propionibacteria. At 3 and 6 months, the probiotic group was significantly more frequently colonized with lactobacilli and propionibacteria ($P < .001$), and fecal counts of all the supplemented microbes, including bifidobacteria, were significantly higher. Group differences at 3 months (data not shown) were consistent with those at 6 months. At 2 years, we observed no differences between study groups in fecal bacterial colonization (Table III).

Side effects of the treatment

In the probiotic and placebo groups abdominal discomfort occurred in, respectively, 35 and 37 infants, vomiting in 7 and 12, and excessive crying in 13 and 9. Of the families, 33 (16 probiotic and 17 placebo) considered the treatment protocol difficult to follow, and 6 (2 probiotic and 4 placebo) infants discontinued the treatment because of major difficulties in swallowing the product.

DISCUSSION

Treatment of pregnant mothers and their high-risk infants with a combination of probiotics and prebiotics showed no overall preventive effect on allergic diseases by age 2 years but tended to reduce IgE-associated (atopic) diseases. Probiotic treatment significantly reduced eczema, from 32% to 26%, and IgE-associated eczema, from

TABLE I. Clinical characteristics and demographic data of infants in the probiotic ($n = 461$) or placebo ($n = 464$) groups

	Probiotic group	Placebo group
Male sex (%)	230 (50)	226 (49)
Birth weight (g)*	3595 $\pm$ 477	3591 $\pm$ 482
Delivery by cesarean section (%)	75 (17) [451]	79 (17) [453]
Maternal allergy (%)	370 (80)	376 (81)
Paternal allergy (%)	266 (58)	266 (57)
Both parents allergic (%)	175 (38)	178 (38)
Exclusively breast-fed (mo)*	2.6 $\pm$ 1.9 [450]	2.4 $\pm$ 2.0 [457]
Breast-fed (duration in mo)*	8.6 $\pm$ 5.4 [454]	8.2 $\pm$ 4.9 [459]
Maternal smoking (%)	73 (16)	59 (13)
Firstborn child in the family (%)	269 (58) [460]	243 (52)
Age (mo) at initiation of day care*	15 $\pm$ 4 [235]	15 $\pm$ 4 [245]
Furred pets at home (%)	92 (20)	88 (19)
Antibiotics during intervention (%)	104 (23)	132 (28) [463]

Sample sizes are shown in brackets if different from those shown in the heading.

*Mean $\pm$ SD.

18% to 12%. The number needed to treat to prevent eczema was 16.

Our large study, with its successfully selected high-risk population and high follow-up rate, elucidates the role of probiotics in allergy prevention. In the first prevention trial on 132 high-risk infants, *L rhamnosus* GG alone halved the incidence of eczema,⁷ whereas in our study its cumulative incidence decreased 20%. In our study eczema was more severe, and its cumulative incidence by age 2 years was lower.

Our main outcome consisted of diseases manifesting at various ages. Eczema is the most common allergic disease in infancy, whereas allergic airway diseases usually manifest later. In the present study scant airway allergies at age 2 years did not yet allow for their comparison.

Our diagnostic criteria were strict and based on confirmation by the study pediatrician, which might have contributed to the lower incidences of allergic diseases in this study than in studies based on parentally reported symptoms or on parental reports of physicians' diagnoses. A large proportion of wheezing before age 2 years is related less to asthma than to viral infections. Our 3.2% incidence of recurrent wheezing with persistent symptoms is in line with the 2.7% incidence of frequent wheezing seen in high-risk infants before age 3 years.²¹

We chose the probiotic mixture to broadly stimulate the mucosal immune system. The diversity of gut microbiota is central to the development of mucosal tolerance,^{1,4} and in animal studies mixtures of gut microbial species have more effectively stimulated the immune system than have single strains.^{22,23} *In vitro*, however, probiotic effects have been strain specific, and various strains have counteracted each other.²⁴ In infants with atopic eczema, treatment with a mixture of 4 probiotic strains showed

TABLE II. Cumulative incidence of allergic diseases and sensitization at age 2 years in infants having received a combination of probiotics and prebiotic galacto-oligosaccharides or a placebo during 6 months from birth

	Probiotic		Placebo		Unadjusted		Adjusted*	
	n	%	n	%	OR (95% CI)	P value†	OR (95% CI)	P value‡
Primary outcome								
Allergic disease‡	145/461	31.5	163/464	35.1	0.85 (0.64-1.12)	.236	0.82 (0.61-1.08)	.159
IgE-associated allergic disease§	64/456	14.0	87/463	18.8	0.71 (0.50-1.00)	.052	0.65 (0.45-0.94)	.022
Secondary outcome								
Eczema	120/461	26.0	150/464	32.3	0.74 (0.55-0.98)	.035	0.69 (0.52-0.93)	.015
Atopic eczema	57/459	12.4	82/463	17.7	0.66 (0.46-0.95)	.025	0.61 (0.42-0.90)	.012
Sensitization¶	127/454	28.0	144/462	31.2	0.86 (0.65-1.14)	.289	0.82 (0.61-1.10)	.184

Values in boldface are statistically significant.

*Adjusted by means of multivariable logistic regression for sex, delivery by means of cesarean section, breast-feeding for at least 6 months, use of antibiotics during the intervention, and regular (daily or ≥ 2 days a week) use of probiotics after the intervention.

†The P value was calculated by using logistic regression analysis.

‡Food allergy, eczema, asthma, or allergic rhinitis.

§Food allergy, eczema, asthma, or allergic rhinitis in infants with positive skin prick test responses or serum antigen-specific IgE levels of greater than 0.7 kU/L.

||Eczema in infants with positive skin prick test responses or serum antigen-specific IgE levels of greater than 0.7 kU/L.

¶Positive skin prick test response or serum antigen-specific IgE level of greater than 0.7 kU/L.

TABLE III. Prevalence and median (interquartile range) of log counts of probiotic bacteria in feces (colony-forming units per gram) in the treatment groups at ages 6 and 24 months

Bacterial strain	Months	Prevalence (%)				Counts*		
		Probiotic	Placebo	RR (95% CI)	P value†	Probiotic	Placebo	P value‡
<i>L. rhamnosus</i> GG	6	91.3	23.1	3.96 (2.39-6.55)	<.001	7.47 (6.17-8.18)	0 (0-0)	<.001
	24	41.5	55.3	0.75 (0.48-1.17)	.195	0 (0-5.10)	3.54 (0-4.99)	.571
<i>L. rhamnosus</i> LC705	6	54.3	0	28.3 (3.98-200)	<.001	4.42 (0-7.00)	0 (0-0)	<.001
	24	24.4	16.7	1.46 (0.64-3.36)	.336	0 (0-3.32)	0 (0-0)	.288
<i>Propionibacterium</i> JS	6	55.3	3.8	14.4 (3.61-57.3)	<.001	3.74 (0-5.98)	0 (0-0)	<.001
	24	14.6	12.5	1.17 (0.41-3.35)	.769	0 (0-0)	0 (0-0)	.966
Bifidobacteria total	6	97.9	86.5	1.13 (1.01-1.27)	.039	9.3 (8.76-9.77)	8.5 (7.12-9.40)	.001
	24	90.2	97.9	0.92 (0.83-1.03)	.117	8.53 (8.00-8.89)	8.06 (7.40-8.98)	.338
Lactobacilli total	6	97.9	55.8	1.75 (1.37-2.24)	<.001	7.84 (6.92-8.45)	4.44 (0-7.78)	<.001
	24	70.7	72.9	0.97 (0.75-1.26)	.819	4.94 (0.0-6.42)	4.29 (0.0-5.34)	.318

Values in boldface are statistically significant.

RR, Risk ratio.

*Values less than the detection limit of 2.7 equal 0.

† χ^2 Test.

‡Mann-Whitney U test.

no effect, whereas *L. rhamnosus* GG induced a low-grade, systemically detectable inflammation and alleviated symptoms.²⁵

Supplemented infants harbored lactobacilli, bifidobacteria, and propionibacteria in their guts significantly more frequently than did those not supplemented, thus indicating successful intervention and good adherence to the treatment. In epidemiologic studies the presence of fewer lactobacilli and bifidobacteria in the neonatal gut flora preceded the development of atopic diseases.³ Our prospective study indicates, for the first time to our knowledge, an inverse association between the prevalence of eczema and the abundance of certain species of the indigenous gut microbiota, thus supporting the important role of commensal bacteria in the pathogenesis of eczema.

Of infants with any allergic disease, 88% exhibited eczema, and of infants with any IgE-associated disease,

92% exhibited eczema, to which the preventive effect seemed confined. Food allergies are central in the pathogenesis of atopic eczema, and targeting by probiotics of the enteric mucosa, the primary route of food antigen contact and sensitization, might influence mechanisms crucial in the pathogenesis of eczema.

The probiotic effect in alleviating eczema has been pronounced in IgE-sensitized (atopic) children.^{9-11,26} Atopic eczema has a strong genetic preference and occurs early in the atopic march to respiratory allergies.²⁷ Nonatopic infants with eczema are a more heterogeneous group, a proportion of which becomes sensitized by age. Sensitized infants with eczema seem to represent the true atopic population with more persistent eczema and more airway allergies.²⁸ Therefore our reduction in atopic eczema by age 2 years might predict fewer allergic diseases later in life.

That probiotics especially affect IgE-associated diseases is also supported by the observation that boys benefited from the treatment more than did girls; the boys' total IgE level was also higher. Compared with girls, boys more frequently produce IgE antibodies²⁹ and have atopic eczema.²⁷ How sex relates to the pathogenesis of atopic diseases remains unknown, and our findings might offer new insight for further studies.

The treatment showed no effect on sensitization, which is consistent with the results of the first prevention trial.⁷ Although early sensitization predicts the development of clinical disease, several sensitized infants remain asymptomatic; hence the role of IgE in the establishment of allergic diseases remains unclear.²⁸ Helminthic infections that intensely induce T_H2 cytokines and IgE synthesis protect IgE-sensitized infants from atopic diseases.^{30,31} Likewise, probiotics seem not to affect sensitization *per se* but regulate the path from sensitization to clinical disease, thus, as this study shows, increasing the proportion of asymptomatic infants.

Gut microbiota contact directly with extensions of dendritic cells, which orchestrate the mucosal immune homeostasis. Commensal bacteria stimulate the innate immune system and contribute to the generation of regulatory lymphocytes, which, through IL-10 and TGF- β , establish and maintain mucosal immune tolerance.²³ *L. rhamnosus* weakly stimulates dendritic cell maturation.³² Its peptidoglycan cell wall binds to Toll-like receptor 2³³ and induces the expression of proinflammatory and anti-inflammatory cytokines.³⁴ Probiotic strains that stimulate the expression of regulatory cytokines have effectively prevented or alleviated eczema.^{7,8,35} Interestingly, in infants with eczema, the same combination of probiotic bacteria as used in this study induced the secretion of IL-10.²⁵

Allergy prevention is undergoing a change from avoidance of environmental antigens to active induction of tolerance, where probiotics might play a role. In high-risk infants probiotics, which are easily accessible, inexpensive, and safe, seem to provide protection against atopic eczema, although weaker than previously reported.⁷ This present large prospective intervention study indicates an inverse association between modification of the indigenous gut microbiota and the prevalence of eczema, especially when IgE associated. Whether this effect endures and extends to airway allergies later in life remains to be explored in follow-up studies on these infants.

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