

Probiotics prevent IgE-associated allergy until age 5 years in cesarean-delivered children but not in the total cohort

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Background: Less microbial exposure in early childhood is associated with more allergic disease later. Allergic children have a different fecal microflora, with less lactobacilli and bifidobacteria. Beneficial effects regarding the development of allergy have been suggested to come through probiotic supplementation.

Objective: We sought to study the effect of probiotic and prebiotic supplementation in preventing allergies.

Methods: In a double-blinded, placebo-controlled study we randomized 1223 mothers with infants at high risk for allergy to receive a probiotic mixture (2 lactobacilli, bifidobacteria, and propionibacteria) or placebo during the last month of pregnancy and their infants to receive it from birth until age 6 months. Infants also received a prebiotic galacto-oligosaccharide or placebo. At 5 years, we evaluated the cumulative incidence of allergic diseases (eczema, food allergy, allergic rhinitis, and asthma) and IgE sensitization.

Results: Of the 1018 intent-to-treat infants, 891 (88%) attended the 5-year visit. Frequencies of allergic and IgE-associated allergic disease and sensitization in the probiotic and placebo groups were similar: 52.6% versus 54.9% and 29.5% versus 26.6%, respectively, and 41.3% in both. No significant difference appeared in frequencies of eczema (39.3% vs 43.3%), atopic eczema (24.0% vs 25.1%), allergic rhinitis (20.7% vs 19.1%), or asthma (13.0% vs 14.1%) between groups. However, less IgE-associated allergic disease occurred in cesarean-delivered children receiving probiotics (24.3% vs 40.5%; odds ratio, 0.47; 95% CI, 0.23 to 0.96%; $P = .035$).

Conclusions: No allergy-preventive effect that extended to age 5 years was achieved with perinatal supplementation of probiotic bacteria to high-risk mothers and children. It conferred

protection only to cesarean-delivered children. (*J Allergy Clin Immunol* ■■■■;■■■:■■■-■■■.)

Key words: Allergy, prevention, high-risk infants, probiotic, prebiotic, eczema, allergic rhinitis, asthma, cesarean

During past decades, the incidence of childhood allergies in the westernized world has hugely increased, making allergies a major health issue. This increase has been attributed to less microbial exposure, especially in early childhood: the hygiene hypothesis. This is demonstrated in many farm studies in which children's exposure to larger numbers of microbes is associated with fewer allergic diseases at school age.^{1,2} Consumption of farm milk might independently protect against development of allergies.³

The large microflora of the gut is important as a stimulatory factor for immune system development. Mice reared in germ-free conditions have an underdeveloped immune system and have no oral tolerance, whereas pathogen-free mice are capable of tolerance development, and reconstitution of the bacterial flora with bifidobacteria restores the ability for tolerance development.⁴ Furthermore, infants with allergy have gut microbiota distinctly different from those of infants remaining nonallergic.⁵⁻⁷ Prevention of allergy by means of probiotic administration to change the infantile gut microbiota was first reported by Kalliomäki et al,⁸ resulting in a marked reduction in atopic eczema persisting to age 7 years.⁹

In a large cohort we earlier demonstrated 29% less atopic disease in the interim analysis at 2 years. This was mainly due to less eczema, the phenotype of allergic disease most prevalent in this age group.¹⁰ Other studies have shown less atopic eczema with supplementation with *Lactobacillus rhamnosus* GG (LGG)⁹ and *Lactobacillus reuteri*.¹¹ Two studies failed, however, to demonstrate less allergic disease with their supplementation of *Lactobacillus acidophilus*¹² and LGG.¹³ A study with prebiotic oligosaccharides showed less atopic eczema at 6 months.¹⁴ Clearly, data on the possibility to prevent the development of allergic diseases, sensitization, or both are contradictory and insufficient to allow recommendations.

In a randomized trial of 1223 high-risk families, the pregnant mothers, before delivery, ingested a probiotic mixture of 4 strains, and their infants received the same probiotics and prebiotics for 6 months from birth. We followed these children until age 5 years for the development of allergic diseases.

METHODS

Pregnant mothers whose fetuses were at high risk for allergy (at least 1 parent with doctor-diagnosed asthma, allergic rhinitis, or atopic eczema) were randomized at 35 weeks of gestation to receive probiotics or placebo in a double-blind manner. From 36 weeks of gestation, mothers in the probiotic

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Abbreviations used

cfu: Colony-forming units
LGG: *Lactobacillus rhamnosus* GG
SPT: Skin prick test

group took a capsule containing freeze-dried LGG (American Type Culture Collection 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) twice daily. Their infants received the same probiotic capsule opened and mixed with 20 drops of syrup containing 0.8 g of galacto-oligosaccharides once daily during the 6 months from birth. The placebo group mothers and infants received capsules containing microcrystalline cellulose, and the infants also received sugar syrup without galacto-oligosaccharides. The capsules and syrups, supplied by Valio, Ltd (Helsinki, Finland), looked, smelled, and tasted identical. The viability of the bacteria was regularly checked. Postrandomization exclusion criteria were birth at less than 37 weeks of gestation, major malformations, and the second born of twins.

A pediatrician examined the children at ages 3 months, 6 months, 2 years, and 5 years. At 3 months, 6 months, and 1, 2, 3, 4, and 5 years, the parents completed and returned questionnaires inquiring into any symptoms related to allergic and infectious diseases, use of antibiotics, and nutrition, socioeconomic, and environmental issues, such as family size, household pets, and day care. The parents were asked to contact the study nurse concerning any allergic symptoms; this led to clinical evaluation by the study pediatrician. Hypoallergenic formulas were not used prophylactically, and commercial infant's formulas contained neither probiotics nor prebiotics. Compliance with the intervention was assessed at the 3- and 6-month visits by queries and by the counting of returned unused capsules. The children's growth was followed. The Ethics Committee of the Hospital for Children and Adolescents of Helsinki University approved the study. Parents provided their written consent.

The primary outcome measure was the cumulative incidence of any allergic disease and any IgE-mediated allergic disease (atopic) until age 5 years. Secondary outcome measures were eczema, food allergy, and asthma with and without IgE sensitization, allergic rhinitis, and IgE sensitization alone.

Eczema was diagnosed according to the Williams UK Working Party's criteria,¹⁵ which meant an itchy skin plus 3 or more of the following: family history of atopic disease, dry skin during the previous 12 months, history of eczema, or visible eczema at typical sites. Food allergy was diagnosed with an open food challenge if food-related symptoms of urticaria, eczema, vomiting, loose stools or diarrhea, or excessive crying had resolved with a 2-week elimination diet.¹⁶ Asthma was diagnosed if the child had 2 doctor-diagnosed wheezing episodes plus continuous cough or exercise-induced symptoms or verified reversible bronchial obstruction in oscillometry. The study pediatrician verified patient records concerning wheezing episodes. Allergic rhinitis was defined according to the Allergic Rhinitis and its Impact on Asthma (www.whiar.org) guidelines as 2 or more symptoms of nasal discharge, blockage, and sneeze/itch recurrently during antigen contact and antigen-specific IgE sensitization.¹⁷ Any allergic disease combined with a positive skin prick test (SPT) response or serum-specific IgE test result (>0.7 kU/L) was IgE associated.

SPTs were performed on the forearm (histamine chloride as a positive and glycerin as a negative control) to cat, dog, birch, timothy, mugwort, *Dermaphagoides pteronyssinus*, cow's milk, egg, wheat, and peanut at 2 and 5 years with commercial solutions (ALK-Abellö, Hørsholm, Denmark, or Stallergenes, Antony, France) or fresh food dilutions with 0.9% sodium chloride. A wheal diameter of 3 mm or greater than the negative control was considered positive according to the European Academy of Allergy and Clinical Immunology recommendations.¹⁸

Blood samples were drawn from consenting subjects at 2 ($n = 687$) and 5 ($n = 737$) years. Specific IgE antibodies were analyzed against milk, egg white, birch, timothy, cat and dog, peanut, and *D pteronyssinus* by using the ImmunoCAP system (Phadia, Uppsala, Sweden). We also analyzed hemoglobin concentration and red blood cell indices and leukocyte and platelet counts.

TABLE I. Characteristics of study children

Characteristic	Probiotic (n = 445)	Placebo (n = 446)
Female	49.7%	51.3%
Birth weight (g), mean (SD)	3592 (472)	3586 (481)
Age, mother (y), mean (SD)	30.8 (4.8)	31.5 (4.6)
Maternal atopy	80.9%	81.6%
Paternal atopy	58.6%	58.1%
Both parents atopic	39.3%	39.7%
Cesarean delivery	15.7%	17.7%
Siblings	41.4%	47.3%
Exclusive breast-feeding >3 mo	47.2%	43.9%
Exclusive breast-feeding >5 mo	4.0%	2.9%
Breast-feeding duration ≥ 6 mo	70.8%	67.3%
Solids started ≥ 4 mo	67.6%	67.0%
Mother smoking	14.4%	11.9%
Smoking at home	31.5%	30.3%
Mother smoking during pregnancy	0.7%	2.0%
Dog/cat in home	16.6%	15.5%
University-educated mother	33.0%	40.2%
University-educated father	34.8%	37.8%

In a randomly selected subsample, we analyzed fecal samples taken at birth ($n = 131$) and at 3 ($n = 98$), 6 ($n = 99$), and 2 years ($n = 89$) for bacterial recovery of supplemented bacteria. Analyses were made for total amount of lactobacilli and bifidobacteria. The *L rhamnosus* strains and the *P freudenreichii* JS strain were further identified by means of random amplified polymorphic DNA.¹⁰

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, 0.64) by probiotics at the 5% significance level and with 90% power, the estimated size of each group was 597, which allowed a 20% dropout rate. The primary variable was any allergic disease (food allergy, eczema, asthma, or allergic rhinitis) by age 5 years. Three additional variables were considered to describe the level of IgE sensitization: an allergic disease combined with (1) a positive SPT response, (2) a serum antigen-specific IgE level of greater than 0.7 kU/L, and (3) a positive SPT response, IgE level of greater than 0.7 kU/L, or both. Univariable logistic regression analysis allowed comparison of the cumulative incidence of allergic diseases with or without IgE sensitization at the end of the 5-year follow-up period. In exploratory analysis the effects of other potential predictive factors (sex, biparental atopy, mode of delivery, number of siblings, duration of total and exclusive breast-feeding, parental education, smoking at home, household pets, antibiotic treatment during the intervention period, and regular use of other probiotic preparations) were analyzed separately. Interactions between these potential predictive factors and the intervention were first assessed by using the Breslow-Day test, with stratified analyses performed when appropriate (mode of delivery and sex). Those potential predicting factors were also submitted to forward stepwise logistic regression analysis.

The study group was forced to the model, and the criteria for entering and removing other potential predictive factors were a P value of .10 or less and a P value of .15 or less, respectively. Interaction terms were not added to the models.

Results of all unadjusted and adjusted logistic regression models are given as odds ratios with 95% CIs. The χ^2 or Fisher exact tests were used to compare the prevalences of bacteria, and the t test was used for independent samples for logarithmically transformed bacterial counts. Data were analyzed with SPSS, version 15.0 (SPSS, Inc, Chicago, Ill).

Throughout the study, the randomization code was kept by the database consultant and revealed to the statistician only. All analysis was made by a statistician with knowledge only of group. Only after all analysis had been made was a decision made to break the code and the database frozen.

RESULTS

Characteristics of study children are depicted in Table I. Initially, 1223 mothers were randomized. The intent-to-treat infants

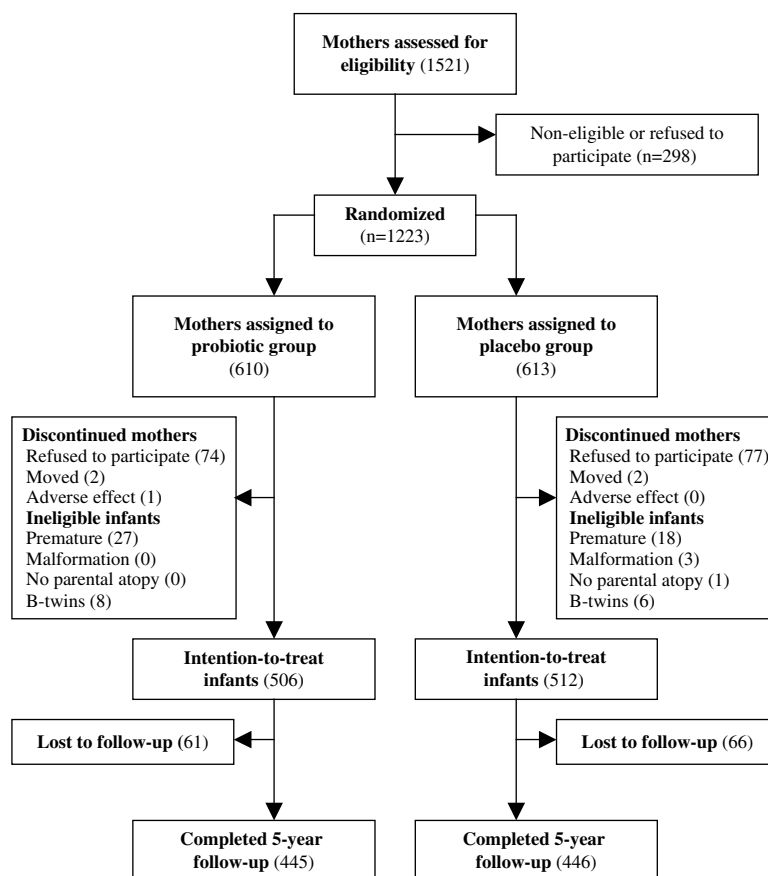


FIG 1. Flow of participants in the probiotic and placebo groups.

numbered 1018. Of these infants, 891 (88%) attended the 5-year visit, and participation was unaffected by probiotic intervention (87.9% among the probiotic group vs 87.1% among the placebo group) or allergy status by age 2 years (96.1% among allergic children vs 95.0% among nonallergic children, Fig 1). Mean (SD) age at the 5-year visit was 4.92 (0.09) years in both groups. At age 5 years, no significant differences in the primary end points of allergic and IgE-associated allergic disease were detectable: 52.6% and 54.9% and 33.0% and 32.7% of the infants in the probiotic and placebo groups, respectively (Table II). No significant differences emerged between the probiotic and placebo groups in the frequencies of eczema (39.3% vs 43.3%), IgE-associated (atopic) eczema (24.0% vs 25.1%), asthma (13.3% vs 14.1%), allergic rhinitis (20.7% vs 19.1%), or atopic sensitization (41.3% vs 41.3%, Table II).

Significant interactions were detectable between mode of delivery and probiotic intervention. Cesarean-delivered children supplemented with probiotics had significantly fewer IgE-associated allergic diseases, particularly eczema, and less IgE sensitization (Table III). In vaginally delivered children no significant differences appeared between treatment groups.

There was also a weak tendency toward interaction between sex and probiotic intervention. Allergic disease in boys developed in 51.3% versus 58.1% and in girls in 53.8% versus 52.0% in the probiotic and placebo groups, with nonsignificant differences. However, after adjustments, still no significant difference appeared in outcomes between intervention groups (Table IV).

The proportions of infants who took more than 80% of the study capsules and syrup were 87.1% and 88.4% in the probiotic and placebo groups, respectively. Compliance, assessed prospectively with a 2-week diary for a subpopulation of 231 infants, was 93%.

Recovery of the supplemented probiotic bacteria was successful (Table V). At 2 years, no significant differences emerged.

In the subgroup of cesarean-delivered children, we noticed a delayed increase in bifidobacteria recovery in placebo-treated children, which was corrected by probiotic and prebiotic supplementation. At 6 months, the prevalences were 57% and 100%, respectively ($P = .036$). In vaginally delivered children the bifidobacteria recovery was not significantly different between groups (Fig 2).

Children's growth was normal, as were key hematologic values (Table VI). By age 5 years, 9 children in the probiotic group and 15 in the placebo group had chronic/severe disease as follows: insulin-dependent diabetes ($n = 2$ and 4), celiac disease ($n = 1$ and 2), inflammatory bowel disease ($n = 0$ and 1), epilepsy ($n = 2$ and 2), other neurologic abnormality (3 and 3), malignancy ($n = 1$ and 2), and death caused by cerebral bleeding ($n = 0$ and 1).

DISCUSSION

One month of prenatal dosage for each mother and 6 months of postnatal supplementation for the infants with a combination of 4 probiotic strains and a prebiotic failed to reduce the prevalence of

TABLE II. Allergic diseases and sensitization at 0 to 5 years in the probiotic and placebo groups

	Probiotic group		Placebo group		OR (95% CI)	P value
Primary end points						
Allergic disease	234/445	52.6%	245/446	54.9%	0.91 (0.70-1.18)	.482
Allergic disease, positive SPT response	138/443	31.2%	137/445	30.8%	1.02 (0.77-1.35)	.906
Allergic disease, specific IgE >0.7 kU/L	124/421	29.5%	106/398	26.6%	1.15 (0.85-1.56)	.370
Allergic disease, positive SPT response and/or specific IgE >0.7 kU/L	147/445	33.0%	146/446	32.7%	1.01 (0.77-1.34)	.925
Secondary end points						
Sensitization						
Any positive SPT response	165/443	37.4%	164/445	36.9%	1.02 (0.78-1.34)	.883
Any specific IgE >0.7 kU/L	152/375	40.5%	137/362	37.8%	1.12 (0.83-1.51)	.455
Any positive SPT response and/or specific IgE >0.7 kU/L	183/443	41.3%	184/445	41.3%	1.00 (0.77-1.33)	.987
Positive food SPT response and/or food-specific IgE >0.7 kU/L	92/443	20.8%	105/446	23.5%	0.85 (0.62-1.17)	.319
Positive inhalant SPT response and/or inhalant specific IgE >0.7 kU/L	169/444	38.1%	162/446	36.3%	1.08 (0.82-1.42)	.573
Allergic disease						
Eczema, all	175/445	39.3%	193/446	43.3%	0.85 (0.65-1.11)	.231
Eczema, IgE associated*	107/445	24.0%	112/446	25.1%	0.94 (0.70-1.28)	.711
Asthma, all	58/445	13.3%	63/446	14.1%	0.91 (0.62-1.34)	.634
Asthma, IgE associated*	43/445	9.7%	40/446	9.0%	1.09 (0.69-1.71)	.393
Rhinitis, IgE associated†	92/445	20.7%	85/446	19.1%	1.11 (0.80-1.54)	.546

Values are presented as the number and percentage of children.

OR, Odds ratio.

*Any positive SPT response, specific IgE level of greater than 0.7 kU/L, or both.

†Positive inhalant SPT response, specific IgE level of greater than 0.7 kU/L, or both.

allergic or atopic disease in this large cohort of almost 900 children followed until age 5 years. It affected neither any single allergic symptom (eczema, allergic rhinitis, or asthma) nor IgE sensitization. At age 2 years, we saw a significant reduction in IgE-associated allergic diseases, particularly in IgE-associated eczema, which decreased by 33%. In contrast to one study using LGG,^{9,19} positive effects from supplementation with probiotics were not sustained in our cohort. In that study only LGG was administered, and administration differed. After birth, mothers were given probiotics, but only after the end of breast-feeding did infants receive probiotics directly. In neither study was sensitization rates affected. In a recent small German study, LGG was given at the same dose as in the study of Kalliomaki et al,¹⁹ and mode of administration was identical, but for reasons difficult to explain, results were quite negative, with no effect on prevalence of atopic eczema.¹³

Another allergy-prevention trial using *L acidophilus* from birth until 6 months without prenatal administration to the mother did not reduce the incidence of atopic eczema at 1 year in a high-risk population of 178 infants but increased IgE sensitization frequency.¹² Prenatal start of supplementation might be crucial to colonize mothers so that they transfer probiotics to their infants during vaginal delivery. A Swedish study also demonstrated no effect from 1 month of prenatal supplementation and 12 months of supplementation with *L reuteri* on eczema at 2 years. In a subgroup of infants with atopic eczema, however, *L reuteri* reduced eczema prevalence during the second follow-up year, and less sensitization occurred in the subgroup of infants with allergic mothers.¹¹

In our study the preventive effect at 2 years was stronger in boys, whereas at 5 years, no effect appeared in boys either. The majority of children with an allergic disease had eczema: 88% at 2 years and 76% at 5 years. Many of the eczema-affected children

also had respiratory allergies up to 5 years of age, for which no preventive effect of probiotics is yet known. Quite variable results regarding both preventive and treatment results from probiotics indicate that the effects of preparations differ markedly,²⁰ and the concept itself is misleading: we should discuss effects of specific bacterial strains or combinations. Furthermore, continuous change in the supplemented strain could lead to continued immunologic stimulus and sustained and stronger effects.

We infer that the transient protection offered by probiotics against IgE-associated allergic diseases is based on stimulation of Toll-like receptors, which produce mediators such as IL-6; these further induce IgA differentiation from naive B cells.²¹ We have shown both these events to occur after probiotic administration to infants with eczema,^{22,23} as well as in infants of the present study who showed increased levels of serum C-reactive protein, IL-10, and IgE at age 6 months.²⁴ We speculate that the probiotic combination causes a paradoxical T_H2 stimulation, much the same as in chronic and balanced helminth infection, which is associated with activation of regulatory T cells.²⁵ Such activation might suppress allergic inflammation, and when helminth infections are treated, the prevalence of allergic sensitization increases rapidly.²⁶ That induction of regulatory T cells is therefore not a permanent event is a plausible explanation also for the fading probiotic effect: because the colonization is transient, these immunologic effects no longer operate, and simultaneously, the clinical effect is lost. Moreover, on the basis of both these studies, immunologic effects expressed as low-grade inflammation were more pronounced with LGG alone rather than with the mixture of 4 strains used in our prevention study. This might explain the more sustained preventive effect observed earlier.⁹ The results of various studies also demonstrate that findings from any one probiotic bacteria cannot be extrapolated to other probiotic bacteria.

TABLE III. Allergic diseases and sensitization at 0 to 5 years and in interim analysis at 0 to 2 years in cesarean-delivered children in the probiotic and placebo groups

	Age (y)	Probiotic group (n = 64-70; %)	Placebo group (n = 69-79; %)	OR (95% CI)
Primary end points				
Allergic disease, all	0-5	57.1	55.7	1.06 (0.55-2.03)
	0-2	26.7	36.7	0.63 (0.32-1.25)
Allergic disease, positive SPT response	0-5	24.3	40.5	0.47 (0.23-0.96)‡
	0-2	14.9	21.5	0.64 (0.28-1.47)
Allergic disease specific IgE >0.7 kU/L	0-5	25.0	30.4	0.76 (0.36-1.64)
	0-2	NA	NA	
Allergic disease, positive SPT response and/or specific IgE >0.7 kU/L	0-5	25.7	40.5	0.51 (0.25-1.02)
	0-2	12.2	22.8	0.47 (0.20-1.12)
Secondary end points				
Sensitization				
Any positive SPT response and/or specific IgE >0.7 kU/L	0-5	31.4	46.8	0.52 (0.27-1.02)
	0-2	23.0	35.4	0.54 (0.27-1.11)
Positive food SPT response and/or food-specific IgE >0.7 kU/L	0-5	10.0	25.3	0.33 (0.12-0.85)‡
	0-2	NA	NA	
Allergic disease				
Eczema, all	0-5	42.9	44.3	0.94 (0.49-1.80)
	0-2	22.7	35.4	0.53 (0.26-1.09)
Eczema, IgE associated*	0-5	15.7	30.4	0.43 (0.19-0.95)‡
	0-2	10.7	21.5	0.44 (0.18-1.08)
Asthma, IgE associated*	0-5	7.1	10.1	0.68 (0.21-2.19)
	0-2	NA	NA	
Rhinitis, IgE associated†	0-5	20.0	25.3	0.74 (0.34-1.60)
	0-2	NA	NA	

OR, Odds ratio; NA, not applicable.

*Any positive SPT response, specific IgE level of greater than 0.7 kU/L, or both.

†Positive inhalant SPT response, specific IgE level of greater than 0.7 kU/L, or both.

‡ $P < .05$.**TABLE IV.** Probiotic intervention and predictive factors in allergic diseases and sensitization at 0 to 5 years

Predictive factors	Primary end points			
	Allergic disease	Allergic disease, positive SPT response	Allergic disease, specific IgE >0.70 kU/L	Allergic disease, positive SPT response and/or specific IgE >0.70 kU/L
Probiotic intervention	0.92 (0.69-1.20)	1.02 (0.76-1.37)	1.14 (0.83-1.56)	1.02 (0.76-1.37)
Biparental atopy	1.98 (1.49-2.64)	1.74 (1.29-2.34)	1.87 (1.35-2.57)	1.76 (1.31-2.36)
Cat/dog in the house	0.67 (0.46-0.99)	NA	NA	0.68 (0.45-1.05)
Male sex	NA	NA	1.33 (0.96-1.82)	NA
Siblings	1.27 (0.96-1.69)	NA	NA	NA

Results, shown as odds ratios and 95% CIs, are based on multivariate forward stepwise logistic regression analysis. The study group was forced to the model, and the criteria for entering and removing other potential predicting factors were a P value of .10 or less and a P value of .15 or less, respectively.

NA, Not applicable.

That cesarean-delivered children supplemented with probiotics had less IgE-associated allergic disease, and less IgE sensitization was already noticeable at 2 years, when this proved allergy-preventive effect tended to be stronger. The concept of giving probiotics to mothers and infants has a sound basis, with neonates in Western societies being exposed to fewer environmental microbes and fewer lactobacilli and bifidobacteria appearing in the feces of allergic infants.²⁷ Children born by means of cesarean section are colonized with bifidobacteria and lactobacilli later than vaginally delivered children^{28,29} and have respiratory allergies more often.³⁰ These children are deprived of the massive

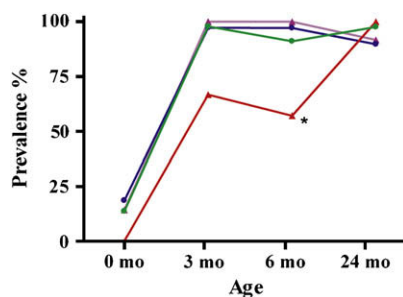
microbial load resulting from vaginal delivery and might particularly benefit from probiotic supplementation, as demonstrated here, where late colonization by bifidobacteria of infants born by means of cesarean section was in fact corrected by probiotic and prebiotic supplementation. The high frequency of cesarean delivery worldwide (as of 2007 up to one third of all deliveries,^{31,32} which is much higher than our 16%) strengthens the effect of our observation.

We were successful in choosing a cohort of families at high risk for allergy because by age 5 years, the placebo-treated children's prevalence of allergic diseases was high (55%), and that of atopic

TABLE V. Prevalence and median of log counts of probiotic bacteria in feces in the treatment groups at 6 months

Bacteria	Probiotic group	Placebo group	P value
LGG			
Prevalence (%)	91	23	<.001
Log counts	7.47	0	<.001
<i>Lactobacillus rhamnosus</i> LC705			
Prevalence (%)	54	0	<.001
Log counts	4.42	0	<.001
<i>Propionibacterium</i> species JS			
Prevalence (%)	55	4	<.001
Log counts	3.74	0	<.001
Total bifidobacteria			
Prevalence (%)	98	86	.039
Log counts	9.30	8.50	.001
Total lactobacilli			
Prevalence (%)	98	56	<.001
Log counts	7.84	4.44	<.001

Medians compared with the χ^2 test and counts with the Mann-Whitney *U* test.

**FIG 2.** Prevalence of fecal bifidobacteria in cesarean (*n* = 22, 17, 19, and 20) and vaginally (*n* = 103, 81, 80, and 69) delivered children at birth, 3, 6, and 24 months of age, respectively. *Cesarean placebo vs probiotic, *p* = 0.036. Purple triangles, Cesarean probiotic group; red triangles, cesarean placebo group; blue circles, vaginal probiotic group; green circles, vaginal placebo group.

disease was 33%. The dropout rate was less than anticipated (ie, only 12%).

We used strict diagnostic criteria for the allergic diseases. Concerning wheezing episodes diagnosed by other doctors, we reviewed all relevant patient records, and only when documentation of wheezing was clear did we include this as a wheezing episode. This cohort of children at high risk for allergy was rigorously followed by our research team. The same physician (KK) met all parents at 5 years and had met most of them at 2 years. All SPTs were performed by 2 specially trained nurses guided by our Global Allergy and Asthma European Network-accredited allergy diagnostic department, which supplied fresh antigens regularly. Throughout the study, use of commercially available probiotics was carefully queried and, in addition, specifically prohibited during the intervention.

This study shows no allergy-preventive effect from supplementation with probiotics during the last month of pregnancy and for infants 6 months after birth when children were evaluated at age 5 years. We earlier demonstrated that the immunologic effects of such supplementation, but with stronger and longer stimuli, possibly by varying the strains of bacteria, might confer better allergy-preventive effects.

TABLE VI. Weight, height, and hematologic values at age 5 years.

Growth		
Weight (kg)	19.7 (2.6)	19.5 (2.5)
Percentage of national reference	2.64 (9.18)	1.58 (8.71)
Height (cm)	111.0 (6.6)	111.0 (6.3)
SD of national reference	0.38 (0.99)	0.38 (0.99)
Hematology	<i>n</i> = 372	<i>n</i> = 362
Hemoglobin (g/L)	126.6 (6.5)	126.6 (6.8)
Hematocrit (%)	37.2 (1.8)	37.3 (1.8)
Red blood cell count (E12/L)	4.6 (0.3)	4.6 (0.3)
Mean corpuscular volume (fl)	80.4 (2.8)	80.4 (3.0)
Mean corpuscular hemoglobin (pg)	27.5 (1.1)	27.4 (1.1)
Leukocyte count (E9/L)	6.8 (1.9)	6.7 (1.8)
Platelet count (E9/L)	301 (62)	306 (60)

Values are presented as means (SDs).

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Clinical implications: One month of prenatal and 6 months of probiotic supplementation of infants appeared insufficient for long-term allergy prevention.

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