

Probiotic supplementation for the first 6 months of life fails to reduce the risk of atopic dermatitis and increases the risk of allergen sensitization in high-risk children: A randomized controlled trial

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Background: Despite preliminary evidence, the role of probiotics in allergy prevention is unclear.

Objective: To determine whether early probiotic supplementation prevents allergic disease in high-risk infants.

Methods: Newborns of women with allergy ($n = 231$) received either *Lactobacillus acidophilus* (LAVRI-A1) or placebo daily for the first 6 months of life. Children were assessed for atopic dermatitis (AD) and other symptoms at 6 and 12 months and had allergen skin prick tests (SPT) at 12 months of age.

Results: A total of 178 infants completed the supplementation period. Those in the probiotic group showed significantly higher rates of *Lactobacillus* colonization ($P = .039$). At 6 months, AD rates were similar in the probiotic ($n = 23/89$; 25.8%) and placebo ($n = 20/88$; 22.7%) groups ($P = .629$). There was also no difference at 12 months, although the proportion of children with SPT+AD was significantly higher in the probiotic group ($P = .045$). At 12 months, the rate of sensitization was significantly higher in the probiotic group ($P = .030$). The presence of culturable *Lactobacilli* or *Bifidobacterium* in stools in the first month of life was not associated with the risk of subsequent sensitization or disease; however, the presence of *Lactobacillus* at 6 months of age was associated with increased risk of subsequent cow's milk sensitization ($P = .012$).

Conclusion: Early probiotic supplementation with *L. acidophilus* did not reduce the risk of AD in high-risk infants and was associated with increased allergen sensitization in infants receiving supplements. The long-term significance of the

increased rate of sensitization needs to be investigated in further studies.

Clinical implications: These findings challenge the role of probiotics in allergy prevention. (J Allergy Clin Immunol 2006;117:1016-1026.)

Key words: Probiotic, infant, atopic dermatitis, sensitization, allergy prevention

Allergic diseases are now the most common chronic disorders of childhood in the developed world.¹ The alarming increase over the last 25 years has highlighted the need to develop more definitive prevention or curative strategies. Growing concern over the adverse immunologic effects of progressively more hygienic environments has led to enormous interest in the role of microbial products such as probiotics in the prevention and treatment of allergic disease.

Our previous studies have contributed to the current paradigm that immature T_H2 -dominant neonatal responses must undergo environment-driven maturation in the early postnatal period.^{2,3} This appears to be associated with gradual inhibition of this early T_H2 propensity in favor of more mature T_H1 -dominant responses. Although the responsible factors are still unclear, experimental models⁴ have provided strong support for the hypothesis that early microbial exposure is critical for normal development (see reviews^{5,6}).

Probiotic intestinal flora contribute to microbial antigen exposure in early life and are one of the most abundant sources of early immune stimulation. Because allergic immune responses manifest early in life, there has been obvious interest in the potential benefits of modifying the gastrointestinal flora by using probiotic supplementation. So far, there has been only 1 published study to address the role of probiotics in primary prevention, with a reported reduction in the incidence of eczema at 2⁷ and 4 years, but no reduction in respiratory allergy, IgE levels, or allergic sensitization.⁸ The role of probiotics in allergy prevention has remained controversial, and there has been an urgent call for similar studies to address this further. In this study, we further examined the role of early probiotic supplementation (with *Lactobacillus acidophilus*, LAVRI-A1)

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Abbreviations used

AD: Atopic dermatitis

OR: Odds ratio

SPT: Skin prick test

for primary allergy prevention in a population of Australian children at high risk of allergic disease.

METHODS

Study design

We used a randomized, double-blind, placebo-controlled design to assess the effect of probiotic supplementation from birth to 6 months on atopic dermatitis (AD) and food allergy (at 6 and 12 months of age) and evidence of allergen sensitization (at 12 months of age).

Participants

We recruited 231 pregnant, atopic women delivering in Perth, Western Australia, between July 2002 and March 2005. We anticipate that with no interventions, this high-risk population would have a 60% to 80% chance of developing atopic disease (70%). We believe that to be of any clinical value, an intervention should reduce the risk of allergy by at least 20% (ie, from 70% to 50% chance of developing allergy), with a relative risk of 0.7. With around 90 subjects in each study group, we had more than 80% power to detect a 20% fall in the expression of atopic disease. We recruited a larger number to allow for an estimated 10% withdrawal rate.

Maternal atopy was defined as a doctor-diagnosed clinical history of asthma, allergic rhinitis, or eczema plus a positive skin prick test (SPT) to 1 or more common allergens (house dust mite, grass pollens, cat, dog, feathers, molds, and cockroach extracts; Hollister-Stier Laboratories, Spokane, Wash). Women were ineligible for the study if they smoked, had other medical problems or pregnancy complications, delivered before 37 weeks of gestation, or were already taking probiotic supplements.

Women were assessed for eligibility during pregnancy, and intervention for the baby commenced within 48 hours of delivery. Infants were reviewed before discharge from hospital and followed up at 1 month, 6 months, and 12 months of age.

Assignment

A computerized randomization schedule was prepared by the hospital biostatistician with allocation and dispensing by the pharmacy department at Princess Margaret Hospital (Perth, Australia). The groups were stratified and block-randomized according to (1) maternal allergy (asthma vs other allergy), (2) parity (first child vs 2 or more children), and (3) paternal allergy (allergy vs no allergy). Infants in the probiotic group received 3×10^9 *L acidophilus* LAVRI-A1 in maltodextrin (Probiomix, Sydney, Australia), whereas those in the control group received maltodextrin alone. Supplements were supplied as stable freeze-dried powder (in sachet packets), dissolved in 1 to 2 mL sterile water and administered orally on a daily basis from birth to 6 months.

Supplement

L acidophilus LAVRI-A1 conformed to the Food and Agriculture Organization (FAO)/World Health Organization expert panel guidelines for probiotics (resistance to acid and bile, adherence to cells of the intestinal epithelium and colonization in the intestinal tract, antagonistic activity toward enteric pathogens, and maintenance of strain identity and viability throughout shelf life),^{9,10} as tested and verified

by the commercial entity Probiomix. Preliminary studies in animals have also shown that coadministration of LAVRI-A1 with allergen is associated with downregulation of immediate hypersensitivity reactions.¹¹

Masking

Probiotic and placebo supplements were image-matched, and participants, research scientists, and pediatricians remained blind to the groups for the duration of the study. Randomization and allocation of supplements occurred at a separate area from participant recruitment. Supplements were dispensed to participants by persons independent from the allocation process.

Compliance

Compliance was monitored by use of a dose chart (completed by parents) and dose counts (returned sachet packets counted by the pharmacy department).

Clinical outcomes

The main clinical outcome measures were incidence of AD, food allergy, and/or sensitization. Infants were clinically evaluated at 12 months of age, which included a detailed history and examination by the same pediatric allergist (S. L. Prescott). Information was also collected on respiratory symptoms, although the limitations of this data at this age are well recognized.

The diagnostic criteria conformed to the recently published clinical guidelines.¹² A diagnosis of AD was made in infants with typical skin lesions¹³ responsive to topical steroids. The severity of AD was determined using the severity score of atopic dermatitis (SCORAD) index as previously described.¹⁴ IgE-mediated food allergy was defined as a history of immediate (within 60 minutes) symptoms after contact with and/or ingestion of food (such as egg, dairy, nut, and so forth) and a positive SPT to the implicated food. Symptoms of acute food allergy included skin reactions (hives, rash or swelling) and/or respiratory symptoms (cough, wheeze, stridor) and/or gastrointestinal symptoms (abdominal pain, vomiting, loose stools) and/or cardiovascular symptoms (collapse).

Allergen SPT

Infants had an SPT at 12 months of age. This was performed using a standardized technique¹⁵ with common allergen extracts (milk, peanut, house dust mite, cat, grass, mold; Hollister-Stier Laboratories) and whole egg, as well as histamine as a positive control and glycerine as a negative control. A wheal diameter of ≥ 3 mm was considered positive.

Assessment of potential confounding factors and interpretation of clinical data

We also collected data on other environmental factors that could confound or influence the relationship of probiotics and allergic disease. Most of these data were collected prospectively (diary cards) and included information about other clinical disease and common exposures (vaccination, infection, day care, diet, and medication use including antibiotics) as well as the home environment (including carpeting, sibship, and pets). This was based on both physician-diagnosed disease and parental recording of symptoms (on diary cards). All parents who noted noisy breathing of any kind were asked to give detailed descriptions of this to the pediatrician (if it had not already been confirmed by another physician). This was then used to determine whether the symptoms were likely to be wheeze, stridor, or a result of secretions in the upper airway. Only children with a convincing history of wheeze (or physician-documented wheeze) were classified in this category. Children were recorded as having upper respiratory infections if they had infections that were limited to coryzal symptoms in the absence of significant chest symptoms.

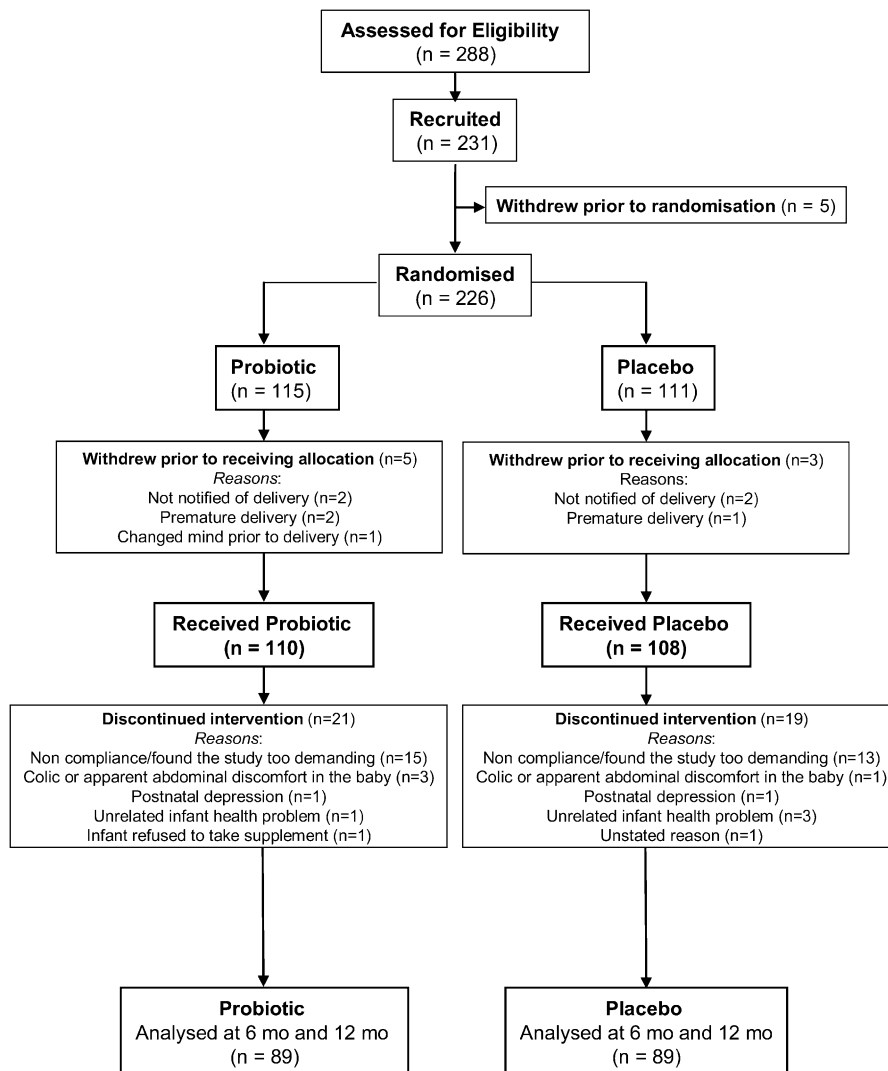


FIG 1. Flow chart showing progress of participants through the trial.

The limitations of all of these data are recognized and have been interpreted accordingly. It was not feasible to perform nasal aspirates and clinical examinations for every reported infectious episode.

Stool analysis

Stool samples were collected 1 and 6 months after commencing the supplement to assess the effect of probiotic intervention on colonization patterns. For logistic reasons (significant geographic separation between the subjects and the laboratory), it was not possible to process samples fresh. All stool samples were generally collected within 5 to 10 minutes of a bowel movement, placed in sterile containers, and frozen immediately. They were all stored at -20°C (in Perth) before they were transported on dry ice (to New South Wales) for anaerobic cultures. Lactobacilli, bifidobacteria, and coliforms were enumerated in these samples as follows: 10-fold diluted homogenates of the stool samples (wt/vol) in half-strength Wilkin-Chalgren broth (Oxoid, Basingstoke, United Kingdom [UK]) were prepared by thawing the frozen samples in an anaerobic chamber and performing the homogenization in the chamber. Homogenates were serially diluted in 10-fold steps, and aliquots (10 μL) were drop-inoculated on Man Rogosa Sharpe (MRS), Prioionic

acid agar, or MacConkey agar for lactobacilli, bifidobacteria, and coliforms, respectively. After incubation at 37°C for 48 hours for lactobacilli and bifidobacteria and 24 hours for coliforms, the colonies were counted and results expressed as the number of colonies per gram wet weight of feces (colony forming units [cfu]/g). There were 163 samples available to assess colonization, 83 in the placebo and 80 in the probiotic group.

Statistical analysis

Differences between the groups for dichotomous data were determined by χ^2 . Continuous data were normally distributed and assessed using the Student t test, expressed as means and SEMs. Logistic regression was used to determine the odds ratio (OR) of developing each clinical outcome in the probiotic group compared with the control. All statistical analyses were performed by using SPSS software (version 11.0 for Apple Macintosh Mac OS X). A P value of $< .05$ was considered statistically significant for all analyses.

Ethics

Ethical approval for the study was granted by Princess Margaret Hospital for Children, King Edward Memorial Hospital, St John of

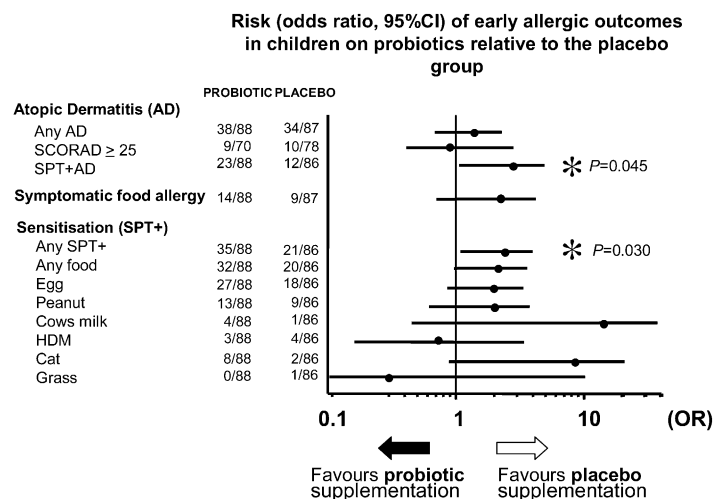


FIG 2. Odds ratios (and 95% CIs) of infants in the probiotic group developing early symptoms of allergic disease or sensitization (SPT-positive) at 1 year of age compared with the placebo group.

God Hospital, and Mercy Hospital ethics committees, and all women gave informed consent.

RESULTS

Characteristics of maternal and neonatal populations

As shown in the trial profile (Fig 1), 288 women registered interest, and 231 women were recruited into the study. Fifty-seven women who registered interest did not meet the inclusion criteria, and 5 eligible subjects withdrew before randomization. A total of 226 women were recruited and randomized for the study, and of these, 48 withdrew from the study (Fig 1) and were excluded from the analysis.

A total of 178 mothers and their healthy full-term infants completed the study, 89 probiotic and 89 control. There were no significant differences in maternal age, parity, or maternal or paternal atopic status between the groups at time of randomization or in those who completed the study. There were no significant differences in gestational age, birth weight, Apgar scores, or sex between the neonates in the 2 groups. However, significant differences were seen with birth length ($P = .013$) and birth head circumference ($P = .023$) between the 2 groups, and these were included as potential confounding factors in subsequent analyses.

Effects of probiotics on atopic outcomes in the first year of life

The main objective of this study was to examine the effects of early probiotic supplementation on early manifestations of allergic disease (namely AD and food allergy) and allergic sensitization in the first year of life. The findings are summarized in Fig 2, which shows the ORs (for the main outcomes) for children in the probiotic group compared with the placebo group.

At 6 months of age, at the end of the supplementation period, the rates of AD were similar in the probiotic ($n = 23/89$; 25.8%) and placebo ($n = 20/88$; 22.7%) groups ($P = .629$), and there were also no differences in other clinical outcomes at this age (Table I).

Similarly, at 12 months of age, no differences were seen in the rate ($P = .581$) or severity (SCORAD; $P = .995$) of AD between the 2 groups. However, the proportion of children with AD and sensitization (positive SPT) was significantly higher in the probiotic group ($P = .045$; Table I). The rate of sensitization to common allergens was significantly higher in the probiotic group ($P = .030$; Table II). There were no differences in the rate of symptomatic (IgE-mediated) food allergy between the groups ($P = .276$; Table I).

Effects of probiotics on other symptoms in the first year of life

There was no evidence that probiotic supplementation protected from respiratory infection during either the first or second 6 months of life. Children receiving probiotics had more ear infections, although this was not statistically significant (Table I). The rates of chest infections were similar in both groups, and rate of wheezing was significantly higher in the probiotic group in the second 6 months of life (OR, 2.45; 95% CI, 1.11 to 5.39; $P = .024$), indicating probiotics were not protective for this outcome.

During the supplementation period, children on probiotics were more likely to be prescribed antibiotics (27.0%) compared with the placebo group (17.0%), although this did not reach statistical significance (OR, 1.80; 95% CI, 0.87 to 3.72; $P = .111$). This was addressed as a confounding factor, because it is possible that this had effects on colonization.

Assessment of confounding factors

Data were collected on potential confounding influences that may also influence atopic propensity. There

TABLE I. Clinical characteristics in the study population in the first year of life; probiotic compared with placebo group at 6 months and 12 months of age†

Characteristics	Control n (%)	Probiotic n (%)	Significance P
0-6 Months of age (n = 177)	n = 88	n = 89	
Any doctor-diagnosed atopic dermatitis	20 (23)	23 (26)	.629
Upper respiratory tract infection	61 (69)	60 (67)	.786
Cough (without cold)	17 (19)	11 (12)	.205
Wheeze	12 (14)	13 (15)	.853
Bronchiolitis	4 (5)	5 (6)	.745
Chest infection	12 (14)	11 (12)	.801
Ear infection (diagnosed)	1 (1)	5 (6)	.099
Gastrointestinal infections	7 (8)	8 (9)	.805
6-12 Months of age (n = 175)	n = 87	n = 88	
Sensitization (SPT ⁺ to any allergen) (see Table II for details)	21 (24)	35 (40)	.030*
AD			
Any doctor-diagnosed AD	34 (39)	38 (43)	.581
SPT ⁺ AD	12 (14)	23 (26)	.045*
Food allergy	9 (10)	14 (16)	.276
Upper respiratory tract infection	53 (62)	54 (61)	.971
Recurrent cough (without cold)	23 (26)	18 (21)	.350
Wheeze	11 (13)	23 (26)	.024*
6-12 mo			
Ever	20 (23)	30 (34)	.104
Recurrent wheeze ≥ 2 episodes	3 (3)	4 (5)	.711
Bronchiolitis	4 (5)	9 (10)	.156
Chest infection	48 (55)	51 (58)	.710
Ear infection (diagnosed)	18 (21)	25 (28)	.253
Gastrointestinal infections	17 (20)	19 (22)	.767

*P < .05 was considered a significant difference.

†Significant differences between the groups were determined by Pearson χ^2 for all nominal data.

were no significant differences between the groups (Table III).

As noted previously, there were differences in antibiotic usage and infant growth parameters (at birth) between the groups. These factors were included in regression modelling to account for possible confounding effects on all of the relationships examined. However, this did not change the key study findings.

Compliance

Approximately 80% of doses were administered with no differences between the groups ($P = .607$).

Stool analysis

At 1 month of age, infants in the probiotic group were almost twice as likely to show culturable levels of *Lactobacillus* species (23%) compared with the placebo group (13%); however, this did not reach statistical significance ($P = .123$). By 6 months of age, the rate of *Lactobacillus* colonization was significantly higher in the probiotic group (36%) compared with the placebo group (21.6%; $P = .039$). There was a trend for lower rates of colonization with coliforms in the probiotic group (38.8% with culturable levels) compared with the placebo group (48.2%; $P = .224$). The rates of colonization with *Bifidobacterium* were similar at 1 month (47% in the placebo group and 51.2% in the probiotic group; $P = .586$) and at 6 months

TABLE II. Sensitization characteristics of the study population at 1 year; probiotic compared with control (maltodextrin) at 6 months and 1 year of age‡

Sensitization	Control n = 86 n (%)	Probiotic n = 88 n (%)	Significance P
Positive SPT (any)	21 (24)	35 (39.8)	.030*
Food (any)	20 (23)	32 (36)	.059†
Egg (whole fresh egg)	18 (21)	27 (31)	.142
Cow's milk	1 (1)	4 (5)	.182
Peanut	9 (11)	13 (15)	.393
House dust mite	4 (5)	3 (3)	.677
Grass mix	1 (1)	0 (0)	.310
Mold mix	1 (1)	1 (1)	.987
Cat pelt	2 (2)	8 (9)	.055†
Rye grass	0 (0)	0 (0)	—

*P < .05 was considered a significant difference.

†Approaching statistical significance.

‡Sensitization at 1 year was significantly correlated with probiotic supplementation as determined by Pearson χ^2 .

(69% in the placebo group and 71% in the probiotic group; $P = .7$). There were significant correlations between *Bifidobacteria* and *Lactobacilli* colonization ($r = 0.335$; $P < .001$).

TABLE III. Characteristics (exposures) of infants in the study population in the first year of life; probiotic compared with control (maltodextrin) at 6 months and 1 year of age*

Characteristics	Control n (%)	Probiotic n (%)	Significance <i>P</i>
0-6 Months of age	n = 88	n = 89	
Breast-fed (ever)	86 (98)	88 (99)	.554
Still breast-fed at 6 months	63 (72)	64 (72)	.962
Maternal antibiotics while breast-feeding	36 (41)	38 (43)	.810
Infant antibiotic administration	15 (17)	24 (27)	.111
Ever received formula	60 (68)	63 (71)	.707
Solids introduced by 6 months	78 (89)	83 (93)	.284
Age of solid introduction (mo)	4.9 ± 0.1	5.0 ± 0.1	.374
Yogurt	16 (18)	17 (19)	.875
Day care attendance	8 (9)	8 (9)	.981
Play group attendance	56 (64)	57 (64)	.955
Older siblings	42 (47)	43 (48)	.881
Pet at home	48 (55)	53 (60)	.501
Cat at home	13 (15)	19 (21)	.256
Dog at home	35 (40)	37 (42)	.807
Height ± SEM (cm)	67.3 ± 0.3	67.8 ± 0.3	.297
Weight ± SEM (kg)	7.9 ± 0.1	7.8 ± 0.1	.540
6 Months to 1 year of age	n = 87	n = 88	
Still breast-fed at 12 months	28 (33)	31 (35)	.710
Maternal antibiotics while breast-feeding	7 (8)	15 (17)	.072
Infant antibiotic administration	34 (40)	40 (46)	.430
Day care attendance	26 (30)	26 (30)	.921
Play group attendance	52 (61)	55 (63)	.783
Height ± SEM (cm)	76.3 ± 0.3	76.1 ± 0.4	.727
Weight ± SEM (kg)	10.2 ± 0.1	10.1 ± 0.14	.523

P < .05 was considered a significant difference.

*There were no significant differences between the groups for any of the variables determined by Student *t* test for continuous data and Pearson χ^2 for all nominal data.

Effects of colonization on atopic outcomes and other symptoms in the first year of life

The detection of culturable *Lactobacillus* species, *Bifidobacterium*, or coliform species in stool samples at 1 month of age was not associated with the frequency of AD, sensitization, or clinical food allergy at 1 year when the population was assessed as a whole (ie, regardless of supplementation) or according to group of supplementation. There were also no relationships between colonization at 6 months at subsequent clinical outcomes, with the only exception that children with culturable *Lactobacilli* at 6 months of age were more likely to develop a positive SPT to milk (*P* = .012). According to the suppliers, the product did not contain any detectable milk protein.

There was also no evidence that presence of culturable *Lactobacillus* species in stool samples at 1 month of age protected from infections during the first year of life.

DISCUSSION

There is a good theoretical basis for using probiotics for disease prevention. Germ-free animal models demonstrate that bacterial gut colonization is essential for maturation of immune function and induction of oral tolerance.⁴ It has

been proposed that a similar but more subtle process may be occurring in human beings with progressively cleaner environments (see review¹⁶). Probiotic intestinal flora are arguably the most abundant source of early immune stimulation and contribute significantly to microbial burden in early life. A number of studies have suggested differences in the early colonization patterns of infants who go on to develop allergic disease (as recently reviewed¹⁶). These differences were already apparent at 1 week of age, with much lower colonization by *Lactobacillus* species (30% compared with 80%) in infants from countries with high and low atopy prevalence, respectively.¹⁷ Other studies have also shown that early colonization with potentially more pathogenic bacteria (such as *Clostridium difficile*¹⁸ and *Staphylococcus aureus*¹⁹) is more likely in children who go on to develop allergy. These studies strongly suggest that the pattern of colonization in the first weeks of life may influence the patterns of immune development. These notions have been supported by observations that gut flora can influence local and systemic immune responses. There has been speculation that intestinal flora may influence the maturing precursor cells that circulate through the gut before they home to other tissues. This may explain how probiotic species can influence systemic immune responses²⁰ and IgA production in distal sites, such as the respiratory tract.²¹ Together with

reported clinical effects in early allergic disease,²²⁻²⁴ this has logically led to a growing interest in the role of probiotics in allergy prevention.

Contrary to this hypothesis, the key finding of our study was that direct probiotic supplementation (with 3×10^9 *L. acidophilus* per day) from birth to 6 months did not achieve any reduction in the risk of early allergic disease (AD or food allergy) in this population, nor any trend in that direction, despite achieving significantly higher *Lactobacillus* colonization at 6 months age. Rather, after probiotic supplementation, we observed a paradoxical increase in sensitization to allergens (Table I and Fig 2), and more children with SPT-positive AD (a group believed to be at greater risk of subsequent inhalant disease).²⁵ This challenges the findings of Kalliomaki et al,⁷ who reported that early probiotic supplementation was associated with a 50% reduction in AD by 2 years of age in a Finnish birth cohort of a similar size. Despite these clinical effects, they did not see any effects on atopic sensitization as measured by both RAST and SPT at 3, 12, and 24 months as well as subsequently at 4 years in a further follow-up analysis. They concluded that the effects of probiotics on AD were independent of T_H1/T_H2 mechanisms, whereas our findings suggest possible adverse effects of T_H2 pathways in the absence of clinical benefits.

Even when we examined the early colonization (regardless of supplementation) in relation to subsequent allergy outcomes, the presence of culturable *Lactobacillus* species or *Bifidobacterium* (at either 1 or 6 months of age) was not associated with reduced risk of sensitization or disease. If anything, the presence of *Lactobacillus* at 6 months was associated with an increased risk of sensitization (to cow's milk) although the probiotic product did not contain detectable milk protein.

The effect of the probiotic supplement on colonization was similar to that now reported by the Finnish group.²⁶ They also noted colonization with *Lactobacillus* species in their placebo group (28%) at 6 months, although this was significantly lower than their probiotic group (58%). In our study, 22% of the placebo group had culturable lactobacilli, which was also significantly lower than our probiotic group (38%; $P = .039$). The slightly lower levels relative to the Scandinavian study could be a result of lower consumption of fermented foods by Australian compared with Scandinavian women.

There are a number of other key differences between these studies that could contribute to the disparity in clinical findings. First, different *Lactobacillus* species were used in the 2 studies: *Lactobacillus rhamnosus* GG (Valio Ltd; Helsinki, Finland) in the Finnish, compared with our *L. acidophilus* species (LAVRI-A1; Probiomix). Although there are noted biological differences between strains,²⁷ our LAVRI strain has been observed to have immunologic effects both *in vivo*²⁸ and *in vitro*.¹¹ It is also worth noting that since the time of this work, another group has failed to show any effect of *L. rhamnosus* and *Lactobacillus* GG on the severity of AD and new sensitization to cow's milk in young infants (1 to 5 months of age).²⁹ Second, Finnish mothers commenced

supplementation during pregnancy, whereas our supplementation began in the first days of life. Because we saw no effects of postnatal supplementation (direct to infants), this would imply a direct immune effect *in utero* rather than any effect on postnatal colonization. However, it seems unlikely that supplementation for only a few weeks in the antenatal period alone would account for such significant differences in study outcomes, although this remains a possibility. Third, in our study, all babies received the supplement directly, regardless of feeding method, whereas in the Finnish study, the mother took the probiotics if babies were breast-fed. Therefore, the Finnish probiotic group included 28 breast-fed infants who did not receive probiotics directly in addition to the bottle-fed infants who received probiotics for 6 months. Concerns about the heterogeneity of this intervention group have been outlined previously in a cautionary editorial.³⁰ If anything, the approach in our study would have led to more consistent probiotic delivery. Fourth, our high-risk population all had maternal allergic disease confirmed by SPT, whereas the Finnish population included children with maternal, paternal, or sibling allergy. This may lead to our population being of slightly higher risk, which is reflected in the higher rate of sensitization in our study (32%) at 12 months compared with the Finnish population (22%) at the same age. Finally, we assessed clinical outcomes at 12 months of age, whereas the effects on AD in the Finnish study were reported at 2⁷ and subsequently at 4 years of age.⁸ Atopic dermatitis typically begins in the first year of life, and 41% of our population already had evidence of this condition by 12 months. It is possible that more children could become affected in their second year of life. However, there is nothing to indicate that this would be less likely in the probiotic group, which showed higher rates of atopic sensitization ($P = .030$).

Our findings also fail to demonstrate any protective effect of supplementation on infection. Rates of respiratory tract infections were similar in both groups; however, children in the probiotic group were significantly more likely to develop associated wheezing (in the second 6 months of life; OR, 2.45; 95% CI, 1.11 to 5.39; $P = .024$). The rate of antibiotic requirement tended to be higher in the probiotic group, particularly in the first 6 months during the supplementation period, although this was not statistically significant ($P = .111$). There was a similar trend for higher antibiotic intake by breast-feeding mothers in the second 6 months of life in the probiotic group ($P = .072$). In both instances, antibiotic usage was transient (typically only 1 or 2 courses of no more than 5 days), although it is possible that this affected colonization. We have attempted to allow for potential confounding effects statistically, but the limitations of this are recognized.

It is also possible that our intervention could have long-term effects on inhalant allergen sensitization and respiratory allergy, which we have not been able to assess here. However, the study by Kalliomaki et al⁸ did not show any evidence of this at long-term follow-up (at 4 years of age).

In summary, within the acceptable limitations of human intervention studies, we have shown that administration of a *L acidophilus* probiotic strain for the first 6 months of life did not reduce the risk of sensitization or allergic disease in this population of high-risk infants. Rather, there was a concerning increase in the rate of sensitization in the intervention group. We also saw no evidence that the presence of culturable *Bifidobacterium* or *Lactobacillus* species in the first month of life (or thereafter) reduced the risk of subsequent allergic disease or sensitization. Although the sensitization to foods in the first years of life is a good indicator of an emerging atopic phenotype, we will continue to assess this cohort for any delayed effects. The findings of this study are of great importance in the context of high public enthusiasm for probiotic use despite a lack of conclusive evidence for benefits. Until this literature is more complete, it is inappropriate to recommend probiotics for allergy prevention.

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