A differential effect of 2 probiotics in the prevention of eczema and atopy: A double-blind, randomized, placebo-controlled trial

Kristin Wickens, PhD,[a] Peter N. Black, FRACP,[e] Thorsten V. Stanley, FRCP,[b] Edwin Mitchell, FRACP, DSc,[f] Penny Fitzharris, FRACP,[g] Gerald W. Tannock, PhD,[d] Gordon Purdie, BSc,[c] Julian Crane, FRACP,[a] and the Probiotic Study Group *Wellington and Auckland, New Zealand*

Background: The role of probiotics in prevention of allergic disease is still not clearly established, although early reports suggested *Lactobacillus GG* halved the risk of eczema at 2 years.
Objective: To determine whether probiotic supplementation in early life could prevent development of eczema and atopy at 2 years.
Methods: Double-blind, randomized placebo-controlled trial of infants at risk of allergic disease. Pregnant women were randomized to take *Lactobacillus rhamnosus* HN001 (*L rhamnosus*), *Bifidobacterium animalis* subsp *lactis* strain HN019 or placebo daily from 35 weeks gestation until 6 months if breast-feeding, and their infants were randomized to receive the same treatment from birth to 2 years (n = 474). The infant's cumulative prevalence of eczema and point prevalence of atopy, using skin prick tests to common allergens, was assessed at 2 years.
Results: Infants receiving *L rhamnosus* had a significantly (*P* = .01) reduced risk of eczema (hazard ratio [HR], 0.51; 95% CI, 0.30-0.85) compared with placebo, but this was not the case for *B animalis* subsp *lactis* (HR, 0.90; 95% CI, 0.58-1.41). There was no significant effect of *L rhamnosus* (HR, 0.74; 95% CI, 0.46-1.18) or *B animalis* subsp *lactis* (HR, 0.82; 95% CI, 0.52-1.28) on atopy. *L rhamnosus* (71.5%) was more likely than *B animalis* subsp *lactis* (22.6%) to be present in the feces at 3 months, although detection rates were similar by 24 months.
Conclusion: We found that supplementation with *L rhamnosus*, but not *B animalis* subsp *lactis*, substantially reduced the cumulative prevalence of eczema, but not atopy, by 2 years. Understanding how Lactobacilli act to prevent eczema requires further investigation. (J Allergy Clin Immunol 2008;122:788-94.)

Key words: *Probiotics, eczema, atopy, allergic disease, infants, allergy prevention, randomized controlled trial*

In 1989, Strachan[1] suggested that decreased exposure to infections could explain the increasing prevalence of allergic disease in Western countries. This has become known as the hygiene hypothesis. Since then, investigations have progressed from the examination of the indirect markers of exposure to infections, such as family position and child care attendance, to measuring direct exposure to microbes and microbial products, such as lactic acid–producing bacteria and endotoxin. The prevalence of lactobacilli was shown to be higher in the feces of infants at 1 year in Estonia, where there is a low prevalence of allergic disease compared with Sweden, which has a higher prevalence of allergic disease.[2] *In vitro* and animal studies have also lent support for a role for organisms such as lactobacilli in immunological maturation.[3,4] Such observations have led to human experimental studies investigating the effect of probiotics on the development of allergic disease. The first of these was a small Finnish study showing that prenatal and postnatal exposure for 6 months to *Lactobacillus rhamnosus GG* halved the frequency of eczema at 2, 4, and 7 years but had no effect on atopic sensitization.[5-7] Since then, 4 other studies have been reported in which lactobacilli were administered to infants from birth to 6 months, but the species used differed between the studies, as have the findings. Kukkonen et al[8] used a combination of 4 probiotics, including 2 *Lactobacillus* species, along with prebiotic galacto-oligosaccharides. This study demonstrated a reduction in eczema that was stronger for the subgroup with atopic eczema. Another Scandinavian study used *Lactobacillus reuteri*[9] and found no overall effect on the cumulative incidence of eczema despite a reduction in

From [a]the Wellington Asthma Research Group, [b]the Department of Paediatrics, and [c]the Department of Public Health, Wellington School of Medicine and Health Sciences, and [d]the Department of Microbiology and Immunology, University of Otago; [e]the Department of Pharmacology and Clinical Pharmacology and [f]the Department of Paediatrics, University of Auckland; and [g]the Immunology Department, Auckland Hospital.

Supported by the Health Research Council of New Zealand and Fonterra Co-operative Group (Auckland, New Zealand). Fonterra Co-operative Group provided the study capsules and maintained quality control of the products throughout the study. All other aspects of the study were conducted independently of Fonterra Co-operative Group.
Disclosure of potential conflict of interest: K. Wickens has received research funding from the Health Research Council and Fonterra Co-operative Group. P. N. Black has received research funding from the Health Research Council of New Zealand, the Child Health Research Foundation, Fonterra Co-operative Group, and Boehringer-Ingelheim. T. V. Stanley has received research funding from the Health Research Council of New Zealand, Fonterra Co-operative Group, the Asthma and Respiratory Foundation of New Zealand, and the Wellington Medical Research Foundation. E. Mitchell has received research funding from the Child Health Research Foundation and the National Heart Foundation of New Zealand and has served as trustee in the past year for the Wilson & Sweet Trust and the Child Health Research Foundation. P. Fitzharris has received research funding from the Marsden Fund and the Foundation for Research, Science & Technology. G. W. Tannock has received research support from the Marsden Fund and the Foundation for Research, Science, and Technology. G. Purdie has received research funding from the Health Research Council of New Zealand, Fonterra Co-operative Group, and the Ministry of Health. J. Crane has received research funding from the Health Research Council of New Zealand, Fonterra Co-operative Group, Rex Medical, the Asthma and Respiratory Foundation of New Zealand, and the National Institute of Environmental Health Sciences.
Received for publication April 9, 2008; revised July 3, 2008; accepted for publication July 14, 2008.
Available online September 2, 2008.
Reprint requests: Kristin Wickens, PhD, Wellington Asthma Research Group, Wellington School of Medicine and Health Sciences, University of Otago, P O Box 7343, Wellington South, Wellington, New Zealand. E-mail: kristin.wickens@otago.ac.nz.
0091-6749/$34.00

doi:10.1016/j.jaci.2008.07.011

Abbreviations used
HR: Hazard ratio
OR: Odds ratio
SCORAD: SCORing Atopic Dermatitis
SPT: Skin prick test

IgE-associated eczema. Other studies have found no effect of *Lactobacillus acidophilus*[10] *or L rhamnosus GG*[11] on atopic dermatitis, with 1 of these studies finding that *L acidophilus* supplementation actually increased the risk of atopic sensitization.[10] The different organisms used and whether there was a prenatal intervention may have influenced the divergent findings.

In this study, we tested the hypothesis that prenatal and postnatal supplementation with *L rhamnosus* strain HN001 or *Bifidobacterium animalis* subsp *lactis* strain HN019 can reduce the prevalence of eczema and allergy during the first 2 years of life in a population of high-risk New Zealand infants. Our study is unique in combining prenatal and postnatal probiotic supplementation, continued use of probiotics for 2 years postnatally, comparison of 2 different probiotics, and fecal sample analysis.

METHODS

Participants

Pregnant women in Auckland and Wellington, New Zealand, were recruited to the study through maternity care providers, antenatal classes, and advertisements. They were invited to take part in the study if they or the infant's father had a history of treated asthma, eczema, or hay fever. Women were ineligible for the study if they planned to move from the study center in the next 2 years, were already taking probiotic supplements long-term, or intended to use these in the child. They were not able to continue in the study if they delivered before 37 weeks gestation, they had not taken the study capsules for $\geq$2 weeks before birth, their infant's weight was <3rd percentile for sex and gestation, or their infant was placed in the neonatal unit for more than 48 hours or had serious congenital abnormalities at birth. If there were twins, only the heavier was included in the study.

Study design

The study was a 2-center, double-blind, randomized, placebo-controlled trial of the effects of probiotic supplementation on the development of eczema and atopic sensitization in infants (Australian New Zealand Clinical Trials Registry: ACTRN12607000518460). There were 2 treatment groups who received either *L rhamnosus* HN001 (6×10^9 colony-forming units/d) or *B animalis* subsp *lactis* HN019 (9×10^9 colony-forming units/d; Fonterra Co-operative Group, Auckland, New Zealand).

The probiotic supplements were manufactured by using aseptic fermentation, concentration, and freeze-drying. The growth media contained skim milk powder, yeast extract, and glucose. After growth, cells of the HN001 and HN019 strains were concentrated by centrifugation and washed twice with sterile saline. During prototype development of the low-allergenic probiotic supplements, the separate ingredients were tested by skin prick test (SPT) on several patients with cow's milk allergy. This work established that after 2 washes, the material had no reaction in the patients with cow's milk allergy. The final washed cells had a cryoprotectant solution, maltodextrin, mixed with the cells. This mix was frozen on trays and freeze-dried. The resulting powder had a particle size of 200 μm or less and was tested for the presence of pathogens before dispatch to a registered pharmaceutical packaging company. The placebo group received a capsule identical in appearance and smell containing dextran, salt, and a yeast extract (Fonterra Co-operative Group). The yeast extract used in the probiotics and the placebo contained no viable cells.

All batches of capsules were tested monthly to ensure viability of the probiotics. Shelf life was managed to ensure minimum cell counts were maintained. In addition, capsules returned from the field were tested for their viability. With very few exceptions, the viability was higher than the minimum required.

At 35 weeks gestation, pregnant women were randomized to receive one of the probiotics or placebo daily, to continue while they were breast-feeding for as long as 6 months postpartum. Infants started the capsules between 2 and 16 days postbirth (median, 6 days), continuing until age 2 years. The capsule powder was either given undiluted to the infant or mixed with water, breast milk, or formula and given via a teaspoon or syringe until solid food was started, when it was sprinkled on food.

Randomization and allocation of supplements were performed by a clinical trials pharmacist at Auckland City Hospital who had no contact with the participants. Randomization was stratified by study center and performed in blocks of 15 according to a computer-generated randomization list. At enrollment, a research study nurse assigned the next study number and provided the participant with the appropriate capsules. All study nurses and participants were blind to treatment assignment for the duration of the study. To evaluate the efficacy of the blinding, the final questionnaire asked participants to indicate whether they believed they were in a probiotic or placebo group.

Information collected at baseline included parental history of allergic disease; sex; ethnicity; household smoking; pet exposure; and length, weight, and head circumference at birth. Eczema prevalence and severity were assessed at follow-up visits at 3, 6, 12, and 18 months and 2 years, and SPTs performed at 2 years to assess atopic sensitization. History of antibiotic use was also collected at these visits.

Ethical approval was granted by a national multiregion ethics committee, covering both study centers.

Outcome measures

Eczema prevalence from birth to 2 years was defined using the UK Working Party's Diagnostic Criteria for atopic dermatitis[12] modified for use in infants. Eczema was determined to be present at each visit if there was a history of scratching or rubbing and 2 or more of the following occurring since birth or the previous visit: (1) a history of involvement of outer arms or legs, (2) a history of a generally dry skin, or (3) visible atopic eczema present on the cheeks or outer arms or legs with no axillary involvement. The research staff were trained in determining eczema by using an internationally recognized training manual for defining atopic eczema.[13]

Eczema severity from birth to 2 years was assessed by using SCORing Atopic Dermatitis (SCORAD)[14] in all children regardless of their eczema diagnosis (as defined). SCORAD was analyzed dichotomously using a cutoff $\geq$10 to exclude those with trivial rash. All staff were trained to apply SCORAD in a standardized way.

After training in the use of a standardized protocol,[15] the study nurse performed SPTs at 2 years to egg white, peanut, cow's milk, cat pelt, *Dermatophagoides pteronyssinus*, and mixed grass pollen (Hollister-Stier, Spokane, Wash). This panel of allergens has been shown to identify 90% of atopic children at 15 months who were tested to a wider range of allergens.[16] Antihistamine medication was withheld for an appropriate period. The allergens and positive (histamine 10 mg/mL) and negative control were applied to the child's arm and pricked vertically for 1 second using Dome-Hollister-Stier lancets (United Kingdom). The histamine response was read at 10 minutes, and allergens and negative control at 15 minutes. A 3-mm or greater mean wheal diameter to 1 or more allergens after subtraction of the negative control wheal diameter and with a positive response to histamine was considered positive. For safety reasons, 6 children who had previously had a severe allergic reaction to a food and a positive SPT response for that food were not retested for the food but considered positive on the basis of the previous test. IgE-associated eczema was defined as eczema plus a positive SPT response, and non–IgE-associated eczema as eczema plus a negative SPT response.

Fecal sample collection

Fecal samples were collected from infants soon after birth and at 3, 12, and 24 months of age. The samples were held in the home freezer until