

SHORT REPORT

Airway inflammation in probiotic-treated children at 5 years

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Early treatment of new-born high-risk children with certain probiotic strains has reduced the risk of atopic eczema. Whether probiotics reduce risk for airway inflammation in long term is not known. We aimed at studying the effect of probiotic treatment during the six first months of life on airway inflammation at age 5 yr. In a randomized double-blind allergy prevention trial between 2000 and 2007 in Helsinki, Finland, we gave a probiotic combination, plus pre-biotics, or placebo, to 1018 children during 6 months from birth. At age 5, we measured exhaled nitric oxide (FE_{NO}) in a randomized sub-population of 160 children. Allergic diseases and IgE-sensitization were assessed in all infants. FE_{NO} did not differ between probiotic and placebo groups, median (interquartile range, IQR) 5.45 (4.3–7.3) vs. 5.70 (3.9–6.8) ppb, $p = 0.22$. FE_{NO} was elevated among those suffering from asthma during the first 5 yr than in healthy non-sensitized children ($p = 0.009$). FE_{NO} correlated positively with serum total and allergen-specific IgE concentrations. Early intervention with probiotics and pre-biotics does not affect airway inflammation later in childhood.

Probiotics have been studied in the prevention of childhood atopic eczema, which is an early event in the atopic march towards airway allergies (1). The strongest effects have been demonstrated in IgE-associated allergic diseases (2). Supplementing infants' diets with *Lactobacillus rhamnosus* GG showed promise in reducing airway inflammation (3), which manifests already in the early stages of asthma (4). Fractional concentration of nitric oxide in exhaled air (FE_{NO}) is assumed to reflect (eosinophilic) airway inflammation and can be non-invasively measured in most pre-school-aged children (4). To study the effect of probiotics in the development of airway inflammation, in a cohort of high-risk children who received probiotics and pre-biotics, or placebo, during the first 6 months of life, we measured FE_{NO} at age 5 yr.

Methods

Between November 2000 and March 2003, we randomized 1223 pregnant mothers carrying children at increased risk for allergy to an allergy-prevention trial on probiotics in

Helsinki, Finland. Of the 1018 intention-to-treat infants, 160 were randomized to have their FE_{NO} measured at age 5.

In the probiotic group, from 36th wk of gestation, mothers took twice daily *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 in capsules supplied by Valio (Helsinki, Finland). Their newborn infants received the same probiotics plus 0.8 g pre-biotic galacto-oligosaccharides once daily for 6 months after birth. Mothers and infants in the control group received placebo. We followed the establishment of any allergic disease (eczema, food allergy, asthma, and allergic rhinitis) and IgE-sensitization. At age 5, skin prick tests were performed on the forearm with allergen extracts of egg, fish, peanut, cat, dog, birch, timothy, mugwort, and *Dermatophagoides pretonyssinus* (ALK-Abellö, Hørsholm, Denmark, or Stallergenes, Antony, France), cow's milk, and wheat grains (diluted in 0.9% NaCl). Weal diameter ≥ 3 mm greater than the negative control was considered positive. We measured serum total IgE and allergen-specific

IgE against cow's milk, egg, peanut, birch, timothy, dog, cat and *D. pretonyssinus* by immunoassay technique (ImmunoCAP® system, Pharmacia diagnostics, Uppsala, Sweden). A concentration of ≥ 0.7 kU/l was considered positive (in detail (2, 5)).

Between November 2006 and December 2007, excluding pollen season (from May to August), FE_{NO} was measured in 5-yr-old children using computerized equipment with a chemiluminescence analyzer (Niox, Aerocrine AB, Sweden). The system was calibrated with a certified NO calibration gas mixture according to the manufacturer's instructions. Children were seated without a nose clip. The study nurse then asked them to fill their lungs completely with NO-free air and to exhale with a mean and instantaneous flow of 50 ml/s. An exhalation time of 6 s was used as a default. Three reproducible FE_{NO} values within 2 ppb or 10% were required, and the mean FE_{NO} was calculated. Parents were advised not to give any inhaled corticosteroids or leukotriene antagonists during the two preceding weeks. In case of acute respiratory tract infection, FE_{NO} measurement was postponed by 2 wk.

The ethics committee of the Hospital for Children and Adolescents of Helsinki University approved the study protocol. One parent signed a written informed consent. The unblinding was performed after the study had been completed.

Sample-size calculation was based on an expected 4 ppb reduction of FE_{NO} in the probiotic group compared to placebo, with 90% power and 5% significance level. The sample size of 160 allowed 20% dropout or failure to perform the measurement. The 160 study patients were extracted from the main population using a table of random numbers according to Fisher and Yates (1963) following Armitage (1971). because of the skewed distribution, FE_{NO} was logarithmically transformed and then inter-group differences were analyzed using independent samples *t*-test. Correlations were made using log-normal FE_{NO} and Pearson's correlation coefficient. We used sums of serum-specific IgE concentrations (food allergens, indoor, and outdoor inhaled allergens). Stepwise logistic regression analyses were used for possible confounding factors and for predictive factors for high FE_{NO}. Qualitative variables were analyzed by Pearson's chi-squared test or Fisher's exact test. Data were analyzed by SPSS, version 17.0 (SPSS Inc, Chicago, IL, USA).

Results

Of the 160 randomized children, 147 attended the 5-yr visit and 131 (64 probiotic, 67 placebo) had acceptable FE_{NO} measurements. Eight children received inhaled corticosteroids (four continuously, four intermittently). One child in the placebo group received inhaled corticosteroids without a 2-wk pause because of persistent symptoms. Her mean FE_{NO} was 30 ppb. We performed intention-to-treat analyses. The baseline and clinical characteristics are shown in Table 1.

The median FE_{NO} (inter-quartile range, IQR) was 5.45 (4.3–7.3) ppb in the probiotic and 5.70 (3.9–6.8) ppb in the

Table 1 Basic and clinical characteristics of the 131 children participating in FE_{NO} measurement at age 5

	Probiotic n = 64	Placebo n = 67
Males (%)	29 (45)	27 (40)
Delivered by cesarean section (%)	9 (14)	16 (24)
Age, yr (s.d.)	4.93 (0.09)	4.93 (0.08)
Height, cm (s.d.)	110.8 (4.6)	111.3 (4.9)
Weight, kg (s.d.)	19.7 (2.3)	19.3 (3.4)
Exposure to tobacco smoke at home (%)	20 (31)	22 (33)
Breast-fed, months (s.d.)	8.1 (5.3)	9.1 (5.2)
Solid foods introduced, months (s.d.)	4.0 (0.7)	4.1 (0.8)
Pets at home (%)	17 (26)	11 (16)
Asthma ever (%) inhaled corticosteroids at 5 yr	6 (9)	10 (15)
Atopic asthma ever (%)	4 (6)	8 (12)
Eczema ever (%)	30 (47)	35 (52)
Atopic eczema ever (%) (eczema in a sensitized individual)	19 (29)	25 (37)
Allergic rhinitis ever (%)	10 (16)	19 (28)
Sensitized at age 2 yr (%)	12 (19)	26 (39)*
Sensitized at age 5 yr (%)	26 (40)	35 (52)
inhaled allergens	23 (36)	32 (48)
Total-IgE kU/l (s.e.)	110 (36)	175 (46)

*Pearson's chi-square $p = 0.015$. No other significant differences between the study groups (Mann-Whitney-*U*, Pearson's correlation, or Fisher's exact test).

placebo group, $p = 0.22$. Adjustment for total duration of breast-feeding, height, and daily exposure to tobacco smoke had no effect on the result.

Compared to healthy non-sensitized children ($n = 37$), whose median (IQR) FE_{NO} was 5.3 (3.6–6.8), the median FE_{NO} was higher in children who were ever (0–5 yr) diagnosed asthma 6.2 (4.9–10.9), $p = 0.009$, and tended to be elevated in children who had eczema 5.9 (4.4–7.2), $p = 0.064$. Compared to healthy non-sensitized children, eczema without asthma did not increase FE_{NO}. FE_{NO} correlated positively with serum total IgE ($r = 0.250$, $p = 0.007$), with the sums of specific-IgE concentrations against food (egg, milk, and peanut; $r = 0.267$, $p = 0.004$), and with inhaled indoor allergens (cat, dog, and house dust-mite; 0.325, $p < 0.001$). FE_{NO} tended to correlate with specific-IgE against pollen allergens (birch and timothy; $r = 0.182$, $p = 0.054$); however, the correlation was not statistically significant.

Conclusions

Treatment with probiotics and pre-biotics during the six first months of life had no effect on airway inflammation at age 5 yr as measured by FE_{NO}. In these same infants, we have previously shown a preventive effect of probiotics on atopic eczema at age 2 (2); however, the effect disappeared by age 5. Nevertheless, no preventive effect of probiotics on respiratory allergies could be shown (5). In a previous study, high-risk

infants treated with probiotic *L. rhamnosus* GG had lowered FENO at age 4 yr (10.8 vs. 14.5 in placebo) (3). Although less airway inflammation was found in the *L. rhamnosus* GG group at age 4 yr, no differences occurred in airway allergies or sensitization at ages 4 nor 7 yr (3) (6). Compared to that study, we measured FENO using a diverse standardized method, and also our probiotic preparation differed. In our high-risk population, the median FENO concentrations were surprisingly low; the median FENO was within normal height-adjusted values in both the probiotic and the placebo group (reference 4.2 ppb at 111 cm) (4). Our sample size was

based on an expected 4 ppb reduction of FENO in the probiotic group (3). However, a bigger sample size may not have changed the main outcome, as we were not able to see a tendency to lower FENO in the probiotic group. In our study, FENO correlated with total IgE and IgE-sensitization. That FENO was not measured during pollen season may explain the less obvious correlation of FENO with pollens than with food or inhalant indoor allergens. Compared to healthy children, FENO was higher among children with doctor-diagnosed asthma during the first 5 yr. This is in line with previous observations (7–9).

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