Probiotics in primary prevention of atopic disease: a randomised placebo-controlled trial

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Summary

Background Reversal of the progressive increase in frequency of atopic disease would be an important breakthrough for health care and wellbeing in Western societies. In the hygiene hypothesis this increase is attributed to reduced microbial exposure in early life. Probiotics are cultures of potentially beneficial bacteria of the healthy gut microflora. We assessed the effect on atopic disease of *Lactobacillus* GG (which is safe at an early age and effective in treatment of allergic inflammation and food allergy).

Methods In a double-blind, randomised placebo-controlled trial we gave *Lactobacillus* GG prenatally to mothers who had at least one first-degree relative (or partner) with atopic eczema, allergic rhinitis, or asthma, and postnatally for 6 months to their infants. Chronic recurring atopic eczema, which is the main sign of atopic disease in the first years of life, was the primary endpoint.

Findings Atopic eczema was diagnosed in 46 of 132 (35%) children aged 2 years. Asthma was diagnosed in six of these children and allergic rhinitis in one. The frequency of atopic eczema in the probiotic group was half that of the placebo group (15/64 [23%] *vs* 31/68 [46%]; relative risk 0·51 [95% CI 0·32–0·84]). The number needed to treat was 4·5 (95% CI 2·6–15·6).

Interpretations *Lactobacillus* GG was effective in prevention of early atopic disease in children at high risk. Thus, gut microflora might be a hitherto unexplored source of natural immunomodulators and probiotics, for prevention of atopic disease.

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Introduction

Allergy, in the form of atopic diseases such as atopic eczema, allergic rhinitis, and asthma, is a chronic disorder of increasing importance in economically more-developed countries.[1] The International Study of Asthma and Allergies in Childhood[2,3] included 11 607 Finnish children aged 13–14 years; 10–20% of the children had symptoms of asthma, 15–23% allergic rhinitis, and 15–19% atopic eczema. Proof of an inverse association between infections early in life and atopy has led to renewed interest in the hygiene hypothesis devised by Strachan[4] a decade ago. The recent rapid rise in atopy might be a result of improved hygiene and reduced family size. Recent epidemiological studies have yielded results both for,[5–7] and against,[8] such a hypothesis.

We propose that specific microbes in the commensal gut microflora are more important than sporadic infections in atopic disease prevention. Gastrointestinal microflora promote potentially antiallergenic processes: (1) T-helper-1-type immunity;[9] (2) generation of transforming growth factor β,[10,11] which has an essential role in suppression of T-helper-2-induced allergic inflammation[12] and induction of oral tolerance;[13] and (3) IgA production,[14] an essential component of mucosal immune defence. The gut microflora might therefore be a major postnatal counter-regulator of the universal T-helper-2-skewed immune system in fetuses and neonates. Confrontation between microbes and their antigens in the gastrointestinal tract begins instantly after birth, and the viable cells of fully established gut microflora outnumber those of the human host by a factor of ten.[15] Consequently, commensal gastrointestinal microbes are the earliest and biggest stimulus for development of gut-associated lymphoid tissue.

Probiotics are cultures of potentially beneficial bacteria of healthy gut microflora[15] and one such strain, *Lactobacillus rhamnosus* (*Lactobacillus* GG, American Type Culture Collection 53103), has proved safe at an early age and effective in treatment of allergic inflammation[11] and food allergy.[16] In a double-blind randomised placebo-controlled trial of prevention of atopic disease, we gave *Lactobacillus* GG prenatally to mothers and postnatally for 6 months to their infants at high risk of these diseases.

Methods

Participants and study design

The only inclusion criterion was a family history of atopic disease—ie, one or more family members (mother, father, or older sibling) with atopic eczema, allergic rhinitis, or asthma. Families were recruited in antenatal clinics in Turku, Finland (population 170 000) between February, 1997, and January, 1998, to avoid the effect of birth month on atopic sensitisation. On the basis of our sample-size calculation before the study, 159 mothers were randomly assigned by computer to receive two capsules of placebo (microcrystalline cellulose) or 1×10^{10} colony-forming units of *Lactobacillus* GG (Valio Ltd; Helsinki, Finland) daily for 2–4 weeks before expected delivery. After delivery, breastfeeding mothers could take the capsules, otherwise children received the agents; in the latter case, capsule

contents were mixed with water then given by spoon. Both these modes of administration have resulted in similar amounts of *Lactobacillus* GG in infant faeces.[16] *Lactobacillus* GG and placebo capsules and contents looked, smelled, and tasted identical. Capsules were taken postnatally for 6 months. Treatment codes were kept by the supplier until data had been collected and analysed—ie, until March, 2000.

Children were examined during the neonatal period and on study visits to a department of paediatrics at ages 3, 6, 12, 18, and 24 months. The outcome measure was atopic disease at 2 years. Since chronic recurring atopic eczema is the main sign of atopic disease in the first years of life,[17] it was the primary study endpoint. Children were grouped as having this disorder (children with atopic eczema) or not (healthy children). The study was approved by the Committees on Ethical Practice in Turku University Hospital and the Health Office of Turku. Written informed consent was obtained from children's parents.

Procedures

The physician (MK) who did the physical examinations, diagnoses of atopic disease, and antiasthma treatments was unaware of the contents of the capsules until March, 2000, when all data had been obtained and analysed. Physical examination included inspection of eyes, ears, nose, and skin, auscultation of heart and lungs, palpation of abdomen, and assessment of growth and neurological development. Parents were asked about their child's signs and symptoms that were possibly related to atopic disease: redness, dryness, oozing, and scratching (itch) of skin; redness, discharge, sneezing, and rubbing (itch) of eyes and nose; and cough, wheeze, and shortness of breath. Sensitisation to common dietary and respiratory antigens was measured by: skin-prick tests at ages 6, 12, and 24 months; and by total and antigen-specific IgE assays in umbilical cord blood and at ages 3, 12, and 24 months.

Atopic eczema was confirmed by pruritis, facial or extensor involvement, or both, and chronic relapsing course.[17] This last criterion was fulfilled if the child had had eczema for 1 month or longer at the 24-month study visit and on at least one previous visit. The SCORAD index[18] was used to assess eczema severity. Allergic rhinitis was diagnosed if the baby had on most days two or more of: nasal discharge, blockage, sneezing, and itching. For diagnosis, temporal relations had to be established between these symptoms of allergic rhinitis, symptoms with allergen exposure, relief of symptoms by antihistamine treatment, and evidence of atopic sensitisation (ie, positive skin-prick test or positive radioallergosorbent assay, or both). Asthma diagnosis was based on an algorithm created by an international paediatric asthma concensus group.[19] Asthma was diagnosed if an infant had chronic or recurrent cough, wheeze or shortness of breath, or both, suggestive of asthma, and if other diagnoses were excluded and trial antiasthma treatment was effective.

Assays for serum total IgE and specific IgE antibodies to milk, egg, cat, and house-dust mite were done with the Pharmacia CAP FEIA immunoassay on a UniCAP 100 automatic analyser (Pharmacia and Upjohn; Uppsala, Sweden) in accordance with manufacturer's instructions. An antigen-specific IgE value of more than 0·35 kU/L was classed as increased. Skin-prick tests were done as previously described,[20] and antigens tested included: milk; wheat and rye flours, both diluted 1/10 (weight/volume) with 0·9% (weight/volume) sodium chloride; gliadin diluted 1/1000 (weight/volume) with 0·9% (weight/volume) sodium chloride; banana, potato, and carrot (all three by prick-prick technique), egg white, cod, soya bean, birch, six local grasses, cat, dog, and *Dermatophagoides pteronyssimus* allergen Der p1 (ALK; Abellò, Denmark); and latex (Stallergens; Marseille, France).

Statistical analysis

The anticipated frequency of atopic disease in the placebo group was 50%. With at least 56 individuals in each group, a reduction of 25% in the frequency of atopic disease could be detected at a 5% level of significance with 80% power. Normally distributed data are expressed as means with 95% CI, and skewed data as geometric means with 95% CI after logarithmic transformation. Values were compared between the groups by unpaired *t* test. χ^2 test was used to compare proportions between the groups. Relative risk and the number needed to treat, both with 95% CI, were used to describe the treatment effect of *Lactobacillus* GG.[21] The proportion (and 95% CI) of children with atopic disease in both groups was calculated with the formulas devised by Gardner and Altman.[22] Total IgE concentration was rated as high if it were greater than the geometric mean concentration of total IgE plus 1 SD in children without atopic disease.[23] A p value less than 0·05 was regarded as statistically significant.

Results

Baseline characteristics and participants

Baseline characteristics were similar in the placebo and *Lactobacillus* GG groups (table 1). The mean (95% CI) start of treatment was 26 (24–28) days before delivery. 132 of 159 (83%) participants completed the 2-year study. The only reason for discontinuation was non-compliance—ie, the family did not arrive for the study visit—and rates were similar in both groups (figure 1). None of the drop-outs had shown signs of atopic disease before discontinuation. The mean (95% CI) times of exclusive and mean total time of breastfeeding were closely similar between children given placebo, 2·7 (2·2–3·1) months and 6·4 (5·4–7·5) months, respectively, and those on *Lactobacillus* GG, 3·0 (2·6–3·4) months and 7·2 (6·4–8·1) months, respectively (p=0·28 and p=0·24, respectively).

Atopic eczema and sensitisation

Atopic eczema was diagnosed in 46 of 132 (35%) children at age 2 years. Six of these children also fulfilled the diagnostic criteria for asthma and one for allergic rhinitis. The mean (95% CI) duration of breastfeeding was very close between infants with atopic eczema and those without—7·0 (5·8–8·2) months and 6·7 (5·9–7·5) months, respectively (p=0·65). In children with atopic eczema the geometric mean (95% CI) age at onset of symptoms was 4·9 (3·9–6·2) months, and objective SCORAD at 24 months was 10·2 (9·3–11·2). Serum total IgE

	Placebo (n=82)	*Lactobacillus* GG (n=77)
Family history		
Maternal atopic disease	63 (77%)	55 (71%)
Atopic eczema in family	35 (43%)	31 (40%)
Older sibling	30 (37%)	26 (34%)
Parental smoking	17 (21%)	9 (12%)
Furry pet at home	9 (11%)	16 (21%)
Birth characteristics		
Weeks of gestation	39 (1·4)	39 (1·3)
Boys	43 (52%)	49 (64%)
Cord blood IgE*	19/63 (30%)	21/53 (40%)
Weight (g)	3612 (466)	3631 (483)
Head circumference (cm)	35 (1·4)	35 (1·3)

Data are mean (SD) or proportion.
*Number of neonates with detectable total IgE (detection limit 0·5 kU/L) in cord blood.

Table 1: **Family history and birth characteristics**

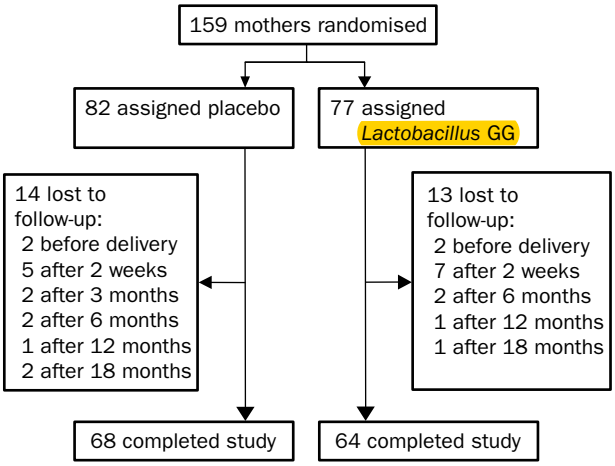

Figure 1: **Trial profile**

concentrations were greater in children with atopic eczema than in those without, at ages 12 and 24 months (p=0·009 and p=0·02, respectively; data not shown). Skin-prick test reactivity to common environmental antigens was more frequent in children with atopic eczema than in healthy children at ages 12 and 24 months (p=0·03 and p=0·01, respectively; data not shown), whereas frequency of increased antigen-specific IgE concentrations in serum was much the same between the groups (p=0·22 and 0·31, respectively; data not shown). The most common antigens that elicited positive reactions by both methods were egg and cow milk.

Effects of probiotics

The frequency of atopic eczema was reduced by half in infants given probiotics compared with those on placebo—15 of 64 (23%) and 31 of 68 (46%), respectively (p=0·008); relative risk (95% CI) 0·51 (0·32–0·84, figure 2). The number needed to treat was 4·5 (2·6–15·6). In affected children the objective SCORAD geometric mean (95% CI) at 24 months was 10·4 (9·3–11·6) in the placebo and 9·8 (8·2–11·8) in the *Lactobacillus* GG group (p=0·60).

Most mothers in both the placebo and the *Lactobacillus* GG group chose to give capsules to infants, 39 of 68 (57%) and 36 of 64 (56%), respectively (p=0·90). Preventive effect did not depend on mode of administration; in the *Lactobacillus* GG group atopic eczema was diagnosed in nine of 36 (25%) infants who consumed the probiotic themselves and six of 28 (21%) infants whose breastfeeding mothers took the capsules (p=0·74). Concentration of total IgE and frequencies of increased antigen-specific IgE

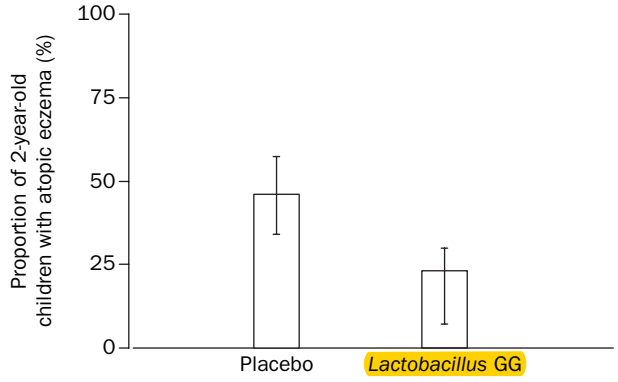

Figure 2: **Treatment effect of *Lactobacillus* GG on atopic disease**
Bars are 95% CI.

	Placebo (n=68)	*Lactobacillus* GG (n=64)	p
Total IgE (kU/L)*			
3 months	3·0 (2·4–3·7)	3·1 (2·5–4·0)	0·79§
12 months	9·7 (7·0–13·4)	11·2 (8·0–15·7)	0·55§
24 months	32·7 (22·6–47·3)	31·3 (22·8–43·0)	0·85§
Increased RAST readings†			
3 months	2/66 (3%)	2/58 (3%)	0·90‖
12 months	15/66 (23%)	16/62 (26%)	0·68‖
24 months	16/64 (25%)	17/62 (27%)	0·76‖
Prick test reaction‡			
6 months	7 (10%)	11 (17%)	0·25‖
12 months	12 (18%)	17/63 (27%)	0·20‖
24 months	9/65 (14%)	11/61 (18%)	0·52‖

*Geometric mean 95% CI. †Number with at least one increased (>0·35 kU/L) antigen-specific IgE concentration in radioallergosorbent (RAST) assay. ‡Number with one positive skin-prick test reaction. §Unpaired *t* test of logarithmically transformed data. ‖χ^2 test (placebo vs *Lactobacillus* GG).

Table 2: **Total IgE antibodies, antigen-specific IgE antibodies, and skin-prick test reaction**

concentrations and of positive reactions in skin-prick tests were very similar between probiotic and placebo groups (table 2). Frequency of children with high total IgE concentration (>93·3 kU/L) was 11 of 61 (18%) in the probiotic and 17 of 63 (27%) in the placebo group at 2 years of age (p=0·23).

Discussion

We have shown prospectively that a specific microbe can prevent atopic disease. Our new insight might provide an opportunity to devise strategies against allergy, the pandemic that affects almost half the population in more-developed countries.[1]

The notion of probiotics use in primary prevention of atopic disease was based on their ability to reverse increased intestinal permeability[24]—characteristic of children with atopic eczema and food allergy.[25] Probiotics also enhance gut-specific IgA responses,[24] which are often defective in children with food allergy.[26] They also help to promote gut barrier function and restore normal gut microecology,[15] alterations in which have been shown in allergic individuals.[27] Some probiotics alleviate changes related to allergic inflammation in vitro and in vivo.[11,16,28] Use of probiotics in allergy prevention is further lent support by results of studies[11,29] showing that oral lactobacilli in atopic children enhance transforming growth factor β and interleukin 10 production in vivo. Findings from clinical and experimental studies[12,30] suggest that these anti-inflammatory cytokines have a crucial role, possibly more essential than that of T-helper-1-type inducers, in prevention and treatment of atopy and atopic diseases. Thus, specific strains in indigenous gut microflora have profound effects on the physiology and immunology of the host.

At birth, the human gastrointestinal tract is sterile, but in the first months and years of life a rapid sequential colonisation occurs until a stable indigenous gut microflora is established.[15] Simultaneously, the T-helper-2-dominant immunity of newborn babies is intensified in atopic individuals, with the subsequent expression of atopic disease.[31] In support of an essential role for indigenous gut microflora in this process, a reduced ratio of bifidobacteria to clostridia in early gut microflora precedes the development of atopy and atopic disease.[32] Dietary antigens also strongly affect the neonatal gastrointestinal system. Results from work in animals indicate that these antigens might provoke atopic-type immunity at mucosal and systemic level.[33] Therefore, treatment for counter-regulation of allergy must work in infancy, and preferably in the first encounters with dietary antigens. Probiotics are

appropriate for the task, not only with respect to timing, but also in their ability to reduce dietary antigen load by degradation and modification of macromolecules.[34] This process of antigen degradation is necessary in development of non-responsiveness to dietary antigens.[35]

A notable overlap occurs in concentrations of total IgE antibodies between atopic and non-atopic children;[36] transient symptomless increases in antigen-specific IgE antibodies happen in up to 80% of healthy children in their first 5 years of life.[37] Furthermore, neonatal concentrations of cytokines that promote production of IgE antibodies are transiently raised in children who do not later develop atopic disease, but not in those who do. Frequency of atopic eczema in our placebo group at age 2 years was within the range that Bergmann and colleagues reported.[38] Because of the natural course of development of IgE antibodies and respiratory allergic diseases,[36–39] we cannot yet estimate the frequencies of other atopic diseases and the effects on them, if any, of probiotics.

Our results suggest that gut microflora have unique, yet largely unexplored, endogenous immunomodulatory properties. These properties might be indispensable in the fight against the increasing frequency of atopic, and possibly other, immunological diseases.

Contributors

Erika Isolauri, Seppo Salminen, Heikki Arvilommi, Pentti Kero, and Marko Kalliomäki developed and designed the study. Pertti Koskinen analysed and interpreted IgE assays. Infants were clinically examined by Marko Kalliomäki, who also analysed and interpreted data with Erika Isolauri. All investigators helped write this report.

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