Allergy 2005: 60: 494–500

ALLERGY
DOI: 10.1111/j.1398-9995.2004.00514.x

Original article

Probiotics in the treatment of atopic eczema/dermatitis syndrome in infants: a double-blind placebo-controlled trial

Background: Probiotic bacteria are suggested to reduce symptoms of the atopic eczema/dermatitis syndrome (AEDS) in food-allergic infants. We aimed to investigate whether probiotic bacteria have any beneficial effect on AEDS.
Methods: Follow-up of severity of AEDS by the Severity Scoring of Atopic Dermatitis (SCORAD) index in 230 infants with suspected cow's milk allergy (CMA) receiving, in a randomized double-blinded manner, concomitant with elimination diet and skin treatment, *Lactobacillus* GG (LGG), a mixture of four probiotic strains, or placebo for 4 weeks. Four weeks after the treatment, CMA was diagnosed with a double-blind placebo-controlled (DBPC) milk challenge in 120 infants.
Results: In the whole group, mean SCORAD (at baseline 32.5) decreased by 65%, but with no differences between treatment groups immediately or 4 weeks after the treatment. No treatment differences were observed in infants with CMA either. In IgE-sensitized infants, however, the LGG group showed a greater reduction in SCORAD than did the placebo group, −26.1 *vs* −19.8 ($P = 0.036$), from baseline to 4 weeks after the treatment. Exclusion of infants who had received antibiotics during the study reinforced the findings in the IgE-sensitized subgroup.
Conclusion: Treatment with LGG may alleviate AEDS symptoms in IgE-sensitized infants but not in non-IgE-sensitized infants.

M. Viljanen[1], E. Savilahti[2], T. Haahtela[1], K. Juntunen-Backman[1], R. Korpela[3,4], T. Poussa[5], T. Tuure[3], M. Kuitunen[1]

[1]The Skin and Allergy Hospital, University of Helsinki, Helsinki; [2]The Hospital for Children and Adolescents, University of Helsinki, Helsinki; [3]Valio Research and Development, Helsinki; [4]Institute of Biomedicine, Pharmacology, University of Helsinki, Helsinki; [5]STAT-Consulting, Tampere, Finland

Key words: atopic dermatitis; food allergy; infants; *Lactobacillus* GG; probiotic; treatment.

Mirva Viljanen
The Skin and Allergy Hospital
University of Helsinki
Meilahdentie 2
00250 Helsinki
Finland

Accepted for publication 12 January 2004

Infantile atopic eczema/dermatitis syndrome (AEDS) is often the first sign of allergies and is associated with food hypersensitivities in a high percentage of cases (1–3). Epidemiological data suggest that changes in gut microbes, reduction of feco-oral infections, and microbes in food products may affect the emergence of allergic diseases (4–7). Indirect evidence for this is the finding that atopic children have more coliforms and clostridia and fewer bifidobacteria and lactobacilli in the gut flora, than do the nonatopic children (4, 7). Some inconsistency, however, exists, as one study demonstrated high prevalence of lactobacilli among allergic children (5). Probiotic bacteria, which beneficially affect the host by improving its microbial balance (8), may mediate antiallergenic effects by stimulating production of Th1-cytokines (9, 10), transforming growth factor-β (11, 12), and gut IgA (13, 14). They may reduce symptoms of AEDS and cow's milk allergy (CMA) in infants, but thus far few clinical trials exist (11, 15, 16).

We investigated the possibility of alleviating symptoms of AEDS with probiotic bacteria. In this double-blind placebo-controlled (DBPC) study, we gave *Lactobacillus* GG (LGG) alone, LGG in combination with three other probiotics, or placebo to AEDS infants suspected to have CMA.

Methods

Participants

This randomized DBPC study was carried out between November 1999 and March 2002 in the Skin and Allergy Hospital of Helsinki University Central Hospital, Helsinki, Finland. Inclusion criteria were: (i) age under 12 months upon entering the study, (ii) symptoms suggestive of CMA, the obligatory symptom being AEDS, (iii) no probiotic preparations used regularly (longer than 1 week, and within 6 weeks before entering the study). Of the 431 infants referred from local health centres, 284 (66%) had parents who wished to participate. Of the 252 infants meeting our inclusion criteria, 230 (aged 1.4–11.9 months, mean 6.4; 62% males) completed the study. One parent of each infant gave written informed consent. The local ethics committee approved the study protocol.

Assessment and treatment of AEDS

At consecutive visits (Fig. 1), the same physician (MV) carefully assessed the severity of AEDS by the Severity Scoring of Atopic Dermatitis (SCORAD) (17). This SCORAD scores the extent (0–100); intensity (estimated by help of reference photographs) as the sum of individual scores for erythema, oedema/papules, oozing/crusts, excoriation, lichenification, and skin dryness (each 0–3, maximum 18); and subjective symptoms including pruritus and sleep-loss (assessed by a parent, scored 0–10-points each, maximum 20). SCORAD was calculated by:

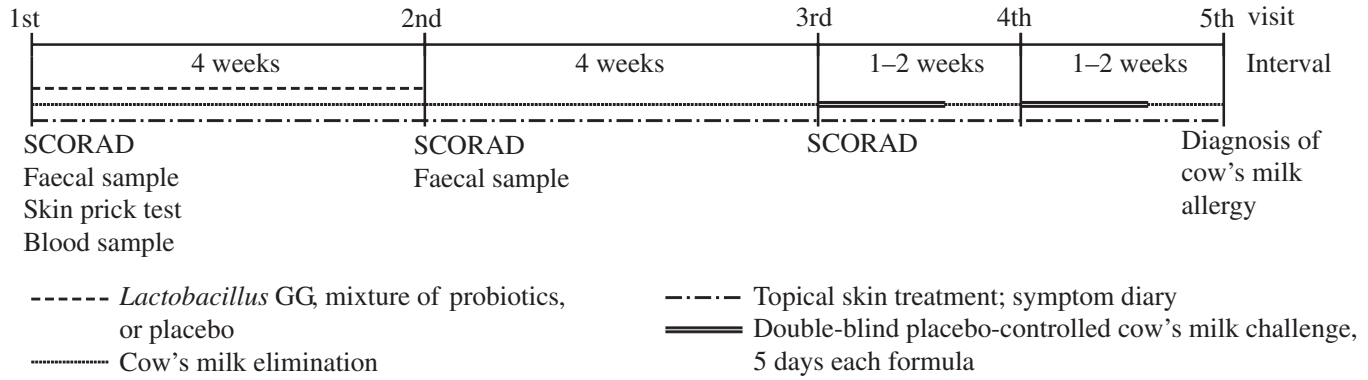

Figure 1. Study protocol.

extent/5 + 3.5 × intensity + subjective symptoms (maximum 103). Parents were instructed to treat the infant's eczematous lesions with emollients continuously and with topical 1% hydrocortisone as needed but for a maximum period of 2 weeks in a row (amount used recorded at every visit).

Skin prick test and serum IgE concentrations

Skin prick tests (SPTs) with commercial allergen extracts of egg-white (Alyostal prick test®, Stallergenes SA, Antony, France), cat, dog, and birch (Soluprick®, ALK-Abellò, Hørsholm, Denmark) were performed according to the standard technique (18). Duplicate tests were performed with fat-free CM; a panel of 10 widely used commercially available adapted CM, extensively hydrolysed, amino acid-, and soy protein-based infant formulas; cereal grains and purified gliadin (19). Any mean weal diameter ≥3 mm greater than the negative control was considered positive. Concentrations of serum, CM, and wheat-specific IgE were measured by the Pharmacia CAP system RAST FEIA (Pharmacia Ltd, Uppsala, Sweden).

Elimination diet

Initially, all products containing CM were eliminated from the infants' and breast-feeding mothers' diets (Fig. 1). All infants received extensively hydrolysed whey formula (EHF) (Peptidi-Tutteli®, Valio Ltd, Helsinki, Finland). If any other foods were suspected to cause symptoms, they were also eliminated.

Treatment with probiotics

Infants were randomized at the first visit according to computer-generated block randomization of six infants to receive one of three products in a double-blinded manner for 4 weeks (Fig. 1). (i) The LGG group ($n = 80$) received capsules containing *L. rhamnosus* GG (ATCC 53103) 5×10^9 colony-forming units (cfu); (ii) the MIX group ($n = 76$), a mixture of probiotics: LGG 5×10^9 cfu, *L. rhamnosus* LC705 (LC705) 5×10^9 cfu, *Bifidobacterium breve* Bbi99 2×10^8 cfu, and *Propionibacterium freudenreichii* ssp. *shermanii* JS (*Propionibacterium* JS) 2×10^9 cfu; and (iii) the placebo group ($n = 74$), only the inert matrix material, microcrystalline cellulose. The capsule content was mixed with food twice daily. These products (supplied by Valio Ltd) looked, smelled and tasted identical. The code was opened after all data were analysed. Parents were urged to give no probiotic preparations to the infants during the study.

Symptom diary

Parents recorded daily in a symptom diary any skin, gastrointestinal, or respiratory symptom; the start of new solid foods; and use of antibiotics.

Diagnosis of cow's milk allergy

When the symptoms had subsided by the third visit (Fig. 1), we started a DBPC CM challenge, in which CM formula (Tutteli®, Valio Ltd) was mixed with the EHF (1 : 2) to make it indistinguishable from the placebo formula (EHF alone). The challenge was started with drops on the skin followed by the same formula given orally in quantities of 2, 10, 50 and 100 ml at 30-min intervals. If no symptoms appeared during the challenge or 2-h follow-up, the infant received the same formula 4–6 dl daily at home for the next 4 days, unless an adverse reaction appeared. At the fourth visit, after a wash-out period of 2–9 days, the challenge formula was changed and this procedure repeated. Thereafter, symptom diaries of the two challenge periods were compared, a decision of symptomatic challenge period was made, and the milk code was opened. CMA was diagnosed in 120 infants who showed urticaria, clear worsening of AEDS, vomiting, diarrhoea, physician-diagnosed wheezing, allergic rhinitis, or conjunctivitis during the challenge with CM-containing formula. In 110 infants the challenge was negative and CMA was excluded. Other food sensitivities were not studied by challenge test.

Definition of IgE-associated AEDS

Any infant with positive SPT or an antigen-specific IgE concentration above 0.7 kU/l to any antigen tested was considered to have IgE-associated AEDS.

Faecal samples

Paired faecal samples were collected from 52 infants at both the first and second visits. All the samples were frozen at −20°C within 15 min, and if taken at home, brought frozen to the hospital, and stored at −70°C until analysis. The samples were analysed on plates for concentrations of anaerobic bacteria (Brain Heart Infusion agar, Lab M, Lancashire, UK), lactic acid bacteria (MRS-agar, Oxoid Ltd, Basingstoke, UK), and bifidobacteria (Raffinose-Bifidobacterium-agar, RB) (20). Concentrations of the two strains of lactobacilli were determined with MRS-vancomycin agar (Oxoid

Ltd) and that of the *Propionibacterium* JS with modified YEL agar (21). Limits of detection were 10^5 cfu/g for bifidobacteria and 10^3 cfu/g for the other bacteria.

Withdrawals

Of the 22 infants not completing the study; two moved away from the area, 11 did not start the elimination diet and skin treatment alleviated the symptoms, four could not tolerate the EHF, three parents found the protocol of the study too difficult, and in two cases infants were hospitalized for severe AEDS, and their study treatment was discontinued. Of the withdrawals, 18 occurred between the first and second visits, four between the second and third; of these 22; five, nine, and eight belonged to the LGG, MIX, and placebo groups.

Statistical analysis

Sample size calculation was based on SCORAD. It was assumed that compared with the placebo, the active treatments (LGG or the mixture of probiotics) would result in a difference of 8-points in SCORAD. Assuming a common SD of 9.5 (15), we estimated that for all paired comparisons a sample size of 40 CMA infants per group has 90% power to detect a difference statistically significant at the 0.05 level. Almost 80 infants per group had to be followed up, as CMA diagnosis was made after the treatment, and only half of the infants that participated eventually had CMA.

The SCORAD was the primary outcome variable. Since the interval after probiotic bacteria administration when possible effects appear in humans is unknown, we decided, before analysis, to calculate the changes from baseline (visit 1) at visit 2, after a 4-week treatment, and at visit 3, 4 weeks after the end of the treatment. ANOVA was performed to compare the treatment groups with respect to the changes in SCORAD. Fisher's LSD test was used for comparisons between probiotic groups *vs* placebo. Treatment differences are given as mean values with 95% confidence intervals.

As an exploratory analysis, ANOVA (with mixed effects) was used to test interactions between treatment and different baseline characteristics. The ANOVA yielded significant ($P < 0.05$) interactions between treatment groups and IgE-association, and an almost significant (< 0.10) interaction between treatment groups and baseline SCORAD; indicating that the treatment effect may be dependent on IgE-association, and baseline SCORAD; subgroup analyses were performed to control for the interactions.

The Kruskal–Wallis test and Mann–Whitney *U*-test were used to test changes in counts of faecal bacteria.

Results

Table 1 shows the main clinical characteristics in different treatment groups.

Symptoms of AEDS improved continuously, as indicated by the decreasing SCORAD during the study in all treatment groups: from baseline (first visit) to time immediately after treatment (second visit) mean change was −15.0, and from baseline to 4 weeks after the end of treatment (third visit) it was −21.3. No difference existed, however, in the decrease in SCORAD between any of the three treatment groups, neither the groups comprising infants with AEDS, nor those comprising infants with CMA, from the first to the second visit (Table 2). From the first to third visit, infants with AEDS treated with LGG showed a nonsignificantly greater decrease than did placebo-treated infants (mean −2.6, 95% CI: −7.4 to 2.1; $P = 0.273$), with no difference between the MIX- and placebo-treated infants (mean −0.1, 95% CI: −5.0 to 4.7; $P = 0.953$). Among infants with CMA, differences between the LGG and placebo (mean −1.3, 95% CI: −8.6 to 6.1; $P = 0.730$) and the MIX and placebo treatments (mean 2.0, 95% CI: −5.3 to 9.4; $P = 0.582$) were also nonsignificant (Table 2).

In subgroup analyses from the first to third visit among infants with IgE-associated AEDS, the LGG group showed a significantly greater reduction in SCORAD than did the placebo group ($P = 0.036$, Table 3). The difference in this subgroup was mainly due to the effect of LGG in those with moderate to severe AEDS (baseline SCORAD ≥ 30), although this difference did not reach significance ($P = 0.075$). We saw no differences between the treatment groups in any of the subgroup analyses from the first to second visit (data not shown).

Since antibiotics interfere with the gut microbe balance, and the number of antibiotic courses was high and unevenly distributed among treatment groups (Table 1), we made a secondary analysis after excluding infants who had received antibiotics between the first and third visits. Among infants with IgE-associated AEDS, SCORAD decreased more for the LGG and MIX groups than for the placebo group, and differences between probiotic and

Table 1. Characteristics in different treatment group of infants with the atopic eczema/dermatitis syndrome (AEDS, whole study population) and with verified cow's milk allergy

	AEDS			Cow's milk allergy		
	LGG ($n = 80$)	MIX ($n = 76$)	Placebo ($n = 74$)	LGG ($n = 44$)	MIX ($n = 44$)	Placebo ($n = 32$)
Age (months)	6.1 (1.8–11.1)	6.3 (1.4–11.4)	6.8 (2.4–11.9)	5.9 (1.8–10.8)	5.8 (1.4–11.4)	6.1 (2.5–11.6)
Exclusive breast-feeding (months)	1.8 (0–5)	1.7 (0–5)	1.8 (0–6)	2.1 (0–5)	2.0 (0–5)	2.3 (0–6)
Age at introduction of cow's milk formula (months)	2.0 (0–10)	1.9 (0–8)	1.8 (0–10)	2.5 (0–10)	2.3 (0–8)	2.4 (0–10)
Age at introduction of solid foods (months)	3.8 (1.5–6.0)	3.7 (2–5.2)	3.8 (1.5–6)	4.0 (1.5–6)	3.8 (2.5–5.2)	4.0 (2.5–6)
Topical hydrocortisone* (g), first to third visit	45 (0–500)	45 (0–300)	38 (0–300)	60 (0–230)	40 (1–300)	50 (5–300)
Antibiotic treatments, first to third visit, *n* (%)	20 (25)	28 (37)	20 (27)	12 (27)	18 (42)	7 (22)
Gastrointestinal infections, first to third visit, *n* (%)	11 (14)	10 (13)	10 (14)	4 (9)	6 (14)	6 (19)

Figures are mean or medians* (ranges) and numbers (percentage) of infants.
LGG, *Lactobacillus* GG.

Table 2. Severity Scoring of Atopic Dermatitis (SCORAD) at the first visit and SCORAD changes from the first to the second and from the first to the third visit in atopic eczema/dermatitis syndrome (AEDS, whole study population), cow's milk-allergic (CMA+), and noncow's milk allergic (CMA–) infants in different treatment groups

		AEDS ($n = 230$)			CMA+ ($n = 120$)			CMA– ($n = 110$)		
		Lactobacillus GG (LGG, $n = 80$)	MIX ($n = 76$)	Placebo ($n = 74$)	LGG ($n = 44$)	MIX ($n = 44$)	Placebo ($n = 32$)	LGG ($n = 36$)	MIX ($n = 32$)	Placebo ($n = 42$)
Visit 1	Mean	34.3	33.3	29.9	33.9	33.4	32.9	34.7	33.1	27.6
	SD	17.2	15	12.2	17.5	14.7	12.3	17.0	15.7	11.8
Change (visit 1–2)	Mean	−16.6	−14.0	−14.2	−15.1	−14.5	−15.2	−18.3	−13.4	−13.4
	SD	13.0	12.9	10.3	14.0	13.7	10.3	11.6	11.8	10.4
Change (visit 1–3)	Mean	−22.9	−20.4	−20.3	−22.7	−19.4	−21.4	−23.2	−21.9	−19.4
	SD	16.1	15.8	12.6	17.5	15.9	13.6	14.5	15.7	11.9

Table 3. Severity Scoring of Atopic Dermatitis (SCORAD) changes from the first to the third visit, SCORAD at baseline, and at visit 3 in different treatment groups in subgroups of atopic eczema/dermatitis syndrome infants

Baseline SCORAD			LGG	MIX	Placebo	anova (*P*-value)	LGG *vs* placebo (*P*-value)*
With IgE-association†		Mean baseline	21.2	21.3	19.5		
		Mean at visit 3	9.5	11.0	9.1		
	<30	Mean change	−11.7	−10.3	−10.4	0.830	0.613
		SD	8.1	6.1	8.3		
		n	18	22	23		
		Mean baseline	48.1	41.2	40.4		
		Mean at visit 3	12.8	14.7	11.2		
	≥30	Mean change	−35.4	−26.6	−29.1	0.037	0.075
		SD	13.7	13.7	8.5		
		n	28	22	23		
		Mean baseline	37.6	31.3	29.9		
		Mean at visit 3	11.5	12.8	10.2		
	All	Mean change	−26.1	−18.4	−19.8	0.027	0.036
		SD	16.6	13.3	12.6		
		n	46	44	46		
Without IgE-association		Mean baseline	29.8	36.1	29.9		
		Mean at visit 3	11.1	12.9	8.8		
	All	Mean change	−18.7	−23.2	−21.1	0.508	0.545
		SD	14.7	18.6	12.8		
		n	34	32	28		

* in Fisher's LSD test.

† any skin prick test (SPT)-positive or any antigen-specific IgE concentration ≥ 0.7 kU/l; LGG, *Lactobacillus* GG.

placebo groups increased compared with the results in the primary analyses (Table 4). For all infants, however, these differences between treatments remained nonsignificant (Table 4).

Before randomization to the treatment groups, the LGG strain was detected in the faeces of six of 18 of the LGG group, two of 17 of the MIX group, and three of 17 of the placebo group infants. Before treatment, faecal counts of the probiotic bacteria in all treatment groups were low (Table 5). Immediately after the LGG treatment, LGG counts were high, and LC705 and *Propionibacterium* JS counts were low; and after the MIX treatment, LGG, LC705 and *Propionibacterium* JS counts were high (Table 5). No changes occurred in the placebo group.

Treatment resulted in increases in total counts of lactobacilli in the probiotic groups and decreases in the placebo group, and differences in changes between probiotic groups and the placebo group were significant (Table 5). Total counts of bifidobacteria showed no major changes.

Discussion

We found no beneficial effect from probiotic bacteria when we looked for SCORAD reductions in a large group of infants with AEDS and in those concomitantly having CMA. When, however, infants with IgE-associated AEDS were considered, the eczema in the LGG group significantly improved, compared with the eczema in the placebo group, but the MIX group showed no effect. The proportion of infants treated with antibiotics during or shortly after consuming the study preparations was large, and the removal of such infants improved the treatment effect of

Table 4. Severity Scoring of Atopic Dermatitis (SCORAD) changes from the first to the third visit, SCORAD at baseline, and at visit 3 in different treatment groups in atopic eczema/dermatitis syndrome (AEDS), cow's milk allergic (CMA), and IgE-associated AEDS infants after exclusion of infants receiving antibiotics between the first and third visits

Baseline SCORAD			LGG	MIX	Placebo	anova (*P*-value)	LGG *vs* placebo (*P*-value)*
AEDS		Mean baseline	37.5	34.1	30.6		
		Mean at visit 3	12.1	11.3	9.8		
	All	Mean change	−25.4	−22.8	−20.9	0.268	0.107
		SD	16.8	14.5	13.2		
		n	60	47	54		
CMA		Mean baseline	38.2	32.7	34.0		
		Mean at visit 3	12.4	12.4	11.8		
	All	Mean change	−25.9	−20.3	−22.2	0.397	0.390
		SD	18.5	12.5	14.5		
		n	32	25	25		
AEDS with IgE-association†		Mean baseline	50.8	42.5	40.6		
		Mean at visit 3	12.4	13.9	12.1		
	≥30	Mean change	−38.4	−28.7	−28.5	0.009	0.008
		SD	13.0	11.7	8.9		
		n	23	17	18		
		Mean baseline	40.6	33.1	30.4		
		Mean at visit 3	12.1	11.0	10.7		
	All	Mean change	−28.5	−22.1	−19.7	0.044	0.016
		SD	17.5	12.8	13.3		
		n	36	28	33		

* Fisher's LSD test.

† Any skin prick test (SPT)-positive or any antigen-specific IgE concentration ≥ 0.7 kU/l; LGG, *Lactobacillus* GG.

Table 5. Median (range) counts (log cfu/g) and prevalence of given probiotic bacteria in faeces at first and second visits in treatment groups

		LGG ($n = 18$)		MIX ($n = 17$)		Placebo ($n = 17$)	
Strains	Visit	Counts	Prevalence	Counts	Prevalence	Counts	Prevalence
Lactobacillus GG	1	3 (3–8.1)	6 (33)	3 (3–7.8)	2 (12)	3 (3–8.4)	3 (18)
	2	6.4 (3–8.5)	13 (72)	6.6 (3–8.9)	15 (88)	3 (3–7.8)	2 (12)
Lactobacillus rhamnosus LC705	1	3 (3–3)	0 (0)	3 (3–3)	0 (0)	3 (3–3)	0 (0)
	2	3 (3–6.3)	2 (11)	5.8 (3–7.5)	9 (53)	3 (3–3)	0 (0)
Propionibacterium JS	1	3 (3–3)	0 (0)	3 (3–6.6)	2 (12)	3 (3–3)	0 (0)
	2	3 (3–3)	0 (0)	5.9 (3–7.2)	11 (65)	3 (3–3)	0 (0)
Bifidobacteria total	1	8.7 (5–10.9)	15 (83)	9.3 (5–11.3)	15 (88)	8.9 (5.3–11.3)	16 (94)
	2	8.3 (5–10.7)	14 (78)	8.9 (6–11.3)	17 (100)	9.1 (5–10.4)	14 (82)
Lactobacilli total	1	6.4* (3–8.4)	12 (67)	5.5* (3–8.7)	9 (53)	6.3 (3–9.5)	11 (65)
	2	6.9 (3–8.5)	14 (78)	6.8 (4.3–9)	17 (100)	4.3 (3–9.4)	9 (53)

Prevalence column shows number (percentages) of infants with detectable levels of corresponding strain.

* Change in lactobacilli counts: Kruskall–Wallis test ($P = 0.009$), *Lactobacillus* GG (LGG) *vs* placebo ($P = 0.029$), MIX *vs* placebo ($P = 0.002$).

both LGG and the MIX in the IgE-associated subgroup. Removal of these infants did not, however, change the SCORAD decreases in the placebo group.

In two previous studies with infants, LGG and bifidobacteria supplementation showed a 9- to 13-points greater reduction in the SCORAD than did the placebo (11, 15). Study populations were, however, small. In a recent study, 1- to 13-year-old children experienced a combination of two *Lactobacillus* strains to improve AEDS symptoms compared with the placebo treatment, but SCORAD did not change in the whole study population (22). In a study concerning primary prevention of allergies with probiotics prevalence of AEDS was lower in the probiotic than in the placebo group, but no differences appeared in severity of AEDS, or in number of positive SPTs or increased IgE concentrations (23).

Unlike findings of previous probiotic studies (11, 15), we saw an alleviation of AEDS symptoms in the placebo group. Elimination of allergenic CM proteins in infants with CMA by use of EHF usually has a marked effect on AEDS symptoms: SCORAD was reduced by 8- to 12-points (47–50%) during 4- and 12-week treatments (24–26). We saw a mean reduction of 21.4 (65%) in SCORAD, and the initial level in our study was higher; surprisingly, the reduction was the same in infants with and without CMA (65 and 70%). As evidenced by the decrease in SCORAD in the non-CMA placebo group, careful instruction in topical care of AEDS was highly

effective. In contrast, a recent study reported only modest efficiency for hydrocortisone acetate in children over 2 years old (27).

Highly effective topical treatment and an elimination diet may have prevented us from fully appreciating the effects of probiotic preparations in this DBPC trial. It could also be argued that treatment or follow-up was too short. CMA was diagnosed after the treatment, and prolonged elimination with EHF might have become stressful for some families. On the other hand, SCORAD was greatly reduced and low after the 8 weeks' follow-up. Randomization did not succeed well, as SCORAD differed at baseline in treatment groups, and we cannot exclude that some of the effect we saw might be due to that.

The LGG had a positive effect only in the IgE-associated subgroup. Also a recent study showed a combination of two *Lactobacillus* strains to decrease SCORAD in IgE-sensitized 1- to 13-year-old children (22). In two previous studies, IgE-sensitization has occurred with 4–37% of antigens (CM, egg and wheat) tested (11, 15). Still, these studies showed the positive effect of probiotics in study populations comprising infants both with and without IgE-sensitization. In a trial, LGG showed no effect on rhinoconjunctivitis-associated oral symptoms against apple in young adults with birch allergy (28). In our study, IgE-mediated CMA infants showed a similar trend in SCORAD changes as was seen in the IgE-associated subgroup, but none of the differences was significant probably because of small group sizes, as only 45% of CMA infants were IgE-mediated. Our findings that treatment with probiotics does not benefit all infants with AEDS, only those with an IgE-association, is in accordance with studies demonstrating a shift of the Th1/Th2 balance towards the Th1 type with lactobacilli treatment (9, 10). The LGG effect in our study was seen 4 weeks after the treatment period, but not immediately after the treatment. Another study in AEDS children showed LGG to increase IL-10 concentration in sera 4 weeks after the treatment, not immediately (29).

We assumed that the mixture of many probiotics might reinforce any beneficial effects of LGG as a combination of two different *Lactobacillus* strains reduced symptoms in IgE-associated AEDS symptoms in children (22). In addition, a mixture of eight different probiotic strains in very high concentrations effectively reduced relapses of chronic pouchitis after ileal pouch-anal anastomosis for ulcerative colitis in adults, and the authors speculated that a composition of many strains might prove most effective (30). In this study, however, combining LGG with other probiotic strains suppressed the effect seen with LGG alone. The suppressive mechanism is not known. An explanation for this might be an interference of immunostimulating effects between the strains (13).

Antibiotics probably interfere with the colonization of probiotics (31), thereby reducing the treatment effect. Antibiotics as such seemed to have no effect on the skin as evidenced by similar changes in SCORAD in the placebo group before and after exclusion of infants treated with antibiotics.

We analysed only a portion of faecal samples, but we believe that this proportion was representative. The treatment succeeded well, as the faecal prevalence of the probiotic strains in the treatment groups was high. There were marked increases in total lactobacilli counts in the probiotic groups, but these changes alone do not explain the better effect of LGG compared with the MIX group on AEDS symptoms, as increases in lactobacilli in faecal samples were similar in both groups. Prior to the study, the prevalence of lactobacilli and bifidobacteria was high, since they belong to the normal gut flora in infants (4, 5). The use of probiotics in families was not forbidden prior to the study, which may have caused the detectable levels of LGG in some infants.

Influencing the gut microflora by administration of probiotic bacteria to treat allergy is a new alternative. Our results suggest that LGG, when administrated as a single probiotic strain, shows a greater SCORAD decrease than do the placebo group in infants suffering from IgE-associated AEDS. Further studies are needed to explore strain-specific effects and mechanisms of different probiotic bacteria on allergic patients.

Acknowledgments

Authors thank Marja Marja-Aho, Tina Ahlfors-Heinonen, and Kirsti Vornanen, MD for assistance during the study; and the Starter Cultures Unit, Valio Ltd for contribution in the bacterial analyses and technical assistance. This study was supported by Valio Limited, Finland; the Foundation for Nutrition Research, Helsinki, Finland; the Foundation for Paediatric Research, Helsinki, Finland; and the Research Foundation of Orion Corporation, Espoo, Finland.

References

1. Johansson SG, Hourihane JO, Bousquet J, Bruijnzeel-Koomen C, Dreborg S, Haahtela T et al.
A revised nomenclature for allergy. An EAACI position statement from the EAACI nomenclature task force.
Allergy 2001;**56**:813–824.

2. Sicherer SH, Sampson HA. Food hypersensitivity and atopic dermatitis: pathophysiology, epidemiology, diagnosis, and management. J Allergy Clin Immunol 1999;**104**:S114–122.

3. Guillet G, Guillet MH. Natural history of sensitizations in atopic dermatitis. A 3-year follow-up in 250 children: food allergy and high risk of respiratory symptoms. Arch Dermatol 1992;**128**:187–192.

4. Björkstén B, Naaber P, Sepp E, Mikelsaar M. The intestinal microflora in allergic Estonian and Swedish 2-year-old children. Clin Exp Allergy 1999;**29**:342–346.
5. Björkstén B, Sepp E, Julge K, Voor T, Mikelsaar M. Allergy development and the intestinal microflora during the first year of life. J Allergy Clin Immunol 2001;**108**:516–520.
6. Matricardi PM, Rosmini F, Riondino S, Fortini M, Ferrigno L, Rapicetta M et al. Exposure to foodborne and orofecal microbes *vs* airborne viruses in relation to atopy and allergic asthma: epidemiological study. BMJ 2000;**320**:412–417.
7. Kalliomäki M, Kirjavainen P, Eerola E, Kero P, Salminen S, Isolauri E. Distinct patterns of neonatal gut microflora in infants in whom atopy was and was not developing. J Allergy Clin Immunol 2001;**107**:129–134.
8. Fuller R. Probiotics in human medicine. Gut 1991;**32**:439–442.
9. Hessle C, Hanson LÅ, Wold AE. Lactobacilli from human gastrointestinal mucosa are strong stimulators of IL-12 production. Clin Exp Immunol 1999;**116**:276–282.
10. Miettinen M, Matikainen S, Vuopio-Varkila J, Pirhonen J, Varkila K, Kurimoto M et al. Lactobacilli and streptococci induce interleukin-12 (IL-12), IL-18, and gamma interferon production in human peripheral blood mononuclear cells. Infect Immun 1998;**66**:6058–6062.
11. Isolauri E, Arvola T, Sütas Y, Moilanen E, Salminen S. Probiotics in the management of atopic eczema. Clin Exp Allergy 2000;**30**:1604–1610.
12. Paganelli R, Ciuffreda S, Verna N, Cavallucci E, Paolini F, Ramondo S et al. Probiotics and food-allergic diseases. Allergy 2002;**57**:97–99.
13. Fukushima Y, Kawata Y, Mizumachi K, Kurisaki J, Mitsuoka T. Effect of bifidobacteria feeding on fecal flora and production of immunoglobulins in lactating mouse. Int J Food Microbiol 1999;**46**:193–197.
14. Kirjavainen PV, Apostolou E, Salminen SJ, Isolauri E. New aspects of probiotics – a novel approach in the management of food allergy. Allergy 1999;**54**:909–915.
15. Majamaa H, Isolauri E. Probiotics: a novel approach in the management of food allergy. J Allergy Clin Immunol 1997;**99**:179–185.
16. Matricardi PM, Björksten B, Bonini S, Bousquet J, Djukanovic R, Dreborg S et al. Microbial products in allergy prevention and therapy. Allergy 2003;**58**:461–471.
17. Severity Scoring of Atopic Dermatitis: the SCORAD Index. Consensus Report of the European Task Force on Atopic Dermatitis. Dermatology 1993;**186**:23–31.
18. Dreborg S, Backman A, Basomba A, Bousquet J, Dieges P, Malling HJ. Skin tests used in type I allergy testing. Position paper. EAACI Sub-committee on skin tests. Allergy 1989;**44**:1–59.
19. Varjonen E, Vainio E, Kalimo K. Life-threatening, recurrent anaphylaxis caused by allergy to gliadin and exercise. Clin Exp Allergy 1997;**27**:162–166.
20. Hartemink R, Kok BJ, Weenk GH, Rombouts FM. Raffinose-Bifidobacterium (RB) agar, a new selective medium for bifidobacteria. J Microbiol Methods 1996;**27**:33–43.
21. Suomalainen TH, Mäyrä-Mäkinen AM. Propionic acid bacteria as protective cultures in fermented milks and breads. Lait 1999;**79**:165–174.
22. Rosenfeldt V, Benfeldt E, Nielsen SD, Michaelsen KF, Jeppesen DL, Valerius NH et al. Effect of probiotic *Lactobacillus* strains in children with atopic dermatitis. J Allergy Clin Immunol 2003;**111**:389–395.
23. Kalliomäki M, Salminen S, Arvilommi H, Kero P, Koskinen P, Isolauri E. Probiotics in primary prevention of atopic disease: a randomised placebo-controlled trial. Lancet 2001;**357**:1076–1079.
24. Høst A, Koletzko B, Dreborg S, Muraro A, Wahn U, Aggett P et al. Dietary products used in infants for treatment and prevention of food allergy. Joint Statement of the European Society for Paediatric Allergology and Clinical Immunology (ESPACI) Committee on Hypoallergenic Formulas and the European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) Committee on Nutrition. Arch Dis Child 1999;**81**:80–84.
25. Isolauri E, Sütas Y, Mäkinen-Kiljunen S, Oja SS, Isosomppi R, Turjanmaa K. Efficacy and safety of hydrolyzed cow milk and amino acid-derived formulas in infants with cow milk allergy. J Pediatr 1995;**127**:550–557.
26. Niggemann B, Binder C, Dupont C, Hadji S, Arvola T, Isolauri E. Prospective, controlled, multi-center study on the effect of an amino acid-based formula in infants with cow's milk allergy/intolerance and atopic dermatitis. Pediatr Allergy Immunol 2001;**12**:78–82.
27. Reitamo S, Van Leent EJ, Ho V, Harper J, Ruzicka T, Kalimo K et al. Efficacy and safety of tacrolimus ointment compared with that of hydrocortisone acetate ointment in children with atopic dermatitis. J Allergy Clin Immunol 2002;**109**:539–546.
28. Helin T, Haahtela S, Haahtela T. No effect of oral treatment with an intestinal bacterial strain, *Lactobacillus rhamnosus* (ATCC 53103), on birch-pollen allergy: a placebo-controlled double-blind study. Allergy 2002;**57**:243–246.
29. Pessi T, Sütas Y, Hurme M, Isolauri E. Interleukin-10 generation in atopic children following oral Lactobacillus rhamnosus GG. Clin Exp Allergy 2000;**30**:1804–1808.
30. Gionchetti P, Rizzello F, Venturi A, Brigidi P, Matteuzzi D, Bazzocchi G et al. Oral bacteriotherapy as maintenance treatment in patients with chronic pouchitis: a double-blind, placebo-controlled trial. Gastroenterology 2000;**119**:305–309.
31. Alm JS, Swartz J, Björkstén B, Engstrand L, Engström J, Kühn I et al. An anthroposophic lifestyle and intestinal microflora in infancy. Pediatr Allergy Immunol 2002;**13**:402–411.