Effect of probiotic *Lactobacillus* strains in children with atopic dermatitis

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Background: Recent studies suggest that oral bacteriotherapy with probiotics might be useful in the management of atopic dermatitis (AD).
Objective: The purpose of this investigation was to evaluate the clinical and anti-inflammatory effect of probiotic supplementation in children with AD.
Methods: In a double-blind, placebo-controlled, crossover study, 2 probiotic *Lactobacillus* strains (lyophilized *Lactobacillus rhamnosus* 19070-2 and *Lactobacillus reuteri* DSM 122460) were given in combination for 6 weeks to 1- to 13-year-old children with AD. The patients' evaluations were registered after each intervention (ie, better, unchanged, or worse). The clinical severity of the eczema was evaluated by using the scoring atopic dermatitis (SCORAD) score. As inflammatory markers, eosinophil cationic protein in serum and cytokine production by PBMCs were measured.
Results: After active treatment, 56% of the patients experienced improvement of the eczema, whereas only 15% believed their symptoms had improved after placebo ($P = .001$). The total SCORAD index, however, did not change significantly. The extent of the eczema decreased during active treatment from a mean of 18.2% to 13.7% ($P = .02$). The treatment response was more pronounced in allergic patients (at least one positive skin prick test response and elevated IgE levels), and in this group the SCORAD score decreased ($P = .02$ compared with nonallergic patients). During active treatment, serum eosinophil cationic protein levels decreased ($P = .03$). No significant changes in the production of the cytokines IL-2, IL-4, IL-10, or IFN-γ were found.
Conclusions: A combination of *L rhamnosus* 19070-2 and *L reuteri* DSM 122460 was beneficial in the management of AD. The effect was more pronounced in patients with a positive skin prick test response and increased IgE levels. (J Allergy Clin Immunol 2003;111:389-95.)

Key words: *Probiotics, atopic dermatitis, clinical trial, inflammation*

Probiotics are live microorganisms that when ingested might have a positive effect in the prevention or treatment of a specific pathologic condition.[1] Recently, a role for oral bacteriotherapy in the prevention of atopic dermatitis (AD) was demonstrated.[2] In 2 previous studies administration of the probiotic *Lactobacillus rhamnosus* GG to infants with AD and cow's milk allergy significantly reduced the severity of the eczema.[3,4] In vitro *L rhamnosus* GG was shown to inhibit antigen-induced IgE production in murine lymphocytes.[5]

The management of AD in childhood is challenging. Administration of topical corticosteroids might control the symptoms, especially in children with mild and moderate eczema. However, relapses are common. Moreover, extensive and prolonged use of corticosteroids implies a risk of systemic side effects and can cause skin atrophy.

The purpose of this study was to evaluate the effect of 2 newly identified probiotics, *Lactobacillus rhamnosus* 19070-2 and *Lactobacillus reuteri* DSM 122460,[6-8] given in combination to children with moderate and severe AD. In addition to a clinical evaluation of the extent and severity of the eczema, we also measured levels of serum eosinophil cationic protein (sECP)[9] and cytokine production from PBMCs.

METHODS

Patients and study design

The study was performed from October 1999 through May 2000. The patients were recruited from the Department of Pediatrics, H:S Hvidovre Hospital, and the Department of Dermatology, Gentofte Hospital. Children aged 1 to 13 years with AD were asked to participate in the study. The diagnosis of AD was made according to standardized criteria.[10]

The patients were randomized in a double-blind crossover design to receive either placebo followed by active treatment (group A) or active treatment followed by placebo (group B). Block randomization with 4 patients in each block was applied. Each intervention period was separated by a 6-week washout period. None of the patients had received or were receiving systemic corticosteroids.

The bacterial preparation consisted of *L rhamnosus* 19070-2 and *L reuteri* DSM 12246. Before selecting these strains, their ability to adhere to the intestinal mucosa had been evaluated in vitro and in healthy volunteers.[6] The study preparation was manufactured by Chr. Hansen A/S (Hørsholm, Denmark). Each dose (weight, 1.0 g) was

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Supported by FØTEK (The Danish Research and Development Programme for Food Technology), The Danish Dairy Board Board, and The Dagmar Marshall Foundation.
Received for publication May 12, 2002; revised October 27, 2002; accepted for publication November 5, 2002.

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0091-6749/2003 $30.00 + 0

TABLE I. Study design and scheduled visits

–2 wk	0 wk	2 wk	4 wk	6 wk	8 wk	10 wk	12 wk	14 wk	16 wk	18 wk
Run-in period	**Placebo or active treatment**						**Placebo or active treatment**			
SCORAD	SCORAD sIgE sECP Cytokines			SCORAD Subjective evaluation sECP Cytokines			SCORAD Subjective evaluation sECP Cytokines			SCORAD Subjective evaluation sECP Cytokines

Abbreviations used
AD: Atopic dermatitis
SCORAD: Scoring atopic dermatitis
sECP: Eosinophil cationic protein in serum
sIgE: IgE in serum
SPT: Skin prick test

stored in airtight alu-bags at –20°C until the first day of the intervention period. A dose of 10^{10} colony-forming units of each strain or an identically appearing placebo preparation was given twice daily. The placebo preparation consisted of skimmed milk powder (0.28 g; bovine protein, 37%) and dextrose anhydrate (0.72 g). When administered to the patients, the bacterial preparation (or the placebo preparation) was dissolved in 2.5 to 5 mL of water or any liquid preferred by the patient. During the 20-week study period, the patients were asked to abstain from any fermented food products containing live microorganisms. Otherwise, none of the patients changed diet during the study period. Issues regarding taste of the test preparation and the child's preferred way to ingest the powder were discussed at each scheduled visit.

During the study period, the patients continued treatment with topical corticosteroids (hydrocortisone and hydrocortisone-butyrate). A quantitative estimate of the use of topical corticosteroids was done at each visit by weighing the medication tubes.

Data on allergic sensitization were obtained from the results of skin prick tests (SPTs) performed with standardized extracts (ALK, Copenhagen, Denmark) of hen's egg, milk, wheat, soy, and airborne allergens (*Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, cat, dog, horse, birch, timothy grass, *Cladosporium herbarum*, and *Alternaria alternata*). In 7 patients SPTs were performed at inclusion, and in 31 patients SPTs had been performed within 2.5 years before the study.

The study protocol included written consent by the parents or guardians of the participating patients and was approved by the Ethical Committee of the Municipality of Copenhagen and Frederiksberg and the Ethical Committee of Copenhagen County (journal no. 02-056/99)

Clinical evaluation

The clinical severity of the eczema was evaluated at inclusion (2 weeks before intervention) and at the beginning and end of each intervention period. A standardized scoring system (scoring atopic dermatitis [SCORAD])[11] developed by the European task Force for Atopic Dermatitis was applied. The SCORAD score combines the clinical evaluation of intensity and extent of the eczema with a subjective itch score indicated by the patient, parents, or both on a visual analogue scale. The range of the SCORAD scores is 0 to 80. As proposed by the European Task Force for Atopic Dermatitis, the eczema was graded as mild (SCORAD score, 0-15), moderate (SCORAD score, 16-40), or severe (SCORAD score, >40). Moreover, the patients were asked at each visit to indicate whether they evaluated the eczema during the latest intervention period to be generally better, unchanged, or worse. Table I shows the study design and the parameters assessed at each scheduled visit.

IgE in serum

Before intervention, a blood sample for analysis of IgE in serum (sIgE) was drawn. Serum IgE was analyzed by using a chemiluminescense assay (Immunolite). The normal reference interval for children 1 to 6 years old was 0 to 100 kIU/L. For children older than 7 years, an sIgE level of less than 150 kIU/L was considered within the normal range.

Eosinophil cationic protein in serum

Blood samples were drawn between 9 AM and 2:30 PM at the beginning and end of each intervention period. Serum ECP was analyzed by means of RIA (Pharmacia CAP system ECP FEIA).[9] All assays were run in duplicate. The measuring range was 2 to 200 μg/L, with a detection limit of 0.5 μg/L.

Production of cytokines from PBMCs

Analysis of the production of cytokines from PBMCs was performed as previously described.[12] Briefly, PBMCs were isolated and incubated with RPMI-650 for 24 hours. The cultures were stimulated with LPS-PHA or left unstimulated for control samples. The supernatants were collected after 24 hours and were stored at –80°C until analysis. Analysis of cytokines was done with the OPTEIA IL-2, IL-4, IL-10, and IFN-γ ELISA (Pharmingen), according to the instructions of the manufacturer. One hundred microliters of plasma was added to a microtiter well coated with mAbs to the cytokine of choice. After incubation for 2 hours, the wells were washed, 100 μL of bionylated antibody to the cytokine of choice and avidin–horseradish peroxidase were added to each well, and the plates were incubated for 1 hour. Finally, the plates were washed, and 100 μL of substrate solution containing hydrogen peroxide was added. The reaction was stopped with phosphoric acid, and the plates were read at 450 nm. Included on each plate was a cytokine standard allowing determination of the concentration of the cytokine in the supernatant.

Statistics

For each parameter, the difference between posttreatment and postplacebo values, the changes observed during active treatment, and the changes observed during placebo treatment were calculated. The changes (Δ values) during active and placebo treatment are presented as means with 95% CIs. *P* values of less than .05 were considered significant. Differences between posttreatment and postplacebo values were compared with changes observed during active and placebo treatment and tested against a zero change. Subsequently, the effect of the treatment was calculated by comparing changes observed during active treatment with changes observed during placebo treatment (paired *t* test). The χ^2 test was used to estimate differences in proportions. Correlations were calculated by using the Spearman bivariate correlation coefficient. A power analysis was performed according to the method of Schødt.[13] To demonstrate a 20% change in the SCORAD score, 40 patients should complete the study.

TABLE II. Demographic data of the patients enrolled in the study

	Group A (placebo→active treatment), n = 20	Group B (active→placebo treatment), n = 23	*P* value
Age (y)	6.0 (1-12)	4.0 (1.5-13)	.18*
SCORAD index	35 (15-66)	40 (18-66)	.42*
sECP (μg/L) before intervention	8.2 (2.6-18.1)	14.9 (2.0-97.4)	.09*
sIgE (IU/L)	108 (13-1442)	118 (21-2035)	.59

Values are presented as median (range).
*Mann-Whitney *U* test.

TABLE III. Clinical characteristics of patients with allergic and nonallergic constitutions

	Clinical classification		
	Allergic,* n = 27	Nonallergic, n = 16	*P* value
Age (y), median (range)	3.0 (1.5-11)	3.5 (1.5-13)	.55
History of asthma-rhinitis, n (%)	10 (43)	1 (6)	.01
History of food allergy, n (%)	3 (13)	0	
Results of SPT† (data available: 38 patients), n			
Pollens	5	0	
House dust mite	6	0	
Molds	1	0	
Pets with fur	5	0	
Cow's milk	3	0	
Egg	5	0	
Soy	1	0	
Wheat	2	0	
sIgE at inclusion (kIU/L)	276 (36-2035)	54 (14-100)	.01
sECP at inclusion (μg/L)	12.4 (2.6-97.4)	9.1 (2.3-21.7)	.18
SCORAD score at inclusion	37 (18-68)	32 (15-66)	.12

*See text for definition.
†See text for details regarding specific allergen extracts.

RESULTS

Study population

Initially, 58 patients were included in the study. Fifteen patients (mean age, 5.1 years; SCORAD score, 18-64) were excluded. The reasons for exclusions were refusal to ingest the powder (3 patients during active treatment and 2 during placebo treatment), nonattendance to scheduled visits (5 patients during placebo treatment), and withdrawal of former informed consent (1 patient during placebo treatment and 1 during active treatment). Two patients were excluded because exacerbation of the eczema requiring hospitalization (1 patient during placebo treatment) or systemic corticosteroids (1 patient during active treatment). Thus 43 patients (42% male patients; mean age, 5.2 years; age range, 1-13 years) completed the study.

At inclusion, the median SCORAD score was 40 (range, 25-51) versus 35 (range, 16-66) at the start of intervention ($P = .08$). Table II shows the clinical and laboratory data as registered before the intervention. There were no significant differences between patients allocated to group A (placebo followed by active treatment) versus those allocated to group B (active treatment followed by placebo, Table II). At inclusion, the mean sECP level was 16.6 μg/L (95% CI, 10.1-23.0 μg/L). No significant association between the SCORAD score and sECP was found (Spearman correlation factor, 0.162; $P = .38$).

The patients were clinically classified as being allergic or nonallergic. Allergic patients had at least one positive SPT response plus sIgE levels of greater than 150 kIU/L (age, >7 years) or greater than 100 kIU/L (age, 1-6 years) or a medical history of asthma, allergic rhinoconjunctivitis, or allergic reactions to food plus elevated sIgE levels. Asthma was defined as at least 2 episodes of wheezing diagnosed by a physician. Allergic rhinoconjunctivitis was defined as rhinitis or conjunctivitis appearing at least twice after exposure to a specific allergen and unrelated to infection. A history of food-induced allergic reactions was defined as parent-reported acute onset of skin eruption, vomiting, or diarrhea more than once after ingestion of a specific food item. Double-blind placebo-controlled food challenges were not performed.

Table III shows the distribution of allergic and nonallergic patients and their clinical characteristics. Three patients had a history of acute skin eruption after ingestion of specific foods (1/5 patients with a positive SPT response for egg and 2/2 patients with a positive SPT response for egg and wheat). Although 3 patients had a positive SPT response to cow's milk, before entering the study, their parents stated their children tolerated milk.

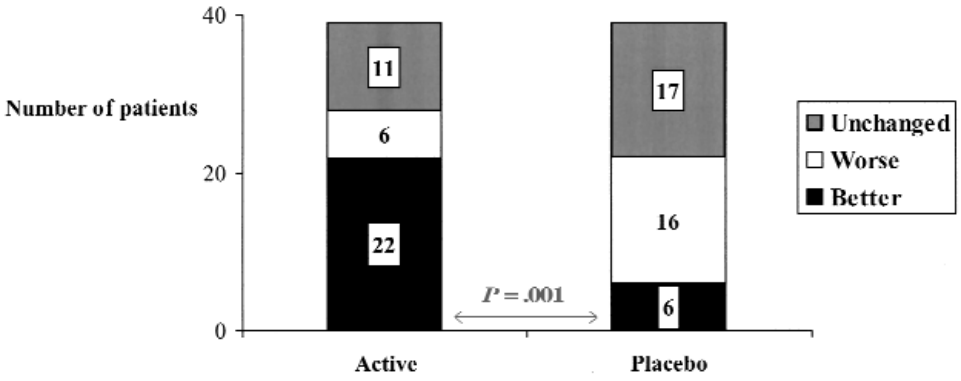

FIG 1. Patients' evaluations during interventions.

TABLE IV. Changes in total SCORAD score and separate SCORAD items during interventions

	I: Posttreatment-postplacebo values	II: Change during active treatment	*P* value (I vs II)	III: Change during placebo	*P* value (I vs III)
SCORAD	–6.2 (–11.4 to –1.1)	–3.4 (–7.9 to 1.1)	.22	0.5 (–3.8 to 3.8)	.09
Intensity	0.6 (–2.5 to 3.6)	–0.8 (–3.8 to 2.2)	.70	–0.6 (–3.2 to 2.0)	.79
Extent	–4.1 (–8.1 to –0.6)	–4.4 (–8.1 to 0.1)	.74	2.4 (–2.1 to 3.8)	.06
Itch score	1.7 (1.5 to 3.9)	0.43 (–1.2 to 2.1)	.36	0.55 (–1.1 to 2.2)	.08

Values are presented as means (95% CIs).

Nonetheless, to reveal any possible allergic reaction to ingredients in the placebo preparation, patients with a positive SPT response to cow's milk received a test dose of the placebo preparation (1 g) before randomization. During the following 48 hours, none of the 3 patients had allergic reactions to the test dose or worsening of eczema.

There were no significant differences in the clinical severity of the eczema between allergic and nonallergic patients.

Clinical effects

Patients' subjective evaluations. Most families preferred the active treatment. After probiotic treatment, 22 (56%) of 39 patients or parents stated the eczema had improved compared with 6 (15%) of 39 after placebo treatment (P = .001). During active treatment, 6 (15%) patients experienced deterioration of the eczema, whereas 16 (41%) believed their eczema had worsened during placebo treatment (P = .04, Fig 1).

Clinical scores. During active treatment, the mean SCORAD index decreased from 35.6 to 31.6 (P = .06), whereas no changes were observed during placebo. These data are presented in Tables IV and V. As shown in Table V, the change in SCORAD score observed during active treatment compared with the change during placebo treatment did not reach the level of significance. In patients indicating that the eczema had improved during active treatment, the mean change in the total SCORAD score was –11.4 (95% CI, –16.2 to –6.5) versus a mean change for patients receiving placebo of 6.1 (95% CI, –1.5 to 13.6; P < .001).

For a closer study of the preferences stated by the patients, each of the SCORAD items (intensity score, itch score, and extent) was analyzed separately (Tables IV and V). Although a trend toward lower itch scores and decreased intensity of the eczema were found after active treatment, these changes did not reach the level of significance. In contrast, the extent of the eczema decreased from a mean of 18.2% to 13.7% (P = .02; ie, a relative reduction of 24.7%).

It appears from Table V that the treatment response was more pronounced in allergic patients compared with in nonallergic patients. In the former group a significant decrease in the total SCORAD score, as well as a substantial decrease in the extent of the eczema, was demonstrated.

Use of topical corticosteroids

Complete data were available from 35 patients. No significant differences in the amount of topical corticosteroids used in the placebo period compared with during the active treatment period were found. Considerable interpersonal variations in the amounts used were noted, and no significant associations to the changes in the SCORAD score during placebo or active treatment were found. The median use of hydrocortisone butyrate during the placebo period was 6.0 g (range, 0-59 g) versus 7.8 g (range, 0-67 g) during the active treatment period (P = .84).

Serum eosinophil cationic protein

During active treatment, sECP levels decreased from 17.4 to 12.6 µg/L but remained stable during placebo treatment. A significant difference between the change in sECP levels during active treatment compared with the change observed during placebo treatment was demonstrated. The mean change in sECP was –6.2 µg/L (95% CI, –13.3 to 0.5 µg/L) versus 2.0 µg/L (95% CI, –0.5 to 4.6 µg/L) during placebo treatment (P = .03).

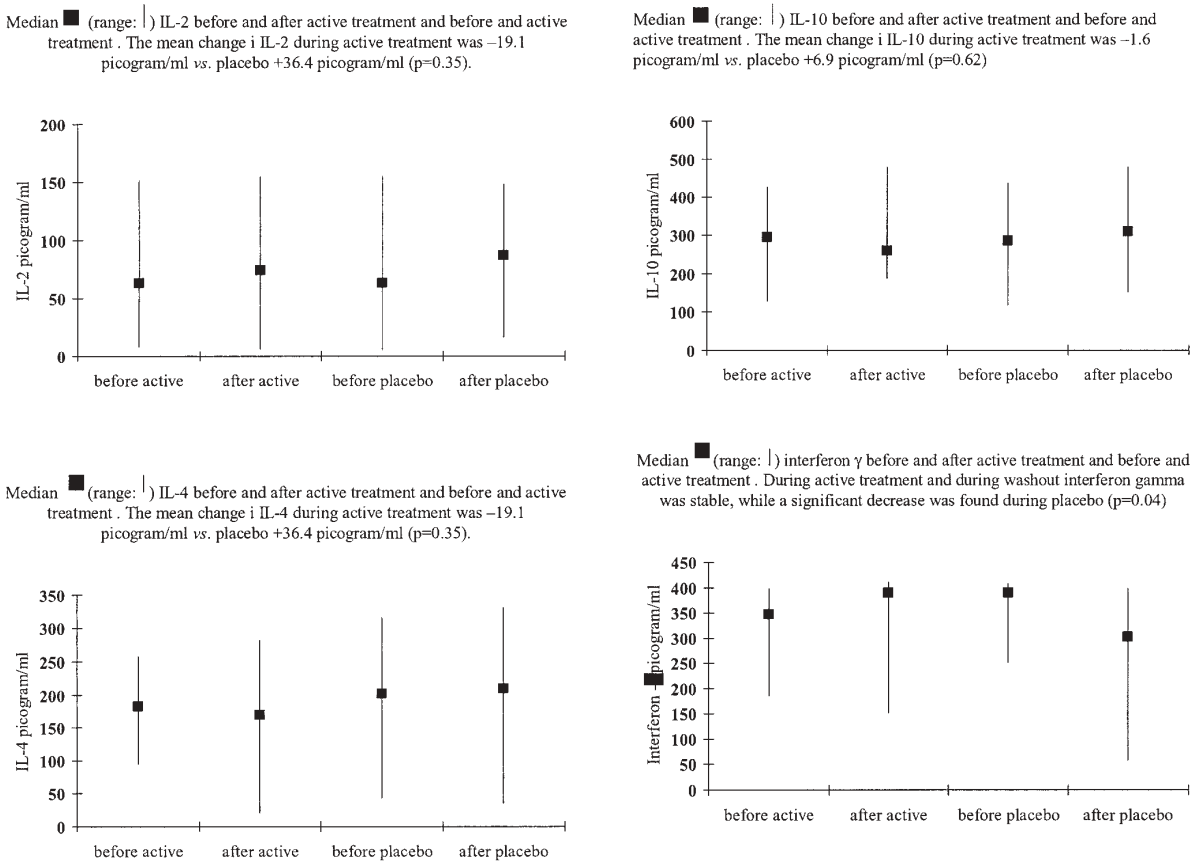

FIG 2. Production of the cytokines IL-2, IL-10, IL-4, and IFN-γ during interventions.

TABLE V. Effect of probiotic treatment on the clinical severity of eczema

	***Lactobacillus* species treatment**	**Placebo treatment**	***P* value**
Total study group, n = 43			
Change in total SCORAD index	–3.4 (–7.9 to 1.1)	0.5 (–3.8 to 3.8)	.12
Change in extent score	–4.4 (–8.1 to 0.1)	2.4 (–2.1 to 3.8)	.02
Change in intensity score	–0.8 (–3.8 to 2.2)	–0.6 (–3.2 to 2.0)	.90
Change in itch score	0.43 (–1.2 to 2.1)	0.55 (–1.1 to 2.2)	.46
Allergic constitution, n = 27			
Change in total SCORAD index	–2.4 (–7.5 to 2.8)	3.2 (–1.6 to 7.9)	.04
Change in extent score	–3.9 (–8.2 to 0.4)	2.5 (–1.7 to 6.8)	.008
Change in intensity score	0.1 (–3.7 to 3.8)	0.9 (–2.3 to 4.1)	.89
Change in itch score	0.8 (–1.3 to 2.9)	1.5 (–0.2 to 3.2)	.42
Nonallergic constitution, n = 16			
Change in total SCORAD index	–5.2 (–14.4 to 4.1)	–5.4 (–11.4 to 0.6)	.95
Change in extent score	–2.5 (–9.0 to 3.9)	–1.8 (–6.0 to 2.4)	.80
Change in intensity score	–2.6 (–7.8 to 2.1)	–3.9 (–8.7 to 0.8)	.63
Change in itch score	0.6 (–2.9 to 4.2)	–1.3 (–4.6 to 2.0)	.73

Values are presented as mean (95% CI).

Cytokines

Production of the cytokines IL-2, IL-4, IL-10, and IFN-γ are shown in Fig 2. No significant changes were found.

A significant association between the level of IL-4 after active treatment and clinical improvement was found (nonparametric correlation factor between IL-4 after active treatment and the decrease in extent score: –0.467; $P = .028$).

DISCUSSION

In a study by Majamaa and Isolauri,[4] the administration of *L rhamnosus* GG to highly selected patients (age <2 years, challenge-proven cow's milk allergy, and mild-to-moderate eczema) significantly improved the total SCORAD score. We examined an unselected group of older children (age range, 1-13 years; mean age, 5.2 years) with chronic moderate-to-severe eczema. Given

the complexity of the pathogenesis of AD, we did not aim specifically to examine eczematous children with food allergy. Although many patients with AD do have an allergic constitution,[14] the contribution of allergy and IgE-mediated hypersensitivity reactions to the pathogenesis and the clinical severity of AD remains controversial. There is some evidence that intestinal inflammatory reaction and disruption of the intestinal barrier function is involved in the pathogenesis of AD.[15,16] Recent studies indicate that an atopic gastroenteropathy might exist in children with AD.[17] Likewise, the frequent occurrence of subtle gastrointestinal symptoms in children with allergic asthma[18] suggests that an atopic gastroenteropathy is common in children with eczema and in children with allergic asthma. In an earlier study small intestinal inflammation with increased counts of immunoreactive IgE cells in duodenal biopsy specimens from patients with AD was demonstrated.[19] These adult patients had multiple positive SPT responses for inhalant, as well as foodborne, allergens. Moreover, a positive correlation between the sIgE level and the number of immunoreactive (anti-IgE–positive) cells in the duodenal biopsy specimens was demonstrated. Thus gastrointestinal inflammatory reactions might be more pronounced in patients with high sIgE levels. Probiotics have been shown in a number of studies to alleviate intestinal inflammation.[20,21] Interestingly, our study suggests a more pronounced effect of probiotic supplementation in patients with an allergic constitution. A clinical observation favoring this theory was done recently, with the demonstration that probiotic supplement could prevent development of atopic eczema in high-risk infants.[2]

The SCORAD score combines an estimation of the intensity and extent of the eczema with a subjective itch score. During active treatment, a significant and clinically relevant decrease in the extent of the eczema was found. We recognize that splitting up the SCORAD index might be problematic. Even so, we consider the extent score to be the most objective of the criteria included in the index. Assessment of the extent of the eczema is performed easily by using the rule of nine, and the result is displayed bedside on standardized evaluation sheets.[11]

In crossover studies the risk of a carry-over effect must be considered. In our initial studies the in vivo colonization capacity of *L rhamnosus* 19070-2 and *L reuteri* DSM 12246,[6] as reported for other probiotic *Lactobacillus* strains, was found to be transient. Five days after administration of the probiotics had stopped, none of the strains were recovered from fecal samples. Ideally, the ability of probiotic strains to adhere to the intestinal mucosa should be assessed by means of identification of ingested test strains in intestinal biopsy specimens. Still, after a 6-week washout period, a persistent effect of the strains seems unlikely.

During *Lactobacillus* species supplementation, a moderate but significant reduction in sECP levels was found. ECP, a cytotoxic protein released from activated eosinophils, has been used to monitor disease activity in AD. In our study we could not confirm a relationship between the clinical score of AD and sECP levels, which has been reported previously.[22] In a study examining changes in sECP levels during allergen challenge, a rapid initial increase in sECP levels during acute exacerbation of the eczema was followed by a rapid reduction.[23] It seems likely that ECP released from activated eosinophils during acute exacerbations might be rapidly eliminated from the circulation. Thus sECP might be a more sensitive marker in acute exacerbations of the eczema than a marker of disease activity per se.

Contrary to previous studies,[24,25] probiotic therapy was accompanied by only moderate changes in production of the cytokines IL-4, IFN-γ IL-10, and IL-2. IL-4 stimulates B lymphocytes to produce IgE. In highly atopic children (sIgE levels >600 kIU/L) with acute exacerbation of severe eczema, increased levels of IL-4 and low levels of IFN-γ have been found.[26] In contrast, normal levels of IL-4 and IFN-γ were found in mildly atopic children (sIgE levels <600 kIU/L) with less severe eczema. In our study an unselected group of patients with sIgE levels ranging between 13 and 2035 kIU/L was examined. Moreover, it is possible that a longer period of treatment might be required to produce significant decreases in IL-4 levels.

In summary, administration of probiotic *Lactobacillus* strains (a mixture of *L rhamnosus* 19070-2 and *L reuteri* DSM 12246) to children with AD was associated with a moderate improvement in the clinical severity of eczema. The study involved a group of older children with severe chronic eczema. In suggesting a more pronounced effect in allergic patients, our results are in accordance with results from experimental studies, as well as those of previous clinical trials. Future studies should address the effects of long-term probiotic supplementation. Moreover, studies should involve comparisons between specific age groups. Our findings suggest they should also stratify patients according to allergic sensitization or total IgE levels.

We thank Tonni Løwe Hansen for technical assistance and loyal support. The test preparations were produced by Chr. Hansen, A/S Hørsholm.

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