

Prospective, randomized controlled trial on *Lactobacillus rhamnosus* in infants with moderate to severe atopic dermatitis

R. Fölster-Holst, F. Müller, N. Schnopp,* D. Abeck,* I. Kreiselmaier, T. Lenz,† U. von Rügen,‡ J. Schrezenmeir,§ E. Christophers and M. Weichenthal

Department of Dermatology, University of Kiel, Schittenhelmstr. 7, D-24105 Kiel, Germany

*Department of Dermatology, Technical University Munich, Munich, Germany

†Statistical Consulting, Mannheim, Germany

‡Federal Centre for Health Education, Cologne, Germany

§Institute for Physiology and Biochemistry of Nutrition, Federal Research Centre for Nutrition and Food, Kiel, Germany

Summary

Correspondence

Regina Fölster-Holst.

E-mail: rfoelsterholst@dermatology.uni-kiel.de

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Key words

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Conflicts of interest

None declared.

Background A reduction of symptoms of atopic dermatitis (AD) in small infants by the administration of *Lactobacillus rhamnosus* has been reported in a few studies. One study with older children and adolescents failed to show any effect.

Objectives We conducted a prospective study to reassess the efficacy of orally administered *L. rhamnosus* strain GG (LGG) in infants with AD.

Methods In a randomized, double-blind, placebo-controlled study, 54 infants aged 1–55 months with moderate to severe AD were randomized to daily 10×10^9 colony-forming units of LGG or to placebo during an 8-week intervention phase. Emollients, class I–II topical corticosteroids and antihistamines were permitted.

Results The treatment with LGG was well tolerated. At the end of treatment there were no significant differences between the groups with respect to clinical symptoms (SCORAD, pruritus, sleep loss), the use of topical corticosteroids and antihistamines, immunological parameters, or health-related quality of life of the parents.

Conclusions Our results could not confirm LGG as an effective treatment of AD in infancy.

Atopic dermatitis (AD) is a common inflammatory skin disease, with an especially high prevalence in infancy. Treatment strategies for AD include continuous adjuvant-based emollient therapy in combination with eczema-related anti-inflammatory treatments using topical steroids or calcineurin inhibitors, and if necessary oral antimicrobial agents, phototherapy or antihistamines. In addition, avoidance of provocation factors such as irritants and allergens is important.

Over the last three decades the prevalence of the disease has increased steadily in industrialized countries.¹ According to the so-called hygiene hypothesis this is attributed to reduced exposure to environmental microbes, including gut-colonizing microbes.² Moreover, the intestinal tract was found to be more frequently colonized by lactobacilli in nonallergic children than in allergic children,³ supporting the idea that appropriate microbial colonization of the gut might lower the risk of developing atopic diseases.^{4,5}

Probiotics such as lactobacilli, defined as live microbial food ingredients beneficial to health, are commensal bacteria of the

human gut microflora. Previously, administration of *Lactobacillus rhamnosus* strain GG (LGG) has been shown to prevent early atopic disease in children at risk⁶ and to improve manifest AD in early infancy.⁷ A 4-year follow up gave strong evidence that the preventive effect of LGG on AD extended beyond infancy.⁸ The effects are attributed to normalization of the increased intestinal permeability and to balancing gut microecology, improvement of the immunological defence barrier of the intestine by the induction of intestinal IgA, an enhanced production of anti-inflammatory factors such as transforming growth factor- β and interleukin (IL)-10, induction of IL-12, IL-18 and interferon (IFN)- γ , and reduction of Th2 cytokine production.^{9–15} Recently, Smits et al.¹⁶ showed that selective probiotic bacteria induce IL-10, producing regulatory T cells and resulting in inhibition of proliferation and cytokine production of immune cells, particularly effector T cells.

Whereas data on prevention of AD by LGG are convincing, the question of whether LGG might have a therapeutic effect in AD is still a matter of controversy. We performed a

randomized, double-blind, placebo-controlled study to examine whether the oral administration of LGG improves clinical and immunological parameters in infants with moderate to severe AD.

Materials and methods

Patients and study design

This study was performed in 2001 and 2002 at two dermatological centres in Germany (Kiel and Munich). In total, 54 children (Kiel 33, Munich 21; M/F 34 : 20) with a median age of 19 months (range 1–55) were recruited. They fulfilled the criteria of Hanifin and Rajka for infantile AD¹⁷ and were randomly allocated to receive either LGG at a dosage of 5×10^9 colony-forming units twice daily or a placebo preparation (microcrystalline cellulose) with identical appearance for up to 8 weeks. The study medication was applied orally in capsules that were mixed in to their bottles of milk directly before feeding or with water as a suspension for older children. The protocol was approved by the Ethics Committees at both centres, and written informed consent was obtained from the parents of all study participants.

Clinical evaluation

The patients were examined at inclusion and at 2, 4, 6 and 8 weeks after the beginning of the study. The primary efficacy endpoint was the severity of disease determined by SCORAD.¹⁸ This severity grading takes into account the extent (applying the rule of nines) and the severity of erythema, oedema/papulation, oozing/crusting, excoriation, lichenification and dryness. The outcome measure also included evaluation of pruritus and sleep loss, which were additionally analysed separately using a visual analogue scale ranging from 1 to 10. Furthermore, a series of secondary outcome measures was assessed, including use of topical corticosteroids and oral antihistamines, which was documented in a diary card. Health-related quality of life of the parents was evaluated by a parent-based score consisting of five dimensions: psychosomatic well-being, social life, satisfaction with medical care, emotional dealing with the disease and acceptance of the disease.¹⁹ Moreover, the parents reported weekly whether in their opinion their child's eczema was improved, unchanged or worse.

Laboratory analyses of blood and faeces

We analysed relative counts of eosinophils as well as total IgE, eosinophil cationic protein (ECP) and soluble CD30 (sCD30) in the serum of the patients. The determination of specific IgE antibodies included food allergens (milk, egg white, wheat), grass allergen mix, and cat and house dust mite allergens. Moreover, we measured faecal α 1-antitrypsin, calprotectin and ECP in patients as compared with healthy children. Blood and faecal measurements were carried out only in the centre of Kiel (n = 28).

The concentrations of IgE, specific IgE antibodies and ECP were measured by CAP-FEIA commercial kits, according to the manufacturer's instructions (Pharmacia, Freiburg, Germany). Plasma levels of sCD30 were determined by radioimmunoassay as described previously.²⁰ The concentrations of α 1-antitrypsin were measured by means of radial immunodiffusion (using LC-Partigen; Date Behring, Marburg, Germany), and calprotectin was assessed by enzyme-linked immunosorbent assay using the Calprest kit (Calpro, Oslo, Norway).

Statistics

Demographic and efficacy parameters are given as rates, as mean and SEM values or as median and range, as appropriate. For statistical comparisons between both treatment groups we used a contingency test for categorical data and Student's t-test or the Wilcoxon-Mann-Whitney U-test for continuous data. The evaluation was done on an intention-to-treat basis. For calculation the statistical software package SPSS v. 9.0 (SPSS, Munich, Germany) was used.

Results

Patient characteristics

In total, 54 patients with AD were included, but one patient dropped out before beginning the intervention (Fig. 1). There were 26 patients allocated to the LGG group and 27 to the placebo group. Six patients terminated early during the treatment phase (four in the LGG group and two in the placebo group) because of personal reasons, lack of efficacy, or intercurrent urticaria. At week 12, five patients were lost to follow up (one in the LGG group and four in the placebo group).

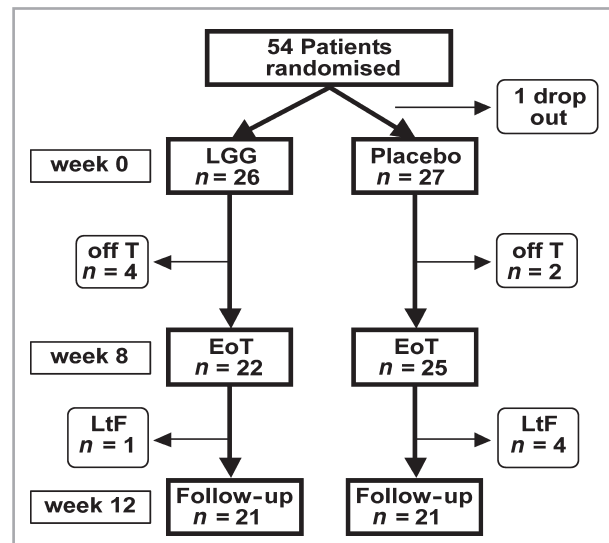


Fig 1. Flow chart of the study. LGG, *Lactobacillus rhamnosus* strain GG; off T, early termination of treatment; EoT, end of treatment; LtF, lost to follow up.

The demographic and clinical characteristics of the patients are summarized in Table 1. There were no significant differences in demographic, disease severity and immunological blood parameters at baseline between the LGG group and the placebo group.

Safety and tolerability

Treatment was generally well tolerated. There were no severe (grade III/IV) adverse events. Mild adverse events included diarrhoea in eight patients (LGG four, placebo four; no significant difference), nausea/vomiting in seven patients (LGG three, placebo four; no significant difference), fever in two patients (LGG one, placebo one; no significant difference) and urticaria in one patient (placebo).

Clinical evaluations

At inclusion, the mean SCORAD score was 43.3 in the LGG group and 41.4 in the placebo group (Fig. 2). An improve-

ment in the severity score occurred in both groups during the treatment phase, with a SCORAD at the end of treatment of 35.3 in the LGG group ($P < 0.001$) and 31.6 in the placebo group ($P < 0.001$). At follow-up, 4 weeks after the end of the intervention, the SCORAD had further decreased, to 32.8 in the LGG group and to 30.1 in the placebo group. However, the difference in response between both groups was not significant at any time (Table 2).

As shown in Table 2, the mean pruritus and sleeplessness decreased with time in both groups, without significant differences. In parents' global evaluation of skin condition and pruritus, which was documented weekly, the LGG group showed no significant advantage compared with the placebo group (data not shown).

Use of corticosteroids and antihistamines

At inclusion, 16 patients in each arm were applying topical corticosteroids. At the end of week 8, 12 patients in each arm were continuing to use topical corticosteroids. The potency of the respective steroids was predominantly mild (class I) or moderate (class II). Only two patients, one in each arm, were using potent (class III) preparations. There were no statistically significant differences between both arms with respect to corticosteroid potency at inclusion or during the study (data not shown). During the whole study period, only two patients in the LGG arm and six patients in the placebo group were able to avoid topical corticosteroids completely ($P = 0.25$; χ^2). Comparing the corticosteroid usage after 8 weeks vs. baseline, the extent of use was reduced on average by 2.7 applications per week in the LGG arm as compared with 0.8 in the placebo group ($P = 0.19$; t -test).

Ten patients in the LGG group and seven patients in the placebo group used antihistamines at inclusion ($P = 0.36$; χ^2). At the end of the active study period (week 8) nine patients in the LGG group and three patients in the placebo group were still taking antihistamines ($P = 0.03$; χ^2).

Health-related quality of life assessed by the parents showed no statistically meaningful differences at the baseline assessment in all five dimensions. No significant differences were found in any of the health-related quality of life dimensions after week 4 and week 8 between both groups (treatment and placebo group).

Laboratory analyses of blood and faeces

Serum IgE levels prior to treatment were not statistically different between LGG-treated patients (341 U mL^{-1}) and placebo controls (339 U mL^{-1}). Comparison of children who tested positive for IgE-mediated sensitizations revealed a reduction of 6.9 SCORAD points in the LGG arm ($n = 8$) and of 8.6 SCORAD points in the placebo group ($n = 12$) which was not significantly different ($P = 0.69$; t -test).

ECP concentrations in faeces correlated with the SCORAD and decreased significantly in the whole study population ($P = 0.04$), but there was no statistically significant difference

Table 1 Demographic data and clinical characteristics at inclusion

	LGG (n = 26)	Placebo (n = 27)	P-value ^a
Median age, months (range)	16.5 (1–53)	21 (5–55)	NS
Gender, F/M	9/17	10/17	NS
Age at onset of AD, months	3.0	5.0	NS
History of asthma (n)	1	1	NS
History of allergic rhinitis (n)	0	1	NS
History of food allergy (n)	9	15	NS
SCORAD ¹⁸ at inclusion	43.3	41.4	NS

LGG, *Lactobacillus rhamnosus* strain GG. ^aStatistical significance by means of χ^2 or t -test, as appropriate; NS, not significant.

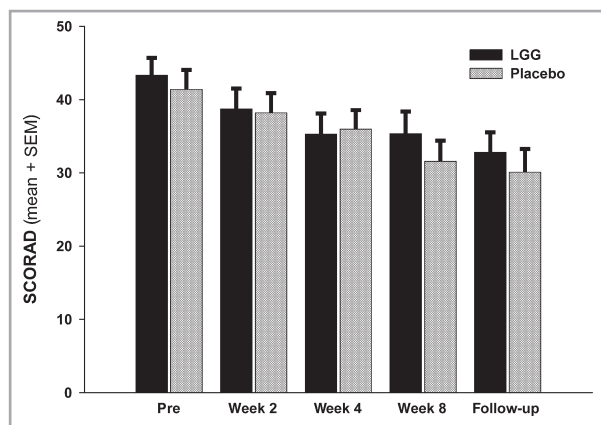


Fig 2. Clinical outcome. LGG, *Lactobacillus rhamnosus* strain GG. Bars denote the mean $\pm$ SEM SCORAD¹⁸ in both treatment groups. The mean SCORAD significantly decreased in children treated with both LGG ($P < 0.001$) and placebo ($P < 0.001$) by the end of treatment. Differences between both groups were not significant at any time.

Table 2 Changes of clinical parameters during the treatment phase

	LGG (n = 26)		Placebo (n = 27)		P-value ^a
	Baseline	Week 8	Baseline	Week 8	
SCORAD, mean $\pm$ SEM	43.3 $\pm$ 2.4	35.3 $\pm$ 3.0	41.4 $\pm$ 2.7	31.6 $\pm$ 2.8	NS
Itching (VAS), mean $\pm$ SEM	4.5 $\pm$ 0.43	4.3 $\pm$ 0.47	5.0 $\pm$ 0.45	3.7 $\pm$ 0.55	NS
Sleeplessness (VAS), mean $\pm$ SEM	2.57 $\pm$ 0.48	1.96 $\pm$ 0.52	2.78 $\pm$ 0.62	1.70 $\pm$ 0.45	NS
Use of topical corticosteroids (applications per week), mean $\pm$ SEM	5.6 $\pm$ 1.0	3.0 $\pm$ 0.6	4.0 $\pm$ 0.7	3.2 $\pm$ 0.9	NS
Use of antihistamines, n	10 (39%)	9 (37%)	7 (26%)	3 (11%)	0.04
QoL score, mean $\pm$ SEM ^b					
Psychosomatic well-being	3.6 $\pm$ 0.8	3.6 $\pm$ 0.7	3.6 $\pm$ 0.8	3.6 $\pm$ 0.8	NS
Effects on social life	4.7 $\pm$ 0.5	4.5 $\pm$ 0.6	4.4 $\pm$ 0.6	4.4 $\pm$ 0.7	NS
Satisfaction with medical care	3.9 $\pm$ 0.6	4.0 $\pm$ 0.7	3.8 $\pm$ 0.8	3.8 $\pm$ 0.8	NS
Dealing emotionally with the illness	3.7 $\pm$ 0.8	3.5 $\pm$ 0.8	3.6 $\pm$ 0.8	3.5 $\pm$ 0.9	NS
Acceptance of the disease	4.0 $\pm$ 0.8	4.3 $\pm$ 0.5	4.0 $\pm$ 0.9	3.8 $\pm$ 1.0	NS

LGG, *Lactobacillus rhamnosus* strain GG; VAS, visual analogue scale. ^aStatistical significance comparing the relative change from baseline to week 8 between both treatment groups; NS, not significant. ^bQuality of life parameter for parents.

between both groups. None of the other immunological serum and faecal parameters was predictive for the severity of disease or responded to treatment. Furthermore, no significant differences were seen between both groups at inclusion or at week 8 of treatment (data not shown).

Discussion

In this randomized, double-blind, placebo-controlled study we could not find any significant difference in the main clinical outcome (SCORAD) by administration of LGG as compared with placebo in children with AD. Additionally, there were no significant differences between the groups in secondary clinical outcomes including use of topical corticosteroids, or health-related quality of life. Although statistically not significant, minor differences in the baseline variables in our study might still be of relevant impact on the outcome measures. However, this seems unlikely as, for example, food allergies were more prevalent in the placebo arm.

Compared with early Finnish studies^{7,21} showing beneficial effects, we included children with more severe AD (median SCORAD score of 43). Additionally, the children in our study had a higher age (mean age 19 months) compared with the Finnish studies. Isolauri et al.⁷ observed 27 exclusively breast-fed infants (median age 4.6 months) with mild AD (median SCORAD score of 16), receiving extensively hydrolysed whey formula with (LGG or *Bifidobacterium* strain) or without probiotics (placebo) for 8 weeks. They showed a reduction in the SCORAD by 15 points (from 16 to 1) for the LGG and by 16 points (from 16 to 0) for the *Bifidobacterium* arm, as compared with a reduction of 2.6 points (from 16 to 13.4) in the placebo arm. The same group²¹ performed another study (open label) with 27 infants (aged 2.5–15.7 months) receiving hydrolysed whey formula with and without LGG. The SCORAD dropped by 42% in the LGG group and by 10% in the placebo group. However, 4 weeks after therapy the SCORAD was sim-

ilar between the groups. A recent randomized, double-blind, placebo-controlled trial from Australia²² showed a significant reduction of SCORAD in infants (aged 6–18 months) with moderate or severe AD (SCORAD score of ≥ 25) in the probiotic (*L. fermentum*) compared with the placebo group. Ninety-two per cent of the children receiving probiotics had a SCORAD better than baseline at week 16 (follow up) compared with 63% in the placebo group. The use of topical corticosteroids measured by potency and number of applications per week showed no difference between the groups.

Looking at older children, an unselected group of 43 patients with more severe AD (median SCORAD 40; age 1–13 years) failed to show significant changes of SCORAD in the total group comparing the probiotic intervention (combination of *L. rhamnosus* and *L. reuteri*; reduction of 4 points) with the placebo group (no change) in a crossover study.²³ However, after active treatment 56% of all patients or their parents stated that the eczema had improved compared with 15% in the placebo phase. Looking only at allergic children (defined as having at least one positive skin prick test response plus elevated serum IgE levels, or a medical history for respiratory atopic disease), there was a significant decrease in SCORAD in the LGG group compared with the placebo group. Other findings confirmed this trend when probiotic administration did not benefit all infants with AD, but only those with an IgE-mediated sensitization.²⁴

In contrast to these results, we did not find a significant benefit in the subgroup of patients with an IgE-mediated sensitization when comparing the LGG group and the placebo group. In fact, classical IgE-mediated diseases such as allergic rhinitis do not show consistent results regarding the efficacy of probiotics.^{25–27} Even though a prevention study⁶ showed fewer children with AD after administration of LGG in comparison with placebo, the induction rates of specific IgE were similar in the active and the control group.⁸ Thus, treatment did not protect against sensitization.

Animal studies provide evidence that sufficient microbial stimuli are necessary for the development of the immune system in infancy, including a maturation of Th1 lymphocytes. In this context it is remarkable that apparently only a special microbial composition prevents development of atopy in later life.²⁸ Studies examining faecal flora in children found that lactobacilli and bifidobacteria were more frequent in nonatopic patients, whereas *Staphylococcus aureus* was more common in atopic patients.²⁹ The proposed rationale for the use of probiotics in AD is their immunomodulatory effects such as inducing IL-10-producing regulatory T cells,^{9,16} reducing IL-5 and IL-4 secretion (Th2 response) and increasing IFN- γ production (Th1 response). Further studies^{11–14,24,30} using the mannitol/lactulose test demonstrated that probiotic supplementation in children with moderate or severe AD improved the disturbed intestinal mucosal barrier which is suggested to determine allergy.¹⁵

In conclusion, our results could not confirm LGG as an effective treatment of AD in infancy. However, there might still be subgroups of patients suitable for a probiotic intervention. In particular, the role of IgE-mediated sensitization as a prerequisite for treatment success remains to be studied. Due to a broad range of prebiotic and probiotic formulas and/or foods it may be difficult to detect therapeutic effects unless particular care is taken to eliminate bias.

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