

ORIGINAL ARTICLE

Efficacy of probiotic *Lactobacillus GG* on allergic sensitization and asthma in infants at risk

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Clinical &
Experimental
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Summary

Background Probiotics are perceived to exert beneficial effects in the prevention and treatment of allergic diseases.**Objective** There are conflicting data from studies as to an impact on allergic sensitization and asthma.**Methods** Our prospective double-blind study randomly assigned 131 children (6–24 months old) with at least two wheezing episodes and a first-degree family history of atopic disease to 6 months of *Lactobacillus rhamnosus* (LGG, 10^{10} colony forming units) or placebo. Atopic dermatitis and asthma-related events (e.g. need of inhalation, symptom-free days) were documented throughout the intervention and 6-month follow-up. We determined IgE, a representative panel of specific IgE, eosinophils, eosinophilic cationic protein, and TGF- β before, at the end of intervention, and after 6 months of follow-up.**Results** There were no significant differences as to atopic dermatitis or asthma-related events. In a subgroup with antecedent allergic sensitizations, asthmatic complaints were even slightly worse. We found fewer sensitizations towards aeroallergens after 6 months of LGG ($P = 0.027$) and after 6 months of follow-up ($P = 0.03$). Supplementation was well-tolerated and no severe adverse events occurred.**Conclusions** In young children with recurrent wheeze and an atopic family history, oral LGG had no clinical effect on atopic dermatitis or asthma-related events, and only mild effects on allergic sensitization. This effect persisted 6 months after the cessation of the supplementation.**Keywords** allergic sensitization, asthma, infants, prevention, probiotics

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Introduction

Allergic diseases are increasing world-wide and result from a complex interaction between genetic susceptibility and environmental factors. Recurrent wheezing in infancy can be the first manifestation of childhood asthma; about 30% of toddlers with more than three episodes of wheezing bronchitis may develop persistent asthma [1]. Children with atopic dermatitis are at an increased risk (up to 80%) of developing persistent asthma [2]. Other predicting factors are a family history of allergic diseases, early sensitization towards aeroallergens, exposition to cigarette smoke, and increased eosinophilic cationic protein (ECP) [3, 4]. Because allergic sensitization may occur early in life,

intervention strategies are requested for this critical period.

Environmental conditions and nutrition appear to have an influence on atopic phenotypes. The hygiene hypothesis tries to explain the increase of allergic diseases by a diminished microbial exposure. Probiotics are cultures of potentially beneficial bacteria of the healthy gut microflora. There is a debate whether probiotics have an impact on atopic dermatitis, hayfever, or bronchial asthma [5, 6].

Our trial examined the impact of probiotic LGG on atopic dermatitis, asthma, and allergic sensitization when given to 6–24-month-old infants with recurrent wheeze and a first line relative with atopic diseases.

Methods

Participants and study design

In our investigator-initiated prospective study, a total of 171 children (6–24-month-old) were recruited from our walk-in clinic from October 2002 to October 2003. This relatively long time was meant to exclude a seasonal selection bias (e.g. upper airway infections or airborne allergies). Inclusion criteria were a history of at least two physician-diagnosed episodes of wheezing (≥ 3 days, necessitating β_2 -bronchodilators or steroids) during the past 12 months, with at least one of these exacerbations in the 3 months immediately before enrolment, and a first-degree family history of atopic disease. Exclusion criteria were preterm birth (< 37 weeks of gestational age), congenital malformations, systemic disorders and chronic diseases, current antibiotic therapy, and prior exposure to and known intolerances towards ingredients of probiotics. After an observation period of 3 weeks to confirm selection criteria, we randomly assigned 131 eligible children to a double-blind dietary supplementation with *Lactobacillus rhamnosus* strain GG ATCC 53103 (LGG; 10^{10} colony forming units; Valio Inc., Helsinki, Finland) or placebo twice daily over 6 months (Fig. 1). The capsules were matched for size, shape, and volume of content, which was reconstituted with 5 mL water and then given by spoon. Compliance was monitored by use of a capsule chart and capsule counts. The parents were instructed not to feed other probiotic preparations during the whole study.

Clinical monitoring was performed for 1 year: before the intervention (visit 1), after 3, 6, 9, and 12 months (visit 2–5). Children underwent a physical examination including determination of the severity scoring of atopic dermatitis (SCORAD) index to assess eczema severity examination on each study visit to our walk-in clinic [7]. To ensure consistency, the same paediatrician, who was blinded to group assignments, performed all SCORAD assessments. The parents were asked to keep a diary,

recording episodes of asthmatic exacerbations (cough and wheeze, numbers and days of associated hospitalizations, symptom-free days, days without use of rescue medication, and associated inhalative steroid- and β -agonist use).

Outcomes and laboratory testing

Primary outcome measures were the SCORAD and asthma-related clinical events (Table 2), including an asthma symptom score defined by cough, wheeze, and need of intervention [8]. Secondary measures were allergic sensitization. Blood samples were taken on visit 1, 3, and 5. As a surrogate marker for allergic sensitization to common dietary and respiratory allergens, we determined the total and antigen-specific serum IgE against hen's egg, cat epithelia, house dust mite (HDM) (Der pt and far), birch pollen, milk protein, lactalbumin, timothy pollen, horse epithelia, and alternaria by chemiluminescence-immunoassay (Immunlite 2000; Biermann Inc., Bad Nauheim, Germany). In the literature, serum levels of specific IgE ≥ 0.2 or ≥ 0.35 IU/mL are considered as positive and predictive for later allergic respiratory disease [9, 10]. Our assay was highly sensitive and FDA-approved especially for the determination of low specific IgE (< 0.35 IU/mL). Because our sensitivity analysis revealed no difference between both thresholds, we limited data presentation to values ≥ 0.2 IU/mL. Additionally, we calculated the cumulative specific IgE as the sum of different aeroallergen-specific IgEs, which has been described as predictive for childhood asthma [4]. We also determined eosinophils, and serum levels of ECP (via CAP; Pharmacia Inc., Freiburg, Germany) and of TGF- β , a profibrotic factor whose expression is increased in asthmatics, indicating airway remodelling [11]. All parents supplied written informed consent before the study. Human experimentation guidelines of Good Clinical Practice, the German Drug Act, and the declaration of Helsinki/Hong Kong were followed in the conduct of clinical research. Local ethical committee approval was obtained. The trial had been registered and published at ClinicalTrials.gov (ID number NCT00490425).

Statistical analysis

Values before and after the intervention were compared using Wilcoxon's test for combined samples without normal distribution. The *U*-test (Mann-Whitney and Kruskal-Wallis) was performed for between-group comparison. We used SPSS for Windows[®] (SPSS Inc., Chicago, IL, USA). Results are expressed as medians with 95% confidence intervals unless indicated otherwise. Probability (*P*) values < 0.05 were considered as statistically significant. The study design was based on a sample size calculation for a two-sided *t*-test, which has a comparable

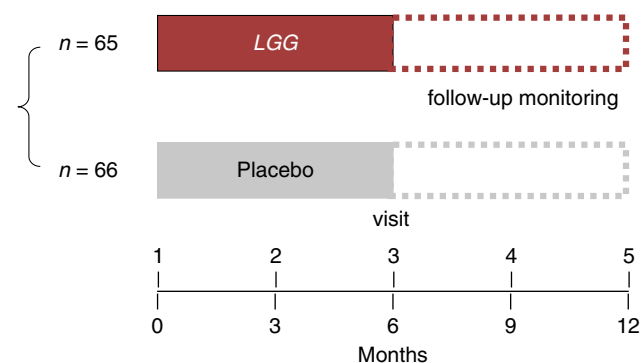


Fig. 1. Study design: Two groups of patients were randomly assigned to probiotic LGG or placebo. Blood samples were taken at 0, 6, and 12 months.

power as the Mann-Whitney *U*-test for normally distributed variables. To achieve a power of 80% (corresponding to a 20% probability of a type 2 error), 64 patients per study arm would be required to reveal a difference in means of half the size of the standard deviation with a significance value of 0.05. We recruited a larger number of subjects to allow for an estimated 10% withdrawal rate.

Results

Baseline characteristics of participants

Of 171 screened infants, 131 (female : male = 51 : 80) could be included into the study after the 3-week observational period, and 65 subjects received LGG (f : m = 23 : 42) vs. 66 placebo (f : m = 28 : 38; Table 1). The remaining 40 subjects (25 male) could not be included, because they appeared too ill for participation, or their parents changed

their mind and withdrew their consent. Mean age (SD) of the participants was 16.7 months (5.52; range: 6.7–23.5) in the LGG group vs. 14.4 (5.83, range: 6.1–23.7) in the placebo group ($P=0.089$; Table 1). In the subgroup sensitized to aeroallergens, mean age (SD) in the LGG group was 17.2 months (5.01) vs. 19.4 months (5.26; $P=0.366$). The average age of LGG-supplemented subjects sensitized to aeroallergens was not different from the whole LGG group ($P=0.791$), but the average age of aeroallergen-sensitized subjects from the placebo group was a bit higher than the whole placebo group ($P=0.041$).

Children belonging to the LGG group tended to be more exposed to cigarette smoke in their household ($n=16$; 24.6% vs. $n=13$; 19.7%; $P=0.41$). In advance of the study, both groups suffered on the average from a similar total number of wheezing episodes: 4.8 (4.4–5.2) in the LGG group vs. 4.3 (3.9–4.6). By chance, significantly more children of the LGG group suffered from more than six episodes of wheezing per year (20.1 vs. 12.2%; $P=0.039$). In the LGG group, 56 subjects (f : m = 22 : 34) completed the whole study and provided clinical data vs. 46 (f : m = 21 : 25) in the placebo group; details are provided by Fig. 2. Sensitivity analysis of the features of the 'intent-to-treat' vs. the 'per-protocol' populations revealed no significant differences between the treatment arms with or without the drop-outs.

Clinical outcome parameters

There were no significant differences of SCORAD indices between the two intervention groups (Tables 1 and 3). We also could not find significant differences of asthma-related events either during the 6 months of food supplementation or during the 6 months of follow-up (Table 2). In the subgroup sensitized to aeroallergens ($n=20$), the asthma symptom score was significantly lower in the placebo group (median 22.9 vs. 42.5; $P=0.04$). In the subgroup sensitized to food allergens ($n=21$),

Table 1. Baseline clinical characteristics of the study population

Characteristics	LGG ($n=65$)	Placebo ($n=66$)
<i>Baseline demographics</i>		
Age (SD) [months]	16.7 (5.52)	14.4 (5.83)
Gender (female, male)	$n=23/42$	$n=28/38$
<i>Risk factors</i>		
Wheezing episodes/year	$n=4.8$ (4.4–5.2)	$n=4.3$ (3.9–4.6)
> 5 wheezing episodes/year	$n=13$ (20.1%)*	$n=8$ (12.2%)*
Household smoke exposure	$n=16$ (24.6%)	$n=13$ (19.7%)
Patients with SCORAD > 15	$n=12$	$n=11$
Total SCORAD index ($\pm$ SEM)	6.2 (4.2–8.3)	4.7 (3.3–6.1)

Values for wheezing episodes are medians (95% confidence intervals) unless indicated otherwise.

Statistical analysis revealed no significant difference except for * $P=0.039$.

LGG, *Lactobacillus* Gasseri et Goldbach; Sensi. aero, subjects sensitized for aeroallergens.

Table 2. Clinical impact of 6 months of LGG or placebo in infants with recurrent wheeze during intervention and 6 months of follow-up (over-all analysis)

	During intervention		During follow-up	
	LGG ($n=56$)	Placebo ($n=46$)	LGG ($n=56$)	Placebo ($n=46$)
No. of wheezing episodes	1.00 (0.66–1.34)	1.00 (0.60–1.40)	1.00 (0.61–1.39)	1.00 (0.66–1.34)
Days with wheeze	7.50 (1.32–13.7)	6.50 (0.80–12.2)	8.00 (3.03–13.0)	11.0 (7.15–14.9)
Days on inhalative betamimetics	13.5 (3.39–23.6)	13.0 (8.11–17.9)	20.7 (11.3–30.2)	13.9 (2.68–15.3)
Days on inhalative steroids	16.0 (7.29–24.7)	9.50 (1.99–17.0)	16.5 (6.38–26.7)	14.8 (4.04–25.6)
No. of rescue-free days	168.5 (158.4–178.6)	177.5 (172.0–183.0)	178.5 (168.9–188.1)	176.0 (169.6–182.4)
No. of symptom-free days	133.0 (123.4–142.6)	126.5 (115.7–137.3)	142.5 (132.6–152.5)	154.0 (146.2–161.8)
Asthma symptom score	19.8 (0.93–38.6)	9.81 (6.34–26.0)	12.5 (0.78–25.8)	13.8 (0.68–26.8)

Per protocol analysis; values are medians ($\pm$ 95% confidence interval). Statistical between group comparisons (Mann-Whitney *U*-test) revealed no significant differences.

LGG, *Lactobacillus* Gasseri et Goldbach; No., number of; rescue medication, steroid suppositories.

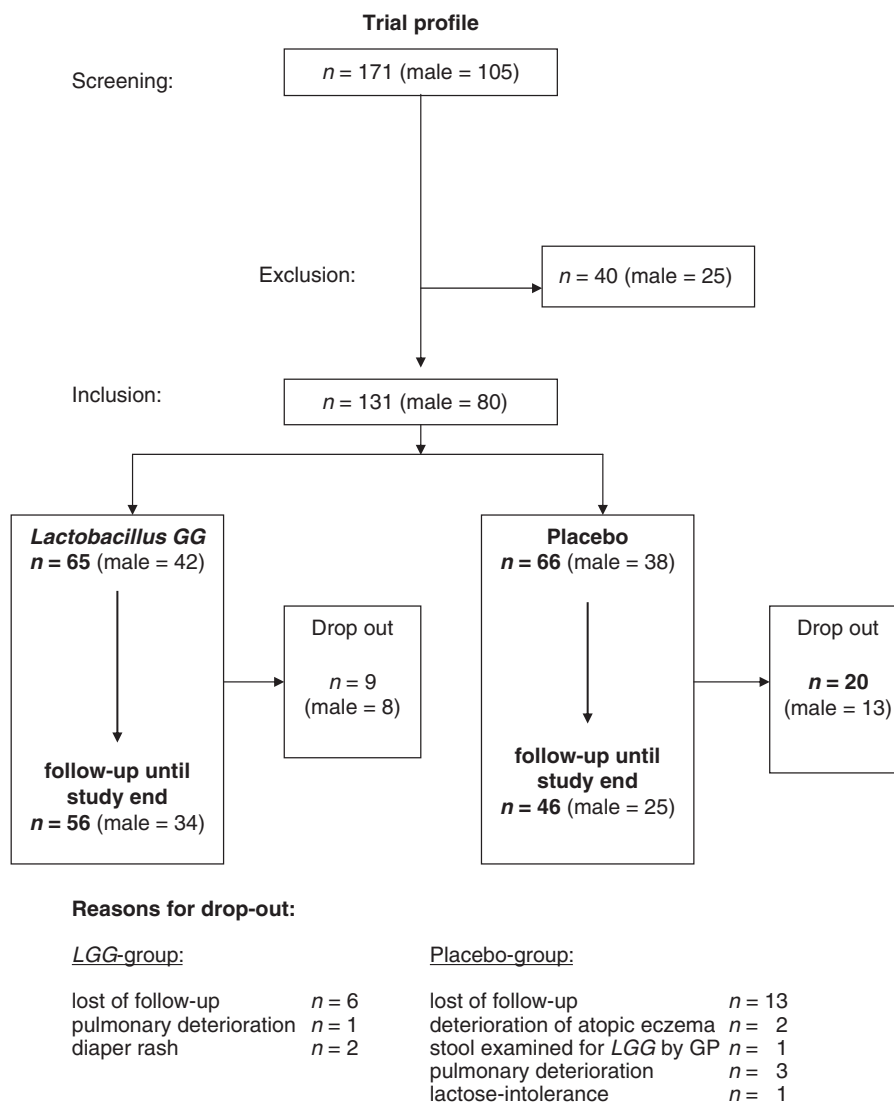


Fig. 2. Trial profile.

Table 3. Skin score and markers of atopy in infants with recurrent wheeze before, after 6 months of LGG or placebo, and after 6 months of follow-up

	<i>LGG</i>			Placebo		
	visit 1	visit 3	visit 5	visit 1	visit 3	visit 5
Total IgE (IU/mL)	16.7 (12.8–56.4)	14.7 (12.3–190.5)	13.6 (6.5–109.1)	12.2 (10.0–60.6)	11.3 (8.7–62.8)	20.2 (16.2–81.3)
Eosinophils (%)	2.8 (2.3–3.2)	3.2 (2.5–4.0)	3.0 (2.2–3.9)	2.8 (2.3–3.4)	3.5 (2.5–4.6)	3.0 (2.5–3.6)
ECP (µg/mL)	22.1 (14.9–29.2)	16.7 (9.89–23.5)	15.6 (1.98–29.2)	19.9 (12.6–27.2)	14.8 (1.91–31.5)	24.4 (16.6–32.1)
SCORAD	3.47 (0.47–14.3)	2.71 (0.71–11.1)	2.22 (0.22–8.49)	4.35 (0.35–18.0)	3.21 (0.21–13.3)	2.08 (0.08–7.19)

Per protocol-analysis; values are medians (95% confidence intervals).

Statistical between-group comparison (Mann–Whitney *U*-test) revealed no significant differences.

Eosinophils, eosinophilic granulocytes; ECP, eosinophilic cationic protein; visit 1, at the beginning of the trial; visit 3, after 6 months of intervention; visit 5, after 6 months of follow-up.

LGG-supplemented individuals needed more inhalative betamimetics and had fewer rescue-free days (median 17.0 vs. 10.0, $P = 0.04$ and 334 vs. 353, $P = 0.03$, respectively).

Laboratory data

Initially, total IgE levels were slightly higher in the *LGG* group than in placebo patients (median 16.7 (95%CI

12.8–56.4) vs. 12.2 U/mL (10.0–60.6; $P=0.051$). There were no significant changes in the course of the study: 14.7 (12.3–190.5) vs. 11.3 (8.7–62.8) on visit 3 ($P=0.533$), and 13.6 (6.5–109.1) vs. 20.2 (16.2–81.3) on visit 5 ($P=0.125$).

As expected, food allergens (e.g. hen's egg, milk protein) played a role in early sensitization. In addition, also aeroallergens (e.g. pollen, HDM, cat's epithelia) were detected already in this young age. A total of 38 subjects were found sensitized (≥ 0.20 IU/mL) to any of the tested allergens. While sensitization to aeroallergens decreased in *LGG* recipients, this was not observed in placebo-supplemented children (Figs 3a and b). When calculating the cumulative specific IgE, levels of aero-allergen specific IgE in serum were lower in the *LGG* group after 6 months of supplementation (0.526 vs. 10.3; $P=0.011$) and after another 6 months of follow-up (1.03 vs. 15.6; $P=0.002$). This observation was confirmed when repeating it for any specific IgE (visit 3: $P=0.07$, visit 5: $P=0.005$, respectively).

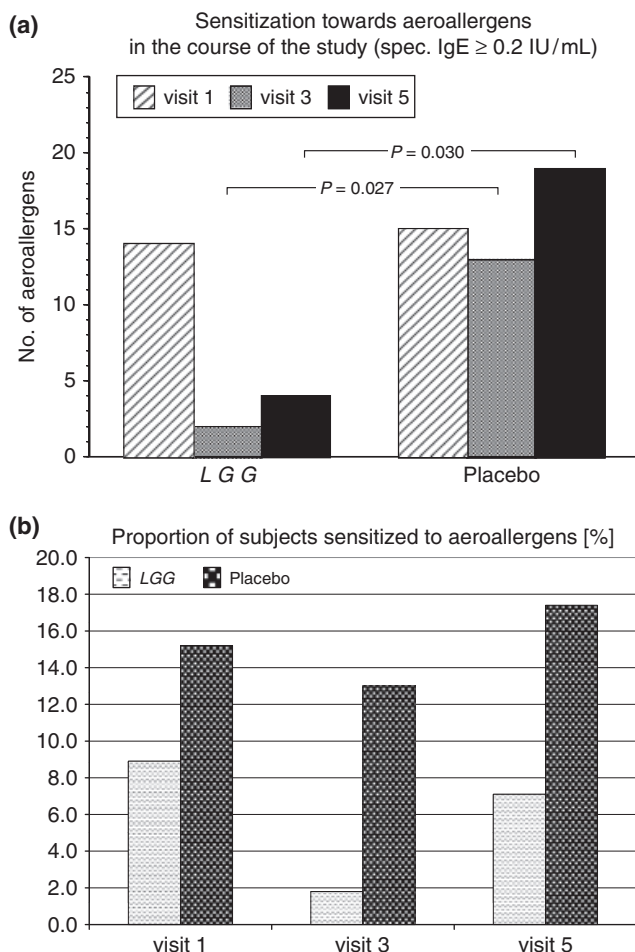


Fig. 3. (a) Sensitization towards aeroallergens in the course of the study (spec. IgE ≥ 0.2 IU/mL) (b) Proportion of subjects sensitized to aeroallergens [%]. visit 1, study start; visit 3, after 6 months of *LGG*; visit 5, after 6 months of follow-up.

Median levels of TGF- β significantly decreased in the *LGG* group (from 33 799 to 21 138 pg/mL; $P=0.002$), while in the placebo group values remained unchanged (28 606 vs. 27 304 pg/mL). When comparing TGF- β levels of *LGG* vs. placebo recipients, we could not find significant differences at any time.

Subgroup analysis: sensitization towards aeroallergens and severe allergic asthma

A subgroup ($n=20$) was found positive for aeroallergen-specific IgE. Because these subjects are expected to have a higher risk to develop an allergic asthma, we additionally analysed them separately. Median serum IgE was 29 (*LGG*) vs. 31 IU/mL (placebo) before intervention ($P=0.268$), 33.5 vs. 33.0 after the supplementation ($P=0.720$), and at the end of the follow-up 31 vs. 59 IU/mL ($P=0.286$). At visit 1, we detected a total of 14 aeroallergen sensitizations in five individuals in the *LGG* group compared with 15 sensitizations in seven individuals in the placebo group. After 6 months of *LGG*, only two sensitizations (in one patient) towards aeroallergens remained compared with 13 (in six patients) in the placebo group. After 6 months of follow-up, this effect persisted with 4 allergens (in four patients) in the *LGG* group vs. 19 allergens in eight placebo recipients.

Median eosinophils (95%CI) were lower after the intervention in the *LGG* group (2.5%; 0.38–5.3) than after placebo (4.4%; 2.1–8.9; $P=0.044$). Median ECP (95%CI) was 22.3 (10.8–38.6) vs. 24.2 (14.2–33.4) before intervention, 16.4 (9.9–41.9) vs. 15.9 (5.43–54.9) after 6 months, and 9.90 (7.85–14.5) vs. 22.0 (15.1–37.0) after 6 months of follow-up ($P=0.01$).

Among the subgroup of children with more severe asthma (i.e. at least five episodes of wheezing annually; $n=21$), *LGG* compared with placebo-supplemented infants had significantly lower levels of IgE specific for birch (0.0 IU/mL vs. 2.62; $P=0.023$), cat (0.0 vs. 15.6 IU/mL; $P=0.035$), and HDM (0.0 vs. 7.14 IU/mL; $P=0.022$) even after 6 months of follow-up.

Discussion

In our study, a 6-month alimentary probiotic food supplementation with *LGG* given to infants with recurrent wheeze and a family history of atopy did not have an impact on eczema or wheezing. Instead of protective effects, in the subgroup sensitized to food allergens, even more inhalative betamimetics and rescue medications had to be used, and in the subgroup sensitized to aeroallergens the asthma severity score was higher. We observed mild immunomodulatory effects; cumulative levels of IgE specific for aeroallergens were significantly lower in the *LGG* group during food supplementation and after 6 months of follow-up. In the small subgroup sensitized to

aeroallergens, median ECP values as a predictive marker for allergic disease were also significantly lower in the LGG group than in the placebo group even after 6 months of the cessation of the intervention.

The profibrotic marker TGF- β was significantly reduced in the LGG group and also lower than in the placebo group 6 months after the end of the intervention. Keeping the ambiguous character of this substance in mind, which has some short-term anti-inflammatory potential in parallel, this might explain in part the negative impact of LGG supplementation in subjects with an allergic sensitization.

As with all studies in young children, we relied on accurate care holders' reporting of the children's symptoms and accepted the limitations of this approach. However, it is unlikely that this has influenced our results, because our study was placebo-controlled. Our study was powered for 64 in each study group to complete the study, which was only slightly more than the number we randomized. But even with the smaller number completing the study ($n = 56$ and 46 , respectively), still a power of 68% would be reached under the assumption of the sample size calculated. Also, the small number of the analysed subgroups (e.g. sensitization towards aeroallergens) might have resulted in type 2 errors. The age structure of our intervention arms and subgroups was balanced; only aeroallergen-sensitized placebo recipients were a bit older than the whole placebo group. Even when controlling for a possible confounding, we could not find significant differences. Overall, we believe in the key findings of our study, which are in line with data from the literature.

Current concepts postulate that the immature immune system of infants is T-helper type 2 (Th2)-cell dominated. In the first years of life, physiologically a change occurs towards Th1, resulting in an effective host defence and immunotolerance. In atopic individuals, this switch of the immune system does not adequately occur or is not maintained in the absence of adequate stimuli. Th2-cells are overrepresented with increased levels of proinflammatory mediators, thus stimulating the synthesis of IgE, activating eosinophils and mastcells [12]. In ovalbumin-sensitized BALB/c and toll-like receptor 9-deficient mice, *Lactobacillus* (*L.*) *reuteri* decreased allergen-induced airway hyperresponsiveness, and significantly attenuated airway eosinophils, and the levels of TNF- α , IL-5, and IL-13 [13].

A few years ago, the first placebo-controlled study on LGG supplementation of high-risk infants demonstrated a significant reduction of atopic eczema up to the age of 7 years [5, 14]. Since then, similar studies with various probiotics gave conflicting results [15–21]. A study performed in New Zealand investigated the role of different probiotics, revealing a significantly reduced risk of eczema in atopic infants only by *L. rhamnosus* [22].

Also data on effects of probiotics on other manifestations of atopic disease are conflicting. Two small studies in adults could not show an impact of LGG on birch pollen allergy [23] or of *Lactobacillus acidophilus* on allergic asthma [24]. On the other hand, clinical improvement was observed in patients suffering from allergic rhinitis after food supplementation with *Lactobacillus paracasei* [25], *L. acidophilus* [26], or *Bifidobacterium longum* [27]. Other studies on probiotics revealed a decrease of allergic symptoms in adults [28] or fewer episodes of rhinitis in children [29].

In our study, the probiotic intervention did not alleviate the clinical symptoms of our patients. LGG recipients with allergic sensitization even tended to need more medical intervention. This might be explained in part because – by chance – our infants randomized to probiotics were more exposed to cigarette smoke and tended to have more wheezing episodes in advance. Our observations are in accordance with another German study, where LGG-food supplementation of atopic pregnant women and their offspring even resulted in an increase of wheezing episodes [17]. Also, the study by Kalliomäki [5] failed to show evidence for protective effects of probiotics on inhalant allergen sensitization and respiratory allergy. In a recent Australian study, probiotic-supplemented infants were even more likely to develop wheezing [16].

Another placebo-controlled study using *L. fermentum* demonstrated a reduction of lower respiratory tract infections in young children [30]. This goes with other international studies, where placebo-controlled probiotic food supplementation in children or pregnant women resulted in reductions of respiratory infections, fever, clinical visits, and antibiotic prescriptions [31–34]. These protective effects might be explained by the observation of *Lactobacilli* producing inhibitory substances against common pathogens (e.g. *Streptococci*, *Enterobacteriaceae*), thus supporting the host in the fight against infections [35].

Conclusion

In young children with atopic disposition and recurrent wheeze, 6 months of oral *Lactobacillus rhamnosus* GG food supplementation had no beneficial effect on airway symptoms and even mild negative clinical effects in children with antecedent allergic sensitization. Overall, laboratory markers of atopy remained unchanged. Only the small sensitized subgroup experienced mild positive immunomodulatory effects, persisting even 6 months after the cessation of the food supplementation. Further studies are needed to investigate long-term benefits.

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