Oral application of bacterial lysate in infancy decreases the risk of atopic dermatitis in children with 1 atopic parent in a randomized, placebo-controlled trial

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Background: Lower prevalence of atopy was found in children with continuous exposure to livestock and thus to microbial compounds. In animal models exposure to endotoxin (LPS) decreases allergic sensitization and airway inflammation.
Objective: We sought to evaluate the effect of orally applied bacterial lysate in infancy on the prevalence of atopic dermatitis (AD) after the treatment phase at 7 months of age.
Methods: This randomized, placebo-controlled trial included 606 newborns with at least single heredity for atopy. From week 5 until the end of month 7, infants were treated orally with bacterial lysate containing heat-killed gram-negative *Escherichia coli* Symbio and gram-positive *Enterococcus faecalis* Symbio or its placebo. Children were followed until 3 years of age.
Results: There was no difference in the primary outcome between the active and placebo groups in the total study group. AD prevalence was significantly reduced at the end of the intervention phase (31 weeks of age) in the subgroup of infants with single heredity for atopy (relative risk, 0.52; 95% CI, 0.3-0.9). Ten percent (15/154) of infants in the active group had AD compared with 19% (27/145, $P = .030$) in the placebo group. This was more pronounced in the group of infants with paternal heredity for atopy (11% vs 32%, $P = .004$; relative risk, 0.34; 95% CI, 0.2-0.7).
Conclusion: Feeding of bacterial lysate might have prevented the development of AD, especially in children with paternal atopy, possibly indicating a preventive property only in subjects with a limited risk for atopy. (J Allergy Clin Immunol 2012;129:1040-7.)

Key words: *Atopic dermatitis, infancy, bacterial lysate, primary prevention*

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Improved hygiene with decreased microbial exposure, lower rates of severe infections, changes in diet, reduced family size, or increased use of antibiotic drugs are probable determinants of the increasing prevalence of atopic diseases.[1,2] The early feeding of cow's milk was identified as a risk factor for atopic dermatitis (AD) or infantile eczema,[3] and the potential benefit of exclusive or extended breast-feeding has been widely discussed in this context.[3,4] Avoidance studies have shown that feeding hydrolyzed formula instead of regular cow's milk formula during the first 4 months of life reduces the incidence of eczema at 12 months by 50%, being comparable with feeding breast milk only.[5] However, avoidance strategies are not feasible for all allergens and have been ineffective in terms of primary prevention of allergies to inhalant allergens.[6] Therefore current studies try to identify protective factors that modulate the immune system and induce tolerance to allergens in a nonspecific fashion. As a prominent example, use of the probiotic *Lactobacillus GG* on the development of AD was intensively studied, with a quite inconsistent outcome.[7] A first report on feeding prebiotic substances (galacto-oligosaccharides or fructo-oligosaccharides) showed protective effects against early infantile eczema in a study population of infants without a family history of atopy.[8] Furthermore, the application of a bacterial lysate containing bacteria often causing upper and lower respiratory tract infections was found to suppress airway inflammation in mice through recruitment of regulatory T cells.[9]

In search of protective factors, epidemiologic studies reliably showed that continuous exposure to microbial compounds (eg, lipopolysaccharides [LPS] of gram-negative bacteria) during pregnancy and early infancy was associated with a lower prevalence of atopy and asthma in children, especially in rural areas ("farm effect")[10] but also in other populations.[11] As proof of this concept, animal models confirmed that prenatally initiated LPS exposure decreased allergic sensitization and airway inflammation.[12] In a recent publication, LPS was identified as the key molecule of *Acinetobacter lwoffii* for inducing protective immune regulation[13] in human subjects. Obviously, maternal exposure to an environment rich in microbes upregulates receptors of innate immunity, so-called Toll-like receptors (TLRs), especially TLR2 and TLR4. Thus the infantile immune system is directed to a T_H1-type affinity and tends toward gradual inhibition of the T_H2 phenotype and finally to postnatal induction of tolerance.[14]

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Abbreviations used
AD: Atopic dermatitis
GINI: German Infant Nutritional Intervention
ITT: Intention to treat
LPS: Lipopolysaccharides
RR: Relative risk
TLR: Toll-like receptor

Furthermore, in a recent birth cohort study maternal contact with farm animals and cats during pregnancy had a significantly protective effect on manifestation of AD during the first 2 years of life associated with increased gene expression of TLR5 and TLR9 in cord blood. A significant interaction between polymorphisms in TLR2, which bind particularly peptidoglycans of gram-positive bacteria and prenatal cat exposure, was additionally observed.[15] In light of these intriguing data, it is reasonable to mimic this protective effect of microbial exposure in an interventional approach. Therefore we hypothesized here that oral application of bacterial lysates of gram-negative and gram-positive bacteria during early infancy would significantly reduce the prevalence of atopic eczema in a high-risk population living in a nonfarming environment.

METHODS

Study population

Approximately 6000 families were informed of the present randomized, placebo-controlled trial through obstetric departments of 32 hospitals in Berlin, Hamburg, Lower Saxony, and Brandenburg within the first week after the infant's birth between May 2002 and September 2007. All visits were performed at Charité Berlin. Of 633 newborns enrolled in the screening phase, 606 could be included in the randomized, placebo-controlled trial. Inclusion criteria were healthy newborns at term, birth weight of 2500 g or greater, 1 or 2 parents with atopic disease (AD, allergic rhinitis, and/or asthma), and informed consent. In a standardized interview parents were asked for atopic and asthmatic symptoms, medication, and doctor's diagnosis by experienced physicians according to the Multicenter Allergy Study[16] and the German Infant Nutritional Intervention (GINI) study.[5] Exclusion criteria were antibiotic treatment or other medication after birth, lymphocytopenia or thrombocytopenia, intensive care after birth, lack of German-language knowledge, and no informed consent. The last treatment of the last patient was performed in April 2008. The study was approved by the Charité ethics committee in 2002.

Study design

After the screening phase (visit 1, age 0-4 weeks), enrolled infants were randomized at visit 2 (age 4-5 weeks).

From week 5 until the end of month 7, infants were treated prophylactically with oral bacterial lysate (Pro-Symbioflor; SymbioPharm, Herborn, Germany) containing heat-killed nonpathogenic gram-negative *E coli* Symbio DSM 17252 and nonpathogenic gram-positive *E faecalis* Symbio DSM 16440 ($1.5\text{-}4.5 \times 10^7$ bacteria/mL) with a daily dosage of 3×0.7 mL. Ten drops of the solution were administered to the infants with a spoon 3 times a day. The autolysate was obtained after heat treatment of the bacterial cultures and contained immunologically active compounds of the bacteria, such as the cell wall, but could no longer replicate. Compliance was estimated by weighing the bottles containing the study medication when parents returned them at the clinic visits. Children were followed until 3 years of age without unmasking the groups (Fig 1).

The primary outcome was the prevalence of AD at the end of the prophylactic treatment period at 7 months of age. AD was clinically assessed by using a score according to the method of Hanifin and Rajka[17] adapted for infants. The diagnosis for AD was made if infants showed a typical itchy rash at flexural/extensor sites, in the face, or both and dryness of the skin for more than 6 weeks. Five hundred twenty-six healthy infants with at least 1 atopic parent completed the study to 7 months of age as per the protocol (completed intervention phase), and 487 children were followed until 3 years of age (completed follow-up phase) (Fig 2).

Infants were seen for clinical examination by a pediatrician during the intervention phase at the ages of 13, 21, and 31 weeks (end of the intervention phase). In the follow-up phase participants were seen for additional visits at 1, 2, and 3 years of age. Clinical visits were comprised of a physical examination, which included obtaining the Scoring Atopic Dermatitis score[18] if signs of AD were present. Furthermore, a detailed questionnaire asked about breastfeeding duration, number of siblings, pet keeping, smoking, and adverse events. At 5 and 31 weeks of age, blood cell counts and liver enzyme and creatinine levels were measured for safety data. Furthermore, specific serum IgE levels to 6 food allergens (cow's milk, hen's egg, wheat, peanut, soya, and cod fish) and 7 inhalant allergens (cat dander, dog dander, *Dermatophagoides pteronyssinus*, birch, grass, mugwort, and *Cladosporium herbarum*; fx5, sx1; Phadia, Freiburg, Germany) and total IgE levels were measured at the ages of 5 and 31 weeks and 1, 2, and 3 years. Stool samples for analyses of gut flora were collected during the intervention phase. Furthermore, detailed immunologic and genetic analyses were performed, and dust samples were collected to analyze allergen and endotoxin contents. In this publication only data on the primary end point, specific sensitization, and AD prevalence at the age of 1, 2, and 3 years will be shown.

The study was registered as a randomized, controlled trial (ISRCTN60475069: "Prophylaxis of atopic and allergic manifestations and activation or modulation of the immune system by Pro-Symbioflor© treatment in newborns/small children from pre-disposed atopic parents").

Randomization and masking

Participants were assigned to the study groups by using simple randomization. This was done centrally at SymbioPharm, giving every participant a randomization number in ascending order according to the date of appearance at the study site. Allocation concealment was provided. A sealed emergency envelope enclosing the treatment information for each participant was stored at the study center. Investigational products were manufactured, labeled, and packed through SymbioPharm in accordance with the German Pharmaceuticals Act (AMG). Bottles were filled in fully randomized order, and blinding was complete for the study personnel. Placebo contained lactose monohydrate (4.9 mg/mL), sodium chloride (8.8 mg/mL), magnesium chloride (0.608 mg/mL), calcium chloride (0.076 mg/mL), potassium chloride (0.133 mg/mL), magnesium sulfate (0.779 mg/mL), and aqua destillata (998.2 mg/mL). Unblinding was performed in November 2010.

Assessment of compliance

Study medication was distributed at every visit from week 5 to week 31. Parents were instructed about the doses and the regular intake. Used bottles were collected and checked for correct intake (weight of bottles). Compliance was similar in both groups. Average intake of the study medication was greater than 95% of recommended doses.

Statistics

Intention-to-treat (ITT) analysis was performed. Missing observations in early discontinued children were imputed by combining the last-value-carried-forward approach with a worst AD prevalence estimate approach using the placebo AD prevalence in completers as the relevant ratio for healthy discontinued children. AD prevalence was tested with the Fisher exact test (global $\alpha = .05$) in the active and placebo groups for efficacy in the total study population and in prespecified subgroups according to atopy heredity. Right-censored cumulative hazard curves were constructed, mean survival times were estimated, and intervention groups were tested for efficacy through Mantel log-rank tests. Survival analysis was carried out by estimating the Kaplan-Meier[19] product limit estimator from the life-time table. The average

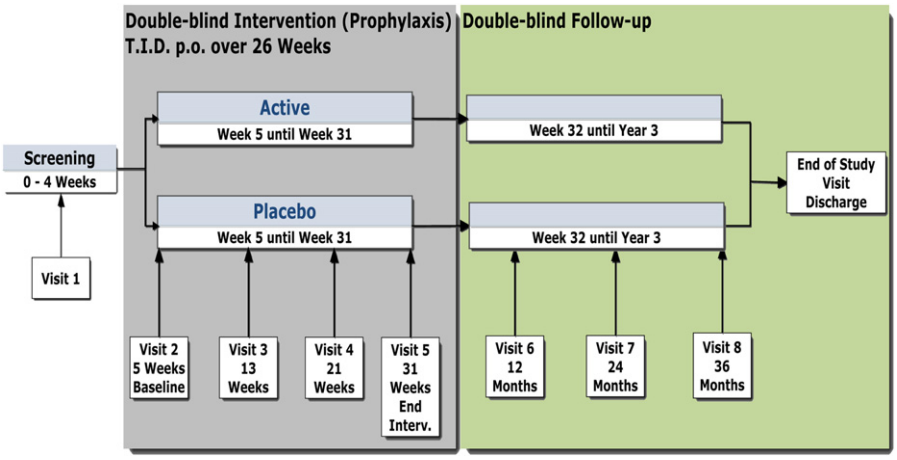

FIG 1. Study design.

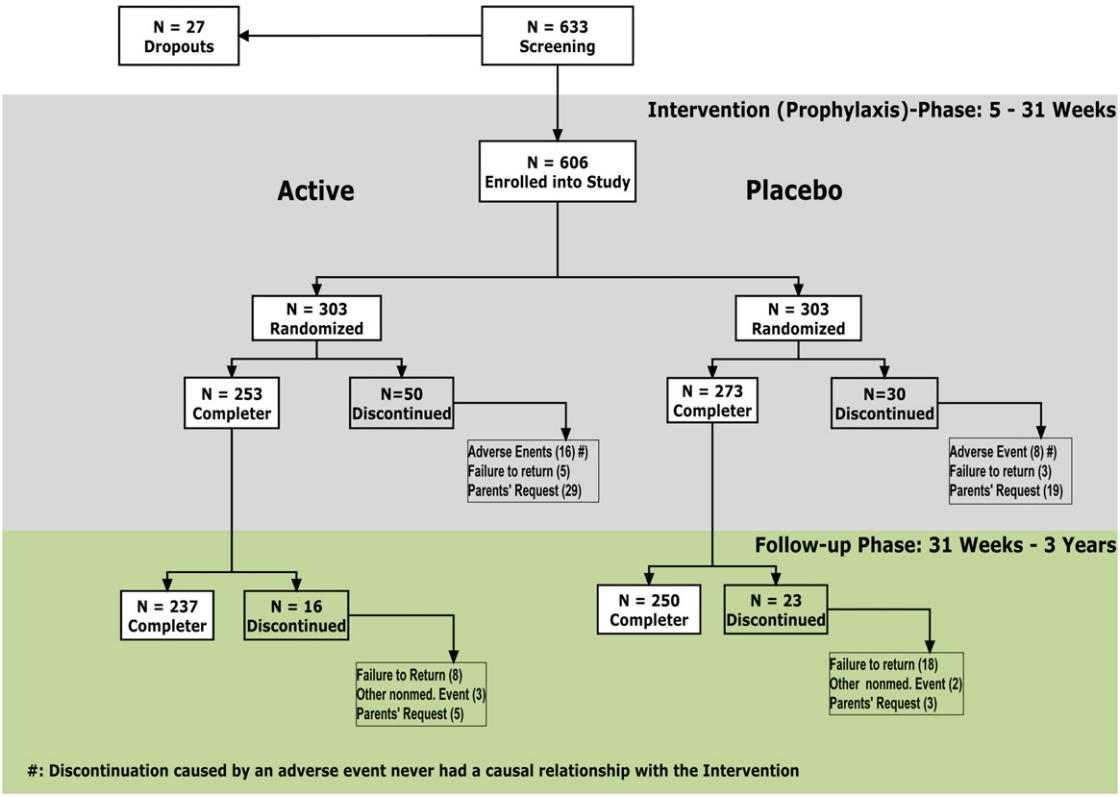

FIG 2. Flow chart of study participants (*light gray,* intervention phase; *green,* follow-up phase).

survival time was estimated from the Kaplan-Meier survivor function, and confidence limits were estimated by using the Greenwood formula.

Sample size estimation with modifications caused by interim analysis

Specifying the type I error to an α value of .05 and the type II error to a β value of .20 (study power, 0.80), the number of children required was estimated before the study as 2×220 planned evaluable subjects. The withdrawal rate was assumed to be 12%, and therefore the planned number of randomized subjects was set at 2×250. In September 2005 (ie, 3 years after study onset), it was mutually agreed between the Federal Institute for Drugs and Medical Devices and SymbioPharm to conduct an interim efficacy evaluation (milestone: randomized until March 9, 2005, and intervention finished). A revision of sample estimation was first carried out and fixed by amendment to the trial protocol to compensate for multiplicity of type I error. On the basis of an α adjustment, the revised sample sizes were evaluated as follows[20]: local significance level of interim analysis was an α_{adj} value of .0150; local significance level of final analysis was an α_{adj} value of .0418 with a global α value of .05; required number of evaluable subjects was 2×282; and required number of subjects to be randomized to cover for 12% prematurely discontinued was 2×316. The ethics committee agreed to the changes, and randomization into the study was likewise conducted.

Statistical analysis was performed with SYSTAT version 13 (SYSTAT Software, Inc, Chicago, Ill) and SAS version 9.2 (SAS Institute, Inc, Cary NC) software.

Role of the funding source

SymbioPharm was involved in the study plan but had no influence on the analyses and interpretation of data. Only Kurt Zimmermann from the research department was involved in the writing of the manuscript.

RESULTS

The study medication was safe and well tolerated by the infants. The most frequently reported possibly related adverse events were abdominal pain (0.8% in both treatment groups), diarrhea (7.5% in the active and 7.4% in the placebo groups), and flatulence (0.1% in both treatment groups). Severe gastrointestinal complaints, such as hemorrhagic colitis or intussusception, were not reported. Reports of adverse events were similar in both treatment groups. In the treatment and placebo groups 2951 versus 2925 adverse events were reported, respectively. In the active 73 and in the placebo group 74 severe adverse events were reported; none of them was assessed as related to the study medication. Severe adverse events were pneumonia, pleural effusion, dehydration caused by viral gastroenteritis, febrile seizures, urinary tract infection, wheezy bronchitis with oxygen supplementation, and allergic purpura.

The dropout rate during the treatment phase was 80 (13%) of 606 for the whole study group and was higher in the active group (50 [18%] of 303) compared with the placebo group (30 [10%] of 303). In the follow-up phase another 6.8% (16/237) in the active group and another 9.2% (23/250) in the placebo group did not complete the follow-up (Fig 2).

Table I shows the baseline characteristics of both treatment groups, which were similar also if performing subgroup analyses (data not shown). A total of 526 infants completed the study to 7 months of age (completed the intervention phase per protocol) and 487 children to 3 years of age (completed the follow-up phase) (Fig 2). At all time points of the intervention and follow-up periods, there was no difference in the adverse event prevalence between the active and placebo groups among the total study group (ITT; Fig 3 and Tables II and III). At the end of 7 months, 114 of 606 infants had adverse events (19% at the end of the intervention phase). The incidence rate between 5 and 13 weeks of age was 39 (3%) of 606, that between 13 and 21 weeks of age was 41 (7%) of 567, and that between 21 and 31 weeks of age was 34 (7%) of 526. In the group of children with maternal or double-positive history for atopy, there was no difference between active and placebo treatment at any time (Table II).

However, there was a significant difference in AD prevalence at the end of the treatment period (31 weeks of age) in the subgroup of infants with single heredity for atopy (Fig 4 and Tables II and III). While 10% of infants in the active group had AD (15/154, ITT analysis), 19% (27/145, $P = .030$) had AD in the placebo group (relative risk [RR], 0.52; 95% CI, 0.3-0.9). This was even more pronounced in the group of infants with only paternal heredity for atopy (11% vs 32%, $P = .004$), showing a reduction of the RR for AD of almost 70% (RR, 0.34; 95% CI, 0.2-0.7; Tables II and III). In this subgroup the significantly different prevalence rates were also observed after the intervention phase at 1 year of age ($P = .020$) and remained borderline until the end of the follow-up phase. In the ITT analysis, 11 children with single heredity or 5 with paternal heredity had to be treated with the study medication to prevent 1 case of AD (Table III). Mean survival times without eczema at the end of the treatment phase were 34.8 weeks in the active group and 34.3 weeks in the placebo group ($P = .035$) among the children with single atopic family history for atopy (Table IV). For children with atopic fathers, the mean survival times were 31.7 and 30.6 weeks at the end of the treatment phase ($P = .003$) and 135 and 114 weeks at the end of the follow-up phase ($P = .003$), respectively.

TABLE I. Baseline characteristics of both treatment groups

Baseline characteristics	Active group (n = 303)	Placebo group (n = 303)
Age of newborns (wk), median (25% to 75% quartile)	5.1 (4.6-5.7)	5.1 (4.6-5.7)
Proportion male	161 (53.1%)	152 (50.2%)
Weight at birth (g), median (25% to 75% quartile)	3480 (3140-3780)	3480 (3230-3800)
Gestational age (wk), median (25% to 75% quartile)	40 (39-40)	40 (38-40)
Cesarean section	75 (24.8%)	76 (25.1%)
No. of siblings, median (25% to 75% quartile)	1 (1-1)	1 (1-1)
Frequency by number		
1	122	110
2	22	28
3	5	3
4	—	—
5	—	1
Smoking mother		
Before pregnancy	81 (26.7%)	73 (24.1%)
During pregnancy	75 (24.8%)	66 (21.8%)
After pregnancy	71 (23.4%)	74 (24.4%)
Households with furry pets	102 (33.7%)	91 (30.1%)
Family history of atopy		
Both parents	148 (48.8%)	157 (51.8%)
One of both parents	154 (50.8%)	143 (47.9%)
Mother	79 (26.1%)	85 (28.1%)
Father	75 (24.8%)	60 (19.8%)
Single mother	2 (0.7%)*	1 (0.3%)†

*Family history of atopy was unknown for 1 father but known for the second father.
†Family history of atopy was unknown for the father.

The treatment effect was strongest for infants with a single parent with allergic rhinitis ($P = .001$, data not shown). If allergic asthma was present in a single parent, the effect was significant but weaker ($P = .040$). No effect was seen if a parent had AD or a combination of atopic symptoms. In the subgroup with paternal atopy only, less parental eczema was reported than in the other subgroups (13% compared with 37% in the group with double heredity and 26% in the group with maternal heredity).

There was no difference in specific serum IgE levels between the intervention and placebo groups at any time (total study group). One hundred twenty-one infants in the placebo group and 114 infants in the active group showed increased levels of serum IgE to food allergens, whereas 3 infants in the placebo group and 4 in the active group already were found to have increased levels of serum IgE to inhalant allergens at the age of 7 months. The most frequent sensitizations observed at the end of the treatment phase were against cow's milk, hen's egg, and peanut. The median total IgE concentration in serum was 5 kU/L for both treatment groups at age 7 months ($P = .824$), 10.0 kU/L in the active group and 9.1 kU/L in the placebo group ($P = .596$) at age 24 months, and 24.3 versus 22.9 kU/L at age 36 months ($P = .283$). In the group with single heredity for atopy, only at age 36 months was there a significant difference between the active and placebo groups (11.8 vs 17.9 kU/L, $P = .016$).

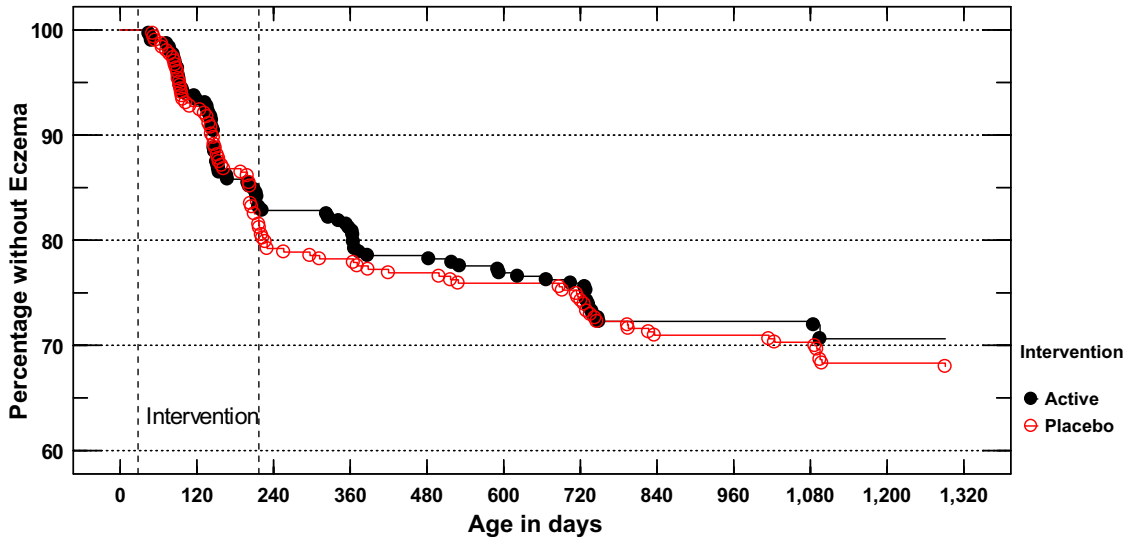

FIG 3. Total study group with double and single parental heredity (n = 606): eczema-free survival rates in the active and placebo intervention groups up to 3 years of life (ITT evaluation).

TABLE II. AD prevalences in the ITT population (n = 303 in the active group and n = 303 in the placebo group)

			Intervention phase				Follow-up phase		
	Visit		2	3	4	5*	6	7	8
Atopy heredity	Age of life		5 wk	13 wk	21 wk	31 wk	12 mo	24 mo	36 mo
Study group or subgroup	Treatment	No.	Atopic dermatitis prevalence (%)						
Total study group	Active	303	0.0	6.3	13.9	**17.2**	21.5	27.7	29.4
	Placebo	303	0.0	6.6	12.9	**20.8**	23.1	28.7	32.0
	P value		–	1.000	.811	**.300**	.696	.857	.538
Double	Active	148	0.0	8.1	18.9	**24.3**	29.1	37.2	39.2
	Placebo	157	0.0	10.2	16.6	**22.9**	24.8	31.8	35.7
	P value		–	.558	.653	**.789**	.440	.338	.555
Single	Active	154	0.0	4.5	8.4	**9.7**	13.6	18.2	19.5
	Placebo	145	0.0	2.8	9.0	**18.6**	21.4	25.5	27.6
	P value		–	.543	1.000	**.031**	.093	.160	.103
Maternal	Active	79	0.0	6.3	8.9	**8.9**	12.7	17.7	20.3
	Placebo	85	0.0	4.7	4.7	**9.4**	14.1	20.0	23.5
	P value		–	.739	.357	**1.000**	.822	.842	.707
Paternal	Active	75	0.0	2.7	8.0	**10.7**	14.7	18.7	18.7
	Placebo	60	0.0	0.0	15.0	**31.7**	31.7	33.3	35.0
	P value		–	.503	.271	**.004**	.022	.072	.047

The Fisher exact test (confirmative at visit 5 only) was used for contingency of AD prevalence and treatment in the total study group and pertinent subgroups according to parental heredity.
*Boldfaced figures denote results in the primary outcome at the end of 31 weeks' intervention.

DISCUSSION

This study reports on an intervention with orally applied bacterial lysates in human subjects for primary prevention of atopic eczema in infancy. Unfortunately, we found no difference between the active and placebo groups for the whole study population. However, in a subgroup with single heredity for atopy, a significant effect was observed, which was most pronounced for infants with atopic fathers. The effect seems to be consistent also at 1 year of age, and there is still a borderline significance at 2 and 3 years of age. The baseline characteristics in this subgroup show no significant difference from the 2 main treatment groups; therefore confounding seems unlikely. However, data from subgroup analyses have to be interpreted with caution. The authors were aware of significant limitations of subgroup analyses in clinical trials. Thus subgroups were only defined because of known important differences in AD prevalence in infants of parents with distinct atopy states.[21]

The classification for family history for atopy was done in a standardized interview by using validated questions from the Multicenter Allergy Study[16] and the GINI study[5] asking for symptoms, antiallergy and antiasthma therapy, and doctor's diagnosis by an experienced study doctor. If parental atopy was not clear, families were not included; therefore misclassification of the main inclusion criterion is unlikely.

The dose of 2.5 to 5.0 ng of LPS/kg body weight was shown to be effective in very recent rat experiments. In the sera of animals

TABLE III. RR for ITT and per-protocol groups (completed treatment and follow-up phase) stratified for atopic family history

		Prevalence at end of intervention phase (age 31 wk)					
	Atopy heredity	Active group		Placebo group		RR	NNT
Evaluation	Study group or subgroup	AD	Healthy	AD	Healthy	RR (95% CI)*	NNT (95% CI)*
ITT	Total study group	52	251	63	240	0.83 (0.6-1.1)	27.5 (10.1-10^6)
	Double	36	113	36	123	1.07 (0.7-1.6)	65.8 (12.6-10^6)
	Single	15	138	27	116	0.52 (0.3-0.9)	11.0 (5.9-89.4)
	Maternal	7	71	8	75	0.93 (0.4-2.5)	150.6 (10.4-10^6)
	Paternal	8	67	19	41	0.34 (0.2-0.7)	4.8 (2.9-13.7)
Per protocol (completed)	Total study group	40	213	55	218	0.78 (0.5-1.1)	23.1 (9.2-10^6)
	Double	30	95	32	114	1.10 (0.7-1.7)	48.0 (12.5-10^6)
	Single	9	118	23	103	0.39 (0.2-0.8)	9.0 (5.2-32.5)
	Maternal	5	56	6	66	0.98 (0.3-3.1)	732 (10.5-10^6)
	Paternal	4	62	17	37	0.19 (0.1-0.5)	3.9 (2.6-8.5)

*Ninety-five percent confidence limits.

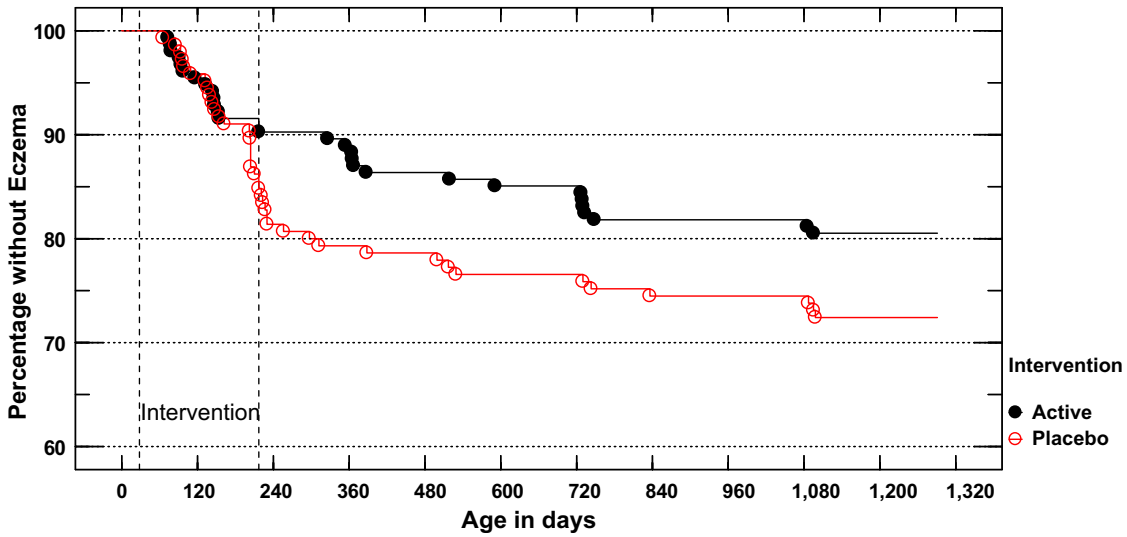

FIG 4. Single parental heredity subgroup (either in the mother or the father, n = 296): eczema-free survival rates in the active and placebo intervention groups up to 3 years of life (ITT evaluation).

that received the bacterial lysate in addition to ovalbumin sensitization, the levels of allergen-specific IgE and IgG were significantly reduced compared with those seen in animals that were not exposed to the bacterial lysate. This was accompanied by an increased cytokine production of IL-10.[22] The dose of the combined bacterial lysate we applied had previously been proved safe in infancy. During the last 40 years, several thousand infants and children have been treated with the product as one approach used in complementary medicine for complaints such as irritable bowel disease or recurrent upper airway infections. However, no studies in infancy and childhood had been conducted. The length of intervention was a compromise between compliance of parents and the assumption that the incidence of AD is highest in the first 6 months of life.[23] However, it is possible that a longer treatment phase or a higher dose given to children with maternal heredity or double-positive heredity for atopy would have been more efficient in the prevention of infantile eczema.

The nonpathogenic inactivated strains of gram-positive *E faecalis* and gram-negative *E coli* (Pro-Symbioflor) were expected mainly to modulate the gut-associated lymphoid tissue, which is known to play a crucial role in development of allergic sensitization and disease in infancy.[24] Briefly, the pathogen-associated molecular patterns lipoteichoic acid and LPS, cell-wall components of respective gram-negative and gram-positive bacteria, activate the innate immune system through their receptors TLR2 and TLR4; therefore they initiate production of proinflammatory cytokines, such as TNF-α and IL-12, and favor T_H1 responses of the adaptive immune system.[25,26] Previous studies in animal models found that systemic LPS application prevented subsequent allergen-induced sensitization and airway inflammation in an IL-12–dependent manner through induction of a general shift toward unspecific T_H1-type immune responses, thus decreasing the susceptibility for sensitization to allergens.[27] However, in the colon TLR2 and TLR4 expression is low. In human subjects intestinal epithelial cells of the small intestine show a polarized pattern of TLR expression. The bacterial lysate used in the prophylactic treatment can activate TLR4 on the basolateral surface, whereas the activation of TLR2 remains uncertain because it has only been shown on the apical and basolateral surfaces of follicle-associated epithelial cells in mice.[28]

TABLE IV. Eczema-free survival time in weeks (ITT evaluation)

			Eczema-free survival time (wk; ITT evaluation)			
			End of intervention (31 wk)		End of follow-up (3 y)	
Atopy heredity	Intervention	No.	Mean (95% CI)	*P* value	Mean (95% CI)	*P* value
Total study group	Active	303	33.8 (33.0-34.6)	.290	144.5 (137.0-151.9)	.502
	Placebo	303	33.6 (32.8-34.4)		141.7 (134.1-149.3)	
Double	Active	148	28.7 (27.8-29.7)	.794	130.2 (118.6-141.8)	.517
	Placebo	157	28.8 (27.8-29.8)		137.1 (131.1-143.2)	
Single	Active	154	34.8 (33.9-35.7)	.035	136.6 (129.5-143.7)	.096
	Placebo	145	34.3 (32.6-35.9)		126.3 (121.4-131.2)	
Mother	Active	79	34.8 (33.5-36.1)	.919	137.6 (128.1-147.2)	.624
	Placebo	85	35.3 (34.3-36.3)		134.8 (125.1-144.4)	
Father	Active	75	31.7 (30.8-32.6)	.003	135.3 (125.0-145.6)	.026
	Placebo	60	30.6 (29.4-31.7)		114.4 (98.9-129.8)	

Values are presented as means and 95% CIs, where shown.

It has been previously reported that infants with eczema during the first 18 months of life show less diversity in fecal microbiota shortly after birth, especially a decreased presence of *E faecalis*.[29] One might assume that the postulated immune-modulating effect on the gut-associated lymphoid tissue in our intervention trial was not strong enough, especially because no colonization could be achieved by bacterial lysates. However, in the subgroup with single heredity for atopy, particularly with paternal allergic rhinitis, a significant effect was observed, although only if no parental eczema was present. Pathophysiologically, it remains unclear why maternal and paternal genetic backgrounds seem to result in different intervention effects on the development of atopic diseases in children. In the group of children with paternal atopy, less parental eczema was reported than in the other subgroups, obviously increasing the chance of a successful intervention addressing the immune system rather than skin-specific barrier dysfunction. Our explanation would be that the weaker the genetic background for eczema, the more successful the intervention. Observational studies have found maternal atopy to be a stronger risk factor for allergic sensitization and development of AD in children than paternal atopy. This might be attributed to programming of the fetal immune system *in utero*.[30] The development of the fetal immune system is guided by parental genetics but even more by interactions with the maternal immune system at the placental barrier. Maternal-fetal interactions are affected by the maternal genetic state and the actual presence of an atopic disease in the mother,[31] potentially delaying fetal lymphoproliferation,[32] as well as by various behavioral and environmental factors, such as the mother's nutrition[30] or smoking status[33] during pregnancy. Therefore interventional approaches have to be strong enough to counteract programming of the fetal immune system based on parental genetic background and the specific immunologic milieu *in utero*. Accordingly, it is not unlikely that the intervention used in this trial might be successful only in terms of prevention in a population with single or paternal atopy, especially with allergic rhinitis and without eczema. Also in the GINI study the partially hydrolyzed formula was not effective in the group of infants with a parental history of eczema, whereas it was preventive in the group of children with a parental history of allergic airway disease.[5] Furthermore, other exposure variables, such as number of siblings,[1] entrance to day care,[34] and early infections,[35] might play a role in the development of atopic eczema and allergic airway disease. However, there was no difference for these variables between the active and placebo groups (data only partly shown). Mechanisms involved in the prevention of AD through our study medication will be analyzed soon.

In addition to trials on hydrolyzed formula targeting early allergen avoidance[5] and the application of lactobacilli or bifidobacteria or prebiotic oligosaccharides[7,8] targeting the gut flora and thus potentially changing early immune regulation, the results of this trial might contribute to a new approach to primary prevention of atopic infantile eczema. Having identified farm milk, *A lwoffii* F78,[13,36] and arabinogalactan[37] from cowshed dust as potentially preventative compounds of the farming environment, further interventional trials are to be expected.

In summary, we conclude from our results that there might be differences based on paternal or maternal heredity of atopic diseases when targeting exposure to microbial compounds early in life as a preventive measure. Data need to be supported by further mechanistic studies and require confirmation in low- and high-risk infants.

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Clinical implications: This study shows a possible approach of primary prevention of infantile atopic eczema in a subgroup of infants with single paternal heredity for atopy.

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