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Received: 6 August 2019 | Revised: 3 November 2019 | Accepted: 21 December 2019

DOI: 10.1111/all.14247

ORIGINAL ARTICLE

Asthma and Lower Airway Disease

Allergy EUROPEAN JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY EAACI WILEY

Orally applied bacterial lysate in infants at risk for atopy does not prevent atopic dermatitis, allergic rhinitis, asthma or allergic sensitization at school age: Follow-up of a randomized trial

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Abstract

Background: The allergy preventive effects of gut immune modulation by bacterial compounds are still not fully understood.

Objective: We sought to evaluate the effect of bacterial lysate applied orally from the second until seventh months of life on the prevalence of allergic diseases at school age.

Methods: In a randomized, placebo-controlled trial, 606 newborns with at least one allergic parent received orally a bacterial lysate consisting of heat-killed Gram-negative *Escherichia coli* Symbio and Gram-positive *Enterococcus faecalis* Symbio or placebo from week 5 until the end of month 7. A total of 402 children were followed until school age (6-11 years) for the assessment of current atopic dermatitis (AD), allergic rhinitis (AR), asthma and sensitization against aeroallergens.

Results: AD was diagnosed in 11.0% (22/200) of children in the active and in 10.4% (21/202) of children in the placebo group. AR was diagnosed in 35% (70/200) of children in the active and in 38.1% (77/202) children in the placebo group. Asthma was diagnosed in 9% (18/199) of children in the active and in 6.6% (13/197) of children in the placebo group. Sensitization occurred in 46.5% (66/142) of participants in the active and 51.7% (76/147) in the placebo group.

Conclusion: An oral bacterial lysate of heat-killed Gram-negative *Escherichia coli* and Gram-positive *Enterococcus faecalis* applied during the first 7 months of life did not influence the development of AD, asthma and AR at school age.

KEYWORDS

asthma, atopic dermatitis, prevention, rhinitis

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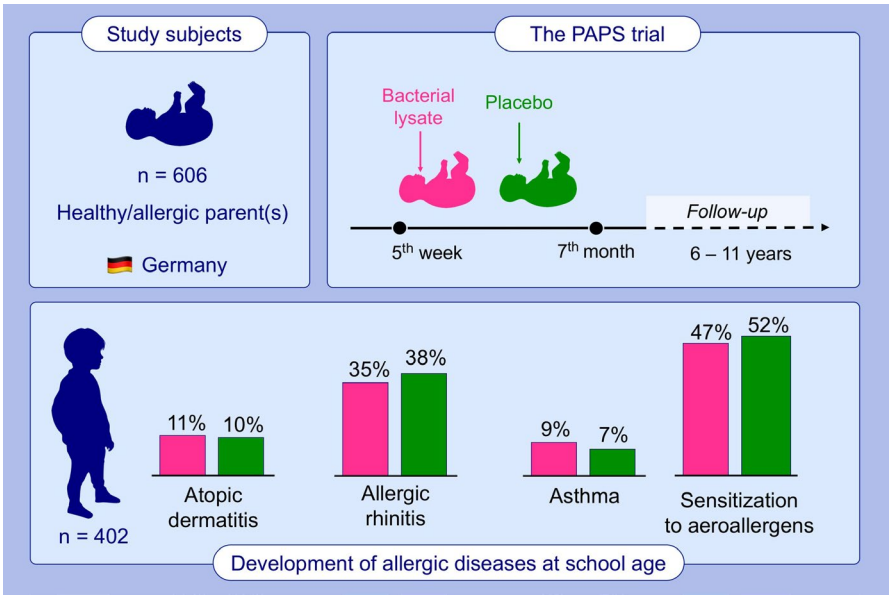

GRAPHICAL ABSTRACT

This study presents data from a large cohort of infants at risk for atopy. Effect of orally applied bacterial lysates during infancy on later allergic manifestations was studied in a long prospective follow-up. The early intervention with a bacterial lysate had no effect on the development of atopic dermatitis, allergic rhinitis, asthma and specific sensitization to aeroallergens at school age.
Abbreviation: PAPS, Pro-Symbioflor® Atopic Dermatitis Prophylaxe bei Säuglingen mit atopischen Eltern

1 | INTRODUCTION

Since epidemiologic studies found a lower prevalence of allergies among children from rural compared with urban background, several protective factors have been suggested. These include prenatal and continuous exposure to life stock thus a diversity of fungi and bacteria, and consumption of unpasteurized farm milk.[1-4] When regularly exposed to microbial compounds in stables during pregnancy such as lipopolysaccharides (LPS) from Gram-negative bacteria, children had significantly less atopic sensitization at school age.[5] Among other factors, LPS has been shown to induce innate toll-like receptors TLR 2 and TLR 4 leading to a protective cytokine shift towards a Th1 response.[5] TLR 2, TLR 4 and TLR 5 expression in infants are inversely associated with the development of AD.[1,5,6] Additionally, results from an atopic mouse model showed protective effects when LPS was given prenatally, allergic sensitization and airway inflammation were less frequent in the sense of a primary prevention.[7] Peptidoglycans of Gram-positive bacteria as another independent marker of microbial exposure were also inversely associated with wheeze.[8] Therefore, a stimulation with a bacterial lysate containing LPS from Gram-negative and cell surface molecules of Gram-positive bacteria seemed a plausible approach for allergy prevention, since immune-modulating effects of decreased specific IgE and IgG, probably via TLR induction, have been shown in animal model.[9]

2 | METHODS

2.1 | Study design and study population

The current analysis was based on a randomized placebo-controlled trial on primary prevention of AD (PAPS) in Berlin, Germany (registration no. ISRCTN60475069, ISRCTN registry Current Controlled Trials UK), which has been described in detail elsewhere.[12] A total of 606 healthy newborns >38 weeks of gestational age weighing ≥2500 g descending from 1 or 2 atopic parents (AD, AR and/or asthma) with informed consent were included in the trial. The current school-age follow-up of the study population (at 6-11 years) took place in 2013 to April 2014. Assessment of parental atopic and asthmatic symptoms was carried out by standardized interview of trained physicians according to the Multicenter Allergy Study (MAS) and the German Infant Nutritional Intervention (GINI) study.[10] Exclusion criteria at inclusion (2002-2008) were antibiotic treatment or other medication after birth, lymphpenia or thrombocytopenia, intensive care after birth, lack of German-language knowledge and no informed consent. The trial was sponsored by Symbiopharm, Herborn, Germany.

2.2 | Description of intervention

Participants received an oral treatment with either a bacterial lysate (Pro-Symbioflor®: heat-killed *Escherichia coli* DSMZ 17 252 and *Enterococcus faecalis* DSMZ 16 440) as active treatment or placebo applied three times daily from 5 weeks until 7 months of life.

2.3 | Outcome assessment

Clinical examination for the development of allergic diseases (AD, AR wheeze/asthma) during the intervention and until the age of 3 years was performed. Primary outcome, the presence of AD at 7 months of life, has previously been reported.[11]

TABLE 1 Baseline characteristics of both treatment groups for children who participated in the school-age follow-up, (n = 402)

Baseline characteristics	Active group (n = 200)	Placebo group (n = 202)
Age of newborns (wk), median (interquartile range)	5 (4.6-5.6)	5 (4.6-5.6)
Birthweight (g), median (interquartile range)	3460 (3120-3745)	3450 (3200-3800)
Proportion of female, n (%)	89 (44.5%)	103 (50.9%)
C-section, n (%)	47 (23.5%)	52 (25.9%)
Maternal smoking, n (%)		
Before pregnancy	40 (20.0%)	44 (21.8%)
During pregnancy	40 (20.0%)	40 (19.8%)
After pregnancy	35 (17.5%)	43 (21.3%)
Atopic family history, n (%)		
Both parents	94 (47.0%)	107 (52.9%)
One of both parents	105 (52.8%)	94 (47.8%)
Mother	45 (22.5%)	55 (27.2%)
Father	60 (30.2%)	39 (19.4%)
Number of siblings, median (interquartile range)	0 (0-1)	0 (0-1)
Day care begin before 24 mo, n (%)	161 (84.7%)	163 (85.8%)
Day care begin after 24 mo, n (%)	29 (15.3%)	27 (14.2%)

The school-age follow-up of the study population included clinical examination, lung function testing, skin prick tests and serum analyses of specific IgE to the most common aeroallergens: house dust mite, dog, cat, mould (alternaria, cladosporium), birch and grass pollen. Data on allergic symptoms were acquired by online questionnaire-based on ISAAC recommendations in order to estimate the prevalence of AD, AR and asthma.[12] The study and follow-up were approved by the hospital's local ethics committee in 2002 and 2012 (application number EA2/023/13). Parents and participants gave written informed consent to participation and this publication.

Current Atopic Dermatitis was defined according to the criteria by Hanifin and Rajka[13] based on a parent-reported doctor's diagnosis in the last 12 months or typical signs or symptoms persisting >6 weeks in the last 12 months.

Current AR was defined when parents reported sneezes and a runny, itchy or stuffed nose without a cold in the last 12 months and/or itchy teared eyes within the last 12 months.

Current Asthma was defined when doctor`s diagnosis was made and any indicative symptom in the last 12 months (wheezing, shortness of breath, norcturnal awakening due to shortness of breath nad/or wheeze) was present.

Sensitization was assessed by skin prick test and serum levels for specific sensitization for allergens house dust mite, dog, cat, alternaria, cladosporium, birch and grass pollen.

2.4 | Statistical methods

We computed descriptive statistics for sociodemographic, lifestyle and medical characteristics using median ± interquartile range (IQR) for continuous variables and absolute and relative frequencies for categorical variables.

Crude risk ratios with 95% asymptotic Wald confidence limits were computed for comparing the risk of AD, asthma and AR between intervention groups. Multivariable Poisson regression was applied for the calculation of adjusted risk ratios (aRR). These analyses were adjusted for sex, atopic parental heredity, duration of breastfeeding, smoking during pregnancy, sensitization to aeroallergens in the first three years, mode of delivery and number of siblings and whether or not the child was attending day care.

3 | RESULTS

A total of 402 participants were followed up at school age. Baseline characteristics were similar between both treatment groups (Table 1). AD was diagnosed in 11.0% (22/200) of children in the active and in 10.4% (21/202) of children in the placebo group (Table 2). AR was diagnosed in 35% (70/200) of children in the active and in 38.1% (77/202) children in the placebo group. Asthma was diagnosed in 9% (18/199) of children in the active and in 6.6% (13/197) of children in the placebo group (Table 2). Allergic sensitization to the most common aeroallergens occurred in 46.5% (66/142) of participants in the active and 51.7% (76/147) in the placebo group (Table 2). Additionally, the duration of breastfeeding was equal between intervention groups (Table 3).

In multivariable analyses adjusting for sex, atopic family history (both parents vs one parent), mode of delivery (C-section vs vaginal), smoking during pregnancy, number of siblings, duration of breastfeeding, allergic sensitization within the first 3 years and attending day care no differences were observed for AD (aRR = 1.00; 95% CI:

TABLE 2 Prevalence of AD (stratified for family history), AR, asthma and allergic sensitization at school age with crude risk ratio estimates and adjusted for sex, atopic family history (both vs one parent), C-section, duration of breastfeeding, smoking during pregnancy, number of siblings, day care and sensitization in first 3 y

	Active	Placebo	RR	(95% CI)	aRR (95% CI)
AD	22/200 (11.0%)	21/202 (10.4%)	1.06	(0.60-1.86)	1.00 (0.87-1.16)
Both parents atopic	16/94 (17.0%)	10/107 (9.4%)	1.82	(0.87-3.82)	
One parent atopic	6/105 (5.7%)	11/94 (11.7%)	0.49	(0.19-1.27)	
Atopic mother	4/45 (8.9%)	8/55 (14.6%)	0.61	(0.20-1.90)	
Atopic father	2/60 (3.3%)	3/39 (7.7%)	0.43	(0.08-2.48)	
AR	70/200 (35.0%)	77/202 (38.1%)	0.92	(0.71-1.19)	0.99 (0.85-1.16)
Asthma	18/199 (9.0%)	13/197 (6.6%)	1.37	(0.69-2.72)	1.01 (0.87-1.17)
Allergic sensitization at school age	66/142 (46.5%)	76/147 (51.7%)	0.90	(0.71-1.14)	0.97 (0.80-1.18)
AD + AR	11/200 (5.5%)	14/202 (6.9%)	0.79	(0.37-1.71)	1.00 (0.87-1.16)
AD + Asthma	5/199 (2.5%)	5/197 (2.5%)	1.0	(0.97-1.03)	1.00 (0.87-1.16)
AR + Asthma	15/199 (7.5%)	10/197 (5.1%)	1.5	(0.68-3.22)	1.01 (0.87-1.17)
AD + sens positive	13/142 (9.2%)	13/147 (8.9%)	1.04	(0.50-2.20)	1.01 (0.85-1.19)
AD + sens negative	4/142 (2.8%)	5/147 (3.4%)	0.83	(0.23-3.02)	1.00 (0.84-1.18)
AR + sens positive	37/142 (26.1%)	47/147 (32.0%)	0.82	(0.57-1.17)	0.99 (0.82-1.18)
AR + sens negative	16/142 (11.3%)	15/147 (10.2%)	1.10	(0.57-2.15)	1.00 (0.84-1.19)
Asthma + sens positive	14/141 (9.9%)	5/145 (3.5%)	2.88	(1.07-7.78)	1.03 (0.87-1.23)
Asthma + sens negative	1/141 (0.7%)	3/145 (2.1%)	0.34	(0.04-3.26)	0.99 (0.83-1.18)
AD + AR + sens positive	7/142 (4.9%)	11/147 (7.5%)	0.66	(0.26-1.65)	0.99 (0.84-1.18)
AD + AR + sens negative	1/142 (0.7%)	1/147 (0.7%)	1.04	(0.07-16.39)	1.00 (0.85-1.18)
AD + Asthma + sens positive	5/141 (3.6%)	4/145 (2.8%)	1.3	(0.35-4.70)	1.01 (0.85-1.19)
AD + Asthma + sens negative	0/141 (0%)	1/145 (0.7%)	—		1.00 (0.84-1.18)
AR + Asthma + sens positive	13/141 (9.2%)	5/145 (3.5%9	2.67	(0.98-7.31)	1.03 (0.87-1.22)
AR + Asthma + sens negative	0/141 (0%)	1/145 (0.7%)	—		1.00 (0.84-1.18)

TABLE 3 Duration of breastfeeding (in weeks) in active and placebo groups

Intervention	N Obs	N	Median	Lower quartile	Upper quartile	Mean	Std Dev
Active	200	200	39.00	29.00	52.00	42.92	27.08
Placebo	202	201	40.00	30.00	54.00	45.50	27.42

0.87-1.17), AR (aRR = 0.99; 95% CI: 0.85-1.15), asthma (aRR = 1.01; 95% CI: 0.87-1.17) and allergic sensitization (aRR = 0.97; 95 CI: 0.80-1.18). Data did not change if we stratified for AD, asthma and AR with proven allergic sensitization versus AD, asthma and AR without sensitization (Table 2).

Assessing the occurrence of comorbidities, no significant differences were observed between treatment groups: AD and AR (aRR = 1.00; 95% CI (0.87-1.16), AD and asthma (aRR = 1.00; 95% CI (0.87-1.15) and AR and asthma (aRR = 1.01; 95% CI (0.87-1.17).

4 | DISCUSSION

The current analyses at school age show that the bacterial lysate applied orally in infancy over a period of 6 months did not influence the development of AD, AR, asthma and aeroallergen sensitization later in life compared to placebo. Potential confounders influencing the crucial immune-modulating gut microbiome like the duration of breastfeeding, rate of C-section, number of siblings and age at entrance to day care were similar in both treatment groups. In a previous publication regarding the incidence of AD in the first year of life during intervention and until the 3rd birthday in the follow-up period of the PAPS cohort, we could show a decreased risk for AD in the subgroup of infants with one atopic parent.[11] However, overall there was no significant difference for the ITT and PP population in the first 3 years of life. This is in contrast to previous studies that suggested preventive effects of probiotics and bacterial lysates regarding the development of AD and sensitization in early childhood.[14-19] Conversely, a recent meta-analysis including 5 RCTs with *Lactobacillus rhamnosus* GG showed no preventive effect for AD.[21] Furthermore, our study complements the results of several systematic reviews examining effects of probiotics showing no benefits regarding any clinical outcome such as asthma, wheeze

and AR.[18-21] An even increased risk for AR under intervention was found in two studies.[22-24] The lack of statistical power to investigate respiratory allergies because of lower prevalence than AD prevalence has been discussed as a limitation in these studies[5] and also that some trials were not primarily focused on asthma and respiratory allergy.[21] In our trial, asthma and respiratory allergy were secondary outcomes, which were monitored prospectively and longitudinally. Although, the cohort included only high-risk children the prevalence of asthma is rather low, while the prevalence of allergic rhinitis and allergic sensitization to aeroallergen is as expected rather high, so that the statistical power seems to be acceptable. One may assume that older children are more likely diagnosed with asthma, possibly missing some asthmatics in the follow-up.[25] Although children were not seen at exactly the same age (6-11 years of age), age distribution was similar in active and placebo groups. Moreover, the Isle of Wight study showed most children who develop asthma are diagnosed already around 6-7 years of age,[26] especially children with persistent wheeze.[27]

It is still a matter of debate if a promising preventive intervention can be achieved regarding questions: when to start, how long to treat, which components, which application and in which dose. Interesting results are to be expected from the German MARTHA trial, introducing only mildly heated raw milk from the 6th month of life on up to 2.5 years of age and the impact on the development of asthma and allergies in early childhood (German Clinical Trials Register DRKS00014781). Consecutively, using an aliment as intervention may put more effort into a healthy nature-related diet to benefit the microbiome with all economic, environmental, social and lifestyle issues related.[15,16]

Due to safety issues, our intervention used a bacterial lysate which was already registered for early infancy in Germany acting as a possible immune-stimulating agent but lacking the ability to colonize, which might have caused a stronger effect.

Our study with sufficient statistical power is so far evidence for not favouring the treatment with this particular bacterial lysate for the prevention of AD and respiratory allergies beyond infancy, especially if the intervention is transient.

5 | CONCLUSION

Applying a bacterial lysate of heat-killed Gram-negative *Escherichia coli* and Gram-positive *Enterococcus faecalis* in early infancy over a period of 6 months does not influence the development of AD, AR, asthma and sensitization at school age.

CONFLICT OF INTEREST STATEMENT

The PAPS trial was sponsored by Symbiopharm, Herborn, from 2002 to 2015 with a grant to Charité. Susanne Lau also received honoraria between 2008 and 2015 from Symbiopharm and from ALK, Allergopharma, Merck, DBV, Boehringer, Nutricia and Sanofi-Genzyme during the last five years. All other authors have no conflict of interest to declare.

ACKNOWLEDGMENT

We thank the participating families, Petra Wagner (study nurse), Thomas Geske (monitor) and Kurt Zimmermann representing the sponsor.

AUTHORS' CONTRIBUTIONS

Susanne Lau is the principal investigator of the PAPS trial, was responsible for the study design of the school follow-up, supervised the clinical investigation and was actively involved in statistical analysis and writing of the manuscript. Thomas Keil supervised the data management and statistical analysis and reviewed the manuscript. Katja Icke and Theresa Keller were performing the data management, clearing of data and the statistical analysis. Siri Roßberg and Valentina Siedmann were study doctors. Siri Roßberg was writing the manuscript as first author and was actively involved in the statistical analysis and interpretation of data. Imke Lau was employed as a student and was actively involved in the clinical follow-up by performing skin prick tests, interviews and in the data management. All authors read and edited the manuscript.

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REFERENCES

1. von Mutius E, Vercelli D. Farm living: effects on childhood asthma and allergy. *Nature Rev Immunol*. 2010;10(12):861-868.
2. Ege MJ, Herzum I, Buchele G, et al. Prenatal exposure to a farm environment modifies atopic sensitization at birth. *J Allergy Clin Immunol*. 2008;122(2):407-412, 12 e1–4.
3. Alfven T, Braun-Fahrlander C, Brunekreef B, et al. Allergic diseases and atopic sensitization in children related to farming and anthroposophic lifestyle–the PARSIFAL study. *Allergy*. 2006;61(4):414-421.
4. Ege MJ. Intestinal microbial diversity in infancy and allergy risk at school age. *J Allergy Clin Immunol*. 2011;128(3):653-654.
5. Ege M, Bieli C, Frei R, et al. Prenatal farm exposure is related to the expression of receptors of the innate immunity and to atopic sensitization in school-age children. *J Allergy Clin Immunol*. 2006;117(4):817-823.
6. Roduit C, Wohlgensinger J, Frei R, et al. Prenatal animal contact and gene expression of innate immunity receptors at birth are associated with atopic dermatitis. *J Allergy Clin Immunol*. 2011;127(1):179-185, 85 e1.
7. Gerhold K, Avagyan A, Seib C, et al. Prenatal initiation of endotoxin airway exposure prevents subsequent allergen-induced sensitization and airway inflammation in mice. *J Allergy Clinic Immunol*. 2006;118(3):666-673.
8. van Strien RT, Engel R, Holst O, et al. Microbial exposure of rural school children, as assessed by levels of N-acetyl-muramic acid in mattress dust, and its association with respiratory health. *J Allergy Clin Immunol*. 2004;113(5):860-867.
9. Ahrens B, Quarcoo D, Buhner S, Matricardi PM, Hamelmann E. Oral administration of bacterial lysates attenuates experimental food allergy. *Int Archives Allergy Immunol*. 2011;156(2):196-204.
10. von Berg A, Koletzko S, Grübl A, et al. The effect of hydrolyzed cow's milk formula for allergy prevention in the first year of life: the German Infant Nutritional Intervention Study, a randomized double-blind trial. *J Allergy Clin Immunol*. 2003;111(3):533-540.

11. Lau S, Gerhold K, Zimmermann K, et al. 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. *J Allergy Clinic Immunol*. 2012;129(4):1040-1047.
12. Asher MI, Keil U, Anderson HR, et al. International Study of Asthma and Allergies in Childhood (ISAAC): rationale and methods. *Eur Respir J*. 1995;8(3):483-491.
13. Hanifin JMRG. Diagnostic features of atopic dermatitis. *Acta Derm Venereol*. 1980;92(suppl):44-47.
14. Osborn DA, Sinn JK. Prebiotics in infants for prevention of allergic disease and food hypersensitivity. *Cochrane Database Syst Rev*. 2007;(4):CD006474. PMID: 17943912.
15. Pfefferle PI, Prescott SL, Kopp M. Microbial influence on tolerance and opportunities for intervention with prebiotics/probiotics and bacterial lysates. *J Allergy Clinic Immunol*. 2013;131(6):1453-1463.
16. West CE, Dzidic M, Prescott SL, Jenmalm MC. Bugging allergy; role of pre-, pro- and synbiotics in allergy prevention. *Allergol Int*. 2017;66(4):529-538.
17. Zhang GQ, Hu HJ, Liu CY, Zhang Q, Shakya S, Li ZY. Probiotics for prevention of atopy and food hypersensitivity in early childhood. *Medicine* 2016;95(8):e2562.
18. Zuccotti G, Meneghin F, Aceti A, et al. Probiotics for prevention of atopic diseases in infants: systematic review and meta-analysis. *Allergy*. 2015;70(11):1356-1371.
19. Cuello-Garcia CA, Brożek JL, Fiocchi A, et al. Probiotics for the prevention of allergy: a systematic review and meta-analysis of randomized controlled trials. *J Allergy Clinic Immunol*. 2015;136(4):952-961.
20. Azad MB, Coneys JG, Kozyrskyj AL, et al. Probiotic supplementation during pregnancy or infancy for the prevention of asthma and wheeze: systematic review and meta-analysis. *BMJ*. 2013;347:f6471.
21. Szajewska H, Horvath A. Lactobacillus rhamnosus GG in the primary prevention of eczema in children: a systematic review and meta-analysis. *Nutrients*. 2018;10(9):1319.
22. Peldan P, Kukkonen AK, Savilahti E, Kuitunen M. Perinatal probiotics decreased eczema up to 10 years of age, but at 5–10 years, allergic rhino-conjunctivitis was increased. *Clin Exp Allergy*. 2017;47(7):975-979.
23. Kalliomaki M, Salminen S, Poussa T, Arvilommi H, Isolauri E. Probiotics and prevention of atopic disease: 4-year follow-up of a randomised placebo-controlled trial. *Lancet*. 2003;361(9372):1869-1871.
24. Kalliomaki M, Salminen S, Poussa T, Isolauri E. Probiotics during the first 7 years of life: a cumulative risk reduction of eczema in a randomized, placebo-controlled trial. *J Allergy Clin Immunol*. 2007;119(4):1019-1021.
25. Grabenhenrich LB, Gough H, Reich A, et al. Early-life determinants of asthma from birth to age 20 years: a German birth cohort study. *The Journal of allergy and clinical immunology*. 2014;133(4):979–988.
26. Scott M, Roberts G, Kurukulaaratchy RJ, Matthews S, Nove A, Arshad SH. Multifaceted allergen avoidance during infancy reduces asthma during childhood with the effect persisting until age 18 years. *Thorax*. 2012;67(12):1046–1051.
27. Oksel C, Granell R, Haider S, Fontanella S, Simpson A, Turner S. Distinguishing Wheezing Phenotypes from Infancy to Adolescence. *A Pooled Analysis of Five Birth Cohorts*. 2019;16(7):868–876.

How to cite this article: Roßberg S, Keller T, Icke K, et al. Orally applied bacterial lysate in infants at risk for atopy does not prevent atopic dermatitis, allergic rhinitis, asthma or allergic sensitization at school age: Follow-up of a randomized trial. *Allergy*. 2020;75:2020–2025. https://doi.org/10.1111/all.14247