

Exposure to endotoxin decreases the risk of atopic eczema in infancy: A cohort study

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Background: Previous studies have shown a protective effect of early exposure to cats and dogs on the development of atopic eczema, asthma, allergic rhinitis, and atopic sensitization in later life. In particular, a higher microbial exposure to endotoxin in early childhood might contribute to this effect.

Objective: We examined the associations between bacterial endotoxin in house dust and atopic eczema, infections, and wheezing during the first year of life in an ongoing birth cohort study (LISA).

Methods: Data of 1884 term and normal-weight neonates with complete information on exposure to biocontaminants and confounding variables were analyzed. House dust from the mothers' and the children's mattresses was sampled 3 months after birth. Endotoxin content was quantified by using a chromogenic kinetic limulus amoebocyte lysate test.

Results: During the first 6 months of life, the risk of atopic eczema was significantly decreased by endotoxin exposure in dust from mothers' mattresses in the fifth quintile (odds ratio [OR], 0.50; 95% CI, 0.28-0.88), whereas the risk was increased for respiratory infections (OR, 1.69; 95% CI, 1.25-2.28) and cough with respiratory infection, bronchitis, or both (OR, 1.73; 95% CI, 1.28-2.33). The risk of wheezing was also significantly increased during the first 6 months of life (OR, 2.37; 95% CI, 1.40-4.03). For the entire first year of life, these associations attenuated, except for the risk of wheezing, which remained significant (OR, 1.60; 95% CI, 1.10-2.30).

Conclusion: Our findings support the hygiene hypothesis that exposure to high concentrations of endotoxin very early in life might protect against the development of atopic eczema within

the first 6 months of life, along with an increased prevalence of nonspecific respiratory diseases. (*J Allergy Clin Immunol* 2001;108:847-54.)

Key words: Endotoxin, house dust, atopic eczema, infections, wheezing, infancy

The prevalence of asthma is increasing worldwide,¹ and large regional differences in asthma prevalence exist.^{2,3} It has been suggested that hygiene standards in modern societies may be partly responsible for the increase of atopic diseases in these populations.⁴⁻⁶ Endotoxin was considered as a potential marker for the hygienic level.^{5,7}

Endotoxins are cell-wall components of the outer membrane of gram-negative bacteria. They are more or less ubiquitous in the environment and present in normal indoor environments as constituents of house dust.⁸⁻¹¹ Inhaled endotoxin can exacerbate airflow obstruction and airway inflammation in individuals with allergic and nonallergic asthma.^{8,12,13} Increased concentrations of house dust endotoxin were found in homes of farming families and in households in which children had regular contact with farm animals¹⁴ and those in which animals were kept indoors.^{7,10,11,15,16} Recently published studies have shown a consistently lower risk of allergic sensitization and hay fever in children who grew up on farms^{17,18} and a protective effect of early exposure to cats and dogs on the development of atopic eczema within the first 6 months of life,¹⁹ as well as the development of atopic sensitization,^{20,21} allergic rhinitis,^{19,20} and asthma²⁰ in later life. It was supposed that the level of environmental exposure to endotoxin and other bacterial wall components early in life might be protective for the development of atopic diseases in childhood.^{5,14,22} The potential of bacterial components to modulate immune reactivity was further demonstrated by Kalliomäki et al,²³ who found that oral administration of probiotics decreased the risk of atopic eczema within the first 2 years of life to one half.

At present, only a few smaller studies can be found in the literature relating objectively measured exposures to endotoxin in house dust to allergies in children. One recent study reported that house dust endotoxin was associated with an increased risk of wheezing during the first year of life for children with a familial predisposition to asthma and allergy.¹¹ Two studies showed an association

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Abbreviation used
OR: Odds ratio

between exposure to endotoxin and exacerbation of asthma.^{9,10} Furthermore, Gereda et al²⁴ found a correlation between house dust endotoxin concentration and IFN- γ -producing T lymphocytes in asthma-prone infants. However, at present, there are no published data describing the role of endotoxin in the development of atopic eczema and infections in the first year of life. We examined the associations between house dust endotoxin, measured 3 months after birth, and atopic eczema, infections, and wheezing during the first year of life using data from an ongoing birth cohort study designed to assess "Influences of Lifestyle-Related Factors on the Immune System and the Development of Allergies in Childhood" (LISA).

METHODS

Subjects and materials

The recruited neonates are from 2 large German cities, Munich (West Germany) and Leipzig (East Germany). Recruitment took place shortly after birth in 6 obstetric clinics in Munich and all 4 obstetric clinics in Leipzig. From December 1997 to January 1999, the target population of the study was defined as newborns from parents who were born in Germany and have German nationality. Neonates fulfilling at least one of the following criteria were excluded from the study: premature birth (maturity at <37 gestational weeks); low birth weight (<2500 g); congenital malformation; symptomatic neonatal infection; antibiotic medication; hospitalization or intensive medical care during the neonatal period; immune-related diseases of the mother, such as autoimmune disorders; diabetes; hepatitis B; long-term medication use; or abuse of drugs or alcohol. The final study population consisted of 2443 neonates (55% of all parents who were contacted and fulfilled the inclusion criteria). Questionnaire-based data were collected for all neonates whose parents consented to participate in the study. House dust from mothers' and children's mattresses was sampled 3 months after birth. Complete data on endotoxin levels and potential confounding variables were available for 1884 of the participants. Thus the final data set for this analysis consisted of data from 1884 children.

The study was approved by the ethics committees of the Landesärztekammer Bavaria and the University of Leipzig and was carried out in accordance with the institutional guidelines for the protection of human subjects. Written informed consent was obtained from all parents of participating children.

Questionnaire data

Parents received a questionnaire at their child's birth and every 6 months thereafter. Data from completed questionnaires comprised information on parental atopy (asthma, hay fever, and/or eczema), parental education, smoking of the mother during pregnancy, breast-feeding, and symptoms and diseases of the child. Data derived from these questionnaires were used for the definition of the primary outcomes, such as physician-diagnosed atopic eczema and respiratory infections, as well as itchy rash, cough, and wheezing.* Data on housing conditions, such as keeping of pets and occupants' behavior, were collected 3 months after birth, the same time the dust samples were taken.

*The exact wording of the questions is given in Appendix 2.

Collection of house dust and extraction

Dust samples were taken from the mothers' and children's mattresses by vacuuming a 1-m² area of mattress surface for 2 minutes. Dust was collected on cellulose filters with ALK sampling devices (ALK Allergen Mouthpiece). Samples were stored at -20°C until extraction. A detailed description of the collection and extraction of house dust is given in the study of Fahlbusch et al.²⁵ No dust sample was taken from mothers' mattresses in cases in which the child did not sleep in the mother's bed (n = 58).

Analysis of endotoxin and allergens in house dust

Endotoxin content was quantified by using a quantitative chromogenic kinetic Limulus amoebocyte lysate test (Kinetic-QCL No. 50-650U; Bio Whittaker; Limulus amoebocyte lysate lot No. 5L3480)⁷ at 37°C. Analyses were performed with an automated microtiter plate reader (anthos ht III, Anthos Labtec Instruments) and WinKQCL 1.1 software. *Escherichia coli* endotoxin (Bio Whittaker, lot No. 5L1570) was used as standard endotoxin. The endotoxin potency of this standard varied between 14 and 16 EU/ng. Diluted samples were initially analyzed in inhibition or enhancement mode, with a dilution rate of 1:1000 being found to be appropriate for further analysis in the routine mode. All measurements were performed in duplicate. Analyses were repeated if the difference between sample duplicates exceeded 10%. The limit of detection was 3.5 ng/g dust.

Analysis of the dust mite allergens *Dermatophagoides pteronyssinus* (Der p 1) and *Dermatophagoides farinae* (Der f 1) and cat allergens (Fel d 1) was done by using a 2-site monoclonal ELISA according to the instructions of the manufacturer (Indoor Biotechnologies).²⁵ The lower limit of detection was 10 ng/g dust for Der p 1 and Der f 1 and 15 ng/g dust for Fel d 1.

Statistical methods

Mean concentrations of endotoxin were expressed as geometric means with a geometric SD. Allergen concentrations below the limit of detection were assigned a value of one half of the detection limit. The Pearson correlation coefficient was used to analyze correlations between endotoxin and allergen levels on the one hand and between endotoxin concentrations in dust from the mothers' mattresses and endotoxin levels in dust from the children's mattresses on the other hand. All correlation analyses were based on natural log-transformed data. Associations between endotoxin concentrations and potential confounding factors were analyzed by means of simple linear regression. Multiple logistic regression analyses were performed to analyze the effect of endotoxin in house dust on symptoms and diseases of the child during the first 6 and 12 months of life, respectively. Because the functional relationship between endotoxin levels and symptoms and diseases is not yet clear, exposure to endotoxin was categorized by using quintiles as cutoff values. We controlled for the influence of potentially confounding factors and other risk factors, such as place of residence, sex, parental atopy, parental education, smoking during the second or third trimester of pregnancy, breast-feeding, keeping of pets, and mite and cat allergens in dust from the mother's mattress, by including them in the regression models. Results are presented as adjusted odds ratios (ORs) with 95% CIs. Statistical significance was defined by a 2-sided α level of 5%. All computations were performed by using the statistical analysis package SAS for Windows version 8.1 (SAS Institute, Cary, NC).

RESULTS

Description of the study population

A description of the study population is given in Table I. Nine percent and 16% of the children had atopic

eczema during the first 6 and 12 months, respectively. Respiratory infections were common, with only 23% of the children not having a physician-diagnosed infection during the first year of life. Fifteen percent of the parents reported more than 3 respiratory infections during the first year of life (data not shown). During the first 6 months of life, 9% of the parents reported wheezing, and at 12 months, the number increased to 21%. Wheezing apart from respiratory infection was rare. In 28% of homes, pets were kept when the child was 3 months old. More than 50% of children had a positive parental history of atopy. Parental education is high (ie, in 72% father, mother, or both have completed >12 grades).

Exposure to endotoxin and allergens

Dust samples from the mothers' mattresses were available for all 1884 children. Eight of the respective children's samples were not available, mainly because of insufficient handling in the laboratory. Endotoxin levels in the dust from the mothers' mattresses ranged from 57 to 1,286,197 EU/g dust, with a geometric mean of 2,910 EU/g dust (geometric SD, 4.2 EU/g). Mite (sum of Der p 1 and Der f 1) and cat allergen levels ranged from below the limit of detection (15% and 12% of the homes, respectively) to 588,624 and 23,702,481 ng/g dust, with geometric means of 1,040 ng/g dust (geometric SD, 13.6 ng/g dust) and 305 ng/g dust (geometric SD, 14.6 ng/g dust), respectively. Correlations between endotoxin and mite ($r = 0.19$) and cat ($r = 0.13$) allergen concentrations were weak but statistically significant ($n = 1884$, $P < .001$). A similar correlation was found for mite and cat allergens ($n = 1884$, $r = 0.12$, $P < .001$).

A substantial correlation was found between endotoxin levels in dust from the mothers' mattresses and endotoxin levels in dust from the children's mattresses ($r = 0.45$, $n = 1876$, $P < .0001$). However, 47% of the children's mattresses were new. This results in much higher amounts of dust sampled from the mothers' mattresses compared with that from the children's mattresses (geometric mean 0.68 g [geometric SD, 2.19 g] vs 0.19 g [geometric SD, 2.09 g]). Extrapolation from these very low amounts of dust resulted in rather high endotoxin levels, ranging from 73 to 690,828 EU/g dust, with a geometric mean of 5523 EU/g dust (geometric SD, 3.8 EU/g dust). Correlations with mite and cat allergens were comparable with those in dust from mothers' mattresses ($r = 0.24$ and $r = 0.11$ [$n = 1876$], respectively).

Endotoxin and confounding variables

Endotoxin concentrations in dust from the mothers' and the children's mattresses related to potentially confounding variables are presented in Table II. Endotoxin levels in dust from the mothers' mattresses were found to be significantly increased in homes where dogs and cats were kept and marginally increased in homes where rabbits, guinea pigs, hamsters, or other pets were kept. Endotoxin levels in dust from the children's mattresses were found to be increased in Leipzig, in homes of parents without a history of atopy, and in homes in which dogs were kept.

TABLE I. Description of the study population

Variable	Frequency
	n/N (%)
Age of 6 mo	
Dermatologic symptoms and diseases*	
Physician-diagnosed atopic eczema	169/1868 (9.1)
Itchy rash	117/1858 (6.3)
Respiratory symptoms and diseases*	
Physician-diagnosed respiratory infection	755/1870 (40.4)
Cough with respiratory infection, bronchitis, or both	862/1866 (46.2)
Wheezing	168/1852 (9.1)
Wheezing apart from respiratory infection	31/1847 (1.7)
Age of 12 mo	
Dermatologic symptoms and diseases*	
Physician-diagnosed atopic eczema	287/1809 (15.9)
Itchy rash	186/1731 (10.8)
Respiratory symptoms and diseases*	
Physician-diagnosed respiratory infection	1415/1841 (76.9)
Cough with respiratory infection, bronchitis, or both	1471/1850 (79.5)
Wheezing	373/1806 (20.7)
Wheezing apart from respiratory infection	56/1750 (3.2)
Keeping of pets (3 mo)	
Any pet	521/1884 (27.7)
Dog	117/1884 (6.2)
Cat	178/1884 (9.5)
Rabbit, guinea pig, and/or hamster	120/1884 (6.4)
Others (eg, fish and birds)	139/1884 (7.4)
Place of residence	
Munich	1215/1884 (64.5)
Leipzig	669/1884 (35.5)
Female sex	919/1884 (48.8)
Positive parental history of atopy	1045/1884 (55.5)
Parental education	
<12 grades	528/1884 (28.2)
≥12 grades	1353/1884 (71.8)
Smoking during pregnancy	171/1884 (9.1)
Exclusively breast-fed for at least 4 months	1137/1884 (60.4)

*Lifetime prevalence.

Associations between endotoxin, atopic symptoms and diseases, and infections

Crude ORs and the prevalence of symptoms and diseases during the first 6 and 12 months of life related to endotoxin concentrations in the dust from the mothers' mattresses are presented in Table III. The number of children for whom wheezing apart from respiratory infection was reported for the first year of life varied among the 5 groups, ranging from 9 to 17 (data not shown). Because of these small numbers, we decided not to analyze associations between exposure to endotoxin and wheezing apart from respiratory infection.

Results of the multiple logistic regression analyses are presented in Table IV. Adjusted ORs differ in most cases only slightly from the crude ORs presented in Table III, indicating that the observed effects were not confounded by place of residence, sex, parental atopy, parental education, smoking during pregnancy, breast-feeding, the

TABLE II. Associations between endotoxin concentrations in dust from the mothers' and children's mattresses and potentially confounding variables

	Endotoxin (EU/g dust)							
	Mothers' mattresses				Children's mattresses			
	n	GM	GSD	P value*	n	GM	GSD	P value*
Place of residence								
Munich	1215	2914	4.1		1212	5045	4.0	
Leipzig	669	2903	4.5	.9584	664	6517	3.4	<.0001
Positive parental history of atopy								
No	1054	3128	4.3		832	6267	3.7	
Yes	830	2746	4.1	.0511	1044	4995	3.9	.0003
Parental education								
<12 grades	528	2782	4.6		527	5505	3.4	
≥12 grades	1353	2962	4.1	.3969	1349	5530	4.0	.9471
Keeping of pets (3 mo)								
Dog								
No	1767	2826	4.2		1760	5374	3.8	
Yes	117	4527	4.3	.0006	116	8384	3.6	.0005
Cat								
No	1706	2847	4.3		1700	5561	3.9	
Yes	178	3590	4.0	.0411	176	5713	3.7	.4966
Rabbit, guinea pig, or both								
No	1764	2866	4.2		1756	5496	3.8	
Yes	120	3635	4.1	.0808	120	5946	3.9	.5344
Others (eg, fish and birds)								
No	1742	2963	4.2		1738	5551	3.8	
Yes	139	2316	4.5	.0526	138	5189	3.9	.5705

GM, Geometric mean; GSD, geometric SD.

**t* test on the basis of log-transformed data.

keeping of pets, or exposure to mite and cat allergens in dust from the mothers' mattresses. For atopic eczema during the first 6 months of life, the risk was significantly decreased in the fifth quintile (OR, 0.50; 95% CI, 0.28-0.88). The risk of physician-diagnosed respiratory infections and cough with respiratory infection, bronchitis, or both was increased in all quintiles compared with in the first quintile, whereas the risk of wheezing during the first 6 months of life was significantly increased in the fourth and fifth quintiles (OR, 1.73; 95% CI, 1.00-2.99 vs OR, 2.37; 95% CI, 1.40-4.03, respectively). For the entire first year of life, these associations attenuated. The risk of wheezing remained as a statistically significant increase in the fifth quintile (OR, 1.60; 95% CI, 1.10-2.30). No association was found between endotoxin concentrations in the dust from the children's mattresses and atopic eczema and respiratory symptoms, neither for the first 6 months nor for the entire first year of life (data not shown). The only exception was cough with respiratory infection, bronchitis, or both in the first 6 months, which was significantly increased by exposure to endotoxin in the third, fourth, and fifth quintiles (OR, 1.62; 95% CI, 1.21-2.18 vs OR, 1.79; 95% CI, 1.33-2.41 vs OR, 1.58; 95% CI, 1.17-2.12, respectively).

The risk of atopic eczema in the first 6 months was found to be significantly decreased for children whose parents reported respiratory infections and cough with respiratory infections, bronchitis, or both. Therefore we looked at the combined effects of exposure to endotoxin and res-

piratory infections on the development of atopic eczema. Interactions between both respiratory infections and cough with respiratory infection and exposure to endotoxin were not statistically significant (data not shown).

DISCUSSION

Endotoxins are known to have strong immunostimulatory and proinflammatory properties. They are believed to promote asthma by inducing airway inflammation on the one hand but to have protective effects on atopy development by activating T_H1 cell responses on the other hand. Our study demonstrates for the first time that exposure to high concentrations of endotoxin is protective with respect to atopic eczema during the first 6 months of life. The observed effect was not confounded by place of residence, sex, parental atopy, parental education, smoking during pregnancy, breast-feeding, the keeping of pets, or exposure to mite and cat allergens.

Associations among endotoxin, atopic symptoms and diseases, and infections

Our results support the hypothesis that the timing of exposure to endotoxin is important. Tulic et al²⁶ showed, in an animal model, that exposure to bacterial LPS immediately before or shortly after primary allergen sensitization inhibits the increase of allergen-specific IgE levels, which is in contrast to the exacerbating effects of LPS exposure several days after allergen challenge on the allergic response.

TABLE III. Crude associations between symptoms and diseases and endotoxin concentrations in dust from the mothers' mattresses

	1st quintile, ≤824 EU/g dust		2nd quintile, 824-1973 EU/g dust		3rd quintile, 1973-4365 EU/g dust		4th quintile, 4365-10,163 EU/g dust		5th quintile, >10,163 EU/g dust	
	n/N (%)	OR	n/N (%)	OR	n/N (%)	OR	n/N (%)	OR	n/N (%)	OR
Age of 6 mo										
Dermatologic symptoms and diseases										
Physician-diagnosed atopic eczema	39/373 (10.5)	1	42/375 (11.2)	1.08	36/371 (9.7)	0.92	32/372 (8.6)	0.81	20/377 (5.3)	0.48
Itchy rash	23/370 (6.2)	1	20/374 (5.3)	0.85	26/370 (7.0)	1.14	26/372 (7.0)	1.13	22/372 (5.9)	0.95
Respiratory symptoms and diseases										
Physician-diagnosed respiratory infection	122/372 (32.8)	1	152/374 (40.6)	1.40	160/374 (42.8)	1.53	153/375 (40.8)	1.41	168/375 (44.8)	1.66
Cough with respiratory infection, bronchitis, or both	138/374 (36.9)	1	178/375 (47.5)	1.55	176/375 (46.9)	1.51	187/369 (50.7)	1.76	183/373 (49.1)	1.65
Wheezing	24/372 (6.5)	1	34/373 (9.1)	1.45	28/368 (7.6)	1.19	36/369 (9.8)	1.57	46/370 (12.4)	2.06
Age of 12 mo										
Dermatologic symptoms and diseases										
Physician-diagnosed atopic eczema	57/367 (15.5)	1	65/368 (17.7)	1.17	58/355 (16.3)	1.06	60/364 (16.5)	1.07	47/353 (13.3)	0.84
Itchy rash	35/357 (9.8)	1	34/355 (9.6)	0.97	38/343 (11.1)	1.15	44/345 (12.8)	1.34	35/331 (10.6)	1.09
Respiratory symptoms and diseases										
Physician-diagnosed respiratory infection	288/370 (77.8)	1	276/371 (74.4)	0.83	288/368 (78.3)	1.02	283/367 (77.1)	0.96	280/365 (76.7)	0.94
Cough with respiratory infection, bronchitis, or both	287/372 (77.2)	1	287/375 (76.5)	0.97	306/371 (82.5)	1.39	302/369 (81.8)	1.33	289/363 (79.6)	1.16
Wheezing	66/368 (17.9)	1	77/366 (21.0)	1.22	66/360 (18.3)	1.03	77/361 (21.3)	1.24	87/351 (24.8)	1.51

The highest incidence of atopic dermatitis is observed in the first 3 months of life,²⁷⁻²⁹ during the time of first contact with environmental allergens. Our results indicate that endotoxin exposure concomitant with first allergen contact could result in lower proportions of atopic eczema. Moreover, atopic dermatitis within the first 3 months of life seems to be a risk factor for sensitization to inhalant allergens during childhood.³⁰⁻³² Therefore we might speculate that exposure to elevated concentrations of house dust endotoxin decreases the risk of atopic sensitization in later life. Whether there are specific consequences for the development of asthma is not known.

We found exposure to elevated concentrations of endotoxin to be associated with an increased risk of physician-diagnosed respiratory infections and cough with respiratory infection, bronchitis, or both during the first 6 months of life. Illi et al³³ suggested that repeated viral infections early in life may stimulate the immature immune system toward the T_H1 phenotype, thereby reducing the risk of asthma and atopy. Therefore we might speculate that exposure to high concentrations of endotoxin early in life, as a proxy for a higher bacterial load and an increased risk of infections, could stimulate the immature immune system toward the T_H1 phenotype and protect against atopy in addition to the probable direct immunostimulatory effects of endotoxin itself.

The results of the present study are in accordance with the findings of Gereda et al,²⁴ who speculated that indoor exposure to endotoxins early in life might promote T_H1 cell differentiation, which might minimize the risk of allergen sensitization. Our findings suggest that children growing up in very hygienic conditions might have an increased risk of atopy. It has been suggested that hygiene standards in modern societies may be partly responsible for the increase in atopic diseases in these populations.^{4,6} Several studies reported increased concentrations of endotoxin in homes in which animals were kept indoors.^{7,10,11,15,16} The association between the presence of pets and increased levels of house dust endotoxin could be confirmed by the present study, in which endotoxin concentrations were found to be significantly increased in homes in which dogs and cats were kept.

At present, only a few studies can be found in the literature relating objectively measured exposures of house dust endotoxin to respiratory health in children. Two studies could show that exposure to endotoxin is associated with an exacerbation of asthma.^{9,10} Douwes et al¹⁰ found peak expiratory flow variability significantly increased with increasing endotoxin levels in atopic and atopic symptomatic children in unadjusted, but not in adjusted, analyses. Rizzo et al⁹ found exposure to endotoxin to be associated with an exacerbation of asthmatic

TABLE IV. Multiple logistic regression between symptoms and diseases and endotoxin in dust from the mothers' mattresses*

	1st quintile, ≤824 EU/g dust	2nd quintile, 824-1973 EU/g dust		3rd quintile, 1973-4365 EU/g dust		4th quintile, 4365-10,163 EU/g dust		5th quintile >10,163 EU/g dust	
	OR	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
Age of 6 mo									
Dermatologic symptoms and diseases									
Physician-diagnosed atopic eczema	1	1.09	0.68-1.74	0.96	0.59-1.56	0.83	0.51-1.37	0.50†	0.28-0.88
Itchy rash	1	0.88	0.47-1.64	1.22	0.68-2.19	1.23	0.68-2.21	1.03	0.56-1.92
Respiratory symptoms and diseases									
Physician-diagnosed respiratory infection	1	1.42‡	1.05-1.92	1.55‡	1.15-2.09	1.43‡	1.06-1.93	1.69‡	1.25-2.28
Cough with respiratory infection, bronchitis, or both	1	1.59‡	1.19-2.14	1.55‡	1.15-2.08	1.83‡	1.36-2.46	1.73‡	1.28-2.33
Wheezing	1	1.58	0.91-2.73	1.27	0.72-2.26	1.73‡	1.00-2.99	2.37‡	1.40-4.03
Age of 12 mo									
Dermatologic symptoms and diseases									
Physician-diagnosed atopic eczema	1	1.17	0.79-1.74	1.10	0.74-1.65	1.13	0.76-1.68	0.87	0.57-1.32
Itchy rash	1	1.01	0.61-1.68	1.24	0.76-2.03	1.44	0.89-2.33	1.19	0.71-1.97
Respiratory symptoms and diseases									
Physician-diagnosed respiratory infection	1	0.83	0.59-1.17	1.03	0.72-1.46	0.98	0.69-1.39	0.95	0.67-1.35
Cough with respiratory infection, bronchitis, or both	1	0.96	0.68-1.36	1.40	0.97-2.01	1.38	0.96-1.98	1.17	0.82-1.68
Wheezing	1	1.25	0.87-1.81	1.06	0.73-1.55	1.31	0.90-1.89	1.60†	1.10-2.30

*ORs are adjusted for place of residence, sex, parental atopy, parental education, smoking during pregnancy, breast-feeding, keeping of pets (dog, cat, hamster, rabbit, guinea pig, and others), and mite (Der p 1 + Der f 1) and cat allergens in dust from mothers' mattresses.

† $P < .05$.

‡ $P < .01$.

symptoms in asthmatic children with mite allergy. Park et al¹¹ recently showed that exposure to elevated concentrations of endotoxin is associated with an increased risk of wheezing during the first year of life for children with a familial predisposition to asthma and allergy, indicating that exposure to endotoxin might induce asthma. The findings of Park et al¹¹ could be confirmed by the present study for a cohort of infants with and without parental history of atopy. For both children with and without a parental history of atopy, the risk of wheezing during the first 12 months of life was increased by exposure to endotoxin in the fifth quintile, but effect estimates were somewhat higher for children with a parental history of atopy (OR, 1.72; 95% CI, 1.06-2.80 vs OR, 1.44; 95% CI, 0.82-2.55). Many infants have episodes of wheezing with viral respiratory illnesses,³⁴ but the majority of infants with wheezing before 3 years of age do not have increased risks of asthma and allergies in later life.³⁵ In the present study not only the risk of wheezing (which is associated with cold in many cases) but also the risk of respiratory infections and cough with respiratory infection, bronchitis, or both was found to increase with increasing exposure to endotoxin. Because the children of the present study are very young and differentiation between wheezing-associated respiratory illness and virus-associated asthma attacks is very difficult in infants of this age,³⁶ the question of whether exposure to endotoxin is associated with the development of asthma cannot as yet be answered.

Mothers' versus children's mattresses

Associations with atopic eczema, respiratory infections, cough, and wheeze were found for endotoxin concentrations in dust from the mothers' mattresses, whereas hardly any significant association was found with endotoxin concentrations in dust from the children's mattresses. We do not have a good explanation for this discrepancy, but we can speculate about potential reasons.

In principle, we consider exposure to endotoxin in the children's mattresses to be of major importance because the infants come into very close contact with endotoxin in their beds. But in taking dust samples from these very young children's mattresses, some serious problems were encountered. Approximately half the children's mattresses were new, and consequently, the amount of dust sampled was very low. Extrapolation from these low amounts of dust to concentrations per gram of dust might be associated with a substantial error, but excluding all new children's mattresses did not change the effect estimates substantially.

Alternatively, concentrations could have been expressed per square meter of mattress surface. However, dust sampling could not be done as highly standardized as necessary because (1) in many cases the mattress surface area was less than 1 m², (2) in some cases dust was taken from other locations than the bed in which the infant slept most frequently (eg, from baby carriages), and (3) in some cases dust was taken from different types

of surfaces on which the child slept (eg, furs). Using concentrations per square meter yielded results very similar to those for concentrations per gram of dust.

Because of these problems, we assumed endotoxin measured in dust from the mothers' mattresses to be a better estimate of exposure to endotoxins in the home environment of the child. This approach is justified by the fact that a dust sample from the mothers' mattresses was taken only when the child was sleeping (at least occasionally) in his or her mother's bed.

Furthermore, one might speculate that prenatal exposure to endotoxin is of major importance. Endotoxin measured in dust from the mothers' mattresses 3 months after birth can be assumed to represent prenatal exposure to endotoxin, which may affect the development of the foetal immune responses prenatally.

Strengths and possible limitations of the study

This study is the first large-scale study relating objectively measured house dust endotoxin concentrations to allergies and infections in infancy. Reporting bias is likely to occur as a result of awareness of exposure. In the present study, however, parents were not informed about the house dust endotoxin levels. Thus this could not have influenced the reporting of symptoms and diseases. Response bias is another source of error. Response bias is likely to occur as a result of a systematic drop out of, for example, nonatopic parents, who might be less interested in the study protocol. In this study proportions of nonatopic parents did not differ substantially among all 2443 included families and the 1884 families included in the analyses (51% vs 56%).

Wheezing apart from respiratory infection occurs rarely during the first year of life, and only a minority of the infants with wheezing before 3 years of age have an increased risk of asthma and allergies in later life. Therefore at present, the children of our cohort are too young to permit the drawing of any conclusions about exposure to endotoxin and a possible predisposition to asthma.

Conclusion

Children exposed to elevated concentrations of endotoxin in house dust were found to have a decreased risk of atopic eczema in the first 6 months of life and an increased risk of respiratory infections. The risk of wheezing during the first year of life was also significantly increased. Our findings support the hygiene hypothesis that exposure to high concentrations of endotoxin very early in life might protect against the development of atopic eczema, along with an increased prevalence of nonspecific respiratory diseases.

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APPENDIX 1.

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APPENDIX 2.

Questions used for the definition of primary outcomes

Outcome variable	Question
Physician-diagnosed atopic eczema	Did a physician diagnose an allergic or atopic eczema in your child in the past 6 months?
Itchy rash	Has your child had a rash (other than diaper rash) in the past 6 months? If yes, was it recurrent and associated with itchiness?
Physician-diagnosed respiratory infections	Did a physician diagnose an infection of the respiratory tract (cold) in your child in the past 6 months?
Cough with respiratory infection, bronchitis, or both	Has your child had a cough in connection with a cold or bronchitis at any time in the past 6 months?
Wheezing	Has your child had wheezing or whistling in the chest at any time in the past 6 months?
Wheezing apart from respiratory infection	Has your child had wheezing or whistling in the chest apart from cold at any time in the past 6 months?