

Early endotoxin exposure and atopy development in infants: results of a birth cohort study

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Summary

Background Exposure to endotoxin in childhood is currently discussed to protect from the development of allergic diseases.

Objective To study the effect of early endotoxin exposure on incidence of atopic sensitization, atopic dermatitis and wheezing until the age of 2 years in infants with different risk status in terms of parental atopy.

Methods Data of 1942 infants of an ongoing birth cohort study were analysed by logistic regression. Endotoxin was measured in settled dust of the mothers' mattresses at infants' age of 3 months. Data on allergic symptoms and physicians' diagnoses were gathered by questionnaire. Sensitization to common food and inhalant allergens was assessed by specific serum IgE.

Results High endotoxin levels increased the risk of repeated wheeze [adjusted odds ratio (OR) for 4th exposure quartile (Q_4) 1.52, 95% confidence interval (CI) 1.08–2.14], but were associated with neither sensitization to food allergens nor atopic dermatitis. Stratification by parental atopy showed that there was an association of endotoxin exposure with incidence of repeated wheeze as well as with sensitization to inhalant allergens (P for trend = 0.008 and 0.044, respectively) only in infants with parental atopy, with the highest risk in the 4th exposure quartile (repeated wheeze: OR_{Q_4} 1.77, 95% CI 1.14–2.73; sensitization to inhalant allergens: OR_{Q_4} 1.69, 95% CI 0.70–4.11).

Conclusion Early endotoxin exposure in terms of mattress dust endotoxin levels seemed to increase the risk of atopic reactions to inhalant allergens at the age of 2 years, especially in infants at risk due to parental atopy. Our data disagree with an early protective effect of endotoxin on atopy development until the age of 2 years.

Keywords allergy, asthma, hygiene hypothesis

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Introduction

Studies on the relationship between living in a farm environment and keeping a pet and atopy development in children suggested that exposure to microbial compounds, especially endotoxin, might confer a protective effect [1–5]. Endotoxin, a lipopolysaccharide and cell-wall component of Gram-negative bacteria, is known to induce T helper 1 (Th1) cell development and production of IFN- γ and IL-12 [reviewed in 6]. So far, only a few studies have investigated the effect of endotoxin in house dust on children's health [7–12]. In accordance with the well-known detrimental effects of endotoxin exposure on respiratory health of adults in occupational environments [e.g. 13, 14], elevated endotoxin levels in house dust increased the risk of repeated wheeze during the first year of life in infants with a

parental history of asthma or allergy [7] and also transiently increased the risk of ever wheeze and repeated wheeze in their elder siblings [10]. Moreover, endotoxin exposure exacerbated asthmatic symptoms in mite-sensitized children with asthma [8]. On the other hand, a recent cross-sectional study in rural areas demonstrated a negative association between endotoxin dust levels and the prevalence of hayfever and atopic asthma in children aged 6–13 years [11].

To date, three cross-sectional studies have suggested that endotoxin exposure may protect from atopic sensitization in children [9, 11, 12]. In the small study with asthma-prone infants by Gereda and co-workers [9], allergen-sensitized infants had lower endotoxin levels in house dust than skin test-negative infants. In addition, endotoxin concentrations correlated with the proportion of Th1 cells. Two studies investigating children aged between 5 and 13 years showed a tendency for negative associations between endotoxin exposure and atopic sensitization defined as specific IgE ≥ 3.5 kU/L [odds ratio (OR) 0.83, 95% confidence interval (CI) 0.63–1.09 [11] and OR 0.82, 95% CI 0.68–0.98 [12], respectively].

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The populations of those studies investigating early endotoxin effects in infants up to 2 years were restricted to children at risk, defined either by parental history of allergies and/or asthma or by high-risk features for asthma development such as previous wheezing episodes and low socioeconomic status [7, 9].

Recent studies supported the hypothesis that genetic variations in the regulation of the receptor system for endotoxin, CD14, may affect endotoxin responsiveness of individuals. A polymorphism in the CD14 gene has been discussed to be associated with serum IgE levels and skin test reactivity to allergens [15]. Thus, individual susceptibility to endotoxin exposure may differ and the relationship between a certain genetic background and endotoxin exposure may represent a model for gene–environment interaction in the development of allergies in children [16].

On this basis, we aimed to disentangle the mixed effects of being at risk due to parental atopy and of early exposure to endotoxin on atopy development in infants. Therefore we studied the relationship between endotoxin exposure during the first months of life and the incidence of atopic sensitization, repeated wheeze and atopic dermatitis until the age of 2 years in a population-based birth cohort, comprising both infants having parents with and without a history of asthma or allergies.

In a preliminary analysis of our cohort considering the first year of life, we found that high endotoxin levels were associated, on one hand, with an increased risk of wheezing during the first 12 months of life and, on the other hand, with a decreased risk of physician-diagnosed, not further specified eczema during the first 6 months of life [17]. The risk of atopic dermatitis was not altered at that age. To our knowledge we are the first to report prospective data on the association of endotoxin dust levels and atopic sensitization in infants until the age of 2 years.

Methods

Subjects

In the ongoing birth cohort study on 'Influences of lifestyle-related factors on the immune system and the development of allergies in childhood' (LISA), 2443 healthy neonates were enrolled in Munich and Leipzig, Germany. From birth until the age of 2 years, data on the infants' health, living conditions and socio-demographic characteristics were gathered by means of questionnaires. In 2108 families (86%), settled house dust on the mothers' mattresses was collected for endotoxin analysis at the infants' age of 3 months. All infants slept at least occasionally in their mothers' beds. At the age of 2 years, blood of 1718 infants (70%) was drawn for serologic analysis. Complete data on endotoxin levels and potential confounding variables for analysis of incident wheezing and atopic dermatitis defined by questionnaire data were available for 1942 infants (79% of the initial cohort). For analysis of atopic sensitization, data for 1553 infants (64%) were available.

The study was approved by the ethics committees of the Landesaerztekammer Bayern (Bavarian General Medical Council) and the University of Leipzig and carried out in accordance with the institutional guidelines for the protection of human subjects. Written informed consent was obtained from all parents of participating infants.

Dust collection and endotoxin analysis

Endotoxin levels in mattress dust were used as proxy for endotoxin exposure. Samples of settled house dust were obtained by vacuuming a 1-m² surface of the mothers' mattresses for 2 min. Dust was collected on cellulose filters with ALK sampling devices. Details of the dust extraction are given elsewhere [18]. Endotoxin concentrations in dust samples were quantified using the chromogenic kinetic Limulus amoebocyte lysate (LAL) test as described before [5]. Values were expressed as endotoxin units (EU) per g dust. All samples were above the detection limit of the assay.

Atopic sensitization

Specific serum IgE to food and inhalant allergens was determined by the RAST–FEIA–CAP system (Pharmacia, Freiburg, Germany). Atopic sensitization to food allergens (egg, cow's milk, wheat, peanut, soy bean, codfish) and to inhalant allergens (house dust mites *Dermatophagoides pteronyssinus* and *D. farinae*, cockroach, cat, mixed grasses, birch pollen, mixed moulds) was defined by a specific IgE value of at least 0.35 kU/L (CAP class ≥ 1).

Atopic dermatitis and respiratory disorders

Questionnaire data were used to assess the incidence of atopic dermatitis and respiratory disorders during the first 2 years of life. For the definition of atopic dermatitis, the parents' reports of symptoms (itchy rash lasting at least 14 days or with recurrent incidence) and physician's diagnosis were applied. Repeated wheeze was defined as having had at least two episodes of wheezing. Furthermore, physician's diagnosis of asthma was used in the analysis.

Statistical analysis

Differences in incidences between girls and boys and between infants with and without parental atopy were assessed by χ^2 test of independence or Fisher's exact test. Data on endotoxin levels are presented as geometric means with 95% CI. Multiple logistic regression models were used to determine adjusted OR with 95% CI for the association between endotoxin exposure and health outcomes. Endotoxin levels were categorized into quartiles and included as dummy variables in the models with the lowest quartile of exposure as reference. OR were adjusted for gender, study region, breastfeeding, elder siblings, parental education, mite and cat allergen levels, frequent respiratory infections and smoking during pregnancy. Trend tests were applied to detect dose–response relations. The Cochran–Armitage test for trend was used for proportions. For analysing trends in multiple logistic regression models, endotoxin exposure quartiles were included as an ordinal variable into the models. Multivariate analyses were stratified by parental atopy to reveal effect modification. The statistical software package SAS for Windows version 8.2 (SAS Institute, Cary, NC, USA) was used for all calculations.

Results

Characteristics of study subjects

The study population comprised 949 girls (49%) and 993 boys (51%) from the two study regions Munich ($n = 1266$, 65%) and

Leipzig ($n = 676$, 35%). The majority of the infants were breast-fed during the first 6 months of life ($n = 1144$, 59%) and had no siblings ($n = 1062$, 55%). Parental atopy defined by a history of asthma, hay fever or atopic dermatitis was reported for 1073 infants (55%); 784 infants (40%) had one affected parent and 289 infants (15%) had both parents being atopic.

Endotoxin exposure

Endotoxin levels ranged from 57 to 1 286 179 EU/g settled mattress dust (interquartile range 1029–7752 EU/g) with a geometric mean of 2893 EU/g (95% CI 2715–3084 EU/g). In homes of atopic parents, endotoxin levels were slightly lower compared to homes of parents without atopic diseases [geometric mean 2765 EU/g (95% CI 2541–3009) versus 3060 EU/g (95% CI 2777–3372)].

Incidence of atopic sensitization and manifestations

Until the age of 2 years, 22% of the infants showed symptoms of atopic dermatitis and 19% had physician-diagnosed atopic dermatitis (Table 1). Repeated wheeze (at least two episodes of wheezing) occurred in 20% of the infants. Physician's diagnosis of asthma was quite rare (0.4%) during the first 2 years of life. At the age of 2 years, 186 infants (12%) were sensitized to any allergen tested (Table 2). Sensitization to food allergens predominated compared to inhalant allergens. Among the 144 infants with specific IgE to food allergens, 81 reacted against egg, 82 against cow's milk and 27 against peanut. Out of the 70 infants reacting to inhalant allergens, 28 had specific IgE to

D. pteronyssinus, 25 to *D. farinae*, 20 to cat allergen, 25 to mixed grasses and birch pollen and nine to mixed moulds.

Incidences of atopic dermatitis, repeated wheeze and atopic sensitization were higher in those infants being at risk due to parental atopy. Boys had higher incidences of repeated wheeze and atopic sensitization, whereas girls had a slightly higher incidence of symptoms of atopic dermatitis (Tables 1 and 2).

Effect of endotoxin exposure on the risk of atopy development

We analysed the relation of endotoxin levels in mattress dust and atopy development in infants by categorizing the exposure into quartiles. Exposure to endotoxin was not associated with incidence of atopic dermatitis until the age of 2 years (Table 3). In contrast, there was a positive relationship between endotoxin exposure $\geq$ 2nd quartile and the risk of repeated wheeze (P for trend = 0.03), with the highest risk of repeated wheeze in the 4th quartile of endotoxin exposure (OR_{Q4} 1.52, 95% CI 1.08–2.14).

Atopic sensitization to any allergen tested or to food allergens was not influenced by endotoxin levels (Table 3). Risk estimates of sensitization to food allergens tended to decrease with elevated endotoxin concentrations (OR_{Q4} 0.66, 95% CI 0.40–1.10) in contrast to risk estimates of sensitization to inhalant allergens (OR_{Q4} 1.36, 95% CI 0.65–2.83).

Influence of parental atopy

To assess further whether risk status of the infants due to parental atopy modified the effect of endotoxin exposure, we

Table 1. Incidence of atopic dermatitis, repeated wheeze and asthma until the age of 2 years [% (n)]

	Atopic dermatitis				Respiratory disorders			
	Symptoms*	P -value§	Physician-diagnosed	P -value§	Repeated wheeze†	P -value§	Physician-diagnosed asthma	P -value§
All ($n = 1942$)	22.3% (403)		19.3% (358)		19.5% (378)		0.4% (8)	
Gender								
Boys ($n = 993$)	20.7% (191)	0.067	18.8% (179)	0.626	21.9% (217)	0.007	0.5% (5)	0.727
Girls ($n = 949$)	24.3% (212)		19.7% (179)		17.0% (161)		0.3% (3)	
Parental atopy‡								
None ($n = 869$)	17.8% (141)	< 0.001	14.2% (117)	< 0.001	16.9% (147)	< 0.001	0.5% (4)	1.000
One parent ($n = 784$)	25.4% (188)		22.3% (168)		18.4% (144)		0.4% (3)	
Both parents ($n = 289$)	28.1% (74)		26.5% (73)		30.1% (87)		0.4% (1)	

Percentages were calculated without including those infants with missing data on a specific outcome. *Itchy rash lasting at least 14 days or with recurrent incidence. †At least two episodes of wheezing. ‡History of asthma, hayfever or atopic dermatitis. § χ^2 -test or Fisher's exact test.

Table 2. Prevalence of atopic sensitization (specific IgE $\geq$ 0.35 kU/L) at the age of 2 years [% (n)]

	Any allergen	P -value†	Food allergens	P -value†	Inhalant allergens	P -value†
All ($n = 1553$)	12% (186)		9% (144)		5% (70)	
Gender						
Boys ($n = 809$)	14% (116)	0.003	11% (88)	0.023	6% (51)	< 0.001
Girls ($n = 744$)	9% (70)		8% (56)		3% (19)	
Parental atopy*						
None ($n = 682$)	10% (69)	0.019	8% (54)	0.247	3% (20)	< 0.001
One parent ($n = 643$)	12% (78)		10% (65)		5% (29)	
Both parents ($n = 228$)	17% (39)		11% (25)		9% (21)	

*History of asthma, hay fever or atopic dermatitis. † χ^2 -test or Fisher's exact test.

stratified analyses by parental atopy. The results for atopic dermatitis and specific sensitization to food allergens remained unchanged (data not shown). But when we compared the incidence of repeated wheeze between infants with and without parental atopy in bivariate analysis, we found a positive dose-response relationship between endotoxin exposure and incidence of repeated wheeze (P for trend = 0.008) as well as sensitization to inhalant allergens (P for trend = 0.044) in infants with parental atopy in contrast to infants without parental atopy (Table 4).

In multivariate analysis, infants at risk due to parental atopy and exposed to high endotoxin levels had a 1.8-fold increased risk of repeated wheeze (OR_{Q4} 1.77, 95% CI 1.14–2.73; P for trend = 0.025). Though not reaching statistical significance, effect estimates of sensitization to inhalant allergens increased with increasing endotoxin exposure in infants with parental atopy (OR_{Q4} 1.69, 95% CI 0.70–4.11), but not in those without parental atopy (Table 4).

By analysing those infants sensitized exclusively to inhalant allergens ($n = 42$) as an extreme group instead of looking at all infants sensitized to inhalant allergens irrespective of food allergen sensitization, we found a more pronounced association between endotoxin exposure and sensitization to inhalant allergens, again confined to infants with parental atopy (OR_{Q2} 2.99, 95% CI 0.59–15.28; OR_{Q3} 4.61, 95% CI 0.96–22.21; OR_{Q4} 4.46, 95% CI 0.92–21.60; P for trend = 0.054).

The incidence of physician-diagnosed asthma in our birth cohort was too low during the first 2 years of life to allow multivariate statistics. Crude OR for asthma in the fourth endotoxin exposure quartile was 3.33 (95% CI 0.34–32.18) in infants with parental atopy and 2.76 (95% CI 0.29–26.80) in infants without parental atopy.

Furthermore, we separately analysed the endotoxin effects for those infants being sensitized and having repeated wheeze or atopic dermatitis and those infants showing symptoms without atopic sensitization. The increased risk of repeated wheeze with

Table 3. Frequency [% (n)] and risk (adjusted OR with 95% CI) of atopic dermatitis, repeated wheeze and atopic sensitization according to exposure to endotoxin in mattress dust in infants at the age of 2 years

	Endotoxin exposure				P for trend
	1st quartile (57–1028 EU/g)	2nd quartile (1029–2987 EU/g)	3rd quartile (2988–7751 EU/g)	4th quartile (7752–1 286 179 EU/g)	
Symptoms of atopic dermatitis*	21% (96)	25% (112)	22% (99)	22% (96)	0.968
	1.00	1.34 (0.98–1.85)	1.12 (0.80–1.56)	1.20 (0.86–1.68)	0.528
Physician-diagnosed atopic dermatitis	19% (87)	22% (101)	18% (84)	19% (86)	0.637
	1.00	1.25 (0.90–1.74)	0.94 (0.67–1.33)	1.04 (0.73–1.47)	0.727
Repeated wheeze†	16% (80)	20% (97)	19% (93)	22% (108)	0.040
	1.00	1.31 (0.93–1.84)	1.23 (0.87–1.75)	1.52 (1.08–2.14)	0.031
Atopic sensitization to any allergen‡	13% (49)	10% (39)	14% (54)	11% (44)	0.937
	1.00	0.80 (0.51–1.26)	1.18 (0.77–1.82)	0.85 (0.54–1.35)	0.904
Sensitization to food allergens‡	11% (44)	7% (28)	11% (41)	8% (31)	0.281
	1.00	0.63 (0.38–1.04)	0.98 (0.61–1.56)	0.66 (0.40–1.10)	0.319
Sensitization to inhalant allergens‡	4% (14)	4% (17)	5% (19)	5% (20)	0.299
	1.00	1.26 (0.60–2.63)	1.41 (0.68–2.92)	1.36 (0.65–2.83)	0.401

*Itchy rash lasting at least 14 days or with recurrent incidence. †At least two episodes of wheezing. ‡Specific serum IgE ≥ 0.35 kU/L.

Table 4. Differences in frequency [% (n)] and risk (adjusted OR with 95% CI) of repeated wheeze and sensitization to inhalant allergens according to exposure to endotoxin in mattress dust in infants at the age of 2 years stratified by parental atopy

	Endotoxin exposure				P for trend
	1st quartile (57–1028 EU/g)	2nd quartile (1029–2987 EU/g)	3rd quartile (2988–7751 EU/g)	4th quartile (7752–1 286 179 EU/g)	
Infants with parental atopy					
Repeated wheeze*	17% (50)	22% (58)	20% (50)	27% (73)	0.008
	1.00	1.35 (0.87–2.09)	1.13 (0.72–1.78)	1.77 (1.14–2.73)	0.025
Sensitization to inhalant allergens†	4% (9)	5% (11)	7% (14)	7% (16)	0.044
	1.00	1.32 (0.53–3.32)	1.54 (0.63–3.76)	1.69 (0.70–4.11)	0.231
Infants without parental atopy					
Repeated wheeze*	15% (30)	17% (39)	19% (43)	16% (35)	0.358
	1.00	1.27 (0.74–2.19)	1.38 (0.80–2.39)	1.22 (0.69–2.15)	0.482
Sensitization to inhalant allergens†	3% (5)	3% (6)	3% (5)	2% (4)	0.275
	1.00	1.27 (0.36–4.43)	1.10 (0.29–4.17)	0.72 (0.18–2.90)	0.603

*At least two episodes of wheezing. †Specific serum IgE ≥ 0.35 kU/L.

high endotoxin exposure was independent of sensitization status in infants with parental atopy. Non-sensitized infants had a risk of OR_{Q4} 1.78 (95% CI 1.07–2.95) and sensitized infants of OR_{Q4} 1.89 (0.59–6.04) for repeated wheeze. Irrespective of sensitization, incidence of atopic dermatitis was not associated with endotoxin levels (data not shown).

Discussion

Current evidence suggests that environmental exposure to endotoxin may be both harmful and beneficial in the context of asthma and allergies. On one hand, inhaled endotoxin induced inflammatory responses in normal and asthmatic subjects and exacerbated airflow obstruction and symptoms in subjects with asthma [8, 19–22]. On the other hand, early exposure to endotoxin before atopic manifestations was proposed to protect against atopy development [9, 11, 12]. Based on observations of an inverse relationship between frequency of respiratory infections and the occurrence of asthma and atopic diseases, it has been hypothesized that diminished contact with microbial antigens would result in a delayed maturation of Th1 immunity, thus supporting Th2-mediated immune reactions against allergens [23]. The bacterial compound endotoxin with its strong immunomodulatory capacities was supposed to be an environmental factor promoting Th1 immunity.

We analysed endotoxin levels in the mothers' mattresses as an indicator for early, and possibly even pre-natal, exposure of infants as experimental studies indicated that the timing of endotoxin exposure in relation to allergen contact is crucial. Nasal provocation of mite-sensitive asthmatic adults with mite allergen, and subsequently with endotoxin, for example, resulted in a significant increase in inflammatory cells in nasal lavage fluid, whereas provocation with endotoxin alone was not effective [24]. Dubin et al. [25] showed in ragweed-sensitized asthmatic subjects that pre-exposure to allergen may enhance the capacity of inhaled lipopolysaccharide to induce airway inflammation. In contrast, inhalation of endotoxin prior or shortly after allergen challenge prevented sensitization in a rat model, whereas endotoxin exposure several days after allergen challenge promoted the inflammatory response [26].

Furthermore, in our analysis, we distinguished between sensitization to food and to inhalant allergens as it is well-known that both the route and the frequency of antigen contact influences IgE synthesis [27]. We chose atopic dermatitis and recurrent wheezing as frequent manifestations of atopy until the age of 2 years for analysis.

Firstly, we demonstrated in our birth cohort that high endotoxin levels in mattress dust during the first months of life were not associated with incidence of specific sensitization to food allergens and of atopic dermatitis until the age of 2 years. This was in accordance with results of a preliminary analysis of this cohort, showing that high endotoxin levels did not influence the risk of symptoms of an itchy rash or physician-diagnosed atopic dermatitis during the first year of life [17]. Endotoxin exposure only lowered the risk of physician-diagnosed, not further specified, eczema at that age.

Oral uptake of endotoxin in settled house dust may be irrelevant for atopic reactions to oral antigens, as bacteria of the intestinal microflora produce cell wall-derived lipopolysaccharides directly at the local site of potential interaction between

food allergens and mucosal immune cells [28]. Moreover, we assume that inhaled endotoxin may not play a role in immune reactions to food allergens since the site of action at the mucosal surface differs, i.e. intestinal versus respiratory tract.

Second, our study revealed that high endotoxin levels in house dust increased the risk of repeated wheezing during the first 2 years of life in infants. Our data suggest that endotoxin has the potential to increase the risk of sensitization to inhalant allergens, but the number of sensitized infants was too low to yield statistical significance. The detrimental endotoxin effect was rather shown in infants with parents having asthma or allergies. Our results are in accordance with data of another birth cohort indicating an increase in inflammatory reactions revealed as repeated wheeze with elevated endotoxin levels in house dust in infants at risk during the first year of life [7]. It has been proposed that endotoxin in the indoor environment could act as an adjuvant to inhaled allergens [16]. Experimental studies with mice demonstrated a promotion of allergen-specific airway inflammation by inhaled endotoxin and suggested that inhaled endotoxin downregulates IgE isotype-specific tolerance to inhalant allergens [29]. Further experimental evidence from a mouse model of asthma showed that Th1 cells did not attenuate Th2 cell-induced airway hyper-reactivity, but caused severe airway inflammation [30]. Thus, an endotoxin-induced shift to type 1 T cell reactivities would be rather detrimental instead of protective concerning immune reactions to inhalant allergens. By analysing chemokine receptor expression on peripheral T cells at an infant's age of 2 years, we could show, in a subsample of 135 infants of our birth cohort, that endotoxin dust levels were positively associated with a high proportion of type 1 $CCR5^+ CD4^+$ T cells [31].

As a matter of fact, our result of an increased risk of repeated wheeze by elevated endotoxin levels has to be interpreted with caution. Several heterogeneous phenotypes of recurrent airway obstruction can be differentiated and transient early wheezing without subsequent asthma development may occur in infants younger than 3 years [32]. By controlling for frequent upper respiratory tract infections, we could exclude that parental reported wheezing episodes were merely a concomitant phenomenon of common colds. We further differentiated between infants with atopic wheezing and those with non-atopic wheezing. The risk of repeated wheeze was increased in both groups with high endotoxin levels. Physician's diagnosis of asthma before the age of 2 years was too uncommon in our birth cohort to allow for further analysis.

Our results indicated that the inherent risk status of an infant, defined by parental history of asthma or allergies, modified the effect of endotoxin exposure on atopic reactions to inhalant allergens. The strong impact of a family history of atopy as a risk factor for atopy development in children has been repeatedly shown [33]. Delayed post-natal maturation of Th1 immune function seems to be an important factor of genetic susceptibility for atopy, leading to an increased risk of early post-natal sensitization to inhalant allergens [34]. In a subsample of our birth cohort, we recently confirmed previous studies that already at birth the proportion of IFN- γ and TNF- α producing type 1 cord blood T cells was lower in newborns with both parents being atopic [35].

All things considered, if one assumes that endotoxin stimulates type 1 T cells in terms of inflammatory reactions and acts as an adjuvant to inhalant allergens as discussed above, then

endotoxin exposure by the inhalative route may further stimulate rather than protect from atopic reactions to inhalant allergens in infants at risk.

The strengths of our study are its prospective design and the inclusion of infants both with and without parental atopy. We analysed endotoxin levels in house dust shortly after birth to assess early exposure. By using dust samples from the mothers' mattresses, we could estimate endotoxin exposure during the first weeks of life more reliably, as dust sampling from the infants' beds or baby carriages could not be performed on the same high standard as from the mothers' beds [17]. In addition to laboratory data on atopic sensitization, we assessed incidences of repeated wheezing and atopic dermatitis using questionnaire data. We did not inform parents about the endotoxin levels measured in the dust samples of their homes, therefore information bias seems unlikely.

Restricted comparability of endotoxin concentrations due to the differences between the studies in the location of dust sampling (e.g. mattress, kitchen or living room floor), analysis methods and reported unit of measurement (e.g. ng/mg or EU/mL) represents a limitation in our study as well as in other studies on endotoxin exposure published thus far [2, 7–12, 22].

In conclusion, our results of a prospective birth cohort study do not indicate a protective potency of endotoxin exposure as measured by endotoxin levels in mattress dust on atopy development until the age of 2 years. Moreover, endotoxin seems to increase the risk of atopic sensitization to inhalant allergens and of repeated wheeze in infants with parental atopy at the age of 2 years. Further follow-up of the infants of our birth cohort will show if those infants already sensitized to inhalant allergens at an age of 2 years are a selective subgroup of atopic children and if the risk of wheezing and atopic sensitization to inhalant allergens associated with elevated endotoxin dust levels decreases as they grow older as suggested by recent studies [10–12].

Concerning the results of reactions to food and inhalant allergens as well as of stratification by parental atopy, our data support the hypothesis that the impact of endotoxin exposure varies with the type and route of concomitant allergen exposure as well as with genetic cofactors [6]. Thus further studies on the association between endotoxin exposure and atopy development in children should not only take into account the timing and dose of endotoxin exposure itself, but also the type and dose of allergen, route of uptake as well as susceptibility characteristics of the children.

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