

Original article

Exposure to house dust endotoxin and allergic sensitization in adults

Background: It has been suggested that exposure to elevated levels of endotoxin decreases the risk of allergic sensitization.

Objective: To examine the associations between current exposure to bacterial endotoxin in house dust and allergic sensitization in adults.

Methods: In 1995–1996, we conducted a nested case–control study following a cross-sectional study performed within the European Community Respiratory Health Survey (ECRHS). Data of 350 adults aged 25–50 years was analysed. Allergic sensitization was assessed by measurement of specific immunoglobulin E (IgE) against several inhalant allergens. Living room floor dust samples were taken. The endotoxin content was quantified using a chromogenic kinetic *Limulus amoebocyte* lysate test.

Results: Multiple logistic regression analysis showed a negative association between exposure to house dust endotoxin and severe allergic sensitization. Odds ratios (95% CI) adjusted for place of residence, gender, age, and ‘caseness’ were 0.80 (0.64–1.00) for sensitization to ≥ 1 allergen and 0.72 (0.56, 0.92) for sensitization to ≥ 2 allergens using 3.5 kU/l as a cut-off value for sensitization. With regard to single allergens, the protective effect of endotoxin was strongest for pollen sensitization [aOR (95% CI) = 0.74 (0.58, 0.93)].

Conclusion: Our results indicate that current exposure to higher levels of house dust endotoxin might be associated with a decreased odds of allergic sensitization in adults.

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Key words: adults; allergic sensitization; endotoxin; house dust.

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Accepted for publication 3 March 2004

Endotoxins are cell wall components of the outer membrane of Gram-negative bacteria. They are known to have strong immune-stimulatory and proinflammatory properties (1). Endotoxins are more or less ubiquitous in the environment and are present in normal indoor environments as constituents of house dust (2). Increased concentrations of house dust endotoxin have been reported for homes of farming families and households where children had regular contact with farm animals (3). Furthermore, elevated endotoxin levels have been found in homes where animals were kept indoors (4–7).

There is some evidence that exposure to endotoxin levels present in the home environment can promote asthma. Two studies (8, 9) found an association between severity of asthma and house dust endotoxin levels in allergic and nonallergic asthmatic adults. Two other

studies (4, 10) found an association between exposure to endotoxin and exacerbation of asthma in school children.

Numerous studies showed consistently that a farming environment and the presence of pets are associated with a decreased prevalence of hay fever and allergic sensitization in children (11–16) and adults (17–23). It was supposed that the exposure to elevated levels of endotoxin might be responsible for this effect. However, Litonjua et al. (24) recently found an association between cat allergens and dog-ownership and wheeze, which was independent of the effect of house dust endotoxin, suggesting different mechanisms. Beyond that, there is some direct evidence for the allergy protective effect of endotoxin from studies with measured endotoxin exposures. Three studies found higher levels of house dust

endotoxin to be associated with a decreased prevalence of allergic sensitization in infants (25) and school children (26, 27). Moreover, exposure to elevated levels of mattress dust endotoxin was found to be associated with a decreased risk of atopic asthma and hay fever (26).

To our knowledge, no study on the association between current exposure to house dust endotoxin and allergic sensitization in adults has been published so far. Although exposure to endotoxin might in particular modify T-cell immunity in (very early) childhood, current exposure to elevated levels of house dust endotoxin might also decrease the risk of allergic sensitization in adults. We used data from a study conducted to assess the influences of INdoor factors and Genetics in Asthma (INGA) in the two German cities of Erfurt and Hamburg.

Methods

Study design

To enrich a population-based sample with atopic subjects this study on INGA was designed as a nested case-control study following a cross-sectional study performed from 1990 to 1992 in the two German cities of Erfurt and Hamburg within the European Community Respiratory Health Survey (ECRHS) (28, 29). The methods of the ECRHS were described in detail elsewhere (30). On the basis of the study results of the ECRHS in 1990–1992 cases and controls were defined. Every subject willing to participate again attended the centre for a detailed questionnaire including respiratory symptoms and determination of specific immunoglobulin E (IgE). The study was performed from June 1995 to November 1996. Identical methods were followed in the two centres. Local ethics committees had approved the study protocol and all subjects gave their informed written consent.

Study population

Cases for the present study were defined as subjects fulfilling at least one of the following four conditions according to the results from the ECRHS (1990–1992): (i) asthma diagnosed by a physician according to the long questionnaire of the ECRHS (30), (ii) at least one positive serum IgE (>0.35 kU/l, CAP system, Pharmacia and Upjohn, Sweden) against grass, birch, cat dander, *Dermatophagoides pteronyssinus* or *Cladosporium herbarum*, (iii) at least one positive skin prick test reaction (mean wheal diameter ≥ 3 mm) against the allergens mentioned above, (iv) $PD_{20}FEV_1 \leq 2.0$ mg methacholine or positive bronchodilator test. The subjects of the control group did not show any of these conditions at the ECRHS.

Based on these criteria 222 cases and 215 controls were recruited from a random list of 901 cases and 989 controls. These cases and controls again attended the study centre for a detailed questionnaire and medical examination including blood sampling. A total of 405 of these subjects (205 cases and 200 controls) agreed with house dust sampling in their homes. We restricted the present analysis to subjects who had not moved between the participation in the ECRHS (1990–1992) and the house dust sampling within the INGA study. Thus, the study population for the present work consists of 350 subjects who were living in their homes for at least 5 years.

IgE measurements and allergic sensitization

Within the framework of the INGA study in 1995–1996 blood samples were taken and measurements of total and allergen-specific serum IgE against *Alternaria alternata* (m6), *Aspergillus fumigatus* (m3), tree pollen (tx5), birch pollen (t3), *C. herbarum* (m2), *D. pteronyssinus* (d1), *D. farinae* (d2), grass pollen (gx1, gx4), timothy pollen (g6), weed pollen (wx3), German cockroach (i6), dog dander (e5), and cat dander (e1) were performed using the IgE CAP system (Pharmacia & Upjohn).

Allergic sensitization was defined as exceeding a specified cut-off serum-specific IgE concentration for at least one of the allergens. Multiple allergic sensitization was defined as exceeding a specified cut-off concentration for at least two allergens. Three different cut-off values were chosen, 0.35 (CAP-class ≥ 1), 0.70 (CAP-class ≥ 2), and 3.5 kU/l (CAP-class ≥ 3). The different cut-off values are considered to represent different degrees of sensitization. Subjects with specific IgE concentrations below the respective cut-off value were defined as nonsensitized. All three cut-offs are commonly used in epidemiological studies. In accordance with other studies, 100 kU/l was used as a cut-off value for total IgE.

Questionnaire

The questionnaire used in the INGA study had been developed according to the validated long questionnaire with 71 items used in the ECRHS (30). The validated German version of the original ECRHS questionnaire was shortened to 40 items without changing the questions and their wording. A checklist on building characteristics and occupant behaviour was developed and a trained interviewer completed the checklist in each dwelling.

Collection, storage and extraction of house dust

Trained personnel visited the homes from June 1995 through November 1996. In each home a dust sample was taken from the living room floor according to a standardized protocol (31). All dust samples were taken using the same type of vacuum cleaner (Flüsterjet Vitall 371, Philips, Hamburg, Germany) by vacuuming an area of 1 m² for 2 min. Dust was collected on cellulose filters using sampling nozzles and filter boxes produced by ALK Laboratories (Hørsholm, Denmark). Samples were stored in filter boxes at -20°C until extraction. Dust samples were not sieved before extraction.

Endotoxin analysis

Endotoxin was extracted from dust samples, including filters, under sterile and pyrogen-free conditions with 0.125 M NH_4HCO_3 + 0.05% Tween (v/v) for 2 h at room temperature with continuous shaking. Extraction ratios between 1 : 10–1 : 100 (w/v) related to the amount of dust per sample. Suspensions were centrifuged at 1000 g. Extracts were stored in three portions at -20°C and analysed for endotoxin within 6 months. Endotoxin was assayed with a quantitative kinetic chromogenic Limulus Amebocyte Lysate (LAL) method (Kinetic-QCL, BioWhittaker, Walkersville, MD; LAL kit lot no. 5L3480) at 37°C . Analyses were performed with an automated microtitre plate reader (anthos ht III, Anthos Labtec Instruments, Wals, Austria) and WinKQCL 1.1 software. *Escherichia coli* endotoxin (BioWhittaker, lot no. 5L1570, bacterial strain *E. coli* 055:B5) was used as standard endotoxin. The

endotoxin potency of this standard (control standard endotoxin, CSE) was 14.5 EU/ng. Fifty CSE units/ml are equivalent to 50 IU of a FDA reference standard endotoxin (RSE, EC-6) per ml with a reconstitution volume of 2.4 ml. Diluted samples were analysed in inhibition/enhancement mode. Analyses were repeated if the difference between sample duplicates exceeded 10%. Endotoxin concentrations were expressed as EU/m² sampled area and as EU/g of dust.

Allergen measurements

Sample allergen content to house dust mites *D. pteronyssinus* (Der p 1) and *D. farinae* (Der f 1) and cat allergen (Fel d 1) was measured by means of a monoclonal enzyme-linked immunosorbent assay (ELISA) (32) with standards UVA 93/03, UVA 93/02, and UVA 94/01 (Indoor Biotechnologies, Clwyd, UK). The lower limit of detection was 1 ng/ml for Der p 1 and Der f 1 and 1.5 ng/ml for Fel d 1. Allergen concentrations were expressed as ng/g of dust.

Statistical methods

Endotoxin and allergen concentrations were best described by a log-normal distribution. Therefore, mean values were expressed as geometric means (GM) with a geometric standard deviation (GSD). Allergen concentrations below the limit of detection were assigned a value of one-half of the detection limit. Correlations between endotoxins on the one hand and mite and cat allergen on the other were expressed as Spearman rank order correlation coefficients.

The definition of 'cases' in the present study was found to be inadequate as it is not a well-defined clinical entity and for this reason outcomes other than case/control were to be analysed. That is case-control data were not analysed as designed but as cross-sectional data. In order to be allowed to do this it is necessary to show that a relation between exposure and the respective outcome is not just a consequence of the selection process. This was approached by controlling for 'caseness'.

Generalized additive models (33) were used to investigate the functional relationship between allergic sensitization and endotoxin. First, the log-odds for sensitization was expressed as a function of natural log-transformed endotoxin levels using nonparametric loess smoothers in SPlus version 6.0 (Insightful Corporation, Seattle, WA, USA). Afterwards, the smoothed relationship was compared with a linear fit by testing for a difference between the linear fit and the smooth fit that includes both linear and smooth terms (33). Since no statistically significant difference was found between the linear fit and the smooth fit, a parametric approach using multiple linear logistic regression analyses (34) with ln-transformed endotoxin levels was applied in subsequent analyses.

We controlled for the influence of potentially confounding factors and other risk factors such as place of residence, gender, age, education, pet ownership, and mite and cat allergen levels in living room floor dust. Results are presented as crude and adjusted odds ratios (OR) with 95% confidence intervals (CI) associated with an increase in exposure of two GSD. Statistical significance was set at a conventional 5% level. We look at the present study as an exploratory study rather than a confirmatory study. Therefore, no adjustment for multiple testing was carried out. To confirm the results of the present study, the corresponding hypotheses have to be tested in further confirmatory studies. Computations were performed using SAS for Windows version 8.2 (SAS Institute, Cary, NC, USA).

Results

Description of the study population and prevalence of allergic sensitization

Table 1 gives a description of the study population. Prevalence of allergic sensitization using different cut-off values are presented in Table 2. Forty percentage of the study population had at least one elevated serum-specific IgE against the allergens tested, 35% had multiple sensitization using 0.35 kU/l as a cut-off value. Sensitization to pollen was most frequent followed by house dust mite. Increasing the cut-off value for sensitization to 3.5 kU/l resulted in sensitization rates of 21% for at least one allergen and 18% for multiple sensitization. Prevalence of allergic sensitization to pollen, mite and cat allergen decreased to 17, 5, and 5%, respectively. Only 1% of the participants had a severe sensitization to dog dander, German cockroach and moulds. Thirty percent-age (103 of 344) of the participants had total IgE levels exceeding 100 kU/l.

Exposure to endotoxin and allergens

Endotoxin levels in living room floor dust ($n = 350$), ranged from 149 to 3 119 999 EU/m² (203–1 661 491 EU/g) with a geometric mean (GSD) of 23 761 (4.4) EU/m² [32 713 (3.1) EU/g]. Mite (sum of Der p 1 and Der f 1) and cat allergen levels ranged from below the limit of detection (27% and 9% of the homes) to 202 804 and 5 273 900 ng/g with geometric mean (GSD) of 254 (13.1) and 606 (22.5) ng/g, respectively. Correlations between endotoxin and cat and mite allergen levels were weak but statistically significant ($r = 0.18$ and $r = 0.22$, respectively, $P < 0.05$).

Associations between endotoxin and allergic sensitization

Crude and adjusted OR describing the association between allergic sensitization and ln-transformed living room floor dust endotoxin are presented in Table 3. Adjusted OR differ only slightly from the crude OR

Table 1. Description of the study population

Variable	Frequency, n/N (%)
Place of residence	
Erfurt	157/350 (44.9)
Hamburg	193/350 (55.1)
Gender female	163/350 (46.6)
Age [median (range)]	40 (25–50)
Education (years)	
≤ 8	70/350 (20.0)
10	145/350 (41.4)
≥ 12	135/350 (38.6)
Pet ownership	
Dog	84/348 (24.1)
Cat	60/356 (17.1)

Table 2. Prevalence of allergic sensitization

	≥0.35 kU/l (CAP-class ≥1), n/N (%)	≥0.70 kU/l (CAP-class ≥2), n/N (%)	≥3.5 kU/l (CAP-class ≥3), n/N (%)
Sensitization to any allergen*			
At least one specific IgE	140/348 (40.2)	113/346 (32.7)	72/346 (20.8)
At least two specific IgE	120/347 (34.6)	98/346 (28.3)	62/346 (17.9)
Allergen-specific sensitization			
Pollen†	106/347 (30.5)	88/346 (25.4)	58/346 (16.8)
House dust mite‡	58/350 (16.6)	38/350 (10.9)	16/350 (4.6)
Cat dander (e1)	35/350 (10.0)	29/350 (8.3)	16/350 (4.6)
Dog dander (e5)	32/350 (9.1)	21/350 (6.0)	3/350 (0.9)
German cockroach (i6)	36/350 (10.3)	19/350 (5.4)	4/350 (1.1)
Moulds§	9/345 (2.6)	7/345 (2.0)	3/345 (0.9)

* *Alternaria alternata* (m6), *Aspergillus fumigatus* (m3), tree pollen (tx5), birch pollen (t3), *Cladosporium herbarum* (m2), *Dermatophagoides pteronyssinus* (d1), *Dermatophagoides farinae* (d2), grass pollen (gx1, gx4), timothy pollen (g6), weed pollen (wx3), German cockroach (i6), dog dander (e5), and/or cat dander (e1).

† Tree pollen (tx5), birch pollen (t3), grass pollen (gx1, gx4), timothy pollen (g6), and/or weed pollen (wx3).

‡ *Dermatophagoides pteronyssinus* (d1) and/or *Dermatophagoides farinae* (d2).

§ *Alternaria alternata* (m6), *Aspergillus fumigatus* (m3), *Cladosporium herbarum* (m2).

Table 3. Crude and adjusted* logistic regression results describing the association between allergic sensitization and ln-transformed living room floor dust endotoxin (expressed as EU/m²)

	RAST ≥ 1		RAST ≥ 2		RAST ≥ 3	
	Crude OR (95% CI)	Adjusted OR (95% CI)	Crude OR (95% CI)	Adjusted OR (95% CI)	Crude OR (95% CI)	Adjusted OR (95% CI)
Sensitization to any allergen†						
At least one specific IgE	0.96 (0.83–1.11)	0.97 (0.82–1.15)	0.95 (0.82–1.11)	0.94 (0.78–1.13)	0.85 (0.71–1.02)	0.80 (0.64–1.00)
At least two specific IgE	0.97 (0.83–1.12)	0.97 (0.81–1.17)	0.90 (0.77–1.05)	0.86 (0.70–1.05)	0.79 (0.65–0.95)	0.72 (0.56–0.92)
Allergen-specific sensitization						
Pollen‡	0.91 (0.78–1.06)	0.89 (0.74–1.08)	0.91 (0.77–1.07)	0.88 (0.71–1.07)	0.79 (0.65–0.96)	0.74 (0.58–0.93)
House dust mite§	1.00 (0.83–1.21)	1.01 (0.82–1.25)	0.95 (0.75–1.19)	0.94 (0.73–1.21)	0.85 (0.60–1.19)	0.88 (0.62–1.26)
Cat dander	0.90 (0.71–1.14)	0.90 (0.70–1.16)	0.90 (0.70–1.17)	0.90 (0.69–1.19)	1.08 (0.78–1.52)	1.10 (0.77–1.57)
Dog dander (e5)	0.98 (0.77–1.25)	1.04 (0.79–1.38)	1.17 (0.87–1.57)	1.26 (0.89–1.78)	NA	NA
German cockroach (i6)	1.11 (0.88–1.40)	1.19 (0.91–1.54)	1.06 (0.78–1.45)	1.15 (0.91–1.64)	NA	NA

Results are presented as odds ratios (95% confidence intervals) associated with a difference of two times geometric standard deviation (GSD) in exposure.

* Adjusted for place of residence, gender, age, and caseness.

† *Alternaria alternata* (m6), *Aspergillus fumigatus* (m3), tree pollen (tx5), birch pollen (t3), *Cladosporium herbarum* (m2), *Dermatophagoides pteronyssinus* (d1), *Dermatophagoides farinae* (d2), grass pollen (gx1, gx4), timothy pollen (g6), weed pollen (wx3), German cockroach (i6), dog dander (e5), and/or cat dander (e1).

‡ Tree pollen (tx5), birch pollen (t3), grass pollen (gx1, gx4), timothy pollen (g6), and/or weed pollen (wx3).

§ *Dermatophagoides pteronyssinus* (d1) and/or *Dermatophagoides farinae* (d2).

NA not analysed, number of sensitized subjects was too small.

indicating that the observed effects were not confounded by place of residence, gender, and age, and 'caseness'. In addition, we carefully checked for confounding by education, pet ownership, and mite and cat allergens in living room floor dust. Since effect estimates for endotoxin remained unchanged by these variables, they were not included in the final model.

Exposure to high levels of endotoxin was associated with a decreases the risk of more severe sensitization [aOR (95% CI) for sensitization to at least one allergen and at least two allergens using 3.5 kU/l as a cut-off value was 0.80 (0.64–1.00) and 0.72 (0.56, 0.92), respectively]. With regard to allergen-specific sensitization, endotoxin was found to protect against pollen sensitization [aOR

(95% CI) using 3.5 kU/l as a cut-off value was 0.74 (0.58, 0.93)]. The effects on sensitization to house dust mite were statistically nonsignificant but showed the same tendency [aOR (95% CI) using 3.5 kU/l as a cut-off value was 0.88 (0.62, 1.26)]. No other association was found to be statistically significant. Due to the small numbers, sensitization to moulds was not analysed. No association was found between total IgE > 100 kU/l and house dust endotoxin [aOR (95% CI) = 0.99 (0.84–1.16)].

We conducted sensitivity analyses comparing subjects with IgE levels exceeding 0.70 kU/l and 3.5 kU/l to subjects with nondetectable IgE (i.e. IgE < 0.35 kU/l), to verify the increasing endotoxin effect with increasing degree of sensitization. The effects were only

slightly different from the comparison of subjects with IgE levels exceeding and above the respective cut-points.

We also analysed associations between allergic sensitization and endotoxin levels expressed as EU/g dust instead of EU/m² (data not shown). The two measures of endotoxin concentration were highly correlated ($r = 0.86$). And therefore their associations with allergic sensitization were rather similar and the same associations were found to be statistically significant.

Discussion

Our study provides evidence that exposure to higher levels of house dust endotoxin is associated with a decreased prevalence of allergic sensitization in adults.

No study on the relationship between endotoxin levels in house dust and allergic sensitization in adults has been published yet. However, the present findings may be supported by studies, which could show that the protective effect of a farm environment and pet ownership against atopy is detectable also in adults (17–23). Most of these studies focused on the effects of (early) childhood exposure, but some of them also studied the role of current exposure. While no association was found between current country living and sensitization in one study (18), two studies (17, 21) showed that not only a farm childhood but also recent and current exposure of adults to a farm environment might contribute to a lower risk of atopic sensitization to common aeroallergens.

Furthermore, the negative association between allergic sensitization and living room floor dust endotoxin for the INGA adult participants is consistent with results from studies on endotoxin exposure and allergies in children (26), in particular with our previous study on school children (27). Again, elevated levels of house dust endotoxin were found to be associated with lower odds of severe sensitization. Furthermore, with regard to allergen-specific sensitization, the protective effect was strongest for sensitization to pollen in schoolchildren and adults. Although consistent with the results of other studies (14, 15, 17), and the study of Kilpeläinen et al. (35), who found the protective effect of farm environment restricted to decreases in sensitization to birch and timothy pollen (and an adverse effect on house dust mite sensitization), the reason for this is not clear.

We think that in particular the similarity between the present results for the INGA adults and our previous results for the school children is noteworthy. It is not only noteworthy because of the age difference, but also, and

probably more important, because of the fact that most of the adults (>95%) moved at any time during their lives, whereas one-third of the school children was still living at their birth address. One interpretation of the latter might be that not only exposure early in life but also exposure in adulthood might influence the production of allergen-specific antibodies. On the contrary, one could also argue that exposure to high levels of house dust endotoxin is associated with other 'life-style' factors such as living in rural environment or pet ownership that might have been more or less unchanged throughout the study participants' life. Thus, current exposure to endotoxin might be representative for exposure earlier in life. To date, there is hardly any knowledge about whether or not a single endotoxin measurement represents exposure for a longer time period. Only five studies performed repeated measurements of house dust endotoxin (2, 10, 25, 36, 37). The maximum period studied was 13 months (2). The correlation between repeated measurements of house dust endotoxin over a period of several years still needs to be analysed. Furthermore, cohort studies are needed to disentangle the role of early and current exposure.

We used different cut-off values for allergic sensitization (0.35, 0.70, and 3.5 kU/l) and found stronger effects for the higher cut-off values indicating that exposure to higher levels of house dust endotoxin in particular decreases the risk of stronger allergic sensitization. This result is consistent with the findings of Gassner-Bachmann et al. (38) who studied serum-specific IgE to timothy pollen, birch pollen, house dust mites, and cat dander in farmer's and nonfarmer's children. They also found the protective 'farming effect' to be increased with increasing cut-off values for the specific IgE levels. The fact that the protective effect of endotoxin was stronger for sensitization to multiple allergens compared with sensitization to one or more allergens points in the same direction of a protective effect in particular against more severe sensitization.

In conclusion, current exposure to higher levels of house dust endotoxin might be associated with decreased odds of severe allergic sensitization in adults. The role of current and past exposure, i.e. exposure during infancy, has to be investigated in the framework of cohort studies.

Acknowledgments

The authors wish to thank all participants in Erfurt and Hamburg; Gabriele Wölke for coordinating the home visits; Alice Houzer and Günter Silbernagl for data management. The INGA study was supported by the Federal Ministry for Education, Science, Research, and Technology, Grant No. 01 EE 93016 and the GSF – National Research Center for Environment and Health.

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Appendix

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