

Relation between house-dust endotoxin exposure, type 1 T-cell development, and allergen sensitisation in infants at high risk of asthma

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Summary

Background Bacterial endotoxin is known to induce interferon γ and interleukin 12 production, and therefore has the potential to decrease allergen sensitisation. To find out the role of early chronic endotoxin exposure in the development of allergen sensitisation and asthma, we compared concentrations of endotoxin in house dust with allergen sensitisation in infants at high risk for developing asthma.

Methods 61 infants 9–24 months old with at least three physician-documented episodes of wheezing were studied. Concentrations of house-dust endotoxin and allergens were measured in the infants' homes. Allergen sensitisation was measured by skin-prick testing with a panel of common inhalant and food allergens. In a subset of these infants, proportions of T lymphocytes producing interferon γ , and interleukins 4, 5, and 13 were calculated by cell-surface and intracellular cytokine staining, with flow cytometry.

Findings House-dust endotoxin concentrations ranged from 104 to 10 000 endotoxin units (EU) per mL (geometric mean 912 EU/mL). Concentrations did not vary significantly over a 6-month interval. Ten infants (16%) were sensitised to at least one allergen. The homes of allergen-sensitised infants contained significantly lower concentrations of house-dust endotoxin than those of non-sensitised infants (mean 468 vs 1035 EU/mL, respectively; $p=0.01$). Increased house-dust endotoxin concentrations correlated with increased proportions of interferon- γ -producing CD4 T cells ($p=0.01$). Such concentrations did not correlate with proportions of cells that produced interleukins 4, 5, or 13.

Interpretation This study may provide the first direct in-vivo evidence that indoor endotoxin exposure early in life may protect against allergen sensitisation by enhancing type 1 immunity.

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Introduction

Endotoxin is derived from the cells walls of gram-negative bacteria, and can be detected in house dust in widely varying concentrations.^{1–3} The potential of endotoxin to abrogate the development of allergy can be appreciated by considering the mechanisms of endotoxin-induced immune responses. In studies on human peripheral blood cells, endotoxin is a potent inducer of the type 1 cytokines interferon γ and interleukin 12.^{4,5} Interferon γ is counter-regulatory to the development of lymphocytes that produce the type 2 cytokines (interleukins 4, 5, 13), that underlie the immune pathogenesis of allergic diseases.⁶

On this basis, chronic endotoxin exposure in infants (ie, before polarised T-cell responses are established), might be expected to protect against allergen sensitisation by continually enhancing type 1 lymphocyte development. Indeed, in young children, increased interferon- γ production in vitro by peripheral-blood mononuclear cells has been associated with a lack of specific allergen sensitisation to mites, and with decreased rates of asthma.⁷ Conversely, decreased interferon- γ production in children, and even newborn babies, has been associated with increased atopy.^{8–11} We aimed to find out whether chronic environmental endotoxin exposure mitigates the development of allergen sensitisation in young asthma-prone children by enhancement of type 1 immunity.

Methods

Participants

61 asthma-prone infants were recruited at enrolment in a separate study on the prevention of chronic asthma in young children, funded by the US National Institutes of Health (#R18-AI41137). Enrolment criteria were: age 9–24 months, three physician-documented wheezing episodes, and low socioeconomic status; these characteristics have been defined as high-risk features for the development of asthma.^{12,13} The study was approved by the National Jewish Medical & Research Center Institutional Review Board for human research, and the Scientific Advisory Committee for the University of Colorado Health Sciences Center General Clinical Research Center. Parental informed consent was obtained before study participation.

Methods

Allergen sensitisation was assessed by allergy skin-prick testing to common indoor inhalant allergens and food allergens by Quintest (Bayer, Spokane, WA, USA). Aeroallergen extracts used for skin testing were dust mite, cat, dog, cockroach, and mouse; food allergen extracts were milk, egg, and soya; the positive control was histamine, and the negative control was glycerol in saline (Bayer, Elkhart, IN, USA). A skin test was regarded as positive if the wheal reaction was at least 3 mm in diameter larger than the negative control.¹⁴

House-dust samples were collected in muslin filters fitted onto the tubing of a canister vacuum cleaner. A house-dust sample was collected from each household by vacuuming the living-room floor and couch, kitchen floor, bedroom floor, and the participant's bed. Dust samples were kept in separate plastic air-tight containers at -20°C until assayed.

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	Skin-test positive (n=10)	Skin-test negative (n=51)	p*
Demographics			
Mean (range) age (years)	1.66 (1.10–2.14)	1.55 (0.87–2.25)	0.46
Sex (male/female)	4/6	35/16	0.15
Ethnicity (white/black/hispanic/other)	3/3/4/1	12/12/26/1	0.56
Parental atopy†	8 (80%)	33 (65%)	0.47
House-dust aeroallergens			
Detectable‡	10 (100%)	48 (94%)	1.0
Above sensitisation threshold§	6 (60%)	25 (49%)	0.73

**t* test for age, otherwise Fisher's exact test. †Mother and/or father with history of asthma, atopic dermatitis, and/or rhinitis/hay fever. ‡At least one house-dust aeroallergen concentration above detection limit. §At least one house-dust aeroallergen concentration above previously reported sensitisation thresholds.

Table 1: Infants' characteristics and allergen exposure

House-dust samples were sieved through a 50-mesh metal sieve (Newark Metal Cloth Company, Newark, NJ, USA) and suspended in pyrogen-free phosphate-buffered saline at a final concentration of 5 g/L. Samples were sonicated for 60 min, with vortexing for 15 s every 15 min, and were diluted in pyrogen-free water. Endotoxin concentration was measured by the Limulus Amebocyte Lysate assay (Bio-Whittaker QCL-1000, Walkersville, MD, USA) under pyrogen-free conditions. Endotoxin concentration is expressed in endotoxin units (EU) per mL, on the basis of the reference standard developed by the US Food and Drug Administration. Serial dilutions of each sample were run in triplicate.

From house-dust samples, concentrations of the major epitopes of the common indoor allergens Der p1, Der f1, Fel d1, Can f1, and Bla g1 were measured by the Reference Laboratory for Dermatology, Allergy & Clinical Immunology (Johns Hopkins University, Baltimore, MD, USA), by sandwich ELISA.¹⁵ The lowest limits of detection for these assays are: 19 ng/g (of dust) for Der p1, 19 ng/g for Der f1, 19 ng/g for Fel d1, 149 ng/g for Can f1, and 0.39 U/g for Bla g1.

Peripheral-blood mononuclear cells were obtained from 3 mL heparinised blood by Ficoll-Hypaque density centrifugation (Amersham Pharmacia Biotech, Piscataway, NJ). The cells were resuspended at 2×10^6 cells/mL in RPMI 1640 with 10% fetal calf serum (Gibco BRL/Life Technologies), and stimulated for 4 h in 5% carbon dioxide at 37°C with 25 µg/L phorbol 12-myristate 13-acetate (Sigma, St Louis, MO, USA), 1 mg/L ionomycin (Sigma), and 10 mg/L brefeldin A (Sigma). This process induced and optimised the production and accumulation of intracellular cytokines for staining, without altering cell-surface staining characteristics. Stimulated peripheral-blood mononuclear cells were then fixed, lysed, rendered permeable, and stained for cell-surface markers and intracellular cytokines with commercially available reagents (Becton Dickinson, San Jose, CA, USA). Cell-surface markers were PerCP-CD4 (SK3) and APC-CD8 (SK1), and cytokine-specific antibodies for intracellular staining were phycoerythrin-interleukin-4 (3010.211), interleukin 5 (JES1-39D10), interleukin 13 (JES10-5A2), and interferon γ (25723.11; Becton Dickinson/Pharmingen). Flow cytometry was done with a five-parameter argon-laser flow cytometer (FacsCalibur, Becton Dickinson) and data were analysed with CellQuest version 3.01 software (Becton Dickinson). We aimed to run 50 000 gated lymphocytes (based on forward/side scatter gating) for each flow-cytometry specimen to quantify accurately rare cytokine-expressing cell populations. Because of the limitations regarding blood sampling from infants, this procedure was not always possible; therefore, only flow-cytometry specimens yielding over 10 000 gated lymphocytes were included in the analysis.

Statistical analysis

Continuous variables were first assessed for normality of their distributions with the Shapiro-Wilk *W* test. Data were log transformed in some circumstances to normalise data distributions and allow for parametric statistical testing (ie, *t* test, pairwise correlation). When data were not normally distributed, or when numbers of participants per group were small, non-parametric analyses were done (eg, Wilcoxon's rank sums test). For differences in proportions, Fisher's exact test was used.

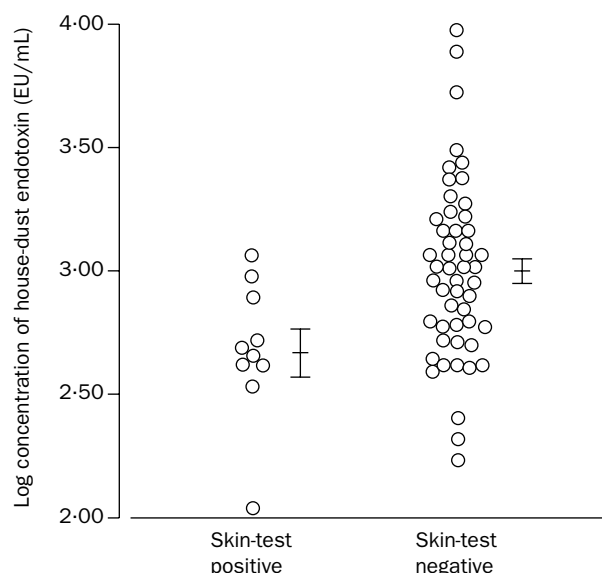


Figure 1: House-dust endotoxin concentrations in allergy skin-test positive and skin-test negative infants

Log transformation of endotoxin values normalised the distribution of the data, allowing for parametric statistical analysis (*t* test).

Results

The 61 asthma-prone infants were divided into those who were positive on the skin-prick test ($n=10$) and those who were negative ($n=51$). The ten allergen-sensitised infants showed no response to the negative control, but at least one positive skin test to major indoor inhalant allergens (nine infants) and/or food allergens (four infants). The nine aeroallergen-sensitive infants were skin-test positive for dust mites (six infants), cat (three), dog (one), and mouse (one); the four food-sensitive infants were sensitised to milk (three) and egg (three). No significant differences between groups with regard to age, sex ratio, ethnicity, or parental atopy were found (table 1).

House-dust samples from all 61 infants' homes were analysed for endotoxin and allergen content. The geometric mean concentration of house-dust endotoxin was 912 EU/mL, and ranged from 104 EU/mL to 10 000 EU/mL in the different households studied. Neither sex nor ethnicity significantly influenced concentrations of house-dust endotoxin (gender: male [$n=39$] *vs* female [22], $p=0.45$ [*t* test]; ethnic groups: white [$n=25$] *vs* black [24] *vs* hispanic [30], $p=0.21$ [ANOVA]). To assess the variation in concentrations of house-dust endotoxin over time, repeat dust sampling was obtained 6 months after initial sampling from 11 households. The differences in endotoxin concentrations over the 6-month period were not significant ($p=0.78$, *t* test), revealing the chronic nature of environmental endotoxin exposure in the home.

To measure the potential effect of environmental endotoxin exposure on allergen sensitisation, we compared concentrations of house-dust endotoxin between skin-test positive and skin-test negative infants. The sensitised infants had significantly lower concentrations of endotoxin in their homes than non-sensitised infants (geometric mean 468 *vs* 1035 EU/mL, respectively; $p=0.01$; figure 1). When infants sensitised to inhalant allergens or food were separately compared with skin-test negative infants, significantly lower concentrations of house-dust endotoxin were also found in the skin-test positive infants (inhalant allergens *vs* skin-test negative, $p=0.01$; food allergens *vs* skin-test negative, $p=0.04$; Wilcoxon's test).

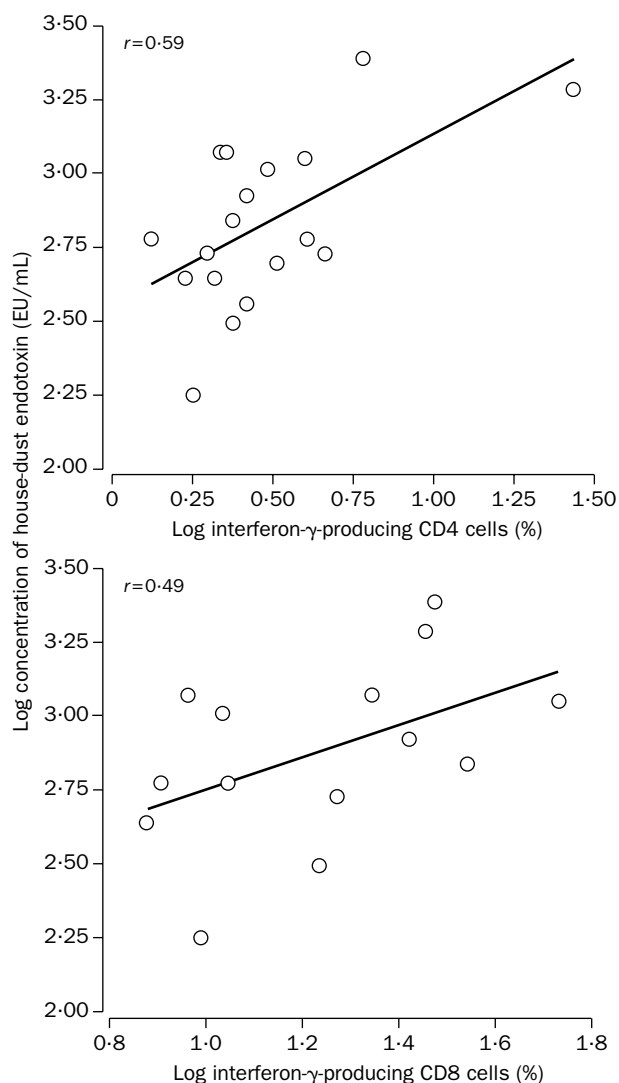


Figure 2: Correlations between concentrations of house-dust endotoxin and proportions of interferon- γ -producing CD4 and CD8 T lymphocytes

Log transformation of endotoxin values and proportions of cells normalised the distributions of the data, allowing for parametric statistical analysis (pairwise correlation).

To further assess whether chronic environmental endotoxin exposure may decrease allergen sensitisation by enhancing type 1 immunity, concentrations of house-dust endotoxin were compared with proportions of interferon- γ -producing T-lymphocytes. Samples of peripheral-blood mononuclear cells were obtained for intracellular cytokine staining and flow cytometry from 20 of the 61 infants. Only specimens yielding more than 10 000 gated cell counts were included in the analysis. This allowed for 13–20 specimens to be included for each cell/stain combination analysed (for all included specimens, the mean cell count was 35 526 [SE 1750]). The percentage of CD4 T cells that were interferon- γ -producing correlated well with concentrations of house-dust endotoxin; a similar trend for interferon- γ -producing CD8 T cells was also noted (figure 2 and table 2). Neither gender nor ethnicity seemed to have confounding effects on interferon- γ -producing T cells, although the number of white infants was small (data not shown). For type 2 cytokine-producing CD4 and CD8 T cells, no significant correlation with concentrations of house-dust endotoxin was found (table 2).

	Number of specimens	Mean (range) proportion T cells expressing cytokine (%)	r^*	p
Type 1 cytokine				
Interferon γ				
CD4	18	3.02 (1.33–27.55)	0.59	0.01
CD8	14	17.37 (7.62–54.03)	0.49	0.07
Type 2 cytokines				
Interleukin 4				
CD4	17	0.50 (0.13–5.18)	–0.10	0.72
CD8	13	0.17 (0.03–0.59)	0.04	0.90
Interleukin 5				
CD4	20	0.76 (0.12–5.46)	–0.01	0.96
CD8	16	0.56 (0.07–3.44)	0.16	0.55
Interleukin 13				
CD4	17	0.37 (0.12–4.98)	–0.18	0.49
CD8	13	0.10 (0.00–0.37)	0.28	0.36

*Pairwise correlation analyses done on log-transformed values.

Table 2: Correlation between house-dust endotoxin concentrations and proportions of T lymphocytes in peripheral blood expressing type 1 or type 2 cytokines

To find out whether differences in aeroallergen exposure explain aeroallergen sensitisation in the skin-test positive infants, house-dust allergen content was evaluated. Concentrations of at least one common indoor allergen (dust mite, cat, dog, cockroach) were increased above previously reported “sensitisation” thresholds in 51% of the households (Der p1 and Der f1 >2 $\mu\text{g/g}$;¹⁶ Fel d1 >8 $\mu\text{g/g}$;¹⁷ Can f1 >10 $\mu\text{g/g}$;¹⁸ Bla g1 >2 U/g¹⁹). However, house-dust allergen concentrations associated with allergen sensitisation in infants and young children can be much lower than those associated with sensitisation in older individuals.^{20,21} At least one indoor allergen was above the detection limits in 95% of the households. Importantly, there were no significant differences in house-dust allergen content, at either threshold or detectable allergen levels, between skin-test positive and skin-test negative infants, implying that differences in household allergen exposure cannot account for differences in aeroallergen sensitisation (table 1). Indeed, for all six infants sensitised to dust mite, only one had detectable house-dust Der p1 (70 ng/g dust [low-level]), and none had detectable house-dust Der f1; thus, aeroallergen sensitisation occurred despite low-level to undetectable house-dust allergen exposure.

Discussion

The potential for environmental exposures in early life to affect the prevalence of atopy has been suggested by epidemiological reports of substantial variation in the prevalence of allergen sensitisation and the atopic diseases in children of common ethnic or genetic descent living under different conditions.^{22–24} Certain microbial infections that are presumably inducers of type 1 immune responses (eg, measles, hepatitis A), have been associated with decreased rates of atopy and asthma.^{25,26} Japanese schoolchildren who mount immune responses to BCG vaccination also have a lower occurrence of allergen sensitisation and atopic disease, lower serum concentrations of interleukins 4, 10, and 13 and increased interferon γ .¹¹ These and other environmental changes in microbial burden have been postulated to influence the predisposition to allergic disease.²⁷ Our investigation provides direct evidence that a chronic environmental exposure capable of inducing type 1 immune responses—household endotoxin—is associated with decreased allergen sensitisation and raised type 1 T-lymphocyte development.

Numerous reports suggest that hygiene measures in modernised societies may be partly responsible for the

increased occurrence of atopic diseases in these populations. Reductions in allergen sensitisation and atopic disease have been found in children of farmers, children with pigs, dogs, or cats in their homes, children raised in day-care from an early age, and children of large families.^{26,28-31} In all of these environmental settings, gram-negative bacteria, and their cell-wall component endotoxin, may exist in abundance. Indeed, occupational disease studies have reported high levels of endotoxin exposure in farming settings.^{32,33}

In conclusion, we have shown a potential role for chronic environmental exposure to endotoxin in the prevention of allergen sensitisation during infancy. Our understanding of atopy-protective environmental exposures, and their influence on the developing immune systems of young children, is in its infancy. Observations suggest that biological differences in type 1 immune responsiveness in early life may bias immune maturation and the development of atopy and asthma.³⁴ Indeed, a specific allele in the promoter region of the gene for the endotoxin receptor/enhancer protein, CD14, is associated with serum concentrations of soluble CD14 and type 1 cytokine skewing.³⁵ In the future, rigorous studies are needed to clarify the role of endotoxin, the variables influencing immune responsiveness to type 1 stimuli in young children, and their potential to provide primary and lasting prevention of atopic disease.

Contributors

Jose Gereda, Donald Leung, and Andy Liu contributed to the background research and development of the study. Jose Gereda and Andy Liu coordinated and executed the study. Mary Klinnert and Marcie Price provided patients, blood, house-dust samples, allergy skin testing, and dust-allergen measurements. Jose Gereda did the endotoxin assays. Akaluck Thatayatikom and Joanne Streib did flow cytometry. Jose Gereda, Donald Leung, and Andy Liu analysed the study results. Andy Liu wrote the paper; Jose Gereda did the figures. All investigators contributed to the critical revision of the paper.

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