

Infant home endotoxin is associated with reduced allergen-stimulated lymphocyte proliferation and IL-13 production in childhood

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Background: Infant endotoxin exposure has been proposed as a factor that might protect against allergy and the early childhood immune responses that increase the risk of IgE production to allergens.

Objective: Using a prospective study design, we tested the hypothesis that early-life endotoxin exposure is associated with allergen- and mitogen-induced cytokine production and proliferative responses of PBMCs isolated from infants with a parental history of physician-diagnosed asthma or allergy.

Methods: We assessed household dust endotoxin at age 2 to 3 months and PBMC proliferative and cytokine responses to cockroach allergen (Bla g 2), dust mite allergen (Der f 1), cat allergen (Fel d 1), and the nonspecific mitogen PHA at age 2 to 3 years.

Results: We found that increased endotoxin levels were associated with decreased IL-13 levels in response to cockroach, dust mite, and cat allergens, but not mitogen stimulation. Endotoxin levels were not correlated with allergen- or mitogen-induced IFN- γ , TNF- α , or IL-10. Increased endotoxin levels were associated with decreased lymphocyte proliferation after cockroach allergen stimulation. An inverse, although nonsignificant, association was also found between endotoxin and proliferation to the other tested stimuli.

Conclusion: Increased early-life exposure to household endotoxin was associated with reduced allergen-induced production of the T_H2 cytokine IL-13 and reduced lymphoproliferative responses at age 2 to 3 years in children at risk for allergy and asthma. Early-life endotoxin-related reduction of IL-13 production might represent one pathway through which increased endotoxin decreases the risk of allergic disease and allergy in later childhood. (*J Allergy Clin Immunol* 2005;116:431-7.)

Key words: Endotoxin, lymphocyte proliferation, cytokine, childhood, allergy

Abbreviations used

EU: Endotoxin units

OR: Odds ratio

SI: Stimulation index

TLR4: Toll-like receptor 4

Childhood allergic diseases, such as asthma and hay fever, are increasing in prevalence, cause chronic ill health, and are a substantial public health concern in developed countries.^{1,2} Measurable childhood sensitization to inhaled allergens occurs primarily after the age of 3 years,³ but the immunologic underpinnings of allergic disease and airway inflammation likely develop far earlier in life.^{4,5} Manifestation of an allergic phenotype likely results from the complex interplay of genetic, developmental, and environmental influences. Adaptive immune processes, including activation of helper T lymphocytes and subsequent B-lymphocyte activation with IgE isotype switching, underlie the process of allergic sensitization in individuals genetically predisposed to development of allergic responses.⁶ Although still controversial,^{7,8} there is mounting evidence from animal models⁹⁻¹³ and the epidemiologic literature^{14,15} suggesting that exposure to endotoxin, a potent activator of innate immunity, might influence subsequent adaptive immune responses to allergen. Furthermore, these studies suggest that the timing and dose of endotoxin exposure influence the nature of the immune response, leading to the hypothesis that early life might be a crucial time window during which endotoxin might reduce the risk of allergy through its influence on innate immunity and downstream T-cell and B-cell regulation of cytokine and IgE expression.

The immunologic pathway linking endotoxin exposure to adaptive immunity and evidence for its effects on the development of allergic diseases have recently been reviewed.^{7,16,17} Briefly, endotoxin is biologically active LPS, a primary component of the outer cell membrane of gram-negative bacteria.^{18,19} Even minute amounts of endotoxin provoke innate immune responses *in vitro* and *in vivo*.²⁰ The nature of that response, which is only partially understood, might depend on the developmental

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stage of the organism (eg, infant or adult) and on the sequence and mode of exposure to endotoxin and allergen. Inhaled endotoxin activates the innate immune system by binding transmembrane toll-like receptors (TLRs) expressed on macrophages and dendritic cells²¹ through IL-12 signaling.²² Given the right timing of exposure and genotype, these endotoxin-activated antigen-presenting cells might stimulate production of IFN- γ and other T_H1 cytokines.²³ Through production of IFN- γ by T_H1-biased lymphocytes or through alternative pathways, endotoxin might downregulate T_H2 cytokine (including IL-13) secretion, IgE production, and consequent allergic disease. Although studied extensively in animal models, few prospective data are available on the effects of home LPS exposure on cytokine production in young children.

Using a prospective birth cohort with a parental history of allergy or asthma, we explored the hypothesis that early-life endotoxin exposure alters immune system responsiveness by examining the relationship between house dust endotoxin and PBMC responses to allergen stimulation. Specifically, we examined associations between house dust endotoxin levels measured 2 to 3 months after the child's birth and allergen- and mitogen-induced cytokine and proliferative responses of mononuclear cells isolated from peripheral blood sampled at age 2 to 3 years. Previously, we had found that increased endotoxin levels in infancy were associated with decreased risk of eczema in the first year of life, suggesting that endotoxin might have a protective effect against allergic disease and the biologic pathways influencing the risk of allergy.²⁴ We hypothesized that endotoxin levels would be positively correlated with levels of IFN- γ , a T_H1 cytokine, and inversely associated with levels of the T_H2 cytokine IL-13, which can mediate isotype switching to IgE.²⁵ We further hypothesized that endotoxin levels would be associated with decreased proliferative responses after allergen stimulation.

METHODS

Description of cohort

The Epidemiology of Home Allergens and Asthma study is a longitudinal birth cohort study of environmental predictors of allergy and asthma development. A description of the recruitment of study participants and study protocol has been previously published.²⁶ In brief, 505 children from 499 families with a parental history of asthma or allergy were enrolled in a birth cohort study designed to examine the effects of allergen exposure in early life on the development of asthma. The Brigham and Women's Hospital Human Research Committee approved the study. Informed consent was obtained from the parents for blood collection and longitudinal follow-up. Mothers in the greater Boston metropolitan area delivered at a large Boston hospital were screened with the following questions: (1) Have you ever had asthma, hay fever, or allergies? (2) Has the biologic father of your child ever had asthma, hay fever, or allergies? Mothers responding yes to either question were asked to complete a screening questionnaire. Families were not approached if the index child was premature, had a major congenital anomaly, or was in the neonatal intensive care unit or if the mother was less than 18 years old or could not speak English or Spanish. Informed consent was obtained from

the parents for blood collection and longitudinal follow-up. The Brigham and Women's Hospital Institutional Review Board approved the study protocol.

Environmental sampling and endotoxin measurements

The home sampling protocol has been described previously.²⁷ A trained research assistant visited participants' homes within 2 to 3 months of the child's birth. During these visits, conducted between 1994 and 1996, detailed demographic, socioeconomic, parental disease history, and home characteristics questionnaires were completed, and standardized dust sampling was conducted in various sites within the home, including the family room. Dust samples to be used for endotoxin assays were stored desiccated at -20°C until extraction.

Endotoxin activity of dust samples was determined by using the kinetic Limulus amebocyte lysate assay with resistant-parallel-line estimation, as previously described.²⁸⁻³⁰ The Limulus amebocyte lysate was supplied by BioWhittaker (Walkersville, Md). Reference standard endotoxin was obtained from the United States Pharmacopoeia, Inc (Rockville, Md), and control standard endotoxin was supplied by Associates of Cape Cod (Woods Hole, Mass). Results were reported in endotoxin units (EU) per milligram of dust adjusted to account for lot-to-lot variation in Limulus amebocyte lysate sensitivity to house dust endotoxin and referenced to the reference standard endotoxin EC5 and EC6 (US Pharmacopoeia, Inc; 1 ng of EC5 and EC6 = 10 EU).³⁰ Because of the prioritization schema for assaying dust samples, the availability of endotoxin measurements was conditional on there being sufficient dust to first assay for home allergens and fungi. As such, for 19 of the 115 subjects with biomarker outcome data, family room dust endotoxin levels were unavailable.

PBMC responses

At 2 to 3 years of age, blood sampling and analysis was conducted in a subgroup of the study participants ($n = 115$). As previously described, selection of this subgroup was based on the home allergen levels measured during the initial home visit.^{31,32} The goal in choosing these subjects was to maximize variability in early-life exposure to the allergens with which their cells were to be stimulated.

PBMCs were isolated from this blood sample by using Ficoll-Hypaque centrifugation.³³ Fresh cells were incubated in media; media containing either 30 $\mu\text{g/mL}$ cockroach allergen (Bla g 2), 30 $\mu\text{g/mL}$ house dust mite allergen (Der f 1), or 1000 U/mL cat allergen (Fel d 1); or media plus 10 $\mu\text{g/mL}$ PHA. Optimal stimulant concentrations used for the assay were determined in a prior dose-response analysis.³¹

At 24 and 60 hours after the initiation of stimulation, supernatants were harvested, and cytokine concentrations were quantified by means of ELISA (Endogen, Woburn, Mass). On the basis of prior optimization for detection of cytokine levels, IL-10 and TNF- α were measured in the 24-hour samples, and IFN- γ and IL-13 were measured in the 60-hour samples. The lower limits of detection for cytokine assays were as follows: IFN- γ , less than 2 pg/mL; IL-13, less than 7 pg/mL; IL-10, less than 3 pg/mL; and TNF- α , less than 5 pg/mL. Because supernatant quantities were limited, cytokine assays were prioritized, with IFN- γ levels being measured first. As a result, fewer subjects have observations of IL-13, IL-10, and TNF- α levels.³²

After incubation of PBMCs with allergen or mitogen for 72 hours, 1 μCi of tritiated thymidine was added to each well. After incubation for an additional 8 hours, the cells were harvested, and tritiated thymidine uptake was determined by means of β -counting.

TABLE I. Characteristics of children in the cohort

Variable	Subjects with measurement of endotoxin and PBMC outcomes* (n = 96)	Subjects with no measurement of both endotoxin and PBMC outcomes (n = 402)	Total (n = 498)
Sex, n			
Male	60 (63%)	208 (52%)	268 (54%)
Female	36 (38%)	194 (48%)	230 (46%)
Race-ethnicity, n			
White or Asian	79 (82%)	324 (81%)	403 (81%)
Other	17 (18%)	78 (19%)	95 (19%)
Income, n			
<\$50,000	27 (28%)	106 (26%)	133 (27%)
≥\$50,000	68 (71%)	283 (70%)	351 (70%)
Family room Bla g (1 or 2), n			
≥0.05 U/g	25 (26%)	84 (21%)	109 (22%)
<0.05 U/g	69 (72%)	291 (72%)	360 (72%)
Family room Der f 1, n			
≥2 μg/g	43 (45%)	190 (47%)	233 (47%)
<2 μg/g	53 (55%)	206 (51%)	259 (52%)
Family room Fel d 1, n			
≥1 μg/g	61 (64%)	234 (58%)	295 (59%)
<1 μg/g	34 (35%)	147 (37%)	181 (36%)
Cold before blood draw, n			
Yes	24 (25%)	—	—
No	71 (75%)	—	—

*The total number varied according to specific outcomes, but 96 children had endotoxin and lymphocyte proliferation data.

Proliferation was quantified by calculating the stimulation index (SI) for each stimulant with the following formula:

$$SI = \frac{(\text{Mean value of tritiated thymidine uptake for stimulated samples})}{(\text{Mean value of tritiated thymidine uptake for unstimulated samples})}^{31}$$

IgE measurements

Serum samples were assayed for total IgE and specific IgE antibodies to dust mite, cat, cockroach, and ovalbumin by using the UNICAP System (Pharmacia, Uppsala, Sweden). IgE increase was defined either as an IgE specific response (≥0.35 IU/mL) to at least one allergen or a total IgE level of greater than 60 IU/mL for age 2 to 3 years.

Statistical methods

All statistical analyses were conducted with SAS version 8.2 (SAS Institute Inc, Cary, NC). In primary analyses investigating lymphoproliferative response as an outcome, both endotoxin and SI were treated as continuous variables. The distribution of SIs and endotoxin levels were log₁₀ transformed for linear regression analyses to improve the normality of residuals and facilitate interpretation of model results. The relationships between log₁₀-transformed SIs, log₁₀-transformed endotoxin, and potential covariates were assessed by using ordinary least-squares univariate and multivariate regression models. The estimates for the relationship of the log of endotoxin to SI were used to calculate the percentage difference in SI for a doubling of endotoxin. This enabled us to translate the estimates into an easier-to-understand outcome that was scaled by a realistic (within the range of our data) increase in endotoxin level. We also considered SI as a dichotomous categorical by using cutoff points that have been considered as positive PBMC responses to allergen or mitogen stimuli in other studies (allergen responses, SI > 3; PHA responses, SI > 153).³¹

Our focus on the relation of endotoxin to IL-13 production presented statistical challenges because of the skewed distribution of the cytokine levels and because of the significant proportion of values below the limit of detection. First, we used nonparametric correlation analyses to relate continuous dust endotoxin with continuous cytokine levels. For nonparametric testing, cytokine observations below the lower limits of detection were assigned very low but nonmissing values (0.001 pg/mL) to be included in the analysis and tested the sensitivity of our findings to the choice of number assigned to the observations below the limit of detection. We then assessed the relation between endotoxin and the odds of having measurable IL-13. Finally, in secondary analyses we tested for trends in the proportion of cytokine observations above the lower limit of detection when grouped by tertile of endotoxin level.

Cockroach (Bla g 1 or 2), dust mite (Der f 1), and cat (Fel d 1) allergen levels were classified as follows: Bla g 1 or 2 of 0.05 U/g or greater, Fel d 1 of 1 μg/g or greater, and Der f 1 of 2 μg/g or greater).³¹ Reported household income was classified as being less than or greater than \$50,000.

RESULTS

Of the 505 children initially enrolled in the study, 7 were followed for less than 5 months in the first year of life. Of the 498 with follow-ups, 115 had lymphocyte proliferation measurements at age 2 to 3 years, of whom 96 also had family room dust endotoxin measurements. Compared with the 402 subjects without both lymphocyte proliferation and endotoxin data, the subsample with both (n = 96) had a greater proportion of boys (Table I). No other selection bias was detected. Endotoxin in the 96 family room dust samples had a geometric mean of 95 EU/mg (geometric SD, 1.8 EU/mg) and a median level of

TABLE II. Distribution of endotoxin, IL-13, and PBMC lymphocyte proliferative response levels*

Variable	Minimum	Median	75th Percentile	Maximum	Detectable, n
Endotoxin (EU/mg), n = 96	19.3	101.2	138.4	518.8	96 (100%)
IL-13 (pg/mg), n = 67					
Bla g 2	<LD	<LD	9.6	207.2	32 (48%)
Der f 1	<LD	8.6	30.5	397.3	47 (70%)
Fel d 1	<LD	<LD	4.7	137.6	26 (39%)
PHA	<LD	1425	2255	3812	64 (96%)
SI					
Bla g 2	0.9	4.1	5.7	53.6	94 (100%)
Der f 1	0.4	3.3	5.9	32.5	95 (100%)
Fel d 1	0.3	2.2	4.4	27.5	96 (100%)
PHA	14.4	138.6	285.5	1205.1	96 (100%)

<LD, Below the limits of detection.

*For the purpose of ranking/nonparametric testing, values below the limits of detection were assigned the value 0.001 pg/mL.

101 EU/mg (Table II). The distributions of lymphocyte proliferation and cytokine levels for this subsample were similar to those previously demonstrated for the larger sample (Table II).^{31,32}

We found no confounders of the association of endotoxin with either the lymphoproliferative response or the cytokine production by stimulated PBMCs after considering living room allergen level, report of dog or cat, race-ethnicity, household income, or cold before blood draw (data not shown). As previously reported for the larger cohort, cockroach allergen (Bla g 1 or 2), cat allergen (Fel d 1), and dust mite allergen (Der f 1) levels measured in family room dust samples were not significantly associated with home endotoxin levels.³⁴

Association of house dust endotoxin and SIs

We observed a consistent association between increased dust endotoxin measured in the first months of a baby's life and decreased SIs at age 2 to 3 years, although this association was only significant for the response to cockroach allergen (Table III). Despite the absence of measurable confounders, to adjust for potential independent influences on the lymphoproliferative responses, we adjusted for indicators of age at the time of blood draw, report of a cold in the week before blood draw, household income level, and house dust allergen levels in the allergen-specific proliferation models in addition to reporting the univariate models.³¹ The multivariate-adjusted relationships between endotoxin and the SIs ranged from -4% to -17%, being strongest and statistically significant for the SI after cockroach allergen stimulation. When dichotomized at previously defined cutoff points,³¹ positive cockroach-induced lymphocyte proliferative responses (SI > 3) were also inversely associated with endotoxin levels (odds ratio [OR] of Bla g 2 SI ≥ 3 for a doubling in family room endotoxin level: OR, 0.53; 95% CI, 0.27-1.049; $P = .07$). Although the estimates for Der f 1 SI were negative, the analyses using SI as a dichotomous outcome were not supportive of a significant association of endotoxin with a lower odds of Der f 1 SI of greater than 3 ($P = .9$) or Fel d 1 SI of greater than 3 ($P = .7$).

Association of house dust endotoxin and stimulation-induced cytokine production

By using rank-based correlations, increasing house dust endotoxin levels measured early in life were inversely correlated with allergen-induced IL-13 but not IL-13 induced by PHA (Table IV). For a doubling of endotoxin levels, children had significantly reduced odds of having measurable-detectable allergen-induced IL-13 levels in response to all 3 allergens, with no association between endotoxin- and PHA-induced IL-13 (Table V). With increasing tertiles of endotoxin, a smaller percentage of children had measurable allergen-induced IL-13 levels (above the limits of detection), although this trend was only statistically significant for the response to Der f 1 (see Fig E1 in the Online Repository in the online version of this article at www.mosby.com/jaci). We did not observe correlations between allergen- or mitogen-induced levels of IFN- γ , TNF- α , and IL-10 in the supernatants of the isolated PBMCs and endotoxin (data not shown). We found no evidence of an association between PHA-induced IL-13 and endotoxin.

DISCUSSION

In this prospective cohort study of young children with a family history of allergic disease, we observed that increased levels of family room dust endotoxin in infancy were associated with decreased allergen-induced IL-13 production by mononuclear cells isolated from peripheral blood at age 2 to 3 years. We also found that endotoxin levels were associated with a downregulation of allergen- and mitogen-stimulated lymphocyte proliferation. Through the interactions of its Lipid A moiety with receptors such as TLR4, endotoxin is hypothesized to modulate the innate immunity pathway and, through those pathways, to influence adaptive immunity^{16,17} with reduced production of cytokines, such as IL-13, as seen in our study. This in turn might result in subsequent reduction of IgE levels, allergy, and allergic disease. In our cohort at this age, we have previously demonstrated an

TABLE III. Percentage change in lymphocyte proliferative response (SI) for a doubling of family room dust endotoxin levels

Stimulus	Univariate*		Multivariate†	
	% Difference	95% CI	% Difference	95% CI
Cockroach allergen (Bla g 2)	−15	−27 to −1	−17	−28 to −4
Dust mite allergen (Der f 1)	−13	−30 to 8	−15	−31 to 5
Cat allergen (Fel d 1)	−5	−21 to 15	−4	−21 to 16
PHA	−11	−28 to 10	−9	−27 to 12

*The percentage difference in SI for a doubling of endotoxin was calculated as follows: $(10^{\beta} [\log_{10}(2) * (\beta)] - 1) * 100$, where β is the endotoxin effect estimate for the univariate model: $\log_{10}(SI) = \beta_0 + \beta_1 [\log_{10}(Endotoxin)]$. Also see the Methods section.

†Same as the univariate model: adjusted for age at blood draw, report of a cold before blood draw, household income, and family room dust allergen level for allergen-induced proliferation models.

association between lower IL-13 levels and reduced risk of allergy/increased IgE levels.³² Increased endotoxin levels were also associated with reduced risk of allergy, but this association was not statistically significant. A doubling of endotoxin in family room dust was associated with an OR of 0.71 (95% CI, 0.39-1.31) for increased IgE levels, as determined by positive RAST results or high total IgE levels among 97 children with data on endotoxin in early life and IgE levels at age 2 to 3 years. In a previous report from this study, we demonstrated that higher infant endotoxin levels were associated with a significantly lower risk of eczema in the first year of life.²⁴ The data we present in this article support the hypothesis that the association of increased home endotoxin exposure with protection against childhood allergic disease might be mediated in part through reduced T_H2 cytokine expression in early life.

Researchers in Europe have observed an association between a farming lifestyle and lower prevalence of allergic disease in children and hypothesized that increased exposure to endotoxin in infancy might be responsible for this association through an early-life influence on innate immune development and its relation to adaptive immunity, cytokine production, and IgE production.^{14,35,36} In a cross-sectional study of German, Austrian, and Swiss children ages 6 to 13 years, endotoxin measured in the dust of children's mattresses was inversely associated with asthma, atopic sensitization, and hay fever.³⁷ Increased bed dust endotoxin levels were associated with decreased LPS-induced production of IFN- γ , IL-10, IL-12, and TNF- α . The researchers interpreted these findings as a global downregulation of the immune response. In contrast, we found a specific endotoxin-related downregulation of a T_H2 cytokine, IL-13, which can mediate isotype switching to IgE^{25,38} without an influence on IFN- γ , IL-10, or TNF- α production. Our study contrasts with the multicenter European study in many ways. We stimulated our PBMCs with allergen, our study was prospective, our cohort was predisposed to allergy by virtue of family history, and our endotoxin levels, measured in infancy, were relatively low. In a small US cross-sectional study of infants with repeated wheeze, allergen-sensitized children came from homes with higher endotoxin levels, and higher home endotoxin levels were correlated with increased

proportions of IFN- γ -producing CD4 T cells.¹⁵ In this study the investigators did not evaluate cytokine secretion of lymphocyte proliferative response of antigen- or mitogen-stimulated PBMCs.

Although this is, to the best of our knowledge, the first report of a prospective association of endotoxin with diminished IL-13 production in children, some animal models have demonstrated similar associations. However, in animal models endotoxin exposure with TLR4 agonists has been shown to both diminish and enhance IFN- γ production, IL-13 production, and allergic responses.¹² The directionality and nature of the immune and allergic responses appears to be dependent on many factors, including the type of model, dose of allergen or TLR4 agonist, timing of allergen or TLR4 agonist, use or not of adjuvant, and type of species or murine strain.^{9,11,39-42} In one murine model prenatal exposure to LPS resulted in increased neonatal IFN- γ secretion and decreased neonatal IL-13 production after OVA exposure, with no protection against airway responsiveness.⁴³ In another murine model, Velasco et al¹² showed that pulmonary administration of Lipid A before allergen sensitization decreased eosinophilia, bronchoalveolar lavage fluid IL-13 levels, serum IgE levels, airway hyperresponsiveness, and the number of CD4⁺ cells in the lung. Lipid A administration during allergen challenge diminished eosinophilia. In contrast, Delayre-Orthez et al⁴² showed that LPS during challenge enhanced allergen-induced eosinophilia but did not enhance allergen-induced IgE or airway hyperresponsiveness. A recent study compared the effects of Lipid A in terms of maturity of the immune system, species, dose, and timing by examination of human cord and adult PBMCs and murine cells *in vitro* and *in vivo*.¹³ Lipid A induced primarily a time- and dose-independent production of IFN- γ .

In both human and animal models, the effects of environmental endotoxin exposure, are likely to be dependent on the dose and timing of exposure and on the developmental stage and genotype.⁴⁴ These factors might explain seemingly contradictory epidemiologic findings of endotoxin effects on allergic disease.^{7,16} As an irritant, in those with or without asthma, endotoxin might increase the severity of symptoms.^{31,45} Previously, we found that endotoxin was associated with increased risk of wheeze

TABLE IV. Correlations between allergen- and mitogen-induced cytokine levels and family room dust endotoxin levels*

Stimulus	Cytokine (pg/mL)	Obs.	Correlation coefficient	P value
Bla g 2	IFN- γ	80	-0.05	.66
	IL-13	67	-0.31	.01
	IL-10	64	0.11	.38
	TNF- α	50	-0.01	.94
Der f 1	IFN- γ	85	0.01	.91
	IL-13	67	-0.27	.02
	IL-10	69	-0.08	.51
	TNF- α	50	-0.09	.54
Fel d 1	IFN- γ	80	0.06	.57
	IL-13	67	-0.21	.08
	IL-10	64	-0.02	.89
	TNF- α	50	-0.03	.83
PHA	IFN- γ	85	0.08	.46
	IL-13	67	0.12	.34
	IL-10	69	-0.05	.67
	TNF- α	50	-0.14	.35

Obs., Number of observations.

*Rank-based (Spearman) correlation coefficients.

and repeated wheeze in the first year of life in this cohort; this might be secondary to its irritant effects.²⁹ In the siblings of the subjects in this cohort, the association of endotoxin with wheeze decreased over time, and as the child grew older, earlier-life endotoxin appears to have a null or even protective effect on wheeze risk, perhaps because the wheeze was more related to allergic inflammation.⁴⁶ This is consistent with the hypothesis that endotoxin might increase the risk of irritant wheeze at the same time as decreasing the risk of allergy and its pulmonary and extrapulmonary manifestations. In their studies of children growing up on farms, Braun-Fahrlander et al³⁷ have found consistent protective effects of endotoxin on allergy but an increased risk of wheeze with increasing endotoxin levels among those without allergy.

We had limited power to fully elucidate pathways leading from endotoxin exposure to cytokine production, early allergy, and subsequent allergic disease expression. In this group of young children, when allergy, particularly allergy to inhaled allergens, is just developing, we found that increased levels of endotoxin were associated with a lower risk of high IgE levels but that association was not statistically significant. This might be due to small numbers (low power) because we were only able to measure both endotoxin and cytokines on a subset of the cohort or due to the age of the children because the allergic phenotype is not fully evolved by age 2 to 3 years. It is quite possible that a skewing toward T_H2 cytokine production is an earlier step in the pathway toward full expression of the allergic phenotype. The associations between observed family room dust endotoxin and allergen- and mitogen-induced proliferation of lymphocytes were small and achieved statistical significance only after stimulation with cockroach allergen. Therefore we cannot

TABLE V. Relative odds of detectable versus nondetectable allergen- and mitogen-induced IL-13 for a doubling of family room endotoxin levels

Cytokine	Stimulus	Obs.	OR	95% CI
IL-13	Bla g 2	67	0.36	0.16-0.81
	Der f 1	67	0.27	0.11-0.70
	Fel d 1	67	0.44	0.20-0.94
	PHA	67	0.89	0.19-4.17

Obs., Number of observations.

exclude the role of chance in determining the observed association between endotoxin and proliferation after stimulation with the other allergens and the mitogen. However, the consistently negative associations suggest that this is not a chance observation. Although confounding bias by unmeasured factors is always a possibility, we found no confounding by other measured exposures (including dog) in our analyses evaluating the association of endotoxin with detectable IL-13, and we adjusted for confounders in our analyses, with SI as our outcome. Ideally, an estimate of each subject's endotoxin exposure would capture the true temporally and spatially integrated exposure that occurs within the first year of life. Our single measure of endotoxin in the home is likely to represent this true exposure with error. Because of the prospective study design, differences between the observed measurements of endotoxin and the subjects' true endotoxin exposure are most likely nondifferential with respect to PBMC responses. This measurement error would most likely have attenuated the exposure effect estimates. A final limitation is that we did not evaluate the allergens that we used to stimulate the PBMCs for the possible presence of endotoxin. Production of IL-13 is likely to be less influenced by trace LPS than production of cytokines involved in innate immunity, such as TNF- α . The possibility remains, however, that increased home allergen levels in infancy are associated with reduced IL-13 production not only in response to allergens but also in response to a combination of allergen and LPS.

In conclusion, in a cohort of children at risk of allergy and asthma, we conducted a prospective study on the relation of home endotoxin levels in infancy to subsequent allergen-stimulated lymphocyte proliferative responses and cytokine production. Our data suggest that increased early-life endotoxin exposure might be associated with a reduction in subsequent allergen-stimulated lymphocyte proliferation and IL-13 secretion. Endotoxin-induced reduction in IL-13 secretion might be one early-life step in the pathway to endotoxin-associated reduction in allergy and allergic disease.

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