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ENVIRONMENTAL EXPOSURE TO ENDOTOXIN AND ITS RELATION TO ASTHMA IN SCHOOL-AGE CHILDREN

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ABSTRACT

Background In early life, the innate immune system can recognize both viable and nonviable parts of microorganisms. Immune activation may direct the immune response, thus conferring tolerance to allergens such as animal dander or tree and grass pollen.

Methods Parents of children who were 6 to 13 years of age and were living in rural areas of Germany, Austria, or Switzerland where there were both farming and nonfarming households completed a standardized questionnaire on asthma and hay fever. Blood samples were obtained from the children and tested for atopic sensitization; peripheral-blood leukocytes were also harvested from the samples for testing. The levels of endotoxin in the bedding used by these children were examined in relation to clinical findings and to the cytokine-production profiles of peripheral-blood leukocytes that had been stimulated with lipopolysaccharide and staphylococcal enterotoxin B. Complete data were available for 812 children.

Results Endotoxin levels in samples of dust from the child's mattress were inversely related to the occurrence of hay fever, atopic asthma, and atopic sensitization. Nonatopic wheeze was not significantly associated with the endotoxin level. Cytokine production by leukocytes (production of tumor necrosis factor α , interferon- γ , interleukin-10, and interleukin-12) was inversely related to the endotoxin level in the bedding, indicating a marked down-regulation of immune responses in exposed children.

Conclusions A subject's environmental exposure to endotoxin may have a crucial role in the development of tolerance to ubiquitous allergens found in natural environments. (N Engl J Med 2002;347:869-77.)

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ASTHMA is the most common chronic disease in childhood and accounts for substantial morbidity and health care costs. Although various environmental factors have been thought to play key parts in the development of asthma and allergies,¹⁻³ the causes of these diseases remain unclear.

One intriguing hypothesis is that changes in the type and degree of stimulation from the microbial environment associated with improvements in public health and hygiene may increase the predisposition to chronic allergic conditions during childhood.⁴ Exposure to microbes can occur in the absence of infection. For example, viable and nonviable parts of microorganisms are found in varying concentrations in many indoor and outdoor environments. These microbial substances are recognized by the innate immune system in the absence of overt infection, and they induce a potent inflammatory response.⁵ Therefore, environmental exposure to microbial products may have a crucial role during the maturation of a child's immune response, causing the development of tolerance to other components of his or her natural environment, such as pollen and animal dander.

We investigated the relation between exposure to microbial products and the occurrence of childhood asthma and allergies in an environment rich in op-

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portunities for such exposure — that is, a rural environment where some families engage in farming. We measured endotoxin — a cell-wall component of gram-negative bacteria — in samples of dust from the mattresses of children and then related the levels of endotoxin to the prevalence of asthma and allergies and to serum levels of specific IgE. We also assessed the cytokine-production profile of peripheral-blood leukocytes after activation of the innate immune system by stimulation with lipopolysaccharide and staphylococcal enterotoxin B.

METHODS

Study Population

This cross-sectional survey was conducted in rural areas of Austria, Germany, and Switzerland, as previously described.⁶ Participating parents (2618 of 3504 potential participants [74.7 percent]) were asked to consent to the measurement of specific IgE in their children's serum, the assessment of the cytokine-production profile of the children's peripheral-blood leukocytes after stimulation with lipopolysaccharide and staphylococcal enterotoxin B, and the collection of dust samples from the children's bedding. The final analysis was restricted to 812 children with complete data and similar ethnic origin (categorized as German, Austrian, or Swiss nationality), in order to avoid potential confounding by ethnic background.⁷

Approval to conduct the survey was obtained from the three local ethics committees for human studies and from the principals of the schools attended by the children. Written informed consent was obtained from the parents of all children.

Dust Sampling

We collected dust by vacuuming each mattress for two minutes per square meter of surface area. The material obtained was divided in two for measurement of endotoxin and allergen content. Dust was collected on special filters provided by the Alleregologisk Laboratorium Copenhagen.⁸ All field workers were centrally trained and certified to ensure similarity of sampling.

Measurements of Endotoxin Levels

One dust sample was stored at room temperature and shipped within one week after collection to the central laboratory (in Munich, Germany). Endotoxin content was measured by a kinetic limulus assay, as described by Hollander et al.⁹ Endotoxin results were expressed as endotoxin units per milligram of dust and as endotoxin units per square meter of surface area of the sampled mattress. All endotoxin levels were within the limits of detection of the assay.

Measurements of Allergen Levels in Dust Samples

The second dust sample was frozen at -20°C for at least two days and then shipped to one central laboratory (University Children's Hospital Charité, Berlin, Germany) and stored at 4°C until it was analyzed for *Dermatophagoides pteromyssinus* (Der p1), *D. farinae* (Der f1), and *Felis domesticus* (Fel d1), as previously described.³ The lower limit of detection was 10 ng per gram of dust for Der p1 and Der f1 and 16 ng per gram of dust for Fel d1; results are expressed in nanograms of major allergen per gram of mattress dust. For allergen levels below the limit of detection (9.7 percent for Der p1, 5.5 percent for Der f1, and 0.2 percent for Fel d1), the mean value between zero and the limit of detection was used.

Questionnaire and Interview

The prevalence of diseases and symptoms and potential explanatory and confounding factors were assessed by a questionnaire giv-

en to the parents that included the questions of the International Study of Asthma and Allergies in Childhood,¹⁰ as described previously.⁶ Farmers' children were defined as children whose parents answered "yes" to the question "Does your child live on a farm?" In an interview with the parents as part of the home visit, we obtained details of the timing of the child's exposure to stables and to farm milk. Exposure to farming during the first year of life was defined as exposure to stables during the first year of life, consumption of milk directly from the farm during the first year of life, or both.

Testing for Specific IgE in Serum

The level of specific IgE against airborne allergens in all serum samples was measured by fluorescence enzyme immunoassay in a central laboratory (University Children's Hospital Charité, Berlin). Atopy was defined by at least one positive test for specific IgE indicating a titer of at least 3.5 kU per liter for one or more of the six airborne allergens (house dust mites, storage mites, grass pollen, birch pollen, cat dander, and cow epithelium).

Assessment of Cytokine Production by Peripheral-Blood Leukocytes

Venous blood was drawn at school from all 812 children. Heparinized blood was diluted in a ratio of 1:8 in RPMI culture medium supplemented with 10 percent heat-inactivated fetal-calf serum to a final volume of 1 ml. Cells were stimulated either with $10\ \mu\text{g}$ of lipopolysaccharide per milliliter for 24 hours or with staphylococcal enterotoxin B for 72 hours at 37°C , in an environment of 5 percent carbon dioxide in humidified air. Cell-free supernatants were stored at -80°C and shipped to the central laboratory for measurement of interferon- γ (limit of detection, 16 pg per milliliter), tumor necrosis factor α (limit of detection, 16 pg per milliliter), interleukin-10 (limit of detection, 8 pg per milliliter), and interleukin-12 (limit of detection, 8 pg per milliliter) by commercially available enzyme-linked immunosorbent assays (OptEIA, Pharmingen). Each sample was tested in duplicate by the serial dilution of a standard supplied by the company with a known cytokine level. Differential blood counts were also performed, and cytokine production was expressed in picograms per 1 million peripheral-blood leukocytes. To ensure consistent performance in sampling and culture procedures, laboratory personnel in the study centers participated in a one-week training and certification program.

Statistical Analysis

Endotoxin levels were \log_{10} -transformed. Multivariate logistic-regression analyses, in which the endotoxin level was treated as a continuous variable, were performed with SAS software,¹¹ with adjustment for age, sex, study area, family history of asthma and hay fever, educational level of the parents, and number of older siblings (the basic model). In addition, potential confounding by farming status, exposure to farming during the first year of life, exposure to cats or dogs during the first year of life, and allergen levels (log-transformed values for Der f1, Der p1, and Fel d1) was evaluated. We included an interaction term to assess whether the effect of endotoxin on asthma and wheeze in children with atopic sensitization (a specific IgE level of at least 0.35 kU per liter) would be different from the effect in children without atopic sensitization.

To evaluate potential threshold values or other nonlinearity in the relation between exposure and response, S-Plus software was used to perform local nonparametric smoothing.¹² The logit of the rates of symptoms was expressed as a continuous function of endotoxin level, obtained by local nonparametric smoothing with control for the covariates mentioned above. The smoothing parameter for each model was determined on the basis of Akaike's information criterion.¹² In the same way, the association between endotoxin levels and cytokine response was assessed. Cytokine levels were log-transformed, and the association of these levels with the level of endo-

toxin exposure was expressed as the ratio of the covariate-adjusted geometric mean cytokine level in children in the highest quartile of endotoxin exposure to the mean level in children in the lowest quartile. The regression analyses were repeated with a restricted sample of children from nonfarming households with adjustment for known allergy-avoidance measures (removal of pets or carpets because of allergies in the family), exposure to cats or dogs during the first year of life, and exposure to farming during the first year of life.

RESULTS

Complete data were available for 812 children, 319 from farming families and 493 from nonfarming families. The mean (\pm SD) age was 9.5 ± 1.2 years. The adjusted odds ratios for asthma and hay-fever symptoms in relation to the farming status did not differ significantly between the group with complete data and the group with only the self-administered questionnaire (0.59 vs. 0.48 for asthma and 0.44 vs. 0.32 for hay-fever symptoms).⁶ The relations between farming status and environmental-exposure variables and health outcomes are shown in Table 1.

The results of multivariate logistic-regression analyses estimating the effect of the mattress endotoxin lev-

el and the endotoxin load on the rates of symptoms and disease, with adjustment for known covariates, are shown in Table 2. The data are presented as adjusted odds ratios for symptoms or disease with an increase from the lowest quartile to the highest quartile of endotoxin exposure. Current endotoxin exposure showed a strong inverse association with hay fever, hay-fever symptoms, and atopic sensitization. Smoothed plots of the prevalence of hay fever, hay-fever symptoms, and atopic sensitization in relation to the level of endotoxin exposure, with control for covariates, showed a largely monotonic decrease in prevalence with an increasing endotoxin load (Fig. 1). Similar results were obtained in analyses in which the endotoxin level was used as the exposure variable (data not shown).

An inverse relation was also found between the level of endotoxin exposure and the capacity of peripheral-blood leukocytes to produce inflammatory and regulatory cytokines after stimulation with lipopolysaccharide (Fig. 2). The associations between endotoxin exposure (in endotoxin units per square meter) and the production of tumor necrosis factor α , interferon- γ , interleukin-10, and interleukin-12, expressed

TABLE 1. ENVIRONMENTAL EXPOSURE AND PREVALENCE OF HEALTH OUTCOMES, ACCORDING TO FARMING STATUS.*

VARIABLE	CHILDREN FROM FARMING HOUSEHOLDS (N=319)	CHILDREN FROM NONFARMING HOUSEHOLDS (N=493)	P VALUE
	geometric mean exposure (5th-95th percentile)		
Environmental exposure			
Endotoxin level (units/mg of dust)	37.8 (14.4-88.9)	22.8 (8.2-62.9)	<0.001
Endotoxin load (units/m ² of mattress surface area)	29,897 (5452-157,208)	14,456 (2915-75,730)	<0.001
Der f1 (ng/g of dust)	528.7 (5-51,990)	610.3 (5-54,160)	0.54
Der p1 (ng/g of dust)	7,092.4 (133-104,110)	1,417.1 (5-104,060)	<0.001
Fel d1 (ng/g of dust)	5,405.6 (356-144,600)	5,744.1 (204-434,460)	0.69
no. [% (95% CI)]			
Health outcomes			
Hay fever	13 (4.1 [1.9-6.2])	52 (10.5 [7.8-13.5])	<0.001
Sneezing and itchy eyes during previous yr	19 (6.0 [3.3-8.7])	62 (12.6 [9.7-16.0])	0.002
Atopic sensitization	55 (17.2 [13.1-21.4])	116 (23.5 [19.8-27.3])	0.03
Atopic asthma	10 (3.1 [1.2-5.0])	29 (5.9 [3.8-8.0])	0.07
Nonatopic asthma	5 (1.6 [0.2-2.9])	13 (2.6 [1.2-5.0])	0.31
Atopic wheeze	15 (4.7 [2.4-7.0])	29 (5.9 [3.8-8.0])	0.47
Nonatopic wheeze	5 (1.6 [0.2-2.9])	30 (6.1 [4.0-8.2])	0.002

*Children were considered to have hay fever if their parents reported a physician's diagnosis of hay fever; to have had sneezing and itchy eyes (symptoms of hay fever) during the previous year if their parents gave a positive response to a question about these symptoms; to have atopic sensitization if they had a specific IgE titer of at least 3.5 kU per liter; to have atopic asthma if their parents reported a physician's diagnosis of asthma or if they had recurrent asthmatic obstruction of the airway or spastic bronchitis and a specific IgE titer of at least 0.35 kU per liter; to have nonatopic asthma if their parents reported a physician's diagnosis of asthma or if they had recurrent asthmatic obstruction of the airway or spastic bronchitis and a specific IgE titer of less than 0.35 kU per liter; to have atopic wheeze if their parents reported that they had had wheezing or whistling in the chest during the previous 12 months and they had a specific IgE titer of at least 0.35 kU per liter; and to have nonatopic wheeze if their parents reported that they had had wheezing or whistling in the chest during the previous 12 months and they had a specific IgE titer of less than 0.35 kU per liter. CI denotes confidence interval, Der f1 *Dermatophagoides farinae*, Der p1 *D. pteronyssinus*, and Fel d1 *Felis domesticus*.

TABLE 2. ASSOCIATIONS BETWEEN CURRENT ENDOTOXIN EXPOSURE (LEVEL AND LOAD) AND ASTHMA, WHEEZE, HAY FEVER, AND ATOPIC SENSITIZATION IN THE TOTAL SAMPLE AND IN THE SUBGROUP OF CHILDREN FROM NONFARMING HOUSEHOLDS.

HEALTH OUTCOME	TOTAL SAMPLE (N=812)		CHILDREN FROM NONFARMING HOUSEHOLDS (N=493)	
	ENDOTOXIN LEVEL	ENDOTOXIN LOAD	ENDOTOXIN LEVEL	ENDOTOXIN LOAD
	adjusted odds ratio (95% CI)*			
Hay fever	0.58 (0.39-0.85)†	0.53 (0.35-0.81)†	0.79 (0.52-1.19)	0.56 (0.33-0.95)†
Sneezing and itchy eyes during previous yr	0.61 (0.43-0.86)†	0.50 (0.34-0.72)†	0.70 (0.47-1.05)	0.46 (0.28-0.76)†
Atopic sensitization‡	0.78 (0.60-1.01)	0.76 (0.58-0.98)†	0.80 (0.59-1.08)	0.73 (0.51-1.04)
Atopic asthma	0.73 (0.44-1.19)	0.48 (0.28-0.81)†	0.68 (0.39-1.19)	0.52 (0.25-1.07)
Nonatopic asthma	1.25 (0.62-2.51)	1.13 (0.57-2.26)	1.29 (0.62-2.68)	1.00 (0.46-2.21)
Atopic wheeze	0.89 (0.57-1.39)	0.62 (0.39-0.99)†	0.79 (0.46-1.33)	0.64 (0.33-1.25)
Nonatopic wheeze	0.97 (0.58-1.61)	1.14 (0.68-1.90)	1.36 (0.86-2.14)	1.82 (1.04-3.18)†

*Odds ratios are for the occurrence of the given symptom or disease with an increase in the endotoxin measure from the lowest quartile to the highest quartile; analyses were adjusted for age, sex, study area, family history of asthma or hay fever, educational level of the parents, and number of older siblings. The analysis of the subgroup of children from nonfarming households was also adjusted for allergen-avoidance measures, exposure to pets during the first year of life, exposure to stables during the first year of life, and consumption of milk directly from a farm during the first year of life.

†P<0.05 for the comparison between children in the lowest quartile of endotoxin exposure and children in the highest quartile.

‡Atopic sensitization was defined by a specific IgE titer of at least 3.5 kU per liter.

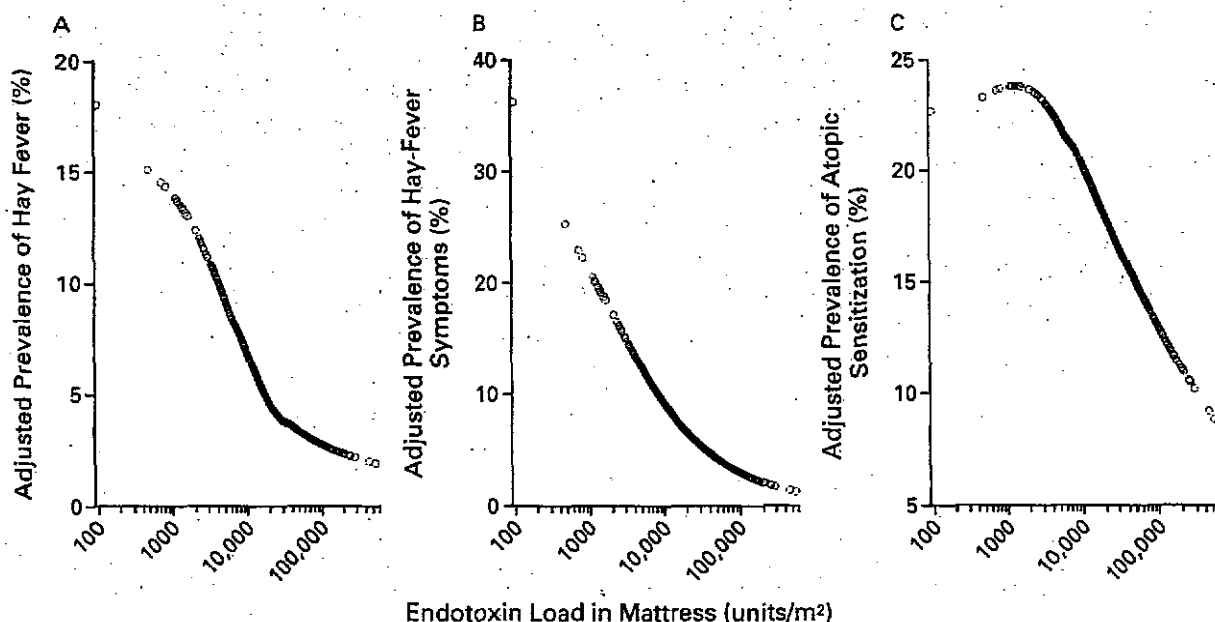


Figure 1. Smoothed Plots of the Prevalence of Hay Fever (Panel A), Hay-Fever Symptoms (Panel B), and Atopic Sensitization (Panel C) in Relation to the Log-Transformed Endotoxin-Load Values.

The analyses controlled for age, sex, study area, family history of asthma and hay fever, educational level of the parents, and number of siblings. For each outcome, there was a monotonic decrease with increasing endotoxin load. A smoothing span of 0.9 was used for all three graphs.

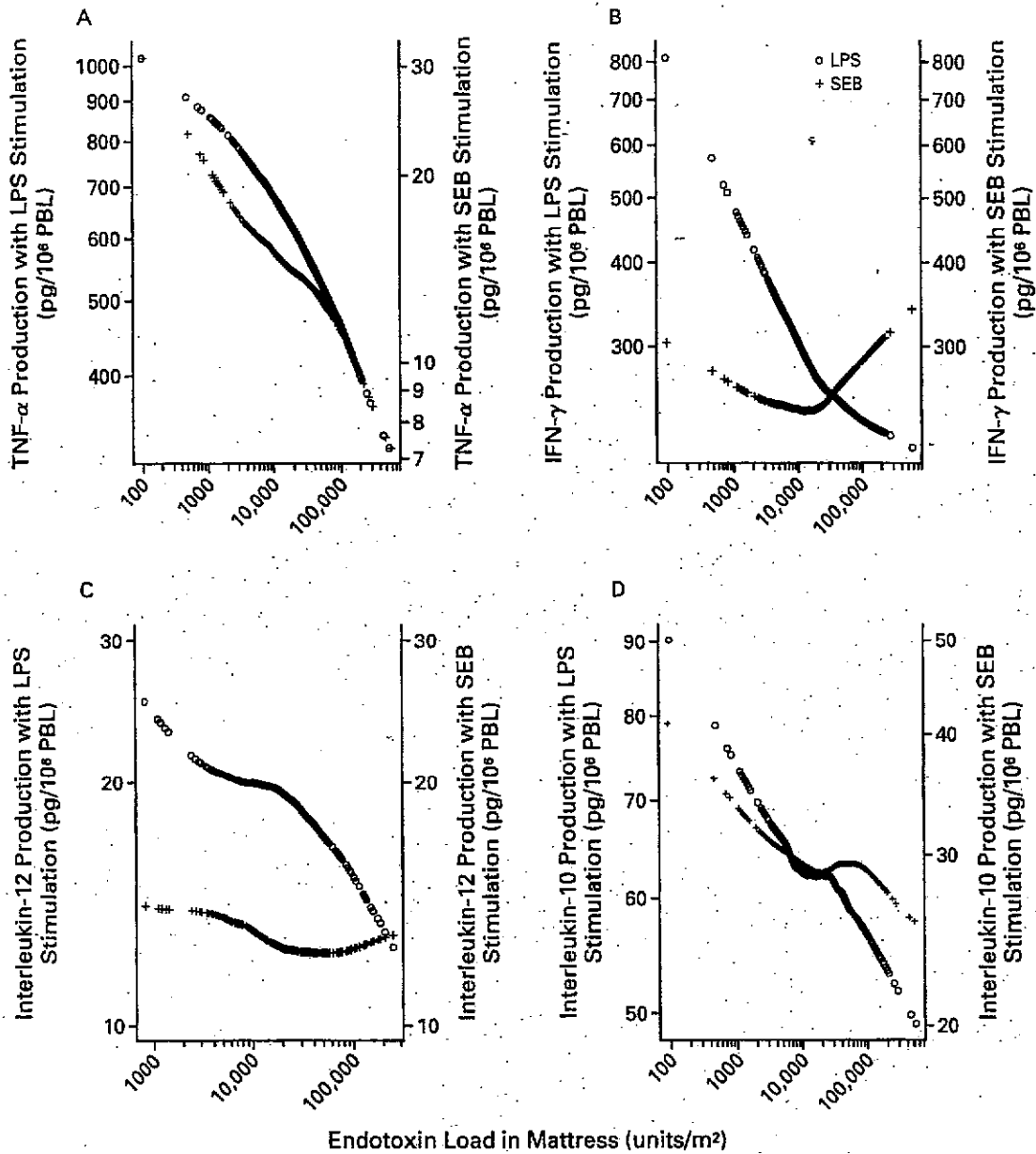


Figure 2. Smoothed Plots of the Log-Transformed Capacity of Peripheral-Blood Leukocytes (PBL) to Produce Tumor Necrosis Factor α (TNF- α) (Panel A), Interferon- γ (IFN- γ) (Panel B), Interleukin-12 (Panel C), and Interleukin-10 (Panel D) after Stimulation with Lipopolysaccharide (LPS) or Staphylococcal Enterotoxin B (SEB) in Relation to the Log-Transformed Endotoxin-Load Values.

Analyses were controlled for age, sex, study area, family history of asthma and hay fever, educational level of the parents, and number of siblings; the analysis shows an inverse relation between the level of endotoxin exposure and cytokine response, except in the case of the production of IFN- γ after SEB stimulation. A smoothing span of 0.9 was used for all four graphs.

as ratios of the mean level of cytokine production for children in the highest quartile of endotoxin exposure to the mean level for children in the lowest quartile, were 0.81 (95 percent confidence interval, 0.74 to 0.89), 0.80 (95 percent confidence interval, 0.70 to 0.92), 0.93 (95 percent confidence interval, 0.81 to 1.07), and 0.87 (95 percent confidence interval, 0.77 to 0.98), respectively. The corresponding results after stimulation with staphylococcal enterotoxin B were 0.83 (95 percent confidence interval, 0.74 to 0.93), 1.05 (95 percent confidence interval, 0.95 to 1.17), 0.97 (95 percent confidence interval, 0.84 to 1.11), and 0.96 (95 percent confidence interval, 0.86 to 1.06), respectively.

The association between endotoxin exposure and wheeze during the past year showed a different exposure-response pattern. There was a strong negative association for atopic wheeze and asthma, whereas for nonatopic wheeze and asthma, there was a nonsignificant trend toward increasing prevalence with increases in the current level of endotoxin exposure (Table 2 and Fig. 3). However, the term for the interaction between the level of endotoxin exposure and atopic status did not reach statistical significance. Exposure to farming in the first year of life showed a strong inverse association with all health outcomes, including non-

atopic wheeze and asthma, independently of the current level of endotoxin exposure (Table 3). Additional adjustment for other potential confounders, including the levels of allergens (Der f1, Der p1, and Fel d1) in mattress dust, farming status, exposure to pets during the first year of life, and exposure to farming during the first year of life, did not change the results. To evaluate whether the results might be generalized to a nonfarming population and to adjust for potential uncontrolled confounding associated with a farming lifestyle, we restricted the sample to children from nonfarming households and also adjusted for exposure to stables and consumption of milk directly from the farm during the first year of life. Again, strong negative associations — albeit not all statistically significant (probably because of the sample size) — between the level of endotoxin exposure and atopic outcomes were observed, whereas positive associations were found for nonatopic wheeze (Table 2).

DISCUSSION

These findings suggest that environmental exposure to microbial products, as measured by the endotoxin levels in mattress dust, is associated with a significant decrease in the risk of hay fever, atopic sensitization, atopic asthma, and atopic wheeze in child-

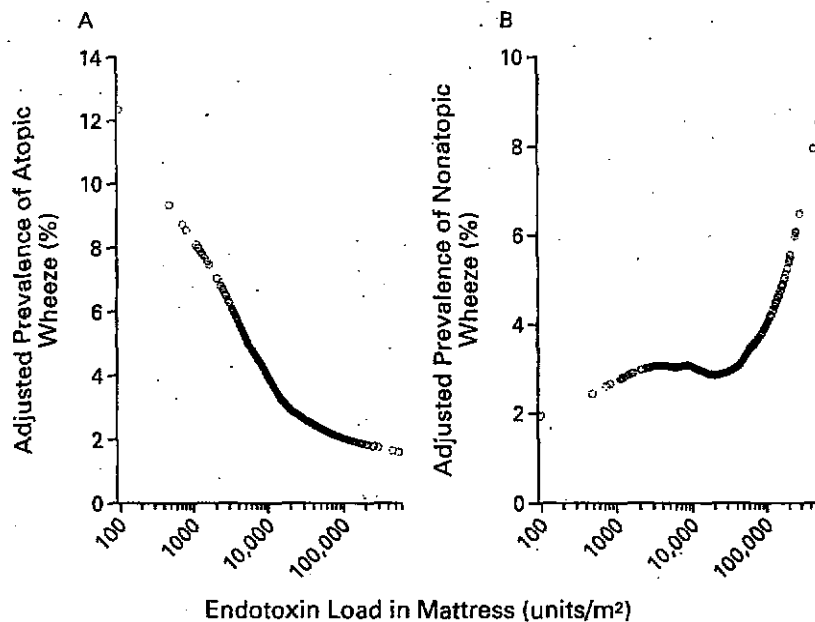


Figure 3. Smoothed Plots of the Prevalence of Atopic Wheeze (Panel A) and Nonatopic Wheeze (Panel B) in Relation to the Log-Transformed Endotoxin-Load Values.

The analyses were controlled for age, sex, study area, family history of asthma and hay fever, educational level of the parents, and number of siblings. There was a negative association for atopic wheeze, whereas for nonatopic wheeze, there was a nonsignificant positive trend with increasing levels of current endotoxin exposure. For Panel A, a smoothing span of 0.9 was used; for Panel B, a span of 0.5 was used.

TABLE 3. ASSOCIATION OF THE PREVALENCE OF SYMPTOMS AND DISEASE WITH THE CURRENT ENDOTOXIN LOAD AND EXPOSURE TO FARMING DURING THE FIRST YEAR OF LIFE.*

HEALTH OUTCOME	EXPOSURE TO FARMING DURING THE FIRST YEAR†	CURRENT ENDOTOXIN EXPOSURE‡
	odds ratio (95% CI)	
Hay fever	0.26 (0.13-0.52)§	0.61 (0.40-0.95)§
Sneezing and itchy eyes after 1 yr of age	0.55 (0.31-0.97)§	0.53 (0.36-0.77)§
Atopic sensitization¶	0.45 (0.30-0.68)§	0.83 (0.63-1.09)
Atopic asthma	0.42 (0.18-0.96)§	0.52 (0.30-0.90)§
Nonatopic asthma	0.48 (0.16-1.41)	1.22 (0.60-2.46)
Atopic wheeze	0.59 (0.28-1.23)	0.66 (0.41-1.07)
Nonatopic wheeze	0.43 (0.19-0.97)§	1.23 (0.73-2.06)

*Odds ratios are for the occurrence of the given symptom or disease with an increase in exposure to farming or the endotoxin load from the lowest quartile to the highest quartile. Analyses were adjusted for age, sex, study area, family history of asthma or hay fever, educational level of the parents, and number of older siblings.

†Odds ratios were also adjusted for current endotoxin exposure.

‡Odds ratios were also adjusted for exposure to farming during the first year of life.

§P ≤ 0.05 for the comparison between children in the lowest quartile of the exposure variable and those in the highest quartile.

¶Atopic sensitization was defined by a specific IgE titer of at least 3.5 kU per liter.

hood. This protective effect was observed in children from farming and nonfarming households, indicating that even the lower levels of exposure that occur in nonfarming environments may favorably influence the risk of atopic diseases in childhood.

The mechanisms by which endotoxin exposure may protect against the development of atopic immune responses and diseases are not fully understood. Our findings suggest that by the time a child reaches school age, high levels of environmental exposure to endotoxin have resulted in a marked suppression of the capacity for cytokine production in response to activation of the innate immune system. Whereas lipopolysaccharide stimulation triggers an innate immune response by activating mainly antigen-presenting cells, staphylococcal enterotoxin B also activates T cells, resulting in a somewhat different pattern of cytokine production. Reduced responsiveness to stimulation with lipopolysaccharide after previous stimulation with lipopolysaccharide is a phenomenon referred to in the literature as lipopolysaccharide tolerance.^{13,14} Our results suggest that such a down-regulation occurs in vivo as a consequence of long-term exposure to environmental endotoxin. Whether this down-regulation is merely a biologic marker of the exposure or is causally related to the decreased rate of atopy cannot be determined on the basis of our data; it is an area in which further exploration is needed. It has been suggested that the innate immune response has an in-

structive role in adaptive immunity.¹⁵ Differential expression of lipopolysaccharide receptors in children from farming and nonfarming households has recently been reported,¹⁶ suggesting that the innate immune system responds to the high microbial burden of the farming environment.

Although only current endotoxin exposure was measured, the levels are likely to reflect long-term exposure. Therefore, long-term, high-level environmental exposure may favor a state of tolerance,¹⁴ which may prevent the development of allergic immune responses. We demonstrated that exposure during the first year of life to stables and other aspects of farm life that are likely to reflect exposure to microbial products has a strong protective effect against the occurrence of asthma and atopy at school age. However, independent of and in addition to this effect, endotoxin exposure at school age was associated with a markedly decreased risk of atopic outcomes. This protective effect was also seen in children with no exposure to farming whose mattress endotoxin levels were similar to levels found in urban homes in the Netherlands¹⁷ and urban areas in the United States,^{18,19} suggesting that exposure to ubiquitous microbial products strongly affects the development of atopy and childhood asthma. The increase in the frequency of asthma in inner-city areas of the United States, by contrast, may be related to other types of environmental exposure.

The protective effect of endotoxin exposure at school age was observed only for atopic wheeze and asthma, not for nonatopic wheeze. Childhood asthma is a complex syndrome with multiple illnesses involving wheezing that develop during the infant, toddler, school-age, and adolescent years, as has been shown in several long-term, prospective surveys in which children were followed from birth to adolescence and adulthood.²⁰⁻²² Although, in many cases, asthma is associated with atopic sensitization to a variety of allergens, illnesses involving wheezing also occur in the absence of increased IgE responses. Variations in genetic background, environmental factors, and the interplay among them are likely to account for the varying clinical presentations of wheeze. In studies of human exposure²³ and in studies of animals,²⁴ endotoxin has been shown to induce airway hyperresponsiveness in healthy, nonatopic subjects but to decrease airway responsiveness in sensitized animals, supporting the notion that the effect is modified by atopy, as we observed. In our study, exposure to farming in the first year of life had a protective effect against nonatopic wheeze, whereas exposure to endotoxin at school age was related to an increased risk. Therefore, not only an exposed subject's atopic status but also the timing of the exposure determines its beneficial or detrimental effects.

Endotoxin was measured in mattress dust, since children come into close contact with the microbial environment of their beds while sleeping and since the reproducibility of repeated endotoxin measurements is greater for dust from the bed than for dust from the floor.²⁵ Endotoxin measurements in dust from the bed have been reported to show little variation over time, with nonsignificant differences over a six-month period.¹⁹ Environmental endotoxin levels are therefore likely to reflect longer-term exposure to microbial compounds. However, the cross-sectional design of our study limited our ability to determine precisely the duration of exposure represented by current endotoxin measurements, and prospective studies are clearly needed. We did not assess other bacterial components, such as nonmethylated cytidine phosphate guanosine dinucleotides specific for prokaryotic DNA (CpG motifs) or cell-wall components from atypical mycobacteria or gram-positive bacteria such as lipoteichoic acid, which are known to affect immune responses in ways similar to that of endotoxin.^{26,27} The observed protective effect associated with endotoxin levels in mattress dust is therefore likely to reflect the effect of exposure to a much broader spectrum of microbial compounds than gram-negative bacteria alone.

The results of our study indicate that environmental exposure to microbial products as assessed by the measurement of endotoxin levels in mattress dust is associated with the development of tolerance toward

ubiquitous allergens found in natural environments. Mechanisms relating to the recognition of these microbial compounds by the innate immune system and the regulation of the resulting inflammatory responses through adaptive immunity are likely to be of key importance for the development of atopic illnesses such as hay fever and childhood asthma and wheeze. These insights may foster the generation of novel strategies aimed at the prevention of these diseases.

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REFERENCES

1. D'Amato G, Liccardi G, D'Amato M. Environmental risk factors (outdoor air pollution and climatic changes) and increased trend of respiratory allergy. *J Invest Allergol Clin Immunol* 2000;10:123-8.
2. Gold DR. Environmental tobacco smoke, indoor allergens, and childhood asthma. *Environ Health Perspect* 2000;108:Suppl 4:643-51.
3. Wahn U, Lau S, Bergmann R, et al. Indoor allergen exposure is a risk factor for sensitization during the first three years of life. *J Allergy Clin Immunol* 1997;99:763-9.
4. Strachan DP. Family size, infection and atopy: the first decade of the "hygiene hypothesis." *Thorax* 2000;55:Suppl 1:S2-S10.
5. Delves PJ, Roitt IM. The immune system. *N Engl J Med* 2000;343:37-49.
6. Riedler J, Braun-Fahrlander C, Eder W, et al. Exposure to farming in early life and development of asthma and allergy: a cross-sectional survey. *Lancet* 2001;358:1129-33.
7. Kabesch M, Schaal W, Nicolai T, von Mutius E. Lower prevalence of asthma and atopy in Turkish children living in Germany. *Eur Respir J* 1999;13:577-82.
8. Phase II modules of the International Study of Asthma and Allergies in Childhood (ISAAC). Münster, Germany: ISAAC, 1998.
9. Hollander A, Heederik D, Versloot P, Douwes J. Inhibition and enhancement in the analysis of airborne endotoxin levels in various occupational environments. *Am Ind Hyg Assoc J* 1993;54:647-53.
10. Asher MI, Keil U, Anderson HR, et al. International Study of Asthma and Allergies in Childhood (ISAAC): rationale and methods. *Eur Respir J* 1995;8:483-91.
11. SAS, release 8.02. Cary, N.C.: SAS Institute, 2001.
12. S-PLUS. Seattle: MathSoft, 2000.
13. West MA, Heagy W. Endotoxin tolerance: a review. *Crit Care Med* 2002;30:Suppl:S64-S73.
14. Schade U, Schlegel J, Hofmann K, Brade H, Flach R. Endotoxin tolerant mice produce an inhibitor of tumor necrosis factor synthesis. *J Endotoxin Res* 1996;3:455-62.
15. Fearon DT, Locksley RM. The instructive role of innate immunity in the acquired immune response. *Science* 1996;272:50-3.
16. Lauener R, Birchler T, Adamski J, et al. Expression of CD14 and toll-like receptor 2 in farmers' and non-farmers' children. *Lancet* 2002;360:465-6.
17. Douwes J, Zuidhof A, Dockes G, et al. (1-3)-Beta-D-glucan and endotoxin in house dust and peak flow variability in children. *Am J Respir Crit Care Med* 2000;162:1348-54.
18. Park JH, Gold DR, Spiegelman DL, Burge HA, Milton DK. House dust endotoxin and wheeze in the first year of life. *Am J Respir Crit Care Med* 2001;163:322-8.

19. Gereda JE, Leung DY, Thatayatikom A, et al. Relation between house-dust endotoxin exposure, type 1 T-cell development, and allergen sensitisation in infants at high risk of asthma. *Lancet* 2000;355:1680-3.
20. Strachan DP, Butland BK, Anderson HR. Incidence and prognosis of asthma and wheezing illness from early childhood to age 33 in a national British cohort. *BMJ* 1996;312:1195-9.
21. Silverman M, Wilson N. Wheezing phenotypes in childhood. *Thorax* 1997;52:936-7.
22. Martinez FD, Wright AL, Taussig LM, et al. Asthma and wheezing in the first six years of life. *N Engl J Med* 1995;332:133-8.
23. Rylander R, Bake B, Fischer JJ, Helander IM. Pulmonary function and symptoms after inhalation of endotoxin. *Am Rev Respir Dis* 1989;140:981-6.
24. Tulic MK, Walc JL, Holt PG, Sly PD. Modification of the inflammatory response to allergen challenge after exposure to bacterial lipopolysaccharide. *Am J Respir Cell Mol Biol* 2000;22:604-12.
25. Park JH, Spiegelman DL, Burge HA, Gold DR, Chew GL, Milton DK. Longitudinal study of dust and airborne endotoxin in the home. *Environ Health Perspect* 2000;108:1023-8.
26. Abou-Zeid C, Gares MP, Inwald J, et al. Induction of a type 1 immune response to a recombinant antigen from *Mycobacterium tuberculosis* in *Mycobacterium vaccae*. *Infect Immun* 1997;65:1856-62.
27. Cleveland MG, Gorham JD, Murphy TL, Thomanen E, Murphy KM. Lipoteichoic acid preparations of gram-positive bacteria induce interleukin-12 through a CD14-dependent pathway. *Infect Immun* 1996;64:1906-12.

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