

ORIGINAL ARTICLE

Food Allergy and Gastrointestinal Disease

Endotoxin, food allergen sensitization, and food allergy: A complementary epidemiologic and experimental study

Angela Tsuang MD, MSc¹  | Alexander Grishin PhD¹ | Galina Grishina MS¹ |Anh N. Do PhD² | Joanne Sordillo ScD³  | Ginger L. Chew ScD⁴ |Supinda Bunyavanich MD, MPH^{1,2} 

¹Division of Allergy & Immunology, Department of Pediatrics, Icahn School of Medicine at Mount Sinai, New York, NY, USA

²Department of Genetics & Genomic Sciences, Icahn School of Medicine at Mount Sinai, New York, NY, USA

³Department of Population Medicine, Harvard Medical School and Harvard Pilgrim Health Care Institute, Boston, MA, USA

⁴Department of Environmental Health Sciences, Mailman School of Public Health, Columbia University, New York, NY, USA

Correspondence

Supinda Bunyavanich, Division of Allergy & Immunology, Department of Pediatrics, Icahn School of Medicine at Mount Sinai, 1425 Madison Avenue, Box 1498, New York, NY 10029, USA.
Email: supinda@post.harvard.edu

Funding information

Funding for this project was awarded in part by the Pediatric Scholars Program from the Department of Pediatrics, Icahn School of Medicine at Mount Sinai. AT is supported in part by The Louis and Rachel Rudin Foundation, Inc SB, AG, GG, and AD are supported in part by NIH R01 AI118833 and U19 AI136053.

Abstract

Background: Household endotoxin levels have been variably associated with risk for asthma and atopy.

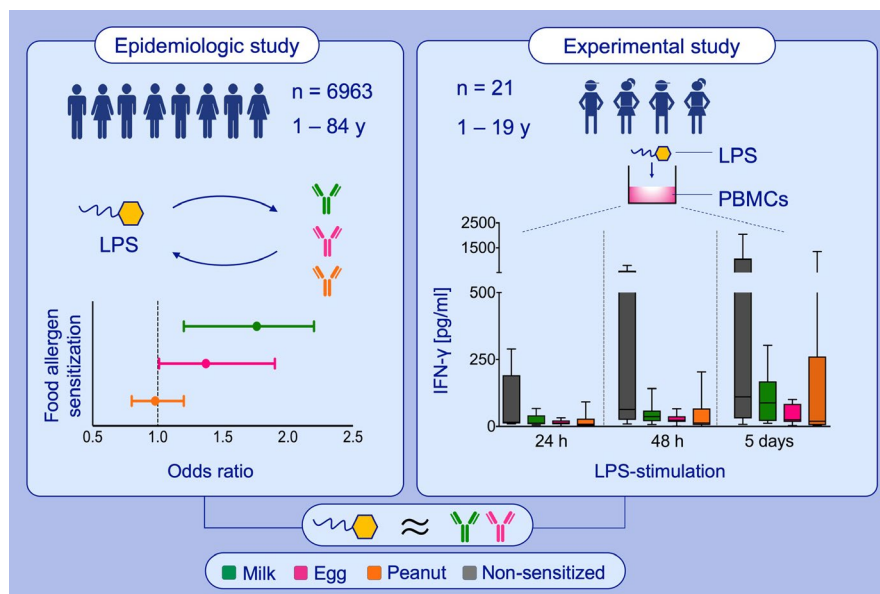
Methods: We studied participants from the 2005-2006 National Health and Nutrition Examination Survey (NHANES, $n = 6963$), a large cohort representative of the US population (aged 1-84 years). We built logistic regression models to test for associations between house dust endotoxin and sensitization to specific foods (milk, egg, and peanut). To experimentally explore the detected epidemiologic associations, peripheral blood mononuclear cells (PBMCs) were collected from 21 children (aged 1-19 years) mono-food allergic (ie, sensitized and clinically reactive) to milk, egg, or peanut and nonallergic controls for stimulation with endotoxin and secreted cytokine measurement. For each food allergy, linear mixed-effects models were built to test the association between endotoxin stimulation and cytokine level.

Results: Among NHANES subjects, the geometric mean household endotoxin level was 15.5 EU/mg (GSE 0.5). Prevalence of food allergen sensitization ($\text{slgE} \geq 0.35 \text{ kU}_A/\text{L}$) varied by food: milk 5.7%, egg 4.0%, and peanut 7.9%. In models adjusted for potential confounders (age, race, country of birth, total people per household, US region, and history of wheezing in the past year), household endotoxin level was associated with sensitization to milk (OR 1.7, 95% CI 1.2-2.1) and egg (OR 1.4, 95% CI 1.01-1.9), but not peanut (OR 0.98, 95% CI 0.8-1.2). Interferon- γ levels of endotoxin-stimulated PBMCs from children allergic to milk or egg, but not peanut, were significantly lower compared to controls in linear mixed-effects models adjusted for repeated measures, experimental variables, age, and inter-individual variability (P -values .007, .018, and .058, respectively). **Conclusion:** Higher household endotoxin is associated with increased odds of milk and egg sensitization. Altered cytokine responsiveness to endotoxin is also observed in PBMCs from individuals with milk and egg allergy.

KEYWORDS

cytokine, endotoxin, food allergy, food sensitization, house dust

Abbreviations: CI, confidence interval; GSE, geometric standard error; IFN, interferon; IL, interleukin; IQR, interquartile range; LPS, lipopolysaccharide; NCHS, National Center for Health Statistics; NHANES, National Health and Nutrition Examination Survey; OR, odds ratio; PBMCs, peripheral blood mononuclear cells; SD, standard deviation; TLR, Toll-like receptor.



GRAPHICAL ABSTRACT

Epidemiologic study of NHANES participants ($n = 6963$) revealed associations between household endotoxin level and sensitization to milk or egg, but not peanut. Complementary experimental study showed attenuated interferon- γ responses from endotoxin stimulation of PBMCs from children with allergy to milk or egg, but not peanut. These complementary findings suggest that endotoxin's effects on food sensitization/allergy may differ by food allergy type.

Abbreviations: NHANES, National Health and Nutrition Examination Survey

1 | INTRODUCTION

The prevalence of food allergy in children has been increasing in recent years and now affects 8% of children.^{1,2} Environmental factors could be contributing to this increase, including household levels of endotoxin. Endotoxins are lipopolysaccharide (LPS) molecules that form a component in the outer cell walls of Gram-negative bacteria. They are found at varying levels in household dust and ambient air.³ Although the relationship between endotoxin and asthma has been frequently studied,^{3–5} there have been fewer studies on endotoxin and food allergy-related outcomes.^{6,7}

Studies of endotoxin and atopic outcomes to date have yielded heterogeneous results. Higher levels of household endotoxin have been associated with increased asthma prevalence and wheezing, suggesting risk from endotoxin exposure.³ In contrast, exposure to endotoxin in early life, particularly in rural areas, has also been associated with lower asthma prevalence.⁵ Other studies of endotoxin level and hay fever or eczema outcomes have shown mixed results.⁴

Within food allergy, the effects reported for endotoxin have also been disparate. In one cohort, higher levels of bedroom endotoxin during the first year of life were found to be protective for the development of overall food allergy and egg allergy.⁶ On the other hand,

a prior study found no significant relationship between endotoxin levels and egg or milk sensitization, with the exception of increased egg sensitization among those 50 years and older with higher household endotoxin levels.⁷

Advancing our understanding of the relationship between endotoxin exposure and food allergy could contribute to prevention efforts and further our mechanistic understanding of environmental influences on food allergy. Here, we present a complementary epidemiologic and experimental study of endotoxin and food allergy outcomes.

We hypothesized that the epidemiologic association between household endotoxin and food allergen sensitization would vary by food allergen. Complementary to this, we also hypothesized that endotoxin stimulation of peripheral blood mononuclear cells from children with food allergy would yield cytokine responses that vary by specific food allergy. We first evaluated the association between house dust endotoxin levels and food allergen sensitization to milk, egg, and peanut among 6963 participants of the National Health and Nutrition Examination Survey (NHANES, $n = 6963$), a large cohort representative of the United States (US) population. We then performed complementary experimental work to assess differences in peripheral blood mononuclear cell (PBMC) response to endotoxin based on food allergy status using PBMCs collected from food-allergic children.

2 | METHODS

2.1 | Epidemiologic study population

We studied participants from the 2005-2006 National Health and Nutrition Examination Survey (NHANES), a US cross-sectional study conducted by the National Center for Health Statistics (NCHS) of the Centers for Disease Control and Prevention. This survey encompassed 10348 US participants, aged 1 year and older. Sampling was designed to be representative of the US population. The study protocol and procedures were approved by the NCHS Institutional Review Board with informed consent obtained from all subjects.⁸ Details on study design and methods can be found on the NHANES website (<http://www.cdc.gov/nchs/nhanes.htm>).⁹

Because prior studies demonstrated variation in food allergen sensitization by US region as well as level of urbanization among NHANES 2005-2006 participants,¹⁰ we obtained access to restricted region-level data for this study by submitting a proposal to the NCHS Research Data Center and then accessing the 2005-2006 NHANES data on-site at the Hyattsville, MD, USA, site of the NCHS. NCHS does not make region-level data publicly available to protect the confidentiality of NHANES participants.

2.2 | Household endotoxin-level measurement

Household endotoxin was collected from each participant's bed and bedroom floor using a standardized protocol and trained technicians.¹¹ A Sanitaire™ Model 3683 vacuum cleaner and Mitest™ Dust Collector (Indoor Biotechnologies, Inc) were used to collect samples. One-square yard of both the bed and adjacent floors were vacuumed for a total of 2 minutes. Dust specimens were sent to a laboratory at the University of Iowa and stored at -80°C , and dust endotoxin levels were measured using Limulus amoebocyte lysate assay. A kinetic chromogenic assay was used to measure dust extracts, and endotoxin from *E coli* O155 was used to establish standard curves.¹¹

House dust endotoxin measurements were available for 6963 NHANES 2005-2006 participants. Endotoxin level in endotoxin units (EU) per milligram of sieved dust (EU/mg) was $\log_{10}$ -transformed for analysis due to its skewed distribution. Summary statistics are reported as the geometric mean per sieved weight of house dust, consistent with many previous studies of endotoxin.^{4,7} While some studies have examined endotoxin load (ie, endotoxin mass per area vacuumed¹²), studies of NHANES and other cohorts^{4,6,7,13-16} have focused on endotoxin mass concentration, which is endotoxin per mass of dust. Perzanowski et al¹² demonstrated that the only allergic health effects (eczema and wheezing) significantly associated with endotoxin were observed with concentration of endotoxin and not load. The following formula was used to calculate the endotoxin amount per sieved dust weight: (Endotoxin EU/mL) $\times$ (1 mL/50 mg dust) = EU/mg dust.¹¹

2.3 | Food allergen sensitization

Serum-specific IgE levels were measured using Thermo Scientific ImmunoCAP Specific IgE.^{17,18} Serum-specific IgE levels for cow's milk, hen's egg white, and peanut were measured for study participants aged 1 year and older. A serum-specific IgE level $\geq 0.35 \text{ kU}_A/\text{L}$ was considered to be positive for food allergen sensitization. Milk, egg, and peanut serum IgE levels were dichotomized for analysis, for example, not milk sensitized (milk serum IgE $< 0.35 \text{ kU}_A/\text{L}$) and milk sensitized (milk serum IgE $\geq 0.35 \text{ kU}_A/\text{L}$). Although food allergen sensitization was measured, clinical symptoms of food allergy were not assessed in NHANES 2005-2006 participants.

2.4 | Statistical analyses of the epidemiologic data

To account for the complex sampling and survey design, survey weights and strata were used in accordance with the NHANES Analytic and Reporting Guidelines.¹⁹ Comparisons among the study population were completed using Student's *t* test to compare means and Pearson's chi-squared test to compare proportions.

To assess the relationship between $\log_{10}$ -transformed endotoxin level and food allergen sensitization (each for milk, egg, and peanut), food allergen sensitization was fitted into logistic regression models as the dependent variable with $\log_{10}$ -transformed endotoxin as a continuous independent variable. Three separate models were created with milk, egg, or peanut sensitization as the dependent variable. Based on prior literature, the following variables (assessed by participant questionnaires) were considered as potential confounders: age⁴ (as a continuous variable), gender, race, country of birth, total number of people per household (as a continuous variable), time in the United States, socioeconomic status, region of United States,¹⁰ level of urbanization, and indicators of other atopic disease (history of wheezing in the past year, doctor's diagnosis of asthma, family history of asthma, hay fever symptoms in the past year, doctor's diagnosis of hay fever, an itchy rash coming and going for 6 or more months, and doctor's diagnosis of eczema). Socioeconomic status was represented by a poverty-index ratio (PIR), which is the ratio of income to the family's poverty threshold. A low PIR of <1.00 is below the official poverty threshold.²⁰ A family with a PIR of <1.85 qualifies for US federal food assistance.⁴ The following variables were found to be associated with both endotoxin concentration and food allergen sensitization (ie, confounders), and they were included in the multivariable logistic regression model to further assess the association between endotoxin concentration and specific food allergen sensitization: age, race, country of birth, total people per household, US region, and history of wheezing in the past year.

Analyses were conducted using Stata 11 and 13 (StataCorp).

2.5 | Study population for the experimental study

To complement findings from our epidemiologic analysis of NHANES subjects, we collected peripheral blood samples from healthy, non-atopic children and children who were mono-food allergic to either milk, egg, or peanut (ie, each was singly food allergic to milk only, egg

TABLE 1 Baseline characteristics of the NHANES 2005-2006 subjects

	All	Milk		Egg			Peanut			
	N = 6963	Sensitized N = 350	Nonsensitized N = 5749	P-value	Sensitized N = 248	Nonsensitized N = 5923	P-value	Sensitized N = 489	Nonsensitized N = 5690	P-value
Age										
1-5 y	480 (7.0%)	72 (20.6%)	259 (4.5%)	<.0001	47 (19.0%)	290 (4.9%)	<.0001	27 (5.5%)	313 (5.5%)	.023
6-17 y	1206 (17.6%)	87 (24.8%)	943 (16.4%)		41 (16.7%)	1007 (17.0%)		109 (22.2%)	939 (16.5%)	
≥18 y	5166 (75.4%)	191 (54.6%)	4547 (79.1%)		159 (64.3%)	4632 (78.2%)		354 (72.3%)	4444 (78.1%)	
Gender—Female	3558 (51.1%)	159 (45.4%)	2949 (51.3%)	.17	127 (51.1%)	3021 (51.0%)	.99	175 (35.7%)	2976 (52.3%)	.0006
Race										
Non-Hispanic white	4742 (68.1%)	215 (61.4%)	3996 (69.5%)	.11	177 (71.2%)	4081 (68.9%)	.56	270 (55.2%)	3989 (70.1%)	.0001
Other Hispanic	251 (3.6%)	17 (4.8%)	201 (3.5%)		7 (2.7%)	213 (3.6%)		25 (5.2%)	193 (3.4%)	
Mexican American	655 (9.4%)	35 (10.0%)	540 (9.4%)		19 (7.7%)	557 (9.4%)		61 (12.5%)	518 (9.1%)	
Non-Hispanic black	856 (12.3%)	53 (15.1%)	673 (11.7%)		27 (10.8%)	711 (12.0%)		99 (20.3%)	637 (11.2%)	
Other	453 (6.5%)	30 (8.7%)	351 (6.1%)		19 (7.6%)	367 (6.2%)		34 (6.9%)	353 (6.2%)	
Region										
Northeast	1010 (14.5%)	40 (12.0%)	795 (14.8%)	.014	29 (12.2%)	807 (14.6%)	.71	55 (12.1%)	781 (14.7%)	.20
Midwest	1769 (25.4%)	74 (22.4%)	1364 (25.4%)		62 (26.3%)	1376 (24.9%)		89 (19.5%)	1350 (25.4%)	
South	2033 (29.2%)	138 (41.9%)	1499 (27.9%)		80 (34.0%)	1609 (29.1%)		153 (33.5%)	1541 (29.0%)	
West	2159 (31.0%)	78 (23.7%)	1719 (32.0%)		65 (27.5%)	1736 (31.4%)		160 (34.9%)	1642 (30.9%)	
Urban	4986 (71.6%)	194 (63.4%)	3644 (71.6%)	.037	141 (68.3%)	3738 (71.2%)	.45	342 (77.7%)	3546 (70.6%)	.037
Number of people per household	3.4 (1.6)	3.6 (1.9)	3.3 (1.6)	.028	3.3 (1.6)	3.3 (1.6)	.99	3.7 (1.8)	3.3 (1.6)	.010
Country of birth										
United States	6119 (87.9%)	337 (96.3%)	5018 (87.3%)	.0003	241 (97.2%)	5177 (87.4%)	.0002	417 (85.2%)	5007 (88.0%)	.20
Mexico	313 (4.5%)	5 (1.5%)	276 (4.8%)		5 (2.0%)	278 (4.7%)		31 (6.3%)	256 (4.5%)	
Elsewhere	529 (7.6%)	8 (2.3%)	454 (7.9%)		2 (0.81%)	462 (7.8%)		42 (8.5%)	427 (7.5%)	
Time in the United States ≥ 4 y	4178 (60.0%)	138 (39.3%)	3570 (62.1%)	.084	151 (61.0%)	3643 (61.5%)	.98	235 (48.1%)	3579 (62.9%)	.036
Family PIR < 1.85 (Poverty)	2182 (32.8%)	135 (38.5%)	1839 (32.0%)	.083	79 (31.8%)	1919 (32.4%)	.88	182 (37.1%)	1822 (32.0%)	.24
Wheezing in chest in past year	1128 (16.2%)	79 (22.5%)	937 (16.3%)	.12	63 (25.6%)	971 (16.4%)	.014	118 (24.1%)	916 (16.1%)	.001

Note: Number (%) or Mean (Standard Deviation).

Sensitized = serum-specific IgE ≥ 0.35 kU_A/L; Nonsensitized = serum-specific IgE < 0.35 kU_A/L.

Means were compared with Student's t test, and proportions were compared with Pearson's chi-squared test.

Abbreviations: PIR, poverty-income ratio (the ratio of income to the family's poverty threshold).²⁰

P < .05 are in bold.

only, or peanut only) for the experimental studies described below. These children were recruited from pediatrics clinics within the Mount Sinai Health System. The study was approved by the Icahn School of Medicine at Mount Sinai Institutional Review Board, with each participant or legal guardian providing written informed consent. Five subjects were recruited for each food-allergic group (milk, egg, and peanut) along with six nonatopic healthy controls of similar age for a total of 21 subjects. Nonatopic healthy controls had no clinical history of food allergy, allergic rhinoconjunctivitis, atopic dermatitis, asthma, drug allergy, or venom allergy, and all healthy controls were each within 1 year of age of food-allergic subjects. Subjects with food allergy to milk, egg, or peanut were diagnosed by a Mount Sinai pediatric allergist as determined by positive oral food challenge or history of convincing symptoms with positive skin prick testing and/or elevated serum-specific IgE levels for that food. Participants with multiple food allergies or a concurrent diagnosis of drug allergy, venom allergy, or the following conditions active within the past 12 months were excluded: allergic rhinoconjunctivitis, atopic dermatitis, or asthma. None of the selected participants had symptoms or signs of any environmental allergy (ie, no reported symptoms or signs of allergic rhinoconjunctivitis in the past 12 months as determined by a pediatric allergist).

2.6 | Endotoxin stimulation of PBMCs from milk-, egg-, and peanut-allergic subjects

Peripheral blood mononuclear cells (PBMCs) were isolated from each study subject's blood sample by Ficoll-Paque PLUS (GE Healthcare) density centrifugation and stimulated with two concentrations of endotoxin (LPS 10 and 100 ng/mL) and media (RPMI/10%FCS), each in duplicate. Supernatants were collected at 24, 48 hours, and 5 days. The following cytokine levels were measured using a multiplex assay (Millipore™ Luminex®, magnetic beads): interferon (IFN)- γ , IL-6, IL-12, IL-4, and IL-5.

Differences in cytokine response were compared between food-allergic subjects (each for milk, egg, and peanut) and nonallergic healthy controls. For each food allergy, data were analyzed using linear mixed-effects models (xtmixed) to allow for repeated measurements of cytokines for each subject and multiple incubation time periods and endotoxin stimulation concentrations to be included within the same model. Cytokine level was modeled as the dependent variable, and the following were included as independent or fixed-effect variables: food allergy status, subject age, endotoxin concentration (LPS 0 ng/mL [media only], LPS 10 ng/mL, and LPS 100 ng/mL), length of incubation (24, 48 hours, and 5 days), and presence of duplicates. To account for inter-individual differences, subject was included as a random-effects parameter.

3 | RESULTS

3.1 | Study population for the epidemiologic analysis

Baseline characteristics of the NHANES 2005-2006 subjects examined in this study ($n = 6963$) are summarized in Table 1. There

TABLE 2 Household endotoxin levels of NHANES 2005-2006 subjects

	Geometric mean (GSE) in EU/mg ^a
Overall	15.5 (0.50)
Age	
1-5 y	29.6 (1.25)
6-17 y	23.0 (1.37)
≥18 y	13.2 (0.41)
Gender	
Female	15.9 (0.56)
Male	15.2 (0.61)
Race	
Non-Hispanic white	14.8 (0.55)
Other Hispanic	14.2 (2.58)
Mexican American	23.5 (1.17)
Non-Hispanic black	15.2 (1.35)
Other	16.3 (1.14)
Region	
Northeast	14.5 (1.35)
Midwest	14.8 (1.12)
South	13.8 (0.85)
West	18.2 (1.07)
Urbanicity	
Rural	16.3 (0.91)
Urban	15.2 (0.65)
Number of people per household	
1	12.9 (0.69)
2	12.6 (0.95)
3	16.6 (1.20)
4	14.5 (0.70)
≥5	20.9 (1.52)
Country of birth	
United States	15.5 (0.50)
Mexico	22.4 (1.84)
Elsewhere	13.2 (1.19)
Time in the United States	
1-3 y	17.8 (2.06)
4+ y	15.5 (1.67)
Family PIR	
PIR < 1.85 (Poverty)	20.0 (0.81)
PIR ≥ 1.85	13.5 (0.53)

^aEU/mg = endotoxin units per milligram of dust; GSE = geometric standard error of the mean. Measures are consistent with previous studies of endotoxin.^{4,7}

were approximately equal proportions of males and females, and the majority of subjects were adults (75.4%) and living above the poverty-income ratio (67.2%). The subjects were largely non-Hispanic white and non-Hispanic black; most Hispanics identified

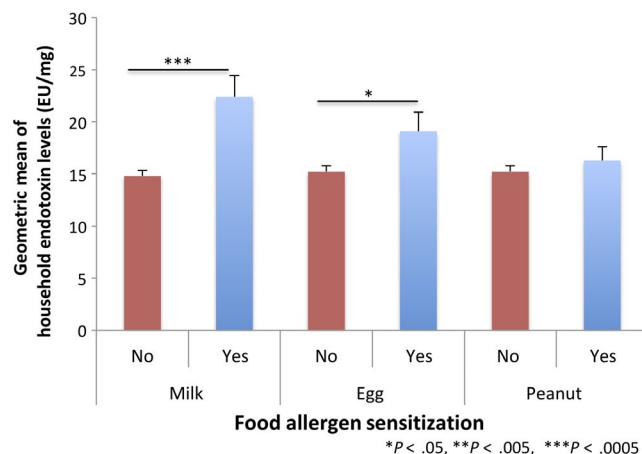


FIGURE 1 Geometric mean of household endotoxin levels by food allergen sensitization among NHANES 2005-2006 subjects ($n = 6963$). Food allergen sensitization defined as serum-specific IgE ≥ 0.35 kU_A/L. Error bars depict geometric standard error

as Mexican American. The number of people per household ranged from 1 (10.7%) and 2 (25.4%) to 3 (20.2%), 4 (20.1%), and ≥ 5 (23.7%). Subjects lived in all regions of the United States, with 71.6% based in urban areas.

3.2 | Household endotoxin levels

Among all subjects, the geometric mean endotoxin level was 15.5 EU/mg (geometric standard error [GSE] 0.50). Household endotoxin levels differed by age, with the highest geometric mean endotoxin level among children aged 1-5 years, followed by older children aged 6-17 years, and then adults aged ≥ 18 years ($P < .0001$) (Table 2). Racial and regional differences were observed, with the higher average household endotoxin levels among those of Mexican American background compared to Non-Hispanic whites ($P < .0001$), and among subjects born in Mexico relative to those born in the United States ($P = .001$) (Table 2). Within the United States, the western region had significantly higher household endotoxin levels compared to the northeast ($P = .045$). Lower socioeconomic status (poverty-income ratio < 1.85) and households

with 5 or more people also had higher average endotoxin levels ($P < .0001$). Because endotoxin has been previously associated with asthma, we also evaluated for wheeze in the past year among the study subjects, finding that those who had wheezed in the past year had higher household endotoxin levels (geometric mean 17.8 EU/mg, GSE 1.27) than those who had not wheezed in the past year (geometric mean 15.2 EU/mg, GSE 0.53) ($P = .04$). There were no differences in household endotoxin level by sex, urbanicity, or time living in the United States (Table 2).

3.3 | Food allergen sensitization

13.5% of subjects were sensitized to at least one food allergen (specific IgE ≥ 0.35 kU_A/L) among milk, egg, and peanut, with 5.7% sensitized to milk, 4.0% sensitized to egg, and 7.9% sensitized to peanut. As expected, sensitization to each of these food allergens was significantly associated with age (milk $P < .0001$, egg $P < .0001$, and peanut $P = .023$), with sensitization most prevalent among children aged 1-5 years (22.0%, 14.2%, and 8.0%) and least prevalent among adults (4.1%, 3.4%, and 7.5%) respectively for milk, egg, and peanut.

Additional baseline characteristics of the subjects stratified by milk, egg, and peanut sensitization are summarized in Table 1. In addition to age ($P < .0001$), milk sensitization was also associated with birth in the United States ($P = .0003$), higher number of people per household ($P = .028$), and residence in the southern United States ($P = .014$) and in a rural area ($P = .037$) (Table 1). There were no significant differences in milk sensitization by gender, race, time in the United States, or poverty level. Similar to milk, egg sensitization was also associated with age ($P < .0001$) and birth in the United States ($P = .0002$), but not with other characteristics. Peanut sensitization was associated with multiple demographic factors, including age ($P = .023$), male gender ($P = .0006$), higher number of people per household ($P = .010$), less time living in the United States ($P = .036$), and residence in an urban area ($P = .037$). Racial categories including "Other Hispanic," "Mexican American," and "Non-Hispanic Black" had a higher proportion of peanut-sensitized patients ($P = .0001$).

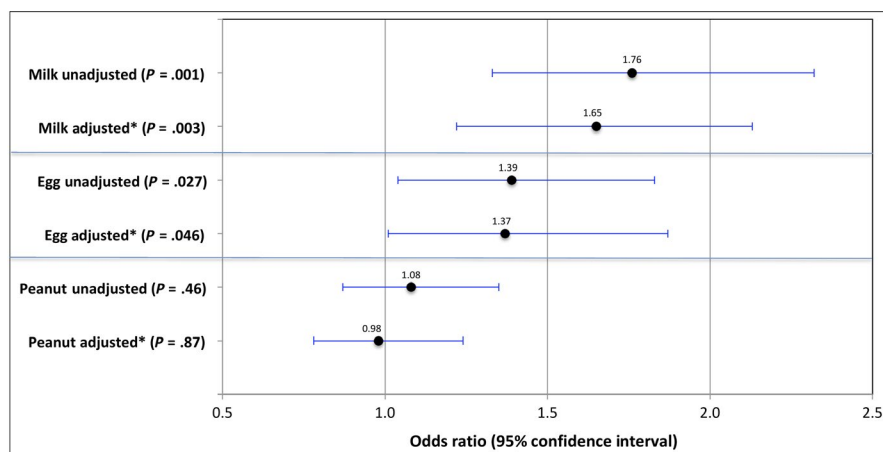


FIGURE 2 Odds of food allergen sensitization with each 10-fold increase in household endotoxin level among NHANES 2005-2006 subjects ($n = 6963$). *Adjusted for: age, race, birth country, total people in household, region of United States, and wheezing in the past year

TABLE 3 Characteristics of the experimental study population

	Healthy control (n = 6)	Peanut allergic (n = 5)	Milk allergic (n = 5)	Egg allergic (n = 5)
Female	4 (67%)	2 (40%)	3 (60%)	2 (40%)
Age: years	7.8 (6.2)	5.6 (3.0)	11.6 (6.6)	7.0 (4.1)
sIgE ⁺ (kU _A /L)	n/a	14.7 (19.0)	44.8 (51.9)	52.2 (48.2)
SPT ⁺ (wheal mm)	n/a	11.3 (4.7)	7.7 (1.5)	8.8 (2.2)
PBMC yield (#cells × 10 ⁶ /1 mL)	1.3 (0.77)	1.5 (0.79)	1.1 (0.36)	1.0 (0.18)

Note: Mean (SD) or number (%) indicated.

*To respective allergen.

3.4 | Association between household endotoxin levels and sensitization to milk or egg but not peanut

Geometric mean household endotoxin levels were significantly higher among subjects sensitized to milk (22.4 EU/mg, GSE 2.02) vs those without milk sensitization (14.8 EU/mg, GSE 0.55) (*t* test $P < .0001$) (Figure 1). Each 10-fold increment in endotoxin level was associated with 1.8-fold odds (95% CI 1.3-2.3) of sensitization to milk (Figure 2). To account for potential confounders, we built multivariable logistic regression models adjusted for age, race, country of birth, total people per household, US region, and history of wheezing in the past year, finding that each 10-fold increment in endotoxin level was associated with 1.7-fold odds (95% CI 1.2-2.2) of milk sensitization in these adjusted models (Figure 2). Results for the association between endotoxin concentration and food sensitization stratified by age are shown in Table S1.

The results for household endotoxin levels among egg-sensitized subjects followed the same direction as for milk sensitization. Geometric mean household endotoxin levels were higher among subjects sensitized to egg (19.1 EU/mg, GSE 1.83) vs those without egg sensitization (15.2 EU/mg, SD 0.56) (*t* test $P = .027$) (Figure 1). Each 10-fold increment in endotoxin level was associated with 1.4-fold odds (95% CI 1.04-1.8) of egg sensitization, and multivariable logistic regression models adjusted for the same potential confounders yield an adjusted OR of 1.4 (95% CI 1.01-1.9) (Figure 2).

Interestingly, household endotoxin levels were not significantly different between those sensitized and not sensitized to peanut (*t* test $P = .46$)

(Figure 1), and there were no significant associations between household endotoxin level and peanut sensitization in the unadjusted (OR 1.1, 95% CI 0.9-1.4) or adjusted (aOR 0.98, 95% CI 0.8-1.2) models (Figure 2).

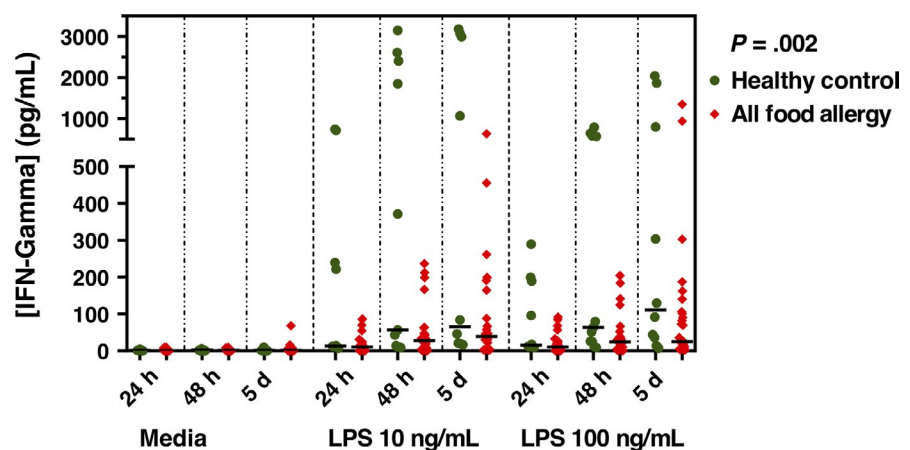
3.5 | Study population for the experimental study

Inspired by our epidemiologic findings of varying associations between household endotoxin level and specific food allergen sensitization, we next performed complementary, laboratory-based experiments to examine the effects of endotoxin stimulation on peripheral blood mononuclear cells (PBMCs) from 21 children mono-food allergic to milk, egg, or peanut, and healthy controls. Specifically, we obtained peripheral blood samples from these 21 children, isolated PBMCs from these samples, and stimulated them with endotoxin (LPS). The baseline characteristics of these subjects are shown in Table 3. Among all 21 subjects, 52% were female and the mean age was 8.0 years (SD 5.4, range 1-19 years). There were no significant differences in age ($P = .93$) or gender ($P = .83$) between the healthy controls and food-allergic subjects.

3.6 | Endotoxin stimulation of PBMCs from milk-, egg-, and peanut-allergic subjects

Among all 21 subjects, the median yield of PBMCs was 1.04×10^6 cells/mL (Interquartile range [IQR] 0.94×10^6 to 1.36×10^6) with no significant difference in yield ($P = .77$) in subjects with any food allergy (mean 1.18×10^6 cells/mL, SD 0.52×10^6) vs

FIGURE 3 Interferon- γ levels (pg/mL) following stimulation with endotoxin of PBMCs from healthy nonallergic children ($n = 6$) and children with food allergy to milk, egg, or peanut ($n = 15$). Hours/days shown are length of incubation with media or endotoxin (lipopolysaccharide [LPS]) at 10 and 100 ng/mL. *P*-value calculated using linear mixed-effects model adjusted for age, length of incubation, stimulus (media, LPS 10 ng/mL, and LPS 100 ng/mL), and presence of duplicates. Medians indicated by black lines



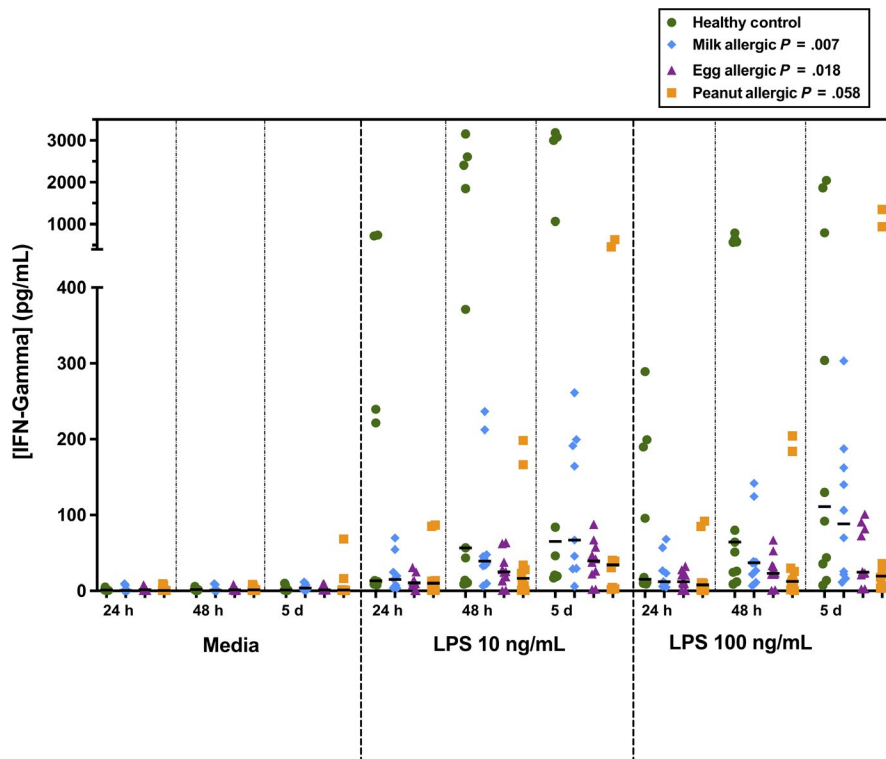


FIGURE 4 Interferon- γ levels (pg/mL) following stimulation with endotoxin of PBMCs from healthy nonallergic children ($n = 6$) and children mono-food allergic to milk (blue points, $n = 5$), egg (purple points, $n = 5$), and peanut (orange points, $n = 5$). Hours/days shown are length of incubation with media or endotoxin (lipopolysaccharide [LPS]) at 10 and 100 ng/mL. P -value calculated using linear mixed-effects model adjusted for age, length of incubation, stimulus (media, LPS 10 ng/mL, LPS 100 ng/mL), and presence of duplicates. Medians indicated by black lines

healthy control (mean 1.26×10^6 cells/mL, SD 0.77×10^6) (Table 3). PBMCs from each subject were stimulated with 0, 10, and 100 ng/mL endotoxin each for 24, 48 hours, and 5 days of incubation, and each in duplicate. The levels of the cytokines in the supernatants under each of these conditions are shown in Table S2. Linear mixed-effects modeling of these cytokine levels accounting for stimulation with variable endotoxin concentration, multiple lengths of incubation, duplicates, as well as subject age and inter-individual effects within each model revealed that higher levels of endotoxin exposure were associated with lower IFN- γ level production by PBMCs from children with any food allergy (milk, egg, or peanut) relative to nonallergic healthy controls (coefficient -347.8 pg/mL, 95% CI -572.4 to -123.2 pg/mL, $P = .002$) (Figure 3).

Linear mixed model results for endotoxin-stimulated cytokine production for each individual food allergy showed significant results for IFN- γ among children with milk and egg allergy, but not those with peanut allergy. Specifically, IFN- γ production by PBMCs from milk-allergic patients (coefficient -415.8 pg/mL, 95% CI -720.5 to -111.1 pg/mL, $P = .007$) and egg-allergic patients (coefficient -349.7 pg/mL, 95% CI -640.1 to -59.4 pg/mL, $P = .018$) was each significantly lower than that by healthy controls (Figure 4). Among peanut-allergic subjects, IFN- γ levels trended toward lower levels than healthy controls but were not significant (coefficient -283.8 pg/mL, 95% CI -577.1 to 9.6 pg/mL, $P = .058$) (Figure 4). The other Th1-related cytokines, IL6 and IL12, trended toward decreased levels in response to endotoxin across milk-, egg-, and peanut-allergic subjects, but these effects did not reach statistical significance in linear mixed-effects models (IL-6: milk $P = .066$, egg

$P = .23$, and peanut $P = .42$ and IL-12: milk $P = .49$, egg $P = .38$, and peanut $P = .87$) (Table S2). Conversely, the Th2 cytokines, IL-4 and IL-5, trended toward increased levels in response to endotoxin across milk-, egg-, and peanut-allergic subjects, but these trends were also not statistically significant (IL-4: milk $P = .63$, egg $P = .68$, and peanut $P = .54$ and IL-5: milk $P = .63$, egg $P = .74$, and peanut $P = .31$) (Table S2).

4 | DISCUSSION

The results from this complementary epidemiologic and experimental study suggest that the associations between endotoxin, food allergen sensitization, and food allergy can differ by individual food allergen. Among 6963 children and adults from the 2005-2006 NHANES cohort, higher household endotoxin levels were associated with increased odds of sensitization to milk and egg, but not peanut, in both unadjusted models and models adjusted for potential confounders. In parallel to these epidemiologic findings, our experimental study of cytokine production by endotoxin-stimulated PBMCs from 21 milk-, egg-, and peanut-allergic children and healthy controls showed that production of IFN- γ was significantly decreased for milk-allergic and egg-allergic children relative to healthy nonallergic controls. While peanut-allergic children trended toward an attenuated IFN- γ response, this difference was not significant. The results from our epidemiologic study of the NHANES population, along with our experimental results from a targeted study of 21 children mono-food allergic to milk, egg, or peanut and controls, particularly

for IFN- γ , prompt consideration that individual food allergen sensitizations and allergies may have differing associations with endotoxin exposure.

Few studies have evaluated the relationship between endotoxin exposure and food allergen sensitization. Our results differ from an earlier study of the NHANES 2005-2006 cohort that reported an association between higher household endotoxin levels and egg sensitization only among adults aged 50 years and older.⁷ The study targeted allergen sensitization to all allergens (environmental and food), and the data examined were limited to approximately 5200 NHANES participants who were 6 years and older, where participants were stratified into 3 age groups (children, adults, and older adults) that were each analyzed separately.⁷ In contrast, we included 6963 NHANES subjects aged 1 year and older and employed multivariable regression adjusted for age and other confounders. As sensitization and food allergy are more prevalent in younger children,²¹ it was important to include these younger subjects in a study of food allergen sensitization, and we controlled for age in our models in addition to considering possible effect modification by age. Furthermore, our focus on food allergen sensitization in particular allowed us to incorporate potential confounders specifically relevant to food allergen sensitization.

In contrast, an independent study of 516 children from an inner-city birth cohort suggested that household endotoxin levels have a protective association with the development of food allergies in children.⁶ Food allergy was defined by positive serum-specific IgE (≥ 0.35 kU_A/L) to milk, egg, or peanut and clinical confirmation, which was met by 51 children out of the 516 children included in the study. Higher bedroom endotoxin levels collected at 3 months of life were significantly associated with decreased odds of overall food allergies (milk, egg, or peanut) and egg allergy by 5 years of age. There was no significant association between endotoxin levels and the development of milk or peanut allergy. As NHANES 2005-2006 does not include children younger than age 1 year, our study does not encompass household endotoxin levels during infancy.

The potential reasons for effects for milk and egg sensitization/allergy distinct from peanut are interesting to consider and merit further study. On a rudimentary level, cow's milk and hen's egg are animal-based, vs peanut, which is plant-based. Clinically, milk and egg allergy are more likely to be outgrown in comparison with peanut allergy.²¹ This is the first study to compare responses to endotoxin between milk-, egg-, and peanut-allergic subjects. There are differences in the epidemiology and natural history of these specific food allergies, and our results suggest that endotoxin response may differ as well. Further experimental studies with larger sample sizes will be needed to investigate the mechanistic underpinnings of these findings.

We found positive associations between household endotoxin level and sensitization to milk or egg, suggesting that endotoxin is a risk-conferring factor. Our finding is both consistent and contrasting to previous studies of endotoxin and allergy-related outcomes more broadly. A Canadian study found that bedroom endotoxin levels

were protective for asthma in children aged 6-12 years.²² This protective effect was not noted in children older than 12 years, however. In a longitudinal birth cohort in the United States, increased home endotoxin levels in infancy were associated with decreased eczema by age 1 year, reduced risk of environmental allergen sensitization by school age, and decreased IL-13 production by allergen-stimulated PBMCs.²³⁻²⁵ In this same cohort, prolonged exposure to endotoxin was also shown to be protective with reduced odds of asthma in children with repeated measurements of high endotoxin levels in infancy and at school age.²⁶ Another birth cohort study of an inner-city population reported that children with higher endotoxin concentrations in the home were less likely to have eczema at age 1 year, but were more like to have wheezed within the past year at age 2 years.¹² In a cross-sectional study of children from rural areas in Austria, Germany, and Switzerland, infant exposure to stables and drinking of farm milk (ie, higher endotoxin levels) was associated with lower frequencies of asthma, hay fever, and atopic sensitization relative to later stable exposure in childhood without infant exposure.⁵ Two-thirds of the mothers of these infants who had been exposed to the farming environment within the first year of life were also active on the farm during their pregnancies.⁵ Thus, endotoxin exposure prenatally and in infancy could be protective, while higher household endotoxin levels later in life could be a risk factor for allergy-related outcomes, including food allergen sensitization, particularly to milk and egg, as we found in this study.

In addition to age of exposure, studies of asthma have shown that differences in the effects of endotoxin can also be attributed to genetic variability.^{27,28} For example, among subjects with a specific genetic polymorphism in ACAA1 (SNP rs156265) exposure to higher household endotoxin levels confers protection against asthma.²⁹ Therefore, varying effects of endotoxin on atopy-related outcomes could be attributed not only to age of exposure but also to gene-by-environment interactions.

Parallel to our epidemiologic results of a positive association between household endotoxin level and milk and egg sensitization among NHANES participants, we further demonstrated that the Th1 cytokine response, IFN- γ , was attenuated in endotoxin-stimulated PBMCs from children with food allergies, particularly those allergic to milk and egg. IFN- γ was the only cytokine for which significant effects were observed. IFN- γ is a key mediator of Th1 response and plays a crucial role in isotype switching and IgE regulation.³⁰ Recent studies evaluating novel therapies for food allergy use IFN- γ as an outcome measure for evaluating response to therapy³¹ and even as adjuvant therapy for food immunotherapy.³²

Several studies have previously shown that allergic children have immature Th1 function in comparison with nonallergic children.^{33,34} Most recently, Tulic et al³⁵ found differences in responsiveness to TLR ligands, including endotoxin, between allergic (eczema, food allergy, asthma, or positive sensitization) and nonallergic children. The innate cytokine response (IL-1 β , IL-6, TNF- α , and IL-12) was found to be initially hyper-responsive at birth (cord blood) among allergic children in comparison with nonallergic children, but the response to endotoxin and other TLR ligands became attenuated

by 5 years of age. IFN- γ responses to allergens and mitogens were also decreased for allergic children during the first 5 years of life, suggesting a lack of appropriate maturation of Th1 function. Even though the inflammatory response to microbes was initially strong in newborns, the Th1 response to allergens became weaker and the Th2 response became predominant with age.³⁶ This increased inflammatory response at birth has also been shown specifically among food-allergic children in comparison with children without food allergies.³⁷ Zhang et al reported that CD14⁺ monocytes from cord blood exposed to endotoxin (LPS) express higher amounts of inflammatory cytokines, specifically IL-1 β , IL-6, and TNF- α in children who later developed confirmed food allergies, demonstrating that these food-allergic children had increased monocyte responsiveness at the time of birth. These inflammatory cytokines have been shown to lead to nonclassic Th2 differentiation and impaired naive T_{reg} function, a possible mechanism in which endotoxin exposure could affect the development of food allergies. As our population was focused specifically on children with food allergies aged 1 year and older and no cord blood was collected, we also demonstrated an attenuated IFN- γ production, representing a weakened Th1 response to endotoxin among food-allergic children compared to nonallergic controls.

There were some limitations to this study. Data from NHANES 2005–2006 are cross-sectional with household endotoxin level and health outcome variables measured at the same time, rendering it challenging to determine causal associations. However, a strength of studying this population is that it is based on a large sample intended to be representative of the US population. While specific assessment of food allergy within NHANES would have been ideal, and food allergen sensitization does not equate a clinical diagnosis of food allergy, there was no information collected in NHANES 2005–2006 on food-related allergic reactions. To partially address this limitation and assess the degree to which findings from NHANES might be relevant to outcomes in children with clinical food allergy, we undertook experimental studies of endotoxin-stimulated PBMCs from twenty-one children with clinical food allergy based on allergist diagnosis and confirmatory allergy testing. Although the NHANES study population included adults and children and was predominantly adult, and our experimental study population included only children and a more limited sample size, the results from both our epidemiologic and experimental studies consistently highlighted differences in those sensitized or allergic to milk or egg specifically.

In summary, this is a unique study evaluating the relationship between endotoxin and sensitization and allergy to specific food allergens (milk, egg, and peanut) using a combined epidemiologic and experimental approach in two different populations. Among NHANES subjects representative of the US population, elevated household endotoxin levels were significantly associated with increased odds of milk and egg sensitization, but not peanut sensitization. In complementary experimental studies of PBMCs from children with food allergy, IFN- γ production in response to endotoxin

stimulation was attenuated among food-allergic children relative to healthy nonallergic controls. Consistent with our epidemiologic results, children with allergy to milk or egg produced significantly less IFN- γ in response to endotoxin, while differences were not significant among peanut-allergic children. The results from this and prior studies suggest that the relationship between endotoxin and food sensitization/allergy is complex with differing effects dependent not only on age of exposure and genetic variation as suggested by prior work, but also on the type of food allergy.

ACKNOWLEDGMENTS

We thank Dieudonne Nahigombeye, MS, statistician for the CDC/National Center for Health Statistics, Research Data Center, for his assistance in the application process to access the restricted NHANES data. We thank Jade Andrade for assistance with subject recruitment. We also thank the children and their parents who participated in this study.

CONFLICTS OF INTEREST

The authors have no conflicts to disclose.

ORCID

Angela Tsuang  <https://orcid.org/0000-0002-9304-0604>

Joanne Sordillo  <https://orcid.org/0000-0002-4966-1796>

Supinda Bunyavanich  <https://orcid.org/0000-0001-6108-439X>

REFERENCES

1. Sicherer SH, Sampson HA. Food allergy: a review and update on epidemiology, pathogenesis, diagnosis, prevention and management. *J Allergy Clin Immunol*. 2018;141:41–58.
2. Gupta RS, Springston EE, Warrier MR, et al. The prevalence, severity, and distribution of childhood food allergy in the United States. *Pediatrics*. 2011;128:9–17.
3. Thorne PS, Cohn RD, Mav D, Arbes SJ, Zeldin DC. Predictors of endotoxin levels in US housing. *Environ Health Perspect*. 2009;117:763–771.
4. Thorne PS, Mendy A, Metwali N, et al. Endotoxin exposure: predictors and prevalence of associated asthma outcomes in the United States. *Am J Respir Crit Care Med*. 2015;192:1287–1297.
5. 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;358:1129–1133.
6. McGowan EC, Bloomberg GR, Gergen PJ, et al. Influence of early-life exposures on food sensitization and food allergy in an inner-city birth cohort. *J Allergy Clin Immunol*. 2015;135:171–178.
7. Min KB, Min JY. Exposure to household endotoxin and total and allergen-specific IgE in the US population. *Environ Pollut*. 2015;199:148–154.
8. National Center for Health Statistics. *NCHS Research Ethics Review Board (ERB) Approval*. Hyattsville, MD: National Center for Health Statistics; 2012. <https://www.cdc.gov/nchs/nhanes/irba98.htm>. Accessed January 1, 2019.

9. National Center for Health Statistics. *Public Data General Release File Documentation*. Hyattsville, MD: National Center for Health Statistics; 2005. https://www.cdc.gov/nchs/data/nhanes/nhanes_05_06/general_data_release_doc_05_06.pdf. Accessed January 1, 2019.
10. Salo PM, Arbes SJ Jr, Jaramillo R, et al. Prevalence of allergic sensitization in the United States: results from the national health and nutrition examination survey (NHANES) 2005-2006. *J Allergy Clin Immunol*. 2014;134:350-359.
11. National Center for Health Statistics. *Allergens-Household Dust (ALDUST_D)*. Hyattsville, MD: National Center for Health Statistics; 2009. https://wwwn.cdc.gov/Nchs/Nhanes/2005-2006/ALDUST_D.htm. Accessed January 1, 2019.
12. Perzanowski MS, Miller RL, Thorne PS, et al. Endotoxin in inner-city homes: associations with wheeze and eczema in early childhood. *J Allergy Clin Immunol*. 2006;117:1082-1089.
13. O'Connor GT, Lynch SV, Bloomberg GR, et al. Early-life home environment and risk of asthma among inner-city children. *J Allergy Clin Immunol*. 2018;141:1468-1475.
14. Lai PS, Sheehan WJ, Gaffin JM, et al. School endotoxin exposure and asthma morbidity in inner-city children. *Chest*. 2015;148:1251-1258.
15. Barnig C, Reboux G, Roussel S, et al. Indoor dust and air concentrations of endotoxin in urban and rural environments. *Lett Appl Microbiol*. 2013;56:161-167.
16. Sheehan WJ, Hoffman EB, Fu C, et al. Endotoxin exposure in inner-city schools and homes of children with asthma. *Ann Allergy Asthma Immunol*. 2012;108:418-422.
17. *Laboratory Procedures Manual*. Elmhurst, IL: CDC Environmental Health. 2008. https://www.cdc.gov/nchs/data/nhanes/nhanes_05_06/al_ige_d_met_specific_ige_total_ige.pdf. Accessed January 1, 2019.
18. National Center for Health Statistics. *Allergen Specific IgE(s) and Total IgE in Serum (AL_IGE_D)*. Hyattsville, MD: National Center for Health Statistics; 2009. https://wwwn.cdc.gov/Nchs/Nhanes/2005-2006/AL_IGE_D.htm. Accessed January 1, 2019.
19. National Center for Health Statistics. *Analytic and Reporting Guidelines*. Hyattsville, MD: National Center for Health Statistics; 2005. https://wwwn.cdc.gov/nchs/data/nhanes/2005-2006/nhanes_analytic_guidelines_dec_2005.pdf. Accessed January 1, 2019.
20. National Center for Health Statistics. *Demographic Variables and Sample Weights (DEMO_D)*. Hyattsville, MD: National Center for Health Statistics; 2009. https://wwwn.cdc.gov/Nchs/Nhanes/2005-2006/DEMO_D.htm. Accessed January 1, 2019.
21. Savage J, Sicherer S, Wood R. The natural history of food allergy. *J Allergy Clin Immunol Pract*. 2016;4:196-203.
22. Lawson JA, Dosman JA, Rennie DC, et al. Endotoxin as a determinant of asthma and wheeze among rural dwelling children and adolescents: a case-control study. *BMC Pulm Med*. 2012;12:56.
23. Phipatanakul W, Celedon JC, Raby BA, et al. Endotoxin exposure and eczema in the first year of life. *Pediatrics*. 2004;114:13-18.
24. Celedon JC, Milton DK, Ramsey CD, et al. Exposure to dust mite allergen and endotoxin in early life and asthma and atopy in childhood. *J Allergy Clin Immunol*. 2007;120:144-149.
25. Abraham JH, Finn PW, Milton DK, Ryan LM, Perkins DL, Gold DR. Infant home endotoxin is associated with reduced allergen-stimulated lymphocyte proliferation and IL-13 production in childhood. *J Allergy Clin Immunol*. 2005;116:431-437.
26. Sordillo JE, Hoffman EB, Celedon JC, Litonjua AA, Milton DK, Gold DR. Multiple microbial exposures in the home may protect against asthma or allergy in childhood. *Clin Exp Allergy*. 2010;40:902-910.
27. Saçkesen C, Karaaslan C, Keskin O, et al. The effect of polymorphisms at the CD14 promoter and the TLR4 gene on asthma phenotypes in Turkish children with asthma. *Allergy*. 2005;60:1485-1492.
28. Zambelli-Weiner A, Ehrlich E, Stockton ML, et al. Evaluation of the CD14/-260 polymorphism and house dust endotoxin exposure in the Barbados asthma genetics study. *J Allergy Clin Immunol*. 2005;115:1203-1209.
29. Sordillo JE, Sharma S, Poon A, et al. Effects of endotoxin exposure on childhood asthma risk are modified by a genetic polymorphism in ACAA1. *BMC Med Genet*. 2011;12:158.
30. Abbas AK, Lichtman AH, Pillai S. *Cellular and Molecular Immunology* (9th ed). Philadelphia, PA: Elsevier; 2018.
31. Srivastava KD, Siefert A, Fahmy TM, Caplan MJ, Li XM, Sampson HA. Investigation of peanut oral immunotherapy with CpG/peanut nanoparticles in a murine model of peanut allergy. *J Allergy Clin Immunol*. 2016;138:536-543.
32. Noh G, Jang EH. Dual specific oral tolerance induction using interferon gamma for IgE-mediated anaphylactic food allergy and the dissociation of local skin allergy and systemic oral allergy: tolerance or desensitization? *J Investig Allergol Clin Immunol*. 2014;24:87-97.
33. Prescott SL, Noakes P, Chow BW, et al. Presymptomatic differences in toll-like receptor function in infants who develop allergy. *J Allergy Clin Immunol*. 2008;122:391-399.
34. Schaub B, Liu J, Höppler S, et al. Impairment of T-regulatory cells in cord blood of atopic mothers. *J Allergy Clin Immunol*. 2008;121:1491-1499.
35. Tulic MK, Hodder M, Forsberg A, et al. Differences in innate immune function between allergic and nonallergic children: new insights into immune ontogeny. *J Allergy Clin Immunol*. 2011;127:470-478.
36. Prescott SL, Martino D, Hodder M, Richman T, Tulic MK. Progress in understanding postnatal immune dysregulation in allergic disease. *World Allergy Organ J*. 2010;3:162-166.
37. Zhang Y, Collier F, Naselli G, et al. Cord blood monocyte-derived inflammatory cytokines suppress IL-2 and induce nonclassic "T(H)2-type" immunity associated with development of food allergy. *Sci Transl Med*. 2016;8:321.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Tsuang A, Grishin A, Grishina G, et al. Endotoxin, food allergen sensitization, and food allergy: A complementary epidemiologic and experimental study. *Allergy*. 2020;75:625-635. <https://doi.org/10.1111/all.14054>

Age Group	Food Sensitization	Odds Ratio	95% CI	P-value
1-5 years (n=480)	Milk	1.20	0.76-1.90	0.41
	Egg	1.23	0.71-2.13	0.43
	Peanut	0.67	0.30-1.50	0.31
6-17 years (n=1206)	Milk	1.06	0.81-1.37	0.66
	Egg	1.12	0.70-1.79	0.61
	Peanut	0.84	0.50-1.40	0.48
18+ years (n=5166)	Milk	1.67	1.12-2.50	0.016
	Egg	1.23	0.85-1.79	0.25
	Peanut	1.14	0.90-1.45	0.25

Table S1: Association between NHANES Household Endotoxin Level and Food Sensitization Stratified by Age

Unadjusted odds of food allergen sensitization with each 10-fold increase in household endotoxin level among NHANES 2005-2006 subjects (n=6963) stratified by age. Food allergen sensitization defined as serum specific IgE ≥ 0.35 kU_A/L. 95% CI = 95% confidence interval.

	Length of Incubation	Allergy	IFN- γ	IL-6	IL-12	IL-4	IL-5
Media	24 hr	Control	1.4 (0.9)	7.0 (3.7)	0.9 (1.0)	1.8 (0.0)	0.9 (0.9)
		Peanut	2.1 (3.4)	5.8 (6.3)	0.7 (0.6)	1.6 (0.3)	1.9 (3.1)
		Egg	2.4 (2.4)	7.2 (3.8)	0.9 (0.6)	1.6 (0.6)	1.3 (1.9)
		Milk	2.0 (3.6)	9.0 (4.0)	0.8 (0.8)	5.6 (8.5)	1.4 (2.3)
	48 hr	Control	1.9 (1.5)	8.4 (4.2)	0.6 (0.2)	1.7 (0.5)	0.8 (0.9)
		Peanut	2.2 (2.7)	8.0 (4.7)	0.6 (0.2)	1.8 (0.2)	1.6 (2.7)
		Egg	2.2 (2.7)	8.4 (3.0)	0.4 (0.1)	1.6 (0.4)	0.5 (0.0)
		Milk	2.3 (3.6)	10.0 (3.2)	0.6 (0.2)	1.8 (0.0)	0.9 (1.0)
	5 days	Control	2.6 (3.0)	8.6 (5.5)	0.6 (0.4)	1.6 (0.4)	0.4 (0.1)
		Peanut	9.1 (18.5)	10.9 (6.5)	1.0 (1.4)	7.3 (12.2)	3.5 (6.8)
		Egg	2.9 (3.4)	10.1 (4.7)	1.0 (1.2)	8.9 (16.6)	1.3 (1.9)
		Milk	4.3 (4.6)	10.4 (3.2)	0.9 (1.2)	10.5 (17.3)	1.8 (2.2)
LPS 10 ng/ml	24 hr	Control	198.5 (311.2)	987.6 (582.7)	7.3 (4.5)	7.0 (7.9)	0.8 (0.4)
		Peanut	22.1 (36.1)	986.5 (1107.8)	7.6 (12.6)	14.9 (25.8)	3.5 (5.7)
		Egg	12.0 (10.0)	787.5 (807.5)	3.7 (3.9)	11.1 (17.6)	1.9 (3.0)
		Milk	21.9 (23.5)	1658.2 (1731.6)	7.6 (12.1)	12.8 (22.3)	2.7 (3.9)
	48 hr	Control	908.6 (1266.0)	4228.9 (7769.0)	12.6 (12.4)	7.8 (8.3)	1.4 (0.7)
		Peanut	47.2 (76.4)	1299.8 (1616.5)	7.3 (10.8)	14.5 (24.3)	3.4 (5.3)
		Egg	27.5 (22.8)	906.3 (773.8)	4.2 (4.6)	11.9 (20.5)	2.2 (3.3)
		Milk	70.3 (87.3)	2623.5 (3047.2)	10.8 (18.2)	14.0 (23.1)	3.6 (5.1)
	5 days	Control	1222.9 (1496.6)	1443.7 (857.0)	15.0 (13.4)	10.9 (16.4)	2.2 (2.0)
		Peanut	124.5 (234.4)	1221.1 (1323.4)	6.9 (9.5)	14.9 (25.4)	3.5 (5.3)
		Egg	39.2 (27.1)	1374.2 (914.6)	4.8 (5.4)	12.7 (21.1)	2.7 (4.0)
		Milk	99.9 (97.9)	6660.6 (10485.8)	7.1 (10.5)	15.4 (23.4)	4.0 (4.8)
LPS 100 ng/ml	24 hr	Control	84.5 (105.7)	1181.4 (787.7)	4.7 (2.8)	3.2 (1.7)	1.3 (0.9)
		Peanut	21.6 (37.5)	1032.3 (1165.5)	6.5 (11.3)	13.8 (23.4)	3.3 (5.6)
		Egg	14.6 (11.3)	790.7 (814.2)	3.0 (3.0)	10.3 (16.5)	1.9 (2.8)
		Milk	22.9 (23.3)	1800.9 (1461.6)	6.6 (9.9)	13.4 (22.1)	3.0 (4.3)
	48 hr	Control	243.8 (315.0)	183021.2 (445789.5)	5.9 (3.2)	9.5 (13.5)	2.0 (1.4)
		Peanut	48.6 (81.9)	1302.1 (1517.4)	7.1 (11.4)	13.6 (23.0)	3.8 (5.9)
		Egg	27.6 (21.3)	1124.3 (887.4)	3.7 (4.4)	13.4 (21.4)	2.3 (3.5)
		Milk	48.3 (48.9)	3388.0 (4035.0)	7.9 (11.6)	15.0 (25.2)	3.5 (4.7)
	5 days	Control	536.1 (753.7)	1845.4 (1337.7)	6.1 (1.9)	11.7 (19.7)	2.2 (1.6)
		Peanut	240.8 (505.6)	1618.6 (1548.4)	6.3 (9.3)	14.2 (23.4)	3.7 (5.4)
		Egg	44.4 (39.9)	1477.0 (877.7)	4.8 (6.2)	14.1 (23.0)	3.3 (4.6)
		Milk	104.3 (93.5)	4369.2 (5163.2)	6.6 (8.5)	14.9 (22.8)	4.5 (4.8)

Table ~~E2~~S2: Mean Production of Cytokines by Length of Incubation and Stimulus

Mean (SD) concentration of cytokine production (pg/mL) following stimulation with endotoxin of PBMCs from healthy non-allergic children (n=6) and children mono-allergic to (A) milk (n=5), (B) egg (n=5), and (C) peanut (n=5) in duplicates. Hours/days shown are length of incubation with media or endotoxin (lipopolysaccharide (LPS)) at 10 ng/ml and 100 ng/ml.