House Dust Endotoxin and Wheeze in the First Year of Life

JU-HYEONG PARK, DIANE R. GOLD, DONNA L. SPIEGELMAN, HARRIET A. BURGE, and DONALD K. MILTON

Department of Environmental Health and Departments of Epidemiology and Biostatistics, Harvard School of Public Health, and The Channing Laboratory, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, Massachusetts

We examined endotoxin exposure and wheezing episodes during the first year of life in a birth cohort of 499 infants with one or both parents having a history of asthma or allergy. We measured endotoxin in settled dust from the baby's bed, bedroom floor, family room, and kitchen floor within the first 3 mo after birth. The primary outcomes were any wheeze (versus no wheeze), and repeated wheeze (versus one or no report of wheeze). We found a significant univariate association of elevated endotoxin ($\geq$ 100 EU/mg) in family room dust with increased risk of any wheeze (Relative Risk = 1.29, 95% CI = 1.03–1.62). The association was not confounded by cockroach allergen, lower respiratory illness (croup, bronchitis, bronchiolitis, and pneumonia), smoking during pregnancy, lower birth weight, maternal asthma, presence of dog, and race/ethnicity in a multivariate model; the multivariate relative risk (RR = 1.33) was marginally significant (95% CI: 1.00–1.76, $p < 0.05$). In a multivariate model, controlling for the above covariates, elevated endotoxin in family room dust was significantly associated with increased risk (RR = 1.56, 95% CI = 1.03–2.38) of repeated wheeze. These results suggest that home endotoxin exposure may independently increase risk of any wheeze and repeated wheeze during the first year of life for children with a familial predisposition to asthma or allergy.

Endotoxin is biologically active lipopolysaccharide, a family of macromolecules that composes the outer membrane of gram-negative bacteria (GNB). Endotoxin is a proinflammatory agent that produces airway inflammation when inhaled (1–3). Environmental endotoxin can be detected in the presence of GNB regardless of whether the bacteria are alive, and because GNB are ubiquitous, everyone is exposed to at least low levels of environmental endotoxin.

It is well known that endotoxin is present in house dust (4–6). Michel and coworkers reported that endotoxin in house dust was associated with increased severity of asthma in mite-sensitized adults exposed to high levels of mite allergen (7, 8). Rizzo and coworkers also recently showed that house dust endotoxin was associated with severity of childhood asthma (9). Thus, exposure to endotoxin in house dust may play an important role in aggravating asthma in adults and children. However, one recent study did not confirm this association. Douwes and coworkers examined house dust endotoxin and peak expiratory flow (PEF) variability in 7- to 11-yr-old school children. Although they observed significant univariate associations, they did not find a consistent association of endotoxin and PEF variability in five subgroups (by symptom, atopy, and asthma) after adjusting for dust mite allergen concentration, presence of pets, and carpets in the home (10).

Wheezing, produced by airways narrowed due to bronchial smooth muscle constriction and airway inflammation, is a frequent symptom for children who are born with small airways and/or when children have upper or lower respiratory infections (11–13). Martinez and coworkers reported that 48.5% of children in a Tucson-based cohort experienced a wheezing episode before age 6, and that 41% of children who wheezed before age 3 continued to wheeze until age 6 (13). The Tucson study also showed that the wheezing episodes without any other respiratory symptoms experienced by children under age 1, who had colds, were not significantly associated with increased risk of asthma. However, there was a significant association with childhood asthma when early wheezing episodes continued until 3 to 4 yr of age or when wheezing episodes started at age 3 to 4 (14).

Previous studies have examined the association of home endotoxin exposure with asthma severity, and of early wheeze with asthma, however, there are no published data describing the role of endotoxin in onset of wheeze in the early years of life. We examined the associations of house dust endotoxin, measured within the first 3 mo after birth, with wheezing during the first year of life in a cohort of children with a familial predisposition to asthma or allergy.

METHODS

Study Cohort

The "Epidemiology of Home Allergens and Asthma" is an ongoing longitudinal closed birth cohort study of children born to parents with histories of allergies and/or asthma. The aim of the study is to examine the role of indoor home allergens exposure in the development of asthma/wheeze and allergic sensitization in early childhood. The "Home Endotoxin and Childhood Asthma" project has supplemented this cohort study by examining the role of endotoxin exposure in these outcomes. A more detailed description of the cohort (505 children from 499 families) was previously published (15). Briefly, between September 1994 and June 1996, we obtained a daily list of all women who had just given birth at a tertiary hospital in Boston (the Brigham and Women's Hospital), who lived in the Greater Boston area (within Route 128). We included mothers in screening only if their maternal age was greater than or equal to 18 yr old and if they did not plan to move in the next 12 mo. We excluded newborn babies with prematurity (< 36 wk), with major congenital abnormalities, or who were hospitalized in the neonatal intensive care unit. Only children of parents with asthma or allergies were included. With those inclusion criteria, of the 1,405 mothers who completed the screening questionnaire, 499 families (505 children) were selected for the cohort and agreed to participate. The 499 families included 6 sets of twins. Based on completed data for wheeze in the first year of life, 499 index children were finally included in data analysis (15).

Home Visit and Exposure Measurements

We visited infants' homes within the first 3 mo after birth of the index child. At the home visit, we collected four dust samples and adminis-

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Correspondence and requests for reprints should be addressed to Dr. Donald K. Milton, Environmental Health, Occupational Health Program, Harvard School of Public Health, 665 Huntington Ave, Boston, MA 02115. E-mail: dmilton@hohp.harvard.edu

tered a detailed home characteristic questionnaire. We used a Eureka Mighty-Mite vacuum cleaner modified to hold 19 × 90-mm cellulose extraction thimble to collect house dust. For bedroom dust, 2 m^2 of the bedroom floor surrounding the baby's crib was vacuumed for 5 min; for bed dust, all layers of bedding in the baby's crib, and in the parents' bed if the index children slept there more than 50% of the time, were vacuumed for 5 min. In the family room, the seat cushion, arms, and back of the upholstered chair most often occupied by the baby were vacuumed for 2.5 min along with 2 m^2 of the surrounding floor (2.5 min). In the kitchen, the edges of the floor under cabinets, around the refrigerator, and under the sink were vacuumed for 5 min. Within 24 h after collection dust was weighed, sifted through a 425-μm mesh sieve, and the fine dust reweighed and divided into aliquots for various analyses—allergens, culturable fungi, and endotoxin. Endotoxin was assayed only if there was sufficient dust after all other assays had been performed. Samples for endotoxin assay were stored desiccated at −20° C until extraction and assay (5, 16).

The endotoxin activity of dust samples was determined by the kinetic Limulus assay with the resistant-parallel-line estimation (KLARE) method, described in previous publications (5, 16). Limulus amebocyte lysate (LAL) was obtained from BioWhittaker (Walkersville, MD), reference standard endotoxin from the United States Pharmacopoeia, Inc. (Rockville, MD), and control standard endotoxin from Associates of Cape Cod (Woods Hole, MA). All glassware was heated to 270° C for 30 min prior to use. Control and reference standards and field samples were serially diluted for endotoxin analysis and extractions were performed in a standard buffer (0.01% triethylamine and 0.05 M potassium phosphate).

Dust samples were extracted immediately before assay by placing 25 mg of sifted dust in endotoxin-free borosilicate glass tubes with 5 ml of buffer and bath sonicationed for 1 h with vortexing at 15-min intervals. An initial 1:25 dilution of dust extracts with suspended particulate was made before the start of the serial dilutions used in the assay, and four serial dilutions with 1:6 dilution factor.

Duplicate 50-μl aliquots of the initial and four serial dilutions of dust extracts and a control standard endotoxin were placed in an endotoxin free 96-well, flat-bottomed polystyrene microplate (Associates of Cape Cod), 50 μl of LAL was added, and the microplate agitated. The optical density (OD) of each well was recorded at 405 nm every 30 s for 120 min during incubation at 37° C. Results are reported in endotoxin units adjusted to account for lot-to-lot variation in LAL sensitivity to house dust associated endotoxin (5, 16) and referenced to the reference standard endotoxins EC5 and EC6 (U.S. Pharmacopoeia, Inc., Rockville, MD; 1 ng EC5 and EC6 = 10 endotoxin units [EU]).

Outcome Definitions and Measurement

We called primary care givers to ask about respiratory symptoms and illnesses in the index child at the end of the first and second months after birth, and every other month thereafter. In this follow-up telephone questionnaire, we asked primary care givers (usually the mother), "Since we last spoke with you on (date given), has your child had symptoms of wheeze?" Therefore, there were seven questionnaires and, thus, up to seven reports of wheeze per child in the first year of life. The outcome variables of interest are no versus any report of wheeze (any wheeze), and no or one versus two or more reports of wheeze (repeated wheeze) during the first year of life. Additional details were described in the previous publications from this cohort study (15). Table 1 shows the prevalence of any wheeze and repeated wheeze by various subgroups.

Data Analysis

Correlation analyses were performed with log transformed data because endotoxin measurements were log normally distributed (Shapiro-Wilk normality test). Pearson correlation coefficients between the endotoxin levels in four different types of samples were computed (PROC CORR, SAS Institute, 1999). The associations between endotoxin in family room dust as a continuous variable and other covariates (lower respiratory illness, smoking in pregnancy, maternal active and inactive asthma, birth weight, presence of dog, race/ethnicity, family income, and home type) were examined using ordinary least-squared regression in both univariate and multivariate models (PROC GLM; SAS Institute, Cary, NC 1999). We chose the endotoxin level in family room dust as our primary exposure index because we had only 26 dust samples available for endotoxin assay from the babies' beds.

Univariate and multivariate regression analyses were performed to examine whether dust endotoxin was associated with wheeze during the first year of life. A generalized linear model (17) with binomial distribution and log link function (SAS PROC GENMOD; SAS Institute, 1999) was used to directly estimate relative risk. Endotoxin in family room dust was the primary estimate of endotoxin exposure because we obtained the largest number of dust samples (n = 404) available for endotoxin assay from the family room. We categorized dust endotoxin at or above the median of the maximum value from each home visit (100 EU/mg) for all available dust samples (all homes and visits) as elevated endotoxin. Samples with no dust available for endotoxin assay from the family room were separately categorized and controlled in both univariate and multivariate analyses. In multivariate analyses, we controlled for cockroach allergen in family room dust (categorized as Bla g 1 or 2 ⩾ 0.05 U/g versus < 0.05 U/g), lower respiratory illness (defined as doctor diagnosed croup, bronchitis, bronchiolitis, or pneumonia), smoking in pregnancy, maternal active and inactive asthma, birth weight, presence of a dog(s) in home, race/

TABLE 1

PERCENT OF WHEEZING EPISODE IN COHORT

	No. of Children	Percent with Wheeze*	
		Any	Repeated
Total	499	42.3	19.2
Sex			
Female	233	43.4	18.9
Male	266	41.4	19.6
Endotoxin, family room dust			
< 100 EU/mg	255	38.4	18.4
⩾ 100 EU/mg	149	49.7	21.5
No dust sample	95	41.1	17.9
Cockroach allergen, family room dust			
Bla g 1 or 2 < 0.05 U/g	359	38.4	15.6
Bla g 1 or 2 ⩾ 0.05 U/g	111	50.5	28.8
No dust sample	26	53.9	26.9
Lower respiratory illness (croup, bronchitis, bronchiolitis, pneumonia)			
No	366	33.6	13.9
Yes	133	66.2	33.8
Smoking during pregnancy			
No	467	41.3	17.8
Yes	32	56.3	40.6
Maternal asthma			
No asthma	345	40.0	16.8
Inactive	48	43.8	18.8
Active†	106	49.1	27.4
Report of presence of dog			
No	420	41.0	17.9
Yes	79	49.4	26.6
Race/ethnicity			
White	375	40.8	16.8
Black	59	52.5	32.2
Hispanic	30	50.0	23.3
Asian	28	32.1	14.3
Other	7	42.9	42.9
Family income			
⩾ $50,000	352	40.3	16.2
$30,000–49,999	88	43.2	22.7
< $30,000	43	55.8	30.2
Unknown	16	43.8	37.5
Poverty level‡			
⩾ 20%	42	64.3	33.3
10%–< 20%	115	36.5	16.5
5%–< 10%	145	37.2	19.3
< 5%	197	44.7	17.8

* Any wheeze: any report of wheeze; repeated wheeze: two or more reports of wheeze.

† Active maternal asthma is defined as doctor-diagnosed asthma with wheeze.

‡ Poverty level: area of Boston by percentage of households below poverty level.

TABLE 2

UNIVARIATE ASSOCIATION OF FAMILY ROOM ENDOTOXIN WITH OTHER VARIABLES

Variables	Mean* (*EU/mg*)	Percentage Difference†	(95% CI)
Bla g 1 or 2, family room dust			
⩾ 0.05 U/g	85	10	(−7, 29)
< 0.05 U/g	78	0	
Presence of dog in home			
Yes	101	32	(9, 59)
No	76	0	
Family income			
⩾ $50,000	86	0	
$30,000–49,999	68	−21	(−34, −5)
< $30,000	61	−29	(−45, −9)
Race/ethnicity			
White	87	0	
Black	51	−41	(−52, −28)
Hispanic	70	−20	(−40, 6)
Asian	72	−17	(−39, 13)
Other	87	−1	(−43, 73)
Living in apartment (⩾ 3 families)			
Yes	69	−18	(−30, −3)
No	84	0	

* Mean endotoxin level (EU/mg) in family room dust for each category of class variables.
† Percentage difference: percentage change in dust endotoxin level relative to reference category.

ethnicity, and family income. Samples with no dust available for cockroach allergen assay from family room were separately categorized and controlled in multivariate analyses. Covariates were chosen to coincide with and to allow comparison with the previous publication analyzing the association of home allergens with repeated wheeze in the first year of life in the same cohort (15). In the multivariate model of repeated wheeze, home type (single- or two-family house versus apartment with more than two units) was also included to examine potential confounding of associations with race-ethnicity and endotoxin exposure. The endotoxin exposure–response relationships for any and for repeated wheeze were also examined using quartiles of endotoxin levels in family room dust, after controlled for other covariates in the final multivariate model.

RESULTS

Association of Endotoxin in Family Room Dust with Other Covariates

The maximum home endotoxin levels from all home visits had a geometric mean (GM) of 100 EU/mg with a geometric standard deviation (GSD) of 2.1 and ranged from 4 to 2,405 EU/mg. We focused analysis on endotoxin levels in 404 family room, 323 bedroom floor, 26 bed, and 245 kitchen floor dust samples collected from the first home visit. The GM and GSD of endotoxin in family room dust were 79 EU/mg and 2.0, respectively (range: $2 \sim 713$ EU/mg). Endotoxin in family room dust was strongly ($r = 0.64$) and significantly associated with endotoxin in baby's bed dust (GM = 50 EU/mg; range = $8 \sim 257$ EU/mg), and weakly but significantly associated with endotoxin in bedroom floor ($r = 0.34$; GM = 63 EU/mg; range = $2 \sim 761$ EU/mg) and kitchen floor dust ($r = 0.30$; GM = 100 EU/mg; range = $9 \sim 1{,}201$ EU/mg).

We also examined the association of endotoxin levels in family room dust samples with covariates used in the analysis of wheeze. High cockroach allergen in the family room was not significantly ($p = 0.26$) associated with measured endotoxin level (Table 2). However, in a multivariate analysis with continuous endotoxin as the dependent variable, adjusting for all covariates as described in METHODS, high cockroach allergen was a significant predictor of family room dust endotoxin ($p = 0.004$); least-squares mean endotoxin levels were 82 EU/mg

TABLE 3

RELATIVE RISK (RR) OF ANY WHEEZE IN RELATION TO FAMILY ROOM DUST ENDOTOXIN

Variable*	Univariate RR (95% CI)	Multivariate RR (95% Confidence Interval)			
		Model 1	Model 2	Model 3	Model 4
Endotoxin					
< 100 EU/mg	1.00	1.00	1.00	1.00	1.00
⩾ 100 EU/mg	1.29 (1.03–1.62)	1.27 (0.98–1.66)	1.33 (1.00–1.76)	1.29 (0.95–1.74)	1.33 (0.99–1.79)
Bla g 1 or 2, family room dust					
< 0.05 U/g	1.00	1.00	1.00	1.00	1.00
⩾ 0.05 U/g	1.31 (1.05–1.65)	1.25 (0.96–1.61)	1.20 (0.92–1.57)	1.22 (0.88–1.69)	1.20 (0.87–1.65)
Lower respiratory illness					
Yes	1.97 (1.63–2.38)	1.97 (1.55–2.50)	1.97 (1.56–2.50)	1.95 (1.50–2.55)	1.96 (1.52–2.53)
Smoking during pregnancy					
Yes	1.36 (0.98–1.88)	1.17 (0.76–1.79)	1.14 (0.73–1.78)	1.17 (0.72–1.90)	1.14 (0.71–1.84)
Low birth weight†	1.15 (1.02–1.30)	1.17 (0.99–1.39)	1.16 (0.97–1.38)	1.16 (0.96–1.40)	1.15 (0.96–1.39)
Maternal asthma					
No	1.00	1.00	1.00	1.00	1.00
Active	1.23 (0.97–1.55)	1.15 (0.87–1.53)	1.15 (0.85–1.54)	1.13 (0.82–1.56)	1.13 (0.82–1.57)
Inactive	1.09 (0.77–1.55)	1.11 (0.74–1.66)	1.06 (0.69–1.63)	1.09 (0.69–1.74)	1.06 (0.67–1.68)
Presence of dog in home					
Yes	1.21 (0.94–1.55)	1.08 (0.80–1.45)	1.10 (0.81–1.51)	1.09 (0.78–1.53)	1.10 (0.79–1.54)
Race/Ethnicity					
White	1.00	—	1.00	—	1.00
Black	1.29 (0.98–1.69)	—	1.24 (0.85–1.80)	—	1.20 (0.75–1.91)
Hispanic	1.23 (0.84–1.79)	—	1.05 (0.65–1.71)	—	1.04 (0.60–1.80)
Asian	0.79 (0.45–1.37)	—	0.88 (0.46–1.69)	—	0.88 (0.44–1.75)
Other	1.05 (0.44–2.49)	—	1.07 (0.38–3.00)	—	1.08 (0.36–3.21)
Family income					
⩾ $50,000	1.00	—	—	1.00	1.00
$30,000–49,999	1.07 (0.82–1.40)	—	—	1.09 (0.76–1.56)	1.05 (0.73–1.51)
< $30,000	1.38 (1.03–1.86)	—	—	1.14 (0.70–1.88)	1.07 (0.62–1.83)

* "No" is reference category for yes/no binary variable.
† Low birth weight is continuous variable and RR is estimated for an interquartile (0.61 kg) difference.

for the high and 64 EU/mg for the low cockroach allergen groups.

The presence of a dog in the home had a strong and significant ($p = 0.004$) univariate association with measured endotoxin in family room dust. Race/ethnicity also showed a significant association with measured endotoxin. Both low- and medium-income families also showed significantly lower endotoxin levels (p value < 0.02) than did high-income families (Table 2). In the multivariate regression, presence of a dog in the home, black (race/ethnicity) (p values < 0.02) as well as high cockroach allergen remained as significant predictors of measured endotoxin but family income did not. The directions and approximate magnitudes of the associations (as percentage difference) with dog and race/ethnicity were the same as in the univariate results.

Certain of the covariates examined in these models appeared to be related to the type of home lived in by the family (i.e., single- or two-family house versus an apartment building with three or more units). In our cohort, 13% of white, 58% of black, and 57% of Hispanic families lived in apartment buildings. Likewise, low- (70%) and medium-income families (34%) tended to live in apartment buildings more often than did high-income families (13%). The type of home was also associated with the presence of dogs. Eighteen percent of families in single- or two-family houses kept a dog inside, but only 9% of families in apartment buildings did so. The univariate regression analysis also indicated that family room dust samples from apartment buildings had significantly ($p = 0.02$) lower endotoxin levels than did those from single- or two-family houses, possibly because 87% of families with dogs lived in single/two family houses. However, home type was not a significant predictor ($p = 0.98$) of measured endotoxin level in family room dust in the multivariate regression model after controlling for other covariates.

Association of Endotoxin in Family Room Dust with Wheezing Episodes

In a univariate analysis, we found that elevated endotoxin level ($\geq$ 100 EU/mg) in family room dust was associated with a significant 29% increased risk of any wheeze during the first year of life. The multivariate model showed a significant ($p = 0.046$) 33% increased risk of any wheeze after we controlled for cockroach allergen, LRI, smoking during pregnancy, lower birth weight, maternal active and inactive asthma, presence of dog, and race/ethnicity (model 2 in Table 3). Table 3 also indicated that lower respiratory illness is the strongest independent risk factor, followed by endotoxin exposure, for any wheeze in the first year of life among covariates in our multivariate models.

Elevated family room endotoxin was not significantly associated with risk of repeated wheeze (RR = 1.17) in a univariate analysis (Table 4). The relative risk was still weak (RR = 1.21) and not significant in a multivariate model controlling for cockroach allergen, LRI, smoking during pregnancy, lower birth weight, maternal active and inactive asthma, and presence of dog. However, elevated endotoxin exposure was associated with a 56% significant increased risk of repeated wheeze when we further controlled for race/ethnicity in the multivariate model (Table 4). An analysis restricted to white infants also showed that elevated endotoxin exposure was associated with a 53% increased risk of repeated wheeze (but not significant due to lack of power: n = 375) after adjusting for the co-

TABLE 4

RELATIVE RISK (RR) OF REPEATED WHEEZE IN RELATION TO FAMILY ROOM DUST ENDOTOXIN

Variable*	Univariate RR (95% CI)	Multivariate RR (95% Confidence Interval)			
		Model 1	Model 2	Model 3	Model 4
Endotoxin					
$<$ 100 EU/mg	1.00	1.00	1.00	1.00	1.00
$\geq$ 100 EU/mg	1.17 (0.78–1.74)	1.21 (0.83–1.77)	1.56 (1.03–2.38)	1.53 (1.01–2.30)	1.55 (1.00–2.42)
Bla g 1 or 2, family room dust					
$<$ 0.05 U/g	1.00	1.00	1.00	1.00	1.00
$\geq$ 0.05 U/g	1.85 (1.27–2.70)	1.79 (1.24–2.59)	1.67 (1.12–2.49)	1.60 (1.03–2.49)	1.63 (1.01–2.63)
Lower respiratory illness					
Yes	2.43 (1.71–3.44)	2.26 (1.60–3.21)	2.47 (1.73–3.52)	2.40 (1.68–3.42)	2.41 (1.64–3.54)
Smoking during pregnancy					
Yes	2.29 (1.44–3.63)	1.82 (1.10–3.01)	1.50 (0.85–2.64)	1.59 (0.89–2.85)	1.68 (0.88–3.19)
Low birth weight†	1.39 (1.12–1.72)	1.32 (1.07–1.62)	1.34 (1.06–1.69)	1.35 (1.07–1.70)	1.34 (1.04–1.73)
Maternal asthma					
No	1.00	1.00	1.00	1.00	1.00
Active	1.63 (1.10–2.40)	1.32 (0.89–1.95)	1.17 (0.78–1.76)	1.27 (0.84–1.92)	1.30 (0.82–2.06)
Inactive	1.12 (0.59–2.10)	1.16 (0.62–2.17)	0.96 (0.49–1.89)	1.06 (0.54–2.08)	1.06 (0.52–2.17)
Presence of dog in home					
Yes	1.49 (0.98–2.27)	1.36 (0.91–2.04)	1.42 (0.91–2.22)	1.47 (0.95–2.29)	1.52 (0.94–2.45)
Race/Ethnicity					
White	1.00	—	1.00	1.00	1.00
Black	1.92 (1.24–2.96)	—	1.73 (1.05–2.86)	1.58 (0.91–2.75)	1.32 (0.69–2.52)
Hispanic	1.39 (0.70–2.76)	—	0.92 (0.45–1.87)	0.84 (0.38–1.85)	0.84 (0.37–1.94)
Asian	0.85 (0.33–2.17)	—	1.05 (0.41–2.67)	1.11 (0.44–2.80)	1.02 (0.38–2.70)
Other	2.55 (1.05–6.18)	—	2.58 (0.81–8.24)	2.70 (0.91–8.00)	2.38 (0.73–7.78)
Family income					
$\geq$ $50,000	1.00	—	—	1.00	1.00
$30,000–49,999	1.40 (0.89–2.21)	—	—	1.29 (0.81–2.06)	1.25 (0.75–2.06)
$<$ $30,000	1.87 (1.12–3.12)	—	—	1.11 (0.55–2.24)	0.95 (0.43–2.08)
Living in apartment ($\geq$ 3 families)					
Yes	1.53 (1.05–2.23)	—	—	—	1.44 (0.88–2.35)

* No is reference category for yes/no binary variable.

† Low birth weight is continuous variable and RR is estimated for an interquartile (0.61 kg) difference.

variates in model 1 of Table 4. The change in relative risk due to race observed after adding home type to the multivariate model (model 4 in Table 4) suggested that a portion of the confounding of endotoxin by race was related to home type.

We examined the endotoxin exposure–response relationship for wheeze (Figure 1) and repeated wheeze (Figure 2) using quartiles of endotoxin level in family room dust from the first home visit, controlled for other covariates in the final model. The quartile with the lowest rate of wheezing was used as the reference group. For any wheeze, the risk was similar in the two lowest and similarly elevated in the two highest quartiles. For repeated wheeze, the second quartile had the lowest risk; the first quartile (RR = 1.59; 95% CI = 0.83–3.05) and the third quartiles (RR = 1.56; 95% CI: 0.82–2.94) had moderately increased risk and the fourth quartile had significantly increased risk (RR = 1.90; 95% CI: 1.03–3.53).

DISCUSSION

Effect of Home Endotoxin Exposure on Wheeze in the First Year of Life

We found that endotoxin is associated with increased risk of wheeze, and may promote persistent wheezing (repeated reports of wheeze) during the first year of life among children with a family history of allergy or asthma. The risks were independent of the effect of lower respiratory infection—one of the strongest risk factors for wheeze in infancy (11–13).

Wheezing is frequent in young children, especially infants with diminished airway function and/or respiratory infection (11–13, 18). Possible mechanisms for wheezing induced by respiratory virus infection have been proposed, some of which are related to inflammation in the airways (19). Exposure to low levels of endotoxin in the first year of life may cause airway inflammation, thus triggering wheeze and promoting persistent wheeze in some infants. Human inhalation challenge studies indicate that endotoxin exposure initiates airway inflammation through nonallergic mechanisms (3, 20–24). It is also well known that endotoxin activates macrophages and monocytes with subsequent release of proinflammatory cytokines including tumor necrosis factor-α (TNF-α) and interleukin-1 and interleukin-8 (IL-1 and IL-8) that promote airway inflammation (25–27).

Our finding that elevated endotoxin exposure was associated with a larger adjusted relative risk for repeated wheeze than for any wheeze suggests that endotoxin may be a stronger independent risk factor for persistent inflammation than for transient inflammation causing wheeze in some children during early life. Exposure–response analysis suggested a nonlinear U-shaped relationship with repeated wheeze. Possible explanations for this observation are unmeasured confounding, that the association arose by chance in these data, or that certain implications of the hygienic hypothesis are responsible. If families with more severe asthma have exacerbations associated with endotoxin exposure, this may have resulted in environmental changes producing lower exposure in the most susceptible infants and thus a peak at both low and high exposures. Alternatively, the hygienic hypothesis posits that early exposure to harmful substances such as endotoxin may cause early transient wheezing, whereas lack of such exposures may predispose to persistent wheezing and development of allergy (28). Thus, the infants with wheeze at the low and high ends of the curve may have different prognoses. The effect, if any, of endotoxin exposure on risk of asthma in this cohort of children with a family history of allergy or asthma remains to be determined.

Michel and coworkers reported an association between house dust endotoxin levels and increased severity of asthma in dust mite-sensitized adults in Belgium, but they did not find an association between dust mite allergen alone and severity of asthma (7, 8). Rizzo and coworkers also reported that house dust endotoxin, but not dust mite antigen, was positively associated with severity of asthma among dust mite-sensitized children between 6 and 16 yr of age (9). These epidemiological studies seem to suggest an adverse effect of low-level home endotoxin exposure on the severity of asthma in dust mite-sensitized adults and children with asthma. However, the impact of early endotoxin exposure on the development of asthma or allergy remains to be determined.

Endotoxin's effects on production of Th1 and Th2 cytokines are likely to be important in understanding the impact of endotoxin on development of asthma and allergy (29). Zimmer and coworkers observed that in humans, an intravenous bolus of 4 ng/kg of *Escherichia coli* lipopolysaccharide (LPS) decreased IL-2 (a Th1 cytokine) but significantly increased IL-10 (a Th2 cytokine) 3 h after the injection (30). They interpreted this as implying that low dose endotoxin exposure promoted Th2 over Th1 responses. Another recent report also showed that subchronic LPS injections (20 injections of 5 μg at 48-h intervals) into gingiva of mice produced a maximum level of interferon-γ (INF-γ) (a Th1 cytokine) at the tenth injection but that INF-γ decreased thereafter. IL-4 (a Th2 cytokine) began to increase after the tenth injection (31). Based on these find-

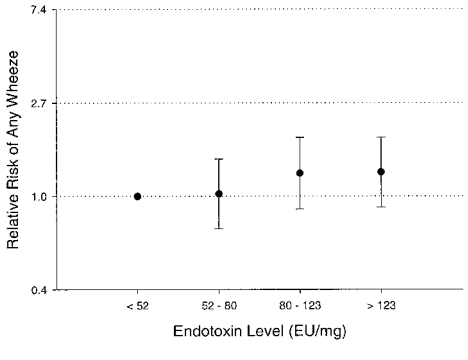

Figure 1. Relative risk of any wheeze using quartiles of endotoxin levels in family room dust, controlling for cockroach allergen, lower respiratory illness (defined as doctor diagnosed croup, bronchitis, bronchiolitis, or pneumonia), smoking in pregnancy, maternal active and inactive asthma, birth weight, presence of a dog(s) in home, race/ethnicity.

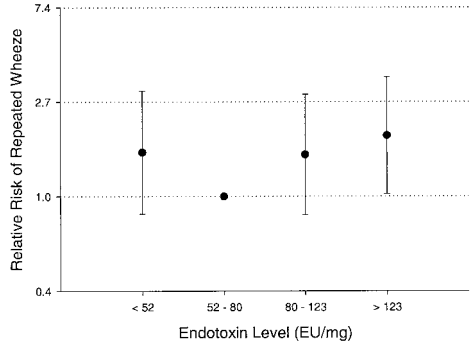

Figure 2. Relative risk of repeated wheeze using quartiles of endotoxin levels in family room dust, controlling for cockroach allergen, lower respiratory illness, smoking in pregnancy, maternal active and inactive asthma, birth weight, presence of a dog(s) in home, race/ethnicity. The second quartile was used as reference and nonlinear exposure–response relationship is shown.

ings, the authors suggested that repeated exposure to endotoxin might induce Th cell type switching from Th1 to Th2. But route of exposure to endotoxin in both studies is quite different from that of exposure to home endotoxin in infants, thus it may not be possible to extrapolate from these studies.

On the other hand, Baldini and coworkers (32) found a polymorphism in the promoter region for the CD14 gene, a key component of the endotoxin receptor (33), associated with high sCD14, low total serum immunoglobulin E (IgE), and reduced risk of hypersensitivity to specific aeroallergens. They also found that sCD14 level was positively associated with IFN-γ (a Th1 cytokine) and negatively associated with IL-4 (a Th2 cytokine) in peripheral blood. Thus, they suggested that endotoxin might play a role in directing Th1 cell development by promoting IL-12 production from dendritic cells activated by sCD14–endotoxin complexes, resulting in a protective effect against atopic disease. A recent report showing an association between house-dust endotoxin and production of Th1 cytokines by peripheral blood mononuclear cells lends support to this hypothesis (34).

Whether endotoxin plays a role in selecting and switching to Th1 or Th2 cells during infancy is yet to be answered. It has been suggested that onset of childhood asthma may be affected during early life by environmental factors that can influence the selection of and switching to Th1 or Th2 cells (35). Therefore, the possibility that endotoxin exposure may increase or decrease the risk of asthma is an intriguing hypothesis. The factors such as level of exposure, route of exposure, time course of exposure, structure of LPS, and preexisting host factors such as family history should also be carefully considered in testing this hypothesis.

Association Between Covariates in Multivariate Models

The association of endotoxin with the risk of any wheeze was not confounded by other covariates including socioeconomic variables in our data. On the other hand, the association of endotoxin exposure with risk of repeated wheeze was negatively confounded by race/ethnicity. The negative confounding by race/ethnicity appeared to result from the lower levels of endotoxin in family room dust collected from the homes of black and Hispanic infants. The association of endotoxin with repeated wheeze was confirmed in an analysis restricted to white infants.

Presence of a dog was an important contributor to increased endotoxin levels in house dust. Our finding that apartments showed significantly lower endotoxin levels than single- or two-family houses may have resulted because landlords often do not allow tenants to keep dogs. In fact, most of the dog owners in our cohort lived in single- or two-family houses. And this relationship might have affected negative confounding of race/ethnicity in association of endotoxin exposure with risk of repeated wheeze because blacks and Hispanics were more likely to live in an apartment than were whites. The association of race/ethnicity with the risk of repeated wheeze was partially confounded by home type, implying that other, unmeasured, environmental factors are responsible for the increased risk among black subjects.

In summary, elevated endotoxin in family room house dust ($\geq$ 100 EU/mg) was associated with increased risk of any wheeze and of repeated wheeze in the first year of life in a cohort of children genetically predisposed to asthma and allergy. The association was stronger with repeated wheeze than with any wheeze. It remains to be determined whether these infants, with wheeze in association with endotoxin exposure, are genetically more susceptible to endotoxin exposure or are at greater or lesser risk of developing asthma.

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