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Multiple Microbial Exposures in the Home May Protect Against Asthma or Allergy in Childhood

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Abstract

Background—Experimental animal data on the gram-negative bacterial biomarker endotoxin suggest that persistence, dose and timing of exposure are likely to influence its effects on allergy and wheeze. In epidemiologic studies, endotoxin may be a sentinel marker for a microbial milieu, including gram-positive as well as gram-negative bacteria, that may influence allergy and asthma through components (pathogen-associated molecular patterns) that signal through innate Toll-like receptor pathways.

Objective—To determine the influence of current gram-negative and gram-positive bacterial exposures on asthma and allergic sensitization in school-aged children.

Methods—We examined the relationship between bacterial biomarkers and current asthma and allergic sensitization in 377 school-aged children in a birth-cohort study. We then evaluated the effects of school-age endotoxin, after controlling for exposure in early life.

Results—Exposure to gram-negative bacteria was inversely associated with asthma and allergic sensitization at school-age (for > median endotoxin: prevalence odds ratio [POR] =0.34 [95% CI=0.2 to 0.7] for current asthma and prevalence ratio [PR]=0.77 [95% CI=0.6 to 0.97] for allergic sensitization). In contrast, elevated gram-positive bacteria in the bed was inversely associated with current asthma (POR= 0.41, 95% CI=0.2 to 0.9) but not with allergic sensitization (POR=1.07, 95% CI=0.8 to 1.4). School-age endotoxin exposure remained protective in models for allergic disease adjusted for early-life endotoxin.

Conclusion—Both gram-negative and gram-positive bacterial exposures are associated with decreased asthma symptoms, but may act through different mechanisms to confer protection. Endotoxin exposure in later childhood is not simply a surrogate of early life exposure; it has independent protective effects on allergic disease.

Keywords

childhood asthma; allergic sensitization; endotoxin; peptidoglycan

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Introduction

Timing as well as persistence of exposure may influence the direction as well as the magnitude of the effects of endotoxin, the biologically active form of lipopolysaccharide (LPS) contained in the outer membrane of gram-negative bacteria (GNB), on the risk of allergy and wheeze [1–6]. Endotoxin has been inversely associated with allergic sensitization, asthma and atopic wheeze in European studies of school-aged farm children [7–9]. Because of the cross-sectional design of these studies, it is not certain whether endotoxin levels measured at school-age represent the relative level of persistent exposure that the children have encountered since infancy. While the documented relation of school-aged endotoxin with retrospectively reported infant parenting and farming practices [10] make it plausible that school-aged endotoxin levels represent the relative level of prior infant exposure in these European studies, it is less likely that school-aged home endotoxin levels would represent prior exposures in urban U.S. homes without a constant source of endotoxin. In a longitudinal urban Northeastern U.S. birth cohort study we had the unique opportunity to investigate the cross-sectional associations of school-aged home endotoxin levels with asthma, wheeze, and allergic sensitization, taking into account endotoxin levels during the critical developmental time window, infancy.

Endotoxin may be a sentinel marker for a complex array of bacterial exposures arising from shared sources (e.g., mammals or dampness) that may influence immune development through shared pathways [9,11]. For example, compared to endotoxin, gram-positive bacteria (GPB) have components (pathogen-associated molecular patterns (PAMPS)) that signal through some shared and some separate Toll-like receptor pathways that may influence the risk of allergy and asthma. While the extent to which endotoxin may be a marker for a more complex milieu of microbial farm exposures is still uncertain, a European cross-sectional study has demonstrated independent associations of higher levels of the gram positive marker muramic acid with reduced wheeze rates [12]. In the urban U.S. setting we had the opportunity to evaluate whether other markers of gram-negative and gram positive bacteria known to work through some shared and some independent mechanisms, partially explained associations of endotoxin with the risk of allergy or wheeze in school children.

METHODS

Study Cohort

The Epidemiology of Home Allergens and Asthma Study is an ongoing longitudinal birth cohort study of the effects of environmental exposures on the risk of allergy and asthma in children born to parents with histories of allergies and/or asthma. [13] A detailed description of subject recruitment and study design has been published previously. [13,14] Briefly, between September 1994 and June 1996, families from metropolitan Boston (MA) were recruited at a major Boston hospital during the immediate post-natal period after the birth of the index child. Of the 505 children enrolled in the study, 7 were excluded because they were followed 4 months during their first year of life. The study was approved by the institutional review board of Brigham and Women's hospital. Exclusion criteria included: premature birth <36 weeks, congenital abnormalities, hospitalization in the neonatal intensive care unit, maternal age <18 years, and planning to move within the next year. Of the original 505 children enrolled, 448 (89%) have health outcome data at school age, and 377 (75%) have both health outcome data and home microbial exposure assessment at school age.

Home visits and dust sample collection

Methods for home dust collection and endotoxin measurement in infancy have been described previously. [13,14] Briefly, family room dust samples collected in infancy consisted of dust vacuumed from both the family room floor and an upholstered chair most often used by the parent while holding the infant. We again collected house family room and bed samples from participating subjects at a mean age of 7 years (range 6.5 to 9.2 years). We used a Eureka Mighty-Mite vacuum cleaner (Model 3621; Eureka Co., Bloomington IN) modified to hold 19 × 90 mm cellulose extraction thimbles. For family room dust samples at school age, we vacuumed both a one-meter² area of the family room floor for 2 minutes and an upholstered chair commonly used by the index child for 3 minutes. For bed dust, all layers of the bedding were vacuumed for 10 min. In order to have sufficient dust for measurement both of allergens and microbial biomarkers, two family room dust samples were collected. For bed dust, when quantities were low, allergen measurement took priority over microbial biomarkers measurement. We weighed and sieved the dust through a 425 µm mesh sieve within 24 hours of collection. We reweighed the fine dust and made aliquots for allergen and microbial biomarker measurement.

Microbial Biomarker Assays

In school-age dust samples, we measured 3 biomarkers of gram-negative bacteria. We determined endotoxin bioactivity using both kinetic Limulus ameocyte lysate (LAL) and recombinant Factor C (rFC) [15] assays with the resistant-parallel-line estimation (KLARE) method as previously described. [16] In our measure of gram-negative bacteria determined by gas chromatography/mass spectrometry (GC/MS), the 3-hydroxy fatty acids (3OHFAs) with the highest correlation to endotoxin (C10:0, C12:0, C14:0 or “mid chain length”) were summed and reported as picomoles (pm) per mg dust. [17] Our gram positive biomarker, peptidoglycan (N-acetyl muramic acid), was also measured by GC/MS [18,19] at school-age, but not in infancy.

Only the Limulus lysate measures of endotoxin were available in infant dust. For each study phase separately (infancy, school-age), Limulus assay results were reported in endotoxin units with references to EC5 or EC6 reference standard endotoxin (U.S. Pharmacopoeia, Inc.; 1ng of EC5 and EC6=10 EU) after adjustment for lot-to-lot differences in LAL sensitivity [20].

Assessment of Allergen Sensitization

Allergy skin testing at school-age was performed at a mean age of 7.4 years (range, 6.5 to 10.1 years) on the volar aspect of the lower arms. The allergens tested included common indoor (cat dander, dog dander, cockroach [*Blattella germanica*], dust mite [*Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*], and mouse epithelial extract, and outdoor (ragweed, mixed trees, *Aspergillus*, *Alternaria*, mixed grasses, *Cladosporium*, and *Penicillium*) allergens (Hollister Steir Labs, Spokane, Wash). Glycerated saline and histamine were used as the negative and positive controls, respectively. Skin tests to specific allergens were considered positive if the mean diameter of the wheal was ≥ 3 mm after subtraction of the control wheal. For the children who assented to allergy measurement but not to skin prick testing [23/271(8%)], allergy was assayed as IgE to the allergens listed, using the UniCAP 250 system (Pharmacia & Upjohn, Kalamazoo, Mich). IgE to specific allergens was considered positive at a level ≥ 0.35 IU/mL.

Definitions of Health Outcome Variables at School-Age

Health outcome data were gathered every 6 months by telephone questionnaire. The disease and symptom data closest to the time of school-age dust sample collection was used in the

cross-sectional analysis. Time between environmental sampling and health outcome assessment by questionnaire was, on average, 2 months (range 1 day to 7 months). Outcomes included (1) current asthma, defined as a doctor's diagnosis of asthma with any wheeze in the past twelve months, including wheeze after exposure to cold air or exercise (current asthma); (2) recurrent wheeze (> 1 wheezing event in the last year); and (3) allergic sensitization, defined as having at least 1 positive skin test or specific IgE to the allergens tested. Outcomes for current asthma and recurrent wheeze were not mutually exclusive (subjects could contribute to both groups).

Data Analysis

To determine correlations among the bacterial biomarkers measured at school-age, we computed Spearman's correlation coefficients. We used logistic regression to determine the cross-sectional associations of bacterial biomarkers levels with symptom/disease prevalence at school age. *A priori*, we expressed the bacterial biomarkers as categorical variables, dichotomized at above vs. below the median level for the specific room sampled at that study phase. In analyses designed to evaluate the sensitivity of our results to the choice of cut points chosen for our dichotomous variables, we expressed the bacterial biomarkers as log-transformed continuous variables or as continuous z-scores. Expression of our exposures either as dichotomous or, alternatively, as continuous variables provided us with the power to evaluate effect modification of school-age endotoxin effects by levels of endotoxin in infancy. Secondly, when preliminary analyses suggested non-linear associations with symptoms (i.e., for muramic acid) we used smoothing splines to explore the shape of those associations. This was done using the gam procedure in R statistical software (www.r-project.org), with automatic smoothing parameter selection (based on UBRE score minimization) to estimate the smoothing parameters for penalized regression splines.

To study modification of the effects of school-age endotoxin by infant endotoxin levels, we created a four level categorical variable: repeatedly high (> median at in early life and school age), high exposure (> median) in early life only, high exposure (> median) at school-age only, and repeatedly low (< median at both time points, reference category). We also evaluated effect modification with models including main terms for school-aged and infant endotoxin levels as log-transformed continuous variables, along with a multiplicative interaction term. We evaluated cleaning behaviors, including the frequency of bedroom cleaning, bed sheet washing (> or < 1x per week), cleaning on the day of or day before dust sample collection; environmental tobacco smoke, household income (less than \$35,000 per year), dog allergen (Can f 1 > 20 µg/g dust), cat allergen (Fel d 1 > 8 µg/g), and dust mite allergen (Der p 1 > 2 µg/g Der f 1 > 2 µg/g) and total collected dust (gm) as potential confounders of microbial biomarker effects (a > 10% change in POR). We controlled for other potential independent predictors of our outcomes, including age, race, sex and maternal asthma. Proc Logistic and Proc Genmod procedures in SAS statistical software (SAS Institute Inc., Cary, North Carolina) were used to compute prevalence odds ratios and prevalence ratios. Prevalence ratios rather than prevalence odds ratios were computed for allergic sensitization, as a conservative effect estimate for this prevalent outcome [21].

RESULTS

Exposure, Demographics and Symptoms

Of the 406 children with family room endotoxin (LAL) levels measured in infancy, 360 also had family room endotoxin and 356 had family room muramic acid measured at school age. The median and range for each of the bacterial biomarkers in family room and bed dust are shown in Table 1. At school age, concentrations of GNB biomarkers in family room dust

were approximately twice as high as those in bed dust, and showed a wider range than biomarkers in bed dust samples (Table 1). Endotoxin measurements above the 89th percentile in family room dust (93.3 to 1003 EU/mg) exceeded the maximum bed dust endotoxin level (93 EU/mg dust) by 10 fold. Muramic acid, mainly derived from GPB, was present at similar levels in family room and bed dust samples. While all bacterial biomarkers measured were significantly ($p < 0.05$) correlated, the correlation coefficients among gram-positive and gram-negative biomarkers were modest ($r = 0.28$ to 0.35).

Children with bacterial measurements at school age were more likely to be white than those who had early-life measurements (Table 1). A higher percentage of white children (80.8%) were tested for allergic sensitization, as compared to the percentage of white children (75.3%) enrolled at baseline [22]. The correlation between family room LAL endotoxin measured at age 1–3 months, and that measured at school age was low ($r = 0.13$, $p=0.01$). Those with a family room sample at school age tended to have endotoxin levels above the median for family room samples at age 1–3 months (Table 1). The prevalence of current asthma was 9.8 %, and the prevalence of recurrent wheeze in the past 12 months was 9.0%, for those with microbial biomarker assessment at school age ($n=377$). Of the children with recurrent wheeze, 74% were diagnosed with asthma. In the subset of children evaluated for allergic sensitization ($n=264$), 73 % with current asthma and 75% with recurrent wheeze were sensitized. Of those with current asthma, 70% used short acting beta-agonists, 24% were on long-term controller medications (e.g.,advair, azamacort, beclovent, flovent, budesonide, pulmicort, vancertil), and 21% were taking leukotriene antagonists. Forty-four percent of those with a prior doctor's diagnosis of asthma (37 of 85) had current active asthma at school age. Current asthma prevalence in children with microbial biomarker assessment at school age was not significantly different from current asthma prevalence in children without exposure assessment at school age. (9.8% vs. 8.5%, respectively, Chi-square $p=0.50$).

School-age home bacterial biomarkers, asthma and allergy

At school age, endotoxin levels above the median in the family room were associated with reduced odds of current asthma, recurrent wheeze and allergic sensitization (Table 2). Similar trends were observed when endotoxin exposure was entered into the models as a log-transformed continuous variable. For a log increase in endotoxin levels, there was a 0.52 reduction in the odds of current asthma (95% CI 0.3 to 0.9), a 0.63 reduction in the odds of recurrent wheeze (95% CI 0.4 to 1.1), and a 0.77 (95% CI 0.61 to 0.97) reduction in the odds of allergic sensitization. Adjusting for infant endotoxin levels did not significantly alter these estimates (Supplementary files, Table E1). For asthma and wheeze, but not for allergic sensitization, associations of bed endotoxin were in the same direction, though weaker and less precise. Other biologic and chemical markers of GNB (rFC, and mid-chain 3OHFAs, respectively) had similar associations with these outcomes (Supplementary files, Table E2).

Bed dust levels of GPB (muramic acid) above the median were more strongly associated with reduced odds of current asthma than dust levels of muramic acid in the family room. A smoothing spline of bed muramic acid and current asthma suggested a more complex dose-response relationship, in the form of an inverse J-shaped curve (data not shown). Muramic acid levels were not associated with allergic sensitization. In general, endotoxin (GNB) and muramic (GPB) biomarker effects were independent of each other in multiple bacterial exposure models, though estimates decreased slightly in precision with both biomarkers in the models (Table 3).

Associations of bacterial biomarker levels with disease, symptom, and sensitization outcomes were not confounded by age, race or sex. Cleaning behavior, low income level, and exposure to environmental tobacco smoke did not substantially change estimates in

logistic regression models. (Online Supplement, Table E3). Likewise, total collected dust (gm), dust mite, cockroach, cat, and dog allergens did not confound the associations between bacterial biomarker levels and symptom outcomes.

Evaluation of Modification of School Age Endotoxin Effects by Early Life Exposure

Early life endotoxin levels did not modify the relation of school age endotoxin to asthma, wheeze or allergy, when both exposures were specified as continuous variables (either log transformed or z-scores) in logistic regression models (p value for interaction term = 0.4 to 0.9). However, compared to those who always had endotoxin below the median, the odds of current asthma were reduced for those either with repeated elevation of endotoxin above the median (in infancy and at school age) or with elevation of endotoxin only at school age. (Table 4) Those with endotoxin levels above the median level only in infancy were not protected against asthma risk at school age.

DISCUSSION

To the best of our knowledge, no other longitudinal birth cohort studies have investigated the independent effects of early life and school age exposures to endotoxin [23–25]. In this U.S. urban setting, we have demonstrated that school-aged home endotoxin exposures have protective influences on active asthma, wheeze and allergy risk that are independent of the effects of infant home endotoxin exposures. While infant endotoxin was not protective against school aged active asthma, in this same cohort, elevated levels of infant home endotoxin predicted subsequent reduced Th2 cytokine IL-13 production, and reduced risk of eczema by age 1 and allergic sensitization by school age [22, 26, 27]. While the prenatal and immediate postnatal period are critical windows for development [28], this is the first urban U.S. longitudinal birth cohort study to suggest that the immunomodulatory protective effects of endotoxin on asthma risk may occur not only in infancy, but also in later childhood.

While there are 3 other longitudinal studies on endotoxin exposure and childhood allergic disease [23–25], ours is the first to report outcomes up to primary school age, and to demonstrate the unique contribution of later childhood exposure, after controlling for exposure in early life.

Although school-aged elevation in endotoxin was associated with protection against wheeze and active asthma at school-age, elevated infant endotoxin levels were associated with increased risk of wheeze in early life [22, 29]. Amongst older siblings close in age to our index children, we have reported associations between endotoxin and increased risk of wheeze at the beginning of follow-up (in early childhood), but with protection against wheeze by the end of the 4-year period, when the children were between 5 and 9 years of age [30]. We hypothesize that these early life findings may be due to the irritant effects of endotoxin, [31] while the later protective associations may occur through endotoxin effects on Toll receptor immunomodulatory pathways. Animal data suggest that endotoxin/LPS effects are stage of life (timing) as well as dose dependent [1–6].

While our data strengthens confidence that later childhood exposure contributes to protection against wheeze and asthma, power limited our confidence in the role of elevated exposure in infancy and in later childhood. We only found effect modification of school-aged endotoxin exposure by infant endotoxin in our analyses using categorical variables, dichotomized at the median, whereas we found no effect modification when we treated endotoxin as a continuous variable. Our positive findings were, thus, sensitive to our a priori cut-point and dependent on the small group of asthmatics in each category (Table 4).

The median for the absolute levels of family room endotoxin at school-age was lower than those reported in infancy, though the range was similar. While it is possible that these differences are real, our data suggest that the primary reason for the drop in mean endotoxin levels observed between infancy and school age may have been lower activity of the LAL reagent lot used for endotoxin assays at school age. When measuring the school-aged endotoxin by LAL, we conducted a lot-to-lot comparison study between the activity of the stored LAL reagent used for the assays of infant endotoxin levels, and the activity of the LAL reagent used for the assays of school-aged endotoxin levels. Our school-age endotoxin measurements incorporate an adjustment for the change in reagent lot. This adjustment may be imperfect, however, because of possible degradation over time of the LAL reagent lot used in infancy. Thus the school age endotoxin values presented here incorporate the best possible lot-to-lot adjustment, given the unknown stability of the reagent over such a lengthy period of time. The issue of lot-to-lot variability in LAL reagent is not unique to our study, and many other laboratory assays are well-known to have significant lot-to-lot as well as lab-to-lab variability [20, 32]. Absolute comparisons of levels of endotoxin measured at different time points in different studies should be made with caution. Nevertheless one can have confidence in the relative ranking of endotoxin levels if done at the same time, particularly if they take into account any lot-to-lot variability if more than one lot is needed for the measurements.

At school age, concentrations of GNB biomarkers in family room dust were approximately twice as high as those in bed dust, and showed a wider range than biomarkers in bed dust samples. Although there was much overlap between the family room and bed dust distributions, endotoxin measurements above the 89th percentile in family room dust exceeded the maximum bed dust endotoxin level by 10 fold. Higher levels observed for this small subset may have resulted from direct contact of the family room floor with footwear carrying dirt (and GNB) in from outdoors, or from the variable effects of home characteristics (pet presence, water damage, air conditioning) on different sampling locations within the home. Muramic acid, mainly derived from GPB, was present at similar levels in family room and bed dust samples.

We can have less confidence in our findings regarding GPB, as the protective associations we found were only statistically significant for the bedroom bed, and the shape of the associations was nonlinear. This could have biologic plausibility, but could also be due to chance. Our study would have also been stronger had we had more repeated measures of endotoxin over time; nevertheless, we have still provided unique longitudinal exposure-response data. Data on the time spent in each room, along with bed dust endotoxin measures in infancy, would have enhanced the accuracy of our exposure assessment. Reported cleaning habits did not confound the associations observed, but it is still possible that they could be explained by unmeasured changes in behavior related to the mother or the child's symptom status. We did not have genetic data, though we are aware that genes may influence endotoxin responses, and the cohort is too small to evaluate gene-by-environmental interactions. Allergic sensitization was assessed by skin prick testing in 92% of participants, and by RAST testing in 8%. However, associations between microbial biomarker exposure and allergic sensitization remained unchanged after removal of the small group of RAST tested subjects from the analysis. A final limitation was that we only had repeated measures data at infancy and at school-age on endotoxin measured by LAL in the family room and not on multiple bacterial biomarkers from multiple rooms in the household. However the home bacterial exposure data gathered at two time points still provides us with unique opportunities to understand their relationships with child health in our urban birth cohort.

Only one other home study and one school study have reported independent protective effects of GPB on wheeze risk [12,33]. As in the ALEX study of school-aged farm children, which reported inverse associations between muramic acid levels in mattress dust and wheeze, the associations were independent of the protective effects of endotoxin [12]. Children in the ALEX cohort had higher levels of muramic acid in the participants' bed (geometric mean, 128 ng/mg) than those observed in our cohort (median, 62.7 ng/mg) but the correlations between endotoxin and muramic acid in bed dust were similar. A cross-sectional study in Chinese schools demonstrated a decreased prevalence of wheeze and daytime breathlessness in subjects exposed to higher levels of muramic acid and mid-chain (C10:0, C12:0, and C14:0) 3OHFAs in dust from classroom floors [33].

While all three of the above studies, including ours, found GPB protective associations with wheeze risk, GPB levels had no relationship with allergic sensitization [12, 33]. Muramic acid from commensal GPB, and endotoxin from GNB are pathogen associated molecular patterns (PAMPs) work through different innate Toll receptor pathways [34]. Muramic acid and its source, peptidoglycan, have been shown to act through the TLR-2 receptor influencing innate immunity [35,36], as well as through increased secretion of IL-10, an anti-inflammatory cytokine produced by T regulatory cells [37]. While it may also promote T-regulatory cell downregulation of inflammatory responses [38], endotoxin has been also demonstrated to bind to the TLR-4 MD2 complex on antigen presenting cells. Downstream, this may result in increased innate IL-12 production, with upregulation of Th1 IFN- γ and consequent downregulation of pro-allergic Th2 cytokine production.

In our cohort and in the ALEX farm children's study, dose response curves for GNB were different compared to GPB. Models for endotoxin exposure as a continuous variable supported a linear dose-response relationship for this gram-negative component. In contrast, as in the ALEX study, muramic acid demonstrated an inverse-J shaped curve in relation to current asthma [12]. While non-linear relationships may be artifacts that relate to chance observation, the replication of our findings suggest that they may have some biologic basis. The finding of protective associations with bed GPB but not with family room floor GPB may also have a basis in room differences in the genera and sources of GPB, as well as their proximity to the child. GPB in the bed may come not only from external sources, but also from the child him/herself. If the child is the primary bacterial source, then the child's flora may reflect his/her health status. Translational animal model studies of protective components of commensal bacteria [39], as well as prospective epidemiologic studies with repeated measures of multiple microbial flora are needed to evaluate potentially protective components of gram-positive and gram-negative bacteria.

Comparison between our findings and those of the European studies must be made cautiously—the exposures of U.S. urban children are likely to differ significantly from their European counterparts in terms of the dose and persistence of endotoxin levels, as well as the composition of the gram-positive bacteria and other PAMPS to which they are exposed.

In conclusion, gram-positive as well as gram-negative commensal microbial exposures in the home may protect against asthma and wheeze in school-aged children. Gram positive and gram-negative bacterial exposures may differ in their influence on allergic sensitization. Independent of infant exposures, elevated endotoxin exposure in later childhood may have a protective effect on allergic disease in school-aged children.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Characteristics of participating children at baseline and at school age

	Age 1–3 months	School Age	
		Family Room Biomarker Assessment (N=360)	Bed Biomarker Assessment (N=299)
Female Sex	230 (46.2%)	160 (44.4%)	130 (43.5%)
Ethnicity			
White	375 (75.3%)	294 (81.7%) *	243 (81.3%) *
Black	60 (12.1%)	26 (7.2%) *	26 (8.7%) *
Hispanic	28 (5.6%)	18 (5.0%)	15 (5.0%)
Asian and Other	35 (7.0%)	22 (6.1%)	15 (5.0%) *
Maternal Asthma	152 (30.5%)	102 (28.3%) *	80 (26.8%) *
Endotoxin, LAL (Family Room), Age 1–3mths [†]			
Median (80 EU/mg)	203 (50%)	136 (46.7%) *	118 (48.6%)
> Median (80 EU/mg)	203 (50%)	155 (53.3%) *	125 (51.4%)
	Biomarker Median (Range)		
	Age 1–3 months Family Room (N=406)	School Age Family Room (N=360)	School Age Bed (N=299)
Endotoxin, LAL (EU/mg)	80 (2 – 713)	39 (2 – 1003)	19 (2 – 93)
Mid Chain 3OHFAs (pmol/mg)	--	60 (7 – 304)	36 (10 – 175)
Muramic Acid (ng/mg)	--	72 (19 – 326)	63 (6 – 510)

* P < 0.05 for the difference in characteristics in the subset with follow-up, compared to the group with microbial measurements at 1–3 months of age.

[†]The subset (N=291) of those with school age biomarker measures who also have family room endotoxin measurement at 1–3 months of age.

Table 2

Relation of microbial biomarker levels (median vs. > median) with current asthma, recurrent wheeze, and allergic sensitization at school age

Endotoxin (LAL)	Current Asthma ^{*,†}		Recurrent Wheeze [*]		Allergic Sensitization ^{*,‡}	
	No	Yes	Yes	No	Yes	PR (95% CI)
<i>Family Room</i>						
median (38.6 EU/mg)	156	24	159	21	52	73
> median (38.6 EU/mg)	169	11	171	9	67	62
						0.39 (0.2 to 0.9) [§]
<i>Bed</i>						
median (18.9 EU/mg)	131	18	129	20	47	53
> median (18.9 EU/mg)	136	14	141	9	49	60
						0.40 (0.2 to 0.9) [§]
<i>Muramic Acid</i>						
median (72.2 ng/mg)	157	20	160	17	58	69
> median (72.2 ng/mg)	165	14	167	12	61	66
						0.66 (0.3 to 1.4) [§]
<i>Bed</i>						
median (62.6 ng/mg)	126	22	129	19	45	55
> median (62.6 ng/mg)	140	10	140	10	51	58
						0.50 (0.2 to 1.1) [§]

* Adjusted for age, race, sex

[†] Adjusted for maternal asthma

[‡] Adjusted for sex, race

[§] p<0.05

POR= prevalence odds ratio; PR = Prevalence ratio

Table 3

Association of microbial biomarkers with respiratory symptoms and allergic sensitization at school age, in dual exposure (GNB + GPB) models

	Current Asthma ^{*†} POR (95% CI)	Recurrent Wheeze [*] POR (95% CI)	Allergic Sensitization ^{*‡} PR (95% CI)
<i>Family Room</i>			
Endotoxin (LAL) (> Median)	0.4 (0.2 to 0.8) [§]	0.4 (0.2 to 0.97) [§]	0.8 (0.6 to 0.97) [§]
Muramic Acid (> Median)	0.7 (0.3 to 1.5)	0.8 (0.3 to 1.7)	1.0 (0.8 to 1.2)
<i>Bed</i>			
Endotoxin (LAL) (> Median)	0.8 (0.3 to 1.8)	0.5 (0.2 to 1.1)	1.03 (0.82 to 1.31)
Muramic Acid (> Median)	0.4 (0.2 to 1.0)	0.6 (0.3 to 1.5)	1.06 (0.81 to 1.39)

* Adjusted for age, race, sex

† Adjusted for maternal asthma

‡ Adjusted for sex, race

§ p < 0.05

POR = prevalence odds ratio; PR = Prevalence ratio

Table 4

Relation of endotoxin in infancy and at school age with current asthma, recurrent wheeze, and allergic sensitization at school age

Endotoxin Assessment in early life + school age (Early Life = Age 1-3months) (School Age= Age 7)	Current Asthma [†]		Recurrent Wheeze [*]		Allergic Sensitization [‡]	
	No	Yes	No	Yes	No	Yes
						PR (95% CI)
Below median at both time points	71	12	71	12	21	35
Above median in early life only	52	10	55	7	22	25
		1.00 (0.38 to 2.67)		0.75 (0.27 to 2.1)		0.89 (0.64 to 1.25)
Above median at school age only	49	4	50	3	21	16
		0.31 (0.09 to 1.1)		0.31 (0.08 to 1.2)		0.68 (0.45 to 1.03)
Above median at both time points	90	3	90	3	31	35
		0.14 (0.04 to 0.56) [§]		0.19 (0.05 to 0.72) [§]		0.86 (0.63 to 1.17)

^{*} Adjusted for age, race, sex

[†] Adjusted for maternal asthma

[‡] Adjusted for sex, race

[§] p< 0.05

POR= prevalence odds ratio; PR = Prevalence ratio