School Endotoxin Exposure and Asthma Morbidity in Inner-city Children

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BACKGROUND: Endotoxin exposure is associated with airway inflammation. Children spend 6 to 8 h/d in school, yet the effect of school-specific endotoxin exposure on asthma morbidity is not well understood.

METHODS: In this longitudinal cohort study, 248 students with asthma, from 38 inner-city schools, underwent baseline phenotyping and follow-up. Clinical outcomes were evaluated throughout the academic school year and linked to classroom-specific dust and air endotoxin levels as well as home dust endotoxin levels. The primary outcome was maximum asthma symptom-days per 2-week period.

RESULTS: Classrooms had higher settled dust endotoxin levels compared with homes (14.3 endotoxin unit/mg vs 11.3 endotoxin unit/mg; $P = .02$). Airborne endotoxin levels exceeding recommended occupational exposure limits for adults were recorded in 22.0% of classrooms. Classroom air endotoxin levels were independently associated with increased maximum symptom-days in children with nonatopic asthma, but not in those with atopic asthma (interaction $P = .03$). Adjusting for home exposures, classroom endotoxin exposure was independently associated with a dose-dependent increase in asthma symptom-days for children with nonatopic asthma (adjusted incidence rate ratio, 1.16 [95% CI, 1.03-1.31]; $P = .02$). In these subjects, maximum symptom-days increased by 1.3 days for each 14-day period when comparing students in classrooms with the lowest endotoxin levels compared with average measured levels.

CONCLUSIONS: Inner-city children with asthma are exposed to high levels of airborne endotoxin at school, resulting in increased asthma symptoms in children with nonatopic asthma. Mitigation of school-related exposures may represent a strategy to decrease asthma morbidity in this population.

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ABBREVIATIONS: EU = endotoxin unit; IRR = incidence rate ratio

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Asthma affects > 300 million adults and children worldwide.¹ Inner-city children share a disproportionately large burden of disease due to asthma, and experience more severe asthma symptoms.² While the reason for this is multifactorial, home-specific exposures have been identified as important,³ with multicenter studies showing that a home environmental intervention to reduce environmental risk factors can reduce asthma morbidity.⁴ Most of these studies have focused on the role of pollutants and allergens in sensitized patients with asthma.

Endotoxin in homes has been demonstrated to increase wheeze,⁵ increase both airflow obstruction and bronchial hyperreactivity in human challenge studies,⁶ and

can potentiate the airway response to allergens in people with asthma.⁷ Endotoxin has also been identified as an important exposure in many occupational settings.⁸ The school is likely an occupational model for children, given that nearly every child spends the majority of his or her day in school. However, hardly anything is known about the role of school-specific endotoxin exposure on asthma morbidity, after adjusting for exposure in the home. In this study, we aimed to test the hypothesis that school endotoxin exposure is associated with increased asthma symptoms independent of home endotoxin exposure and to test whether the effect of school endotoxin exposure differs between children with atopic asthma and those with nonatopic asthma.

Materials and Methods

Study Population

The School Inner-City Asthma Study (SICAS) is a single-center, prospective cohort study of children with persistent asthma who attended inner-city elementary schools in a northeastern US city from 2008 to 2013. Children with persistent asthma attending these schools were recruited based on established inclusion and exclusion criteria modeled from other urban studies⁹ and adapted for SICAS. The criteria included (1) history of physician-diagnosed asthma and either current symptoms defined as cough, wheezing, shortness of breath, or whistling in the chest in the past 12 months; daily controller medication use; or unscheduled medical visits for asthma in the past year; and (2) attendance in grades kindergarten through sixth grade at a school where permission for environmental sampling had been obtained. The exclusion criteria included any significant pulmonary disease other than asthma. Written informed consent was obtained from each participant's parent or legal guardian, and assent was obtained from each participant. The protocol was approved by the institutional review board (protocol No. 07-11-0465) as well as the participating school system.

Study Procedures

Study procedures were as previously described¹⁰ and as depicted in Figure 1. Screening questionnaires were collected each spring in participating schools to identify eligible children with asthma. Eligible subjects were invited to participate in a baseline, comprehensive clinical phenotyping evaluation during the summer, which included a detailed questionnaire, allergy skin testing (MultiTest device; Lincoln Diagnostics Inc) or specific IgE (ImmunoCAP; Phadia AB) if skin testing was contraindicated; spirometry according to American Thoracic Society guidelines (Koko spirometer; Ferraris Respiratory Inc); and exhaled nitric oxide (Niox MINO; Aerocrine AB). Home and classroom exposure samples were subsequently collected throughout the academic year and linked to enrolled students and their longitudinally collected health outcomes. Follow-up telephone surveys were administered by study staff approximately 3, 6, 9, and 12 months after the initial visit.

School exposure assessment was performed in the fall and spring of the school year. Classroom settled dust was collected with an Oreck XL vacuum (model BB870-AD; Oreck, LLC) fitted on the inlet hose with

a dust collector filter (Dermatology, Allergy and Clinical Immunology Reference Laboratory, Johns Hopkins University) using a standardized protocol.⁹ One-week classroom airborne dust samples were collected using charged particle samplers (Quadra; Sharper Image/Camelot Venture Group) based on a validated protocol.^{11,12} Collected dust was extracted in buffered saline and the endotoxin content was measured using the Limulus amoebocyte lysate assay (BioWhittaker Inc).

Every school year, approximately 75 eligible students were recruited from 8 to 10 participating schools, with 351 students completing baseline clinical phenotypic screening evaluations from 38 schools over the course of the study. Of the 351 children with asthma who participated in the baseline screening clinical evaluation, 280 eligible students had complete information on all relevant study variables (allergy testing, home, school exposure) and were eligible for follow-up in this study. Of the 280 subjects eligible for follow-up visits, 32 had missing relevant health outcome data during the academic school year of interest, leaving 248 subjects in the final analysis (Fig 2).

Outcome Measures

The primary outcome was maximum asthma symptom-days, as used in prior urban asthma studies.^{3,4,13} This is defined as the largest of the following variables in the 2 weeks prior to each follow-up survey: (1) daytime wheezing, chest tightness, or cough; or (2) days on which the child had to slow down or discontinue play activities due to wheezing, chest tightness, or cough; or (3) nights with wheezing, chest tightness, or cough leading to disturbed sleep (e-Appendix 1).

Sensitization Definition

Atopy was defined as any positive skin prick test to 14 common aeroallergens (with positive defined as a wheal diameter ≥ 3 mm larger than saline control) on intracutaneous testing or a specific IgE level ≥ 0.35 kU/L.

Statistical Analysis

Outcomes were linked to the temporally closest measured endotoxin level during the academic school year. Endotoxin levels were log transformed to minimize the impact of extreme values. All models included both classroom airborne and settled dust endotoxin as well as home settled dust endotoxin exposure. Age, sex, race, allergic sensitization, use of asthma controller medications, income (modeled as above vs below \$25,000/y), school, and home settled-dust mouse allergen were included in the final statistical models. Additional covariates such as BMI were tested and were not significantly associated with the outcome or did not confound the exposure-response relationship. To adjust for the known nonlinear variation in asthma symptoms due to season, a penalized spline term for time since school start was fit using a generalized additive mixed-effects model.^{13,14} Subject- and school-specific random intercepts were included to account for within-subject and within-school correlation.

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Figure 1 – Typical annual schema for subject recruitment, screening, enrollment, and study procedures. Fall was defined as the time period between September 1 to February 28, and spring as March 2 to the end of the academic school year.

Classroom-specific random intercepts were not included, because 54.8% of classrooms had only one study participant. For count outcomes, Poisson additive mixed-effects models accounting for overdispersion were applied. Interaction terms between school endotoxin levels and atopy were used to determine whether the effect of exposure differed by atopic status.

Prior reports have suggested that the relationship between airborne endotoxin and asthma symptoms may have an inverted U shape,¹⁵ with

both very low and very high endotoxin levels associated with fewer asthma symptoms. Thus, school air endotoxin was also modeled using a penalized spline term on non-log-transformed airborne endotoxin levels.¹⁶

All statistical analyses were performed in R 3.1.0. Generalized additive mixed-effects models were fit using the gamm4 package.¹⁷ A two-sided P value $< .05$ was considered statistically significant.

Results

The average age of the children was 8 years (range, 4–13 years), and the children were predominantly Hispanic or black (Table 1¹⁸). Most (70%) lived in a single-parent household, and 57.3% had an annual household income of $\leq \$25,000$. Most of the subjects (69.0%) were sensitized to at least one of the allergens tested, with the most common allergens being cat

(37.1%), dust mite (33.1%), and mouse (29.8%).

One-half of the subjects were taking either an inhaled corticosteroid or a leukotriene modifier for asthma control. At the baseline screening visit, the average number of days with asthma symptoms in the prior 2 weeks was 2.7, although there was a seasonal rise in asthma symptoms peaking in the wintertime (e-Fig 1).

School dust endotoxin levels were significantly higher than home levels (14.3 endotoxin units [EU]/mg vs 11.2 EU/mg; $P = .02$) (Table 2¹⁸). The average (geometric mean) school air endotoxin concentration was 24.7 EU/m³, whereas prior studies in urban north-eastern US homes using a different sampling strategy reported average air endotoxin levels of 0.8 EU/m³.¹⁹ In this study, 22.0% of classroom air endotoxin levels exceeded 90 EU/m³, a recommended occupational exposure limit for adults.²⁰ Classroom air and dust endotoxin levels were not well correlated (Pearson correlation, 0.02; Spearman correlation, 0.14). There was significant variation of both air and settled dust endotoxin levels both within and between schools (e-Fig 2).

Figure 2 – Flow diagram of subject inclusion and exclusion.

TABLE 1 Baseline Characteristics of the Study Population (N = 248)

Characteristic	No. (%)
Demographic	
Age, median (range), y	8 (4-13)
Male sex	129 (52.0)
Race or ethnic group	
White	11 (4.4)
Black	88 (35.5)
Hispanic	91 (36.7)
Mixed race	19 (7.7)
Other	39 (15.7)
Single-parent household	173 (69.8)
Annual household income <\$25,000	142 (57.3)
Clinical	
General	
Family members with asthma	200 (80.6)
Physician diagnosis of hay fever or allergic rhinitis	29 (11.7)
Physician diagnosis of eczema	114 (46.0)
Healthcare and medication use	
Hospitalization in past 12 mo	18 (7.3)
Unscheduled doctor's visit in past 12 mo	94 (37.9)
Systemic steroid use in past 12 mo	24 (9.6)
Controller medication use ^a	138 (55.6)
Use of rescue inhaler ≥ 1/wk	89 (35.9)
Pulmonary testing	
FEV ₁ %, ^b mean (SD)	102.7 (18.9)
FEV ₁ /FVC %, ^b mean (SD)	99.0 (8.0)
FENO, ^c ppb	
≤ 25	62 (68.1)
26-44	20 (22.0)
> 45	9 (9.9)
Allergic sensitization	
≥ 1 allergen	171 (69.0)
Cat	92 (37.1)
Cockroach	54 (21.8)
Dust mite	82 (33.1)
Dog	28 (11.3)
Mold	45 (18.1)
Mouse	74 (29.8)
Symptoms	
Maximum symptom-d, mean (SD)	2.7 (4.0)

FENO = fractional exhaled nitric oxide; ppb = parts per billion.

^aUse of inhaled corticosteroid or leukotriene modifier, as ascertained by review of active prescriptions during baseline screening visit.

^bn = 212, prebronchodilator. Both FEV₁ and FEV₁/FVC are reported as % predicted based on reference equations from the general US population.¹⁸

^cn = 91.

TABLE 2 School and Home Endotoxin Levels^a

Sampling Method	Location ^b	Geometric Mean (Range)	Detectable, %
Air ^c	School	24.7 (0.2-780.0) EU/m ³	100
Settled dust ^d	School	14.3 (0.7-463.5) EU/mg	100
Settled dust	Home	11.3 (0.1-1,697.7) EU/mg	97.6

EU = endotoxin unit.

^aThere were 187 fall and 188 spring airborne endotoxin measurements, 188 fall and 188 spring settled dust endotoxin measurements, obtained from 188 distinct classrooms in 38 schools. There were 248 fall settled dust measurements obtained from 248 homes.

^bTwo hundred forty-eight homes, 188 classrooms, 38 schools.

^cHome air sampling not performed. As a comparison, previous studies for urban northeastern US home report air endotoxin levels of 0.8 EU/m³ (0.01-30.2 EU/m³),¹⁸ although the air sampling strategy and laboratory where endotoxin analysis was performed differed from our study and may contribute to variability in measured levels.

^dSchool settled dust levels higher than home settled dust levels, *P* = .02.

After adjusting for school, there was no significant seasonal variation in either air or settled dust endotoxin levels.

In models simultaneously adjusting for school air, school dust, and home dust endotoxin levels, only school air endotoxin concentration was associated with asthma symptoms (Table 3). Although children with atopic asthma experience higher maximum symptom-days than those with nonatopic asthma (incidence rate ratio [IRR], 1.86 [95% CI, 1.09-3.17]), school air endotoxin levels increased asthma symptom-days only in children with nonatopic asthma (nonatopic asthma adjusted IRR, 1.16 [95% CI, 1.03-1.31]; atopic asthma adjusted IRR, 1.00 [95% CI, 0.93-1.07]; atopy*endotoxin interaction term, *P* = .03).

When looking at the component outcomes that contribute to asthma symptoms, school air endotoxin exposure was associated with higher daytime wheeze and exercise-related symptoms, but was not associated with nighttime wheeze, in children with nonatopic asthma. Children with atopic asthma had significantly more nighttime wheeze overall than those with nonatopic asthma, but school airborne endotoxin exposure was associated with decreased nighttime wheeze. For all symptom outcomes, the effect of school air endotoxin differed by atopy.

The effect of school air endotoxin exposure on maximum symptom-days is depicted in Figure 3. On average, children with atopic asthma had higher maximum symptom-days than those with nonatopic asthma. However, school air endotoxin exposure was

TABLE 3] Effect of School Air Endotoxin^a on Asthma Outcomes in School-age Children With Atopic Asthma and Those With Nonatopic Asthma

	Atopic		Nonatopic		Main Effect P Value ^{c,d}	Interaction P Value ^{c,d}
	Crude IRR ^b (95% CI)	Adjusted ^c IRR (95% CI)	Crude IRR (95% CI)	Adjusted ^c IRR [95% CI]		
Maximum symptom-d ^e	1.05 (0.99-1.12)	1.00 (0.93-1.08)	1.12 (1.02-1.23)	1.16 (1.03-1.31)	.016	.033
Daytime wheeze	1.06 (0.99-1.14)	1.04 (0.89-1.21)	1.10 (0.99-1.21)	1.21 (1.06-1.38)	.005	.046
Exercise-related symptoms	1.05 (0.96-1.16)	1.03 (0.92-1.14)	1.15 (1.01-1.31)	1.45 (1.19-1.77)	< .001	.003
Nighttime wheeze	0.94 (0.85-1.05)	0.83 (0.73-0.94)	1.13 (0.95-1.35)	1.07 (0.88-1.31)	.501	.030

IRR = incidence rate ratio. See Table 2 legend for expansion of other abbreviation.

^aSchool and home settled dust endotoxin levels were not a significant predictor of asthma symptoms.

^bIRR depicted are for a 1 unit change in log-transformed endotoxin units per cubic meter (log EU/m³). Numerically, this translates to an increase in maximum asthma symptom-d of 1.3 d for each 14-d period when school air endotoxin levels increased from 0.2 EU/m³ (lowest measured classroom level) to 24.7 EU/m³ (average measured classroom level).

^cAdjusted for age, sex, race, annual income (above or below \$25,000), controller medication, home settled dust endotoxin, school settled dust endotoxin, home settled dust mouse allergen, school mouse settled dust allergen, and season.

^dAll models included the main effects of school airborne endotoxin concentration (log transformed) as well as the interaction term between atopy × school airborne endotoxin concentrations (log transformed).

^eMaximum number of days over prior 2 wk with (1) daytime wheezing, chest tightness, or cough; or (2) days on which child had to slow down or discontinue play activities due to wheezing, chest tightness, or cough; or (3) nights with wheezing, chest tightness, or cough leading to disturbed sleep.

associated with increased maximum symptom-days only in subjects with nonatopic asthma. An increase in school air endotoxin from 0.2 EU/m³ (lowest measured classroom level) to 24.7 EU/m³ (average measured classroom level) was associated with a dose-dependent increase in maximum asthma symptom-days of 1.3 days for each 14-day period.

To identify the functional form of the endotoxin exposure-response relationship, school air endotoxin was also modeled using a penalized spline term (Fig 4). In subjects who were nonatopic, higher concentrations of school air endotoxin was associated with increased maximum symptom-days. In contrast, for children with atopic asthma, there was an inverted U-shaped relationship between school air endotoxin and maximum symptom-days, where symptoms appeared to plateau at 230 EU/m³, then decrease at higher levels.

Discussion

Through nearly a decade of community relationships, our study team has accomplished an unprecedented infrastructure, linking rigorous and extensive school/classroom and home-specific environmental sampling with carefully phenotyped health effects in children with asthma, allowing us to fully tease out school-specific environmental effects on health outcomes. Although we have previously identified higher settled-dust school endotoxin levels compared with homes,²¹ here we first report that airborne endotoxin levels are high in this inner-city setting and, in fact, 22% of classrooms have

airborne endotoxin levels exceeding recommended occupational limits for adults.²⁰ In this longitudinal study, we found that classroom-specific airborne endotoxin levels were independently associated with increased asthma symptoms in children with nonatopic asthma, after adjusting for home exposures. More important is the magnitude of effect, which has not previously been reported. Our study suggests that if classroom-specific airborne endotoxin levels are reduced from the average measured to the lowest measured levels, maximum asthma symptom-days can be reduced by 1.4 days per 2-week period. This translates to 34 fewer days per year of symptoms for each affected child, which is comparable to the effect seen with inhaled corticosteroids in the Childhood Asthma Management Program,²² and is approximately three times the effect size as that previously described for the addition of omalizumab to guideline-based asthma therapy in inner-city children.¹³ High levels of school endotoxin exposure may explain why inner-city children experience a higher burden of disease and higher morbidity due to asthma, and represents an important potential area of intervention

In nearly all countries, school attendance is mandatory and is, in essence, the occupation of children, who spend 6 to 8 h daily in school. However, occupational limits of exposure currently do not apply to children in the school setting. Several non-US-based studies have identified elevated airborne endotoxin levels in schools.^{23,24} A small fraction had levels exceeding recommended occupational exposure limits for adults²³; but

Figure 3 – Adjusted effect of school air endotoxin exposure on maximum symptom-d, stratified by atopic status. Rug plot along the bottom of the graph indicates values of school air endotoxin for which an observation was present. Increased school air endotoxin is associated with increased number of maximum symptom-d in children with nonatopic asthma but not those with atopic asthma. Note that subjects with atopic asthma have an overall higher number of maximum symptom-d, even though school air endotoxin is not associated with increased asthma symptom-d in subjects with atopy. EU = endotoxin unit; max = maximum.

overall levels in these non-inner-city schools were lower than we report. A cross-sectional study based in The Netherlands did identify a trend toward an association between school endotoxin exposure and asthma-like symptoms in children without atopy¹⁵; however, the association did not reach statistical significance. The investigators used passive air samplers, making it difficult to compare results to most occupational studies, which use active air-sampling techniques.

It is not surprising that high endotoxin exposure in the school setting increases symptoms in people with established asthma. In the laboratory setting, endotoxin induces potent host inflammatory responses, with increased airflow obstruction and bronchial hyperreactivity lasting > 48 h.⁶ What is more intriguing are the observed differences in the response of subjects with atopic asthma vs those with nonatopic asthma to school endotoxin exposure. This observation was reported previously in a landmark study evaluating the effect of

home endotoxin on asthma and allergy.²⁵ Opposing effects of endotoxin were seen in atopic vs nonatopic children, with a trend toward decreased wheeze in atopic children but a significant increase in wheeze in nonatopic children from nonfarming households. Bronchial challenge studies in healthy subjects further demonstrate that atopy modifies the human response to endotoxin, with atopic subjects showing significantly decreased systemic inflammatory responses to inhaled endotoxin compared with nonatopic subjects.²⁶

It may seem puzzling that classroom-specific airborne endotoxin levels were associated with asthma morbidity whereas settled dust levels were not. The correlation between classroom-specific air and dust endotoxin levels was poor, consistent with other studies performed in the urban setting.¹⁹ Settled dust likely represents larger particles tracked in on the footwear of students, and are too large to become airborne and subsequently inhaled.

Figure 4 – Use of a penalized spline to determine the correct functional form of the relationship between school air endotoxin and maximum symptom-d. Increased school air endotoxin levels is associated with increased number of maximum symptom-d in subjects with nonatopic asthma. An inverted U-shaped relationship exists between school air endotoxin and number of maximum symptom-d for subjects with atopic asthma, where the fewest symptom-d are seen at very low and very high endotoxin levels. See Figure 3 legend for expansion of abbreviations.

This study has several strengths. Repeated sampling in a school environment is extremely difficult to achieve, given the need for environmental sampling procedures that do not interfere with classroom activities. The study design was longitudinal as opposed to cross-sectional, allowing us to infer temporal associations. Although it is possible that endotoxin is a surrogate for other exposures, such as pest infestation, we adjusted for mouse allergen exposure and found an independent effect of endotoxin on asthma morbidity. We also adjusted for potential unmeasured confounders exhibiting a seasonal variation, such as rhinovirus infections, strengthening the validity of our findings.¹³

This study has some limitations. Home endotoxin exposure was limited to settled dust levels, given feasibility. It is possible that home airborne endotoxin levels, if measured, are associated with differences in asthma morbidity. Air sampling was performed with an ion-charging device because of the need for unobtrusive air sampling during classes. We did not make

any direct comparisons of our sampling techniques against other sampling methods, although this method has previously been shown to yield results comparable to pump-based sampling methods.^{11,12} Not all of the subjects who underwent a baseline screening evaluation in the summertime were eligible for participation in the study. There were no significant differences in baseline characteristics between those who had complete data and those who did not (e-Table 1), suggesting that a selection bias due to missing data was unlikely to have affected our conclusions.

Conclusions

In conclusion, we found that classroom-specific airborne endotoxin levels frequently exceeded recommended adult occupational limits. Higher school airborne endotoxin levels are associated with increased asthma symptoms in children with nonatopic asthma. Mitigation of school-specific endotoxin exposures may be associated with decreased asthma morbidity in this population.

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Author contributions: P. S. L. and W. P. had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis, including and especially any adverse effects. P. S. L. wrote the manuscript and served as principal author. P. S. L. and W. P. contributed to the study concept and design; W. J. S., J. M. G., and W. P. participated in the study implementation and data acquisition; W. P. established the cohort on which this study is based; P. S. L., C. R. P., and B. A. C. contributed to data analysis; P. S. L., W. J. S., J. M. G., C. R. P., B. A. C., D. R. G., and W. P. contributed to interpretation of the results; and P. S. L., W. J. S., J. M. G., C. R. P., B. A. C., D. R. G., and W. P. contributed to critical revision of the manuscript.

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Additional information: The e-Appendix, e-Figures, and e-Table can be found in the Supplemental Materials section of the online article.

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