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The association between endotoxin in house dust with atopy and exercise-induced bronchospasm in children with asthma

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ABSTRACT

Background: Studies have reported protective and adverse associations between microbial exposure and childhood asthma. However, among children with asthma the relationships between endotoxin and exercise-induced bronchospasm (EIB) is less clear.

Objective: We investigated the association between exposure to endotoxin in house dust with atopy and EIB in children with asthma.

Methods: A cross-sectional survey was conducted among schoolchildren (aged 7–17 years) in the province of Saskatchewan, Canada. A subpopulation with asthma (n = 116) were identified from 335 participants using a validated asthma algorithm. We determined atopy among the asthma subpopulation by skin prick testing (SPT) while EIB was evaluated using exercise challenge testing (ECT). Dust samples were collected from mattress and play area floors, and endotoxin was measured in dust extracts. Logistic regression analyses were used to explore associations between endotoxin with atopy and EIB.

Results: Among the 116 children with asthma, 99 completed SPT and all had completed ECT. Of these, 71/99 (71.7%) were atopic and 26/116 (22.4%) had EIB. Exposure to high play area endotoxin concentration [adjusted odds ratio (aOR) = 0.15, 95% CI: 0.03–0.85] and load (aOR = 0.11, 95% CI: 0.02–0.73) were negatively associated with atopy. In contrast, EIB was positively associated with high mattress endotoxin concentration (aOR = 6.01, 95% CI: 1.20–30.13).

Conclusion: Indoor microbial endotoxin exposure has varied associations with atopy and exercise-induced bronchospasm among children with asthma.

1. Introduction

Indoor microbial exposure has been suggested to influence the presence of respiratory disorders, including childhood asthma (Kanchongkittiphon et al., 2015) but the associations are conflicting. Bacterial endotoxin has been reported to have protective (Tischer et al., 2011; Lawson et al., 2012), adverse (Tavernier et al., 2005; Thorne et al., 2005; Chinn and Williams, 2007) as well as no association (Perzanowski et al., 2006; Gehring et al., 2008) for childhood asthma. Reasons for the paradoxical effects are unclear but could be linked to different presentations of the disease in children with asthma now presenting in allergic and non-allergic forms.

Previous studies of endotoxin have shown more consistent associations with allergic sensitization (Gehring et al., 2002, 2007; Tischer et al., 2011). Studies have also shown that exposure to endotoxin is inversely associated with atopic asthma (Braun-Fahrlander et al., 2002; Schram-Bijkerk et al., 2005a) and atopic wheeze (Braun-Fahrlander et al., 2002; Schram-Bijkerk et al., 2005a) among schoolchildren in the general population. However, it is less clear if this relationship also persists among children with asthma since only half of asthma cases in the general population can be attributed to allergic sensitization (Douwes et al., 2002).

Furthermore, children with allergic or non-allergic asthma may also demonstrate bronchial hyperressponsiveness (BHR) in response to

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environmental stimuli such as exercise (Novak and Bieber, 2003; Weiler et al., 2016) or have varied degree of symptom severity associated with microbial exposures (Dannemiller et al., 2016). Exercise-induced bronchospasm (EIB) is one method of assessing BHR, but currently there are limited studies investigating the effects of house dust endotoxin exposures on EIB among children with asthma.

In the present study, we investigated the association between house dust endotoxin exposure with atopic sensitization or EIB among children with asthma. We hypothesized that exposure to high levels of house dust endotoxin will be inversely related to atopy but positively related to EIB. This may provide some insight into the relevant effects of indoor endotoxin exposures and associated response among children with asthma for better childhood asthma management.

2. Methods

2.1. Study population, selection, and recruitment

We conducted a cross-sectional study among schoolchildren with asthma (aged 7–17 years) in the province of Saskatchewan, Canada from 2015 to 2016. Participants in this study were recruited from a 2013 cross-sectional survey ($n = 3509$) as previously described (Lawson et al., 2017). Those who consented to participate in further testing based on a question from the 2013 survey ($n = 1348$) were re-approached in 2015. At this time, we repeated the survey and completed clinical testing (spirometry, exercise challenge testing, and skin prick test) as well as home dust sample collection.

The study was approved by the University of Saskatchewan Biomedical Research Ethics Board (Bio #: 14–162). Completion and return of the survey implied voluntary consent for the questionnaire portion. Children and parents provided written assent and consent, respectively, prior to clinical testing and home dust collection. Furthermore, all school divisions involved approved the study.

2.2. Survey questionnaire

We used standardized and validated questions from the International Study of Asthma and Allergy in Childhood (ISAAC) (Asher et al., 1995, 1998), the American Thoracic Society Children's Respiratory Disease (Ferris, 1978), and questionnaires used previously in the Saskatchewan Lung Health studies (Rennie et al., 2008; Lawson et al., 2012) to obtain information on respiratory health (including physician-diagnosed asthma), general health, parental health history, environmental exposure, sociodemographic factors as well as housing characteristics. A total of 335 schoolchildren completed and returned the 2015 survey questionnaire.

2.3. Spirometry and exercise challenge testing (ECT)

Of the 335 subjects with survey responses, 288 (86%) performed spirometry and ECT. During home or school visits, trained field technicians performed spirometry assessments according to recommended standards for children (Pellegrino et al., 2005; Coates et al., 2013). Measurements of forced expiratory volume in one second (FEV_1), forced vital capacity (FVC), FEV_1/FVC ratio, and forced expiratory flow ($FEF_{25\%–75\%}$) were done using the Easy-on PC spirometer (ndd Medical Technologies, Zurich, Switzerland). Some subjects were excluded from testing because they were unable to perform the test due to existing medical conditions ($n = 3$).

ECT was also completed based on recommended protocols (Gerald et al., 2004). Briefly, children stepped up and down on a 6 in. step at a heart rate between 150 and 200 beats per minute for 5 min. Heart rates were monitored throughout the exercise with a Polar heart rate monitor (Polar Electro, Woodbury, NY). Spirometry was repeated 3 and 10 min after cessation of exercise.

2.4. Skin prick test (SPT)

All SPTs were completed at the school or during home visits. Tests were completed on the forearm using a panel of six common and standardized allergen extracts according to recommended protocol (Joint Task Force on Practice Parameters, 2007): cat, local grass (meadow, timothy weed, rye, Kentucky blue, orchard, and red top), *Aspergillus, Alternaria, Cladosporium*, and house dust mite (Omega Laboratory, Montreal QC, Canada). Two controls: a histamine positive control and a saline negative control were used to reduce false positives and false negatives. The wheal size diameter was measured after 15 min. Subjects was considered positive for atopy if a positive reaction to at least one of the applied allergens is raised $\geq$ 3 mm compared to the saline control (Joint Task Force on Practice Parameters, 2007). All SPTs in the study were performed by trained technicians who were blinded to the asthma status of each child.

2.5. Identification of subpopulation of children with asthma among survey participants

The asthma classification criteria were based on a validated asthma case detection algorithm (Gerald et al., 2004). Based on the parental response to the questionnaire, children were classified as “diagnosed asthma” if they had positive responses to the questions: “Has this child ever been diagnosed as having asthma by a doctor?” and/or “Has this child taken prescribed asthma medication in the past 12 months?” Children who were otherwise classified as “no asthma” based on survey responses but who had FEV_1/FVC ratio $<$ 80% upon spirometry testing; or demonstrated a $>$ 15% decrease in FEV_1 or a $\geq$ 25% decrease in $FEF_{25\%–75\%}$ from baseline after cessation of exercise were considered to be positive for asthma (Lougheed et al., 2012; Parsons et al., 2013). Overall, a total of 116 children were identified to have asthma.

2.6. Classification of atopy and exercise-induced bronchospasm among children with asthma

Within the subpopulation of children with asthma ($n = 116$), we identified two outcomes. This included: 1) atopy outcome based on atopic vs. non-atopic status, and 2) EIB status using the ECT results (EIB vs. no EIB). Atopy was defined as sensitization ($\geq$ 3 mm in wheal diameter compared to saline control after 15 min) to one or more allergens from SPT in the presence of diagnosed asthma. EIB was defined as $>$ 15% fall in FEV_1 from baseline after cessation of exercise.

2.7. Collection and analysis of dust samples to quantify endotoxin exposure

Dust samples were obtained from the floor of the play area and from the mattress by trained personnel using a Solaris Turbo Plus vacuum cleaner (Model: Miele S514, Germany), set at 950 W according to the ISSAC protocol (Weiland et al., 2004). Samples were collected into X-Cell-100 dust collection filter socks with pore size between 4.0 and 12.3 μm (Midwest Filtration LLC, OH, USA). Vacuum cleaners were calibrated for flow rate and static pressure pre and post data collection ($R^2 > 0.99$). A separate, pre-weighed and labelled filter sock was used for each of the play area and mattress sample collections. The filter sock was placed into the distal end of the extension tube of vacuum cleaner and sealed with a clean crevice collecting tool placed over the distal end of the extension tube. For each sample collected, a cleaned crevice tool was used. The crevice device was also wiped clean with alcohol swab prior to any dust collection procedure to prevent cross contamination between locations and homes. Vacuum cleaners are further cleaned after each day sample collection procedures. All crevice devices were also cleaned with soapy warm water and treated with alcohol solution in the laboratory at the end of each day sample collection task.

Sampling area and time for dust collection followed the

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