Characterization of Lipopolysaccharides Present in Settled House Dust
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The 3-hydroxy fatty acids (3-OHFAs) in lipopolysaccharides (LPS) play an important role in determining endotoxin activity, and childhood exposure to endotoxin has recently been associated with reduced risk of atopic diseases. To characterize the 3-OHFAs in house dust (HD), we used gas chromatography-mass spectrometry to assay 190 HD samples. Dust from beds, bedroom floors, family rooms, and kitchen floors was collected as part of a birth cohort study of childhood asthma (study 1) and a longitudinal study of home allergen and endotoxin (study 2). We also measured endotoxin activity with a Limulus assay and computed specific activity (endotoxin activity per nanomole of LPS). Longer-chain (C16:0 and C18:0) 3-OHFAs were predominant in HD compared with short-chain (C10:0, C12:0, and C14:0) acids. Endotoxin activity was positively correlated with short-chain 3-OHFAs in both studies. In study 2, 3-OH C16:0 was negatively correlated and 3-OH C18:0 was not correlated with endotoxin activity, consistent with previous findings that the Limulus assay responds preferentially to LPS containing short-chain 3-OHFAs. Kitchen dust contained the highest concentrations of 3-OH C16:0, the highest endotoxin activities, and the highest specific activities (P < 0.03). Bed dust contained the largest amounts of long-chain 3-OHFAs, the highest concentrations of LPS, and the lowest specific activities. Apartments had significantly different types of LPS (P < 0.03) compared with single-family homes in study 2. These data suggest that the Limulus assay may underestimate exposure to certain types of LPS. Because nontoxic LPS may have immune modulating effects, analysis of 3-OHFAs may be useful in epidemiologic studies.

Endotoxin is biologically active lipopolysaccharide (LPS), a family of macromolecules with similar chemical structures that are the major lipid of the outer membrane of gram-negative bacteria. Environmental endotoxin is ubiquitous and has been detected in settled house dust and home air (7, 10, 15, 22). Thus, everyone is constantly exposed to at least low levels of environmental endotoxin.

House dust endotoxin has been associated with asthma severity in adults and children (14, 15, 25). Park et al. reported that early-life exposure to house dust endotoxin is associated with an increased risk of repeated wheezing during the first year of life (20). These observations indicate that exposure to low levels of home endotoxin induces airway inflammation and may aggravate or contribute to the development of airway disease in susceptible individuals. Recently, a possible protective effect of early-life endotoxin exposure on the risk of childhood asthma has also been attracting considerable attention (3, 13, 29, 30). Several reports suggest that early-life endotoxin exposure may induce immune polarization toward a Th1 cytokine profile that may reduce the risk of atopic diseases in later life. However, more information on the critical timing and routes of exposure, the necessary dose, and a characterization of the LPS encountered in the environment may still be required to understand the biological impacts of endotoxin exposure.

LPS consists of polysaccharide and lipid A components. Lipid A, the endotoxic component, shows a unique structure with bisphosphorylated β(1-6)-N-glucosamine disaccharide as a backbone. This backbone structure typically carries 4 mol equivalents of 3-hydroxy fatty acids (3-OHFAs) with nonhydroxylated fatty acids ester linked to one or more of the 3-hydroxyl groups (24). The 3-OHFAs are a unique component of the lipid A molecule, making them well suited as a chemical marker for LPS (11, 12). Gram-negative bacteria from different genera may contain 3-OHFAs of differing chain lengths (31). Furthermore, the biological activity of endotoxin is dependent on the structure of lipid A (19, 23, 26–28). Takada et al. (28) demonstrated that the presence of 3-OHFA groups on the bisphosphorylated β(1-6)-N-glucosamine disaccharide backbone is required for endotoxin activity with Limulus amoebocyte lysate. Qureshi et al. (23) showed that the fatty acid composition determines, in part, the endotoxin activity of lipid A in mammals, and recent observations suggest that lipid A structure may determine specificity for Toll-like receptors 2 and 4 (19). Therefore, data about the quantity and quality of LPS in environmental samples, in addition to their activity in the Limulus assay, may be critical to understanding the biological effects of environmental endotoxin exposure.

Our objective in this study was to characterize the LPS in house dust samples. We analyzed the quantity and distribution of different chain lengths of 3-OHFAs, determined by gas chromatography-mass spectrometry (GC-MS), in house dust samples collected in the Boston area. We used the 3-OHFA distribution as an indicator of variations in microbial flora characteristic of differing environments and examined the specific activity (endotoxin units [EU] per nanomole of LPS) of dust samples by comparing the Limulus assay activity of the
samples with their LPS content determined by assay for 3-OHFA. We hypothesized that each type of dust sample (bed, bedroom, family room, and kitchen), samples from different seasons, and samples associated with certain home characters (e.g., pets) would have characteristic flora and that this would be reflected in differences in 3-OHFA distribution and specific activity among the samples.

MATERIALS AND METHODS

Origin of house dust samples. We analyzed settled house dust collected for two observational studies: a birth cohort study of home allergens and endotoxin and development of childhood asthma (study 1) (9, 20) and a longitudinal study of home allergens and endotoxin (study 2) (4, 21). Study 1 is an ongoing, longitudinal, closed birth cohort study of children born to parents with histories of allergies and/or asthma. Recruitment criteria and methods have been previously detailed (9). Between September 1994 and June 1996, families were recruited within 24 to 48 h of the birth of the index child. We collected dust samples from the baby’s bed, the bedroom floor, the family room, and the kitchen floor in each home and administered a home characteristic questionnaire within the first 3 months of life by trained interviewers previously described (9). Study 2 was designed to characterize seasonal variation in home allergen, fungus, and endotoxin levels. We recruited 20 subjects from the faculty, staff, and students at the Harvard School of Public Health who lived in the greater Boston, Mass., area. Each participant in the 20 homes answered a home characteristic questionnaire and collected three dust samples (bedroom bed, bedroom floor, and kitchen floor) on prescheduled days every month from April 1995 through July 1996 as previously described (4, 21). Thus, we had four types of samples (bed, bedroom floor, family room, and kitchen) from study 1 and three types (bed, bedroom floor, and kitchen) from study 2.

All samples were collected by using the previously described protocol (5, 9). We used a Eureka Mighty-Mite vacuum cleaner (model 3621; The Eureka Co., St. Louis). The vacuum cleaner was equipped with a nylon-silica gel column (30 m by 0.25 mm [inside diameter]) coated with CP-Sil 8 CB-MS, 0.25-μm film thickness (Chrompack, Middelburg, The Netherlands). Injections were made with a Hewlett-Packard 7673 autosampler in the splitless mode; the split valve was opened 1 min after injection. Helium was used as the carrier gas, at an inlet pressure of 7 lb/in², and the temperature of the oven was programmed to increase from 90 to 280°C at 10°C/min. The ion source temperature was 200°C, and electron impact ionization was used at 70 eV. Analyses were made in the selected ion monitoring mode.

Data analysis. Specific activity of LPS in house dust samples was computed by dividing the endotoxin activity level (endotoxin units per milligram) by the LPS concentration (nanomoles per milligram) for each sample. The distributions of measured endotoxin activity levels and 3-OHFA and LPS concentrations within each study were positively skewed. Thus, we performed log transformations to obtain symmetrical distributions.

We computed the Spearman correlation coefficient (SAS Proc Corr; SAS Institute Inc., Cary, N.C.) to examine the correlation between endotoxin activity (endotoxin units per milligram of dust) and nanomoles of 3-OHFA per milligram of dust. We used multivariate mixed models with a random effect of home to examine the fixed effect of the study (controlled for fixed effects of season and sample type) on the concentrations of 3-OHFA in house dust samples. We tested the linearity of the log transformation by including a cubic term of log-transformed 3-OHFA. We computed the Spearman correlation coefficient of LPS concentration (nanomoles per milligram) and endotoxin activity (endotoxin units per milligram of dust). In study 2, we observed positive, statistically significant (P < 0.05) correlations between endotoxin activity and the various concentrations of 3-OHFA and LPS.

RESULTS

We analyzed a total of 190 dust samples from the two studies for both endotoxin activity and 3-OHFA concentrations. There were no samples that fell below the LOD of the Limulus assay; four samples were below the LOD for C16:0 3-OHFA, and none were below the LOD for the remaining 3-OHFA concentrations in the GC-MS analysis. The numbers of homes and samples in each study by sample type and season are shown in Table 1. One hundred thirty-seven dust samples were from study 1, and 53 were from study 2. Study 1 included four types of dust samples (bed, bedroom floor, kitchen, and family room floor); however, because only one bed dust sample from study 1 was assayed for 3-OHFA, the results for that sample are not included in the remaining tables. Of the 120 homes in study 1, 19 (15.8%) had dogs at the time of sample collection and 20 (16.7%) with no dog at the time of sample collection reported having had dogs previously. Twenty-six homes (21.7%) were apartments in buildings with three or more units, and none of the apartments had dogs at the time of sample collection or previously. Study 2 included only three types of dust samples since we did not collect dust samples from family room floors; 13 homes (65.0%) were apartments, and none of the homes in study 2 kept a dog.

Table 2 shows the coefficients of correlation between the concentrations of 3-OHFA (nanomoles per milligram) and endotoxin activity (endotoxin units per milligram of dust). In study 1, we observed positive, statistically significant (P < 0.05) correlations between endotoxin activity and the various
TABLE 1. Number of homes and samples by sample type, season, and study

<table>
<thead>
<tr>
<th>Sample type or season</th>
<th>Study 1*</th>
<th>Study 2*</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Homes</td>
<td>Samples</td>
<td>Homes</td>
</tr>
<tr>
<td>Bedroom floor</td>
<td>52</td>
<td>53</td>
<td>15</td>
</tr>
<tr>
<td>Kitchen floor</td>
<td>21</td>
<td>22</td>
<td>7</td>
</tr>
<tr>
<td>Bed</td>
<td>1</td>
<td>1</td>
<td>8</td>
</tr>
<tr>
<td>Family room</td>
<td>60</td>
<td>61</td>
<td>15</td>
</tr>
<tr>
<td>Spring</td>
<td>17</td>
<td>18</td>
<td>10</td>
</tr>
<tr>
<td>Summer</td>
<td>44</td>
<td>51</td>
<td>7</td>
</tr>
<tr>
<td>Fall</td>
<td>38</td>
<td>40</td>
<td>6</td>
</tr>
<tr>
<td>Winter</td>
<td>28</td>
<td>28</td>
<td>15</td>
</tr>
<tr>
<td>Total</td>
<td>120</td>
<td>137</td>
<td>19</td>
</tr>
</tbody>
</table>

* Study 1 is a Boston-based longitudinal birth cohort study of childhood asthma.

TABLE 2. Correlation between concentrations of 3-OHFA and endotoxin activity

<table>
<thead>
<tr>
<th>Endotoxin and carbon length of 3-OHFA parameter</th>
<th>Correlation coefficient a</th>
</tr>
</thead>
<tbody>
<tr>
<td>C 10:0 (nmol/mg of dust)</td>
<td>0.27 a</td>
</tr>
<tr>
<td>C 12:0 (nmol/mg of dust)</td>
<td>0.39 a</td>
</tr>
<tr>
<td>C 14:0 (nmol/mg of dust)</td>
<td>0.55 a</td>
</tr>
<tr>
<td>C 16:0 (nmol/mg of dust)</td>
<td>0.20 a</td>
</tr>
<tr>
<td>C 18:0 (nmol/mg of dust)</td>
<td>0.27 a</td>
</tr>
</tbody>
</table>

Study 1 (n = 137)

Endotoxin activity (EU/mg of dust) | Correlation coefficient a |
-----------------------------------|---------------------------|
C 10:0 (nmol/mg of dust)           | 0.62 a                    |
C 12:0 (nmol/mg of dust)           | 0.35 a                    |
C 14:0 (nmol/mg of dust)           | 0.68 a                    |
C 16:0 (nmol/mg of dust)           | 0.66 a                    |
C 18:0 (nmol/mg of dust)           | 0.77 a                    |

Study 2 (n = 53)

Endotoxin activity (EU/mg of dust) | Correlation coefficient a |
-----------------------------------|---------------------------|
C 10:0 (nmol/mg of dust)           | 0.16                      |
C 12:0 (nmol/mg of dust)           | 0.24                      |
C 14:0 (nmol/mg of dust)           | 0.02                      |
C 16:0 (nmol/mg of dust)           | 0.03                      |

a Spearman correlation.

Table 3 shows the adjusted geometric mean concentration of the 3-OHFA (nanomoles per milligram) in house dust samples. The concentration of C 10:0 was consistently among the lowest, and the concentration of C 16:0 was consistently the highest, of the five measured 3-OHFA. The level of C 10:0 3-OHFA was highest in kitchen dust in both studies, but the difference was not statistically significant. In study 2, both bed and bedroom floor dust samples had significantly higher concentrations of 3-OH C 16:0 (P < 0.02) than did kitchen dust; bed dust had significantly greater and bedroom floor dust had borderline significantly greater (P = 0.06) 3-OH C 18:0 concentrations than did kitchen dust. The concentrations of C 16:0 and C 18:0 were not significantly different between bed dust and bedroom floor dust. These results indicate that there is within-home variation in the concentrations of different chain length 3-OHFA in house dust, which suggests that the type of LPS, and thus the microbial flora, in house dust varies with the area sampled within a home.

Samples from study 2 had significantly greater concentrations of C 16:0 (P = 0.015) and C 18:0 (P = 0.003) 3-OHFA and of total LPS (P = 0.03) than did those from study 1. There was no study-by-sample type interaction for C 16:0 and C 18:0 3-OHFA and total LPS. The study-by-sample type interaction for LPS specific activity, suggesting that the effect of sample type on specific activity was different by study. Therefore, we performed the analyses of season and sample type with separate models for each study.

The mixed model for study 1 also showed significant overall seasonal variation in 3-OH C 12:0 (P = 0.04) and 3-OH C 18:0
from that of endotoxin activity. Both bed and bedroom floor dust samples had greater amounts of total LPS than did kitchen dust. Adjusted multiple comparisons showed that the total amount of LPS was significantly ($P = 0.008$) higher in bed dust than in kitchen floor dust while endotoxin activity was significantly ($P < 0.0001$) lower in bed dust than in kitchen floor dust (Table 4). LPS concentration variation with season was borderline significant in study 1 ($P = 0.059$), with fall greater than winter ($P = 0.054$). LPS concentration did not vary significantly with season in study 2 ($P = 0.76$).

The specific activity of LPS in house dust (Table 4) varied significantly with sample type in both studies, with kitchen dust significantly more active per nanomole of LPS than any other dust (study 1, comparison with bedroom floor [$P = 0.03$] and family room [$P = 0.02$]; study 2, comparisons with bed dust and bedroom floor dust [$P < 0.001$]). Also in study 2, LPS in bed dust had significantly ($P = 0.01$) lower specific activity than that in bedroom floor dust. LPS in family room and bedroom floor dust had similar specific activities. Seasonal variation of the specific activity of LPS in dust was not significant in study 1 and was borderline significant in study 2 ($P = 0.08$, spring $>$ winter).

In study 2, 3-OH C$_{18:0}$ was significantly ($P = 0.03$) lower in apartments (0.09 nmol/mg) than in other homes (0.16 nmol/mg), controlling for season and sample type. Presence of dogs or cats at home was not associated with significant changes in the amount of specific 3-OHFA.

**DISCUSSION**

We found that LPS in bed dust had a predominance of longer-chain 3-OHFAs, while kitchen floor dust was characterized by increased amounts of short-chain 3-OHFAs. Bedroom floor and family room dust resembled bed dust more closely than kitchen dust. Similarly, kitchen dust was more active in the Limulus assay than was bed dust, and bedroom floor and family room dust samples were intermediate. These data demonstrate that LPS in house dust varies qualitatively by location within homes.

We observed that concentrations of longer chain length 3-OHFAs and of total LPS were highest in the fall. This finding indicates that LPS in house dust may vary qualitatively across seasons, suggesting different microbial flora in dust from different seasons.

Our results confirm our previous observation (27) that different chain lengths of 3-OHFAs in LPS are differently correlated with endotoxin activity detected by the Limulus assay. Shorter-chain (C$_{10:0}$, C$_{12:0}$, and C$_{14:0}$) 3-OHFAs are positively correlated with endotoxin activity, while longer-chain (C$_{16:0}$ and C$_{18:0}$) 3-OHFAs tend to have lower, no, or even negative correlations with endotoxin activity in the Limulus assay. The predominance of short-chain fatty acids in kitchen dust therefore accounts for the otherwise paradoxical finding that kitchen dust contained the smallest amounts of LPS but the largest amounts of endotoxin bioactivity.

The observation that kitchen samples had significantly more endotoxin activity and higher LPS specific activities and had the highest fraction of C$_{10:0}$ relative to those from other rooms suggests that the kitchen may be different from other environments within the house so that it supports different microbial
flora. It is likely that the increase in C_{100} is an indication of increased organisms that grow in pooled water or plumbing, such as pseudomonas-like organisms that are rich in C_{100} and C_{120} (1).

We did not observe that the presence of pets such as dogs and cats at home changes the microbial flora in house dust, as we had expected on the basis of previous reports of higher endotoxin levels in the presence of pets. Andersson et al. (1) demonstrated that dust collected from cattle barns and swine confinement buildings had different microbial flora from that collected from schools and day care centers, suggesting that animal and human sources have characteristic flora. However, our failure to find different gram-negative flora between homes with and without pets may result from the small number of homes with pets in the data analyzed. On the other hand, our data showed that apartments in buildings with three or more units had significantly decreased amount of 3-OH C_{14:0} compared with single-family or duplex houses. These data suggest that apartment dwellers may be exposed to different types of LPS compared with people living in single-family or duplex homes.

It is known that biological activities of LPS from different species of bacteria may vary qualitatively. For example, Rhodopseudomonas sphaeroides LPS is nontoxic but retains significant immunostimulatory activity and is capable of inactivating suppressor T-cell activity and is capable of preventing tolerance to polysaccharide immunostimulatory activity and is capable of inactivating

REFERENCES


