Characterization of Lipopolysaccharides Present in Settled House Dust

Ju-Hyeong Park,1,4 Bogumila Szponar,2 Lennart Larsson,2 Diane R. Gold,3,4 and Donald K. Milton3,4*

Division of Respiratory Disease Studies, National Institute for Occupational Safety and Health, Morgantown, West Virginia;2 University of Lund, Lund, Sweden;2 and The Channing Laboratory, Department of Medicine, Brigham and Women’s Hospital and Harvard Medical School3 and Harvard School of Public Health,4 Boston, Massachusetts

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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)-D-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)-D-glucosamine disaccharide backbone is required for endotoxin activity with Limulus amoebocyte lysate. Oureshi 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
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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 characteristics (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 to hold cellulose extraction thimbles (19 by 90 mm) in each home and administered a home characteristic questionnaire with frequent vortexing to maintain particulates in suspension. The response parameter for the LAL reaction was the maximum rate of centrifugation and with frequent vortexing to maintain particulates in suspension. The limit of detection (LOD) was 0.5 pmol/mg of dust for each of the 3-OHFA. Samples with a 3-OHFA concentration below the LOD were, for statistical analyses, assigned a concentration for that particular 3-OHFA equal to the LOD divided by the square root of 2.

A VG (Manchester, United Kingdom) Trio-1 S system was used for GC-MS. The gas chromatograph, a model 5890 (Hewlett-Packard, Avondale, Pa.), was equipped with a fused-silica capillary 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 20°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.

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; for four samples were below the LOD for C16:0 3-OHFA, and none were below the LOD for the remaining 3-OHFA 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 the first 3 months of life of cohort members as previously described (9). 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...
3-OHFA (range, 0.20 to 0.55). The correlation was strongest for the C\textsubscript{12:0} and C\textsubscript{14:0} 3-OHFA. In study 2, positive correlations of endotoxin activity with short-chain 3-OHFA were also observed, but the strongest and only significant positive correlation was with the C\textsubscript{10:0} 3-OHFA at α = 0.05. However, the C\textsubscript{14:0} 3-OHFA was significantly (\textit{P} = 0.08) and positively correlated with endotoxin activity at α = 0.1. The C\textsubscript{16:0} 3-OHFA was significantly (\textit{P} = 0.04) negatively correlated with endotoxin activity, and the C\textsubscript{18:0} 3-OHFA was not correlated with endotoxin activity. In general, the correlations of short-chain 3-OHFA with endotoxin activity were consistently positive while those of long-chain 3-OHFA were more weakly positive or negative.

There was no difference in the mean levels of C\textsubscript{10:0} to C\textsubscript{14:0} 3-OHFA between studies in the mixed model with random effect of home and fixed effects of season and sample type.

### 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\textsuperscript{a}</th>
<th>\textit{C}_{\textsc{10:0}}</th>
<th>\textit{C}_{\textsc{12:0}}</th>
<th>\textit{C}_{\textsc{14:0}}</th>
<th>\textit{C}_{\textsc{16:0}}</th>
<th>\textit{C}_{\textsc{18:0}}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study 1 ((n = 137))</td>
<td></td>
<td>0.27\textsuperscript{b}</td>
<td>0.39\textsuperscript{b}</td>
<td>0.55\textsuperscript{b}</td>
<td>0.20\textsuperscript{b}</td>
<td>0.27\textsuperscript{b}</td>
</tr>
<tr>
<td>Endotoxin activity (EU/mg of dust)</td>
<td></td>
<td>\textit{C}_{\textsc{10:0}} (nmol/mg of dust)</td>
<td>0.62\textsuperscript{b}</td>
<td>0.35\textsuperscript{b}</td>
<td>0.30\textsuperscript{b}</td>
<td>0.30\textsuperscript{b}</td>
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<tr>
<td></td>
<td></td>
<td>\textit{C}_{\textsc{12:0}} (nmol/mg of dust)</td>
<td>0.68\textsuperscript{b}</td>
<td>0.50\textsuperscript{b}</td>
<td>0.52\textsuperscript{b}</td>
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<tr>
<td></td>
<td></td>
<td>\textit{C}_{\textsc{14:0}} (nmol/mg of dust)</td>
<td>0.66\textsuperscript{b}</td>
<td>0.63\textsuperscript{b}</td>
<td>0.63\textsuperscript{b}</td>
<td>0.63\textsuperscript{b}</td>
</tr>
<tr>
<td></td>
<td></td>
<td>\textit{C}_{\textsc{16:0}} (nmol/mg of dust)</td>
<td>0.77\textsuperscript{b}</td>
<td>0.77\textsuperscript{b}</td>
<td>0.77\textsuperscript{b}</td>
<td>0.77\textsuperscript{b}</td>
</tr>
<tr>
<td>Study 2 ((n = 53))</td>
<td></td>
<td>0.28\textsuperscript{b}</td>
<td>0.16</td>
<td>0.24</td>
<td>-0.29</td>
<td>0.03</td>
</tr>
<tr>
<td>Endotoxin activity (EU/mg of dust)</td>
<td></td>
<td>\textit{C}_{\textsc{10:0}} (nmol/mg of dust)</td>
<td>0.73\textsuperscript{b}</td>
<td>0.35\textsuperscript{b}</td>
<td>0.10</td>
<td>0.28\textsuperscript{b}</td>
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<tr>
<td></td>
<td></td>
<td>\textit{C}_{\textsc{12:0}} (nmol/mg of dust)</td>
<td>0.67\textsuperscript{b}</td>
<td>0.30\textsuperscript{b}</td>
<td>0.61\textsuperscript{b}</td>
<td>0.61\textsuperscript{b}</td>
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<tr>
<td></td>
<td></td>
<td>\textit{C}_{\textsc{14:0}} (nmol/mg of dust)</td>
<td>0.46\textsuperscript{b}</td>
<td>0.71\textsuperscript{b}</td>
<td>0.71\textsuperscript{b}</td>
<td>0.71\textsuperscript{b}</td>
</tr>
<tr>
<td></td>
<td></td>
<td>\textit{C}_{\textsc{16:0}} (nmol/mg of dust)</td>
<td>0.56\textsuperscript{b}</td>
<td>0.56\textsuperscript{b}</td>
<td>0.56\textsuperscript{b}</td>
<td>0.56\textsuperscript{b}</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Spearman correlation.
\textsuperscript{b} \textit{P} < 0.05.
\textsuperscript{c} \textit{P} = 0.08.
3-OHFA ($P = 0.03$) concentrations. Adjusted for multiple comparisons, the 3-OH C$_{12:0}$ 3-OHFA concentration in summer was marginally significantly ($P = 0.053$) higher than that in the winter, and dust collected in the fall contained significantly ($P = 0.03$) more, and dust collected in the summer contained borderline significantly ($P = 0.056$) more, 3-OH C$_{18:0}$ than did dust collected in the winter (Table 3). The seasonal pattern of 3-OH C$_{12:0}$ and 3-OH C$_{18:0}$ was similar in study 2 but did not reach statistical significance, possibly because of the smaller sample size.

Mean endotoxin activity in dust (Table 4) varied significantly with sample type in both studies. Endotoxin activity was significantly ($P < 0.021$) lower in bedroom dust than in kitchen floor dust in both studies and was significantly ($P < 0.0001$) lower in bed dust than that in kitchen floor dust in study 2. Bedroom floor and family room dust samples had similar activity levels ($P = 0.63$) in study 1, while bedroom floor dust had marginally ($P = 0.049$) higher endotoxin activity than did bed dust in study 2. Seasonal variation was marginally significant ($P = 0.05$) for study 1, and adjusted multiple comparison suggested that the summer level was significantly higher than the winter level ($P = 0.04$). In study 2, seasonal variation was significant ($P = 0.01$); spring, summer, and fall had similar endotoxin activity levels, but only spring had significantly ($P = 0.01$) greater endotoxin activity than winter.

The amount of total LPS (nanomoles per milligram of house dust) also varied significantly ($P = 0.01$) with sample type in study 2. However, the order of the total LPS level was reversed 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-OHFA, while kitchen floor dust was characterized by increased amounts of short-chain 3-OHFA. 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-OHFA 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-OHFA in LPS are differentially correlated with endotoxin activity detected by the Limulus assay. Shorter-chain (C$_{10:0}$, C$_{12:0}$, and C$_{14:0}$) 3-OHFA are positively correlated with endotoxin activity, while longer-chain (C$_{16:0}$ and C$_{18:0}$) 3-OHFA 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$_{10:0}$ is an indication of increased organisms that grow in pooled water or plumbing, such as pseudomonas-like organisms that are rich in C$_{10:0}$ and C$_{12:0}$ (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$_{16:0}$ 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 cells and of preventing tolerance to polysaccharide antigens (2, 23). Thus, nontoxic, as well as toxic, LPS may suppressor T cells and of preventing tolerance to polysaccharide antigens. Thus, nontoxic LPS is nontoxic but retains significant immunostimulatory activity and is capable of inactivating suppressor T cells and of preventing tolerance to polysaccharide antigens (2, 23).

References


