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FRACTIONATION OF SWINE BARN DUST AND ASSESSMENT OF ITS IMPACT ON THE RESPIRATORY TRACT FOLLOWING REPEATED AIRWAY EXPOSURE

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The effects of repeated exposure to a range of doses of swine barn dust (SBD) on airway hyperresponsiveness (AHR) and inflammation were evaluated using a mouse model system. A number of components, including endotoxin and a number of feed proteins, were identified in SBD, and mice were exposed 20 min/d for 14 d to a log dilution series of nebulized SBD suspensions. AHR to methacholine was measured using head-out whole-body plethysmography, and the methacholine concentration inducing a 20% decrease in pulmonary airflow (PC_{20} MCh) was calculated. At the end of the 14-d exposure period, bronchoalveolar lavage (BAL) fluids were recovered, cytokines (interleukin [IL]-1β, IL-6, keratinocyte-derived chemokine [KC], and tumor necrosis factor [TNF]) in BAL were measured by enzyme-linked immunosorbent assay (ELISA), and leukocytes in BAL were counted. The PC_{20} MCh was significantly lower in the group of mice that were exposed to the highest concentration of SBD than in controls or the group exposed to the lowest level of dust. Likewise, the group that was exposed to the highest level of SBD had significantly higher levels of IL-1β, KC, and TNF than controls and some other groups. There were substantially more lymphocytes and monocytes in the BAL from mice that were exposed to the higher levels of SBD for the 14-d period, but neutrophils were not a part of this response. The SBD exposures used in these experiments induced chronic inflammatory phenotype responses, as indicated by the predominance of lymphocytes and monocytes, but not neutrophils, in BAL and by inflammatory cytokines detected. The association between the PC_{20}MCh and dose of SBD suggests that a threshold of susceptibility occurs after a relatively low, chronic exposure to SBD.

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Inhalation of pure endotoxin, lipopolysaccharide (LPS), or of organic dusts containing endotoxin induces intense acute lung inflammatory responses that involve neutrophils and macrophages (Michel et al., 1997), as well as clinically apparent symptoms, including fever and chills. In addition, significant changes in lung function, characterized by bronchoconstriction and airway hyperresponsiveness (AHR), were reported

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(Sundblad et al., 2002). Endotoxins, constituent components of the cell walls of gram-negative bacteria, are ubiquitous in the environment and often present in high concentrations in organic dusts (100–5000 EU/m^3 in swine barn dust [SBD]) (Dosman et al., 2000). Endotoxins are potent proinflammatory substances and are thought to be one of the primary agents in organic dusts that produce changes in airway physiology and airway inflammation (Mayeux, 1997). LPS certainly comprises a biologically important part of animal confinement facility airborne dusts (Dosman et al., 2006), but by the same token it has been reported that removal of LPS from SBD extracts does not eliminate their proinflammatory activities (Romberger et al., 2002).

In contrast to naive subjects who suffer from acute inflammatory reactions to SBD exposures, surveys of workers who are exposed daily to such dusts show that they present a moderate airway infiltration of inflammatory cells but not characteristic acute responses (Pedersen et al., 1996). This loss of acute responsiveness in swine barn worker populations might be explained as simple attrition from the workforce of those that do suffer such responses, but a more plausible explanation is that persistent swine barn workers undergo a physiologic adaptation process that dampens their acute responsiveness (Chénard et al., 2007; Sundblad et al., 2009). Despite some levels of tolerance to airborne contaminants in long-term swine barn workers, they still present with increased AHR (Zhou et al., 1991), which may lead to decreased pulmonary function over time. Indeed, a trend of accelerated annual decreases in lung function in swine barn workers was reported (Senthilselvan et al., 1997). Recurrent airway inflammation and injury may hasten the decline in lung function and place swine barn workers at increased risk of developing chronic lung disease.

To date, most studies assessing adverse human health effects after exposure to swine barn contaminants addressed only the acute effects of such exposures. However, swine barn workers often work 6–8 h/d, 5 d/wk, for many years. Therefore, studies modeling more prolonged or chronic exposure would be valuable in determining the long-term effects of inhaling airborne contaminants present in swine barns. Five-day, but not 20-d, exposures(8 h/d) of rats to swine barn air with endotoxin levels greater than 15,000 EU/m^3 were shown to induce lung inflammation and AHR (Charavaryamath et al., 2005). The inflammatory response of mice following 4-h exposure to SBD was examined (Mueller-Anneling et al., 2006), but the impact of repeated exposure to various levels of SBD has not been rigorously evaluated, to our knowledge. Thus, the objective of the present study was to characterize the biologically active constituents of SBD and to evaluate the effects on AHR and inflammation of repeated exposures to SBD over a biologically relevant dose range in a murine model.

MATERIALS AND METHODS

Swine Facility and Dust Collection

Settled dust was obtained from undisturbed ledges in a grower/finisher room at Prairie Swine Centre, Inc. (Floral, Saskatchewan, Canada), in June 2008. This facility uses management and production methods that are consistent with those commonly used by the pork industry in North America and described previously (Senthilselvan et al., 2009). The dust was scraped into sterile Whirl-Pak bags with a stainless-steel spatula and was stored in the presence of a desiccant at 4°C until use. The dust was filtered using a size USA#50 mesh screen and mixed in saline at 400 mg/ml. This stock was sonicated in a jeweler-type sonicator for 20 min on ice. The stock was then diluted to 0.00004–0.4mg/ml in USP saline, aliquoted, and stored at −20°C until use.

Endotoxin Assay

Serial twofold dilutions of the swine barn dust (SBD) suspensions (0.4 mg/ml) were analyzed for gram-negative bacterial endotoxin using an endpoint *Limulus* amebocyte lysate

assay (number N184-06, Cambrex BioScience, Inc., Walkersville, MD) in which the *Escherichia coli* O111:B4 endotoxin supplied by the manufacturer was employed as a standard. The endotoxin concentration in the samples was determined using the method recommended by the manufacturer, which involves visual detection of a clotting reaction.

Identification of Swine Barn Dust Components

The SBD extracts were analyzed using a number of approaches. To assess arsenic (a component of some swine feed additives), the SBD was processed by the Saskatchewan Research Council Analytical Laboratory (Saskatoon, SK) using a high-pressure microwave digestion technique with nitric acid. The resulting solution was analyzed using inductively coupled plasma mass spectrometry (ICP-MS) to identify and quantitatively measure the arsenic concentration.

For identification of major organic components, an aqueous extract was prepared using a bead beater (Fastprep FB120, ThermoScientific, Waltham, MA) 3 times for 30 s each on the maximum setting and sterilized by passing through a 0.2−μm filter (Sarstedt, Montreal, QC) The major components were separated by fast protein liquid chromatography (FPLC; AKTA, GE Lifesciences, Bain d'Urfe, Quebec). Nine FPLC fractions were assayed for their abilities to induce interleukin (IL)-6 and IL-8 release from A549 cells (a human bronchial adenocarcinoma epithelial cell line, ATTC CCL185) using methods described previously (Zhao et al., 2009). The components of each fraction were visualized using sodium dodecyl sulfate (SDS) polyacrylamide gel eletrophoresis (PAGE) gels (Figure 1) that were stained with deep purple dye (GE Lifesciences, Bain d'Urfe, QC). Four prominent bands were further analyzed by mass spectroscopy at the Proteomics Centre, Genome BC, University of Victoria, Victoria, BC, Canada. The results were used to search the NCBInr database.

Dust Suspension Preparation

In order to produce an exposure level in mice that would be representative of the exposure of humans in the barn, scaling factors were used that accounted for the respiration rate of mice as a variable that influences dust deposition in the airways. Ventilation rates were assumed for 25 g mice of 25 ml/min (i.e., 1 ml/min/g tissue), and for a 70-kg human of 5 L/min (i.e., 0.07 ml/min/g of tissue; Salem & Katz, 2006). Accordingly, under equivalent dust conditions a mouse would experience a calculated 14-fold greater airway dust exposure than a human. Dust levels in a swine barn are approximately 20 mg/m^3 (Larsson et al., 1992, 1994; Mackiewicz, 1998; O'Sullivan et al., 1998; Sundblad et al., 2002; Wang et al., 1996, 1997), such that the equivalent mouse exposure would be (20 mg/m^3)/14 = 1.4 mg/m^3. The dust exposure levels noted later in this article were designed to bracket this representative exposure level.

Animals and Experimental Design

Adult male CD1 mice, 23 to 25 g body weight and 6 wk of age, were obtained from the University of Saskatchewan Animal Resource Centre and were housed in our institutional Animal Care Unit (Western College Veterinary Medicine, University of Saskatchewan, Canada). They were randomly assigned to one of six treatment groups (n = 5) and were given food and water ad libitum. The individual groups of mice were exposed en masse in enclosed chambers to nebulized suspensions of SBD in sterile saline for 20 min/d daily for 14 d. The volume of the exposure chamber was approximately 5.7 L, and approximately 10 ml of SBD suspension was aerosolized during each exposure. The aerosols were generated using an ultrasonic nebulizer (Ultra-Neb 99; DeVilbiss Co., Somerset, PA) set to deliver 0.5 ml/min, and calibrated to produce aerosol particles ≤3 μm in size. The mice were exposed to either sterile water (controls; group 1), or aerosols containing 0.00004 (group 2), 0.0004 (group 3), 0.004 (group 4),

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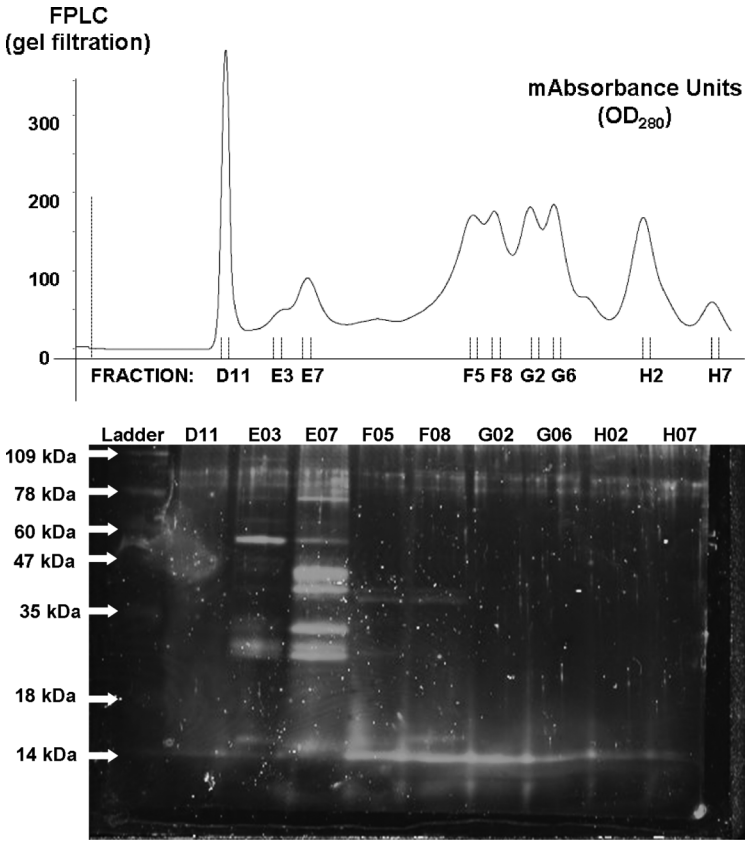

FIGURE 1. FPLC (upper panel, A) and PAGE (lower panel, B) analysis of the components contained within SBD extracts. Aqueous extracts of SBD were subjected to gel filtration FPLC (upper panel) and the indicated fractions (black arrows) were then further fractionated using SDS-PAGE. The PAGE gels were stained with deep purple. The four bands within the boxes on the gel were excised and submitted for mass spectroscopy for identification.

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0.04 (group 5), or 0.4 (group 6) mg/ml SBD. The experiments were performed in accord with recommendations of the Canadian Council on Animal Care Guidelines and were approved by the University of Saskatchewan Campus Committee on Animal Care.

Assements of Airway Hyperresponsiveness

Airway hyperresponsiveness (AHR) was assessed in conscious mice by head-out, whole-body plethysmography, as previously described in detail (Gordon et al., 2005; Schneider et al., 2001). Briefly, air was supplied to the head and body compartments of a plethysmograph via a small-animal ventilator and changes in the air flow through the body compartment were monitored using a flow sensor linked to a computer-driven real-time data acquisition/analysis system (DasyLab 5.5; DasyTec USA, Amherst, NH). Doubling doses of nebulized methacholine (MCh) aerosols (0.75–25 mg/ml) were delivered to the head compartment of the plethysmograph and bronchoconstriction data were gathered as running 1-s means of the air flow at the 50% point in the expiratory cycle (Flow@50%TVe1). This parameter accurately reflects bronchiolar constriction, as opposed to alveolar constriction or airway occlusion (Vijayaraghavan et al., 1994). Each mouse was sequentially exposed to aerosols of saline and then doubling doses of MCh (0.75, 1.5, 3, 6, 12, 25 mg/ml) over approximately 15 min. The concentration of MCh provoking a 20% drop in Flow@

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50%TVe1 (PC_{20}MCh) was determined for each animal from the resultant MCh dose-response curve.

Blood Collection, Bronchoalveolar Lavages, and Cell Counts

Blood and bronchoalveolar lavage (BAL) samples were collected from the mice 48 h after the last dust aerosol exposure. Each mouse was exanguinated under isofluorane anaesthesia and anticoagulated blood was collected in evacuated blood collection tubes containing heparin. Total white blood cells (WBC) counts were determined by direct counting using a hemocytometer. BAL was performed on each mouse as described previously (Schneider et al., 2001). Differential counts were made from Wright's-stained smears (100–200 cells/smear).

Cytokine Measurement Procedure

The following cytokines/chemokines in BAL fluid were measured: interleukin-1-beta (IL-1β), interleukin-6 (IL-6), tumor necrosis factor (TNF) and keratinocyte-derived chemokine (KC). The cytokine enzyme-linked immunnosorbent assay (ELISA) protocols have been reported in detail previously (Schneider et al., 2001). Briefly, the BAL fluids were not diluted for cytokine assay, and quantification is based on a recombinant protein standard curve. All cytokine ELISA were sensitive to 5–10 pg/ml recombinant cytokine standards.

Statistical Analysis

Linear regression was used to evaluate the association of base 4 log-transformed dust exposure levels to AHR. The value of 1 (pg/ml) was added to each of the dust exposure levels in order to facilitate log-transformation. Total leukocyte counts in blood were analyzed using a Kruskal–Wallis one-way analysis of variance (two-tailed test). Additionally, to assess treatment group differences a comparison of mean ranks was conducted. For correlation analysis of cytokine levels the data were ranked and then analyzed by linear regression. Normality of the data was determined using descriptive statistics and the Shapiro–Wilk normality test. After analysis of variance of normally distributed data (cytokine levels in BAL), group means were compared using the Bonferroni test. The analyses were completed using Statistix7 analytical software (Tallahassee, FL). Statistical significance was set at $p < .05$ with 95% confidence and 80% power.

RESULTS

Characteristics of Settled Dust From a Large Swine Barn Operation

SBD had an endotoxin concentration of 2400 EU/mg and a negligible arsenic concentration (0.2 ng/mg). FPLC gel filtration chromatography of aqueous extracts of this dust yielded a characteristic elution profile such that we routinely collected nine individual peaks (arrows, Figure 1A) for further analyses. These included PAGE (Figure 1B), the endotoxin content of selected fractions, and their abilities to induce IL-6 and IL-8 release from human A549 bronchial epithelial cells (Figure 2). The D11 FPLC fraction induced the strongest A549 cell IL-8 response, while the F08 FPLC fraction contained the majority of the LPS in the sample (1.2×10^4 EU/ml) and induced a relatively robust IL-8 response. FPLC fraction E07 contained the bulk of the readily discernible protein bands in the PAGE gels. However, this fraction contained little LPS ($<1.5 \times 10^3$ EU/ml), weakly induced IL-6 expression by A549 cells, but induced a marked IL-8 response

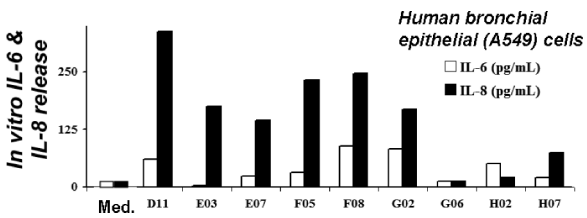

FIGURE 2. LPS is one of multiple inflammatory components in SBD aqueous extracts. The FPLC fractions of SBD generated in Figure 1 were assessed for their abilities to stimulate inflammatory cytokine (IL-6, IL-8) expression by A549 human bronchial epithelial cells.

by these cells. Data suggests that the endotoxin(s) within organic SBD are only one of a number of biologically relevant components of these occupational irritants.

There were a number of bands readily identifiable within the PAGE gel analyses of the FPLC peaks obtained from our SBD (Figure 1). Thus, from this gel two bands were excised from peak E03, and two from peak E07, and each of these was submitted for mass spectroscopy (Figure 1). Four of these were identified as having identity or extensive homology (ion score > 781) with major swine feed components. Bands 1 and 2 were identified as glycinin G1 and Gy5 (glycine soja), both soluble soybean storage glycoproteins. Bands 3 and 4 were identified as subunits of cruciferin, a major storage protein of canola.

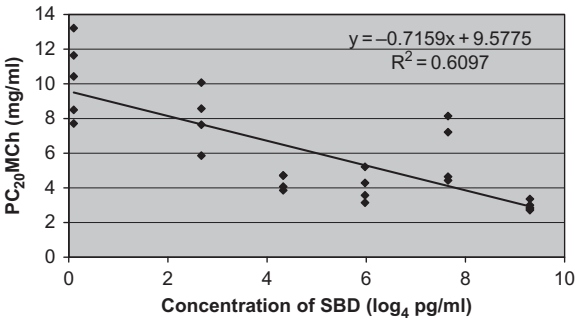

FIGURE 3. Dose-dependent effect of SBD Inhalation on airway hyperresponsiveness (AHR) to methacholine (MCh). Mice (n = 5) were exposed daily for 14 d to a range of doses of SBD administered as nebulized aerosols, and then their AHR to MCh was assessed by plethysmography, as indicated in the Materials and Methods section. The results show that repeated exposures of the mice to increasing levels of SBD led to dose-dependent susceptibility to MCh.

Impact of Swine Barn Dust Exposure on Airway Hyperresponsiveness in Outbred Mice

The degree of AHR to MCh in mice exposed for 2 wk to nebulized aerosols of SBD clearly correlated with the concentration of dust to which the mice were exposed (Figure 3; $R^2 = .6097$). Overall, four of the mice (one in each of groups 2, 3, 4 and 5) were too large to fit into the plethysmography chamber and were excluded. The median PC_{20}MCh for the group of mice exposed to the highest concentration of SBD was 2.8 mg/ml, which was significantly lower than the median PC_{20}MCh of the groups that were exposed to the lowest concentration of dust or saline (8.1 or 10.4 mg/ml, respectively). Linear regression shows that the PC_{20} was negatively correlated with the $\log_4$ of the dust exposure level (Table 1), indicating that for every 1 unit $\log_4$ increase in dust exposure there is a 0.72-mg/ml decrease in the dose of MCh required to provoke a 20% drop in airflow.

TABLE 1. Swine Barn Dust Proteins Identified by LC-MS/MS Analysis

FPLC fraction	Band	Top hits	Hit score
E3	1	Glycinin G1 precursor	432
E3	2	Gy5 (glycine soja)	510
E7	3	Cruciferin subunit	435
E7	4	Cruciferin subunit	781

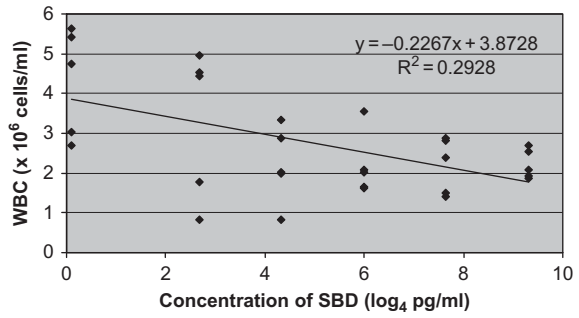

FIGURE 4. Mice were exposed to SBD for 14 d as in Figure 3, and the number of circulating white blood cells was determined by direct counting. Repeated exposures of the mice to greater concentration of SBD led to dose-dependent decreases in circulating leukocyte numbers.

Leukocytes in Bronchial Alveolar Lavage

As expected in nonasthmatic mice, no eosinophils were found in the airways of these animals. Interestingly, few neutrophils were observed in the airways of these mice, and no neutrophils in those animals exposed for 14 d to ≥4 μg/ml SBD. In contrast, there was a significant association between exposure to SBD and lymphocytes in BAL. Linear

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TABLE 2. Leukocytes Detected in Bronchial Alveolar Lavage

SBD exposure (mg/ml)	Leukocytes in bronchial alveolar lavage (mean (SEM); cells/ml)				
	Eosinophils	Neutrophils ($\times 10^2$)	Monocytes ($\times 10^4$)	Lymphocytes ($\times 10^4$)	Total leukocytes ($\times 10^4$)
0	ND	5.8 (5.8)	12.6 (0.4)	2.4 (0.3)	15.0 (0.6)
0.00004	ND	5.3 (5.3)	13.0 (2.9)	3.7 (0.9)	16.8 (3.7)
0.0004	ND	6.8 (6.8)	15.7 (5.1)	4.2 (1.7)	20.4 (5.2)
0.004	ND	ND	14.2 (10.9)	2.7 (2.0)	15.2 (9.3)
0.04	ND	ND	21.0 (7.0)	7.4 (3.3)	28.3 (7.8)
0.4	ND	ND	27.8 (4.6)	13.6 (2.0)	41.5 (6.6)

Note. ND signifies that no cells of this type were observed. Totals are not identical to the sum of the columns to the left because some blood smears were unsuitable for an accurate differential count.

regression showed that lymphocytes in BAL were positively correlated with the log of the dust exposure level, indicating that for every 1 unit $\log_4$ increase in dust exposure there is a 1.1×10^4 cells/ml rise in the lymphocyte numbers in the BAL. Similarly, monocytes in the BAL were positively correlated with the log of the dust exposure level, indicating that for every 1 unit $\log_4$ rise in dust exposure there is a 1.6×10^4 cells/ml increase in the monocyte concentration in BAL. As expected with the rise in lymphocyte and monocyte numbers, total leukocytes in the BAL were positively correlated with the log of the dust exposure level, indicating that for every 1 unit $\log_4$ increase in dust exposure there is a 2.6×10^4 cells/ml elevation in the total leukocyte concentration in BAL.

Repeated Exposure to Swine Barn Dust for 14 d Induces Airway Inflammation

The levels of IL-1β, TNF, and KC in the BAL fluid from controls were significantly lower than levels in the mice that were exposed to the highest concentration of SBD (Table 3). One-way analysis of variance showed a significant effect of group on the level of IL-6, with control mice having lower IL-6 levels than most of the groups that were exposed to SBD, although the comparison of means showed that these differences were not statistically significant. The analysis of cytokine production was performed to evaluate the pulmonary inflammatory response resulting from a 14-d exposure of mice to SBD.

Impact of Swine Barn Dust Exposure on Circulating Leukocyte Profiles of Outbred Mice

Intravenous administration of inflammatory mediators such as the ELR-CXC chemokines (e.g., IL-8) induces neutrophil margination in the pulmonary vascular bed and thereby an apparent circulating neutropenia (Van Zee et al., 1992). Similarly, when the SBD-exposed CD-1 mice were compared to the control group, they had significantly lower (8.53×10^7 cells/ml) total white cell counts than control mice exposed to saline alone (1.94×10^8 cells/ml). A dose-dependent effect was not observed, since there was no marked difference in the total white blood cell counts for the groups of mice that were exposed to $\geq$0.0004 mg/ml of SBD (groups 3–6) (Figure 3).

Linear regression showed that total leukocyte count in blood was negatively correlated with the $\log_4$ of the dust exposure level (Table 1), indicating that for every 1 unit $\log_4$ increase in dust exposure there was a 1.5×10^6 cells/ml decrease in the concentration of circulating leukocytes.

DISCUSSION

In this study the components present in settled SBD were physically and biologically characterized. The dust samples were obtained from ledges that were 5–8 feet from the floor in a large swine operation, indicating that the dust certainly had been airborne and

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TABLE 3. Cytokines Detected in Bronchial Alveolar Lavage Fluids From Mice Chronically Exposed to a Range of Doses of Nebulized SBD

SBD exposure (mg/ml)	Cytokines in bronchial alveolar lavage (mean (SEM); pg/ml)[a]			
	Interleukin-1β	Interleukin-6	TNF-α	KC
p (ANOVA)	.0043	.042	0.024	.0069
0 mg/ml	194.4 (30.4) ^{A}	550.1 (66.2)	86.4 (51.7) ^{A}	390.2 (69.7) ^{A}
0.00004 mg/ml	287.7 (37.4) ^{AB}	954.4 (185.3)	658.9 (385.5) ^{AB}	581.4 (78.2) ^{AB}
0.0004 mg/ml	230.3 (35.7) ^{A}	750.1 (133.4)	342.5 (199.5) ^{A}	468.0 (67.1) ^{AB}
0.004 mg/ml	183.8 (47.3) ^{A}	509.7 (130.7)	520.7 (473.2) ^{AB}	288.4 (66.3) ^{A}
0.04 mg/ml	310.8 (41.6) ^{AB}	946.8 (189.5)	1847.5 (1066.5) ^{AB}	619.1 (99.6) ^{AB}
0.4 mg/ml	452.1 (73.0) ^{B}	1168.0 (184.6)	6160.9 (2918.5) ^{B}	839.8 (154.4) ^{B}

[a]Within each column, values with the same capital letter superscript are not significantly different (Tukey comparison of means).

therefore would have been inspired by workers. Their size was not assessed so one cannot predict where in the respiratory tract of workers these particulates may have lodged following inspiration. Nevertheless, it is possible that they induced potent and dose-dependent pulmonary inflammatory responses in mice exposed daily to nebulized aerosols thereof for 14 d. The outbred mice used in this study (CD1) were relatively hyporesponsive when compared to 17 other strains of mice genetically characterized for LPS sensitivity (Cook et al., 2003); in this regard CD1 mice could be considered representative of the general human population, in which approximately 10% have polymorphic TLR4 alleles. It has been reported previously that loss of lung function in swine barn workers is correlated with the duration of their employment in swine operations and their daily exposure times, indicating that increased exposure leads to increased loss of lung function (Zejda et al., 1993; Senthilselvan et al., 1997). Increased AHR in swine barn workers has also been reported (Sundblad et al., 2002). Presumably this is attributable to a progressive change in lung architecture associated with the chronic inflammatory state.

Our data suggest that there was a dose-dependent induction of chronic inflammation in the lungs of outbred mice exposed daily to SBD for 2 wk. Thus, a dose-dependent increase was observed in the airway levels of a number of inflammatory cytokines, including IL-1, TNF, and the ELR-CXC chemokine KC, and this was associated with an apparent leukopenia. It is clear that numerous agonists activate airway epithelial cells to express inflammatory cytokines important in neutrophil recruitment (Zhao et al., 2009), but these cells would also express monocyte- and lymphocyte-specific chemokines (e.g., RANTES, monocyte chemoattractant proteins [MCP], IP-10; Baggiolini, 1998). Just as an increase in lymphocyte/monocyte response in the lavage fluid of mice chronically exposed to SBD was noted, others also reported significant accumulations of lymphocytes (both B and T cells) in the lung parenchyma of animals given SBD extracts for 2 wk (Poole et al., 2009). However, it is noted that this other study utilizing SBD extract found a predominance of neutrophils in the lavage fluid (Poole et al., 2009). It is possible that in our study, 2 wk of SBD exposure led to our animals developing a hypersensitivity pneumonitis or farmer's lung-like syndrome, although data to support this are not available.

It is also well known that exposure of blood leukocytes to ELR-CXC chemokines (e.g., IL-8) induces neutrophil margination in the vascular bed that is seen clinically as an apparent neutropenia (Van Zee et al., 1992) and that pulmonary vascular neutrophil margination is characteristic of more long-term pulmonary inflammatory events, such as in bacterial pneumonia (Doyle et al., 1997; Doerschuk et al., 1996). Interestingly, a previous assessment of

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the impact of swine barn air exposure on the lungs of mice found no significant airway inflammatory cytokine response or distinct blood neutropenia in mice exposed to dust-contaminated swine barn air for 1, 5, or 20 d (Charavaryamath et al., 2008), while rats similarly exposed to such air develop an exaggerated blood, but not airway, neutrophilia after 20 d of exposure (Charavaryamath et al., 2005). As in both of these reports, no discernible airway neutrophil responses were observed in the mice we used, despite the high levels of KC present in the lungs. It was reported previously that neutrophil agonists, including endotoxin, C5a, and IL-1, among many such mediators, induced neutrophil tachyphylaxis, such that these cells became unresponsive to their agonists (Colditz & Movat, 1984; Cybulsky,et al., 1988). On the other hand, when aqueous extracts of SBD (i.e., without the presence of particulate matter) are administered to mice repeatedly, there is no neutrophil tachyphylaxis observed, such that the animals develop progressive airway neutrophil responses associated with a progressive attrition (or perhaps tachyphylaxis) of their airway inflammatory cytokine responses (Poole et al., 2009). This raises the question of whether the inhaled particulates in SBD themselves carry biological activities above and beyond those extracted by aqueous or other solvents.

Certainly, our data clearly show that SBD contains multiple components that are able to activate airway epithelial cells. Although endotoxin is an important component of SBD that is associated with respiratory disease in exposed workers, it is not the sole component of the dust that produces adverse respiratory effects (Wyatt et al., 2008). Further, it is readily apparent from our data that some FPLC fractions that contained little endotoxin were able to activate these epithelial cells. Whether the feed proteins identified as prominent components in our SBD samples were themselves biologically active was not determined, but they were present in FPLC fractions that carried significant pro-inflammatory activities. As an example, glycinin G1, which was identified in FPLC peak E3, has multiple epitopes that cross-react with peanut allergens and thereby bind immunoglobulin (Ig) E antibodies, indicating that it potentially carries significant activity (Zeece, 1999). Thus, this is one of the few studies of respiratory exposures and SBD that identifies other nonendotoxin dust components.

There was a clear relationship between the concentration of dust to which mice were exposed in this study and their AHR to MCh after 2 wk of daily exposure. The mechanisms by which organic dusts induce AHR are not fully understood (Park et al., 1999), but others have also shown that short-term exposures to organic dusts augment airway responses to MCh challenge (Charavaryamath et al., 2005, 2008; Poole et al., 2009). However, unlike what was observed in the present study, Poole et al. (2009) observed tachyphylaxis of this response following 2 wk of daily exposure to extracts of SBD. This again suggests that there are significant differences between responses to particulate matter that is laden with biologically active mediators and responses to the mediators in the absences of such particulates. It has been reported, however, that 3 wk of exposure to dusty swine barn air leads to tachyphylaxis of MCh hyperresponsiveness, and that this is not attributable to the endotoxin(s) present in the dust (Charavaryamath et al., 2008). Interestingly, data suggest not only that 3 wk of exposure makes the animals less responsive to MCh challenge, but also that exposed mice become much less responsive than animals never exposed to organic dusts (i.e., the normal controls). The mechanisms of such hyporesponsiveness or how that would reconcile with the observed loss of lung function in swine farmers across time is not clear. Nevertheless, the fact that a dose-dependent increase was found in AHR to MCh (at 14 d) suggests that further studies in the lower exposure range would be valuable in determining a no-observable-adverse-effect level (NOAEL), which might be used for the establishment of occupational exposure limits for total dust and endotoxins in swine barns.

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