

# Effects of Hardwood Smoke Exposure on Allergic Airway Inflammation in Mice

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Hardwood smoke (HWS) from wood burning stoves and fireplaces can be a significant contributor to the composition of ambient air pollution. We hypothesize that the inhalation of HWS by ovalbumin (OVA)-sensitized mice with preexisting lung inflammation leads to the exacerbation of allergic airway responses. Two different models were employed to characterize the effects of inhaled wood smoke on allergic airway inflammation. In both models, male BALB/c mice were sensitized by injection with OVA and alum. In one model, mice were challenged by inhalation with OVA 1 day prior to exposure to HWS (30, 100, 300, or 1000  $\mu\text{g}$  particulate matter [PM]/ $\text{m}^3$ ) for 6 h/day on 3 consecutive days. In the other model, mice were exposed by inhalation to OVA, rested for 11 days, were exposed to HWS for 3 consecutive days, and then were exposed to OVA immediately after the final HWS exposure. Bronchoalveolar lavage (BAL), and blood collection were performed  $\sim 18$  h after the last HWS or OVA exposure. HWS exposure after the final allergen challenge (first model) led to a significant increase in BAL eosinophils only at the 300  $\mu\text{g}/\text{m}^3$  level. In contrast, changes in BAL cells did not reach statistical significance in the second model. There were no HWS-induced changes in BAL interleukin (IL)-2, IL-4, IL-13, and interferon (IFN) $\gamma$  levels in either model following OVA challenge. These results suggest that acute HWS exposure can minimally exacerbate some indices of allergic airway inflammation when a final OVA challenge precedes HWS exposure, but does not alter Th1/Th2 cytokine levels.

Despite the use of alternate energy sources for heating and power generation in the industrialized world, the use of wood, primarily in residential stoves and fireplaces, still contributes significantly to indoor and outdoor air pollution. Wood-burning stoves and fireplaces emit a complex mixture of inorganic gases, volatile organic hydrocarbons, aldehydes, polycyclic aromatic compounds, and particulate matter (PM) (Boman et al., 2003; Zelikoff et al., 2002). During the winter months up to 80–90% of the ambient particle concentration in Seattle, WA, 45% in Santa Clara County, CA, and 20–30% in Denver, CO, comes from residential wood combustion (Larson & Koenig, 1994; Muhlbaier Dasch, 1982). Epidemiology studies indicate that there is an association between wood smoke exposure and the development

of respiratory illness in children and adults (Koenig et al., 1993; Lipsett et al., 1997; Norris et al., 1999; Schwartz et al., 1993; Sheppard et al., 1999; Yu et al., 2000).

Only a limited number of experimental studies have investigated the effects of inhaled wood smoke emissions on pulmonary function and immunity, with no specific studies examining the effects of wood smoke on the exacerbation of asthma (Zelikoff et al., 2002). Subchronic exposure of rats to wood smoke at a PM concentration of 10  $\text{mg}/\text{m}^3$  leads to minor changes in carbon monoxide diffusing capacity, pulmonary resistance, inflammation, and squamous-cell metaplasia in the larynx (Tesfaigzi et al., 2002). Acute inhalation exposure to wood smoke produces airway hyperresponsiveness (AHR) to substance P, capsaicin, and prostaglandin  $\text{F}_{2\alpha}$ , but not to histamine or acetylcholine, in guinea pigs (Hsu & Kou, 2001). Other studies show that acute exposure to wood smoke impairs bacterial clearance from the lung and impairs macrophage function (Demarest et al., 1979; Fick et al., 1984; Loke et al., 1984; Zelikoff et al., 2002).

We used a common experimental sensitization approach (intraperitoneal ovalbumin [OVA] plus aluminum hydroxide) to determine whether hardwood smoke (HWS) exposure in the presence (e.g., induction of allergic lung response) or absence (e.g., systemic allergen-specific antibodies [atopic] but no lung

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## MATERIALS AND METHODS

Male BALB/c mice (8–10 wk old; 15–16 animals per treatment group) were purchased from Charles River Laboratories (Wilmington, MA). Mice were housed in wire rack cages inside whole-body exposure chambers (H-2000; Hazleton Systems, Inc., Maywood, NJ). Chamber temperatures were maintained at  $26 \pm 2^\circ\text{C}$  and lights were on a 12-h on/off cycle. Mice were given food (Teklad certified rodent diet, Harlan Teklad, Madison, WI) ad libitum except during the 6-h exposure period. Water was available ad libitum throughout the duration of the study.

Mice were exposed to allergen and HWS according to two different protocols. For both protocols, mice were sensitized by injection with OVA (10  $\mu$ g) and aluminum hydroxide (2 mg) in sterile water on days 0 and 7. In protocol 1 (Figure 1A;

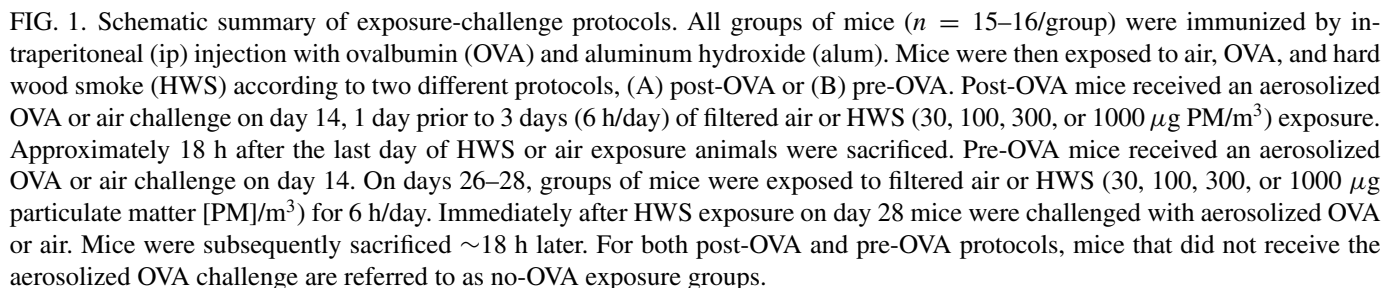


TABLE 1  
Clean air and HWS exposure atmosphere characterization

| Particle mass target             | Units                          | 0                 | 30                | 100               | 300               | 1000               |
|----------------------------------|--------------------------------|-------------------|-------------------|-------------------|-------------------|--------------------|
| Particle mass                    |                                |                   |                   |                   |                   |                    |
| Particle mass                    | $\mu\text{g}/\text{m}^3$       | $6.4 \pm 6.9$     | $40.5 \pm 9.2$    | $113.3 \pm 21.8$  | $319.7 \pm 43.9$  | $1041.1 \pm 123.5$ |
| HWS particle mass                | $\mu\text{g}/\text{m}^3$       |                   | 34.1              | 106.9             | 313.3             | 1034.7             |
| Particle size                    |                                |                   |                   |                   |                   |                    |
| Mass median aerodynamic diameter | $\mu\text{m} \pm \text{GSD}^a$ | <LOQ <sup>b</sup> | $0.25 \pm 3.3$    | $0.35 \pm 2.5$    | $0.35 \pm 2.0$    | $0.36 \pm 2.1$     |
| Gases                            |                                |                   |                   |                   |                   |                    |
| Nitrogen monoxide                | ppm                            | <LOQ <sup>b</sup> | <LOQ <sup>b</sup> | <LOQ <sup>b</sup> | <LOQ <sup>b</sup> | <LOQ <sup>b</sup>  |
| Nitrogen dioxide                 | ppm                            | <LOQ <sup>b</sup> | <LOQ <sup>b</sup> | <LOQ <sup>b</sup> | <LOQ <sup>b</sup> | <LOQ <sup>b</sup>  |
| Carbon monoxide                  | ppm                            | $0.2 \pm 0.0$     | $0.7 \pm 0.1$     | $1.6 \pm 0.3$     | $4.0 \pm 0.7$     | $13.0 \pm 1.6$     |
| Total vapor hydrocarbon          | ppm                            | $0.1 \pm 0.4$     | $0.3 \pm 0.1$     | $0.6 \pm 0.2$     | $1.3 \pm 0.1$     | $3.1 \pm 0.5$      |

<sup>a</sup>Geometric standard deviation.

<sup>b</sup>Below limit of quantitation.

post-OVA), an OVA aerosol challenge was given on day 14, 1 day prior to 3 days (6 h/day) of filtered air or HWS (30, 100, 300, or 1000  $\mu\text{g PM}/\text{m}^3$ ) exposure. In protocol 2 (Figure 1B; pre-OVA), mice received an OVA aerosol or air challenge on day 14. On days 26–28, groups of mice were exposed to filtered air or HWS (30, 100, 300, or 1000  $\mu\text{g PM}/\text{m}^3$ ) 6 h/day. Immediately after HWS exposure on day 28, mice were challenged with aerosolized OVA or air. For both protocols, mice were sacrificed ~18 after the final exposure (HWS or OVA). Additionally, for both protocols, mice that did not receive the aerosolized OVA challenge are referred to as no-OVA exposure groups. All animal procedures for this study were reviewed and approved by the Lovelace Respiratory Research Institute (LRRI) Institutional Animal Care and Use Committee.

### Hardwood Smoke Generation and Exposures

HWS was generated from a conventional, uncertified wood stove manufactured by Pineridge. The stove was placed in an ~600-ft<sup>2</sup> single-room building adjacent to the exposure laboratory. A mixture of black (~60%) and white (~40%) oak species from the Missouri area was obtained and stored in a temperature- and humidity-controlled environment to maintain the humidity of the wood at ~20–25 %.

The stove was operated over a three-phase burn cycle that spanned the 6-h exposure period. The fire was started (which initiated exposures) with unprinted/unbleached newspaper (end rolls obtained from a local printer) and split hardwood (same wood type used for entire test). The phases included kindling (~15–20 min), a high burn rate (4–6 kg of wood for ~90 min), and a low burn rate (4–6 kg of wood for remainder of exposure period), controlled by the setting of the air intake damper. Smoke was extracted, routed to the dilution/distribution/exposure system, and diluted with ambient air that had been scrubbed by passage through high-efficiency particulate air (HEPA) and charcoal-impregnated prefilters to remove particles and volatile organics.

Exposures were conducted in exposure chambers (H2000, Hazleton Systems, Maywood, NJ). The target exposure levels were 6-h average concentrations yielding 30, 100, 300, and 1000  $\mu\text{g}/\text{m}^3$  of HWS PM. The variation of the PM within these chambers was determined to be less than 10% when determined using previously described methods (McDonald et al., 2004).

### Exposure Characterization

The composition of the exposure atmospheres at all exposure levels was analyzed in detail (not reported here) as described previously (McDonald et al., 2004). The concentrations of PM and other components comprising a majority of the exposure mass are summarized in this article. The PM, PM size distribution, nitrogen monoxide/nitrogen dioxide (NO/NO<sub>2</sub>), total hydrocarbon, and CO in the exposure atmospheres are reported in Table 1. PM concentration was measured daily. HWS PM was calculated as the total PM minus the PM measured in the control chamber. Particle size was determined (~0.05–10  $\mu\text{m}$ ) using a rotating 10-stage micro-orifice uniform deposit impactor (MOUDI; MSP Corp.). CO was determined using a photoacoustic gas analyzer (Innova 1312, California Analytical Instruments, Irvine, CA). NO and NO<sub>2</sub> were measured using a chemiluminescent analyzer (model 200A NOx Analyzer, Pollution Instruments, San Diego, CA).

### OVA Exposures

Exposure to aerosolized OVA was carried out using a whole-body plethysmography chamber exposure system (Buxco Electronics, Sharon, CT). OVA aerosols were generated by ultrasonic nebulization (DeVilbiss Pulmosonic, model 5500) (10 min) of 1% heat-aggregated OVA (chicken egg, grade V; Sigma, St. Louis, MO) in water that passed directly through a 25-cm diffusion dryer before the Buxco distribution plenum and then the exposure chambers. The total mass concentration of OVA

was determined by gravimetric analysis of filter samples taken during test runs. The average mass concentration of OVA was  $\sim 225 \text{ mg/m}^3$  during a 10-min exposure. OVA particle size was measured (gravimetrically) directly from the exposure chamber with a Mercer-style impactor (size range 0.6–12  $\mu\text{m}$ ). The mass median aerodynamic diameter ( $\mu\text{m}$ ) and geometric standard deviation were  $1.1 \pm 2.0$ .

### Pathologic Analysis

Approximately 18 h following the last exposure, animals were euthanized by injection with a lethal dose of a pentobarbital-based euthanasia solution. Blood was collected by cutting the renal artery, and serum was prepared. Bronchoalveolar lavage (BAL) cells were obtained from 10 animals by inserting a catheter into the trachea and lavaging the lung three times with 0.8 ml phosphate-buffered saline (PBS without calcium chloride or magnesium chloride). Total BAL cells were determined using a hemacytometer. BAL cells were spun onto slides by cytocentrifugation and stained with a modified Wright–Giemsa stain. Four hundred cells were counted and the percentage of specific cell types was determined for each animal. Of the 10 samples prepared for each HWS exposure, the smallest number that was available for the statistical analysis was eight. Lungs from 5 separate animals that were not lavaged were infused via the trachea with 10% neutral buffered formalin, the trachea ligated, and lungs immersed in formalin. The lungs were removed and fixed in the same solution. After paraffin embedding, 5- $\mu\text{m}$ -thick sections for microscopic analysis were stained with hematoxylin and eosin.

### Analysis of OVA-Specific IgG<sub>1</sub> and IgG<sub>2a</sub>

Anti-OVA-specific immunoglobulin (Ig) G<sub>1</sub> and IgG<sub>2a</sub> antibody was measured with enzyme-linked immunosorbent assay (ELISA). Briefly, 96-well plates were coated with 5  $\mu\text{g/ml}$  heat-aggregated OVA (Sigma) in PBS and incubated overnight at 4°C. Plates were washed 3 $\times$  (0.1% Tween 20 in PBS) and then blocked with 2.5% skim milk for 1 h. Samples diluted in skim milk were added (100  $\mu\text{l}$ ) and incubated for 1 h at 37°C. Plates were washed 3 $\times$  and goat anti-mouse IgG<sub>1</sub>-HRP or IgG<sub>2a</sub>-HRP (Pharmingen, San Diego, CA) was added (1:2000; incubation 1 h at 37°C). Next, plates were washed 5 $\times$  and ABTS peroxidase substrate (KPL, Gaithersburg, MD) was added (incubation 30 min at 37°C). Finally, peroxidase stop solution (KPL) was added, and the plates were read in a microplate reader at 410 nm. Results are presented as units/per milliliter, where the relative units represent the optical density of a sample multiplied by the sample dilution factor.

### Analysis of OVA-Specific IgE

OVA-specific IgE antibody was measured by ELISA. Briefly, 96-well plates were coated overnight at 4°C with 2  $\mu\text{g/ml}$  anti-mouse IgE (Pharmingen). Plates were washed 3 $\times$  (0.1% Tween 20 in PBS) and then blocked with 10% fetal bovine serum (Hyclone, Logan, UT) in PBS for 1 h. Plates were washed 3 $\times$  and

then 100  $\mu\text{l}$  of sample was added and incubated for 2 h at room temperature. Again, plates were washed 5 $\times$  and then 100  $\mu\text{l}$  of OVA conjugated with biotin (EZ-link Biotinylation Kit; Pierce Biotechnology, Rockford, IL; 4  $\mu\text{g/ml}$ ) plus avidin–horseradish peroxidase (HRP) (1/1000; Pharmingen) was added to each well and incubated at room temperature for 1 h. Plates were washed 7 $\times$ , and 100  $\mu\text{l}$  of TMB substrate (Pharmingen) was added (incubation for 30 min in the dark at room temperature). Finally, stop solution (2 N H<sub>2</sub>SO<sub>4</sub>) was added and the plates were read at 450 nm on a microplate reader. Results are presented as units per milliliter. Total IgE in serum samples was measured using an ELISA kit (IgE BD OptEIA; Pharmingen) following the manufacturer's protocol. Apart from OVA-exposed animals with post-OVA challenge (where antibody measurements from 9–10 animals per HWS exposure group were taken), antibody measurements from 11–16 animals per HWS exposure group were taken.

### Cytokine Analysis

Cytokine levels for IL-2, -4, and -5 and IFN $\gamma$  in the BAL were measured in 8–11 randomly chosen animals per HWS exposure group by ELISA kit following the manufacturer's protocol (OptEIA; Pharmingen). Levels of IL-13 in BAL fluid were determined using monoclonal anti-mouse IL-13, biotinylated anti-mouse IL-13, and recombinant murine IL-13 in an ELISA following the manufacturer's recommended protocol (R&D Systems, Minneapolis, MN).

### Statistical Analysis

One-way analysis of variance (ANOVA) was used to evaluate HWS-related effects on BAL parameters, cytokine, and antibody measurements. When an ANOVA indicated significant ( $p < .05$ ) differences in mean responses across experimental groups, Dunnett's multiple comparison procedure was performed to compare HWS-exposed group means to those of controls (Dunnett, 1955, 1980). The linear term of the ANOVA (with equal dose spacing) was used to assess evidence of exposure-related trends in response across control and exposed groups. Because of differing variance structures, four separate ANOVAs were applied for each endpoint, corresponding to the two different allergen challenges (OVA and air) in the two protocols (pre- and post-OVA).

For several of the endpoints that were examined, there was significant heteroscedasticity, with exposure group variances functionally related to their associated sample means. Prior to statistical analysis, logarithmic transformations were used to stabilize the variances of BAL cell numbers associated with post-OVA exposure (adding 0.001 to zero cell counts) and IFN $\gamma$  and IL-13 measurements. Square-root transformations were applied to IL-2 and IL-5 measurements.

Data are summarized and plotted as mean  $\pm$  standard error of the mean, and all reported  $p$  values are two-sided. Statistical computations were performed with the SAS software system (Cary, NC).

## RESULTS

### Post OVA

OVA challenge alone led to a significant ( $p < .05$ ) increase in BAL eosinophils. HWS exposure led to a significant ( $p < .05$ ) increase in BAL eosinophils only at the  $300 \mu\text{g}/\text{m}^3$  HWS concentration in OVA-challenged mice, although there is a significant increasing linear trend across all exposure levels. Additionally, while OVA challenge alone led to a significant ( $p < .05$ ) increase in BAL macrophages and lymphocytes, there was no effect of HWS exposure (Figure 2). There was no effect of HWS exposure on BAL cell numbers in the no-OVA groups. While HWS exposure did not significantly alter BAL fluid cytokine levels in OVA-challenged mice there was evidence of HWS exposure-dependent decreases (linear trend) in IL-2, -4, and IFN $\gamma$  for the no-OVA groups (Figure 3). HWS exposure led to a significant increasing trend in OVA-specific IgE, but there was no substantive evidence of HWS effects on total IgE, OVA-specific IgG $_1$ , or OVA-specific IgG $_{2a}$  in OVA challenged mice (Figure 4). Histologic assessment of lung tissue demonstrated that OVA exposure alone led to mild peribronchiolar and perivascular inflammation comprised primarily of eosinophils with

some neutrophils and lymphocytes present (Figure 5). However, there was no discernable difference in tissue inflammation as a result of HWS exposure.

### Pre OVA

As in the post-OVA model, there was very little effect of HWS on BAL parameters, cytokine, and antibody measurements. HWS-induced changes in BAL cell numbers did not reach the level of statistical significance (Figure 6). There was no effect of HWS exposure on BAL cell numbers in OVA-sensitized mice that were not exposed to OVA by inhalation. While all HWS exposure concentrations tended to increase IL-5 levels in OVA-challenged mice, no individual exposure group differed significantly ( $p < .05$ ) from air controls, and there was no evidence of an exposure-dependent response (Figure 7). In contrast, IL-13 levels were decreased at the highest HWS exposure level in OVA-challenged mice, with no evidence of an exposure-dependent response. There was no evidence that HWS exposure affected BAL fluid IL-2, IL-4, or IFN $\gamma$  levels in OVA-challenged mice. There were no statistically significant effects of HWS exposure on serum total IgE, OVA-specific

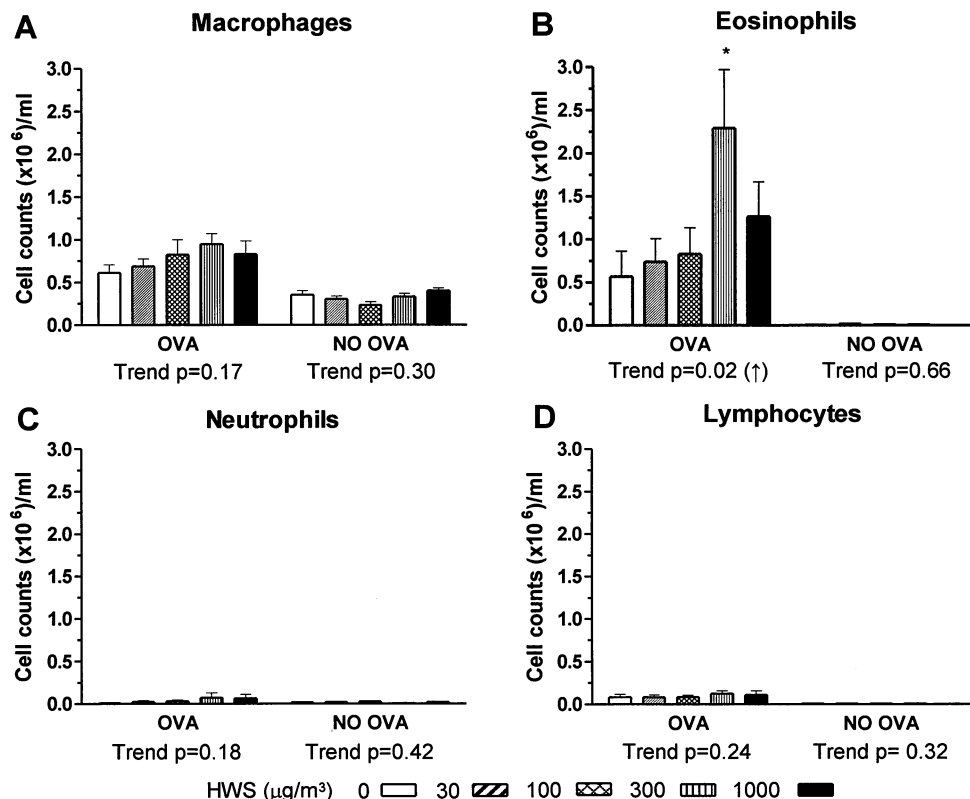


FIG. 2. Bronchoalveolar lavage cell numbers following post-OVA exposure protocol: (A) macrophages, (B) eosinophils, (C) neutrophils, and (D) lymphocytes. Asterisk indicates significant at  $p < .05$ , vs.  $0 \text{ mg}/\text{m}^3$ . Results of linear trend analyses examining dose-dependent trends are indicated below each treatment group with an arrow indicating the direction of the trend. OVA challenge alone led to a significant ( $p < .05$ ) increase in macrophages, eosinophils, and lymphocytes when compared to the no-OVA exposure groups.

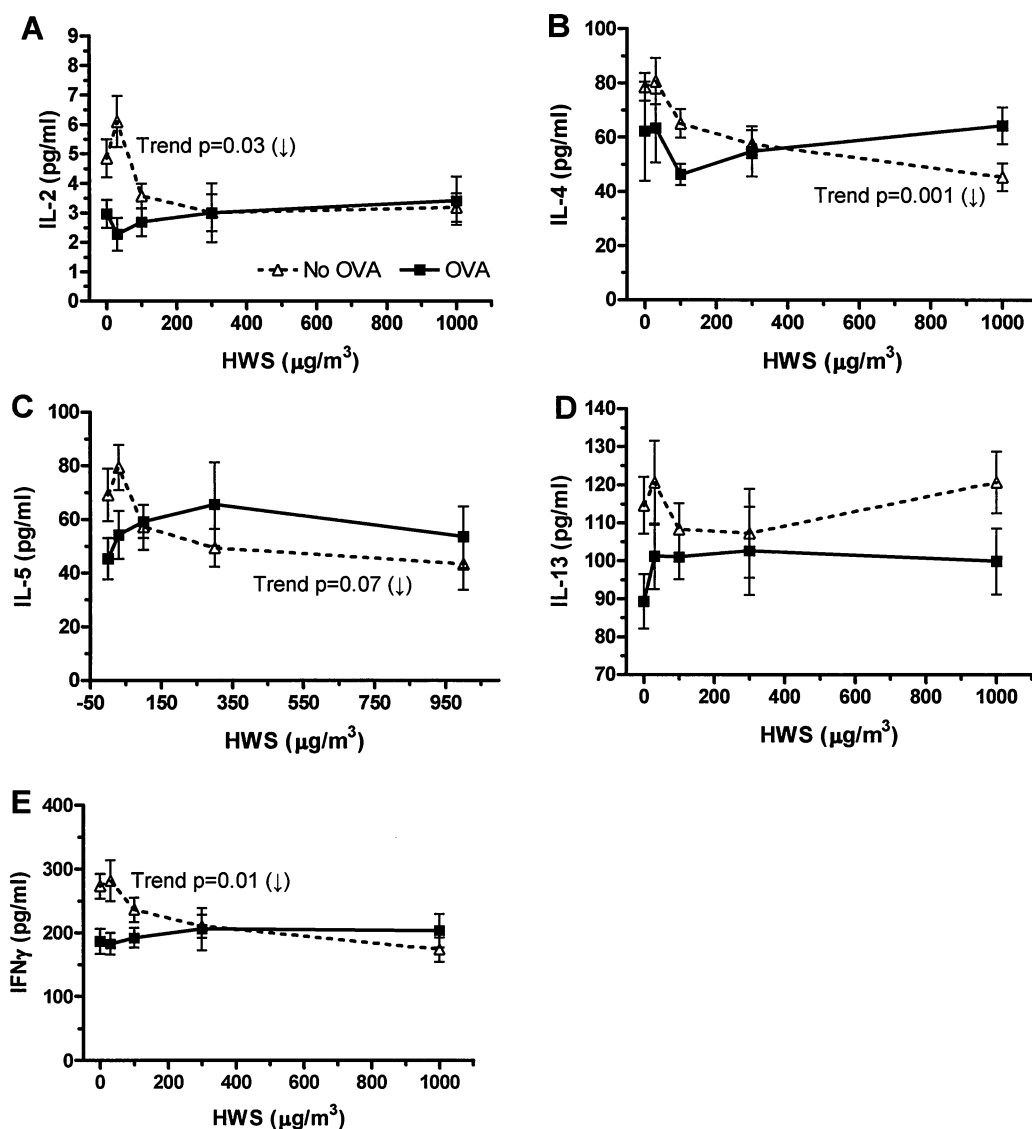


FIG. 3. Cytokine levels in bronchoalveolar lavage fluid following post-OVA exposure protocol: (A) IL-2, (B) IL-4, (C) IL-5, (D) IL-13, and (E) IFN $\gamma$ . Results of linear trend analyses examining dose-dependent trends are indicated below each treatment group with an arrow indicating the direction of the trend.

IgE, or OVA-specific IgG<sub>2a</sub> in OVA challenged mice (Figure 8). There was an increasing trend in serum OVA-specific IgG<sub>1</sub> levels with HWS exposure. Histologic assessment of lung tissue indicated that OVA exposure alone led to mild peribronchiolar and perivascular inflammation comprised primarily of eosinophils with some neutrophils and lymphocytes present (Figure 9). There was no discernable difference in tissue inflammation as a result of HWS exposure.

## DISCUSSION

We demonstrate herein that short-term exposure to HWS can minimally exacerbate some indices (e.g., BAL eosinophils and serum OVA-specific IgE) of OVA-induced airway inflammation

when the final OVA challenge precedes HWS exposure (post-OVA protocol). The same endpoints did not reach the level of statistical significance in the pre-OVA protocol. In the pre-OVA protocol, animals were initially challenged with OVA in order to establish inflammation in the lung and then rested for 11 days in order to allow the initial inflammatory response to subside prior to HWS exposure and final OVA challenge. Evidence suggests that inflammatory events (i.e., increased inflammatory cells) can last up to 21 days in the lung following a single acute OVA challenge; however, the peak response occurs ~48–72 h after challenge (Ohkawara et al., 1997). Cytokine levels in the BAL fluid peak at ~24 h after a single OVA challenge and return to background levels by 5 days after challenge (Ohkawara et al.,

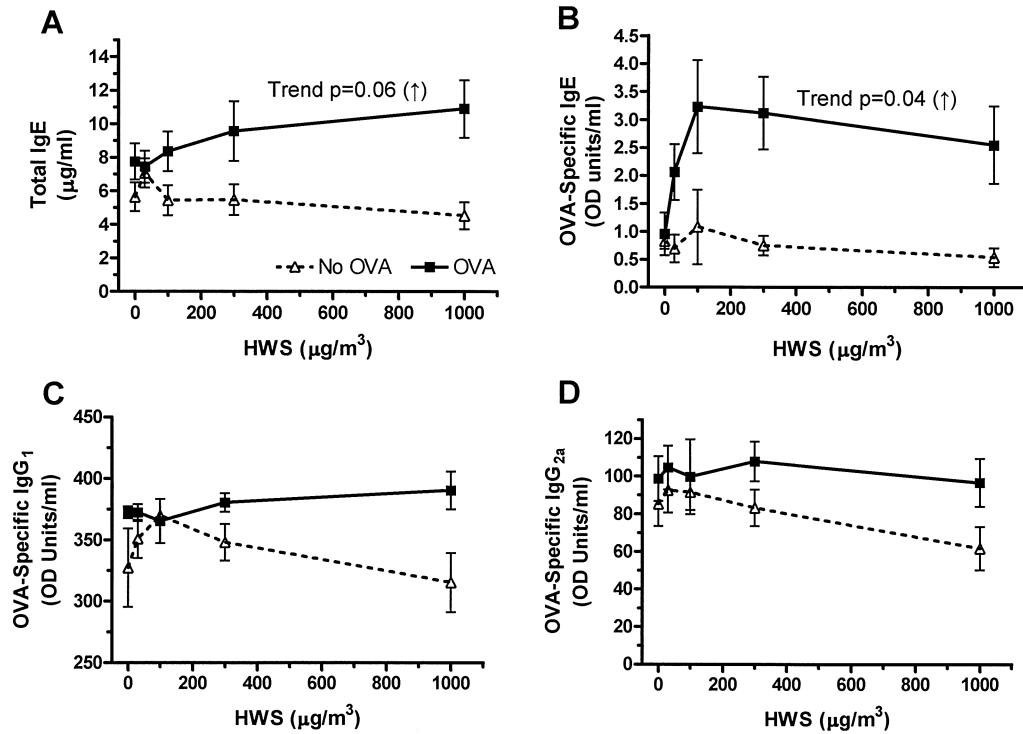


FIG. 4. Serum antibody levels following post-OVA exposure protocol: (A) total IgE, (B) OVA-specific IgE, (C) OVA-specific IgG<sub>1</sub>, and (D) OVA-specific IgG<sub>2a</sub>. Results of linear trend analyses examining dose-dependent trends are indicated below each treatment group with an arrow indicating the direction of the trend.

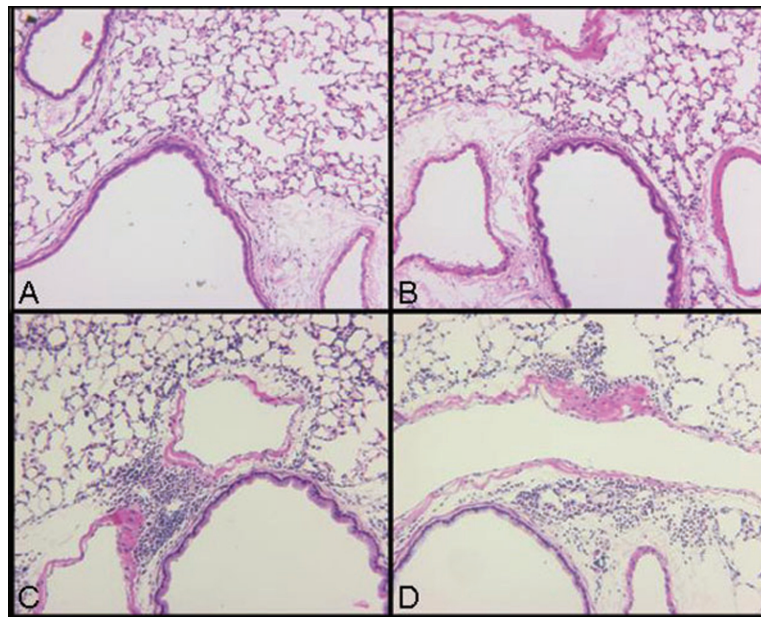


FIG. 5. Representative photomicrographs from mice exposed to (A) air, (B) air + 1000  $\mu\text{g}/\text{m}^3$  HWS, (C) OVA, and (D) OVA + 1000  $\mu\text{g}/\text{m}^3$  HWS following the post-OVA exposure protocol. Histologic analysis of lung tissue indicates that increased inflammation in OVA-exposed animals was primarily due to increased peribronchiolar and perivascular inflammatory cell infiltrates. HWS exposure did not further enhance tissue inflammation. Magnification = 200 $\times$ .

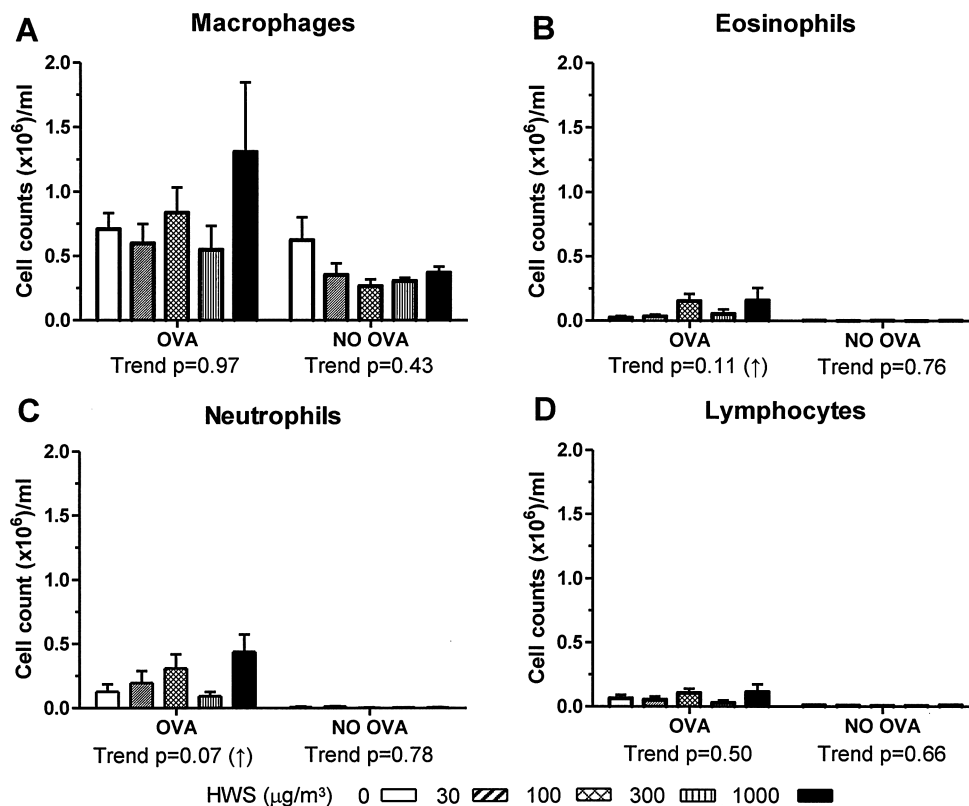


FIG. 6. Bronchoalveolar lavage cell numbers following pre-OVA exposure protocol: (A) macrophages, (B) eosinophils, (C) neutrophils, and (D) lymphocytes. OVA challenge alone led to a significant ( $p < .05$ ) increase in eosinophils, neutrophils, and lymphocytes when compared to the no-OVA exposure groups.

1997). The greater increase in BAL neutrophils in the pre-OVA protocol and conversely the larger increase in eosinophils in the post OVA protocol were likely due to the timing of the animal sacrifice after OVA challenge. Following acute (single) OVA exposure, BAL neutrophils typically peak at 24–48 h and eosinophils peak at 48–72 h (Ohkawara et al., 1997). Thus, while both exposure protocols (post and pre) used in this study presumably had established inflammation in the lung at the time of HWS exposure, the degree of inflammation in the pre-OVA protocol was likely less. This may explain the lack of a significant exposure effect following HWS in the pre-OVA exposure protocol.

The small increase in BAL eosinophils was not associated with any discernable differences in lung tissue inflammation as determined by histopathological assessment or significant increases in the Th1 (IL-2 and IFN $\gamma$ ) or Th2 (IL-4 and IL-13) cytokines measured following hardwood smoke exposure. Further, while BAL fluid IL-5 levels tended to increase following HWS exposure, they did not reach statistical significance. Increased levels of IL-5 in the lung are associated with the enhancement of differentiation, recruitment, and activation of eosinophils (Adamko et al., 2004). Significant increases in BAL eosinophils and IL-5 levels are associated with allergen–diesel engine exhaust exposure, although at high levels (3 mg/m $^3$ )

(Miyabara et al., 1998). However, other experimental allergen–diesel engine exhaust studies performed at lower exposure levels (0.3 and 1 mg/m $^3$ ) have produced changes in BAL eosinophils, lung histopathology, and BAL fluid IL-5 levels similar to those in the present HWS study (Takano et al., 1998).

HWS exposure did not induce lung inflammation in animals that were not challenged with OVA (no-OVA groups). Further, despite being sensitized and having the presence of serum OVA-specific IgE and IgG, mice in the no-OVA groups did not exhibit increased serum antibody levels following HWS exposure. Mice similarly sensitized to OVA (e.g., with no inhalation exposure) and exposed to diesel exhaust particulate matter also show no change in serum antibody levels (Hao et al., 2003). In contrast, those mice that were sensitized, challenged with OVA (post OVA), and exposed to HWS showed an increasing linear trend for serum OVA-specific IgE. The absence of an increase in serum OVA-specific IgE in the pre-OVA exposure protocol may have been due to similar protocol differences (e.g., time of sacrifice) that resulted in different BAL eosinophil numbers. Interestingly, in a similar OVA sensitization–exposure protocol (e.g., post-OVA) with exposure to OVA–diesel exhaust PM (2 mg/m $^3$ ) there was no subsequent increase in serum OVA-specific IgE despite the existence of significant pulmonary allergic inflammation (Hao et al., 2003).

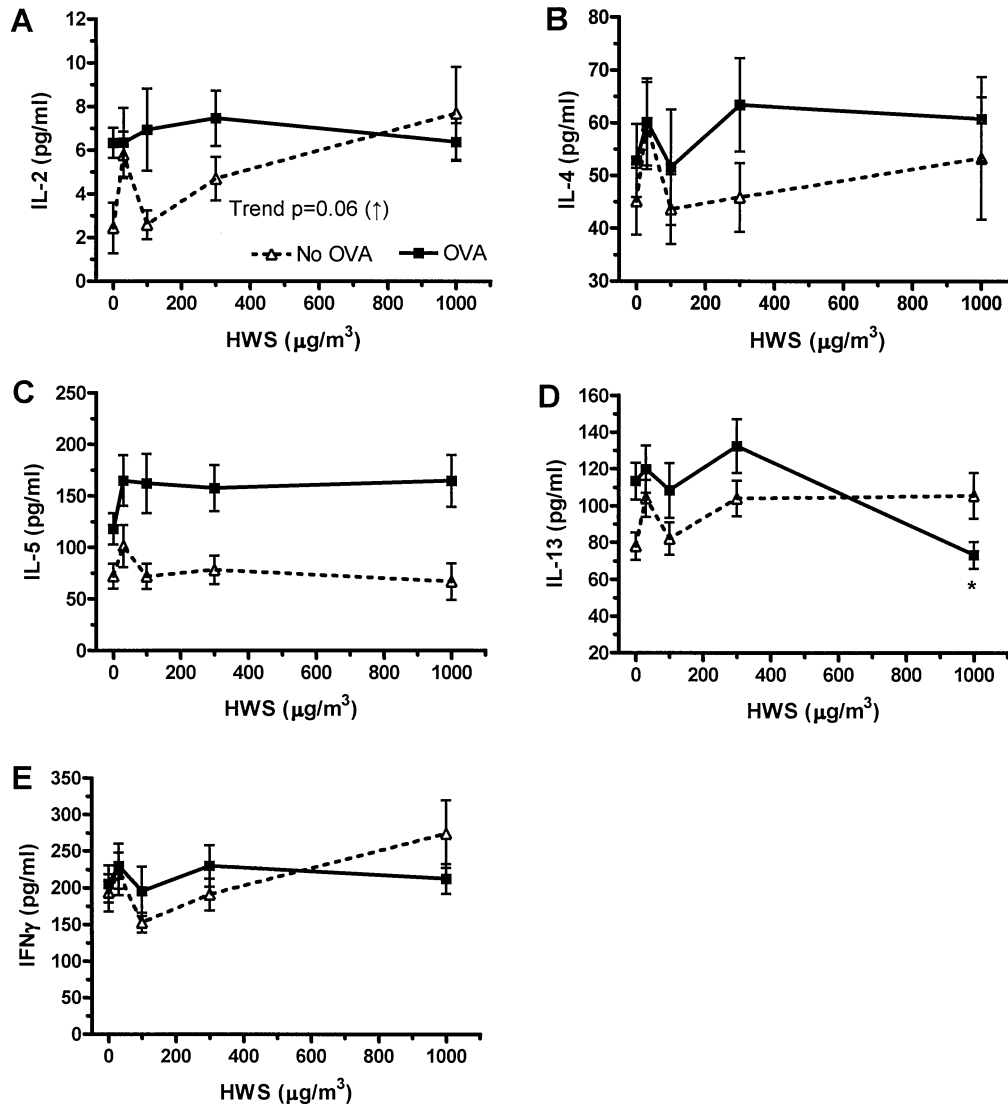


FIG. 7. Cytokine levels in bronchoalveolar lavage fluid following pre-OVA exposure protocol: (A) IL-2, (B) IL-4, (C) IL-5, (D) IL-13, and (E) IFN $\gamma$ . Asterisk indicates significant at  $p < .05$  vs.  $0 \mu\text{g}/\text{m}^3$ .

HWS exposure led to a significant decreasing trend in BALF cytokine (IL-2, IL-4, and IFN $\gamma$ ) levels in the no-OVA groups from the post-OVA protocol. However, these trends were not observed in the no-OVA groups in the pre-OVA protocol. The primary difference between the pre and post protocols with respect to the no-OVA groups is the time of HWS exposure after the first OVA immunization, 26 or 15 days, respectively. Melgert and colleagues have shown a reduction in allergic airway responses (e.g., reduction in lung eosinophilia and Penh response) in OVA sensitized-challenged mice following mainstream cigarette smoke exposure (Melgert et al., 2004). However, there was no effect on BAL cytokine levels or serum antibody levels, and OVA-sensitized nonchallenged mice were not included in the study. IL-13 levels were decreased at the highest HWS exposure level in OVA-challenged (pre-OVA protocol) mice, with no evidence of an exposure-dependent response.

The HWS exposure concentrations used in this study represent a range of exposure levels that may be experienced in the indoor or outdoor environment. Concentrations of PM as great as  $820 \mu\text{g}/\text{m}^3$  over a 24-h period have been measured indoors where a non-airtight wood stove is located (Traynor et al., 1987). The lower exposure concentrations are in a range that may be experienced in areas, such as Washington state, where wood burning can contribute to up to 90% of the ambient PM (Larson & Koenig, 1994; Muhlbaier Dasch, 1982). The composition of the exposures in the current studies is consistent with that of previous wood smoke exposures generated in the laboratory setting (Tesfaigzi et al., 2002).

In the present studies, short-term exposure to HWS was associated with an increase in OVA-induced BAL eosinophils and serum IgE levels when allergen challenge immediately ( $\sim 24$  h) preceded HWS exposure. However, HWS exposure in the

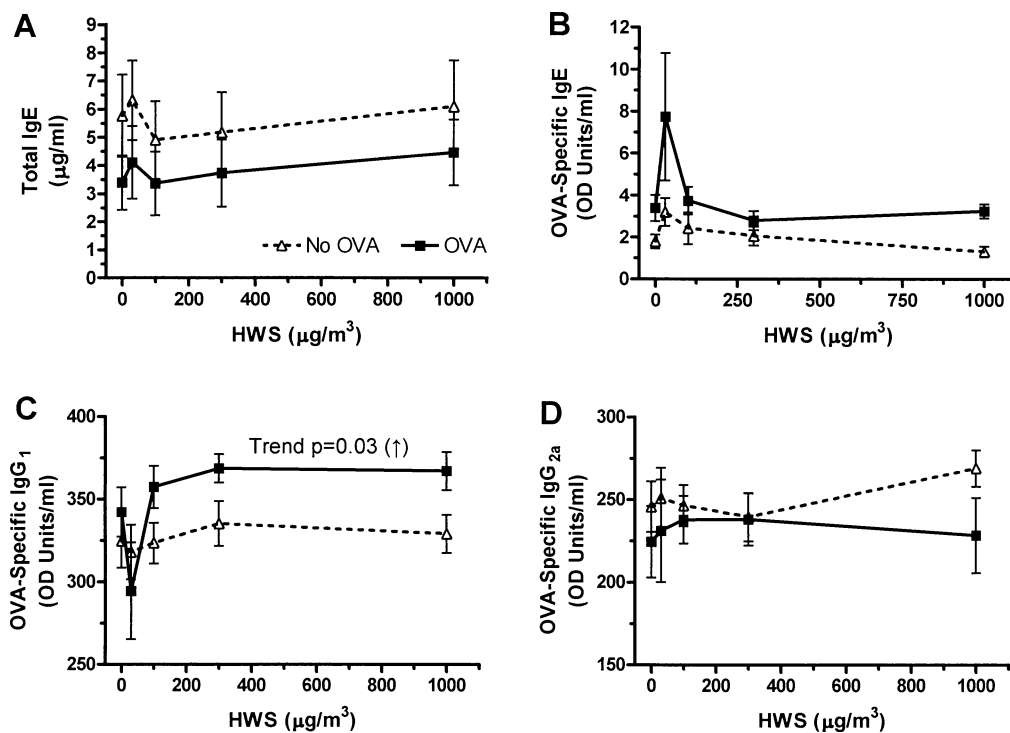


FIG. 8. Serum antibody levels following pre-OVA exposure protocol: (A) total IgE, (B) OVA-specific IgE, (C) OVA-specific IgG<sub>1</sub>, and (D) OVA-specific IgG<sub>2a</sub>.

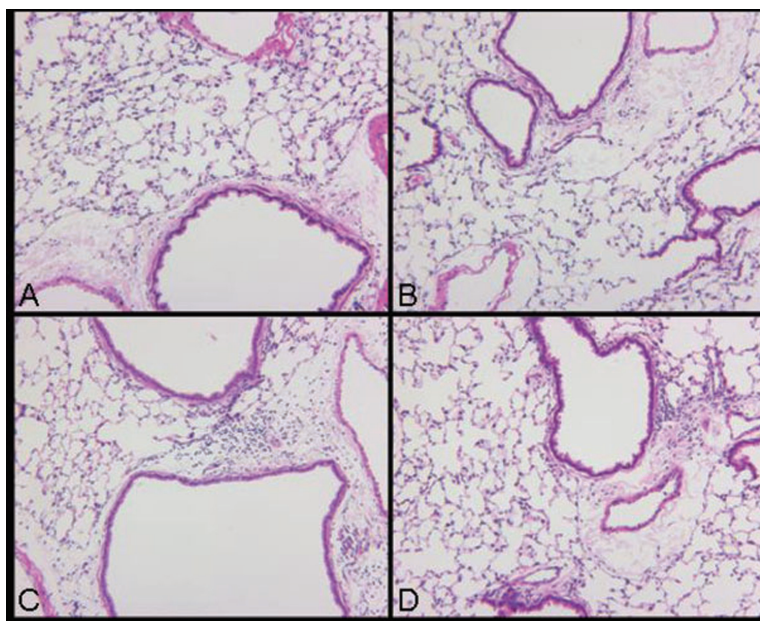


FIG. 9. Representative photomicrographs from mice exposed to (A) air, (B) air + 1000  $\mu\text{g}/\text{m}^3$  HWS, (C) OVA, and (D) OVA + 1000  $\mu\text{g}/\text{m}^3$  HWS following the pre-OVA exposure protocol. Histologic analysis of lung tissue indicates that increased inflammation in OVA-exposed animals was primarily due to increased peribronchiolar and perivascular inflammatory cell infiltrates. HWS exposure did not further enhance tissue inflammation. Magnification  $p = 200\times$ .

presence of allergen-specific serum antibodies alone (e.g., absence of allergen challenge) was not sufficient to exacerbate allergic responses and in fact reduced both BALF Th1 and Th2 cytokine levels. While the effects of HWS exposure were relatively mild, they were comparable to responses observed with other ambient pollutants, such diesel exhaust, in model systems with similar exposure paradigms (Hao et al., 2003; Takano et al., 1998).

## REFERENCES

- Adamko, D., Lacy, P., and Moqbel, R. 2004. Eosinophil function in allergic inflammation: from bone marrow to tissue response. *Curr. Allergy Asthma Rep.* 4:149–158.
- Boman, B. C., Forsberg, A. B., and Jarvholm, B. G. 2003. Adverse health effects from ambient air pollution in relation to residential wood combustion in modern society. *Scand. J. Work Environ. Health* 29:251–260.
- Demarest, G. B., Hudson, L. D., and Altman, L. C. 1979. Impaired alveolar macrophage chemotaxis in patients with acute smoke inhalation. *Am. Rev. Respir. Dis.* 119:279–286.
- Dunnett, C. W. 1955. A multiple comparison procedure for comparing several treatments with a control. *J. Am. Stat. Assoc.* 50:1096–1121.
- Dunnett, C. W. 1980. Pairwise multiple comparisons in the unequal variance case. *J. Am. Stat. Assoc.* 75:796–800.
- Fick, R. B., Jr., Paul, E. S., Merrill, W. W., Reynolds, H. Y., and Loke, J. S. 1984. Alterations in the antibacterial properties of rabbit pulmonary macrophages exposed to wood smoke. *Am. Rev. Respir. Dis.* 129:76–81.
- Hao, M., Comier, S., Wang, M., Lee, J. J., and Nel, A. 2003. Diesel exhaust particles exert acute effects on airway inflammation and function in murine allergen provocation models. *J. Allergy Clin. Immunol.* 112:905–914.
- Hsu, T. H., and Kou, Y. R. 2001. Airway hyperresponsiveness to bronchoconstrictor challenge after wood smoke exposure in guinea pigs. *Life Sci.* 68:2945–2956.
- Koenig, J. Q., Larson, T. V., Hanley, Q. S., Rebolledo, V., Dumler, K., Checkoway, H., Wang, S. Z., Lin, D., and Pierson, W. E. 1993. Pulmonary function changes in children associated with fine particulate matter. *Environ. Res.* 63:26–38.
- Larson, T. V., and Koenig, J. Q. 1994. Wood smoke: Emissions and noncancer respiratory effects. *Annu. Rev. Public Health* 15:133–156.
- Lipsett, M., Hurley, S., and Ostro, B. 1997. Air pollution and emergency room visits for asthma in Santa Clara County, California. *Environ. Health Perspect.* 105:216–222.
- Loke, J., Paul, E., Virgulto, J. A., and Smith, G. J. 1984. Rabbit lung after acute smoke inhalation. Cellular responses and scanning electron microscopy. *Arch. Surg.* 119:956–959.
- McDonald, J. D., Barr, E. B., White, R. K., Chow, J. C., Schauer, J. J., Zielinska, B., and Grosjean, E. 2004. Generation and characterization of four dilutions of diesel engine exhaust for a subchronic inhalation study. *Environ. Sci. Technol.* 38:2513–2522.
- Melgert, B. N., Postma, D. S., Geerlings, M., Luinge, M. A., Kiok, P. A., van der Strate, B. W. A., Kersjens, H. A. M., Timens, W., and Hylkema, M. N. 2004. Short-term smoke exposure attenuates ovalbumin-induced airway inflammation in allergic mice. *Am. J. Respir. Cell Mol. Biol.* 30:880–885.
- Miyabara, Y., Takano, H., Ichinose, T., Lim, H. B., and Sagai, M. 1998. Diesel exhaust enhances allergic airway inflammation and hyperresponsiveness in mice. *Am. J. Respir. Crit. Care Med.* 157:1138–1144.
- Muhlbaier Dasch, J. 1982. Particulate and gaseous emissions from wood-burning fireplaces. *Environ. Sci. Technol.* 16:639–645.
- Norris, G., YoungPong, S. N., Koenig, J. Q., Larson, T. V., Sheppard, L., and Stout, J. W. 1999. An association between fine particles and asthma emergency department visits for children in Seattle. *Environ. Health Perspect.* 107:489–493.
- Ohkawara, Y., Lei, X. F., Stampfli, M. R., Marshall, J. S., Xing, Z., and Jordana, M. 1997. Cytokine and eosinophil responses in the lung, peripheral blood, and bone marrow compartments in a murine model of allergen-induced airways inflammation. *Am. J. Respir. Cell Mol. Biol.* 16:510–520.
- Schwartz, J., Slater, D., Larson, T. V., Pierson, W. E., and Koenig, J. Q. 1993. Particulate air pollution and hospital emergency room visits for asthma in Seattle. *Am. Rev. Respir. Dis.* 147:826–831.
- Sheppard, L., Levy, D., Norris, G., Larson, T. V., and Koenig, J. Q. 1999. Effects of ambient pollution on nonelderly asthma hospital admissions in Seattle, Washington, 1987–1994. *Epidemiology* 10:23–30.
- Takano, H., Ichinose, T., Miyabara, Y., Shibuya, T., Lim, H. B., Yoshikawa, T., and Sagai, M. 1998. Inhalation of diesel exhaust enhances allergen-related eosinophil recruitment and airway hyperresponsiveness in mice. *Toxicol. Appl. Pharmacol.* 150:328–337.
- Tesfaigzi, Y., Singh, S. P., Foster, J. E., Kubatko, J., Barr, E. B., Fine, P. M., McDonald, J. D., Hahn, F. F., and Mauderly, J. L. 2002. Health effects of subchronic exposure to low levels of wood smoke in rats. *Toxicol. Sci.* 65:115–125.
- Traynor, G. W., Apte, M. G., Carruthers, A. R., Dillworth, J. F., Grimrud, D. T., and Gundel, L. A. 1987. Indoor air pollution due to emissions from wood-burning stoves. *Environ. Sci. Technol.* 21:691–697.
- Yu, O., Sheppard, L., Lumley, T., Koenig, J. Q., and Shapiro, G. G. 2000. Effects of ambient air pollution on symptoms of asthma in Seattle-area children enrolled in the CAMP study. *Environ. Health Perspect.* 108:1209–1214.
- Zelikoff, J. T., Chen, L. C., Cohen, M. D., and Schlesinger, R. B. 2002. The toxicology of inhaled woodsmoke. *J. Toxicol. Environ. Health B. Crit. Rev.* 5:269–282.