# Ambient Urban Baltimore Particulate-induced Airway Hyperresponsiveness and Inflammation in Mice

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**Airborne particulate matter (PM) is hypothesized to play a role in increases in asthma prevalence, although a causal relationship has yet to be established. To investigate the effects of real-world PM exposure on airway reactivity (AHR) and bronchoalveolar lavage (BAL) cellularity, we exposed naive mice to a single dose (0.5 mg/mouse) of ambient PM, coal fly ash, or diesel PM. We found that ambient PM exposure induced increases in AHR and BAL cellularity, whereas diesel PM induced significant increases in BAL cellularity, but not AHR. On the other hand, coal fly ash exposure did not elicit significant changes in either of these parameters. We further examined ambient PM-induced temporal changes in AHR, BAL cells, and lung cytokine levels over a 2-wk period. Ambient PM-induced AHR was sustained over 7 d. The increase in AHR was preceded by dramatic increases in BAL eosinophils, whereas a decline in AHR was associated with increases in macrophages. A Th2 cytokine pattern (IL-5, IL-13, eotaxin) was observed early on with a shift toward a Th1 pattern (IFN-γ). In additional studies, we found that the active component(s) of ambient PM are not water-soluble and that ambient PM-induced AHR and inflammation are dose-dependent. We conclude that ambient PM can induce asthmalike parameters in naive mice, suggesting that PM exposure may be an important factor in increases in asthma prevalence.**

**Keywords:** air pollution; allergy; asthma; inflammation

There has been an alarming increase in the prevalence of asthma over the past several decades (1, 2). Although there is a recognized genetic component of asthma, the continuing increases in prevalence cannot be adequately explained by changes in the gene pool of the population. Therefore, environmental factors are thought to play a role in these observed increases. It has been hypothesized that $PM_{10}$ and $PM_{2.5}$ may contribute to the increasing incidence, morbidity, and mortality of asthma, particularly in urban areas. Episodes of increased PM air pollution have been associated with increases in mortality, hospital admissions, respiratory symptoms, decreases in lung function, and exacerbations of asthma in both adults and children (3, 4). Such epidemiologic evidence clearly indicates that increases in PM levels can exacerbate existing airway disease; however, there are relatively few studies that show an association between PM levels and induction of asthma symptoms. In one of the few studies that have examined this association, Abbey and colleagues (5) found an increase in new cases of asthma associated with increases in long-term levels of total suspended particulate. Despite such studies, establishment of causality in epidemiologic studies is difficult because of confounding copollutants (i.e., ozone, $NO_2$, etc.), emphasizing the need for controlled animal studies.

Recently, various animal models have been employed to examine the relationship between PM (residual oil fly ash [ROFA], diesel exhaust particles) and airway responsiveness and inflammation of the airways (6–11). Although studies using specific sources of particulate air pollution (i.e., ROFA, diesel PM) may help elucidate mechanisms by which various compounds elicit their effects, they do not address the complex mixture of substances found in ambient PM.

To investigate a possible causal relationship between PM exposure and induction of asthmalike symptoms, we have examined the dose and time relationship between exposure of naive mice to ambient particulate collected in urban Baltimore and development of asthmalike airway responses.

## METHODS

### Animals

Specific pathogen-free, male A/J mice 6 to 7 wk of age (NCI, Frederick, MD) were housed in laminar flow hoods in an environmentally controlled animal facility for the duration of the experiment. The studies reported here conformed to the principles for laboratory animal research outlined by the Animal Welfare Act and the Department of Health, Education, and Welfare (National Institutes of Health) guidelines for the experimental use of animals.

### Particulate Matter

Ambient PM was collected from a sixth floor window in urban Baltimore using a high-volume cyclone collector with a theoretical cut point of 0.85 μm aerodynamic diameter when operated at a flow rate of 0.6 $m^3$/min (12). The cyclone was intermittently operated over a period of months at a flow rate of 0.6 $m^3$/min. Collected PM was pooled and refrigerated until use.

Coal fly ash was obtained from a local Baltimore power plant burning bituminous coal. A reference source of bituminous coal fly ash (SRM1633b) was purchased from the National Institute of Standards and Technology (Gaithersburg, MD). Diesel PM (1650a) was also purchased from the National Institute of Standards and Technology.

The particle size distributions of ambient PM, local coal fly ash, and reference coal fly ash were determined using phase contrast light microscopy at a magnification of ×400. Particles were counted and sized according to the method of Hinds (13). The count median diameter (CMD) of ambient PM was 1.78 μm with a geometric standard deviation (GSD) of 2.21. CMD (GSD) of local and reference coal fly ash were 1.85 μm (2.23) and 1.57 μm (1.99), respectively. The mean diameter of the number distribution for diesel PM was reported as 1.55 ± 0.04 μm in a certificate of analysis accompanying the product.

Total protein in the ambient PM sample was measured using Coomassie Plus Total Protein reagent (Pierce Chemical, Rockford, IL) according to the manufacturer's recommendations. Endotoxin content was measured semiquantitatively using a Limulus Amebocyte Lysate agglutination assay kit (BioWhittaker, Inc., Walkersville, MD).

Metal and organic components in ambient PM were analyzed by Research Triangle Institute (Research Triangle Park, NC). Metal content was measured using inductively coupled plasma mass spectrometry. Organic components were analyzed using gas chromatographic mass spectrometry. A report of metal content of the reference coal fly ash (SRM1633b) was provided with the product, and a report of organic compounds was provided with the diesel PM (SRM 1650a).

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### Particulate Exposure

To ensure equal exposure, PM was delivered via aspiration challenge as previously described by Wills-Karp and colleagues (14). Briefly, anesthetized mice (45 mg/kg ketamine and 8 mg/kg xylazine) were suspended on a 60-degree incline board. With the tongue gently extended, a 50 μl aliquot of PM suspended in PBS (10 mg/ml) placed in the back of the oral cavity was aspirated by the animal. Using this technique, approximately 80% of 0.5 mg PM delivered is deposited in the lungs of each mouse (15).

### Leaching and Washing Ambient PM

An aqueous leachate was prepared by incubating 10 mg/ml ambient PM in PBS at 37° C for 4 h. The suspension was then centrifuged (21,000 × *g* for 10 min) to remove the particulate from suspension. The supernatant was filtered through a 0.2-μm filter (Acrodisc Syringe Filters; Pall Corp., Ann Arbor, MI) to produce the leachate. The remaining pellet was resuspended in 1 ml fresh PBS and centrifuged again. The supernatant from this wash was discarded and the pellet was resuspended with 1 ml fresh PBS; 50 μl of leachate or washed PM were delivered to mice via aspiration as described previously.

### Airway Responsiveness Measurements

Airway responsiveness to intravenously administered acetylcholine (ACh) was assessed as previously described (16). Briefly, mice were anesthetized (80 to 90 mg/kg sodium pentobarbital), cannulated, and ventilated at 120 breaths/min with a tidal volume of 0.2 ml. The mice were then paralyzed with decamethonium bromide (25 mg/kg). ACh (50 μg/kg) was injected into the inferior vena cava and changes in airway pressure were recorded.

### Bronchoalveolar Lavage

Immediately after AHR measurements, mice were exsanguinated and the lungs were lavaged three times with a single 1.0-ml aliquot of cold Hanks' balanced salt solution (Biofluids, Rockville, MD). Recovered lavage fluid (70 to 80%) was centrifuged (300 × *g* for 8 min) and the supernatant was stored at −80° C for later measurement of cytokine protein levels. The cell pellet was resuspended in 1.0 ml of 10% fetal bovine serum in PBS. Total cells were counted with a hemacytometer. Slides were prepared by cytocentrifugation (Cytospin 3; Shandon Instruments, Pittsburgh, PA), and stained with Diff-Quik (Dade Behring, Düdingen, Switzerland). BAL cell differential counts were determined using morphologic criteria under a light microscope with evaluation of ⩾ 500 cells/slide.

### Quantitation of Cytokine Protein Levels in BAL Fluid

Interleukin-13 (IL-13), interleukin-5 (IL-5), eotaxin, and interferon-gamma (IFN-γ) protein levels were measured in unconcentrated BAL fluid by enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's recommendations. Optical density (OD) readings of samples were converted to picograms per milliliter using values obtained from standard curves generated with serial dilutions of each recombinant cytokine (15 to 1,000 pg/ml).

### Data Analysis

One-way analysis of variance (ANOVA) was used to determine differences between groups with *post hoc* comparisons using Fisher's method. Significance was assumed at $p < 0.05$.

## RESULTS

### PM Characterization

A metal analysis of ambient PM and reference coal fly ash (Table 1) reveals that the most abundant metals found in ambient Baltimore PM were iron, aluminum, titanium, magnesium, copper, manganese, and zinc. When metal content in the ambient PM was compared with that found in reference coal fly ash, we noted dramatically higher concentrations of copper, manganese, and zinc in ambient PM. No metal analysis was available for the local coal fly ash or diesel PM. Organic components in highest concentration in ambient PM included fluoranthene, pyrene, and acenaphthalene; however, all organic components measured in diesel PM were present in higher concentrations than in ambient PM, with the exception of ideno[1234c,d]pyrene (Table 2). The ambient particulate was also analyzed for total protein and endotoxin content. It was found to contain approximately 150 μg/ml protein and < 10 EU/ml endotoxin, resulting in 7.5 μg protein/mouse, and < 0.5 EU/mouse, respectively. Although endotoxin was detected in the sample (< 10 EU/ml), it did not likely play a major role in the responses seen since C3H/HeJ mice, an endotoxin-resistant strain, exhibited responses similar to those of the endotoxin-sensitive A/J strain (unpublished data).

**TABLE 1. METAL CONCENTRATIONS (μg/g DUST) IN AUB AND SRM 1633b**

| Metal | AUB | SRM 1633b |
|---|---|---|
| Aluminum | 3,782.000 | 150,500.000 |
| Antimony | 35.370 | 6.000 |
| Arsenic | 6.759 | 136.200 |
| Barium | 299.600 | 709.000 |
| Beryllium | 0.295 | . |
| Cadmium | 4.079 | 0.784 |
| Chromium | 130.700 | 198.200 |
| Cobalt | 9.252 | 50.000 |
| Copper | 1,444.000 | 112.800 |
| Europium | 0.193 | 4.100 |
| Holmium | 0.131 | 3.500 |
| Iron | 21,600.000 | 77,800.000 |
| Lanthanum | 8.046 | 9.400 |
| Lead | 231.000 | 68.200 |
| Lithium | 4.280 | . |
| Magnesium | 2,310.000 | 4,820.000 |
| Manganese | 1,220.000 | 131.800 |
| Molybdenum | 16.690 | 85.000 |
| Nickel | 67.560 | 10.260 |
| Scandium | NA | 41.000 |
| Selenium | 4.979 | 10.260 |
| Silver | 0.709 | . |
| Strontium | 100.100 | 1,041.000 |
| Thalllium | 0.384 | 5.900 |
| Thorium | 0.876 | 25.700 |
| Titanium | 2,755.000 | 7,910.000 |
| Uranium | 1.342 | 8.790 |
| Vanadium | 84.420 | 295.700 |
| Ytterbium | 0.464 | 7.600 |
| Zinc | 1,088.000 | 210.000 |

### PM Comparison

In an initial experiment, we compared the effects of ambient PM collected in Baltimore city, diesel PM, and local and reference sources of coal fly ash on airway responsiveness with intravenously administered ACh 48 h after exposure in naive A/J mice. We found that exposure to ambient PM induced a marked increase in AHR when compared with PBS-treated controls (810 ± 67 versus 530 ± 41 cm $H_2O$/s). Surprisingly, neither source of coal fly ash induced any change in AHR (local: 421 ± 94; reference: 439 ± 83 cm $H_2O$/s), whereas diesel PM induced only a small, insignificant increase in AHR (590 ± 37). Furthermore, ambient PM induced a greater degree of eosinophilic and neutrophilic inflammation than did either source of coal fly ash. Although all sources of PM induced increases in the number of eosinophils and neutrophils recovered from BAL fluid, only ambient PM significantly increased eosinophil numbers compared with PBS controls ($3.69 \pm 1.26 \times 10^4$ versus $0.01 \pm 0.01 \times 10^4$).

**TABLE 2. ORGANIC COMPONENTS (ng/g) IN AMBIENT URBAN BALTIMORE PM OR DIESEL PARTICULATE MATTER**

| Compound | AUB | DPM |
|---|---|---|
| Acenaphthalene | 2,238.7 | . |
| Anthracene | 161.3 | 1,500 |
| Anthracene, 2-methyl | < LOD | . |
| Benzo(a)anthracene | 1,200.0 | 6,300 |
| Benzo(a)pyrene | 848.0 | 1,330 |
| Benzo(b)flouroanthene | 1,556.5 | 8,810 |
| Benzo(e)pyrene | 1,170.5 | 7,440 |
| Benzo(ghi)perylene | 735.5 | 6,500 |
| Benzo(j)flouroanthene | 1,557.0 | 3,520 |
| Benzo(k)flouroanthene | 650.0 | 2,640 |
| Chrysene | 2,168.5 | 14,500 |
| Cyclopenta[c,d]pyrene | 242.0 | |
| Flouranthene | 6,514.6 | 49,900 |
| Flourene | 67.0 | . |
| Indeno[1234c,d]pyrene | 696.5 | 316 |
| Methyl phenanthrene | < LOD | 34,000 |
| Methylnaphthalene | < LOD | . |
| Naphthalene | 277.0 | . |
| Phenanthrene | 1,596.5 | 68,400 |
| Pyrene | 2,886.2 | 47,500 |
| Retene | 227.5 | . |

### Time Course of Airway Responses

On the basis of the observation that ambient PM, but not diesel PM or coal fly ash, induced AHR in our initial study, we subsequently assessed the kinetics of airway responses to ambient PM exposure. Measurements of AHR and BAL cellularity were conducted at intervals for 2 wk after administration of 0.5 mg ambient PM or PBS. The response to ACh was elevated above control values as early as 6 h and reached maximum values from 1 to 3 d after AUB exposure (Figure 1A). This response was sustained for at least 7 d after challenge and only returned to control values by Day 14.

### Kinetics of BAL Cellular Changes

BAL cellularity increased after ambient PM exposure; however, increases in various cell types did not follow the same kinetic pattern as PM-induced AHR (Figure 1B). The number of eosinophils, neutrophils, and epithelial cells in BAL fluid was elevated 6 h after PM exposure and peaked at 12 h, with a time-dependent decline and return to control values thereafter. Interestingly, the peak number of eosinophils in BAL fluid was approximately 10 times greater than neutrophil numbers 12 h after PM aspiration.

In contrast to the trends seen in eosinophils, neutrophils, and epithelial cells, macrophage and lymphocyte numbers increased 2 to 3 d after PM exposure. Macrophage numbers significantly increased 2 to 3 d after challenge and remained elevated above the control range for the duration of the 2-wk period examined. BAL lymphocytes increased after PM exposure in a less consistent manner at 3 and at 7 d after PM challenge.

### BAL Cytokine Levels

We examined the Th2 cytokines IL-13, IL-5, and eotaxin, as well as the Th1 cytokine IFN-$\gamma$ in unconcentrated BAL fluid. A decrease in IL-13 levels in BAL fluid can be seen in Figure 2A beginning 12 h after PM exposure and returning to control values by 7 d with a significant decrease from 1 to 3 d. Although IL-5 levels exhibited a decrease similar to that seen with IL-13 at 1, 2, and 3 d, there is an earlier significant increase in IL-5 at 6 and 12 h (Figure 2B). Likewise, eotaxin levels were significantly increased at 6 h and decreased thereafter, although only significantly so at 2 and 3 d (Figure 2C). In contrast, IFN-$\gamma$ levels were initially depressed, but increased 2 and 3 d after PM challenge, concomitant with the increase in BAL macrophages (Figure 2D).

### Aqueous Leaching of Ambient PM

In order to address the hypothesis that soluble metal content of PM may be responsible for the increases in airway responsiveness and inflammation observed, we exposed mice to leachate or washed particles (*see* METHODS). The leachate did not induce any AHR or inflammation (data not shown). However, washed particles appeared to induce a greater increase in AHR than did unwashed particles (516 $\pm$ 60 versus 389 $\pm$ 42 cm $H_2O$/s, respectively). The washed particles also induced an inflammatory cell profile similar to that seen with unwashed PM with a 20- to 25-fold increase in eosinophils and approximately 2-fold increases in neutrophils and macrophages. Additionally, aqueous leachate did not induce any change in cytokine levels compared with PBS controls, whereas washed PM induced decreases in Th2 cytokine levels similar to those seen after exposure to unwashed particles.

### Ambient PM Dose–Response

In order to address whether the type and degree of lung injury induced by ambient PM are dose-dependent, we conducted a dose-response study ranging from 10 to 1,000 $\mu$g PM/mouse. AHR was significantly increased above PBS control values, beginning at the 50-$\mu$g dose of PM, and continued to increase dose-dependently (Figure 3A).

A dose-dependent increase in BAL cells was also observed (Figure 3B). Although all cell types exhibited some increase in numbers with increasing doses of PM, the trend is most pronounced in eosinophil and neutrophil numbers. Increases in eosinophil and neutrophil numbers become significant at the 250-$\mu$g dose compared with PBS. Macrophage and epithelial cell numbers were significantly increased above those of PBS controls at the 50-$\mu$g dose; however, the trend is not entirely consistent at the higher doses of PM (500 to 1,000 $\mu$g).

## DISCUSSION

In the present study, we show that ambient PM collected in urban Baltimore can induce dramatic and sustained AHR and pulmonary inflammation, as well as local changes in cytokine levels in naive mice. This AHR and inflammation is not seen with exposure to an equivalent mass of either local or a reference source of coal fly ash. The effects of ambient PM do not appear to be associated with the water-soluble fraction at neutral pH but remain with the washed particles. Finally, PM-induced increases in AHR and BAL cellularity are dose-dependent with regard to severity; however, the type of inflammation remains consistent across the two-log range of doses examined.

A single aspiration of 0.5 mg ($\sim$ 20 mg/kg) of ambient Baltimore PM induced striking increases in AHR beginning 6 h after exposure and continuing at least 7 d. The increases in AHR after PM exposure observed here are consistent with the findings of Gavett and colleagues (6) who reported increases in pulmonary opening pressure and expiratory resistance 4 d after a single instillation of ROFA in rats. Additionally, Pritchard and colleagues (17) found that airway resistance increased with exposure to several sources of PM, including ambient particulate as well as ROFA.

Exposure to ambient PM induced a significant influx of inflammatory cells into the murine lung, although the kinetics of individual cell types found in the lavage fluid varied. The early peak in eosinophil number at 12 h preceded the greatest in-

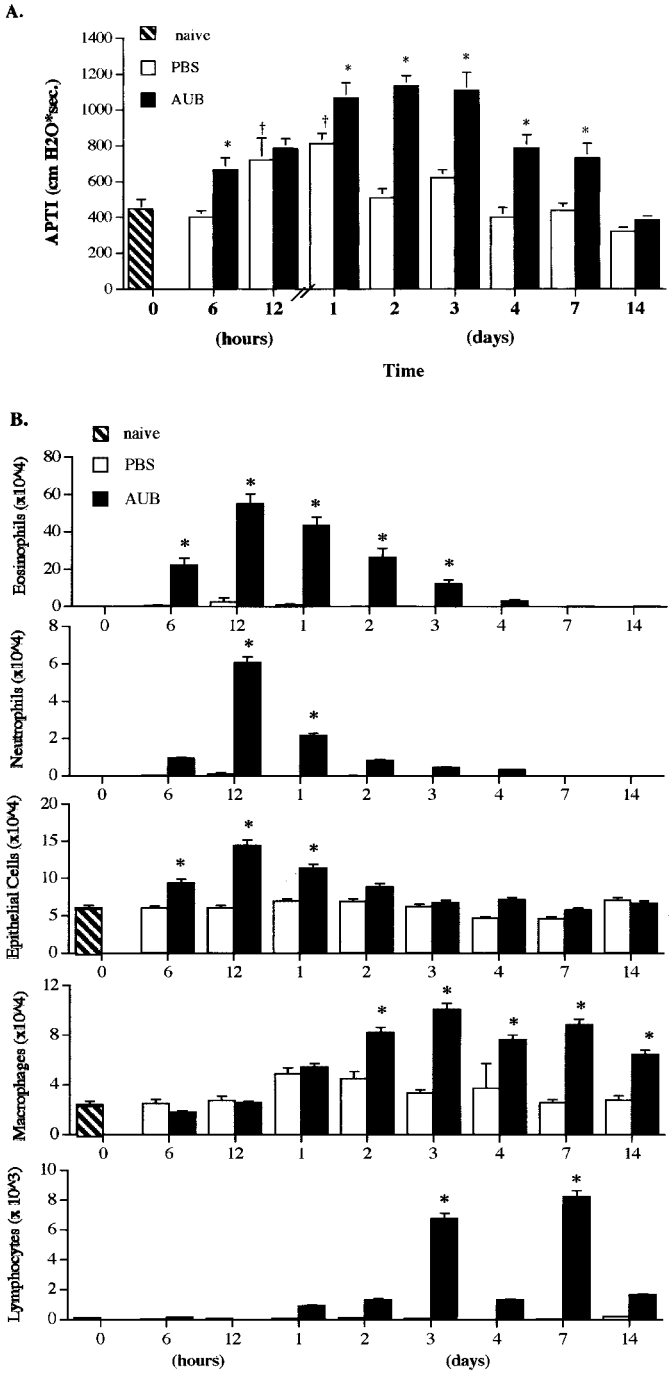


*Figure 1.* (*A*) Airway responsiveness to intravenously administered acetylcholine and (*B*) BAL cells in A/J mice (n = 8 to 12/group). AHR increased at 6 h and remained elevated at least 7 d after exposure to ambient PM. Airway responsiveness is expressed as the time-integrated change in airway pressure over baseline pressure (APTI). PM exposure induced significant increases in all BAL cells, but at different times. Granulocytes and epithelial cell numbers peaked at 12 h and declined temporally, whereas macrophage and lymphocyte numbers increased at later time points. Values are mean ± SEM. *Significant increase over PBS control for each time point. †Significant increase over naive controls ($p < 0.05$).

creases in AHR, suggesting that these cells may play a role in PM-induced AHR. Eosinophilic inflammation is a hallmark of asthma; however, the contribution to disease symptoms is not entirely clear. Although the predominantly eosinophilic inflammation is somewhat surprising, as PM exposure is typically thought to elicit a neutrophilic response, an influx of eosinophils in response to PM exposure has been reported by others (6–8). In fact, Costa and Dreher (8) suggested that the presence of eosinophils may be used as a marker of metal exposure. In the present study, we found a 10-fold greater increase in eosinophils than in neutrophils; however, this degree of eosinophilia may be strain-specific, as A/J mice are known to exhibit a strong allergic phenotype.

In contrast to the early rise in granulocytes, the mononuclear fraction of cells exhibited a delayed increase in numbers that preceded the decreases in AHR from peak values (1 to 3 d). This finding suggests a possible role of these cells in the resolution of AHR. Macrophage number remained elevated from 2 d onward, and PM-containing macrophages are seen throughout the time course in histologic sections. Inability to completely clear the particles may be a result of decreases in macrophage function resulting from particle toxicity or macrophage overloading, which reduces mobility (18). In either case, the inability to clear all PM delivered may have further enhanced or prolonged the effects of ambient PM. Increases in lymphocyte numbers were seen at 3 and at 7 d, despite the fact that naive mice were used. These lymphocytes may be recruited to survey foreign antigens and mount a specific immune response to protect the lung from future exposure; however, we have no information about the subset of lymphocytes present or their activation state.

The shift from a Th2-like response to a Th1-like response in cytokine expression over the time course was consistent with the change in type of inflammation seen. The early peak in eosinophil numbers was preceded by an elevation of IL-5 and eotaxin levels in BAL fluid. IL-5 plays an important role in eosinophil development, maturation, and survival, whereas eotaxin is chemotactic for eosinophils. As levels of these Th2 cytokines decreased over Days 1 to 3, the level of the Th1 cytokine, IFN-γ, increased. IFN-γ is known to activate macrophages, and histologic sections show a change from a predominantly granulocytic inflammation to predominantly monocytic inflammation beginning 2 d after PM exposure.

Ambient PM collected in urban Baltimore contains a variety of both anthropogenic and natural components. Local anthropogenic sources include a variety of combustion products such as coal fly ash from local power plants and diesel exhaust from heavy trucks and buses. Ambient PM had a count median diameter of 1.78 with a geometric standard deviation of 2.21. Other emission-source particulates had similar diameters, indicating that all PM sources used contained a large fraction of particles within the respirable range. Protein was detected in the ambient PM, indicating the presence of biologic material that may include plant debris, allergens, and/or bacteria.

Although the mechanism(s) by which ambient particulates induce AHR are unknown, several groups have suggested that the effects of PM are dependent on metal concentrations (6–11, 17). Transition metals are of particular interest because they have the capacity to participate in electron cycling (19), which may contribute to AHR and pulmonary injury through the production of reactive oxygen species (ROS) by neutrophils, eosinophils, macrophages, and even epithelial cells. A comparison of metal content in ambient Baltimore PM and a reference coal fly ash reveals substantially greater concentrations of the transition metals copper and manganese in the Baltimore PM. The ambient PM is also very high in zinc content compared with coal fly ash. Unlike copper and manganese, zinc does not have multiple valence states and thus does not participate in electron cycling. Gavett and colleagues (6)

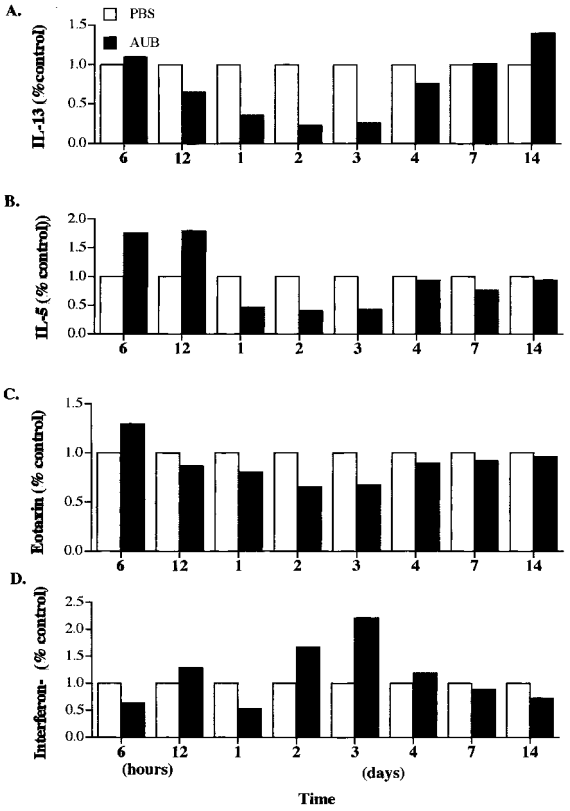


*Figure 2.* (*A*) IL-13, (*B*) IL-5, (*C*) eotaxin, and (*D*) IFN-γ protein levels in unconcentrated BAL fluid from A/J mice (n = 8 to 12/group) were measured by ELISA. All Th2 cytokine levels measured were depressed 1, 2, and 3 d after AUB exposure. IL-5 levels were significantly increased at 6 and 12 h, whereas eotaxin levels were significantly elevated 12 h after AUB exposure. In contrast, IFN-γ levels were significantly elevated only at 3 d. Values are percent of PBS control. *Significant change from PBS control values for time point ($p < 0.05$).

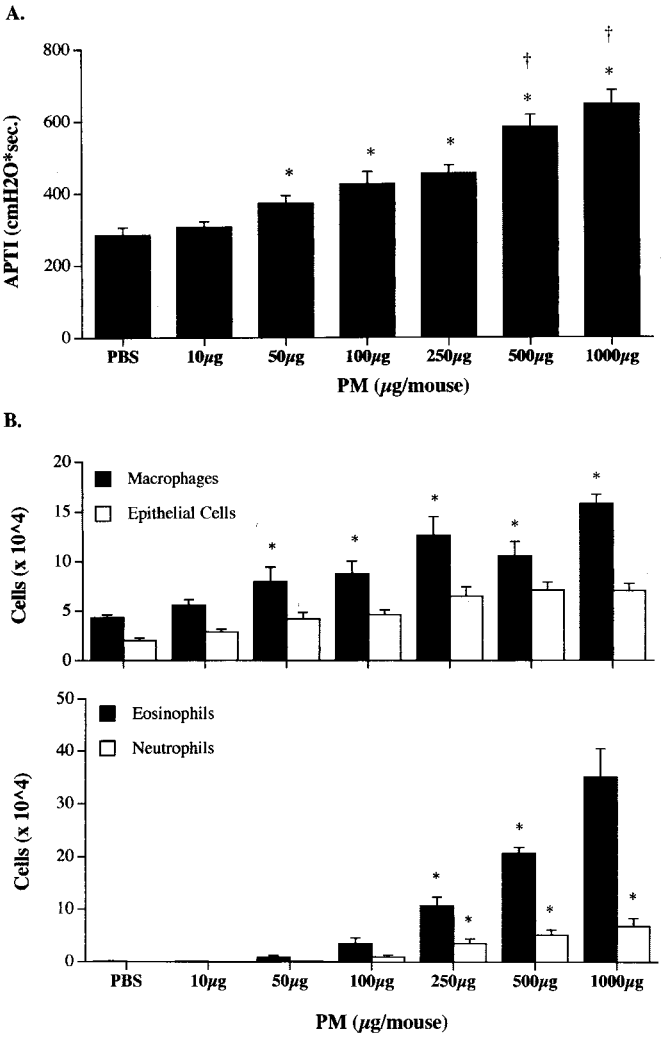


*Figure 3.* Ambient PM dose–response for (*A*) airway responsiveness to intravenously administered acetylcholine and (*B*) differential cells in BAL fluid in A/J mice (n = 6 to 8/dose); 50 μg or greater dose of PM induced a significant increase in AHR and in BAL macrophage and epithelial cell numbers. In contrast, eosinophil and neutrophil numbers exhibited a significant increase over PBS control only at doses of 250 μg and greater. Values are mean ± SEM. *Significant increase over PBS control ($p < 0.05$). †Significant increase compared with 250 μg and lower doses of PM ($p < 0.05$).

found that in rats, ROFA containing high concentrations of zinc induced greater airway hyperresponsiveness and neutrophilic inflammation compared with a sample that contained a low level of zinc, but relatively higher concentrations of the transition metals iron, vanadium, and nickel. However, other studies with ROFA implicate iron and vanadium as potential culprits in the induction of airway injury (7). Our studies of coal fly ash contradict this finding as both iron and vanadium are found in higher concentrations in the reference coal fly ash than in Baltimore PM, yet the fly ash does not induce AHR or as great a degree of inflammation.

The lack of responses after exposure to coal fly ash may be explained by the findings of Costa and Dreher (8) who reported that metals in coal fly ash have low water-solubility. This may also explain the absence of a response to the aqueous leachate of ambient PM. In support of our finding that the active component remains particle-bound, Ghio and colleagues (20) reported that metals associated with the insoluble fraction of ambient PM may also catalyze an oxidative stress and in turn lead to inflammation and AHR.

Another component of Baltimore PM that may contribute to the induction of AHR and inflammation is diesel particles. Several investigators have shown that exposure to diesel PM induces an increase in AHR in mice (11, 21). Diesel PM is thought to act through organic substances such as polyaromatic hydrocarbons (PAHs) bound to the surface of the particle, which can initiate oxidative stress (22) and lead to alterations in normal cell functions. Many PAHs are thought to be immunosuppressive, which may explain the decrease in Th2 cytokines and initial depression of IFN-γ observed. On the other hand, it has been shown that nasal challenge with diesel PM can induce an increase in mRNA levels of cytokines (IFN-γ, IL-2, IL-4, IL-5, IL-6, IL-10, and IL-13) in humans (23). Other studies in both animal models and in humans indicate that diesel PM, when given with allergen or in presensitized subjects, can skew cytokine production toward a Th2 pattern (10, 24) as we observed at 6 and at 12 h after PM challenge.

In conclusion, ambient PM can induce sustained asthma-like parameters of AHR and pulmonary inflammation, which are not seen after exposure to other source-specific particulates, suggesting that such sources may not be adequate surrogates for studies of real-world PM exposures. Future studies will be aimed at determining the mechanism(s) by which ambient PM induces the physiologic and immunologic changes noted here.

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