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Effects of Concentrated Ambient Particles and Diesel Engine Exhaust on Allergic Airway Disease in Brown Norway Rats

Jack R. Harkema, James G. Wagner, Norbert E. Kaminski, Masako Morishita, Gerald J. Keeler, Jacob D. McDonald, and Edward G. Barrett

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with a Critique by the HEI Health Review Committee

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ABOUT HEI

The Health Effects Institute is a nonprofit corporation chartered in 1980 as an independent research organization to provide high-quality, impartial, and relevant science on the effects of air pollution on health. To accomplish its mission, the institute

- Identifies the highest-priority areas for health effects research;
- Competitively funds and oversees research projects;
- Provides intensive independent review of HEI-supported studies and related research;
- Integrates HEI's research results with those of other institutions into broader evaluations; and
- Communicates the results of HEI research and analyses to public and private decision makers.

HEI receives half of its core funds from the U.S. Environmental Protection Agency and half from the worldwide motor vehicle industry. Frequently, other public and private organizations in the United States and around the world also support major projects or certain research programs. HEI has funded more than 280 research projects in North America, Europe, Asia, and Latin America, the results of which have informed decisions regarding carbon monoxide, air toxics, nitrogen oxides, diesel exhaust, ozone, particulate matter, and other pollutants. These results have appeared in the peer-reviewed literature and in more than 200 comprehensive reports published by HEI.

HEI's independent Board of Directors consists of leaders in science and policy who are committed to fostering the public–private partnership that is central to the organization. The Health Research Committee solicits input from HEI sponsors and other stakeholders and works with scientific staff to develop a Five-Year Strategic Plan, select research projects for funding, and oversee their conduct. The Health Review Committee, which has no role in selecting or overseeing studies, works with staff to evaluate and interpret the results of funded studies and related research.

All project results and accompanying comments by the Health Review Committee are widely disseminated through HEI's Web site (*www.healtheffects.org*), printed reports, newsletters, and other publications, annual conferences, and presentations to legislative bodies and public agencies.

ABOUT THIS REPORT

Research Report 145, *Effects of Concentrated Ambient Particles and Diesel Engine Exhaust on Allergic Airway Disease in Brown Norway Rats*, presents a research project funded by the Health Effects Institute and conducted by Dr. Jack R. Harkema of the Department of Pathobiology and Diagnostic Investigation, Michigan State University, East Lansing, Michigan, and his colleagues. This report contains three main sections.

The HEI Statement, prepared by staff at HEI, is a brief, nontechnical summary of the study and its findings; it also briefly describes the Health Review Committee's comments on the study.

The Investigators' Report, prepared by Harkema and colleagues, describes the scientific background, aims, methods, results, and conclusions of the study.

The Critique is prepared by members of the Health Review Committee with the assistance of HEI staff; it places the study in a broader scientific context, points out its strengths and limitations, and discusses remaining uncertainties and implications of the study's findings for public health and future research.

This report has gone through HEI's rigorous review process. When an HEI-funded study is completed, the investigators submit a draft final report presenting the background and results of the study. This draft report is first examined by outside technical reviewers and a biostatistician. The report and the reviewers' comments are then evaluated by members of the Health Review Committee, an independent panel of distinguished scientists who have no involvement in selecting or overseeing HEI studies. During the review process, the investigators have an opportunity to exchange comments with the Review Committee and, as necessary, to revise their report. The Critique reflects the information provided in the final version of the report.

PREFACE

HEI's Research on Particles and the Exacerbation of Allergy and Asthma

Particles emitted from a number of sources (e.g., diesel and gasoline vehicles, electric power plants, and factories), and formed from gases also emitted from these sources, contribute to the exposure of urban populations to particulate matter (PM). Epidemiologic and experimental studies published in the 1990s suggested the possibility of adverse effects on the respiratory and immune systems of people exposed to diesel exhaust and other particles on a short-term basis; these effects could be more severe in persons with asthma and other allergic diseases. Some of these studies raised the question of whether various constituents of traffic-generated air pollution (including diesel exhaust particles) might contribute to an increase in symptoms of asthma or rhinitis or might enhance immune responses to allergens. In reviewing this literature, however, the HEI Health Research Committee noted that the epidemiologic studies did not measure ambient concentrations of individual pollutants, and many of the experimental studies did not use relevant exposure routes and particle doses. Thus the Committee considered that additional research with improved measures of exposure and outcomes was needed to elucidate dose–response relationships.

In 2000, HEI issued Request for Applications (RFA) 00-1, *Effects of Diesel Exhaust and Other Particles on the Exacerbation of Asthma and Other Allergic Diseases*. The RFA sought studies that would provide a better understanding of whether, and by what mechanism, particles from diesel engines and other sources may contribute to the frequency and severity of asthma attacks and to the exacerbation of allergic responses — especially in susceptible groups such as children, older people, and people with chronic airway diseases. The Committee was interested in research proposals for epidemiologic studies, studies of controlled exposure in humans, and animal studies.

RFA 00-1 was part of a larger HEI effort to study the effects on human health of exposure to current and future diesel particulate emissions and to investigate how the toxicity of diesel particles compares with that of other ambient particles. HEI's earlier efforts included a comprehensive review of the health effects associated with diesel emissions (HEI Diesel Working Group 1995) and a project initiated in 1998 to examine more closely the epidemiologic studies used in quantitative risk assessment (HEI Diesel Epidemiology Expert Panel 1999).

Four studies have been funded under RFA 00-1; one is presented in this Research Report, one was published in 2008, one in 2009, and one is under review by HEI. All four are described briefly below. For additional information on this research program, see the HEI Program Summary *Research on Diesel Exhaust and Other Particles* (2003).

"Fine Airborne Particles and Allergic Airway Disease," Jack Harkema, Michigan State University

The study by Harkema and colleagues that is presented in this report (HEI Research Report 145, *Effects of Concentrated Ambient Particles and Diesel Engine Exhaust on Allergic Airway Disease in Brown Norway Rats*) is the third of the four studies to be completed and published (Harkema et al. 2009). The investigators evaluated the effects on epithelial cell remodeling and inflammation in the airways of Brown Norway rats inhaling either whole diesel exhaust or concentrated ambient particles during the initial (sensitization phase) or subsequent (challenge phase) exposure to an airborne allergen. The underlying hypothesis was that inhalation of particles during either sensitization or challenge causes airway remodeling and exacerbates airway inflammation.

"The Relationship Between Pollutant Particles in Alveolar Macrophages from Normal Children and Proxy Markers of PM_{10} Exposure," Jonathan Grigg, University of Leicester, Leicester, United Kingdom

The study by Grigg and colleagues examined whether the detection of PM (which was not specific to diesel) in macrophages obtained from sputum could be used as a biomarker of PM exposure in children. The investigators also explored the relationship between PM load in macrophages and markers of inflammation in children's lungs. This study was published as HEI Research Report 134, *Black-Pigmented Material in Airway Macrophages from Healthy Children: Association with Lung Function and Modeled PM_{10}* (Grigg et al. 2008).

"Health Effects of Diesel Exhaust in Asthmatics: A Real-World Study in a London Street," Junfeng (Jim) Zhang, University of Medicine and Dentistry of New Jersey–School of Public Health

The study by Junfeng (Jim) Zhang and colleagues used two locations in London, United Kingdom, to study the effects of a real-world exposure to urban diesel traffic in persons with mild or moderate asthma. The study tested the hypotheses that exposure to diesel exhaust leads to worsening of asthma symptoms, with a reduction in lung function and an increase in oxidative stress and inflammation in the airways, and that such effects are dependent on the severity of asthma. Each subject participated in an experimental exposure session and a control exposure session by walking in each site for two hours while portable monitors determined concentrations of selected air pollutants. The exposure site was a busy street (Oxford Street) where motor-vehicle traffic was restricted to taxis and buses, the majority of which were powered by diesel engines; the control site was a nearby park with no motor-vehicle traffic (Hyde Park). The subjects' asthma symptoms, pulmonary function, and indicators of inflammation and oxidative stress were subsequently measured. This study was published as HEI Research Report 138, *Health Effects of Real-World Exposure to Diesel Exhaust in Persons with Asthma* (Zhang et al. 2009).

"Exacerbation of Allergic Inflammation in the Lower Respiratory Tract by Diesel Exhaust Particles," Mark A. Riedl, University of California–Los Angeles School of Medicine (original Principal Investigator, David Diaz-Sanchez, now at the U.S. Environmental Protection Agency)

The goal of the study by Effros and colleagues was to evaluate the effects on the immune system and lung function of the inhalation of diesel exhaust emissions in asthmatic subjects. The study compared the effects of exposure to whole diesel exhaust and to NO_2, which is a major component of the gas phase of diesel exhaust, in asthmatic subjects and in asthmatic subjects who were also allergic to cat dander. The final report for this study is currently being reviewed at HEI.

ADDITIONAL STUDIES

While the studies funded by this RFA were in progress, the U.S. Environmental Protection Agency introduced lower PM emission standards for heavy-duty diesel engines, which are expected to result in lower concentrations of several pollutants in ambient air. As part of an effort to assess how emissions and health effects of diesel engines may be changing with the introduction of new technologies developed to meet the new standards, HEI initiated the Advanced Collaborative Emissions Study (ACES), in collaboration with the Coordinating Research Council, a nonprofit organization that directs research on automotive technologies and fuels. The study has two main components: a recently completed characterization of the emissions from four heavy-duty diesel engines meeting the 2007 PM standards (Coordinating Research Council 2009), and a chronic inhalation bioassay in rodents to evaluate both short- and long-term health effects of the emissions of one of the four engines. As the new diesel fleet slowly replaces older models, it will also be important to document the associated changes in air quality and health.

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HEI STATEMENT

Synopsis of Research Report 145

Effects of Concentrated Ambient Particles and Diesel Emissions on Rat Airways

BACKGROUND

In the 1990s, results from several epidemiologic and controlled-exposure studies suggested an association between exposure to air pollution from traffic-derived particulate matter (PM) and increases in symptoms of airway diseases, including exacerbation of asthma. Some results also suggested that exhaust from diesel engines contributed to these effects. To address some of the questions raised by these findings, HEI issued Request for Applications 00-1, "Effects of Diesel Exhaust and Other Particles on the Exacerbation of Asthma and Other Allergic Diseases." In response, Dr. Jack R. Harkema, Michigan State University (MSU), and colleagues proposed a study to investigate how the inhalation of $PM_{2.5}$ (PM with an aerodynamic diameter $\leq$ 2.5 µm) concentrated from ambient air (concentrated ambient particles, CAPs) or diesel engine exhaust (DEE) would affect the airway inflammatory and allergic responses to the airborne allergen ovalbumin (OVA) in a rat model of asthma. Harkema and his colleagues proposed to expose rats to the pollutants at critical points in the induction of the allergic response, namely, the initial or sensitization phase and the second or challenge phase. The investigators hypothesized that inhalation of CAPs or DEE during sensitization or challenge would enhance inflammatory responses in the airways. The HEI Health Research Committee recommended the proposal for funding.

APPROACH

The study was conducted in two different locations: the CAPs study in Michigan (exposures in Detroit and analysis at MSU in East Lansing) and the DEE study at the Lovelace Respiratory Research Institute (LRRI) in Albuquerque, New Mexico. The same OVA sensitization and challenge regimen was used in both the CAPs and DEE studies: Brown Norway rats were *sensitized* by intranasal instillation of a 0.5% solution of OVA (or saline as a control) on days 1 through 3; they rested for days 4 through 14 and were *challenged* intranasally with a 1.0% solution of OVA (or saline) on days 15 through 17.

In Detroit, Harkema and colleagues used a mobile research facility that they had used in a previously funded HEI study to collect CAPs from an area of the city with a higher-than-average prevalence of asthma. On the same 3 days that groups of rats were challenged with OVA or saline (days 15–17), the investigators exposed the rats to CAPs for 8 hours/day in the mobile facility. The two 3-day exposures, conducted in two different weeks, are referred to as CAPs Experiments 1 and 2 in this document and the Critique prepared by the HEI Health Review Committee in its independent review of the study. The investigators made measurements of PM mass; elemental carbon and organic carbon (EC and OC); sulfate, nitrate, and ammonium ions; pollutant gases (ozone, carbon monoxide [CO], sulfur dioxide [SO_2], and total nitrogen oxides [NO_x]); several organic species including polycyclic aromatic hydrocarbons (PAHs); and several trace elements in both ambient air and CAPs. The mobile laboratory also collected meteorological measurements.

At the LRRI, a team led by Dr. Joe Mauderly produced DEE from a single-cylinder 5500-watt diesel engine generator using number 2 nationally certified diesel fuel. The PM and gaseous components of DEE were characterized physically and chemically as in the CAPs substudy. Whole-body exposures to DEE at either 30 or 300 µg/m^3 PM (or to filtered air as a control) were conducted for 8 hours/day for 3 days on the same days as either sensitization (days 1–3) or challenge (days 15–17) with OVA or saline.

This Statement, prepared by the Health Effects Institute, summarizes a research project funded by HEI and conducted by Dr. Jack R. Harkema, Department of Pathobiology and Diagnostic Investigation, Room 218, National Food Safety and Toxicology Building, East Lansing, MI 48864, and colleagues. Research Report 145 contains both the detailed Investigators' Report and a Critique of the study prepared by the Institute's Health Review Committee.

In both the CAPs and DEE studies, rats were killed on day 18 and airway tissues from the nose and lung were harvested and bronchoalveolar lavage fluid (BALF) was collected. Total and differential cell counts and levels of secreted mucin glycoprotein (Muc5AC), total protein, elastase, β-glucuronidase, multiple cytokines, and other soluble mediators including OVA-specific immunoglobulin E (IgE) were evaluated in the BALF. Fixed nasal, and proximal and distal axial pulmonary airways were examined morphometrically to assess characteristics of inflammation in the airways, which included mucus cell metaplasia and an increase in stored intraepithelial mucosubstances (IM) in airway epithelial cells. The investigators also used bromodeoxyuridine (BrdU) labeling to assess the number of surface epithelial cells synthesizing DNA in the axial airways. Levels of RNA specific for mucin and multiple cytokines were also assessed in lung tissue.

RESULTS

The concentrator in the mobile laboratory in Detroit preferentially concentrated particles around 0.6 μm and smaller in diameter from ambient air, and by 20- to 31-fold based on $PM_{2.5}$ mass. Some PM components in the CAPs (e.g., some trace metals) were concentrated in proportion to their mass in ambient air, but others, including EC and OC, showed some variation. In both CAPs Experiments 1 and 2 the proportions of the major identifiable components of CAPs — OC, sulfate, nitrate, and ammonium ions — were similar, but levels of several trace metals — including Rb, Ni, Fe, and Al — were higher during CAPs Experiment 1 than Experiment 2. Ambient concentrations of several components of the PM mix that were not concentrated — ultrafine particles (diameter ≤ 0.1 μm), PAHs, and the pollutant gases SO_2 and NO_x, were also higher during CAPs Experiment 1 than Experiment 2.

At the LRRI most of the particles derived from the diesel generator were in the ultrafine range, and the major chemical component was EC, followed by OC (6%–14%), with small amounts of inorganic ions and trace metals. Levels of NO_x and CO in the high-diesel-exposure atmosphere were approximately 4 ppm.

At both study sites OVA sensitization and challenge of rats resulted in several features of an inflammatory response in the nasal and pulmonary areas. In the absence of sensitization and challenge with OVA, exposure to either CAPs alone or DEE alone had few biologic effects.

Exposure to CAPs during OVA challenge in CAPs Experiment 1 increased some features of the OVA-induced inflammatory response in the lungs — the severity and extent of distribution of detected pathology in the bronchi and alveoli; total number of cells (particularly eosinophils and lymphocytes); levels of total protein, Muc5AC, and tumor necrosis factor α in BALF; and levels of IM in proximal and distal pulmonary airways — but decreased levels of several cytokine-specific RNAs in lung tissue. Levels of nearly all measured cytokines and OVA-specific IgE (a characteristic of an allergic immune response) in BALF were not affected. Few, if any, effects of CAPs during OVA challenge on inflammatory or immune endpoints were detected in CAPs Experiment 2. No effects of CAPs during OVA challenge were detected in the nose in either CAPs experiment.

The effects of DEE exposure on OVA sensitization and OVA challenge were mild, and the pattern of DEE-associated changes was quite complicated. First, in both sensitization and challenge substudies, greater effects were observed at the lower DEE exposure concentration, 30 $\mu g/m^3$ PM, than at the higher, 300 $\mu g/m^3$ PM; few effects were detected at the high-level DEE exposure. Second, exposure to the low-level DEE during OVA sensitization predominantly *enhanced* inflammatory endpoints induced by OVA alone, whereas exposure to the same level of DEE during OVA challenge *attenuated* inflammatory endpoints induced by OVA alone. No changes in levels of OVA-specific IgE were detected, and no effects of DEE exposure during either OVA sensitization or challenge were detected in the nose.

SUMMARY AND CONCLUSIONS

In its independent review of the study, HEI's Health Review Committee thought that Harkema and colleagues successfully designed and conducted a descriptive study to evaluate the effects of two pollutants — CAPs (concentrated $PM_{2.5}$) and DEE — in a rodent model of asthma. The model had some but not all characteristics of the human disease, so caution should be used in extrapolating data obtained in the model to humans.

The most surprising findings were the relative lack of effect of high-level DEE (300 $\mu g/m^3$ PM) exposure in the model, which used rats sensitized

and challenged with the airborne allergen OVA. In addition, low-level DEE exposure (30 μg/m^3 PM) during allergen sensitization mildly enhanced inflammatory responses, but the same exposure during allergen challenge attenuated several effects of exposure to OVA alone.

These findings differ from those of previous diesel-exposure studies, which have reported enhancement of inflammatory and allergic responses in humans when exposed and in animal models. Differences in findings could be the result of the lower levels of diesel emissions used in the current study compared with some previous studies. Another possible explanation is that in some prior diesel-exposure studies rodents and humans were administered diesel particles, rather than the whole emissions used in the current study. Thus, the presence of gases in the DEE used in the present study could be hypothesized to have inhibited responses to the diesel particulate fraction. On the other hand, some controlled human studies of exposure to DEE — which clearly contained both gaseous and particulate components — have shown limited *enhancement* of allergic and inflammatory responses, findings inconsistent with the notion that gases found in diesel emissions might have inhibited a diesel-particulate-mediated enhancement of allergic and inflammatory responses that would otherwise have occurred.

The Committee also thought that the investigators made good use of a mobile air research laboratory to expose rats to CAPs at a site where the prevalence of asthma in the population is higher than average. In CAPs Experiment 1, exposure to CAPs during the 3 days of OVA sensitization enhanced some allergic and inflammatory endpoints, a finding consistent with data from previous studies, but that was not observed in CAPs Experiment 2. Differences in the findings of the two CAPs experiments suggest that the observed differences in composition of CAPs, and hence sources of $PM_{2.5}$, in the different weeks that CAPs were collected may be factors in determining the pattern of response obtained. However, the investigators did not perform analyses to identify sources of pollutants. In addition, in contrast to the investigators, the Committee was not convinced that the multiple elements whose levels were elevated in CAPs in Experiment 1 were associated specifically with local stationary sources. Thus, the Committee did not believe that positive effects of CAPs in Experiment 1 could be attributed easily to any one set of PM components or type of source.

The Committee also cautioned that in this study, as in all others that use CAPs, the concentrated particles may not be representative of particles in ambient air. Concentrated and ambient particles may differ in either physical characteristics — selective concentration of a particular size of particle — or chemical composition — selective concentration of particular components. In a similar note of caution about the diesel-exposure results, the Committee noted that the diesel emissions emitted by the generator used in the current study differ from emissions derived from new diesel-powered vehicles, which are subject to recent regulations to reduce particulate emissions. Future studies to explore the effects of exposure to airborne pollutants will need to use more relevant exposure atmospheres and better models of human disease.

INVESTIGATORS' REPORT

Effects of Concentrated Ambient Particles and Diesel Engine Exhaust on Allergic Airway Disease in Brown Norway Rats

Jack R. Harkema, James G. Wagner, Norbert E. Kaminski, Masako Morishita, Gerald J. Keeler, Jacob D. McDonald, and Edward G. Barrett

Departments of Pathobiology and Diagnostic Investigation (J.R.H., J.G.W.) and Department of Pharmacology and Toxicology (N.E.K.), Michigan State University, East Lansing, Michigan; Department of Environmental Health Sciences and Atmospheric, Oceanic, and Space Sciences, University of Michigan, Ann Arbor, Michigan (M.M., G.J.K.); and Lovelace Respiratory Research Institute, Albuquerque, New Mexico (J.D.M., E.G.B.)

ABSTRACT

Increased concentrations of airborne fine particulate matter ($PM_{2.5}$; particulate matter with an aerodynamic diameter ≤ 2.5 μm)* are associated with increases in emergency room visits and hospitalizations of asthmatic patients. Emissions from local stationary combustion sources (e.g., coal-burning power plants) or mobile motor vehicles (e.g., diesel-powered trucks) have been identified as potential contributors to the development or exacerbation of allergic airway disease. In the present study, a rodent model of allergic airway disease was used to study the effects of concentrated ambient particles (CAPs) or diesel engine exhaust (DEE) on the development of allergic airway disease in rats sensitized to the allergen ovalbumin (OVA). The overall objective of our project was to understand the effects of $PM_{2.5}$ on the development of OVA-induced allergic airway disease. Our specific aims were to test the following hypotheses: (1) exposure to CAPs during OVA challenge enhances epithelial remodeling of the airway and inflammation in rats previously sensitized to the allergen; and (2) exposure to DEE during OVA sensitization, or during OVA challenge, exacerbates epithelial remodeling of the airway and inflammation in rats.

In the DEE studies, Brown Norway (BN) rats were sensitized with three daily intranasal (IN) instillations of 0.5% OVA, and then two weeks later were challenged with IN OVA or saline for 3 consecutive days. Rats were exposed to DEE diluted to mass concentrations of 30 or 300 $\mu g/m^3$ diesel exhaust particles (DEPs) or to filtered air during either the sensitization or challenge periods. For the CAPs studies, the same OVA sensitization and challenge rat model was used but exposures to Detroit, Michigan, CAPs were limited to the OVA challenge period. Two separate 3-day CAPs exposures were conducted (week 1, high mean mass concentration = 595 $\mu g/m^3$; week 2, low mean mass concentration = 356 $\mu g/m^3$) during OVA challenge. In both the DEE and CAPs studies, rats were killed 24 hours after the last OVA challenge, bronchoalveolar lavage fluid (BALF) was collected and analyzed for cellularity and secreted mediators, and lungs and nose were processed for histopathologic examination and morphometric analysis of intraepithelial mucosubstances (IM).

The results of our animal inhalation studies in the southwest (SW) Detroit community, an area with elevated ambient $PM_{2.5}$ concentrations, suggested that, during allergen challenge, exposure to CAPs that were predominantly associated with emissions from combustion sources markedly enhanced the OVA-induced allergic airway disease, which was characterized by an increased infiltration in the lungs of eosinophilic and lymphocytic inflammation, increased IM in conducting airways, and increased concentrations in BALF of mucin-specific proteins and inflammatory cytokines. These findings suggest that urban airborne $PM_{2.5}$ derived from stationary combustion sources (e.g.,

This Investigators' Report is one part of Health Effects Institute Research Report 145, which also includes a Critique by the Health Review Committee and an HEI Statement about the research project. Correspondence concerning the Investigators' Report may be addressed to Dr. Jack R. Harkema, Department of Pathobiology and Diagnostic Investigation, Room 218, National Food Safety and Toxicology Building, East Lansing, MI 48864; 517-353-8627; harkemaj@msu.edu.

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* A list of abbreviations and other terms appears at the end of the Investigators' Report.

refineries, coal-burning power plants, waste-treatment plants) may enhance the development of human allergic airway diseases like childhood asthma. Previous animal inhalation studies in this community have also suggested that these fine, ambient combustion-derived particles may also exacerbate preexisting allergic airway disease.

In contrast to our CAPs studies in Detroit, the controlled DEE exposures of allergen-sensitized BN rats, during either allergen sensitization or challenge periods, caused only a few mild modifications in the character of the allergen-induced disease. This finding contrasts with other reported studies that indicate that DEPs at relatively higher exposure doses do enhance allergic airway disease in some rodent models. The reasons for these disparities between studies likely reflect differences in exposure dose, animal models, the timing of exposures to the allergens and DEP exposures, the methods of allergen sensitization and challenge, or physicochemical differences among DEEs.

INTRODUCTION

Air pollution is a major health concern in heavily populated urban centers, where ambient levels of $PM_{2.5}$ and gaseous pollutants (e.g., ozone [O_3]) are generally high. Individuals with chronic airway diseases (e.g., children with asthma) living in these urban environments may be particularly vulnerable to the toxic effects of $PM_{2.5}$ and other air pollutants. Though the results of several human and animal studies (Harkema et al. 2004; McCreanor et al. 2007; Miyabara et al. 1998; Rabinovitch et al. 2006; Vagaggini et al. 2002; Wagner et al. 2002) suggest that exposure to common urban air pollutants such as O_3 and particulate matter (PM) may exacerbate preexisting allergic asthma and rhinitis, the effects of these air pollutants on the development of allergic airway diseases are unclear. Furthermore, the underlying cellular and molecular events (pathogenesis) responsible for any exacerbation of allergic airway disease, particularly the remodeling of the airway structure, by exposure to air pollutants, have not been thoroughly investigated.

According to recent estimates, approximately 5% to 10% of the population in the United States has asthma, and 20% has some form of respiratory allergy (Peden 1996). Asthma is the most common chronic disease of childhood, affecting approximately 10 million children under the age of 16 years in the United States (Gergen et al. 1988). Between 1982 and 1992, the prevalence of pediatric asthma (children under age 18) in the United States increased by 58% (American Lung Association, Epidemiology and Statistics Unit 1992). There is a growing concern that populations in urban settings appear to be particularly at risk, with minority populations showing disproportionate rates of this disease (Nelson et al. 1997). The national trend of increasing incidence of asthma is reflected in Michigan urban communities such as Detroit, where it has been reported that 17.4% of 230 children studied had physician-diagnosed asthma (Joseph et al. 1996). The rising trends for asthma also suggest that more of our urban population may suffer from disease related to air pollution in the future. Multiple and complex factors cause and aggravate pediatric asthma, including genetic disposition, demographic variables, psychosocial stressors, and environmental exposures (Weiss et al. 1992; Bates 1995; Beggs and Curson 1995). The last category includes exposures to particulate air pollution.

Increased ambient air pollution levels, particularly of respirable particulate matter (Pope et al. 1991; Tseng et al. 1992; Koenig et al. 1993; Schwartz et al. 1993; Dockery and Pope 1994; Walters et al. 1994; Peters et al. 1997; Ostro and Chestnut 1998) and O_3 (Koenig et al. 1987; Ponka 1991; Cody et al. 1992; Thurston et al. 1992; Abbey et al. 1993; White et al. 1994; Kesten et al. 1995; Ostro et al. 1995; Romieu et al. 1995), have been associated with symptoms of asthma (Pope et al. 1991; Abbey et al. 1993) and an increase in emergency department visits and hospitalizations for asthma (Koenig et al. 1987; Ponka 1991; Cody et al. 1992; Thurston et al. 1992; Schwartz et al. 1993; Walters et al. 1994; White et al. 1994; Kesten et al. 1995; Ostro et al. 1995; Romieu et al. 1995). Studies in the United States and Europe reported an association between increased morbidity and mortality and ambient PM concentrations that were below the limits specified in the U.S. National Ambient Air Quality Standards (Dockery and Pope 1994; Schwartz 1994, 1995). Highly sensitive subpopulations, such as persons with asthma, are at increased risk, and exposure to PM and copollutants in the ambient environment may be a critical factor in increased morbidity and mortality in these individuals in urban centers (Schwartz 1993; Hoek and Brunekreef 1995; U.S. Environmental Protection Agency [EPA] 1996).

Several experimental studies have been conducted to examine the effects of diesel exhaust and other components of $PM_{2.5}$ in exacerbating allergic airway disease. Diaz-Sanchez and his colleagues have conducted several in vivo studies of human subjects who were intranasally administered DEPs or ragweed allergen or both. These investigators demonstrated that IN administration of DEPs markedly enhanced ragweed-specific immunoglobulin E (IgE) and the production of messenger RNA (mRNA) for Th2-type cytokines (cytokines associated with allergic rhinitis and asthma) in the nasal mucosa (Diaz-Sanchez et al. 1997). Furthermore, Diaz-Sanchez and his colleagues have demonstrated that intranasally administered DEPs

can act as mucosal adjuvants to a de novo IgE response and increase allergic sensitization (Diaz-Sanchez et al. 1999). They also conducted in vitro studies to demonstrate that the organic constituents of DEPs, and in particular phenanthrene, a major polyaromatic hydrocarbon constituent of DEPs, can enhance IgE production by IgE-secreting Epstein-Barr virus–transformed human B cells (Tsien et al. 1997). Nel and colleagues (2001) have suggested that organic extracts of DEPs induce the production and release of oxygen species by macrophages and bronchial epithelial cells and that these reactive species of oxygen may be responsible for the promotion of cytokines and chemokines involved in the exacerbation of allergic inflammation.

Results from experimental studies using laboratory rodents also suggest that DEPs and other potential constituents of $PM_{2.5}$ play an important role in the increasing prevalence of allergic airway disease. Mice that were intranasally instilled with DEPs and the commonly used experimental aeroallergen OVA over several weeks also had greater IgE antibody production compared to mice that received only OVA (Takafuji et al. 1987). Similarly, Takano and colleagues (Takano et al. 1997) demonstrated that intranasally instilled DEPs enhance OVA-induced airway inflammation in mice. Fujimaki and associates (1997) exposed mice by inhalation to 3 and 6 mg/m^3 of DEPs for six weeks and intranasally instilled OVA before, during, and after the exposures to DEPs. They found that the OVA-specific IgE antibody titers of mice exposed to DEPs and OVA were significantly higher than those of mice exposed to OVA alone.

Lambert and coworkers (1999) showed in a BN rat model of house-dust-mite allergy that preexposure to particles of residual oil fly ash (ROFA) enhances the sensitization period such that the secondary immune responses increased after allergen challenge. Furthermore, these investigators conducted additional studies with BN rats, and the results from these studies suggest that the metallic constituents of ROFA mediate the enhancement of sensitization to the house-dust-mite antigen (Lambert et al. 2000). They suggest that pulmonary inflammation induced by ROFA and its metallic constituents may play a key role in the mediation of the adjuvant effect.

Though data from recent laboratory studies strongly suggest that exposures to high concentrations of DEPs or other constituents of particulate air pollution may enhance the induction of allergic airway disease in humans or laboratory animals, the effects of $PM_{2.5}$ from *real-world* urban atmospheres have not been adequately investigated. Well-designed studies are needed to investigate specifically the effects of $PM_{2.5}$, from urban communities with high daytime concentrations of particulate air pollution, on the induction of allergic airway disease. In addition, the effects of $PM_{2.5}$ exposure on the remodeling of airway epithelium (measured by, for example, mucus cell metaplasia [MCM] and associated mucin-gene expression) caused by allergen sensitization and challenge have not been thoroughly investigated. The present study used community- and laboratory-based animal inhalation studies to address these two specific gaps in our present data.

SPECIFIC OBJECTIVES

The overall objective of this project was to understand the effects of fine CAPs, CAPs with a mass median aerodynamic diameter 0.1–2.5 μm, and DEE on the induction of antigen-induced allergic airway disease (e.g., allergic asthma). The primary focus of these studies was to determine the effects of exposures to these airborne particles on allergen-induced airway inflammation and the remodeling of the surface epithelium lining the respiratory airways. In addition, our studies were designed to better understand the underlying cellular and molecular mechanisms responsible for CAPs- and DEE-related modulation (either an increase or a decrease) of inflammatory responses resulting from the exposure of the airway epithelium to allergens.

An integrated investigative team of environmental and biomedical scientists from Michigan State University (MSU) in East Lansing, Michigan, and the University of Michigan (UM) in Ann Arbor, Michigan, conducted toxicologic and atmospheric research of CAPs in a SW Detroit community that has a high incidence of childhood asthma and high concentrations of particulate air pollution during the summer months. The team used a state-of-the-art mobile air research laboratory (AirCARE 1), located in the community, to conduct ambient monitoring, concentrate ambient particles, and perform concurrent inhalation toxicology studies with CAPs in laboratory rats. The airways of rats exposed to CAPs and the airway allergen OVA were examined for cellular or molecular markers, or both, of airway epithelial injury and remodeling caused by OVA challenge and for potential alterations resulting from CAPs exposure during OVA challenge. These experiments, which examined the effects of CAPs on the induction of allergic airway disease, extended our previous studies that were designed to determine the effects of CAPs exposure on rats with only preexisting allergic airway disease (Harkema et al. 2004).

Using the same rat model of allergic airway disease, MSU and UM investigators along with scientists at the Lovelace Respiratory Research Institute (LRRI; Albuquerque, NM) also studied the effects of DEPs on the induction of allergic airway disease. These inhalation studies were conducted under controlled exposure conditions at the LRRI.

The two specific aims for the entire project were to test the following hypotheses:

Aim 1: Exposure to CAPs during OVA challenge will exacerbate airway epithelial remodeling (e.g., MCM) and airway inflammation in the airways of rats previously sensitized to the antigen.

Aim 2: Exposure to DEE during OVA sensitization or challenge will exacerbate airway epithelial remodeling and airway inflammation in rats.

STUDY DESIGNS, METHODS, AND MATERIALS

DESIGN OF INHALATION STUDIES OF CAPs IN DETROIT

Laboratory Animals

Male BN rats (Harlan Laboratories; Indianapolis, IN), age 10 to 12 weeks, were assigned to one of four experimental groups. Male rats were chosen for these experiments to avoid hormonal changes during the estrous cycle that have been shown to influence epithelial cell populations and the mucus apparatus in the airways of female rodents, and that thus might complicate the interpretation of results (Hayashi et al. 1979). Prior to the start of this study, the laboratory rodents were housed in polycarbonate cages in the University Research Containment Facility at MSU. Rats were free of pathogens and respiratory disease, and all treatment protocols were approved by the MSU Institutional Animal Care and Use Committee. All laboratory animal facilities at MSU (including the mobile laboratory used in our study) are accredited by the Association of Assessment and Accreditation of Laboratory Animal Care International.

Inhalation Exposures (Air or CAPs) and IN Instillations (Saline or OVA)

Two separate 3-day inhalation exposure studies were conducted in July 2003. The first inhalation study was conducted during week 1 (July 14–16). The second was conducted during week 2 (July 21–23). Different laboratory rats were used for each study. Both studies followed the same exposure and treatment protocols. In each study, 32 male BN rats were sensitized daily for 3 consecutive days to OVA, a protein allergen derived from chicken eggs, by IN instillation of a 0.5% solution of OVA in saline (150 µL/nasal passage). The airway sensitization of these animals was conducted in the laboratory of Dr. Harkema at MSU. Twelve days after the end of the IN sensitization, all the rats were moved to AirCARE 1, which was parked at Maybury Elementary School in SW Detroit (AirCARE 1 and the exposure site are described in detail below). Rats were exposed to CAPs or filtered air in whole-body, stainless steel and glass inhalation-exposure chambers, located within AirCARE 1. Inhalation exposures were for 3 consecutive days, 8 hours/day (7:30 AM to 3:30 PM). A Harvard/EPA ambient fine particle concentrator was used to generate the CAPs. One hour after the end of each daily inhalation exposure, the rats were intranasally challenged with a 1.0% solution of OVA in saline (150 µL/nasal passage; allergen challenge) or were challenged with saline alone (controls; no allergen challenge). Twenty-four hours after the last IN challenge, each rat was killed and airway tissues from the nose and lungs were removed for analysis. In the MSU laboratory, standard analytical techniques were used to identify exposure-related alterations in the nasal and pulmonary airway tissues and in BALF from the exposed rodents (specific details provided below).

In summary, the four experimental groups of rats for both weeks 1 and 2 were (1) control rats exposed to filtered air and challenged with saline (Air/Saline group), (2) rats exposed to CAPs and challenged with saline (CAPs/Saline group), (3) rats exposed to filtered air challenged with OVA (Air/OVA group), and (4) rats exposed to CAPs and challenged with OVA (CAPs/OVA group). Figure 1 summarizes the experimental groups and exposure/instillation protocols for these studies.

AirCARE 1 and Ambient Fine Particle Concentrator

AirCARE 1 is a unique and specially designed mobile air research laboratory constructed inside the confines of a 16.2-m semitrailer (Figure 2). The design and operation of this mobile lab for inhalation toxicology studies of "real-world" airborne particles have been previously described in detail (Harkema et al. 2004). In brief, AirCARE 1 contains

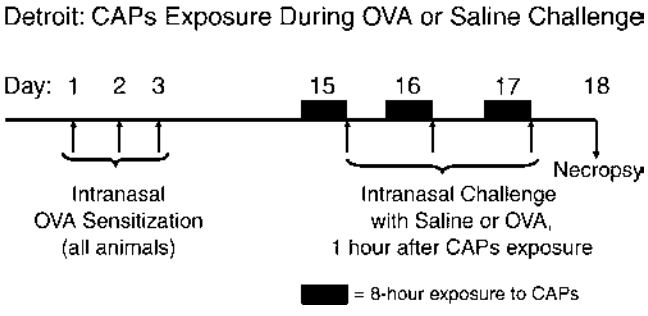

Figure 1. CAPs exposure and OVA sensitization and challenge protocols. Rats were sensitized with OVA by IN instillation on days 1 through 3, and challenged on days 15 through 17. Prior to IN challenge, rats were exposed to CAPs or filtered air on days 15 through 17. All necropsies were conducted on day 18. Black bars represent 8-hour exposures to CAPs.

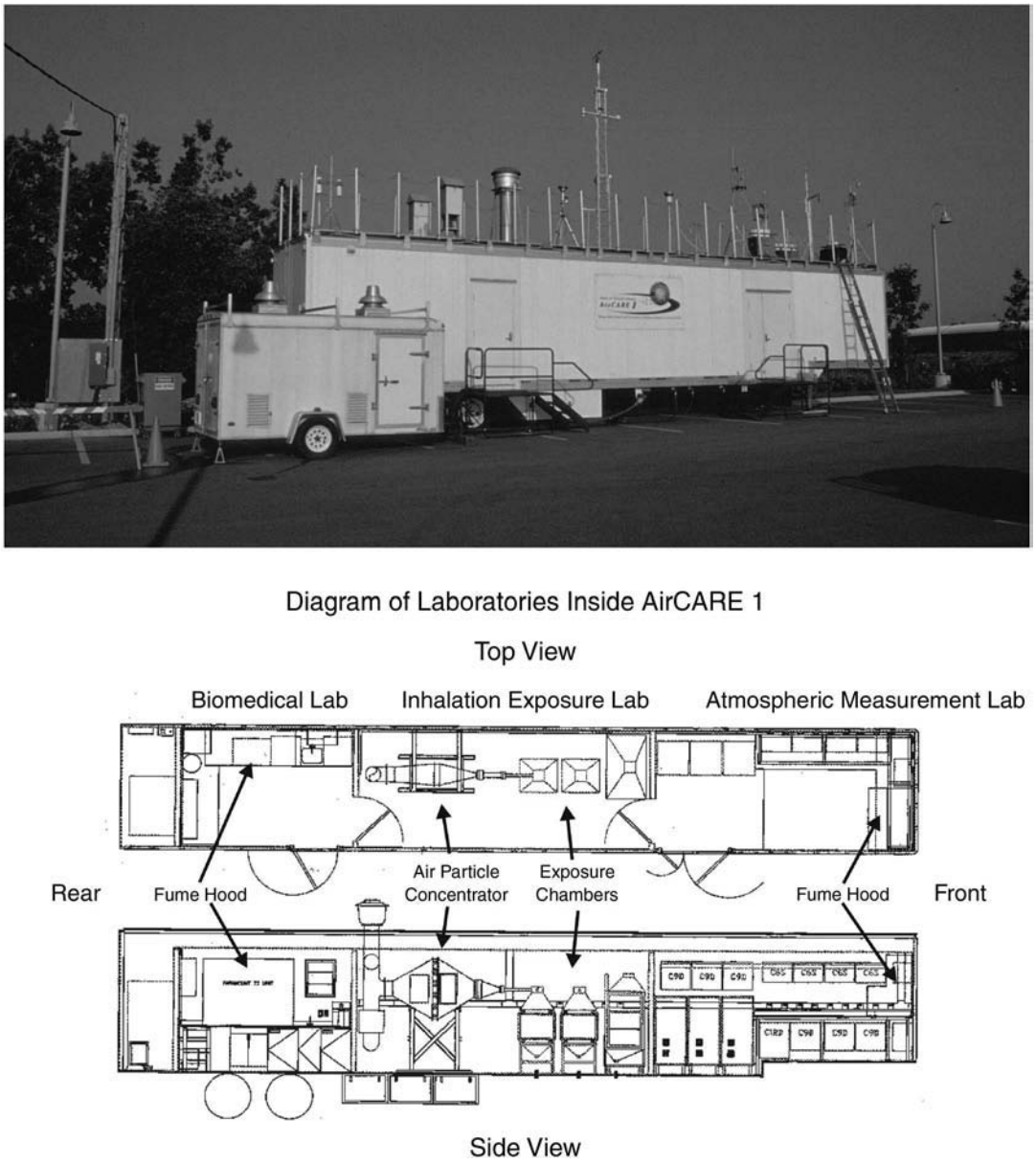

Figure 2. The layout and design of the AirCARE 1 laboratories.

on-board electric power, water, and heating, ventilation, and air conditioning (HVAC) systems. It contains three distinct rooms that provide over 37 m^2 of laboratory space. These three rooms are (1) a biomedical laboratory for animal necropsies and surgical procedures, (2) an inhalation exposure laboratory, and (3) an atmospheric measurement laboratory (Figure 2). Each laboratory has its own separate HVAC control, which allows for careful monitoring of temperature within each room. A 10-m telescoping meteorological tower attached to the outside of AirCARE 1 was used to collect routine meteorological measurements of temperature, relative humidity, solar radiation, wind speed, and wind direction.

The inhalation exposure laboratory within AirCARE 1 contains an ambient fine particle concentrator, two reinforced, stainless-steel Hinners-type whole-body inhalation chambers (CAPs and filtered air), and a Hazelton H1000 chamber to house laboratory rodents during nonexposure periods. The concentrator is a three-stage aerosol concentrator that utilizes virtual impactors to increase

the concentration of particles (size range 0.1–2.5 μm) by a factor of approximately 30 (Sioutas et al. 1995; Harkema et al. 2004). Therefore, when the ambient concentration of $PM_{2.5}$ was 25 μg/m^3, the chamber concentration was approximately 750 μg/m^3 with 7 to 8 air changes per hour. The $PM_{2.5}$-size-selective inlet to the concentrator is mounted on top of the trailer roof approximately 1.5 m above the grated, aluminum mezzanine platform. A series of stainless-steel ducts 0.31 m in diameter connect the inlet to the concentrator.

The two inhalation chambers are located directly in-line with the outlet of the concentrator. These chambers have a volume of 0.32 m^3 and hold a single level of 16 rats. One chamber was used to expose the rats to CAPs, and the other served as a control chamber providing only filtered air.

The SW Detroit Community: Site for Inhalation Exposures

The site selected for this inhalation toxicology study was an urban, residential neighborhood in SW Detroit, which has reported moderately high to very high hospitalization rates in children with asthma (Joseph et al. 1996; Lippman et al. 2000). AirCARE 1 was parked at Maybury Elementary School, located in a community in which human epidemiologic and asthma intervention studies have been recently conducted (Keeler et al. 2002; Lewis et al. 2005). The densest industrial community in Detroit is in this area, which contains iron and steel manufacturing plants, coke ovens, chemical plants, refineries, sewage-sludge incinerators, and coal-fired utilities (Keeler et al. 2002). In addition, SW Detroit experiences heavy motor vehicle traffic, including both passenger-car and diesel-truck traffic, due to its proximity to major interstate highways and the entrance to the Ambassador Bridge to Windsor, Ontario, Canada. Recent studies, which used detailed chemical and physical characterization of $PM_{2.5}$ measured in SW Detroit coupled with receptor modeling (positive matrix factorization), identified six major sources of ambient $PM_{2.5}$ mass during the summer months (Morishita et al. 2006). The major $PM_{2.5}$ mass contributors included (1) coal combustion and secondary sulfate aerosol, (2) motor vehicle and urban road dust, (3) municipal waste incinerators, (4) oil combustion and oil refineries, (5) sewage-sludge incinerators, and (6) iron and steel manufacturing facilities.

DESIGN OF INHALATION STUDIES OF DEE

Study 1: Exposure to DEE During Allergen (OVA) Sensitization

Male, 10-to-14-week-old BN rats (8 rats/exposure group) were exposed to two concentrations of DEE or to filtered air in whole-body exposure chambers at the LRRI. DEE exposure studies occurred during February and March 2003. Rats were exposed for 3 consecutive days, 8 hours/day (7:30 AM to 3:30 PM), to filtered air alone (0 μg/m^3 DEE; controls), a low concentration of DEE (30 μg/m^3), or a high concentration of DEE (300 μg/m^3). DEE was generated using a single-cylinder, 5500-watt diesel engine generator using number 2 nationally certified diesel fuel (see more details below). One hour after the end of each daily inhalation exposure, rats were intranasally instilled with a 0.5% solution of OVA in saline (150 μL/nasal passage for airway sensitization) or with saline alone (no OVA sensitization). At the end of the 3-day inhalation exposures, the rats were moved back to the animal housing facilities at the LRRI. Twelve days after the end of the last exposure, all the rats were intranasally challenged with 300 μL of a 1% solution of OVA in saline (150 μL/nasal passage per day for 3 consecutive days). Twenty-four hours after the last IN challenge, each rat was killed and airway tissues from the nose and lungs were removed for analysis. All necropsies were conducted at the LRRI. The same tissue and fluid analyses described for the CAPs study were conducted for the DEE studies. All of these analyses were conducted at MSU in the laboratory of Dr. Harkema.

A summary of the experimental groups and exposure and instillation protocols in this first DEE study is presented in Figure 3. The six experimental groups in study 1 were (1) rats exposed to filtered air and sensitized with saline (Air/Saline), (2) rats exposed to 30 μg/m^3 DEE and sensitized with saline (30 DEE/Saline), (3) rats exposed to 300 μg/m^3 DEE and sensitized with saline (300 DEE/Saline), (4) rats exposed to filtered air and sensitized with OVA (Air/OVA), (5) rats exposed to 30 μg/m^3 DEE and sensitized with OVA (30 DEE/OVA), and (6) rats exposed to 300 μg/m^3 DEE and sensitized with OVA (300 DEE/OVA).

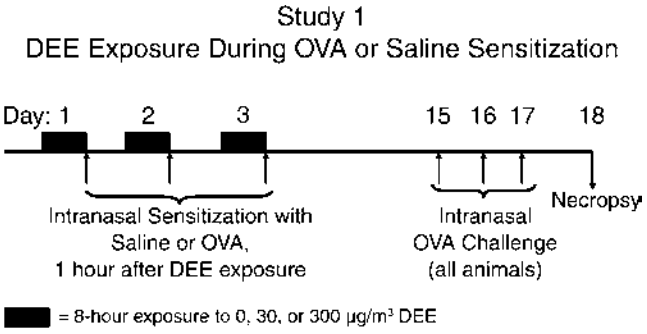

Figure 3. Protocol for study 1, DEE exposure during OVA (or saline) sensitization. Rats were exposed to 0, 30, or 300 μg/m^3 DEE for 8 hours on days 1 through 3 and then sensitized with OVA (or saline) by IN instillation 1 hour after DEE exposure. They were challenged with OVA on days 15 through 17. All necropsies were conducted on day 18. Black bars represent 8-hour exposure to 0, 30, or 300 μg/m^3 DEE.

Study 2. Inhalation Exposure to DEE During Allergen (OVA) Challenge

Male, 10-to-14-week-old BN rats (8 rats/exposure group) were sensitized to OVA by IN instillation with a 0.5% solution of OVA in saline (150 µL/nasal passage) for 3 consecutive days. Airway sensitization procedures and the housing of these animals were also conducted at the LRRI. Twelve days after the end of the IN sensitization, rats were exposed to three concentrations of DEE in whole-body inhalation exposure chambers at the LRRI. As in study 1, rats were exposed for 3 consecutive days, 8 hours/day (7:30 AM to 3:30 PM) to DEE concentrations of 0, 30, or 300 µg/m^3. DEE was generated as it was in study 1.

One hour after the end of each daily inhalation exposure, rats were intranasally challenged with a 1% solution of OVA in saline (150 µL/nasal passage) or with saline alone (no OVA challenge). At the end of the 3-day inhalation exposures, the rats were moved back to the animal housing facilities at the LRRI. Twenty-four hours after the last IN OVA challenge, each rat was killed and airway tissues from the nose and lungs were removed for analysis, as was described for study 1. Study 2 was also conducted in February and March 2003.

Figure 4 summarizes the experimental groups and the exposure and instillation protocols in study 2. The six experimental groups in study 1 were (1) rats exposed to filtered air and challenged with saline (Air/Saline); (2) rats exposed to 30 µg/m^3 DEE and challenged with saline (30 DEE/Saline); (3) rats exposed to 300 µg/m^3 DEE and challenged with saline (300 DEE/Saline); (4) rats exposed to filtered air and challenged with OVA (Air/OVA); (5) rats exposed to 30 µg/m^3 DEE and challenged with OVA (30 DEE/OVA); and (6) rats exposed to 300 µg/m^3 DEE and challenged with OVA (300 DEE/OVA).

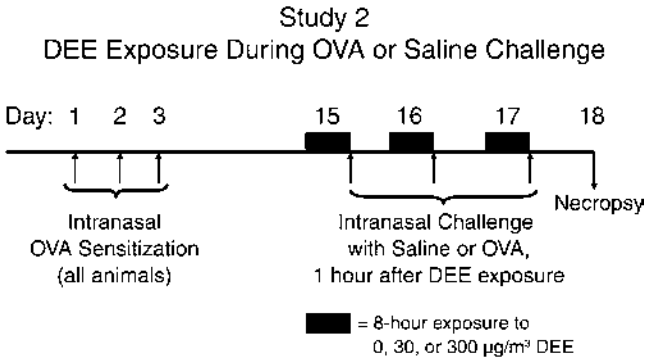

Figure 4. Protocol for study 2, DEE exposure during OVA (or saline) challenge. Rats were sensitized with OVA by IN instillation on days 1 through 3. They were exposed to 0, 30, or 300 µg/m^3 DEE for 8 hours on days 15 through 17 and then challenged with OVA (or saline) by IN instillation 1 hour after DEE exposure. All necropsies were conducted on day 18. Black bars represent 8-hour exposure to 0, 30, or 300 µg/m^3 DEE.

Generation of DEE

DEE was produced by a 406-cc displacement, air-cooled single-cylinder diesel engine generator (5500-watt Yanmar, Model YDG5500E; Yanmar America Corp., Buffalo Grove, IL) operated with a number 2 diesel certification fuel (Phillips Chemical Company, Borger, TX) and commercially available crankcase oil (Rotella T 40-weight motor oil; Shell, The Netherlands). Electrical current was pulled from the engine to provide a constant load during operation. The engine was operated in a steady-state configuration at 100% capacity. Single-cylinder-engine emissions have been used to study the composition and characteristics of exhaust from diesel engines operating with alternative fuels and lubricants, and have also been used in a study that assessed differences in mutagenicity associated with different diesel fuels (McDonald et al. 2004). This system was a surrogate for on-road diesel emissions, and the composition of the material emitted from this engine system showed reasonable similarities to on-road diesel emissions.

DEE Exposure of Laboratory Rats

Exposure concentrations were targeted to mass concentrations of diesel particulate of 30 and 300 µg/m^3. Dilution was achieved with ambient air that had been passed through activated charcoal followed by high efficiency particulate air (HEPA) filtration. Details on the configuration of the exposure system and the approach for characterizing the exposure atmosphere have been previously reported (McDonald et al. 2004). The animal inhalation exposure chambers were conventional 1-m^3 whole-body inhalation exposure chambers (Hazleton H-1000; Lab Products, Maywood, NJ) operated at a flow rate of approximately 250 L/min. Temperature, relative humidity, and flow (as measured by an orifice plate mated to electronic pressure transducer) in the whole-body exposure system were monitored and recorded at all times by LabVIEW (version 6) software. DEE concentrations were adjusted manually for dilution in the air transit system prior to the animal exposures.

Total nitrogen oxides (NO_x = NO + NO_2) were measured using a chemiluminescence analyzer (API Model 200A) that was zeroed with ultra zero air and calibrated with National Institute of Standards and Technology (NIST)–traceable span gases. Carbon monoxide (CO) concentrations were determined using a photoacoustic multigas analyzer (Innove 1312, LumaSense Technologies, Denmark). The manufacturer performed the initial calibration, and calibration was checked on-site against NIST-traceable span gases. Total hydrocarbons (THC) were measured using a real-time flame ionization detector (model 300H;

California Analytical Instruments, Orange, CA) calibrated against a certified propane standard. Sampling and characterization methods for DEPs are described in detail below.

The Hazelton H1000 whole-body exposure chambers were equipped with individual animal cages, an automated watering system, and food bins. Experience has shown that DEE distributes evenly within these exposure chambers, but to assure that animals whose cages were at different levels were exposed to equal amounts of the same material, the animals' cages were rotated daily. The welfare of the animals during exposure and nonexposure periods was maintained and monitored by an attending certified veterinarian. The temperature of the animals' cages was maintained at between 18 and 27°C, and the humidity at between 30% and 70%.

SAMPLING METHODS FOR CAPs AND DEPs IN INHALATION EXPOSURE CHAMBERS

Samples were collected directly from the exposure chambers as described by McDonald and colleagues (2004) for the DEE exposure study, and by Harkema and associates (2004) for the CAPs exposure study in SW Detroit. In brief, during the DEE exposure periods, the mass of particulate matter in the exposure chambers was determined by placing 47-mm Teflon (polytetrafluoroethylene [PTFE]) filters (Gelman Sciences, Ann Arbor, MI) in holders attached to the exhaust manifold of the exposure system, which was operating at a flow rate of 10 L/min. Prebaked quartz filters (Gelman Sciences) were also placed in filter packs and were sampled at a flow rate of 10 L/min. During the exposure study in Detroit, the mass of CAPs was determined by placing 47-mm Teflon filters in Teflon-coated aluminum filter packs (URG Inc., Chapel Hill, NC) attached to the back of the animal exposure chambers and downstream of the concentrator, which was operating at a flow rate of 2 L/min. Backup filters coated with sodium carbonate were placed behind the Teflon filters to correct for the loss of nitrate from the Teflon filters as a result of volatilization during sampling. Prebaked quartz filters were placed in URG filter packs mounted on the exposure chamber and sampled at a flow rate of 2 L/min. The size distribution of DEPs and CAPs was measured using a 10-stage micro-orifice uniform deposit impactor (MOUDI) (MSP Corp., Shoreview, MN) with Teflon filters at a 30-L/min sampling flow rate. The MOUDI was also used to obtain the size-fractionated chemical characteristics of DEPs and CAPs. Flow rates were measured with a calibrated rotameter (Matheson, Montgomeryville, PA). In addition, continuous measurement of the mass of CAPs was performed directly from the exposure chamber. A TEOM (tapered element oscillating microbalance) series 1400a monitor (Rupprecht & Patashnick Co, Inc., Albany, NY) was placed in-line to continuously measure the concentrated fine mass concentration during the 8-hour exposure periods. The TEOM sampled CAPs at a flow rate of 3 L/min, and the TEOM filter was heated to 40°C to match the ambient TEOM measurements. Particulate material for subsequent speciated particle and semivolatile organic analysis was collected on sorbent-impregnated filters (SIFs). To ensure that target analytes were trapped efficiently, two filters were collected in series and analyzed separately. SIFs were made with glass fiber filters (47 mm Pallflex; Pall-Gelman, East Hills, NY) impregnated with finely ground XAD-4 sorbent (Sigma-Aldrich, St. Louis, MO) to trap vapor-phase, semivolatile organic compounds that would desorb or pass through the filters without the inclusion of the sorbent, and prevent particle-phase compounds from desorbing from the filter during collection. XAD-4 was finely ground and precleaned by Soxhlet solvent extraction (1:1 methanol/dichloromethane) for 24 hours prior to use. Filters were dipped into a slurry of the finely ground XAD-4, air dried, and dipped again using procedures adapted from Gundel and Hering (1998). Samples were also collected from the clean-air control chamber on several monitoring days, and field blanks, which included SIF filters that went through transit and sample handling but were not sampled, were also included.

ANALYTICAL METHODS FOR AMBIENT PARTICLES, CAPs, AND DEPs

A brief overview of the analytical methods used for this project is provided below.

GRAVIMETRIC ANALYSIS

All gravimetric determinations were made using a microbalance (MT5; Mettler-Toledo, Columbus, OH) in a temperature-and-humidity-controlled clean laboratory as described in Federal Reference Method (EPA 1997). Measurements of field blanks, filter-lot blanks, replicate analyses, and externally certified standard weights were incorporated into all gravimetric analyses for quality assurance (QA) and quality control (QC) purposes. A minimum of 10% of the preweighed and postweighed filters was reweighed to replicate the analysis, and all the values were within ± 15 µg.

ION ANALYSIS

Teflon filter samples were analyzed for sulfate ions (SO_4^{2-}), nitrate ions (NO_3^-), and ammonium ions (NH_4^+) by ion chromatography (IC) (Model DX-600; DIONEX Corp., Sunnyvale, CA). A detailed description of the analytical

methods we used was previously documented (Keeler et al. 1991, 2002). The IC method incorporates routine daily QA and QC measures, including the use of field blanks, ultrapurified water blanks (Milli-Q, Billerica, MA), replicate analyses, and external standards (Simulated Rainwater ≤ 2, ICA and ICB; High-Purity Standards, Charleston, SC).

CARBON ANALYSIS

Quartz filters were prebaked at 550°C for 2 hours prior to sampling. PM samples were collected onto quartz filters and were stored at −40°C prior to analysis for carbonaceous aerosols using a thermal-optical analyzer (Sunset Laboratory, Forest Grove, OR). The thermal-optical-reflectance method developed by Huntzicker and coworkers (1982) was used to measure organic and elemental carbon (EC) by converting particles to gases under different temperature and oxidation conditions. First, organic carbon (OC) was measured by the reflectance of a sample that was heated in helium up to 550°C. Then, oxygen was added to the helium at temperatures higher than 550°C and desorbed gases were oxidized to carbon dioxide (CO_2) by catalytic conversion and reduced to methane, which was quantified by a flame ionization detector. To account for pyrolytic carbon, a laser monitored the reflectance of the sample.

TRACE ELEMENT ANALYSIS

All equipment and supplies used in sampling were rigorously acid cleaned, and all filters were placed in 50-mm, acid-cleaned petri dishes, sealed with Teflon tape and triple bagged in the field. Sample handling, processing, and analysis of PM samples took place in a class 100 ultraclean laboratory at the UM Air Quality Laboratory (UMAQL), which is designed for ultra-trace-element analysis with an emphasis on the measurement of low-level elements in the environment (Long and Martin, 1992; Keeler et al. 2002). After gravimetric analysis of the Teflon sample filters was completed, the filters were placed in 15-mL centrifuge tubes and wetted with 150 µL of ethanol before the particles were extracted into 10 mL of a solution of 10% nitric acid (HNO_3), which was made on the same day it was used. The extraction solution was then sonicated for 48 hours in an ultrasonic bath and, after that, allowed to passively acid digest for two weeks. Sample extracts were analyzed for a suite of trace elements using inductively coupled plasma–mass spectrometry (ICP–MS) (Finnigan ELEMENT2; Thermo, San Jose, CA). Calibration curves were created using multielement standards (SPEX CertiPrep, Metuchen, NJ) in a 10% HNO_3 solution, to match the sample solution. A set of standard solutions was analyzed, and the software for the Finnigan ELEMENT2 calculated a standard curve, with its r^2 value for each element. The UMAQL routine analysis method incorporated daily QA and QC measures such as the use of field blanks, acid blanks, laboratory blanks, and replicate analyses. Throughout the analysis run, check standards were analyzed as samples to evaluate the instrument stability. Check standards that were not within 15% of the calibration standards resulted in recalibration of the instrument with mass-calibration software. NIST (Gaithersburg, MD) Standard Reference Material (SRM) 1643d was used as a QC standard to check the calibration of the instrument. If measured values were not within ± 20% of the expected values, the calibration procedure was repeated. Minimum detection limits for each element were calculated as three times the standard deviation (SD) of seven consecutive measurements of a spiked blank.

LUNG TISSUE DIGESTION PROCEDURE AND RETAINED ELEMENT ANALYSIS

A complete protocol for digestion of lung tissues (right middle lobe; see Figure 5) and for analysis of trace elements by ICP–MS was developed from biopsy mineral analysis (Braselton et al. 1997) and has been reported elsewhere (Morishita et al. 2004). For QA and QC purposes, blanks and NIST SRM 1577b Bovine Liver were digested with all the samples. The diluted extracts were analyzed directly for trace elements using ICP–MS, as described above.

SPECIATED ORGANIC ANALYSIS

SIF extractions were conducted using microwave-assisted solvent extraction (MARS-X; CEM Corp., Matthews, NC) with approximately 20 mL of analytical grade dichloromethane. Prior to extraction, samples were spiked with a suite of deuterated internal standards closely resembling the chemical structures and behavior of the analytes of interest. These internal standards mimicked the behavior of the target analytes throughout the extraction process. The internal standards used included deuterated phenanthrene, acenaphthene, chrysene, benzo[*a*]pyrene, and dibenzo[*a,h*]anthracene. After extraction, solvents were concentrated by rotary evaporation and filtered through a 0.2 µm Acrodisc filter (Waters Corp.). Extracts were then evaporated to approximately 50 µL under a gentle nitrogen stream and brought to 200 µL with acetonitrile prior to analysis.

Sample extracts were injected (1 µL injections with the injector operated at 300°C) into a gas chromatograph (HP 5890 GC) equipped with a phenylmethylsilicone fused-silica capillary column (30 m, 0.25 mm × 0.25 mm; DB-5 ms; J&W Scientific) and coupled to a mass spectrometer

(5972 MS). Samples were quantified by comparing the response of the deuterated internal standards to the analyte of interest. Analyte response was referenced to calibration curves created from standard solutions made with authentic standards (Sigma-Aldrich) and the NIST SRM 2260. Several blank SIFs were analyzed in the same batch as the samples, and the concentrations of the blanks were subtracted from the sample extract concentrations. The precision of the analysis was approximately 10% among all analytes. The limits of quantitation in solution were approximately 30 pg/µL. This translated to a lower limit of quantitation of approximately 5 ng/m^3 with the low volume collected (5 m^3), and a limit of detection of 1 ng/m^3.

URBAN AMBIENT AEROSOL AND GASEOUS POLLUTANT MEASUREMENTS

Aerosol sampling of size-segregated pollutants was performed for each exposure period using multiorifice impactors (MOIs) as well as $PM_{2.5}$ cyclone samplers. The volume sampled was determined using calibrated dry test meters (DTMs) (Schlumberger, Owenton, KY). The DTMs were calibrated against a spirometer, a primary calibration standard, before and after being deployed in the field. In addition, calibrated rotameters were used to check the flow rate at the beginning and end of the sampling period. Continuous measurements of air pollutants and meteorological parameters, including wind speed, wind direction, temperature, relative humidity, and solar radiation, were recorded from the exposure trailer.

$PM_{2.5}$ AND MOI

Fine particle mass was sampled onto 47-mm Teflon membrane filters. Vacuum pumps were used to draw air through a Teflon-coated cyclone inlet (URG Inc.) at a flow rate of 16.7 L/min. A six-stage MOI (MSP Corp., Shoreview, MN) was used to collect size-fractionated samples. Particles were collected on 37-mm Teflon filters on each stage of the MOI at a flow rate of 30 L/min. The sizes of the particles collected on the six stages were as follows: first stage, > 5 µm; second stage, 5–2.5 µm; third stage, 2.5–1 µm; fourth stage, 1–0.6 µm; fifth stage, 0.6–0.18 µm; and last stage, < 0.18 µm.

ANNULAR DENUDER/FILTER PACK SYSTEMS

Annular denuder/filter pack systems were employed to collect the acidic gaseous species, including nitrous acid (HNO_2), HNO_3, sulfur dioxide (SO_2), ammonia (NH_3), and inorganic fine particulate ions (SO_4^{2-}, NO_3^-, NH_4^+, H^+). A detailed description of the annular denuder sampling system has been previously reported (Keeler et al. 1991).

TEOM

Levels of $PM_{2.5}$ were monitored at 30-minute intervals continuously with a Rupprecht & Patashnick Series 1400a TEOM equipped with a sharp-cut cyclone inlet (BGI Inc., Waltham, MA). The TEOM filter was heated to 40°C to minimize interference from particle-bound water and semivolatile components.

SCANNING MOBILITY PARTICLE SIZER (SMPS)

A Series 3936 SMPS (TSI, Shoreview, MN) system continuously measured the concentrations of submicrometer aerosols, in the range from 10 to 800 nm in diameter, and output the average concentrations for 5-minute intervals. The SMPS consisted of an electrostatic classifier, to determine particle size, and a condensation particle counter, to determine particle concentrations.

O_3

Ambient O_3 was measured using a continuous UV photometric analyzer (TECO 49; Teco Diagnostics, Anaheim, CA). The analyzer was calibrated before and after the exposure studies using an O_3 calibrator (TECO 5009). Ambient air was sampled at a flow rate of 2.0 L/min. Voltage from the analyzer that corresponded to the concentration was transmitted to a data logger. The average O_3 concentration was recorded every 30 minutes. The instrument specifications listed the limit of detection (LOD) as 1 part per billion (ppb) and the precision as ± 1 ppb.

CO

Continuous ambient CO measurements were made using a nondispersive infrared analyzer (TECO 48S). The instrument specifications listed the LOD as 0.04 ppm and the precision as ± 0.1 ppm.

TOTAL NITROGEN OXIDES (NO_x)

Ambient NO_x concentrations were measured using a commercial chemiluminescence detector (TECO 42S). The instrument has a single photomultiplier tube that automatically cycles between the NO and NO_x modes. Signals from the photomultiplier tube were conditioned and then sent to the microprocessor where a mathematical algorithm was utilized to calculate three independent outputs —NO, NO_2, and NO_x. The instrument specifications listed the LOD as 0.4 ppb and the precision as ± 0.4 ppb.

SO_2

Ambient SO_2 concentrations were measured using a pulsed fluorescence technique (TECO 43S). The instrument specifications listed the LOD as 0.6 ppm and the precision as ± 1 ppb.

STATISTICAL ANALYSES FOR AMBIENT AIR, CAPS, AND DEPS

Statistical analyses, including correlation, multiple regression, and analysis of variance (ANOVA), for all of the gaseous pollutants and PM components were performed using a statistical analysis system (SAS 9.1; SAS Institute, Inc., Cary, NC). Data describing the parameters of particle number, mass, and components were expressed as the mean value ± the SD. Statistical significance was tested using the Tukey-Kramer post-hoc test. The criterion for statistical significance was $P \leq 0.05$.

ANIMAL NECROPSIES AND TISSUE SELECTION FOR ANALYSIS

In both the CAPs and DEE inhalation studies, rodents were killed 24 hours after the last IN challenge. At the time of death, the animals were deeply anesthetized and exsanguinated via the abdominal aorta. After death, the trachea was cannulated, and the heart and lung block was removed. The right extrapulmonary bronchus was ligated with suture, and the right lung lobes were removed. The entire right cranial lobe and the microdissected main axial airway from the right caudal lobe were processed for isolation of total RNA (Figure 5). The right-middle and accessory lobes were placed in a Teflon container, frozen in liquid nitrogen, and stored at −80°C. The head of each rat was removed, and the lower jaw and skin were removed. The nasal airways were flushed retrograde through the nasal pharyngeal meatus with 3 mL of 10% neutral buffered formalin prior to immersion and storage in a larger volume of the same fixative.

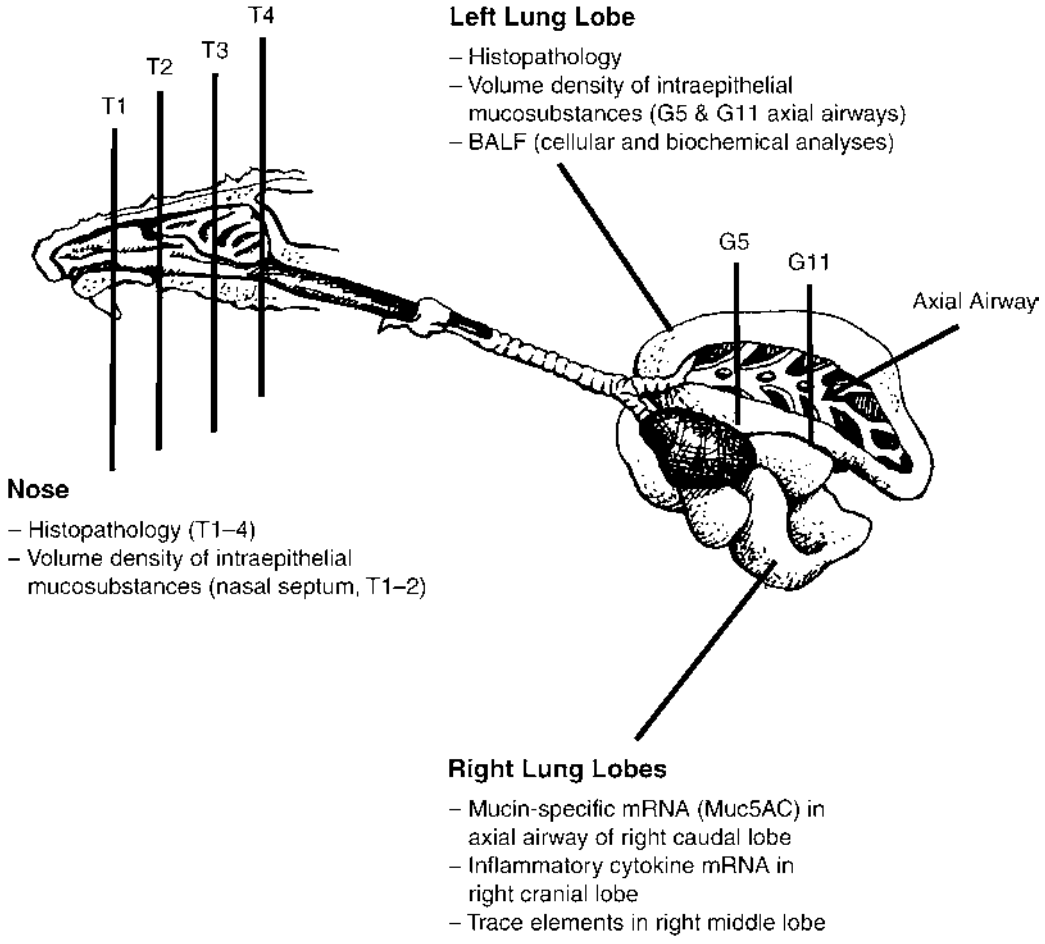

Figure 5. Sites of rat nasal and pulmonary tissues selected for morphometric and biochemical analyses.

The left lung lobe was instilled with 4 mL sterile saline through the tracheal cannula which was withdrawn to recover BALF (Figure 5). A second lavage was performed and combined with the first. The BALF samples from each rat were analyzed for total and differential cell counts, secreted mucins, total protein, cytokines, and soluble mediators.

After bronchoalveolar lavage, the left lung lobe was perfusion-fixed with a 10% neutral-buffered formalin via the trachea at a constant pressure of 30 cm of fixative. After 2 hours of perfusion fixation, the trachea was ligated and the lung lobe was immersed in a large volume of the same fixative. Forty-eight hours later, the left lung lobe was processed for light microscopy as described below. Figure 5 summarizes the sites in the rat respiratory tract selected for analysis.

AIRWAY TISSUE SELECTION AND PROCESSING FOR AIRWAY MORPHOMETRY

The intrapulmonary airways of the fixed left lung lobe from each rodent were microdissected according to a modified version of the technique of Plopper and colleagues (1983), which is fully described in our previous publication (Steiger et al. 1995). Beginning at the lobar bronchus, airways were split down the long axis of the largest daughter branches (i.e., main axial airway, the large-diameter conducting airway) through the twelfth airway generation. Tissue blocks that transverse the entire lobe of the lung at the levels of the fifth and eleventh airway generations of the main axial airway (proximal and distal axial airway generations 5 and 11, respectively) were excised and processed for light microscopy and morphometric analyses (Figure 5). The lung tissue blocks were embedded in paraffin, and 5-µm sections from the proximal face of each block were cut and placed on charged slides (Probe-on Plus; Fisher Scientific, Pittsburg, PA). Tissue sections were histochemically stained with (1) hematoxylin and eosin for routine histopathologic examination or (2) Alcian Blue (pH 2.5)/Periodic Acid Schiff Sequence (AB/PAS) to detect acidic and neutral mucosubstances for the quantitation of stored mucosubstances within the airway epithelium.

After nasal lavage, the nasal airways were fixed in 10% neutral-buffered formalin for at least 24 hours prior to further processing. After fixation, the head was decalcified in 13% formic acid for 4 days, and then rinsed in distilled water for 4 hours. After decalcification, the nasal airways were sectioned at four specific anatomical locations, using the following gross dental and palatine landmarks previously described by Young (1981): The most proximal nasal tissue section (T1) was taken immediately posterior to the upper incisor teeth; the second section (T2) was taken at the incisive papilla of the hard palate; the third nasal tissue section (T3) was taken at the level of the second palatal ridge; and the most distal section (T4) was taken at the level of the first upper molar teeth (Figure 5). Tissue blocks were embedded, sectioned, and stained as described above for the pulmonary tissues.

The following airway levels of the respiratory tract were selected for morphometric determination of the amount of AB/PAS-stained mucosubstances in the surface epithelium lining the airway lumen (intraepithelial mucosubstances [IM]): (1) the proximal nasal septum in T1, (2) the distal nasal septum in T2, (3) the proximal axial airway in the left lung lobe (generation 5), and (4) the distal axial airway in the left lung lobe (generation 11) (Figure 5). All morphometric analyses were conducted in Dr. Harkema's laboratory at MSU according to procedures that are well documented in the literature (Harkema et al. 1997a,b) and briefly described below.

MORPHOMETRIC ANALYSIS OF IM IN AIRWAY EPITHELIUM

The volume densities of stored IM in the surface epithelium lining the nasal and pulmonary airways were determined using image analysis and standard morphometric techniques previously described in detail (Harkema et al. 1997a,b). The quantity of stored mucosubstances per unit area was determined as described by Harkema and associates (1987a,b) and was expressed as the mean volume (nL) of IM/mm^2 of basal lamina ± SEM (n = 8/group).

NUMERIC EPITHELIAL CELL DENSITY

The numeric epithelial cell density was determined by counting the number of epithelial cell nuclear profiles in the surface epithelium and dividing by the length of the underlying basal lamina. The length of the basal lamina was calculated from its contour length in a digitized image using the U.S. National Institutes of Health image system as described in Harkema et al. (1997a,b). The data are expressed as the mean number of epithelial cells present in the surface epithelium lining the axial airway per millimeter of basal lamina ± SEM (n = 8/group).

EPITHELIAL CELL LABELING INDEX AND SYSTEMIC DELIVERY OF BROMODEOXYURIDINE (BRDU)

The number of surface epithelial cells in the axial airway of the left lung lobe that were engaged in DNA synthesis was assessed by positive immunoreactivity with BrdU (Sigma Chemical Co., St. Louis, MO). During the 3 days of inhalation exposure, BrdU (50 mg/kg body weight) was delivered to each rat in vivo at a constant rate (10 µL/hr) via an Alzet osmotic pump (ALZA Corp., Palo Alto, CA),

which was implanted subcutaneously along the dorsal midline of the rat approximately 24 hours before the start of the inhalation exposures to filtered air, CAPs, or DEE. The osmotic pumps were removed at the time of necropsy, approximately 24 hours after the end of the inhalation exposures.

The cumulative epithelial cell labeling index (LI; percentage of BrdU-labeled epithelial cells) in the axial airway was determined using lung sections immunohistochemically stained to label cells with BrdU incorporated into nuclear DNA (i.e., cells that have undergone DNA synthesis) (Henderson et al. 1993; Hotchkiss et al. 1997). BrdU labeling was also standardized per the length of epithelium surface of conducting airways. A unit-length labeling index (ULLI) was determined by dividing the LI by millimeters of basal lamina counted. The data are expressed as LI and ULLI ± SEM (n = 8/group).

ANALYSES OF BALF

CELLULARITY

Total cells recovered by nasal and bronchoalveolar lavages were counted manually using a hemacytometer. Cell smears were made with a cytocentrifuge and stained with Diff-Quick. Differential cell counts that included neutrophils, macrophages, eosinophils, and lymphocytes were determined by counting 200 cells per slide (Hotchkiss et al. 1989a,b). Cell smears, hemacytometer counts, and other cytological analyses were performed in Dr. Harkema's laboratory at MSU. The BALF was centrifuged to remove cells and debris, and the supernatant was stored at −80°C until enzyme-linked immunosorbent assays (ELISAs) were performed.

TOTAL PROTEIN

Protein concentration in the BALF was determined by the bicinchonic acid method using a microplate assay (Pierce Kit #23225). Absorbance readings of protein standards and experimental samples were read at 550 nm using a BioTek ELx808 (BioTek Instruments, Winooski, VT) plate reader.

MUCIN GLYCOPROTEIN

The amount of rMuc5AC mucin glycoprotein in the BALF was monitored by ELISA using a monoclonal antibody (Mucin 5AC Ab-1; NeoMarkers, Fremont, CA) specific for this mucin gene product (Steiger et al. 1992, 1994, 1995). ELISAs were performed in 96-well plates using horseradish-peroxidase-conjugated avidin-biotin complex (ABC Reagent; Vector Laboratories, Burlingame, CA) and a fluorescent substrate. Results for this assay were expressed as units/sec (V_{max}).

OVA-SPECIFIC IgE

Concentrations of OVA-specific IgE in the BALF were estimated by an ELISA using a rabbit monoclonal antibody specific for this gene product (Molecular Probes, Eugene, OR). ELISAs were performed in 96-well plates using horseradish-peroxidase-conjugated avidin-biotin complex and a fluorescent substrate.

ELASTASE AND β-GLUCURONIDASE

Estimates of the amounts of elastase and β-glucuronidase recovered in the BALF were determined by an ELISA using a rabbit monoclonal antibody to either human elastase (Calbiochem, San Diego, CA) or β-glucuronidase (Molecular Probes, Eugene, OR). ELISAs were performed in 96-well plates using horseradish-peroxidase-conjugated avidin-biotin complex (ABC Reagent; Vector Laboratories, Burlingame, CA) and a fluorescent substrate.

CYTOKINES

Concentrations of the rat inflammatory cytokines tumor necrosis factor (TNF)α, interferon (INF)γ, interleukin (IL)-4, IL-6, and IL-10 in the BALF were determined with a BD Cytometric Bead Array (CBA) Rat Protein Flex Set (BD Biosciences, San Diego, CA), which was used, according to the manufacturer's instructions, with a BD FACSCalibur flow cytometer and BD CBA Analysis Software (BD Biosciences). These analyses were also conducted in Dr. Harkema's laboratory at MSU.

GENE EXPRESSION ANALYSIS OF AIRWAY MUCIN AND INFLAMMATORY CYTOKINES

Allergic or asthmatic airway disease has several hallmark features of immunologic involvement as evidenced by the presence of inflammatory cells and associated soluble mediators (e.g., cytokines). The cytokines IL-4 and IL-5 play an essential role in this disease process (Broide et al. 1992; Hogg 1997; Mori et al. 1997; Hogan et al. 1998). Repeated exposure to airway allergens leads to clonal expansion of T cells that secrete IL-4 and IL-5 (Walker et al. 1991; Hogg 1997; Hogan et al. 1998). In light of this, a ribonuclease protection assay (rCK-1; PharMingen, San Diego, CA) and a sensitive quantitative reverse transcriptase–polymerase chain reaction (RT–PCR) method (Vanden Heuvel et al. 1993), requiring minimal amounts of tissue, were employed to measure the steady state mRNA expression of IL-4 and IL-5 and other inflammatory or anti-inflammatory cytokines (e.g., IL-1α, IL-1β, TNFα, TNFβ, INFγ, IL-2, IL-9, IL-10, and IL-13) in pulmonary airways.

We used a similar quantitative RT–PCR assay to determine steady-state levels of airway-specific mucin mRNA

(i.e., rMuc5AC) in pulmonary tissues, as an early indicator of exposure-induced MCM. These molecular analyses were performed in the laboratory of Dr. Harkema using techniques well described in the literature (Wagner et al. 2001).

STATISTICAL ANALYSES OF BIOLOGIC ENDPOINTS

Data describing the type and magnitude of the pulmonary inflammatory response (e.g., from the analyses of the BALF) and airway epithelial alteration (e.g., changes in mucosubstances in the airway epithelium) were expressed as the mean group value ± SEM SD$\sqrt{n}$. The data were subjected to ANOVA for the type of inhalation exposure (e.g., CAPs or filtered air) and airway sensitization or challenge (OVA or saline). Significant differences between experimental groups were identified using appropriate post-hoc tests (e.g., Tukey omega procedure). Transformation of data (e.g., usually log or arcsin^{-1}) was performed, if needed, to render variances homogeneous. When analytical measurements were below the level of detection, these samples were given a value of zero, and ANOVA on ranks was the statistical method used to determine differences between groups. The criterion for statistical significance was $P \leq 0.05$ in all studies.

RESULTS

CHEMICAL AND PHYSICAL CHARACTERIZATION OF AMBIENT $PM_{2.5}$

In July 2003, the levels of fine particulate matter and gaseous pollutants (e.g., O_3, NO_x) were measured in the atmosphere of SW Detroit, at the site of the Maybury Elementary School. The arithmetic average and SD of the ambient $PM_{2.5}$ mass concentration measured by TEOM during the exposure studies in July 2003 was 16.2 ± 10.7 μg/m^3.

Figure 6 shows the average mass size distribution of ambient particles measured during our study. Ambient $PM_{2.5}$ was dominated by submicron particles (PM_1). Our past studies also found that, on average, $PM_{2.5}$ in Detroit accounted for 60% of PM_{10} (up to 80% on some days).

Detailed chemical characterization of the ambient $PM_{2.5}$ collected during the exposure period was also conducted (Table 1). This allowed us to determine that the CAPs to which the laboratory rats were exposed reflected the complex mixture of ambient $PM_{2.5}$. Figure 7 illustrates the day-to-day variation of Fe and La in ambient $PM_{2.5}$ and CAPs. These trace elements measured in CAPs accurately

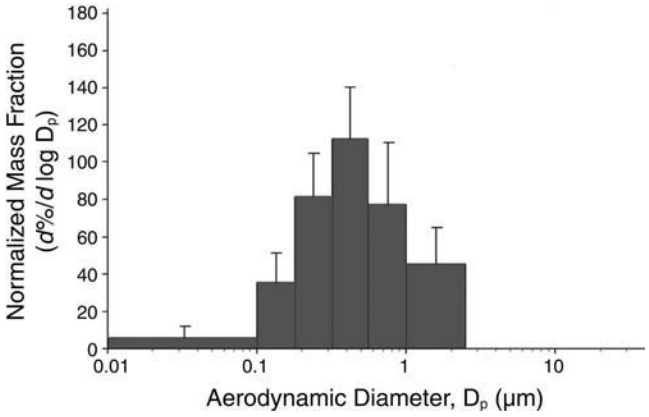

Figure 6. Average mass size distribution of ambient particles in Detroit, July 2003. Bars represent the mean and standard deviations ($N = 21$).

reflected the concentrations of elements in the ambient particles, with only minor variability observed as a result of daily fluctuations in the performance of the concentrator.

Table 2 summarizes the average ultrafine particle number concentrations in ambient air for each day during the two 3-day exposure periods. As shown in the table, the maximum number concentrations occurred on July 14 and 15. Analyses of ambient gaseous pollutants showed SO_2 peaked on these days as well (Table 3), while the highest NO_x concentration was on July 14. Overall, the ambient concentrations of pollutant gases were greater during the first exposure week than the second.

CHEMICAL AND PHYSICAL CHARACTERIZATION OF CAPs

Daily average ambient $PM_{2.5}$ and CAPs mass concentrations during the two 3-day exposure studies in July are shown in Table 4. The average CAPs mass concentration for the first and second exposure periods were 596 ± 563 μg/m^3 and 356 ± 222 μg/m^3, respectively. As discussed in the section Biologic Changes Related to CAPs Exposures in Detroit, the first 3-day exposure period (July 14–16) was the only period during which pathobiologic effects were identified in the CAPs-exposed OVA-challenged rats. Therefore, the chemical and physical characteristics of PM and of its sources were investigated to determine if specific components may be responsible for these observed biologic effects.

First, the chemical composition of the major components of CAPs during the two 3-day exposure studies was examined. As previously observed in SW Detroit, sulfate and OC dominated the CAPs during the summer months (Figure 8). Although, on average, the major components of the CAPs mass during the two 3-day exposure periods in July

Table 1. Ambient $PM_{2.5}$ Composition During 8-Hour Exposure Periods in SW Detroit

	3-Day 8-Hour Exposure Study 1				3-Day 8-Hour Exposure Study 2			
	7/14	7/15	7/16	TWA	7/21	7/22	7/23	TWA
Mass ($\mu g/m^3$)	24	41	3	23 ± 19	18	13	6	13 ± 6
Carbon ($\mu g/m^3$)								
OC	4.2	3.9	1.2	3.1 ± 1.7	3.0	2.2	2.1	2.4 ± 0.5
EC	1.3	0.7	0.3	0.8 ± 0.5	1.2	1.4	0.5	1.1 ± 0.5
Inorganic ions ($\mu g/m^3$)								
SO_4^{2-}	5.4	8.5	0.1	4.7 ± 4.3	1.7	1.3	0.2	1.1 ± 0.8
NO_3^-	3.0	3.2	0.1	2.1 ± 1.7	0.4	0.1	0.2	0.2 ± 0.1
NH_4^+	2.6	4.0	0.1	2.2 ± 2.0	1.2	1.0	0.2	0.8 ± 0.5
Elements (ng/m^3)								
Mg	49	44	15	36 ± 18	42	14	20	25 ± 15
Al	37	36	10	28 ± 15	34	27	19	27 ± 8
S	2149	4146	73	2123 ± 2037	1139	807	193	713 ± 480
V	2.2	1.4	0.1	1.2 ± 1.1	0.6	1.8	0.4	0.9 ± 0.8
Cr	0.9	1.0	1.2	1.1 ± 0.2	4.4	0.8	0.8	2.0 ± 2.1
Mn	21.5	5.0	1.5	9.3 ± 10.7	11.5	3.8	2.7	6.0 ± 4.8
Fe	356	134	38	176 ± 163	181	73	72	109 ± 63
Co	0.09	0.05	0.02	0.05 ± 0.04	0.05	0.03	0.03	0.04 ± 0.01
Ni	1.2	1.5	0.1	1.0 ± 1	0.5	0.4	0.2	0.4 ± 0.2
Cu	9.6	5.0	5.4	6.7 ± 2.5	8.2	5.7	7.0	7.0 ± 1.3
Zn	65	27	8	33 ± 29	77	24	26	43 ± 30
As	8.8	4.8	0.8	4.8 ± 4.0	2.9	4.5	2.9	3.4 ± 0.9
Se	11.8	18.9	0.4	10.3 ± 9.3	5.0	2.3	1.5	2.9 ± 1.8
Rb	0.7	0.2	0.0	0.3 ± 0.3	0.2	0.1	0.0	0.1 ± 0.1
Sr	1.1	1.6	0.2	1.0 ± 0.7	0.8	0.3	0.4	0.5 ± 0.3
Mo	1.7	0.4	1.1	1.1 ± 0.7	0.7	0.4	0.2	0.4 ± 0.3
Cd	0.3	0.2	0.1	0.2 ± 0.1	0.5	0.1	0.1	0.3 ± 0.2
Ba	9.2	3.8	1.9	5.0 ± 3.8	4.5	4.8	4.2	4.5 ± 0.3
La	0.37	0.22	0.01	0.20 ± 0.18	0.87	0.05	0.03	0.31 ± 0.48
Sm	0.01	0.01	0.00	0.00 ± 0.00	0.01	0.00	0.00	0.00 ± 0.00
Pb	9.9	3.7	1.4	5.0 ± 4.4	4.3	2.5	1.9	2.9 ± 1.3

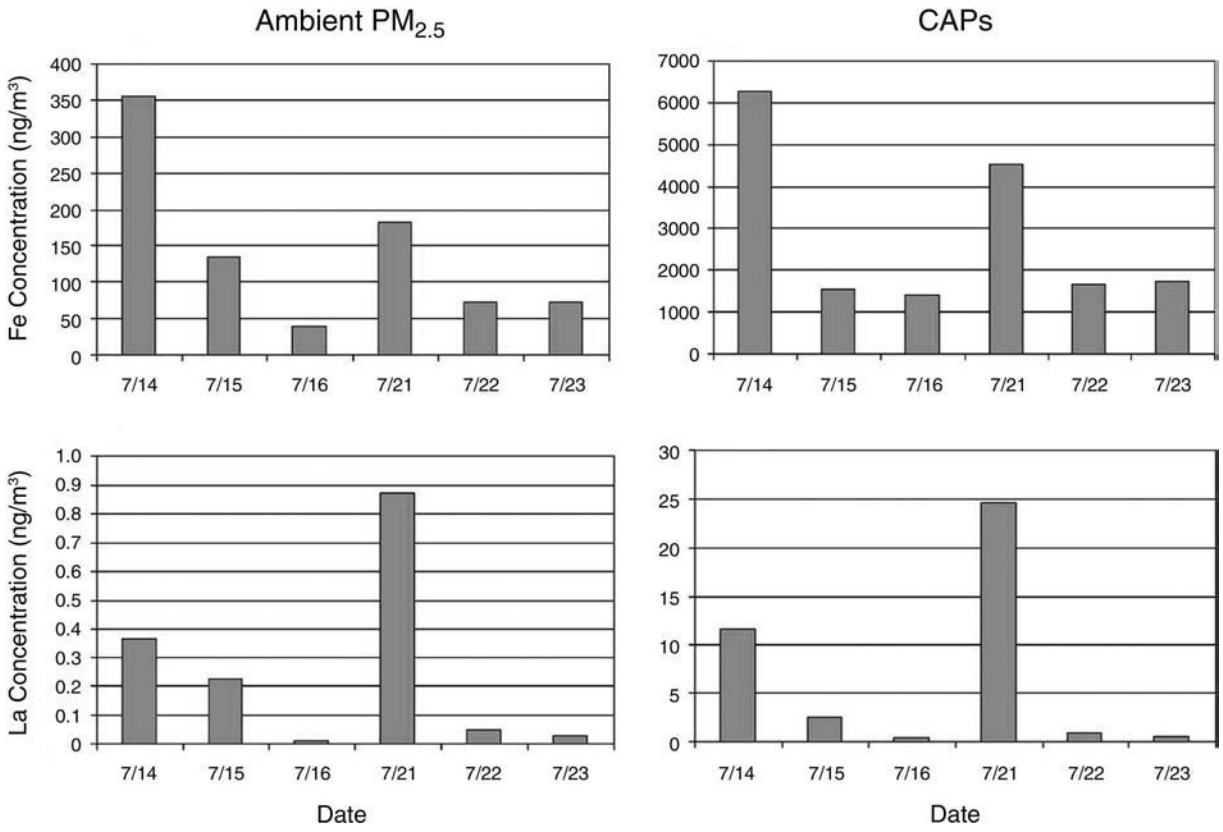

Figure 7. Daily variations of the trace elements Fe and La in ambient $PM_{2.5}$ and CAPs during the inhalation exposure periods in July 2003.

Table 2. Average Ultrafine (< 0.1 μm) Particle Number Concentration During 8-Hour Exposure Periods in SW Detroit

Date	*N*	Mean ($\times 10^4$/cm^3)	Median	Min	Max	STD
7/14	97	2.8	2.7	0.9	5.9	0.8
7/15	97	2.5	2.3	0.9	6.4	1.1
7/16	97	1.5	1.7	0.3	2.6	0.6
7/21	96	2.8	2.8	0.7	4.7	0.7
7/22	97	1.8	2.1	0.5	3.2	0.8
7/23	97	2.2	2.0	1.1	5.0	0.8

Table 3. Average Concentrations of Primary Gaseous Pollutants During 8-Hour Exposure Periods in SW Detroit

	O_3 (ppb)		CO (ppm)		SO_2 (ppb)		NO_x (ppb)	
Date	Mean	Max	Mean	Max	Mean	Max	Mean	Max
7/14	41	78	1.7	3.3	14	47	26	141
7/15	44	62	1.2	1.5	18	42	4	12
7/16	38	61	1.1	1.1	1	1	4	11
7/21	17	33	1.3	2.9	4	13	10	31
7/22	28	53	1.1	1.3	4	7	9	51
7/23	24	39	1.1	1.4	1	3	11	51

Table 4. CAPs and Ambient $PM_{2.5}$ Mass Concentrations During the Two 3-Day Exposure Studies in SW Detroit

3-Day Exposure	Concentration Efficiency Factors	CAPs ($\mu g/m^3$)	Ambient $PM_{2.5}$ ($\mu g/m^3$)
7/14	21	495	24
7/15	30	1202	41
7/16	26	89	3
TWA	26	596	23
7/21	31	570	18
7/22	29	372	13
7/23	20	127	6
TWA	27	356	13

were similar, the details of the trace element composition and the meteorological data indicated $PM_{2.5}$ contributions were from local combustion sources on July 14 and 15, which was during the first 3-day exposure period.

As shown in Table 5, the concentrations of the following trace elements in the CAPs reached their highest levels on July 14 (week 1): Mg, Al, Cr, Mn, Fe, Co, Ni, As, Rb, Sr, Mo, Cd, Ba, Sm, and Pb. In particular, Rb reached the highest level that we have ever observed during our exposure studies in SW Detroit. This element has been found to be associated with the emissions from sewage-sludge incinerators.

A summary of the concentrations of polycyclic aromatic hydrocarbons (PAHs) in the CAPs is shown in Table 6. This list represents a broad range in PAH volatilities (and molecular weights), from phenanthrene (molecular weight 178) to benzo[*ghi*]perylene (molecular weight 276). While there were detectable amounts of PAHs during all exposure periods, the PAH concentrations measured on July 14 and 15 were significantly higher (> 60%) than those during the remainder of the exposure days.

TRACE ELEMENTS FROM PARTICLES RETAINED IN LUNG TISSUES OF CAPs-EXPOSED RATS

A suite of trace elements was successfully quantified in rat lung tissues after inhalation exposure to CAPs generated from urban Detroit air. During the first 3-day exposure period in July 2003, elevated amounts of La, Rb, and Mo were recovered from the lungs of rats exposed to CAPs. Figure 9 shows Mo was recovered from lungs of OVA-challenged rats exposed to CAPs. It is interesting to note that the concentrations of these anthropogenic trace elements (La, Rb, and Mo) recovered from the lung tissues were also elevated in ambient $PM_{2.5}$ on July 14.

BIOLOGIC CHANGES RELATED TO CAPs EXPOSURES IN DETROIT

Nasal and Pulmonary Histopathology

In week 1 and week 2, Air/Saline and CAPs/Saline rats had no exposure-related histopathology in nasal or pulmonary tissue sections examined by light microscopy. OVA-challenged rats had a mild-to-moderate allergic rhinitis. This nasal response was characterized by a mixed inflammatory response as evidenced by eosinophils, lymphocytes, plasma cells, and occasional neutrophils found in the nasal mucosa, which were principally lined by transitional epithelium (in the T1 section) or respiratory epithelium (in the T1 through T4 sections). Interestingly, this inflammatory response was minimal or absent in the mucosa lined by olfactory epithelium (in the T2 through T4 sections). There was mild mucus cell hyperplasia and hypertrophy in the respiratory epithelium lining the proximal

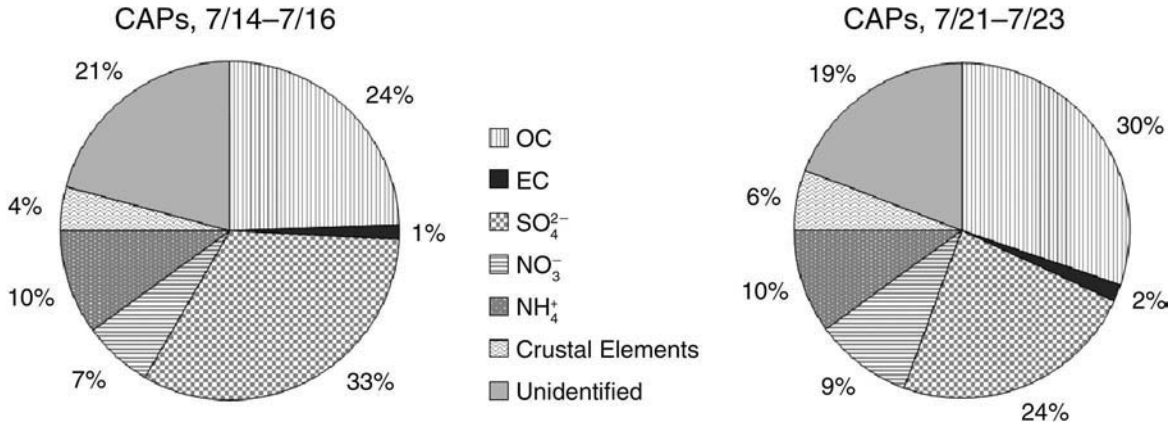

Figure 8. Average chemical composition of the major components of CAPs during the two 3-day exposure studies in SW Detroit.

Table 5. Composition of CAPs During 8-Hour Exposure Periods in SW Detroit

	3-Day 8-Hour Exposure Study 1				3-Day 8-Hour Exposure Study 2			
	7/14	7/15	7/16	TWA	7/21	7/22	7/23	TWA
Mass ($\mu g/m^3$)	495	1,202	89	596 ± 563	570	372	127	356 ± 222
Carbon ($\mu g/m^3$)								
OC	206	186	37	143 ± 92	123	140	62	108 ± 41
EC	13	7	4	8 ± 5	8	5	5	6 ± 2
Inorganic ions ($\mu g/m^3$)								
SO_4^{2-}	56	355	2	137 ± 190	123	47	7	59 ± 59
NO_3^-	64	22	6	29 ± 30	45	22	3	23 ± 21
NH_4^+	51	75	3	42 ± 37	45	24	7	25 ± 19
Elements (ng/m^3)								
Mg	1,086	377	421	628 ± 397	907	265	507	560 ± 324
Al	1,058	348	308	571 ± 422	814	244	461	506 ± 287
S	24,906	42,190	1,050	22,715 ± 20,657	34,419	16,697	3,506	18,208 ± 15,512
V	26	10	2	12 ± 13	12	31	4	16 ± 14
Cr	18	9	5	10 ± 7	13	8	13	12 ± 3
Mn	587	57	47	230 ± 309	316	89	71	158 ± 137
Fe	6,268	1,526	1,396	3,063 ± 2,776	4,535	1,650	1,723	2,636 ± 1,645
Co	1.7	0.5	1.1	1.1 ± 0.6	1.0	0.5	0.6	0.7 ± 0.3
Ni	16	6	1	8 ± 7	10	6	3	7 ± 4
Cu	252	47	209	170 ± 108	289	230	259	259 ± 30
Zn	1,494	337	154	662 ± 727	1,811	526	195	844 ± 854
As	68	25	4	32 ± 32	44	50	52	49 ± 4
Se	130	147	3	93 ± 79	133	41	24	66 ± 59
Rb	14	3	1	6 ± 7	5	1	1	2 ± 2
Sr	30	18	10	20 ± 10	21	9	10	13 ± 7
Mo	15	4	6	8 ± 6	10	5	2	6 ± 4
Cd	4	2	1	2 ± 2	4	1	2	2 ± 1
Ba	273	49	76	133 ± 122	134	132	117	128 ± 9
La	11.7	2.5	0.4	4.8 ± 6.0	24.6	1.0	0.5	8.7 ± 13.8
Sm	0.2	0.1	0.1	0.1 ± 0.1	0.2	0.0	0.1	0.1 ± 0.1
Pb	213	54	42	103 ± 95	87	58	36	60 ± 26

Table 6. Average PAH Concentrations in CAPs During 8-Hour Exposure Periods in SW Detroit*

	7/14	7/15	7/16	7/21	7/22	7/23
Sum of Measured PAHs	281.9	185.6	29.0	93.9	72.1	108.5
Speciated PAHs						
Phenanthrene	46.7	31.7	21.7	40.9	28.9	27.9
Anthracene	2.2	1.3	0.2	1.8	1.3	17.8
Methylphenanthrenes						
a-Methylphenanthrene	9.0	6.8	2.1	3.7	4.4	3.8
b-Methylphenanthrene	9.0	5.5	2.6	5.2	5.6	6.2
c-Methylphenanthrene	1.5	0.3	<1.0	0.6	<1.0	<1.0
4-Methylphenanthrene	6.0	4.0	1.0	2.6	3.8	4.0
1-Methylphenanthrene	4.1	3.1	0.3	1.8	3.3	2.3
Dimethylphenanthrenes						
3,6-Dimethylphenanthrene	8.2	6.8	<1.0	<1.0	<1.0	<1.0
2,6-Dimethylphenanthrene	4.8	3.5	<1.0	<1.0	<1.0	<1.0
2,7-Dimethylphenanthrene	3.2	2.2	<1.0	<1.0	<1.0	<1.0
1,3+2,10+3,9-Dimethylphenanthrene	1.7	1.3	<1.0	<1.0	<1.0	<1.0
1,6+2,9-Dimethylphenanthrene	35.9	20.5	<1.0	<1.0	<1.0	<1.0
1,7-Dimethylphenanthrene	4.4	0.0	<1.0	<1.0	<1.0	<1.0
2,3-Dimethylphenanthrene	0.0	0.0	<1.0	<1.0	<1.0	<1.0
Molecular Weight 202-228 PAHs						
Fluoranthene	20.1	8.5	<1.0	9.7	<1.0	<1.0
Pyrene	15.5	7.0	<1.0	8.0	<1.0	<1.0
Fluorine	15.4	39.4	<1.0	0.0	<1.0	12.3
Benzo[*a*]anthracene	7.5	5.2	<1.0	4.2	<1.0	<1.0
Chrysene/triphenylene	16.1	6.8	<1.0	5.7	17.4	<1.0
Molecular Weight 252-278 PAHs						
Benzo[*b*+*j*+*k*]fluoranthene	4.8	4.6	<1.0	<1.0	<1.0	<1.0
Benzo[*e*]pyrene	11.3	5.0	<1.0	3.8	<1.0	<1.0
Perylene	0.8	0.6	<1.0	0.4	<1.0	<1.0
Benzo[*a*]pyrene	9.0	3.8	<1.0	1.2	4.3	<1.0
Indeno[1,2,3-*cd*]pyrene	31.5	11.8	<1.0	<1.0	0.6	<1.0
Benzo[*ghi*]perylene	8.5	3.0	<1.0	<1.0	<1.0	<1.0
Sulfur-Containing PAHs						
Dibenzothiophene	4.6	3.1	1.2	4.2	2.4	34.2

* Concentration units: ng/m^3.

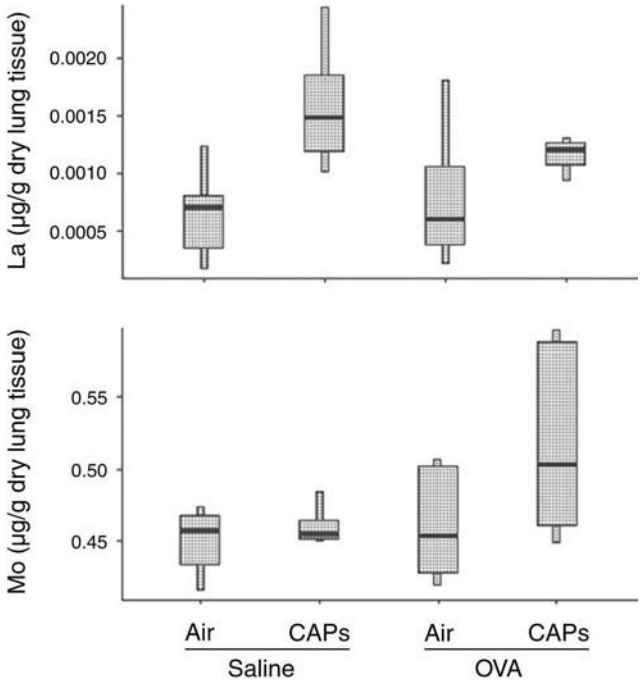

Figure 9. Amounts of La and Mo in lung tissues of rats exposed to filtered air or CAPs and challenged with saline or OVA during week 1 of the study (July 14–16, 2003). Concentrations are in µg of trace elements per gram of dry lung tissue. The central line in each box marks the median value; the top and bottom edges of the box represent the 25th and 75th percentiles. The bars extending from the top and bottom of the boxes show the highest and lowest values.

and distal septum (in the T1 and T2 sections, respectively) and lining the nasopharyngeal meatus (in the T3 and T4 sections). In addition, there was marked lymphoid hyperplasia in the nasal-associated lymphoid tissues lining the nasopharyngeal meatus in the T3 section.

In weeks 1 and 2, the magnitude of all of these OVA-induced nasal lesions was similar in the Air/OVA and CAPs/OVA rats, suggesting that CAPs exposure had no microscopically detectable effect on the nasal epithelial and inflammatory responses to OVA.

The principal morphologic lesions in the lungs of BN rats intranasally challenged with OVA were an allergic bronchiolitis and alveolitis (allergic bronchopneumonia). There was a noticeable proximal to distal decrease in the severity of the bronchopneumonia in the left lung lobe of each animal, with more inflammatory lesions in the proximal lung section (G5 axial-airway level, closest to the hilus) compared to the more distal section (G11 axial-airway level) (Figure 10).

OVA-induced inflammatory and epithelial lesions in the conducting airways involved the large-diameter, proximal axial airways and the small-diameter, distal preterminal and terminal airways. Inflammatory and epithelial lesions were usually more severe in the more proximal axial bronchioles compared to those in the more distal preterminal and terminal bronchioles. OVA-induced bronchiolitis was characterized by peribronchiolar edema associated with a mixed inflammatory cell influx of eosinophils, lymphocytes, plasma cells, and occasional neutrophils. Peribronchiolar inflammation was principally located in the subepithelial interstitial tissues (e.g., the lamina propria and submucosa) with markedly fewer inflammatory cells in the surface epithelium lining these airways.

Bronchiole-associated lymphoid tissues in these OVA-exposed airways were also enlarged as a result of lymphoid hyperplasia. Perivascular, interstitial accumulation of a similar mixture of eosinophils and mononuclear cells, along with perivascular edema, was also present in the lungs of OVA-challenged rats (i.e., surrounding pulmonary arteries adjacent to bronchioles and pulmonary veins scattered throughout the alveolar parenchyma).

OVA-challenged rats exposed either to air or CAPs had MCM and hyperplasia with increased amounts of AB/PAS-stained mucosubstances (i.e., IM) in the surface epithelium lining the affected large-diameter bronchioles, including the proximal and distal axial airways (Figure 11; see morphometric results in the first section below entitled Changes in Airway Epithelial Cell Density and Labeling Indices). Saline-challenged rats exposed to filtered air or CAPs had significantly fewer mucus cells and IM compared to the OVA-challenged animals. The amounts of IM in saline-challenged rats exposed to CAPs and saline-challenged rats exposed to filtered air were not significantly different.

In addition to the perivascular and peribronchiolar lesions, there were varying-sized focal areas of allergic alveolitis in the lung parenchyma (Figure 12). These alveolar lesions were characterized by accumulation of large numbers of alveolar macrophages (AM), epithelioid cells, and eosinophils, with lesser numbers of lymphocytes, monocytes, and plasma cells in the alveolar airspace. Often the alveolar septa in these areas of alveolitis were thickened due to type II pneumocyte hyperplasia and hypertrophy, intracapillary accumulation of inflammatory cells, and capillary congestion.

During week 1, OVA-challenged rats exposed to CAPs (CAPs/OVA group) had a more severe allergic bronchopneumonia than OVA-challenged rats exposed only to filtered air (Figure 12). This was reflected in both the severity and distribution of the allergic bronchiolitis and alveolitis. Air/OVA rats had a mild-to-moderate allergic bronchopneumonia, with inflammatory and epithelial lesions in approximately one-fourth to one-third of the lung lobe.

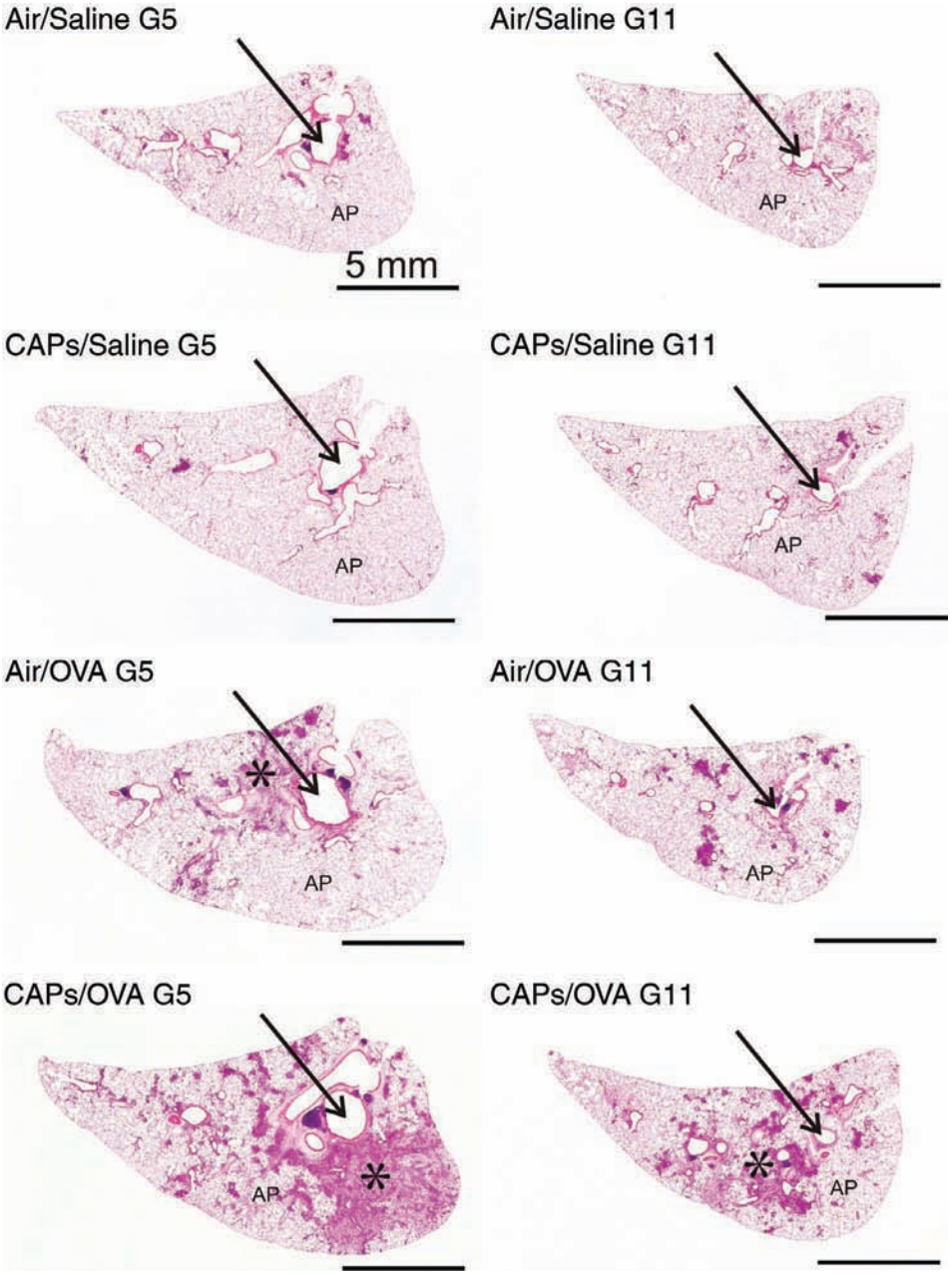

Figure 10. Light photomicrographs of tissue sections taken from the left lung lobe perpendicular to the main axial airway (arrows) at the level of the proximal airway generation 5 (G5) and the distal airway generation 11 (G11) in rats exposed to filtered air or CAPs and intranasally challenged with saline or OVA during week 1. Allergic bronchopneumonia (represented by an asterisk) is evident only in the lungs of Air/OVA and CAPs/OVA rats. Lung lesions are more severe in the tissue sections from CAPs/OVA rats compared to those from Air/OVA rats. Tissues were stained with hematoxylin and eosin. Scale bars are 5 mm. AP = alveolar parenchyma.

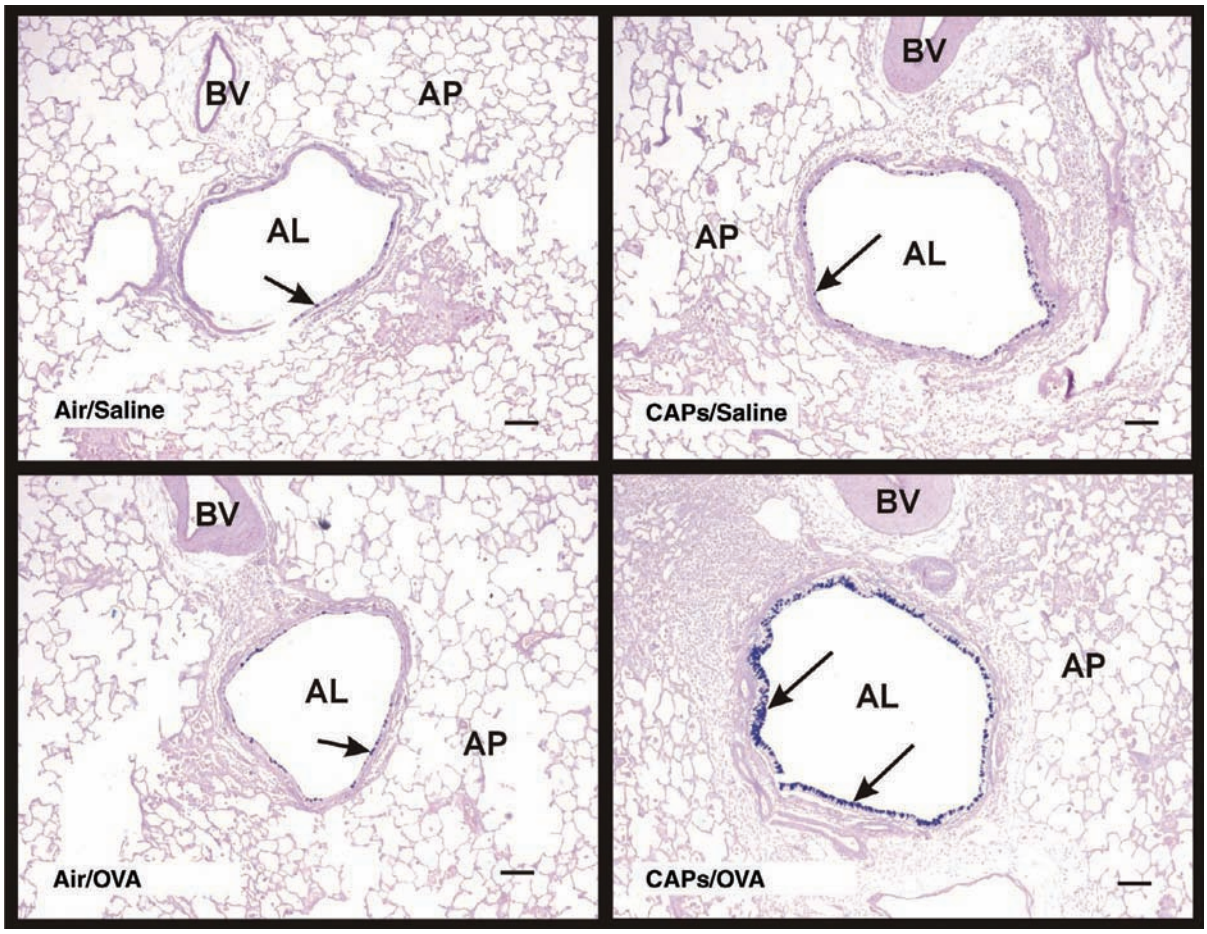

Figure 11. Light photomicrographs of the respiratory airway epithelium lining the intrapulmonary axial airway (generation 11) in the left lung lobe of OVA-challenged rats exposed either to air or CAPs during week 1. Tissues were stained with AB/PAS for specific identification of IM (dark magenta stain identified by arrows). AL = airway lumen; AP = alveolar parenchyma; and BV = blood vessel. Scale bars are 100 µm.

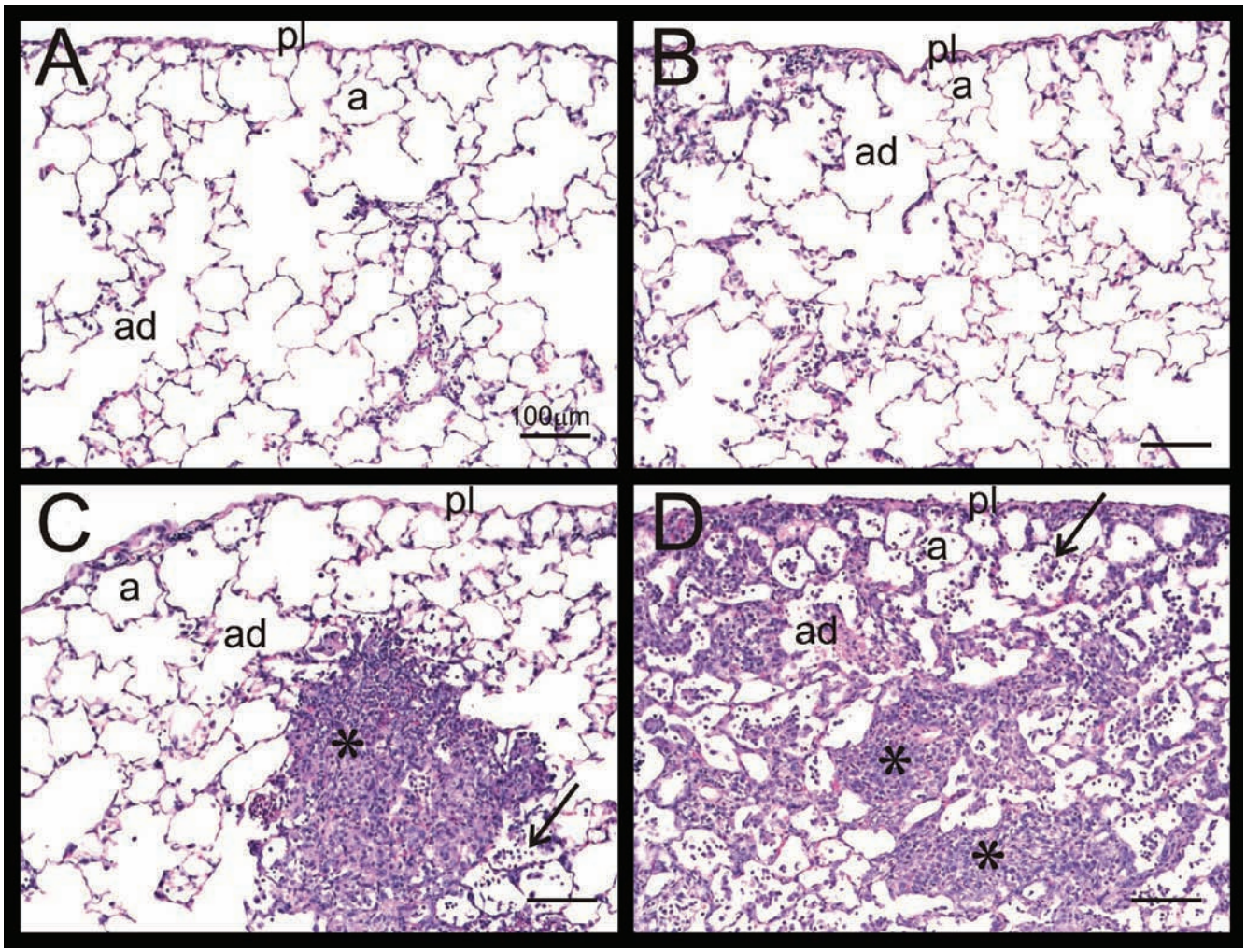

Figure 12. Light photomicrographs of the alveolar parenchyma from the left lung lobe of rats exposed to and challenged with (A) Air/Saline, (B) CAPs/Saline, (C) Air/OVA, or (D) CAPs/OVA during week 1. Focal areas of alveolitis composed mainly of macrophages, epithelioid cells, lymphocytes, and eosinophils (indicated with asterisks) that obscure alveolar airspaces, along with increased numbers of inflammatory cells and macrophages in adjacent alveolar lumens (indicated by arrows), are evident in C and D, but not in A and B. The most severe allergic alveolitis is evident in the lungs from the CAPs/OVA rats (D). Tissues were stained with hematoxylin and eosin. pl = pleura; a = alveolus; and ad = alveolar duct. Scale bars are 100 µm.

In contrast, CAPs/OVA rats had a moderate-to-marked bronchopneumonia, with lesions in approximately one-half or more of the lung lobe. CAPs/OVA rats also had more severe MCM in the epithelium lining the large-diameter axial airways compared to OVA-challenged rats exposed only to filtered air (Figure 12). This was reflected in the mean volume densities of IM in the axial airways (see morphometric results in Table 13).

Lung lesions in the OVA-challenged rats of week 1 and week 2 were similar in character and severity, with the marked exception that CAPs/OVA rats in week two did not have an enhanced allergic bronchopneumonia as compared to the Air/OVA rats. There were no microscopic differences, between the air-exposed and CAPs-exposed rats, in the severity or distribution of the inflammatory and epithelial lesions induced by OVA.

Changes in IM Lining Pulmonary Airways

CAPs exposure during weeks 1 and 2 had no effect on the amounts of IM in the main axial airways of saline-challenged rats (Figure 13). In week 1, IM were not statistically increased in Air/OVA rats compared to those in the Air/Saline control rats. However, IM in the proximal and distal pulmonary airways of CAPs/OVA rats were significantly increased compared to those in Air/OVA control rats (33% and 200% increases, respectively). In week 2, however, CAPs exposure did not enhance the IM induced by OVA alone.

CAPs exposures of saline-challenged rats had no effects on IM in the epithelium lining the nasal septum (Figure 14). In both studies, OVA challenge induced increases of IM in both the proximal and distal septum. IM in the proximal nasal septum were not altered by CAPs exposure in either week 1 or 2. IM in the distal septum of CAPs-exposed, OVA-challenged rats were not different from those of air-exposed animals, in either week 1 or 2.

Changes in Airway Epithelial Cell Density and Labeling Indices

The morphometry of the pulmonary airway epithelium that reflects cell proliferation (i.e., numeric cell densities and the BrdU labeling indices) is presented in Table 7.

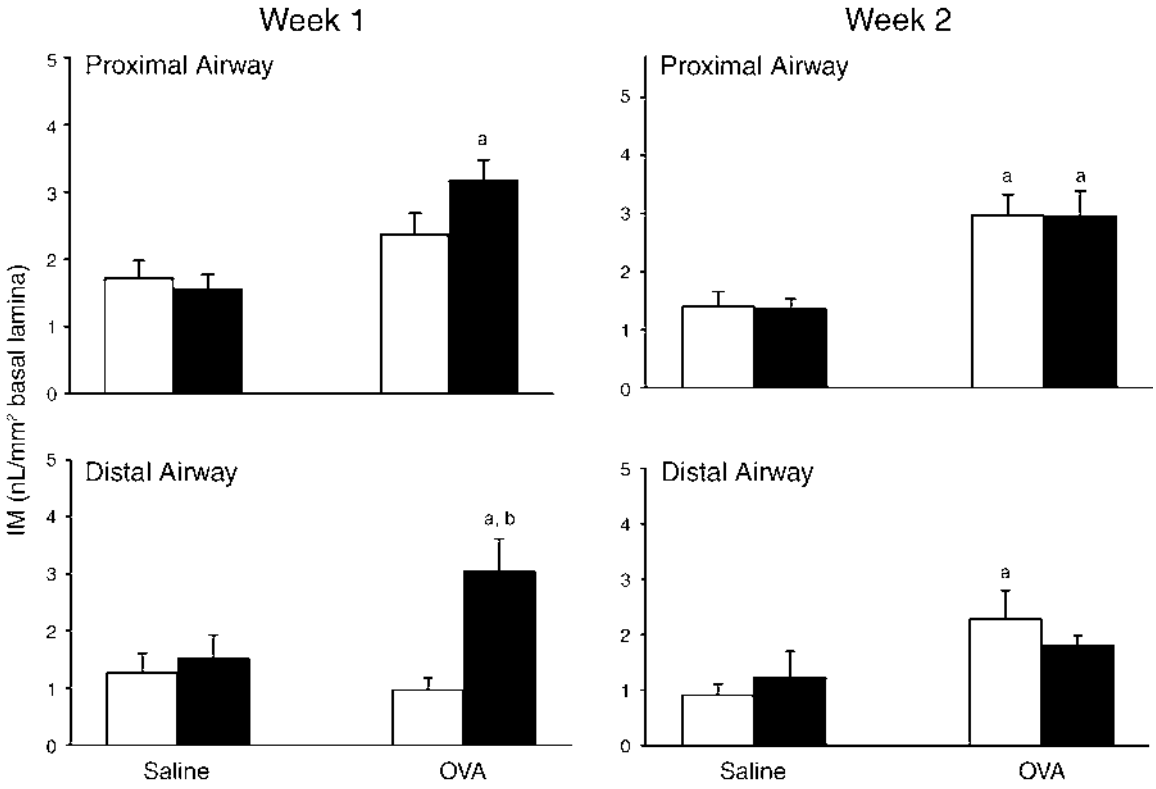

Figure 13. IM in pulmonary proximal (top graphs) and distal (lower graphs) conducting airways. Rats were exposed to CAPs and challenged with saline or OVA for 3 consecutive days during week 1 (left graphs) or week 2 (right graphs). Twenty-four hours later lungs were collected, processed, and stained with AB/PAS for specific identification of IM as described in the text. a = significantly different from respective group challenged with saline; b = significantly different from respective group exposed to filtered air; $P \leq 0.05$. Clear bars represent filtered air; black bars represent CAPs.

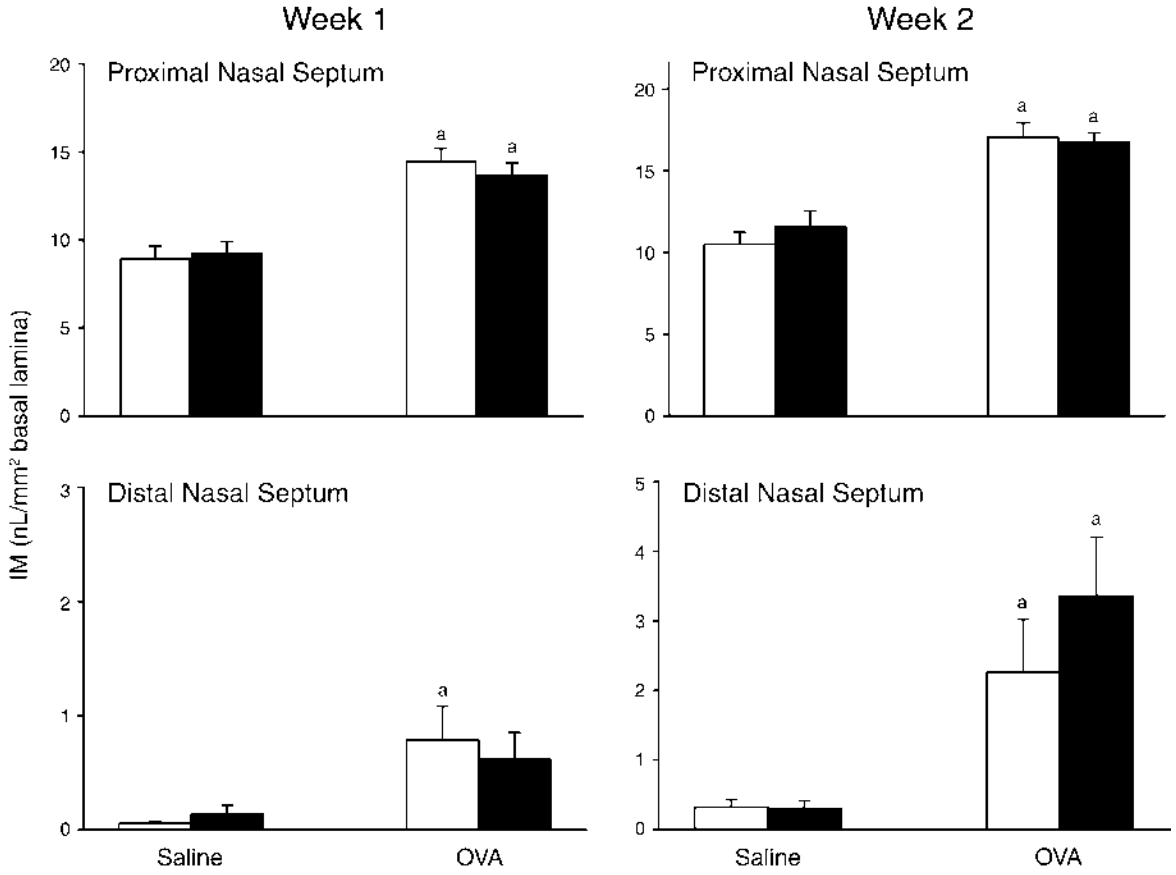

Figure 14. IM in the proximal and distal nasal septum. Rats were exposed to CAPs and challenged with saline or OVA for 3 consecutive days. Twenty-four hours later the nasal septum was collected and processed and stained with AB/PAS for specific identification of IM as described in the text. a = significantly different from respective group challenged with saline; $P \leq 0.05$. Clear bars represent filtered air; black bars represent CAPs. Note the use of different y-axis scales in the bottom two figures.

Table 7. Effects of CAPs Exposure and OVA Challenge on Epithelial Cell Proliferation in Pulmonary Axial Airways

	Proximal Airways			Distal Airways		
Group	LI (%)	ULLI (%/mm)	Numeric Cell Density (total cells/mm basal lamina)	LI (%)	ULLI (%/mm)	Numeric Cell Density (total cells/mm basal lamina)
Week 1						
Air/Saline	1.93 ± 0.32	2.93 ± 0.56	170.74 ± 12.36	2.58 ± 0.44	5.72 ± 1.11	214.71 ± 11.46
CAPs/Saline	1.64 ± 0.31	2.64 ± 0.54	156.95 ± 10.86	2.49 ± 0.83	5.74 ± 2.15	210.03 ± 15.05
Air/OVA	9.57 ± 0.95[a]	17.67 ± 2.74[a]	178.18 ± 11.77	21.39 ± 3.81[a]	46.25 ± 7.87[a]	222.13 ± 12.51
CAPs/OVA	9.42 ± 0.78[a]	18.05 ± 1.74[a]	190.37 ± 3.47	20.12 ± 3.35[a]	41.47 ± 6.78[a]	208.47 ± 7.15
Week 2						
Air/Saline	4.08 ± 0.81	6.32 ± 1.30	154.26 ± 9.43	2.85 ± 0.35	6.01 ± 0.87	207.73 ± 8.08
CAPs/Saline	3.28 ± 0.53	5.34 ± 1.01	156.77 ± 9.37	2.33 ± 0.42	4.76 ± 1.05	195.01 ± 14.43
Air/OVA	11.22 ± 1.75[a]	20.17 ± 3.90[a]	175.04 ± 9.14	14.90 ± 2.44[a]	31.64 ± 4.96[a]	214.26 ± 9.65
CAPs/OVA	11.75 ± 1.49[a]	21.17 ± 2.72[a]	182.04 ± 9.28	15.43 ± 2.87[a]	32.73 ± 6.52[a]	209.70 ± 7.40

[a] Significantly different from respective group challenged with saline alone; $P \leq 0.05$.

Table 8. Effects of CAPs Exposure and OVA Challenge on Bronchoalveolar Lavage Cellularity (cells/mL BALF)

Group	Total Cells	Macrophage Monocytes	Neutrophils	Eosinophils	Lymphocytes
Week 1					
Air/Saline	103,928 ± 20,859	89,725 ± 13,867	2,973 ± 1,621	10,353 ± 9,391	890 ± 857
CAPs/Saline	117,143 ± 21,407	93,177 ± 13,286	3,159 ± 922	19,743 ± 12,604	1,077 ± 371
Air/OVA	237,500 ± 49,654[a]	125,848 ± 44,499[a]	65,576 ± 12,297[a]	43,262 ± 11,978[a]	2,813 ± 1,030[a]
CAPs/OVA	319,688 ± 53,969[a,b]	84,108 ± 11,825	85,810 ± 12,696[a]	142,849 ± 36,187[a,b]	7,023 ± 1,670[a,b]
Week 2					
Air/Saline	83,125 ± 8,703	73,605 ± 7,952	1,581 ± 428	7,764 ± 4,108	175 ± 174
CAPs/Saline	93,571 ± 12,656	73,243 ± 6,372	3,850 ± 858	15,874 ± 8,373	602 ± 323
Air/OVA	319,688 ± 65,859[a]	100,561 ± 17,915	113,357 ± 30,060[a]	101,534 ± 35,604[a]	1,818 ± 1,410[a]
CAPs/OVA	339,687 ± 65,859[a]	106,180 ± 18,284	123,121 ± 39,510[a]	108,498 ± 26,881[a]	1,996 ± 1,106[a]

[a] Significantly different from respective group challenged with saline (i.e., not challenged with OVA [control]); $P \leq 0.05$.
[b] Significantly different from respective group exposed to filtered air; $P \leq 0.05$.

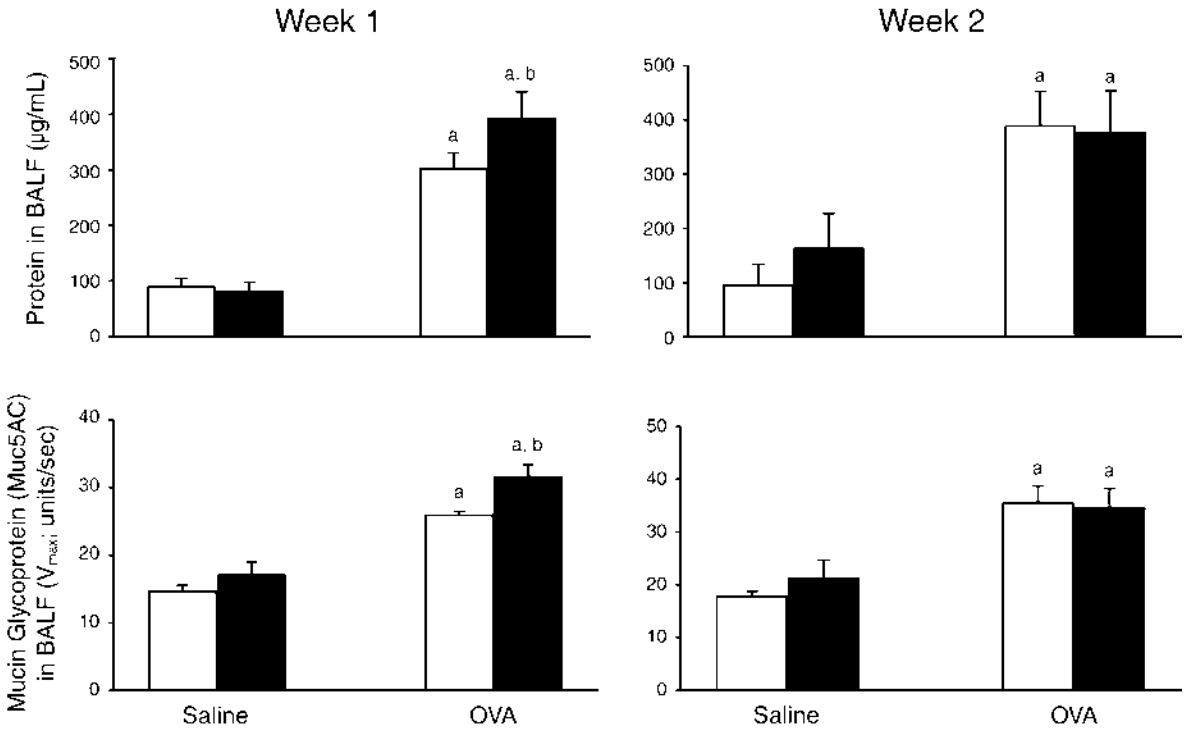

Figure 15. Protein (top graphs) and mucin glycoprotein (Muc5AC) (lower graphs) in BALF. Rats were exposed to CAPs and challenged with saline or OVA for 3 consecutive days, and BALF was collected 24 hours later. Protein and mucins in BALF were analyzed as described in the text. a = significantly different from respective group challenged with saline; b = significantly different from respective group exposed to filtered air; $P \leq 0.05$. Clear bars represent filtered air; black bars represent CAPs. Note the use of different y-axis scales in the bottom two figures.

Compared to the Air/Saline controls, OVA challenge and/or CAPs exposure did not induce changes in the total numbers of epithelial cells lining the proximal or distal axial airways (G5 and G11, respectively) in the left lung lobe of exposed rats. OVA-challenged rats, however, in both weeks 1 and 2, did have significant increases in both the BrdU LI and ULLI in the airway epithelium lining both proximal and distal axial airways. CAPs exposures did not alter these OVA-induced increases in the BrdU labeling indices in either axial airway location. In addition, CAPs exposures alone did not alter these parameters of airway epithelial proliferation in saline-challenged rats in weeks 1 or 2.

Table 9. Effects of CAPs Exposure and OVA Challenge on BALF Cytokines (ng/mL)*

Group	TNFα	IFNγ	IL-4	IL-6	IL-10
Week 1					
Air/Saline	ND	ND	ND	ND	ND
CAPs/Saline	ND	ND	ND	ND	ND
Air/OVA	13.82 ± 1.87[a]	ND	ND	28.88 ± 6.27[a]	ND
CAPs/OVA	25.85 ± 5.6[a]	ND	ND	25.46 ± 5.17[a]	ND
Week 2					
Air/Saline	ND	ND	ND	ND	ND
CAPs/Saline	ND	ND	ND	ND	ND
Air/OVA	10.55 ± 3.64[a]	ND	ND	10.72 ± 3.35[a]	ND
CAPs/OVA	14.72 ± 4.93[a]	ND	ND	9.78 ± 3.25[a]	ND

* ND indicates not detected.

[a] Significantly different from respective group challenged with saline (i.e., not challenged with OVA [control]); $P \leq 0.05$.

Changes in BALF

Exposure to CAPs during either week 1 or 2 had no significant effects on the cellularity of the BALF in rats not challenged with OVA (CAPs/Saline group) (Table 8). In contrast, OVA challenge caused significant increases in total cells (250%–300% increase), macrophages (40%), neutrophils (2000%–3000%), lymphocytes (200%–900%), and eosinophils (300%–1000%) in BALF during both week 1 and week 2. In week 1, CAPs exposure enhanced OVA-induced total BALF cells (34% increase), eosinophils (250%), lymphocytes (150%), and neutrophils (30%) compared to OVA-challenged rats exposed only to filtered air (Air/OVA). Conversely, the number of macrophages in the BALF of CAPs/OVA rats was similar to that of the control animals (Air/Saline). In week 2, CAPs exposures had no effect on OVA-induced increases in the numbers of BALF cells.

Total protein and secreted mucosubstances in BALF were unaffected by CAPs exposures in saline-challenged rats during both exposure periods (Figure 15). In both studies, challenge with OVA caused significant increases in both protein (250%–300% increases) and mucus hypersecretion (90%–100%). During week 1, CAPs exposure enhanced BALF protein (40%) and secreted mucins (50%) in OVA-challenged rats. By comparison, CAPs exposure during week 2 had no effects.

BALF concentrations of TNFα and IL-6 were increased in OVA-challenged rats exposed to filtered air or CAPs during both weeks 1 and 2 (Table 9). Treatment-related effects were not detected in other BALF cytokines of rats exposed to CAPs or challenged with OVA.

Sensitization and challenge with OVA induced significant accumulation of OVA-specific IgE in BALF that was unaffected by CAPs exposures in either week 1 or 2 (Figure 16). The BALF IgE levels of animals challenged with saline were not affected by CAPs exposure.

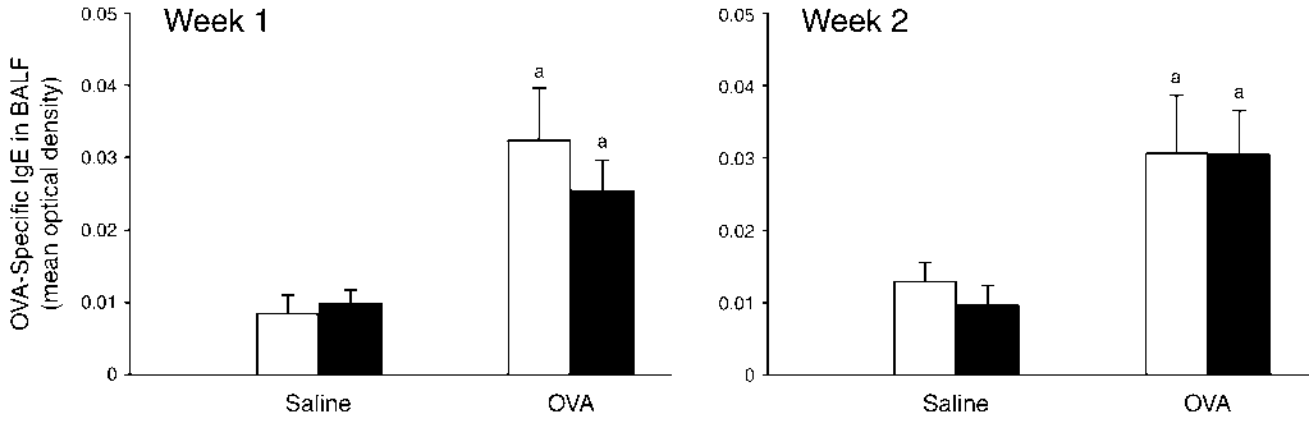

Figure 16. OVA-specific IgE in the BALF. Rats were exposed to CAPs and challenged with saline or OVA for 3 consecutive days, and BALF was collected 24 hours later. IgE in BALF was analyzed as described in the text. a = significantly different from respective group challenged with saline; $P \leq 0.05$.

Table 10. Effects of CAPs Exposure and OVA Challenge on Pulmonary Gene Expression*

Group	Muc5AC	IL-5	IL-10	IL-12p35	IL-12p40	IL-13	IFNγ	TNFα
Week 1								
Air/Saline	1.000 ± 0.301	1.000 ± 0.302	1.000 ± 0.242	1.000 ± 0.206	1.000 ± 0.344	1.000 ± 0.280	1.000 ± 0.152	1.000 ± 0.242
CAPs/Saline	1.325 ± 0.213	0.560 ± 0.095	0.699 ± 0.152	1.041 ± 0.253	1.005 ± 0.312	0.516 ± 0.095	1.657 ± 0.711	0.699 ± 0.128
Air/OVA	0.289 ± 0.028[a]	0.585 ± 0.148	4.343 ± 1.033[a]	0.692 ± 0.157	0.614 ± 0.171	0.697 ± 0.064	2.988 ± 0.714[a]	4.343 ± 1.104[a]
CAPs/OVA	0.318 ± 0.063[a]	0.344 ± 0.053	1.934 ± 0.962[b]	0.563 ± 0.150	0.350 ± 0.132[a]	0.473 ± 0.094	1.092 ± 0.321[b]	1.943 ± 1.028[b]
Week 2								
Air/Saline	1.000 ± 0.401	1.000 ± 0.580	1.000 ± 0.252	1.000 ± 0.211	1.000 ± 0.373	1.000 ± 0.405	1.000 ± 0.233	1.000 ± 0.165
CAPs/Saline	1.203 ± 0.386	0.989 ± 0.213	0.715 ± 0.171	1.237 ± 0.241	1.141 ± 0.430	1.112 ± 0.285	0.894 ± 0.224	0.592 ± 0.105
Air/OVA	0.655 ± 0.198	0.951 ± 0.391	1.811 ± 0.635	0.723 ± 0.174	0.414 ± 0.144	0.685 ± 0.103	1.333 ± 0.484	0.815 ± 0.222
CAPs/OVA	0.368 ± 0.069[a]	0.707 ± 0.178	1.477 ± 0.447	0.933 ± 0.213	0.467 ± 0.118	0.812 ± 0.196	1.317 ± 0.285	0.689 ± 0.124

* Reported as fold increase relative to Air/Saline control.

[a] Significantly different from respective group challenged with saline (i.e., not challenged with OVA [control]); $P \leq 0.05$.

[b] Significantly different from respective group exposed to filtered air; $P \leq 0.05$.

Gene Expression of Airway Mucin and Inflammatory Cytokines

During both weeks 1 and 2, CAPs exposures had no effect on mucin or inflammatory cytokine gene expression in lung lobes from rats challenged with saline (Table 10). During week 1, we detected an OVA-induced decrease in Muc5AC, and increases in IL-10, IFNγ, and TNFα in air-exposed rats. Muc5AC and IL-12p40 were also decreased in OVA-challenged, CAPs-exposed animals, but CAPs inhibited the OVA-induced increase in IL-10, IFNγ, and TNFα. The only significant treatment-related effect during week 2 was suppression of Muc5AC in the lungs of OVA-challenged, CAPs-exposed animals.

RESULTS OF INHALATION STUDIES OF DEE

DEE Exposure Concentrations

The concentrations of DEPs, CO, NO_x, and THC are shown in Table 11. The target concentrations for the low- and high-exposure levels were 30 and 300 μg/m^3 DEPs, respectively. However, the system operator set a target of DEPs plus 12 μg/m^3, which was an historical value for the mass particulate matter concentration in clean air chambers that results from animal dander. It was assumed that there were DEPs plus clean air control particulate matter in the exposure chambers. To assess the DEPs exposure precisely, the concentrations of particulate matter measured directly in the clean air chambers were subtracted from the exposure chamber concentrations. The concentrations of particulate matter in the clean air chamber were only approximately 5.6 μg/m^3, so after subtraction of the background matter, the concentrations of DEPs were slightly above target despite being within a couple percent (on average) of the target of DEPs plus 12 μg/m^3.

The gaseous copollutants in the exposure chambers had an exposure-related difference in concentrations between the low and high chambers. However, the differences in the concentrations of CO and THC between the clean air chamber and the control chamber were not large, because in both chambers the dilution of DEE was great and thus the concentrations of its constituents were close to background concentrations. CO was not efficiently scrubbed by the carbon scrubber on the dilution air, and the most volatile portions of the THC, especially methane, might not have been completely scrubbed, which likely contributed to the bulk of the THC mass. Since speciation of the individual volatile organics in these chambers was not conducted, it was not possible to assess whether the compositions of the bulk THC in the low and the control chambers were different, but it was assumed that they were because of the presence of DEE in the low chamber.

Table 11. Particle and Gas Concentrations in Clean Air, Low- (Target 42 μg/m^3), and High- (Target 312 μg/m^3) DEE Exposure Atmospheres*

	Control	Low	High
Particle mass (μg/m^3)	5.6 ± 1.2	38.7 ± 5.4	311.7 ± 11.6
DEPs[a] (μg/m^3)	NA	33.0	306.0
NO_x (ppm)	ND	0.3 ± 0.1	3.8 ± 0.5
CO (ppm)	0.5 ± 0.1	0.4 ± 0.1	3.1 ± 0.5
THC (ppm)	0.2 ± 0.1	0.2 ± 0.1	0.5 ± 0.1

* NA indicates not applicable; ND indicates not detected.

[a] DEPs = exposure chamber particle mass concentration − concentration in the clean air chamber (control) (TEOM used to measure particle mass).

CHEMICAL AND PHYSICAL CHARACTERIZATION OF DEPs

Table 12 summarizes the major chemical composition of the DEPs observed in the single-cylinder-engine exposure system. DEPs during this study consisted of EC (68%–77%), OC (6%–14%), small amounts of inorganic ions, including SO_4^{2-}, NO_3^-, and NH_4^+ (0.3%–1.4%), and trace metals (0.2%–1.4%). The organic component was estimated by applying a correction factor of 1.2 to account for unmeasured hydrogen and oxygen. As other studies have reported, EC and OC composed most of the DEE PM during the exposure studies (Rogge et al. 1993; Lowenthal et al. 1994; McDonald et al. 2004). Measured elements found to be above detection limits included Al, P, Ca, Ti, V, Mn, Fe, Co, Cu, Zn, As, Mo, Ba, and Pb. Among these, the most abundant element was Ca, followed by Zn and then P. These three elements — Ca, P, and Zn — are used as additives in fuel and lubrication oil (EPA 2002).

The particle mass size distribution for the diluted DEE was measured by an MOI. The mass median diameter was determined to be 0.1 μm with a geometric standard deviation (GSD) of 1.9. The mass distribution revealed that approximately 18% of the total mass was attributed to ultrafine particles, and more than 85% of the diesel-engine particle mass was associated with particles below 1 μm in aerodynamic diameter.

The number size distribution ranged from a median of 66 nm (1.5 GSD), for the low-exposure concentration, to 79 nm (1.5 GSD) for the high-exposure concentration. As expected, the number size distribution of particles was slightly smaller than that reported for mass. Data were obtained using a fast mobility particle sizer (FMPS; TSI Inc., St Paul, MN) at the DEE concentration of 300 μg/m^3 during typical operation of the engine exposure system.

Table 12. Average Chemical Composition of Diluted DEPs During 8-Hour Exposure Periods

	High Chamber	Low Chamber
Mass (μg/m^3)	304.2 ± 19.5	34.9 ± 3.5
Carbon (μg/m^3)		
OC	27.2 ± 9.4	3.0 ± 3.0
EC	215.3 ± 16.6	24.4 ± 4.3
Inorganic ions (μg/m^3)		
SO_4^{2-}	0.44 ± 0.21	0.08 ± 0.08
NO_3^-	0.27 ± 0.11	0.17 ± 0.11
NH_4^+	0.08 ± 0.29	0.19 ± 0.02
Trace elements (ng/m^3)		
Al	18.6 ± 17.4	3.6 ± 3.8
P	157 ± 91	18 ± 10
Ca	425 ± 208	123 ± 11
Ti	0.29 ± 0.20	0.33 ± 0.34
V	0.23 ± 0.15	0.02 ± 0.02
Mn	1.39 ± 0.67	0.34 ± 0.16
Fe	61 ± 33	52 ± 22
Co	0.20 ± 0.01	0.07 ± 0.09
Cu	65 ± 70	34 ± 31
Zn	256 ± 111	33 ± 28
As	0.11 ± 0.02	0.05 ± 0.02
Mo	0.57 ± 0.53	0.20 ± 0.35
Ba	0.86 ± 0.78	0.59 ± 0.23
Pb	10.3 ± 12.0	4.0 ± 5.1

TRACE ELEMENTS IN LUNG TISSUE OF DEE-EXPOSED RATS

Understanding the relationship between what was in the air and what was deposited in the respiratory system is important because this link may show specific chemical or elemental components that could be related to the observed effects or indicative of the source of those effects. Vincent (1990) assessed the risk posed by considering the total harm inflicted by the particles retained in the lungs of aerosol-exposed subjects. Since the severity of the inflammatory responses in the lungs depends on the physical and chemical characteristics of the retained particles, identification of the types of retained particles in the lungs is important. During this study, a subset of those elements observed in DEPs was above the detection limits in lung tissues (data not shown); these included Al, Ba, Cd, C, Cr, Co, Cu, Fe, Pb, Mg, Mn, Mo, Sr, P, Ti, and V. Among these elements, several likely originated from DEPs and not from nutrients and essential elements in rat chow since the concentrations of these elements in the lung tissues of the DEE-exposed groups were significantly different from those of the groups exposed to filtered air. Figure 17 shows that concentrations of two trace elements, V and Fe, recovered from the lung tissues of DEE-exposed rats are elevated compared to concentrations recovered from the lungs of control rats. The central line in each box marks the median value of the element, and the top and bottom edges of each box mark the first (25th percentile) and third quartiles (75th percentile), respectively. Groups 0, 30, and 300 represent rats that were exposed to HEPA-filtered air as the control group, 30 μg/m^3 DEPs, and 300 μg/m^3 DEPs, respectively. A statistically significant difference between the filtered-air control group and the 300 μg/m^3 DEP group was found based on a P value ≤ 0.05. The data for Mn also showed a statistically significant difference between the control and exposed groups.

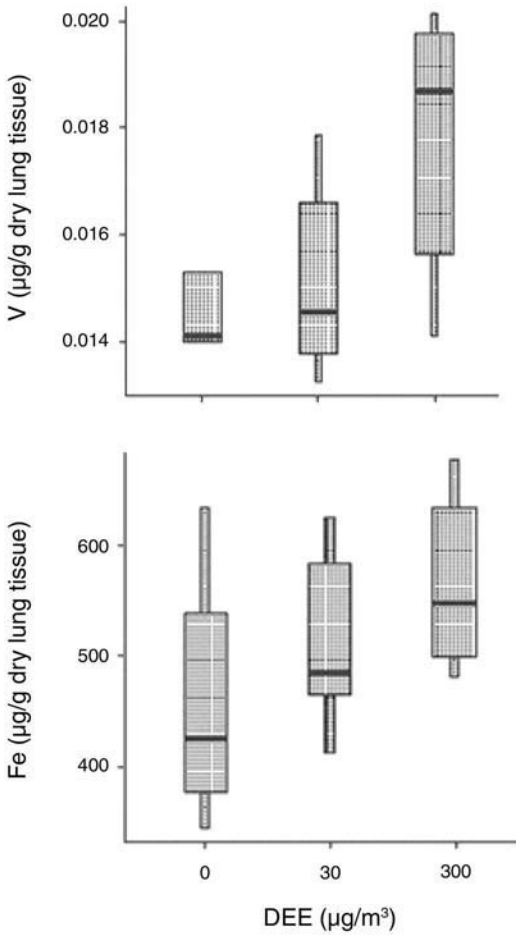

Figure 17. Amounts of V and Fe in the lung tissues of OVA-sensitized rats that were exposed for 3 days to DEE immediately before challenge with OVA. Groups: 0 = Air/OVA; 30 = 30 µg/m^3 DEE/OVA; 300 = 300 µg/m^3 DEE/OVA. Concentrations are in µg of trace elements per gram of dry lung tissue. The central line in each box marks the median value; the top and bottom edges of the box represent the 25th and 75th percentiles. The bars extending from the top and bottom of the boxes show the highest and lowest values.

The data revealed direct evidence of deposition of DEP metals, primarily V, Fe, and Mn, in the lung. The mass size distributions of these elements as measured by a MOUDI are shown in Figure 18, which reveals that over 10% of the total mass was attributed to these ultrafine particles, and approximately 70% of these elements were below 1 µm in aerodynamic diameter. In addition, V, Fe, and Mn are well-known transition metals that could undergo the Fenton reaction, which is a stimulator of oxidant generation in macrophages (Han et al. 2001; Valko et al. 2005). Although further studies are required to understand a direct link between the deposited metals and observed health effects, these results suggest specific chemical or elemental components that may be related to the effects.

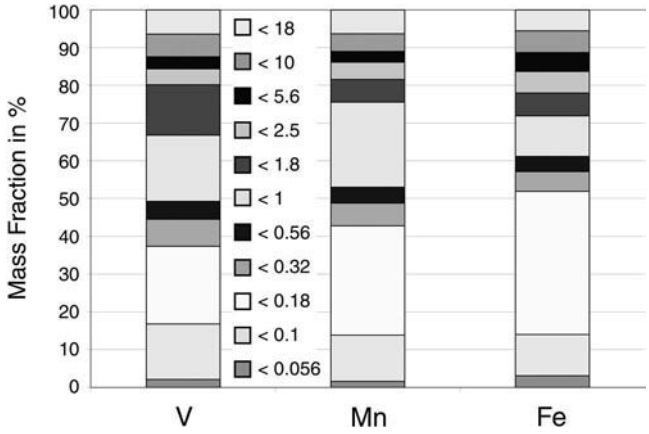

Figure 18. Mass-based size (µm) distribution of V, Mn, and Fe in DEPs measured by the MOIDI from the high-exposure chamber (300 µg/m^3 DEE).

BIOLOGIC CHANGES RELATED TO DEE EXPOSURES

STUDY 1: EXPOSURE TO DEE DURING ALLERGEN SENSITIZATION

Nasal and Pulmonary Histopathology

In study 1, Air/Saline, 30 µg/m^3 DEE/Saline, and 300 µg/m^3 DEE/Saline rats had no exposure-related histopathology in nasal or pulmonary tissue sections, with one exception: a mild dose-dependent increase in AM and monocytes scattered throughout the airspaces in the alveolar parenchyma of DEE-exposed rats compared to filtered-air-exposed animals.

As in the CAPs inhalation study, OVA-sensitized-and-challenged rats had a mild-to-moderate allergic rhinitis. This nasal response was characterized by a mixed inflammatory response composed of eosinophils, lymphocytes, plasma cells, and occasional neutrophils in the nasal mucosa lined by transitional or respiratory epithelium. There was also mild mucus cell hyperplasia and hypertrophy in the respiratory epithelium lining the proximal and distal septum and the nasopharyngeal meatus. In addition, there was marked lymphoid hyperplasia in the nasal-associated lymphoid tissues lining the nasopharyngeal meatus. DEE exposures had no affect on any of these OVA-induced nasal lesions.

The principal morphologic lesions in the lungs of OVA-sensitized-and-challenged BN rats were an allergic bronchiolitis and alveolitis (allergic bronchopneumonia) with mucus cell hyperplasia and hypertrophy in the respiratory epithelium lining the large-diameter conducting airways,

similar to that described previously for the OVA-sensitized-and-challenged rats in the CAPs inhalation study. The high dose of 300 μg/m^3 DEE during sensitization had no effect on the magnitude of these OVA-induced lung lesions. However, the low dose of 30 μg/m^3 DEE during sensitization caused a slight increase in the severity of the bronchiolitis and alveolitis in most of the OVA-sensitized rats. This was reflected in the total number of inflammatory cells in the BALF from these animals (see results below).

Changes in BALF

In saline-sensitized rats, inhalation exposure to 30 μg/m^3 DEE had no effect on the cellularity of the BALF, but exposure to 300 μg/m^3 DEE caused significant accumulation of macrophages in BALF (Figure 19). OVA sensitization and challenge in filtered-air-exposed rats (Air/OVA) caused significant increases in total inflammatory cells (2.4-fold), eosinophils (20-fold), macrophage/monocytes (1.7-fold), and neutrophils (3.4-fold). In OVA-sensitized rats, exposure to 30 μg/m^3 DEE significantly enhanced total cells in BALF (62% increase), with contributions from increased eosinophils (52%), macrophages (35%), and neutrophils (120%). By contrast, exposure to 300 μg/m^3 DEE had no effect on OVA-induced cellularity.

OVA challenge of OVA-sensitized rats caused significant increases in total protein (4-fold) and Muc5AC (2-fold) in BALF that was unaffected by DEE at either concentration (Figure 20, top-right and top-left panels). Elastase and β-glucuronidase in BALF were also increased in animals sensitized to OVA (Figure 20, bottom-left and bottom-right panels). However, DEE exposure inhibited OVA-induced increases in β-glucuronidase, and a similar trend was suggested in elastase levels of DEE-exposed rats.

Levels of IL-6 protein were elevated in animals sensitized with OVA and exposed to air, and were further increased (2.2-fold) by exposure to 30 μg/m^3 DEE, but unaffected by exposure to 300 μg/m^3 DEE (Table 13). Other cytokines analyzed in BALF (TNFα, IFNγ, IL-4, and IL-10) were not significantly affected by OVA sensitization or DEE exposure.

Sensitization and challenge with OVA induced significant accumulation of OVA-specific IgE in BALF that was unaffected by exposure to either 30 or 300 μg/m^3 DEE (Figure 21).

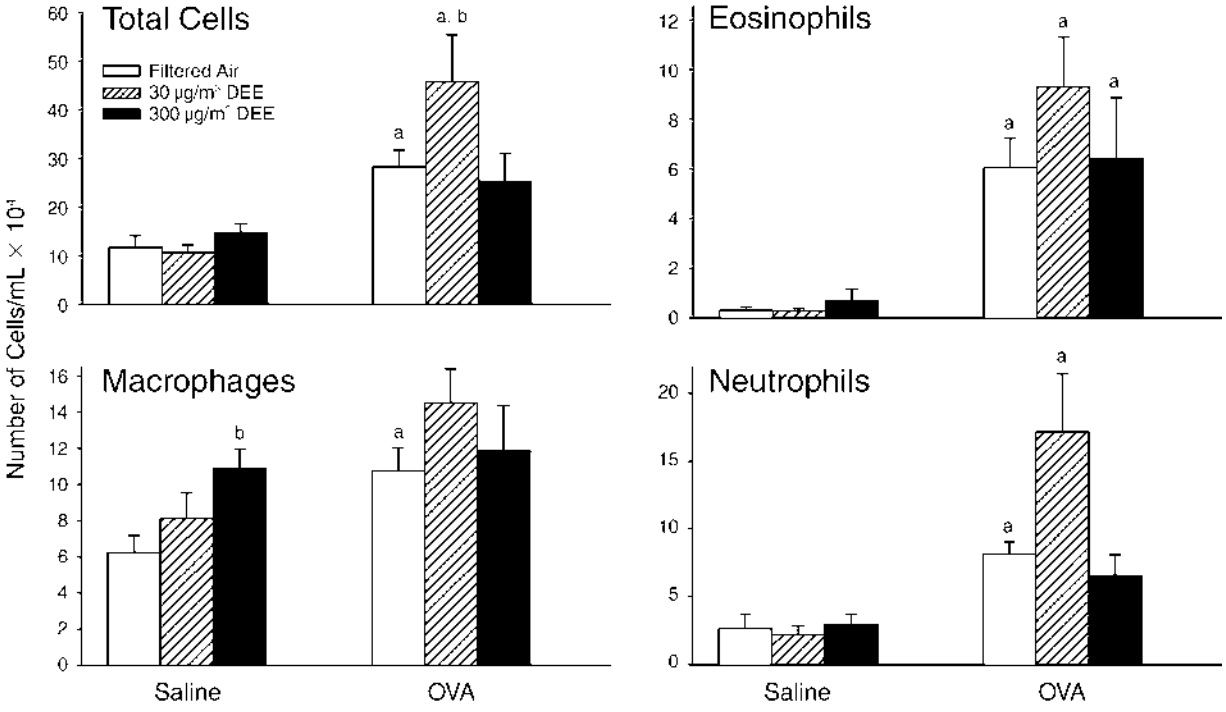

Figure 19. Cellularity of BALF. In DEE study 1, rats were exposed to DEE during sensitization with saline or OVA, and 2 weeks later were all challenged with OVA for 3 consecutive days. BALF was collected 24 hours later, and total cells, eosinophils, macrophages, and neutrophils were determined as described in the text. a = significantly different from respective group sensitized with saline; b = significantly different from respective group exposed to filtered air; $P \leq 0.05$. Clear bars represent filtered air; striped bars represent 30 μg/m^3 DEE; black bars represent 300 μg/m^3 DEE. Note the use of different y-axis scales.

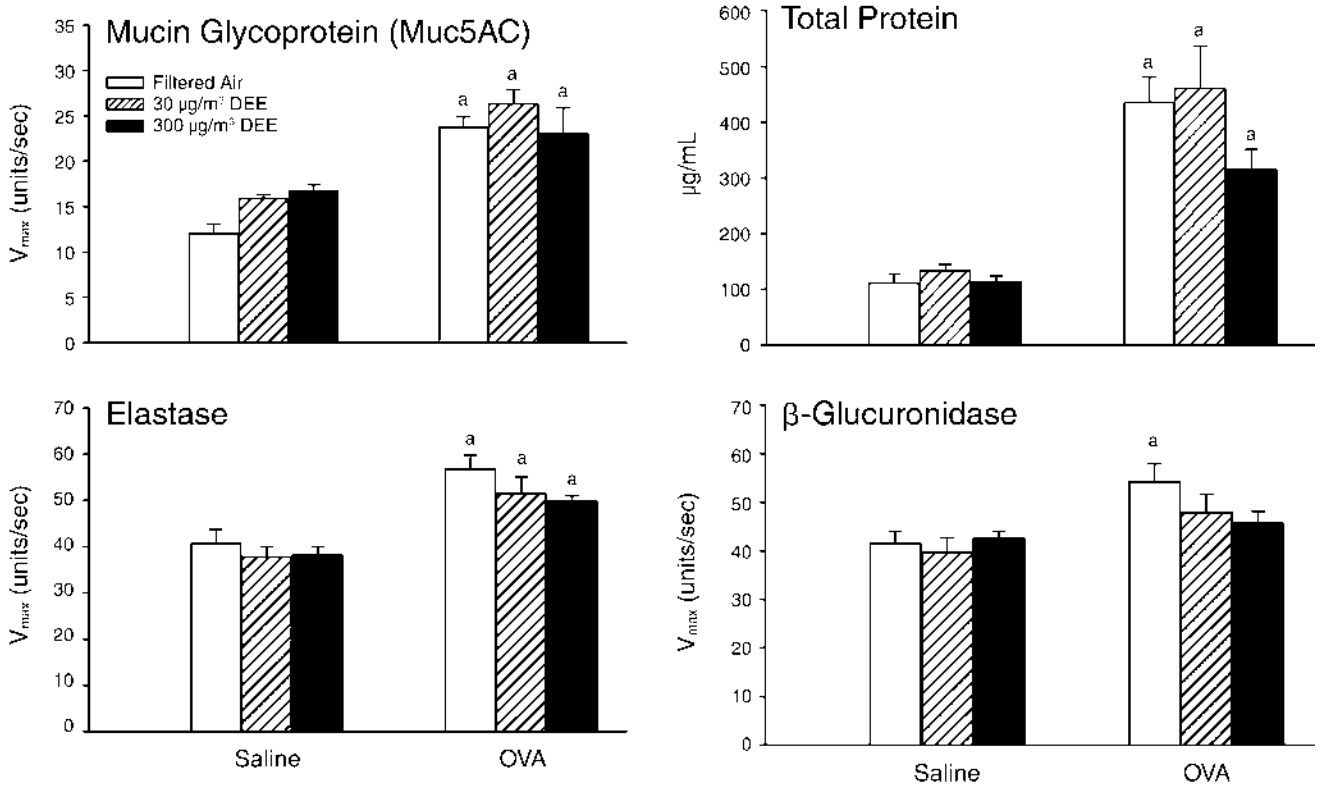

Figure 20. Mucin glycoprotein (Muc5AC), total protein, elastase, and β-glucuronidase content in BALF. In DEE study 1, rats were exposed to DEE during sensitization to saline or OVA, and 2 weeks later were all challenged with OVA for 3 consecutive days. BALF was collected 24 hours later and analyzed as described in the text. a = significantly different from respective group sensitized with saline. Clear bars represent filtered air; striped bars represent 30 µg/m^3 DEE; black bars represent 300 µg/m^3 DEE. Note the use of different y-axis scales for the top two figures.

Table 13. Study 1: DEE Exposure During OVA Sensitization — Effects of DEE and OVA on BALF Cytokines (ng/mL)*

Group	TNFα	IL-4	IFNγ	IL-6	IL-10
Air/Saline	3.41 ± 2.25	ND	ND	4.97 ± 2.52	5.77 ± 3.47
30 DEE/Saline	ND	ND	ND	ND	ND
300 DEE/Saline	ND	ND	ND	ND	ND
Air/OVA	ND	ND	ND	13.69 ± 3.41	ND
30 DEE/OVA	5.41 ± 2.43	ND	ND	30.09 ± 11.16[a]	3.55 ± 1.70
300 DEE/OVA	ND	ND	ND	9.35 ± 3.39[a]	ND

* ND indicates not detected.

[a] Significantly different from respective group sensitized with saline (i.e., the control, which was not sensitized with OVA); $P \leq 0.05$.

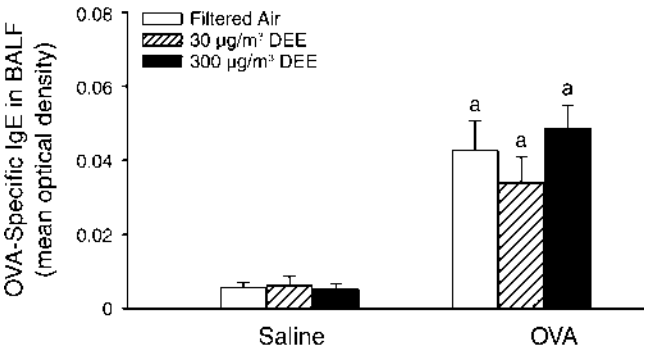

Figure 21. OVA-specific IgE in BALF. In DEE study 1, rats were exposed to DEE during sensitization with saline or OVA, and 2 weeks later were all challenged with OVA for 3 consecutive days. Twenty-four hours after the last challenge, the lungs were lavaged. IgE in BALF was analyzed as described in the text. a = significantly different from respective group sensitized with saline; $P \leq 0.05$.

IM in Pulmonary Axial Airways

Both levels of exposure to DEE (30 or 300 µg/m^3) had no effect on the amount of stored IM in axial conducting airways of saline-sensitized animals (Figure 22). OVA sensitization and challenge caused an increase in IM in both proximal (2.4-fold) and distal (9-fold) conducting airways. Apparent decreases of OVA-induced IM in the proximal airways of rats exposed to 30 µg/m^3 DEE were not statistically significant. A trend toward increased IM associated with 300 µg/m^3 DEE exposure was evident in both proximal and distal airways.

Levels of DEE exposure (30 or 300 µg/m^3) during sensitization had no effect on IM in the nasal septum of the Air/Saline rats (Figure 23). OVA challenge of OVA-sensitized rats caused significant accumulation of IM in the proximal septum that was unaltered by DEE exposures. Increases of IM in the distal septum were not statistically significant.

Changes in Airway Epithelial Cell Density and the Labeling Indices

Morphometric changes in the epithelium of the pulmonary airway that reflect treatment-induced alterations in cell proliferation (as indicated by numeric cell densities and BrdU labeling indices) are presented in the top section of Table 14. Neither OVA sensitization nor DEE exposures or both caused significant changes (compared to the control rats sensitized to saline and exposed to air [the Air/Saline controls]) in the total numbers of epithelial cells (numeric cell density) lining the proximal or distal axial airways (G5 and G11, respectively) in the left lung lobe. In addition, exposure to either high or low concentrations of DEE did not alter the BrdU LI or the ULLI of proximal or distal airway epithelium in rats sensitized with only saline. Air/OVA rats did have significant increases in both the LI and ULLI of the proximal airway epithelium and in the ULLI of the distal airway epithelium, as compared to the control Air/Saline rats (Table 14). The level of DEE exposure (i.e., 30 or 300 µg/m^3) did not alter these OVA-induced increases in the LI or ULLI for the epithelium lining the proximal axial airway. Interestingly, exposure to the high, but not the low, concentration of DEE appeared to attenuate the OVA-induced increase in ULLI in the distal airway epithelium.

Gene Expression of Airway Mucin and Inflammatory Cytokines

Exposure to 30 or 300 µg/m^3 DEE during sensitization with saline had no effect on mucin or cytokine gene

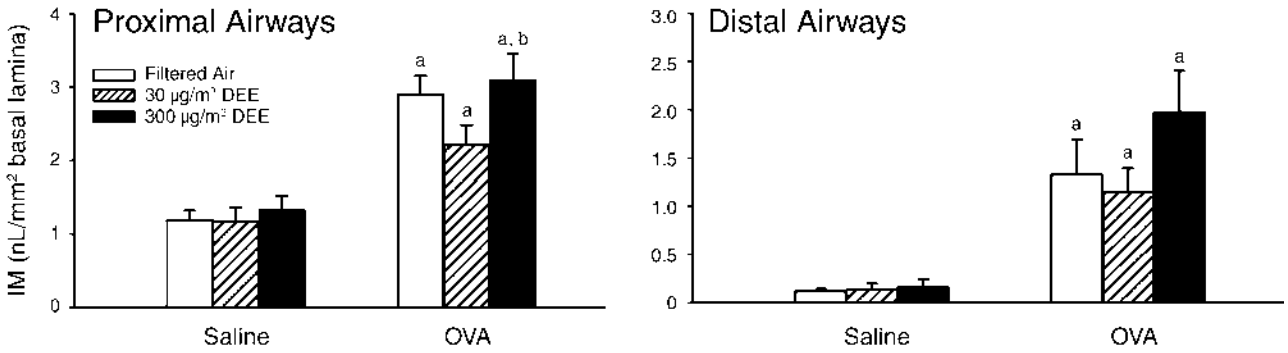

Figure 22. IM in pulmonary proximal and distal conducting airways. In DEE study 1, rats were exposed to DEE during sensitization with saline or OVA, and 2 weeks later were all challenged with OVA for 3 consecutive days. Twenty-four hours after the last challenge, the lungs were collected, processed, and stained with AB/PAS for specific identification of IM as described in the text. a = significantly different from respective group sensitized with saline; b = significantly different from respective group exposed to 30 µg/m^3 DEE; $P \leq 0.05$. Clear bars represent filtered air; striped bars represent 30 µg/m^3 DEE; black bars represent 300 µg/m^3 DEE. Note the use of different y-axis scales.

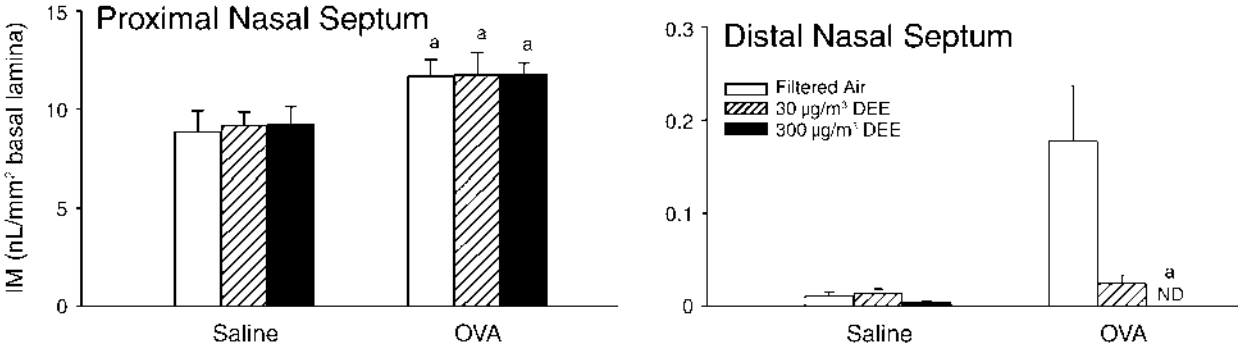

Figure 23. IM in the nasal septum. In DEE study 1, rats were exposed to DEE during sensitization with saline or OVA, and 2 weeks later were all challenged with OVA for 3 consecutive days. Twenty-four hours later the nasal septum was collected, processed, and stained with AB/PAS for specific identification of IM as described in the text. ND = not detectable; a = significantly different from respective group sensitized with saline; $P \leq 0.05$. Clear bars represent filtered air; striped bars represent 30 µg/m^3 DEE; black bars represent 300 µg/m^3 DEE. Note the use of different y-axis scales.

Table 14. Effects of DEE and OVA in DEE Studies 1 and 2 on Epithelial Cell Proliferation in Pulmonary Axial Airways

	Proximal Airways			Distal Airways		
Group	LI (%)	ULLI (%/mm)	Numeric Cell Density (total cells/mm basal lamina)	LI (%)	ULLI (%/mm)	Numeric Cell Density (total cells/mm basal lamina)
Sensitization						
Air/Saline	3.89 ± 0.73	4.80 ± 1.12	121.04 ± 14.43	10.33 ± 1.81	15.32 ± 3.05	144.14 ± 11.00
30 DEE/Saline	3.97 ± 0.57	4.92 ± 0.77	124.12 ± 7.44	6.68 ± 1.03	9.89 ± 2.36	151.32 ± 13.30
300 DEE/Saline	4.92 ± 0.72	5.86 ± 0.97	120.07 ± 12.61	7.36 ± 1.38	11.25 ± 2.41	152.44 ± 15.76
Air/OVA	8.62 ± 1.26[a]	8.91 ± 0.97[a]	120.84 ± 4.75	14.09 ± 1.29	25.64 ± 2.96[a]	183.88 ± 17.36
30 DEE/OVA	11.56 ± 1.88[a]	16.75 ± 3.65[a]	121.93 ± 8.16	15.66 ± 2.01	24.74 ± 3.66[a]	156.13 ± 10.01
300 DEE/OVA	11.32 ± 1.18[a]	13.53 ± 1.72[a]	118.39 ± 7.00	11.49 ± 1.86	16.07 ± 2.40	144.54 ± 10.50
Challenge						
Air/Saline	0.35 ± 0.14	0.61 ± 0.20	129.35 ± 15.41	0.00 ± 0.00	0.00 ± 0.00	147.93 ± 23.29
30 DEE/Saline	0.60 ± 0.17	0.52 ± 0.13	97.13 ± 8.52	0.17 ± 0.17	0.28 ± 0.28	129.16 ± 20.16
300 DEE/Saline	0.08 ± 0.08	0.08 ± 0.08	100.07 ± 15.77	0.17 ± 0.09	0.33 ± 0.17	123.60 ± 19.02
Air/OVA	2.15 ± 0.42[a]	2.02 ± 0.46[a]	96.57 ± 10.23	4.05 ± 0.16[a]	5.29 ± 1.55[a]	117.63 ± 9.55
30 DEE/OVA	1.46 ± 0.43[a]	1.53 ± 0.51[a]	105.10 ± 17.67	7.95 ± 1.59[a]	9.78 ± 1.84[a]	129.65 ± 14.85
300 DEE/OVA	1.49 ± 0.35[a]	1.41 ± 0.38[a]	92.15 ± 7.971	5.16 ± 0.74[a]	9.15 ± 2.08[a]	172.27 ± 23.96

[a] Significantly different from respective group challenged with saline alone; $P \leq 0.05$.

Table 15. Study 1: DEE Exposure During OVA Sensitization — Effects of DEE and OVA on Pulmonary Gene Expression*

Group	Muc5AC	IL-5	IL-10	IL-12p35	IL-12p40	IL-13	IFNγ	TNFα
Air/Saline	1.000 ± 0.245	1.000 ± 0.148	1.000 ± 0.179	1.000 ± 0.151	1.000 ± 0.362	1.000 ± 0.161	1.000 ± 0.175	1.000 ± 0.163
30 DEE/Saline	0.676 ± 0.159	1.148 ± 0.391	1.019 ± 0.130	0.817 ± 0.185	0.940 ± 0.260	0.826 ± 0.211	0.673 ± 0.198	0.628 ± 0.124
300 DEE/Saline	0.374 ± 0.093	1.569 ± 0.571	1.602 ± 0.199	1.406 ± 0.224	1.049 ± 0.132	1.330 ± 0.433	1.624 ± 0.324	1.286 ± 0.181
Air/OVA	0.881 ± 0.174	1.670 ± 0.482	6.137 ± 1.306[a]	0.363 ± 0.091[a]	0.761 ± 0.275	3.598 ± 0.851[a]	1.797 ± 0.615	0.905 ± 0.338
30 DEE/OVA	0.903 ± 0.203	1.922 ± 0.475	5.656 ± 1.076[a]	0.599 ± 0.081	0.975 ± 0.165	3.345 ± 0.701[a]	2.118 ± 0.557[a]	0.869 ± 0.160
300 DEE/OVA	0.858 ± 0.229	1.668 ± 0.589	10.600 ± 2.337[a,b,c]	0.946 ± 0.295[b]	0.946 ± 0.266	3.389 ± 1.119[a]	2.813 ± 0.585	1.149 ± 0.480

* Reported as fold increase relative to Air/Saline controls.

[a] Significantly different from respective group sensitized with saline (i.e., the control, which was not sensitized with OVA); $P \leq 0.05$.

[b] Significantly different from respective group exposed to filtered air; $P \leq 0.05$.

[c] Significantly different from respective group exposed to 30 DEE; $P \leq 0.05$.

expression in lungs from rats challenged with OVA two weeks later (Table 15). However, trends toward decreased Muc5AC and an increase in some of the cytokines (e.g., IL-5, IL-10) were noted. OVA sensitization and challenge induced significant increases in IL-13 (3.6-fold) and IL-10 (6-fold) expression, and significantly depressed expression of IL-12p35. Enhancement of IL-10 and IL-12p35 in the lungs of rats exposed to 300 μg/m^3 DEE and of IFNγ in the lungs of rats exposed to 30 μg/m^3 DEE were the only exposure-related alterations of OVA-induced gene responses detected in the lungs.

STUDY 2: EXPOSURE TO DEE DURING ALLERGEN CHALLENGE

Nasal and Pulmonary Histopathology

In study 2, Air/Saline, 30 μg/m^3 DEE/Saline, and 300 μg/m^3 DEE/Saline rats had no exposure-related histopathology in nasal or pulmonary tissue sections. As in study 1, OVA-sensitized-and-challenged rats had a mild-to-moderate allergic rhinitis, bronchiolitis, and alveolitis. Exposure to 300 μg/m^3 DEE at the time of OVA challenge had no effect on any of the OVA-induced nasal or pulmonary lesions. By contrast, rats exposed to 30 μg/m^3 DEE at the time of allergen challenge, as compared to the Air/OVA rats, had a mild attenuation of the severity and distribution of the OVA-induced allergic bronchopneumonia. This was reflected in the reduced cellularity and protein concentrations in the BALF of the 30 μg/m^3 DEE/OVA rats compared to those in the BALF of Air/OVA control rats (see results below).

Changes in BALF

In saline-challenged rats, inhalation exposure to DEE had no effect on the number or type of cells in the BALF (Figure 24). OVA sensitization and challenge caused significant increases in total cells (7-fold), eosinophils (8-fold), macrophage/monocytes (2-fold), and neutrophils (90-fold) as compared to air exposure and saline challenge (Air/Saline controls). In rats exposed to 30 μg/m^3 DEE during OVA challenge, the OVA-induced increase in total BALF cells was attenuated (60% decrease) and the OVA-induced increase in macrophages showed a similar trend of attenuation (54% reduction). Though not statistically significant, there was some suggestion of a 30 μg/m^3 DEE–induced decrease in the elevation of neutrophils (44% reduction) and eosinophils (60% reduction) caused by OVA challenge. Increases in eosinophils in BALF from OVA-challenged rats exposed to 300 μg/m^3 DEE were not significant relative to those of the Air/OVA group, and BALF neutrophils were depressed in this group.

OVA challenge of sensitized rats caused significant increases in total protein (5-fold), mucin glycoprotein (80%), elastase (40%), and β-glucuronidase (70%) in BALF (Figure 25). Exposure to 30 μg/m^3 DEE attenuated the OVA-induced increase in total protein by 82% and in β-glucuronidase by 40%. A trend toward depressed concentrations of mucin glycoprotein and elastase in BALF was also evident after exposure to 30 or 300 μg/m^3 DEE.

IL-6 was significantly increased in BALF from Air/OVA rats. Exposure of rats to either 30 or 300 μg/m^3 DEE markedly attenuated this increase in IL-6 (Table 16). No other changes in inflammatory cytokines were detected in the BALF of any exposure group.

Exposure to DEE had no effect on OVA-specific IgE in the BALF from saline-challenged rats. Sensitization and challenge with OVA induced significant accumulation of OVA-specific IgE in BALF, but that amount was unaffected by a DEE exposure of either 30 or 300 μg/m^3 (Figure 26).

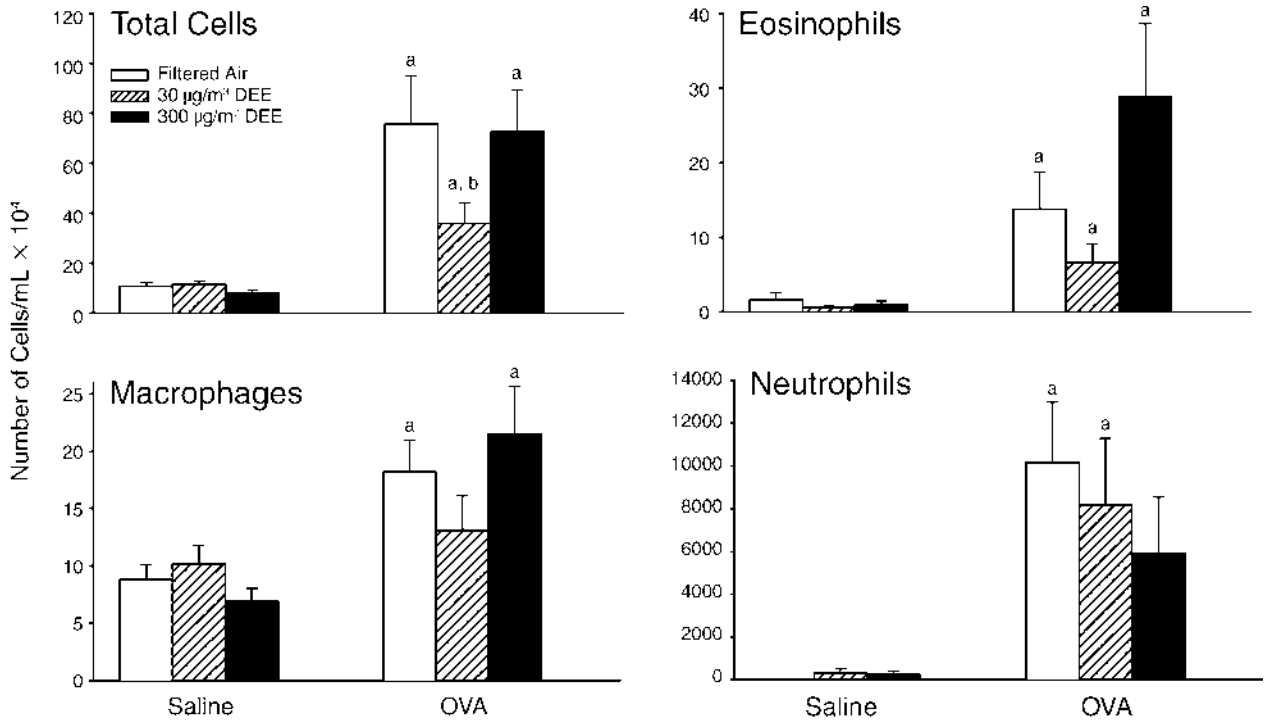

Figure 24. Cellularity of BALF. In DEE study 2, OVA-sensitized rats were exposed to DEE during challenge with saline or OVA for 3 consecutive days. BALF was collected 24 hours after the last challenge, and the total cells, eosinophils, macrophages, and neutrophils were determined as described in the text. a = significantly different from respective group challenged with saline; b = significantly different from respective group exposed to filtered air; $P \leq 0.05$. Clear bars represent filtered air; striped bars represent 30 µg/m^3 DEE; black bars represent 300 µg/m^3 DEE. Note the use of different y-axis scales.

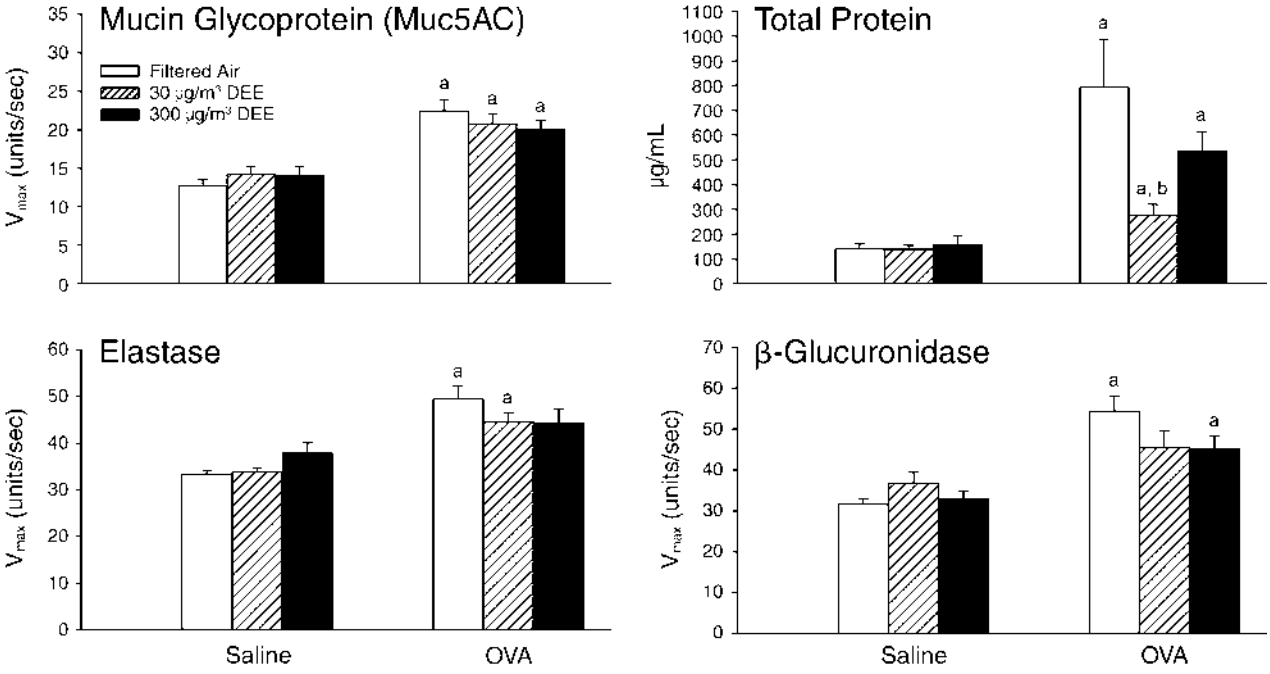

Figure 25. Mucin glycoprotein (Muc5AC), total protein, elastase, and β-glucuronidase content in BALF. In DEE study 2, OVA-sensitized rats were exposed to DEE during challenge with saline or OVA for 3 consecutive days. BALF was collected 24 hours later and analyzed as described in the text. a = significantly different from respective group challenged with saline; b = significantly different from respective group exposed to filtered air; $P \leq 0.05$. Clear bars represent filtered air; striped bars represent 30 µg/m^3 DEE; black bars represent 300 µg/m^3 DEE. Note the use of different y-axis scales.

Table 16. DEE Exposure During OVA Challenge — Effects of DEE and OVA on BALF Cytokines (ng/mL)*

Group	TNFα	IL-4	IFNγ	IL-6	IL-10
Air/Saline	ND	ND	ND	ND	ND
30 DEE/Saline	ND	ND	ND	ND	ND
300 DEE/Saline	ND	ND	ND	ND	ND
Air/OVA	ND	ND	ND	361.28 ± 113.69[a]	ND
30 DEE/OVA	ND	ND	ND	9.24 ± 9.24[a,b]	ND
300 DEE/OVA	ND	ND	ND	100.75 ± 33.81[a]	ND

* ND indicates not detected.

[a] Significantly different from respective group challenged with saline (i.e., the control, which was not challenged with OVA); $P \leq 0.05$.

[b] Significantly different from Air/OVA group; $P \leq 0.05$.

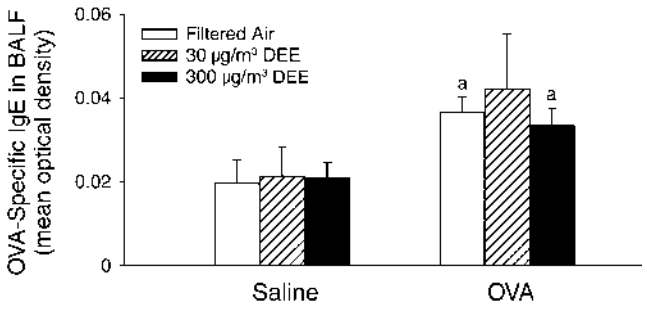

Figure 26. OVA-specific IgE in BALF. In DEE study 2, OVA-sensitized rats were exposed to DEE during challenge with saline or OVA for 3 consecutive days. BALF was collected 24 hours later and analyzed for IgE as described in the text. a = significantly different from respective group challenged with saline; $P \leq 0.05$.

IM in Pulmonary Axial Airways

Exposure to DEE (30 or 300 µg/m^3) had no effect on the amounts of IM in the axial conducting airways of saline-challenged animals (Figure 27). OVA sensitization and challenge caused an increase in IM in the proximal (2-fold) and distal (11-fold) axial airways. While exposure to DEE had no effect on IM increases in proximal airways during OVA challenge, exposures of both 30 and 300 µg/m^3 DEE during OVA challenge decreased OVA-induced increases in IM by 54% in the distal airways.

DEE exposures during saline challenge had no effect on IM in the nasal septum (Figure 28). In the proximal septum, OVA challenge increased IM, and exposure to DEE at 30 or 300 µg/m^3 had no effect on OVA-induced increases in IM. In the distal nasal septum, OVA caused minor increases in IM that were not significantly enhanced by DEE.

Changes in Airway Epithelial Cell Density and Labeling Indices

Morphometric changes in the epithelium of the pulmonary airways reflecting induced alterations in cell proliferation (numeric cell densities and BrdU labeling indices) in rats exposed to DEE during OVA challenge are shown in the bottom half of Table 14. Similar to study 1, neither OVA challenge nor DEE exposure caused significant changes in the numbers of total epithelial cells (numeric cell density) lining the proximal or distal axial airways (G5 and G11, respectively). In addition, neither exposure to high nor low concentrations of DEE during OVA or saline challenge altered the BrdU LI or ULLI of epithelial cells lining the proximal or distal axial airways of rats. Air/OVA rats did have significant increases in both LI and ULLI of the proximal and distal airway epithelium, as compared to the control (Air/Saline) rats.

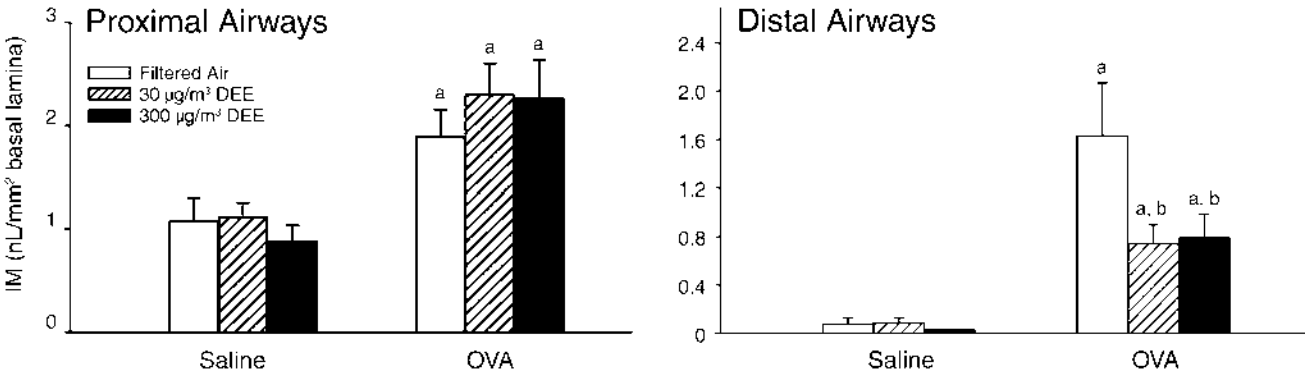

Figure 27. IM in pulmonary proximal and distal conducting airways. In DEE study 2, OVA-sensitized rats were exposed to DEE during challenge with saline or OVA for 3 consecutive days. Twenty-four hours later lungs were collected, processed, and stained with AB/PAS for specific identification of IM as described in the text. a = significantly different from respective group challenged with saline; b = significantly different from respective group exposed to filtered air; $P \leq 0.05$. Clear bars represent filtered air; striped bars represent 30 µg/m^3 DEE; black bars represent 300 µg/m^3 DEE. Note the use of different y-axis scales.

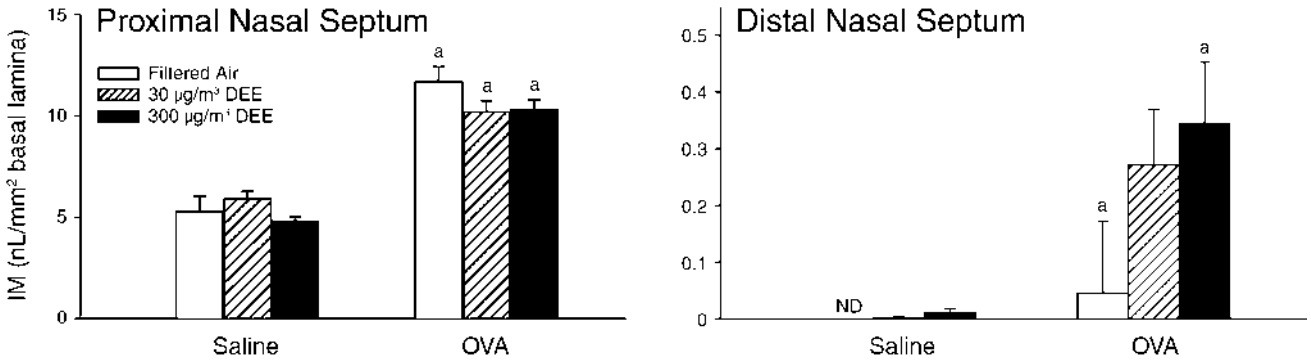

Figure 28. IM in the nasal septum. In DEE study 2, OVA sensitized rats were exposed to DEE during challenge with saline or OVA for 3 consecutive days. Twenty-four hours later the nasal septum was collected, processed, and stained with AB/PAS for specific identification of IM as described in the text. ND = not detectable; a = significantly different from respective group challenged with saline; $P \leq 0.05$. Clear bars represent filtered air; striped bars represent 30 µg/m^3 DEE; black bars represent 300 µg/m^3 DEE. Note the use of different y-axis scales.

Table 17. Study 2: DEE Exposure During OVA Challenge — Effects of DEE and OVA on Pulmonary Gene Expression*

Group	Muc5AC	IL-5	IL-10	IL-12p35	IL-12p40	IL-13	IFNγ	TNFα
Air/Saline	1.000 ± 0.239	1.000 ± 0.356	1.000 ± 0.329	1.000 ± 0.264	1.000 ± 0.302	1.000 ± 0.412	1.000 ± 0.328	1.000 ± 0.349
30 DEE Saline	1.329 ± 0.415	0.937 ± 0.267	0.897 ± 0.260	0.848 ± 0.113	1.379 ± 0.677	0.626 ± 0.158	0.991 ± 0.217	0.918 ± 0.189
300 DEE/Saline	1.359 ± 0.302	0.482 ± 0.125	0.443 ± 0.113	0.399 ± 0.054[b,c]	0.689 ± 0.207	0.549 ± 0.175	0.739 ± 0.167	0.746 ± 0.093
Air/OVA	2.179 ± 0.498	1.009 ± 0.253	2.866 ± 0.877	0.684 ± 0.155	1.684 ± 0.532	1.186 ± 0.212	2.630 ± 0.605[a]	1.231 ± 0.335
30 DEE/OVA	2.232 ± 0.596	1.761 ± 0.482	3.628 ± 1.030[a]	0.659 ± 0.137	2.290 ± 1.386	1.656 ± 0.659	2.216 ± 0.469[a]	1.418 ± 0.485
300 DEE/OVA	1.801 ± 0.201	0.694 ± 0.116	2.751 ± 0.662[a]	0.417 ± 0.073	0.360 ± 0.094	0.820 ± 0.111	2.036 ± 0.447[a]	0.727 ± 0.131

* Reported as fold increase relative to Air/Saline control.

[a] Significantly different from respective group challenged with saline (i.e., the control, which was not challenged with OVA); $P \leq 0.05$.

[b] Significantly different from respective group exposed to filtered air; $P \leq 0.05$.

[c] Significantly different from respective group exposed to 30 DEE; $P \leq 0.05$.

Gene Expression of Airway Mucin and Inflammatory Cytokines

Exposures to DEE during challenge with saline showed trends for depressed gene expression of cytokines in rats, though only the IL-12p35 protein was significantly decreased by the DEE exposure of 300 µg/m^3 (Table 17). There was a significant increase in the expression of IFNγ in the Air/OVA rats compared to the Air/Saline rats, and though not significant, there was also increased expression of Muc5AC and IL-10. Increases in IL-10 were only observed when rats were exposed to DEE and challenged with OVA.

DISCUSSION

CAPs INHALATION STUDIES IN SW DETROIT

The principal pathologic features of asthma and other chronic airway diseases, such as chronic bronchitis, are airway inflammation (e.g., airway infiltration of lymphocytes and eosinophils in allergic asthma) and excessive amounts of mucus in the airway lumens, which are associated with increased numbers of mucus (goblet) cells in the respiratory epithelium lining the intrapulmonary conducting airways (Reid 1954; Glynn and Michaels 1960; Esterly and Oppenheimer 1968; Dunnill et al. 1969; Cutz et al. 1978; Reynolds and Merrill 1981; Aikawa et al. 1992).

The results of our CAPs inhalation study in SW Detroit indicated that the high concentrations of CAPs, during the first exposure period in July, were likely the result of emissions from stationary combustion sources, and that the magnitude of airway inflammation was enhanced, as evidenced by the histopathology and changes in BALF (e.g., an increase in lavaged eosinophils and lymphocytes) in our laboratory rat model of allergic airway disease. CAPs also enhanced OVA-induced mucus production, as indicated by (1) a morphometrically detected increase in IM in the intrapulmonary conducting airways (which is a quantitative

indicator of MCM and intracellularly stored mucus product) and by (2) an increased concentration of mucin-specific protein in the BALF (which is an indicator of mucus hypersecretion). In these rats, the CAPs-induced enhancement of the allergen (OVA)-induced allergic bronchopneumonia, which results in excessive production of airway mucus, appeared to be dose-dependent: CAPs-related effects occurred after exposure to a high average mass concentration of CAPs in week 1 (i.e., 596 $\mu g/m^3$), but not after exposure to the markedly lower average mass concentration in week 2 (i.e., 356 $\mu g/m^3$). These findings confirmed our specific hypothesis in aim 1; namely, that CAPs exposure during allergen challenge will exacerbate airway epithelial remodeling (e.g., MCM) and airway inflammation in BN rats previously sensitized to the allergen.

We have previously demonstrated that CAPs exposures in this same SW Detroit community enhanced preexisting, allergen-induced, airway epithelial and inflammatory lesions in BN rats (Harkema et al. 2004). The experimental design of this previous study compared to that of our present study differed primarily in when OVA-sensitized BN rats were exposed to CAPs in relation to the OVA challenges. In our previous study, OVA-sensitized BN rats were challenged with OVA 48 hours before the start of the daily CAPs inhalation exposures for 4 or 5 consecutive days. In that study, the allergic inflammatory and epithelial responses in rats were ongoing (they had preexisting airway disease) prior to the CAPs exposure. In contrast, OVA-sensitized rats in our present study were challenged with OVA on the same days that they were exposed to CAPs. Here we were examining the effects of CAPs on allergen-induced airway disease, rather than its effect on ongoing preexisting disease.

Our CAPs inhalation study was not designed to precisely determine the specific toxicologic mechanism(s) by which CAPs enhance the allergic hypersensitivity reaction to OVA. However, the physiochemical components of ambient PM and CAPs that we identified, the concurrent meteorological data, and the profile of trace elements in the lungs of the CAPs/OVA rats indicated that the CAPs that induced an exaggerated allergic response in week 1, in mid-July, were predominantly associated with emissions from stationary combustion sources and less with motor vehicle emissions (e.g., diesel or gasoline engine exhaust).

Southeastern Michigan's air quality is typically dominated by air pollution emitted by heavy industry and motor vehicles within the Detroit metropolitan area together with regionally transported secondary pollutants that include O_3 and $PM_{2.5}$ (Keeler et al. 2002; Morishita et al. 2006). In our present study, $PM_{2.5}$ accounted for 60% of PM_{10} (daily max 80%) in Detroit, and the temporal correlation between $PM_{2.5}$ and PM_{10} mass was $r = 0.90$, suggesting that much of the larger-particle effects may be driven by temporally covarying smaller $PM_{2.5}$ particles (Lippmann et al. 2000). Taken together, these results indicated that the inhalable fraction of PM (PM_{10}) in SW Detroit was driven by much smaller particles (PM_1), and stationary combustion sources were likely the major culprit for the frequent air pollution events in this area.

Ultrafine particle concentrations measured in urban areas provide a good indication of the spatial and temporal scale for the emissions that are transported (Young and Keeler 2004, 2007). Observations of elevated number concentrations of these smallest of particles suggest that local combustion sources are important, as the lifetimes in the atmosphere of these are typically very short. The maximum ultrafine number concentrations occurred on July 14 and 15 during week 1 of our CAPs inhalation study. The number concentration peaks on July 15 coincided with elevated SO_2 concentrations, which previously have indicated contributions from local combustion sources (Young and Keeler 2004, 2007).

The predominant wind direction during July 22 and 23 in week 2 was northerly. Days with northerly winds were associated with low pollutant concentrations since there was only a limited number of large stationary sources to the north of our site. The average ambient $PM_{2.5}$ concentration was only 11 $\mu g/m^3$ for the two days with flow from the north. The predominant wind direction on July 21 of that second week was southwesterly. However, several rain events, which occurred just prior to this exposure, lowered particulate air pollutant levels through the deposition of wet particles and the scavenging of particles. The relative humidity on that day averaged 81% with a minimum of 75%.

As described in the Study Designs, Methods, and Materials section, the highest density of industrial activity in the city is in SW Detroit and includes iron and steel manufacturing plants, coke ovens, chemical plants, refineries, sewage-sludge incinerators, and coal-fired utility plants in and around Zug Island and along the Detroit River (Keeler et al. 2002; Morishita et al. 2006). Meteorological measurements on July 14 revealed that the pollution event was associated with south-southeast winds. Winds from the southerly direction significantly increase the likelihood of locally generated pollutant transport, which was also evident by the elevated SO_2 concentration measured at the site (Table 3).

A previous study in Detroit reported that Rb and Zn (which were also elevated on July 14) were associated with emissions from sludge incinerators that were located close to our sampling site (Morishita et al. 2006). The City of

Detroit Water and Sewerage Department operates one of the largest sewage-sludge incinerators in the United States. It is located between the site and Zug Island on Jefferson Avenue. The EPA AirData NET Facility Emissions report (EPA, 2000a) estimated that approximately seven tons of $PM_{2.5}$ were emitted from this facility in 1999. The observed elevated levels of Pb and Cd on July 14 were likely associated with municipal incinerators. Elevated levels of Mo, Fe, and Mn on that day were most likely associated with emissions from iron and steel manufacturing.

Previous studies have shown that trace elements, including Zn, Pb, Mg, Al, Fe, Mn, and Ba, are commonly detected in emissions from mobile sources, including diesel engine emissions (Cadle et al. 1998; EPA 2000b). We anticipated that total emissions would include a relatively large proportion of motor-vehicle-related particles since the SW Detroit community experiences heavy car and truck traffic because of the presence of two major interstates and the Ambassador Bridge, the largest international border crossing that connects Detroit to Windsor, Canada. The Michigan Department of Transportation (2002) reported that the average daily volume of traffic on major interstate highways around the Ambassador Bridge in 2002 was close to 100,000 vehicles/day.

To further evaluate the impact of diesel sources, the levels of PAHs in CAPs collected during the exposure periods were compared to the values reported for the PAH composition of emissions from diesel and other emission sources. The PAH data indicated strong impacts from the combustion sources on July 14 and July 15, during the first 3-day exposure period.

Studies have shown an increased emission of high-molecular-weight PAHs (PAHs with molecular weights greater than or equal to 202) from gasoline-powered and diesel-powered light-duty vehicles (Cadle et al. 1998), and dimethylphenanthrene measured in motor vehicle exhaust is typically enriched in the 1,3+2,10+3,9-dimethylphenanthrene isomers (McDonald et al. 2000). For ambient PAH concentrations, Adonis and colleagues (2003) investigated 24-hour average concentrations of carcinogenic PAHs adsorbed onto PM_{10} collected in diesel revision plants and in an urban area, and they reported that PAHs adsorbed onto PM_{10} in the diesel plants showed a high loading for low-molecular-weight PAHs, such as pyrene and chrysene, as well as for high-molecular-weight PAHs, such as benzo[*a*]anthracene and benzo[*k*]fluoranthene.

Reviews of the available literature summarized the ratios of PAHs for the characterization of their sources in environmental samples (Yunker et al. 2002; Vasilakos et al. 2007). For example, the ratio of indeno[1,2,3,-*cd*]pyrene to indeno[1,2,3,-*cd*]pyrene plus benzo[*ghi*]perylene is reported to be 0.35 for diesel emissions. In contrast, values in the literature above 0.5 are associated with wood, grass, and coal combustion aerosols. For the CAPs samples collected during the first 3-day exposure period in SW Detroit, the predominance of emissions from coal combustion and gasoline-powered motor vehicles, over those from diesel-powered vehicles, was indicated by the ratio of indeno [1,2,3,-*cd*]pyrene to indeno[1,2,3,-*cd*]pyrene plus benzo [*ghi*]perylene, which was > 0.7 (Table 6).

In contrast, during the second 3-day exposure period, most of the PAH concentrations were below detection limits, suggesting that fewer combustion sources had an impact on the site than during the first 3-day exposure period. In addition, the ratio of benzo[*a*]anthracene to benzo[*a*] anthracene plus chrysene was reported to be 0.49 for gasoline emissions and 0.68 for diesel engines (Vasilakos et al. 2007). The corresponding ratio during our Detroit exposure periods was less than 0.5, again suggesting that the relative impact of diesel-engine emissions was not strong. The fluoranthene to fluoranthene plus pyrene ratio is below 0.5 for gasoline, diesel, and fuel-oil combustion and emissions from cars and diesel trucks, but this ratio is above 0.5 in most coal- and wood-combustion samples (Yunker et al. 2002). The ratio observed during both of the 3-day exposure periods was below 0.5. Together, these results suggest that the higher levels of PAH concentrations observed during the exposures in this community were more likely to be attributed to combustion sources (specifically coal combustion) than to diesel-emitting sources.

The combined findings related to trace elements, PAHs, gaseous pollutants, meteorological parameters, and ultrafine particle numbers revealed that emissions from local combustion sources, including incinerators and coal-combustion sources, were dominant contributors to the $PM_{2.5}$ during the first 3-day exposure period in SW Detroit. Furthermore, an enhancement of the allergic reaction in the lungs of the CAPs/OVA rats in week 1 was observed during this period.

It is interesting that in both this study and our previously reported study of CAPs (Harkema et al. 2004) in this SW Detroit community, we determined that CAPs enhancement of OVA-induced allergic airway disease only occurred when the sources of $PM_{2.5}$ were predominantly coal-combustion utilities, local sewage-treatment facilities, and refineries, rather than diesel- or gasoline-powered motor vehicles. In both studies, when elevated concentrations of specific trace elements (as well as other source indicators) associated with these local combustion sources were observed in the ambient air pollution, in the CAPs inhalation chambers, and in lung tissues, there was a measured enhancement of allergic airway disease in the CAPs/OVA

rats. These exposure "dose"/response relationships suggested that the enhancement of allergic airway disease was principally due to the rats' exposures to CAPs derived from anthropogenic combustion sources. Future in vivo and in vitro studies are needed to determine which specific component(s) in the PM from these local combustion sources may be responsible for the enhanced allergic airway disease.

Though there have been several studies in recent years investigating the effects of combustion-derived particles collected from industrial and motor-vehicle emission sources (e.g., ROFA and DEE particles, respectively) on allergic airway disease in laboratory animals, there are only a few reports in the scientific literature of animal inhalation studies that have investigated the effects of airborne CAPs derived from real-world air pollution on the development or exacerbation of allergic airway disease. The present investigation is the first study to demonstrate that exposure to airborne CAPs derived from fossil fuel and other local combustion sources can enhance the development of allergic airway disease in the lungs of rodents (BN rats).

Other studies have shown that ROFA particles enhance the allergic sensitization of BN rats to house-dust-mite antigen (Lambert et al. 1999, 2000). Goldsmith and associates (1999) tested the hypothesis that, in mice, exposure to CAPs or the leachate of ROFA particles can cause an amplification of pulmonary inflammation and airway hyperresponsiveness induced by OVA. They found that OVA-challenged mice, when further exposed to CAPs, did not exhibit any additional airway hyperresponsiveness or pulmonary inflammation. However, for OVA-challenged mice, subsequent exposure to the ROFA leachate did result in enhanced airway hyperresponsiveness as compared to the controls. Kobzik and colleagues (Kobzik et al 2001; Goldsmith et al. 2002) investigated the effects of the coexposure of O_3 and CAPs on mice with and without OVA-induced allergic airway disease. In these studies, they found these pollutants had no effect on airway inflammation, but they did observe an immediate transient increase in airway hyperresponsiveness in mice with allergic airway disease exposed to both O_3 and CAPs that was associated with the Al–Si fraction of the particles.

More recently, Kleinman and coworkers (2005), in a study showing averages over a two-year period, demonstrated that OVA-sensitized BALB/c mice exposed to ultrafine or fine CAPs along a roadway in southern California had increases in biomarkers that are associated with airway allergic responses (IL-5, IgE, immunoglobulin G1, and eosinophils). In addition, mice exposed to CAPs 50 m downwind of the roadway had, on average, greater allergic responses and showed greater indications of inflammation than did mice exposed to CAPs 150 m downwind. The investigators concluded that their study was consistent with the hypothesis that exposure to CAPs close to a heavily trafficked roadway influenced allergic airway responses.

Unlike murine models of OVA-induced allergic airway disease in which inflammatory cell infiltrates are confined to perivascular and peribronchiolar locations and do not affect alveolar gas-exchange regions of the lung, the OVA-induced allergic airway disease in our BN rats extended the mainly airway-centered disease into adjacent alveolar parenchyma, which caused an allergen-induced bronchopneumonia. Interestingly, CAPs exposure during OVA challenge in the current study enhanced the allergic inflammatory response in both the bronchiolar and alveolar compartments of this hypersensitivity lung disease in rats.

CAPs exposure did not enhance the observed OVA-induced inflammatory or epithelial responses (e.g., allergic rhinitis with MCM along the nasal septum) in the nasal airways of rats. Since it is estimated that few inhaled particles in the range of 0.1 to 2.5 microns in diameter are likely to deposit in the nasal region of the respiratory tract (ICRP 1994), it is not surprising that CAPs exposure did not have an effect on the severity of the OVA-induced rhinitis. We have previously shown, however, that exposure to O_3, a common gaseous air pollutant that causes both nasal and pulmonary toxicity, did enhance OVA-induced rhinitis in BN rats (Wagner et al. 2002).

There is strong evidence that mucus impaction of the pulmonary airways contributes in an important way to morbidity and mortality from asthma (Aikawa et al. 1992). Excessive production of sputum is a common symptom in asthma and chronic bronchitis, and mucus plugging of the airways is consistently reported in fatal asthma cases, when the airways are examined post-mortem. The results of our CAPs inhalation study in SW Detroit indicated that exposure to high concentrations of CAPs during OVA challenge caused significant OVA-induced increases in the amount of stored mucosubstances in the epithelium of the intrapulmonary airways and an increase in the concentration of mucin-specific protein in the BALF of CAPs/OVA rats.

Several animal studies from our laboratory and others have indicated that common ambient air pollutants (e.g., O_3, SO_2, and sulfur oxide particles) can significantly alter the mucus apparatus of the conducting airways by increasing the number of mucus cells in the luminal surface epithelium and thereby increasing secreted mucus in the airway lumen (Reid 1963; Harkema et al. 1987a,b; Gearhart and Schlesinger 1989; Harkema et al. 1989; Schlesinger et al. 1992; Harkema et al. 1997b). Harkema and colleagues (1987a,b) reported MCM in the nasal airways of bonnet monkeys after acute and chronic exposures (8 hours/day

for 6 or 90 days) to ambient concentrations of O_3 (0.15 or 0.30 ppm). We have also reported that O_3-induced MCM in the nasal epithelium can be induced in the F344 rat after acute (6 hours/day for 3 or 7 days) or chronic (6 hours/day, 5 days/week, for 20 months) exposures to 0.5–1.0 ppm O_3 (Harkema et al. 1989, 1994; Hotchkiss et al. 1991; Harkema and Hotchkiss 1994).

Gearhart and Schlesinger (1989) reported that rabbits repeatedly exposed to acidic aerosols (250 μg/m^3 sulfuric acid [H_2SO_4], 1 hour/day, 5 days/week for up to 52 weeks) have marked increases in mucus secretory cells, especially in small pulmonary airways, the likely consequence of which would be an increase in production and hypersecretion of airway mucus. It has also been reported that rats exposed to ROFA had conspicuous increases in mucus (goblet) cells in pulmonary airways along with airway inflammation and epithelial hyperplasia (Dreher et al. 1997). Miyabara and associates (1998a,b,c) have also demonstrated that diesel exhaust enhances antigen-induced eosinophilic inflammation and mucus cell proliferation in the murine airway. Dreher and coworkers (1997) have previously reported that ROFA-induced pulmonary inflammation, airway epithelial alterations, and cytokine and chemokine expression are positively correlated with the presence of several soluble (leachable) metals associated with these particles.

We did not determine if CAPs contained measurable levels of biogenic substances such as endotoxin that might modulate allergic responses. Endotoxin has been shown to inhibit allergic responses if administered during sensitization procedures (Tulic et al. 2000; Delayre-Orthez et al. 2004). Conversely, endotoxin given during allergen challenge can either enhance or inhibit responses, depending on the endpoint or dosing regimen. While most studies involve delivery of endotoxin by IN or intraperitoneal routes, Hollingsworth and coworkers conducted aerosol coexposures with OVA and endotoxin, an approach more comparable to our CAPs inhalation exposures (Hollingsworth et al. 2006). They found that OVA-induced airway eosinophils were increased 3-fold when aerosol challenge also contained 0.18 μg/m^3 endotoxin. While this mass concentration would be well within the organic and carbonaceous fractions of our CAPs samples, the Detroit airshed is not typically rich in biogenic substances such as endotoxin.

In summary, our observations were that Detroit CAPs exposure during OVA challenge enhanced many of the pathologic features of allergic airway disease (e.g., MCM, mucus hypersecretion, and the mixed-cell inflammatory influx of eosinophils and lymphocytes) in the lungs of OVA-sensitized rats. These findings suggest that fine urban particulate matter may contribute to the development of human allergic asthma. Future studies are needed to further identify the specific toxic components in SW Detroit $PM_{2.5}$, and the underlying biologic mechanisms responsible for this particle-induced enhancement of allergic responses in the lung.

DEE STUDIES

Laboratory-animal models of allergic airway disease, as that for BN rats, require a sensitization period to the allergen prior to intra-airway challenge, for the animal to develop an allergic airway immune response and manifest the full pathological features of this disease. The aim of our DEE studies using our BN rat model of allergic airway disease was to test our hypothesis that DEE, when delivered to the rats during their allergen (OVA) sensitization or challenge periods, would enhance the allergic inflammatory and epithelial responses in the respiratory tract of rats after allergen challenge. Unlike our study using this rat model and CAPs inhalation exposure, in which we found a clear enhancement of inflammatory and epithelial responses to OVA when CAPs exposure was during OVA challenge, the effects of DEE during OVA challenge did not significantly alter OVA-induced allergic changes in the lungs or nose of BN rats at the high DEP concentration of 300 μg/m^3. At the low DEP concentration of 30 μg/m^3, exposure actually attenuated some of the OVA-induced pulmonary inflammatory responses (e.g., total inflammatory cells in BALF) and epithelial responses (e.g., amount of IM and MCM in distal axial airways). When BN rats were exposed to the high-concentration DEE during the sensitization period and then challenged with OVA, there were likewise no significant changes in the measured OVA-induced allergic responses in the nose or lungs. Though most measured parameters in DEE/OVA rats exposed to the low concentration of DEPs during sensitization did not indicate an enhanced OVA-induced response, there was a modest increase in total inflammatory cells in the BALF compared to that in Air/OVA rats. Overall, the results of our DEE inhalation studies did not confirm our hypothesis in aim 2, that DEE, given during OVA sensitization or challenge, would enhance the OVA-induced allergic airway disease in BN rats.

Previous animal studies have been designed to investigate the effects of DEPs on allergic airway disease and not the effects of DEE, which contains both gaseous and particulate components, as in our present study. This difference in exposures may explain, in part, some of the differences in our results compared to those of other laboratories. However, there are also major interlaboratory differences in the methods and experimental designs used in these studies, such as the type of rodent used (e.g., differences in species or strain), the type, amount, and delivery of the

allergen, and the time when DEE or DEP exposures were given with respect to allergen sensitization or challenge. Therefore, it is extremely difficult to compare the results of similar studies among laboratories that do not use exactly the same animal models and exposure conditions.

Several rodent studies have shown that DEPs enhance allergen-induced airway disease (Ichinose et al. 1997, 2002; Takano et al. 1997, 1998; Miyabara et al. 1998a,b,c; Dong et al. 2005a,b). Exposure to DEPs was also shown to enhance OVA-induced airway hyperresponsiveness in mice when these animals were challenged with OVA at the same time as the DEP exposure (Miyabara et al. 1998c; Takano et al. 1998). Inoue and colleagues (2007) have reported that exposure of mice to naphthoquinone, one of the extractable chemical compounds of DEPs, aggravated OVA-induced airway inflammation in a dose-dependent manner. However, other studies have shown that organic components of inhaled DEPs and other pollutants inhibit allergen-induced airway inflammatory responses (Dong et al. 2005a; Melgert et al. 2004).

Most of the studies on the effect of DEPs on allergic airway disease in rodents have been done with mice (Ichinose et al. 1997, 2002; Takano et al. 1997, 1998; Miyabara et al. 1998a,b,c). Dong and colleagues (2005a,b) reported on two studies they performed that used BN rats to examine the effects of DEPs on OVA-induced allergic airway disease. Their model of allergic airway disease involved a much longer OVA-sensitization period than was used in our present study, and they used high concentrations of nebulized DEPs (22.7 ± 2.5 mg/m^3). In their first reported study, these investigators found that DEP exposure prior to the sensitization period enhanced IgE and immunoglobulin G (IgG) antibody production, but inhibited airway inflammation induced by OVA challenge (Dong et al. 2005a). In contrast, when DEPs were given 1 day prior to the last day of OVA challenge, the investigators found that the DEPs enhanced both antibody production and pulmonary inflammatory infiltration characterized by marked increases in T lymphocytes and infiltration of eosinophils. They also found in this study that these in vivo findings correlated with the interactive effects of DEPs with OVA: markedly increased production of reactive oxygen species (ROS), increased production of nitric oxide (NO) by AM and alveolar type II cells (ATII cells), enhanced NO levels in BALF, the induction of inducible NO synthase expression in AM and ATII cells, and the depletion of intracellular glutathione (GSH) in AM and lymphocytes. They concluded that DEP-induced enhancement of allergic airway disease may be due in part to the elevated generation of ROS in AM and ATII cells, a depletion of GSH in AM and lymphocytes, and an increase in AM and ATII cell production of NO. These effects together reflect an enhanced oxidative stress response in the lungs. This is in agreement with similar findings of many other investigators who have suggested that the enhancement of allergic airway responses caused by DEPs, urban airborne particles, and other combustion-derived airborne particles (e.g., carbon black) are likely to be a result, at least partially, of an additive or a synergistic increase in the generation of ROS and an attenuation of protective antioxidants such as GSH (Pandya et al. 2002; Li et al. 2004; Donaldson et al. 2005; Nel 2005; Xia et al. 2006).

It is not clear why DEE in our study did not significantly modify the OVA-induced allergic airway disease in the BN rats as it did in the previously reported DEP study by Dong and coworkers (2005a,b). As mentioned previously, there were marked differences in the exposure regimens for both OVA and DEPs. However, the most probable reason for the observed differences in pulmonary responses between the two studies was the amount of DEPs to which the BN rats were exposed. Dong and colleagues exposed rats to a daily concentration of airborne particles that was 76 times higher than the highest concentration used in our studies. Even at markedly higher particulate concentrations, Dong and colleagues reported only moderate neutrophilic inflammation and no observable cytotoxicity in the lungs of rats exposed to DEPs alone (without OVA). Our rats were exposed to much lower daily particulate concentrations and had no measurable toxicant-related lung injury. In light of the marked differences in exposure concentrations between our studies and those of Dong and colleagues, it is not surprising that the low, but more environmentally relevant, concentrations of DEE used in our study did not exacerbate OVA-induced allergic airway disease. In addition, the chemical makeup of the DEPs used in our studies may have been considerably different and less toxic than that used by Dong and colleagues. Our present findings and those previously reported by others suggest that the dose, time of exposure, and chemical composition of inhaled DEPs are all important factors determining whether or not this component of particulate air pollution will enhance the development or severity of allergic airway disease.

Since DEE also contains some NO_x and other gases (e.g., CO), it is possible that these gaseous components may contribute to the effects (or lack of effects) of DEE on the rat's allergic responses to OVA. It has been reported by Hubbard and coworkers (2002) that exposure of OVA-sensitized mice to NO_2 (0.7 ppm) markedly attenuated the allergic pulmonary inflammation in mice that were exposed 2 hours prior to each daily challenge with OVA. This response was similar to what we observed in our OVA-sensitized BN rats that were exposed to DEE during OVA challenge. However, it is possible that DEE (and DEPs) may exert varied and

complex effects on experimental allergic asthma depending on the animal models, the timing of DEE exposure, and the allergen sensitization and challenge process.

CONCLUSION

The results of our animal inhalation studies in a SW Detroit community with high ambient $PM_{2.5}$ concentrations demonstrated that exposures to CAPs, derived from combustion (point) sources, during allergen challenge, markedly enhanced allergic airway disease in BN rats. This finding suggests that urban airborne $PM_{2.5}$ derived from combustion sources (e.g., refineries, coal-burning power plants, and waste-treatment plants) may enhance the development of human allergic airway diseases like childhood asthma. Previous animal inhalation studies in this community have also suggested that these fine, ambient combustion-derived particles may also exacerbate preexisting allergic airway diseases.

In contrast to our CAPs studies in Detroit, the controlled DEE exposures of allergen-sensitized BN rats, during either allergen sensitization or challenge periods, caused only a few mild modifications in the character of the allergen-induced disease. This finding is in contrast to other reported studies that indicate that DEPs alone (without gaseous components), but at relatively higher exposure doses, do enhance allergic airway disease in some rodent models. The reasons for these disparities between studies are not known, but likely reflect differences in exposure dose, animal models, timing of allergen and DEP exposures, the methods of allergen sensitization and challenge, or physicochemical differences between DEE and DEPs.

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ABBREVIATIONS AND OTHER TERMS

AB/PAS	Alcian Blue (pH 2.5)/Periodic Acid Schiff Sequence
ACES	Advanced Collaborative Emissions Study
AM	alveolar macrophages
ANOVA	analysis of variance
ATII	alveolar type II cells
BALF	bronchoalveolar lavage fluid
BN	Brown Norway
BrdU	bromodeoxyuridine
CAPs	concentrated ambient particles
CO	carbon monoxide
CO_2	carbon dioxide
DEE	diesel engine exhaust
DEP	diesel exhaust particle
EC	elemental carbon
ELISA	enzyme-linked immunosorbent assay
GSD	geometric standard deviation
GSH	glutathione
H^+	hydrogen ion
HEPA	high efficiency particulate air
HNO_2	nitrous acid
HNO_3	nitric acid
H_2SO_4	sulfuric acid
ICP–MS	inductively coupled plasma–mass spectrometry
IgE	immunoglobulin E
IgG1	immunoglobulin G
IL	interleukin
IM	intraepithelial mucosubstances
IN	intranasal
INF	interferon
IM	intraepithelial mucosubstances
LI	labeling index
LOD	limit of detection
LRRI	Lovelace Respiratory Research Institute
MCM	mucus cell metaplasia
MOI	multiorifice impactor
mRNA	messenger RNA
MSU	Michigan State University
Muc5AC	mucin glycoprotein
NH_3	ammonia
NH_4^+	ammonium
NIST	National Institute of Standards and Technology
NO_2	nitrogen dioxide
NO_3^-	nitrate ion
NO_x	total nitrogen oxides
O_3	ozone
OC	organic carbon
OVA	ovalbumin
PAH	polycyclic aromatic hydrocarbon
PM	particulate matter
$PM_{2.5}$	PM with an aerodynamic diameter $\leq$ 2.5 μm
PTFE	polytetrafluoroethylene (Teflon)
QA	quality assurance
QC	quality control
ROFA	residual oil fly ash
ROS	reactive oxygen species
RT–PCR	reverse transcriptase–polymerase chain reaction
SD	standard deviation
SIF	sorbent impregnated filter
SMPS	scanning mobility particle sizer
SO_2	sulfur dioxide
SO_4^{2-}	sulfate ion
SRM	Standard Reference Material
SW	southwest
Th2	T-helper cell [interleukin-2]
THC	total hydrocarbons
TEOM	tapered element oscillating microbalance
TNF	tumor necrosis factor
TWA	time-weighted average
ULLI	unit-length labeling index

ELEMENTS

Al	aluminun
As	arsenic
Ba	barium
Br	bromine
Ca	calcium
Cd	cadmium
Co	cobalt
Cr	chromium
Cu	copper
Fe	iron
La	lanthanum
Mg	magnesium
Mn	manganese
Mo	molybdenum
Ni	nickel
P	phosphorus
Pb	lead
Rb	rubidium
S	sulfur
Se	selenium
Sm	samarium
Sr	strontium
Ti	titanium
V	vanadium
Zn	zinc

Research Report 145, *Effects of Concentrated Ambient Particles and Diesel Engine Exhaust on Allergic Airway Disease in Brown Norway Rats*, J.R. Harkema et al.

INTRODUCTION

As outlined in the Preface, epidemiologic studies published in the 1990s suggested that short-term exposure to traffic in urban areas adversely affected the respiratory and immune systems, particularly in individuals with asthma and other allergic diseases. In these studies, diesel engine exhaust (DEE*) was a major contributor to particulate matter (PM) in urban air. In addition, experimental studies in the 1990s suggested that exposure to diesel exhaust particles (DEPs) affected the respiratory and immune systems of people with asthma. However, the epidemiologic studies did not measure ambient concentrations of individual pollutants, and many of the experimental studies did not use relevant exposure routes and particle doses. To provide a better understanding of whether particles from diesel engines and other sources contribute to the frequency and severity of asthma attacks and to the exacerbation of allergic responses, and if so by what mechanism, HEI issued Request for Applications (RFA) 00-1, *Effects of Diesel Exhaust and Other Particles on the Exacerbation of Asthma and Other Allergic Diseases*, in 2001. The RFA sought proposals for epidemiologic studies and studies of controlled inhalation exposures to particles in humans and other species, especially in groups considered susceptible to particle effects.

In response, Dr. Jack R. Harkema, Michigan State University (MSU), and colleagues submitted a proposal to evaluate the effects of exposure of rats to concentrated ambient particles (CAPs) of particulate matter (PM) with an aerodynamic diameter $\leq$ 2.5 μm (fine particles, $PM_{2.5}$); CAPs would be derived from an area in southwest Detroit, Michigan, that had a higher-than-average prevalence of childhood asthma. Harkema and colleagues had previously evaluated the effects of CAPs from this area in an earlier study of a rodent model of asthma — responses of rats to the airway-administered allergen ovalbumin (OVA) — that was funded by HEI (Harkema et al. 2004); they now proposed to use the same model to evaluate the effects in the airways of rats of exposure to CAPs during different phases of the administration of allergen. The HEI Research Committee considered Dr. Harkema's application a logical extension of his group's previous study. However, because the concentrator would sample PM from a location where DEE was only one of many sources, the Committee thought the application did not specifically address exposures to diesel. After discussions with HEI, Dr. Harkema included an evaluation of the effects of exposure to DEE in the same rat model. The diesel study would be carried out at the Lovelace Respiratory Research Institute (LRRI) in Albuquerque, New Mexico, where a system for exposing rodents to diesel exhaust was already in place. The Research Committee recommended the study for funding.

BACKGROUND

ASTHMA

Asthma is a chronic disease of the lower airways characterized by inflammation, mucus hypersecretion, reversible airway obstruction, and airway hyperresponsiveness. There are several subgroups among patients with asthma, each with somewhat different pathologies. In a major subgroup, asthma has an immunologic basis, most frequently associated with allergic reactions to inhaled allergens. Numerous early epidemiologic studies reported that short-term exposure to particulate pollution exacerbates the symptoms of asthma (e.g., Schwartz et al. 1993; Lipsett et al. 1997) and decreases lung function in people with asthma (e.g., Pope et al. 1991; Peters et al. 1997). The mechanism or mechanisms by which particles exacerbate asthma symptoms were not well defined, however.

EXPOSURE TO CAPs IN ANIMAL MODELS OF HUMAN AIRWAY DISEASE

Harkema and colleagues' previous HEI study was the first to evaluate the effects of exposure to CAPs in

Dr. Jack R. Harkema's 2-year study, "Fine Airborne Particles and Allergic Airway Disease," began in February 2003. Total expenditures were $438,886. The draft Investigators' Report from Harkema and colleagues was received for review in May 2007. A revised report, received in January 2008, was accepted for publication in February 2008. During the review process, the HEI Health Review Committee and the investigators had the opportunity to exchange comments and to clarify issues in both the Investigators' Report and the Review Committee's Critique.

* A list of abbreviations and other terms appears at the end of the Investigators' Report.

OVA-sensitized-and-challenged rats, a model with some but not all of the characteristics of human asthma (Harkema et al. 2004). As in the current study, the investigators used a mobile air research laboratory to collect PM and concentrate CAPs from the air in southwest Detroit, a site where the rate of asthma is three times higher than the national average. The PM collection site was at a school located within 2.5 km of a major border crossing between the United States and Canada for diesel trucks, steel and chemical refineries, and the intersection of two large highways.

In the earlier study, in rats previously sensitized and challenged with OVA, exposure to CAPs — for either 1 day or 4 to 5 days in two separate exposure campaigns (July and September) — was associated with changes in a limited number of airway inflammatory endpoints (Harkema et al. 2004). Compared with control animals, more effects were noted after CAPs exposures in September — including higher numbers of leukocytes in bronchoalveolar lavage fluid (BALF) and increased levels of mucus cell metaplasia (levels of intraepithelial mucosubstances [IM] and epithelial cell density) in the pulmonary airways — than in July. After the 5-day September exposures, the investigators detected higher levels of the trace metals V and La in lung tissues in CAPs-exposed animals than in animals exposed to filtered air. In a follow-up study (Morishita et al. 2004), the investigators reported increased recovery of the $PM_{2.5}$ trace elements V, La, Mn, and S from the lung tissues of CAPs-exposed rats, which they attributed to emissions from local refineries and other combustion sources.

In addition to the prior studies of Harkema and colleagues in the allergen model, other studies had evaluated the effects of exposure to CAPs in the airways both in healthy rodents and in a different model of airway inflammatory disease, chronic bronchitis. Gordon and colleagues (1998, 2000) found no changes in the airways of a small number of healthy rats inhaling CAPs collected in New York City. They did find modest changes in the distribution of peripheral blood leukocytes — an increase in the percentage of neutrophils and a decrease in the percentage of lymphocytes (Gordon et al. 1998, 2000). Clarke and colleagues (1999) showed that CAPs derived from Boston air enhanced tidal volume and increased inflammatory cell numbers in healthy rats and rats with bronchitis; CAPs induced more potent effects in the rats with bronchitis. The same group (Saldiva et al. 2002) found that exposure to CAPs increased neutrophil numbers in healthy rats and rats with chronic bronchitis, and it linked these changes with the PM components V and Br. Batalha and colleagues (2002) reported that CAPs exposures induced vasoconstriction of small pulmonary arteries in healthy and bronchitic rats, which was correlated particularly with the silicon component of PM. Using CAPs derived from another site, Research Triangle Park, North Carolina, Kodavanti and colleagues (2000) found that inflammatory responses of bronchitic rats varied with different exposure campaigns. In a different model of chronic bronchitis in rats, induced by administration of endotoxin rather than sulfur dioxide (SO_2), as was used in the studies cited above, Harkema and colleagues (2004) found no effects of exposure to Detroit CAPs. Moreover, in mice sensitized and challenged with OVA, Kobzik and colleagues (2001) found no effects of short-term exposure to Boston-derived CAPs on inflammatory endpoints in the airways. CAPs had no effect on control mice that were not sensitized or challenged with OVA.

Overall, these studies indicate that inhalation of CAPs had limited inflammatory effects in the airways and circulation of healthy rodents and rodents in different models of airway inflammatory diseases such as asthma and bronchitis.

EXPOSURE TO DIESEL EXHAUST IN HUMANS AND OTHER SPECIES

Before the current study, studies in both humans and rodents suggested that administration of DEPs enhanced features characteristic of an allergic pattern of immune response. Diaz-Sanchez and colleagues (1994) found that administering 300 µg of DEPs into the noses of healthy and asthmatic subjects enhanced the total number of immunoglobulin E (IgE)-secreting B cells in the upper airways of both groups. They also found (Diaz-Sanchez et al. 1997) that administering DEPs in combination with ragweed allergen enhanced the production of messenger RNA specific for interleukin-4 (IL-4) and IL-13 — cytokines associated with the Th2 subset of $CD4^+$ T lymphocytes and the development of key features of an allergic response, including IgE synthesis. Several early studies in rodents (e.g., Takano et al. 1997; Fujimaki et al. 1997) reported that the administration of DEPs with allergens such as OVA enhanced Th2-cell cytokine production and antigen-specific IgE synthesis. Mice sensitized to allergen showed increased inflammatory responses — mucus cell proliferation and enhanced numbers of eosinophils — after exposure to DEPs via inhalation (Miyabara et al. 1998).

Some human controlled-exposure studies had evaluated immunologic and inflammatory endpoints in healthy and asthmatic human volunteers exposed to diesel exhaust. Salvi and colleagues (1999, 2000) reported an increase in neutrophil and B cell numbers and in levels of IL-8, fibronectin, and histamine in BALF from healthy participants. Rudell and colleagues (1999) also found an increase in neutrophil numbers in BALF of healthy volunteers, but not in

their fibronectin or myeloperoxidase levels. Nightingale and colleagues (2000) found a significant increase in sputum neutrophils and myeloperoxidase. In a study of inflammatory responses in asthmatic people exposed to DEE, Nordenhäll and colleagues (2001) found a significant increase in IL-6 but not in myeloperoxidase, eosinophil cationic protein, or IL-8. Overall, these studies suggested that controlled exposure to diesel exhaust had limited inflammatory effects in healthy people and people with asthma.

OBJECTIVES AND SPECIFIC AIMS

The goals of the present study were to evaluate the effects in the airways of rats of exposure to DEE or CAPs at critical phases in the development of the allergic response, namely during *sensitization*—the first administration of allergen—and during *challenge*—the second administration of the same allergen.

The specific aims tested the following hypotheses:

1. Exposure of rats to CAPs during allergen challenge exacerbates airway epithelial remodeling (measured by, for example, mucus cell metaplasia) and airway inflammation.
2. Exposure of rats to DEE during either allergen sensitization or challenge exacerbates airway epithelial remodeling and airway inflammation.

STUDY DESIGN

The study was conducted in two different locations: the CAPs study in Michigan (exposures in Detroit and analysis at MSU in East Lansing) and the DEE study at the Lovelace Respiratory Research Institute (LRRI) in Albuquerque, New Mexico. The laboratories followed the same OVA sensitization and challenge regimen for CAPs and DEE studies: Brown Norway rats were *sensitized* by intranasal instillation of a 0.5% solution of OVA (or saline as a control) on days 1 through 3, they rested for days 4 through 14, and were *challenged* intranasally with a 1.0% solution of OVA (or saline) on days 15 through 17.

For the CAPs exposures, rats previously sensitized to OVA (or saline) were exposed to CAPs on days 15–17, that is, on the same days the animals were *challenged* with OVA. For the DEE exposures, in one experiment ("study 1" in the Investigators' Report) rats were exposed to DEE on the same days as OVA *sensitization* (days 1–3), and in a second experiment ("study 2") previously sensitized rats were exposed on the same days as OVA *challenge* (days 15–17).

CAPs COLLECTION AND EXPOSURES

The investigators used a mobile research facility to collect ambient air and expose rats to CAPs. The mobile laboratory was located at a school in southwest Detroit, Michigan. In addition to concentrating particles approximately 30-fold, the mobile lab collected meteorologic measurements including temperature, relative humidity, solar radiation, wind speed, and wind direction. Harkema and colleagues made measurements, in both ambient air and CAPs, of PM mass; elemental carbon and organic carbon (EC and OC); sulfate, nitrate, and ammonium ions; pollutant gases (ozone, carbon monoxide [CO], SO_2, and total nitrogen oxides [NO_x]); several organic species, including polycyclic aromatic hydrocarbons (PAHs); and several trace elements.

In the study of *CAPs during OVA challenge*, groups of eight rats were exposed via whole-body inhalation to CAPs or filtered air on days 15–17 for 8 hours/day; the OVA challenge was performed on the same days 1 hour after the end of the CAPs exposure. This substudy resulted in four groups of rats differing in OVA (or saline) challenge and CAPs (or filtered air) exposure: Air/Saline, CAPs/Saline, Air/OVA, and CAPs/OVA.

CAPs exposures were performed during two different weeks, July 14–16 and July 21–23, 2003. As described below in the Results section, the investigators found that CAPs concentration and composition differed in these two weeks; thus, they considered each week's 3-day exposure a separate experiment. Note that the investigators refer to these in the Investigators' Report as "week 1" and "week 2." To avoid confusion, this Critique refers to "CAPs Experiment 1" and "CAPs Experiment 2."

EXPOSURE TO DEE

Whole-body DEE exposure studies were conducted at the LRRI during February and March 2003. DEE was generated using a single-cylinder, 5500-watt diesel engine generator using number 2 nationally certified diesel fuel. In addition to PM mass, the investigators measured total hydrocarbons and the pollutant gases NO_x and CO.

In the study of *DEE during OVA sensitization* ("study 1"), rats were exposed to 30 $\mu g/m^3$ PM (low concentration) or 300 $\mu g/m^3$ PM (high concentration) DEE or filtered air for 8 hours/day on days 1–3 (see Figure 3 in the Investigators' Report). OVA *sensitization* was performed on the same days 1 hour after the end of the DEE exposure. Thus this substudy resulted in six groups, each with eight treated rats, that differed in OVA (or saline) sensitization and DEE (or air) exposure: Air/Saline, 30 DEE/Saline, 300 DEE/Saline; Air/OVA, 30 DEE/OVA, 300 DEE/OVA.

In the study of *DEE during OVA challenge* ("study 2"), rats were exposed to filtered air or the same concentrations of DEE (30 and 300 μg/m^3 PM) but on days 15–17 (see Figure 4 in the Investigators' Report). OVA *challenge* was performed on the same days 1 hour after the end of the DEE exposure. This substudy also resulted in six groups of treated rats differing in OVA (or saline) challenge and DEE (or air) exposure.

BIOLOGIC ENDPOINTS

Twenty-four hours after the last intranasal OVA challenge, each rat was killed and airway tissues from the nose and lung were removed. The left lung lobe was used to prepare BALF, which was assayed for total and differential cell counts and levels of the secreted mucin glycoprotein (Muc5AC), total protein, elastase, β-glucuronidase, multiple cytokines, and other soluble mediators including OVA-specific IgE. The left lung lobe was then fixed and processed, and Harkema and colleagues dissected out the proximal and distal axial airways. The nasal airways were also fixed and processed. The right lobe of the lung and main axial airway were used for isolation of total RNA; using reverse transcriptase–polymerase chain reaction, the investigators assessed the expression of airway mucin and multiple cytokines.

To assess changes in mucus cells in the airways, Harkema and colleagues conducted morphometric analyses of IM at four different regions of the respiratory tract: the proximal and distal nasal septum and the proximal and distal axial airway in the left lung lobe. The morphometric assessments included quantity and volume densities of stored IM in the surface epithelial lining of nasal and pulmonary airways, and numeric epithelial cell density. The investigators also used bromodeoxyuridine (BrdU) labeling to assess the number of surface epithelial cells synthesizing DNA in the axial airways: Values were assessed as percent labeled epithelial cells (labeling index [LI]) and labeling index per unit length of epithelium surface (unit-length labeling index [ULLI]).

DETECTION OF TRACE ELEMENTS

The investigators measured trace elements in ambient air, the exposure atmospheres, and the lung tissue of rats using inductively coupled plasma–mass spectrometry (ICP–MS). Levels of trace elements in lung tissue in Detroit were determined after CAPs Experiment 1; trace elements in the lungs at the LRRI were also evaluated after rats had been exposed to 30 or 300 μg/m^3 PM DEE for 3 days immediately prior to OVA challenge.

STATISTICAL ANALYSIS

Biologic endpoints were expressed as the mean ± the SEM and analyzed by analysis of variance for the type of pollutant exposure (CAPs, DEE, or air) and airway sensitization (allergen or saline). Samples with no detectable level of the molecule measured were analyzed as zero values. Significant differences were identified using post-hoc tests such as the Tukey omega procedure. The criterion for statistical significance was $P \leq 0.05$.

RESULTS

PHYSICAL AND CHEMICAL CHARACTERIZATION OF EXPOSURE ATMOSPHERES

Ambient $PM_{2.5}$, CAPs, and Gases

The average ambient $PM_{2.5}$ concentration measured at the Detroit mobile lab site was 16.2 ± 10.7 μg/m^3 in July 2003, with over 80% of the particles having a diameter ≤ 1 μm. The CAPs concentration was higher on average during the 3-day exposure period in CAPs Experiment 1 (July 14–16) than in CAPs Experiment 2 (July 21–23): 596 ± 563 μg/m^3 versus 356 ± 222 μg/m^3, respectively. The composition of the major identifiable components of CAPs — OC; sulfate, nitrate, and ammonium ions — was similar in both Experiments 1 and 2, but the composition of trace elements in CAPs and meteorologic data differed between the two experiment weeks. Levels of several trace metals — including Rb, Ni, Fe, and Al — were at their highest during CAPs Experiment 1.

Ambient concentrations of ultrafine particles (diameter ≤ 0.1 μm), PAHs, and the pollutant gases SO_2 and NO_x were higher during CAPs Experiment 1 than during CAPs Experiment 2.

The concentrator concentrated ambient $PM_{2.5}$ mass 20- to 31-fold on different days. The investigators showed (Figure 7 in the Investigators' Report) that for the PM components Fe and La the concentration factor, i.e., the enhancement in CAPs concentration over the concentration in ambient air, was consistent — 10- to 20-fold — over several days of concentrator use. However, a comparison of the data in Tables 1 and 5 of the Investigators' Report shows that the concentration factor for OC was approximately 30% to 140% higher than the concentration factor for PM mass on 5 of 6 days, and the concentration factors for EC and Mo were 50% or less than the concentration factor for PM mass on all 6 days, and were 67% or less for V, Ni, and As on at least 5 of the 6 exposure days.

DEE

At the LRRI, the investigators achieved concentrations close to the targets of 30 and 300 μg/m^3 PM diesel particle mass in the exposure atmospheres. Most of the particles were in the ultrafine range, contributing approximately 18% of the particle mass. The major chemical components of the diesel particles were EC (68%–77%) and OC (6%–14%), with small amounts of inorganic ions and trace metals. The investigators indicated that the most abundant of the detected trace metals — Ca, Zn, and P — were components of additives in fuel and lubrication oils. Levels of NO_x and CO in the high-diesel-exhaust exposure atmosphere were approximately 4 ppm; these levels were approximately 10-fold lower in the low-diesel-exhaust atmosphere.

BIOLOGIC ENDPOINTS

Characteristics of the OVA-Sensitization-and-Challenge Model

OVA sensitization and challenge of rats at MSU and at the LRRI resulted in many similar features:

- a mild-to-moderate rhinitis, characterized by an inflammatory response in the nasal mucosa comprising a mixture of leukocytes, with mild mucus cell hyperplasia and hypertrophy in the respiratory epithelium, and lymphoid hyperplasia in the nasal-associated lymphoid tissue.
- bronchiolitis and alveolitis in the lungs, which the investigators referred to as "allergic bronchopneumonia"; the bronchiolitis was characterized as an inflammatory response of infiltrating leukocytes of different types with mucus cell hyperplasia and hypertrophy in the respiratory epithelium lining the large-diameter conducting airways. Bronchiole-associated lymphoid tissue was also enlarged. Alveolar lesions were characterized by accumulation of macrophages, epithelioid cells, and eosinophils, with thickening of alveolar septa. Increased mucus cells and IM in surface epithelial cells lining the large-diameter bronchioles of the axial airways were also detected, as were increases in BrdU LI and ULLI of the airway epithelial lining of both proximal and distal airways.

BALF showed evidence of an inflammatory response: increases in total cells and multiple types of leukocytes (neutrophils, lymphocytes, eosinophils, and macrophages), as well as increases in levels of OVA-specific IgE, total protein, Muc5AC, and IL-6. However, apart from IL-6, levels of other cytokines assessed in BALF (tumor necrosis factor [TNF]α, IL-4, IL-10, and interferon [IFN]γ) were mostly undetectable. In addition, of the multiple cytokine-specific RNAs assessed in lung tissue after OVA sensitization and challenge, levels of only IL-10 were enhanced; levels of IL-12 and Muc5AC were decreased.

Some responses to OVA differed in the different weeks of the study at MSU and between animals evaluated at MSU and the LRRI; these included differences in numbers of total cells, proportions of various types of leukocytes, and levels of cytokines, cytokine-specific RNAs, and OVA-specific IgE. For example, the mean level of IL-6 measured in BALF in OVA-sensitized-and-challenged rats exposed to air was approximately 29 ng/mL in CAPs Experiment 1 at MSU, 11 ng/mL in CAPs Experiment 2 at MSU, 14 ng/mL in study 1 at the LRRI, and 361 ng/mL (with a large error) in study 2 at the LRRI. In addition, levels of OVA-specific IgE were increased by sensitization and priming only about 3-fold at MSU but over 10-fold at the LRRI.

Changes Associated with Exposure to CAPs or DEE

The focus of the study was to evaluate effects of pollutant exposure during OVA sensitization or challenge in rats. Few effects of CAPs or DEE exposure were detected in the absence of OVA sensitization and challenge. In addition, exposure to CAPs or DEE had no histologic or other biologic effects in the nose above those seen in rats sensitized and challenged with OVA.

Some effects of CAPs and DEE were detected in the lungs of OVA-sensitized-and-challenged rats, and these are summarized in Critique Table 1. Overall, Critique Table 1 indicates the following:

- Exposure to CAPs during OVA challenge in CAPs Experiment 1 *increased* some features of the OVA-induced inflammatory response in the lungs — the severity and distribution of bronchiolitis and alveolitis; numbers of cells; levels of total protein, Muc5AC, and TNFα in BALF; and IM in proximal and distal pulmonary airways. Levels of several cytokine-specific RNAs *decreased* after exposure to CAPs in CAPs Experiment 1. Few, if any, effects of CAPs during OVA challenge on inflammatory endpoints were detected in CAPs Experiment 2.
- The effects of DEE exposure on OVA sensitization and OVA challenge were quite mild, and the pattern of DEE-associated changes was quite complicated. First, in both studies more changes in OVA-associated endpoints were observed at the lower exposure concentration, 30 μg/m^3 PM, than at the higher, 300 μg/m^3 PM. Second, exposure to 30 μg/m^3 DEE during OVA sensitization predominantly *enhanced* OVA-associated inflammatory endpoints, whereas exposure to 30 μg/m^3 DEE during OVA challenge *attenuated* OVA-associated inflammatory endpoints.

Critique Table 1. Effects Associated with CAPs or DEE Exposure in the Lungs of Rats Sensitized and Challenged with OVA (Effect of Pollutant Compared with Filtered Air Control)[a]

Lung Endpoint	CAPs Exposure During OVA Challenge		Low DEE During OVA Sensitization	High DEE During OVA Sensitization	Low DEE During OVA Challenge	High DEE During OVA Challenge
	Experiment 1	Experiment 2				
Bronchiolitis and alveolitis, severity and distribution	↑	No effect	Slight ↑	No effect	Mild ↓	No effect
BALF cells	↑ Total cells (34%), predominantly eosinophils (200%), lymphocytes (150%), and neutrophils	No effect	↑ Total cells (62%), predominantly neutrophils (120%), eosinophils (52%), and macrophages (35%)	No effect	↓ Total cells (60%) and macrophages (54%)	No effect
Soluble products[b]	↑ Total protein (40%) and Muc5AC (20%), TNFα level (100%) No effect on IL-6 (all other cytokines ND)	No effect on total protein or Muc5AC, or on IL-6 and IL-10 (all other cytokines ND)	No effect on total protein or Muc5AC ↓ β-Glucuronidase ↑ IL-6, TNFα, and IL-10[c]	No effect on total protein or Muc5AC ↓ β-Glucuronidase No difference from control IL-6 (all other cytokines ND)	↓ Total protein (82%), β-Glucuronidase (40%), and IL-6 (98%) (all other cytokines ND)	No effect on total protein, Muc5AC, elastase, or β-Glucuronidase ↓ IL-6 (67%) (all other cytokines ND)
Gene expression (lung cells)	↓ IL-10, IFNγ, TNFα, IL-5, and IL-12 No effect on Muc5AC	No effect on any cytokine ↓ Muc5AC	Small ↑ in IL-12p35 but no other cytokines No effect on Muc5AC	↑ IL-10, IL-12p35, but no other cytokines or Muc5AC	No effect on any cytokine or Muc5AC	No effect on any cytokine or Muc5AC
Morphometry IM in pulmonary axial airways[d]	↑	No effect	No effect	No effect	No effect in proximal airways ↓ in distal airways	No effect in proximal airways ↓ in distal airways
Cell labeling indices	No change in LI or ULLI or numeric density in either proximal or distal airways	No change in LI or ULLI or numeric density in either proximal or distal airways	Small ↑ in ULLI but not LI or numeric density in proximal airways; no changes in distal airways	Small ↑ in ULLI but no effect on LI or numeric density in proximal airways ↓ in ULLI but no effect on density in distal airways	No effect on LI or ULLI in proximal airways Small ↑ in LI or ULLI in distal airways	No effect on LI, ULLI, or numeric cell density in proximal airways Small ↑ in ULLI and numeric cell density in distal airways

[a] ND indicates not detected.

[b] Levels of OVA-specific IgE not affected by any pollutant exposure. No pollutant effects on elastase or β-glucuronidase unless indicated in the table.

[c] Control values for all cytokines except IL-6 were nondetectable.

[d] Effects in proximal and distal airways, unless otherwise indicated.

Levels of Trace Elements in Lung After Exposure to CAPs or DEE

The investigators detected slightly higher levels of the elements La and Mo (and Rb — data not shown) in the lungs of rats exposed to CAPs in Experiment 1 than in the lungs of control rats not exposed to CAPs (Figure 9 in the Investigators' Report). No data on levels of trace elements were shown for CAPs Experiment 2.

The investigators also detected increases in several trace elements in the lungs of animals exposed to 30 or 300 μg/m^3 PM DEE for 3 days immediately prior to OVA challenge (data not shown). Figure 17 in the Investigators' Report shows very small increases in the levels of the trace elements V and Fe in the lungs of rats exposed to 30 or 300 μg/m^3 PM DEE compared to controls.

HEI EVALUATION

In its independent review of the study, the HEI Health Review Committee considered that this study addressed an important issue — investigating the effects in the airways of rats of exposures to relevant environmental pollutants at key stages in the response to an airborne allergen. The experimental design was well conceived and well carried out and the investigators' interpretation of data was generally appropriate.

The investigators made good use of their previously developed OVA-sensitization-and challenge model in rats that exhibited some characteristics of human asthma (Harkema et al. 2004). The features of the model were reasonably consistent at the two study sites, MSU and the LRRI. However, as the investigators acknowledge, although the model exhibited some features of human asthma, such as inflammation of nasal and bronchial airways and mucus cell metaplasia, other features — in particular, the observed alveolitis and increases in IL-6 levels — are not characteristic of human asthma. Moreover, the Committee noted that the investigators did not investigate submucosal changes, such as smooth muscle cell hyperplasia and hypertrophy, which are important pathologic features of allergic airway disease, and they did not detect levels of other cytokines that are associated with asthma. Thus, these differences limit the ability to extrapolate the results in this model to human asthma.

Although exposure to a low level of DEE mildly enhanced inflammatory responses when exposures were conducted concomitantly with allergen sensitization, the most surprising findings from the study were the relative lack of effect of the higher-level DEE exposure in the OVA model and findings suggesting that the low-level DEE exposure attenuated several effects of OVA when the exposures occurred during OVA challenge.

The inconsistency of changes after exposure to diesel emissions in this study appears to contrast with studies in humans and rodents that showed enhancement of different facets of the allergic response after exposure to diesel (e.g., Diaz-Sanchez et al. 1994, 1997; Fujimaki et al. 1997; Takano et al. 1997). One prior study in this rat OVA model (Dong et al 2005) reported inhibitory effects of inhaled DEPs before sensitization with OVA but used very high levels (20 mg DEPs, that is, 100- to 1000-fold higher than used in the current study), so the results are difficult to compare with those of the current study. As the investigators discuss in the report, some of the differences in findings could be the result of the lower levels of diesel emissions used in the current study compared with other studies; alternatively, differences in findings may possibly result from the use of DEPs in previous studies, in contrast to the DEE used in the current study that contained both particles and gases. The lack of effects of DEE observed in the current study could possibly be attributed to an inhibitory effect of the gaseous components of diesel on the stimulatory effects of DEPs. This possible interpretation is supported by the finding that exposure to nitrogen dioxide (NO_2) inhibited eosinophilia and inflammatory responses in a mouse OVA model (Hubbard et al. 2002). By contrast, in another mouse OVA study, NO_2 had no effect on the response to OVA (Husain et al 2004).

However, although the Committee considered that the inhibition of OVA effects by exposure to gases in DEE is not implausible, several controlled exposure studies in humans that used whole DEE found enhancement of airway inflammatory responses in healthy people and people with asthma (Rudell et al. 1999; Salvi et al. 1999, 2000; Nightingale et al. 2000; and Nordenhäll et al. 2001). In addition, in a real-world study on a street in London, United Kingdom (McCreanor et al. 2007; Zhang et al. 2008), people with asthma who were exposed to a diesel-emissions-rich environment showed enhanced inflammatory responses (as well as mild impairment of pulmonary function). These findings are inconsistent with the notion that gases found in diesel emissions might have inhibited a diesel-particulate-mediated enhancement of allergic and inflammatory responses that would otherwise have occurred.

The Committee also considered that the decreases in some of the endpoints in DEE-exposed animals, such as the decrease in total numbers of cells recovered in BALF, might have been the result of cell activation, and that these activated cells might have adhered to and been sequestered by airway surfaces or connective tissue components. In the absence of more detailed information in the report

about retrieval efficiency and numbers of inflammatory cells remaining in the lungs after lavage, this scenario was difficult to evaluate, however.

As in their previous HEI-funded study (Harkema et al. 2004), Harkema and colleagues made good use of a valuable resource at their disposal — the mobile air research laboratory — to expose rats to CAPs at a site where the prevalence of asthma is higher than average and where individuals may be exposed to high levels of pollutants from stationary and mobile sources. Harkema and colleagues at MSU and the LRRI also used state-of-the-art analytic methods to characterize the physical and chemical complexity of CAPs and DEE composition.

Overall, the Committee agreed with the investigators that responses to CAPs exposure differed in the two CAPs experiments, and that in the experiment in which CAPs exposure affected airway endpoints — the week in which levels of CAPs mass and some CAPs components were higher — the reported changes in this experiment were consistent with the results of earlier studies (Harkema et al. 2004; Morishita et al. 2004). In addition, the inflammatory effects of CAPs on cell numbers in the airways in Experiment 1 were generally consistent with those of more recent studies conducted in Los Angeles, California (Kleinman et al. 2005, 2007). Using a mobile research facility similar to the one used in the current study, Kleinman and colleagues exposed OVA-sensitized–and-challenged mice to CAPs downwind from a major roadway. In contrast to the current study, however, Kleinman and colleagues found that CAPs exposure enhanced measures of the immunologic response to OVA — levels of OVA-specific IgE and IgG1, both of which are associated with allergic, Th2-type responses in mice; however, this enhancement of the allergic response was noted only in mice exposed 50 m or less from the roadway (Kleinman et al. 2005, 2007).

Interpretation of the effects of pollutants on levels of cytokines in the airways in this study was challenging. Presumably the investigators expected to find increases in protein or RNA levels of at least some cytokines associated with the induction of allergic or inflammatory responses. However, exposure to either CAPs or DEE in the OVA model increased protein and RNA levels of few of the cytokines assessed; protein levels of most cytokines were undetectable, and pollutant exposures decreased some cytokine RNA and protein levels compared with controls. The Committee considered that the investigators might have chosen a time for cytokine analysis that missed augmentation of RNA or protein levels; alternatively, the levels of some other unmeasured cytokines might have been enhanced by exposure to pollutants. In this light, the Committee noted Harkema and colleagues' recent experiments (Heidenfelder et al. 2009) with CAPs inhalation in the same rat model. In that study, rats were exposed to CAPs between sensitization and challenge with OVA. CAPs-associated changes in histologic and biochemical endpoints were similar to those noted in the current study; however, gene-expression experiments using microarrays suggested CAPs-associated changes in levels of the cytokine transforming growth factor β, which plays an important role in repair mechanisms and remodeling in the lung, but which was not investigated in the current report.

Why effects of CAPs exposure were detected in CAPs Experiment 1 but not CAPs Experiment 2 can only be speculated upon. CAPs mass and levels of some trace elements were higher on average in CAPs Experiment 1 than in CAPs Experiment 2. Thus, as the investigators argue, the differences in biologic endpoints resulting from exposures in the different weeks of the current study could have resulted from differences in composition and concentrations of individual PM components, sources of emissions of components, or differences in meteorology between the two weeks. However, the Committee was not convinced by the investigators' interpretation that the effects of CAPs in Experiment 1 were attributable to local stationary sources — the investigators did not perform analyses to identify such sources, and the Committee was not convinced that the multiple elements whose levels were elevated in CAPs in Experiment 1 were associated specifically with local stationary sources. In addition, experiments showing levels of trace elements detected in the lungs after exposures to CAPs (or to DEE at the LRRI) were difficult to interpret because the differences in levels among the exposure groups were very small.

The Committee also noted some issues inherent in extrapolating results from the current studies in which CAPs and DEE emissions from a diesel generator were used. First, as in all studies that concentrate particles from ambient air, the physical characteristics of the concentrated particles do not reflect the composition of particles in ambient air. By definition, the concentrator preferentially concentrates fine particles, particularly those in the range of 0.6 to 1 μm in mass median aerodynamic diameter, and does not similarly concentrate ultrafine or coarse particles ($\leq$ 0.1 μm and $>$ 2.5 μm, respectively), which are outside the limits of the concentrator's size range but which may also have effects in this system. However, as the Committee recognized, the investigators used the concentrator to focus the study on the effects of exposure to higher-than-ambient levels of fine particles at a site of interest, an area in which the prevalence of asthma is high.

A second issue in using a concentrator is that the chemical composition of concentrated particles may also differ

from the composition of ambient particles. For example, although the investigators indicated that some trace elements were concentrated uniformly over several days and were proportional to their concentrations in ambient air, some constituents of the PM mix (such as OC) were preferentially concentrated, whereas other constituents of the mix (EC and several trace elements) were reduced in proportion to their concentration in ambient air.

In addition, the properties of the particles and gases emitted by the diesel generator used in the current study differ from those emitted by heavy- and light-duty diesel engines, which are of greater public health concern. For example, in previous work from the LRRI group (Reed et al. 2004), the emissions from a light-duty diesel engine emitting 300 $\mu g/m^3$ PM were composed of similar levels of EC, but 2.5-fold more OC, 5-fold more NO_x, and 67% lower CO levels than were in the emissions from the generator used in the current study. The Committee also noted that the current study was conducted before the United States (as well as Europe and other countries) introduced low-sulfur diesel fuel and implemented tighter diesel PM emission standards for both light-duty and heavy-duty vehicles (reviewed in HEI 2009). These changes have resulted in the introduction of new PM control devices — primarily filters and catalysts — for diesel vehicles.

Although the particles used in the current study were not "real world," the concentrator and diesel generator did generate exposure atmospheres with some characteristics of real-world aerosols. Future studies to explore the effects of exposure to airborne pollutants will need to use more relevant exposure atmospheres and models of human disease.

SUMMARY AND CONCLUSIONS

HEI's Review Committee thought that Harkema and colleagues successfully designed and conducted a descriptive study to evaluate the effects of two pollutants — DEE and CAPs (concentrated $PM_{2.5}$) — in a rodent model of asthma. The model had some but not all characteristics of the human disease, so caution should be used in extrapolating data obtained in the model to humans.

The most surprising findings were the relative lack of effect of high-level DEE (300 $\mu g/m^3$ PM) exposure in the model, which used rats sensitized and challenged with the airborne allergen OVA. In addition, low-level DEE exposure (30 $\mu g/m^3$ PM) during allergen sensitization mildly enhanced inflammatory responses, but the same exposure during allergen challenge attenuated several effects of exposure to OVA alone.

These findings differ from those of previous diesel-exposure studies, which have reported enhancement of inflammatory and allergic responses in human exposures and animal models. Differences in findings could be the result of the lower levels of diesel emissions used in the current study compared with some previous studies. Another possible explanation is that in some prior diesel-exposure studies rodents and humans were administered diesel particles, rather than the whole emissions used in the current study. Thus, the presence of gases in the DEE used in the present study could be hypothesized to have inhibited responses to the diesel particulate fraction. On the other hand, some studies of controlled human exposure to DEE — which clearly contained both gaseous and particulate components — have shown limited *enhancement* of allergic and inflammatory responses, findings inconsistent with the notion that gases found in diesel emissions might have inhibited a diesel-particulate-mediated enhancement of allergic and inflammatory responses that would otherwise have occurred.

The Committee also thought that the investigators made good use of a mobile air research laboratory to expose rats to CAPs at a site where the prevalence of asthma in the population is higher than average. In CAPs Experiment 1, exposure to CAPs during the 3 days of OVA sensitization enhanced some allergic and inflammatory endpoints, a finding consistent with data from previous studies, but that was not observed in CAPs Experiment 2. Differences in the findings of the two CAPs experiments suggest that the observed differences in composition of CAPs and hence sources of $PM_{2.5}$ in the different weeks CAPs were collected may be factors in determining the pattern of response obtained. However, the investigators did not perform analyses to identify sources of pollutants. In addition, in contrast to the investigators, the Committee was not convinced that the multiple elements whose levels were elevated in CAPs in Experiment 1 were associated specifically with local stationary sources. Thus, the Committee did not believe that positive effects of CAPs in Experiment 1 could be attributed easily to any one set of PM components or type of source.

The Committee also cautioned that in this study, as in all others that use CAPs, the concentrated particles may not be representative of particles in ambient air. Concentrated and ambient particles may differ in either physical characteristics — selective concentration of a particular size of particle — or chemical composition — selective concentration of particular components. In a similar note of caution about the diesel-exposure results, the Committee noted that the diesel emissions emitted by the generator used in the current study differ from emissions derived from new diesel-powered vehicles, which are subject to recent regulations to reduce particulate emissions. Future

studies to explore the effects of exposure to airborne pollutants will need to use more relevant exposure atmospheres and better models of human disease.

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