Lung Inflammation Induced by Concentrated Ambient Air Particles Is Related to Particle Composition

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The objectives of this study were (*1*) to determine whether short-term exposures to concentrated air particles (CAPs) cause pulmonary inflammation in normal rats and rats with chronic bronchitis (CB); (*2*) to identify the site within the lung parenchyma where CAPs-induced inflammation occurs; and (*3*) to characterize the component(s) of CAPs that is significantly associated with the development of the inflammatory reaction. Four groups of animals were studied: (*1*) air treated, filtered air exposed (air-sham); (*2*) sulfur dioxide treated (CB), filtered air exposed (CB-sham); (*3*) air treated, CAPs exposed (air-CAPs); and (*4*) sulfur dioxide treated, CAPs exposed (CB-CAPs). CB and normal rats were exposed by inhalation either to filtered air or CAPs during 3 consecutive days (5 hours/day). Pulmonary inflammation was assessed by bronchoalveolar lavage (BAL) and by measuring the numerical density of neutrophils (Nn) in the alveolar walls at the bronchoalveolar junction and in more peripheral alveoli. CAPs (as a binary exposure term) and CAPs mass (in regression correlations) induced a significant increase in BAL neutrophils and in normal and CB animals. Nn in the lung tissue significantly increased with CAPs in normal animals only. Greater Nn was observed in the central compared with peripheral regions of the lung. A significant dose-dependent association was found between many CAPs components and BAL neutrophils or lymphocytes, but only vanadium and bromine concentrations had significant associations with both BAL neutrophils and Nn in CAPs-exposed groups analyzed together. Results demonstrate that short-term exposures to CAPs from Boston induce a significant inflammatory reaction in rat lungs, with this reaction influenced by particle composition.

Keywords: ambient particles; neutrophils; inflammation; rats

Epidemiologic studies have consistently demonstrated associations between ambient air particle concentrations and morbidity and mortality, particularly among those with chronic respiratory and cardiovascular diseases (1–5). Associations have been demonstrated for a wide range of concentrations in different geographic locations, generally shown for short time lags and without a safety threshold (1–5).

Pulmonary inflammation, particularly in susceptible individuals, may be an important mechanism by which ambient particles may adversely affect human health. Recently, we used a rat model of chronic bronchitis (CB) (6) to examine further whether the observed increases in morbidity and mortality are associated with chronic respiratory disease. Using bronchoalveolar lavage (BAL), we demonstrated that concentrated air particles (CAPs) induce a detectable pulmonary inflammatory reaction in animals (6). These results are consistent with those observed in humans (7), as humans exposed to CAPs were also shown to exhibit pulmonary inflammation. Furthermore, autopsy studies on individuals living in areas with high ambient particle concentrations have shown inflammatory alterations of the airways and pulmonary parenchyma, with evidence of particle accumulation within the lungs (8, 9).

Although health effects have been related to mass concentrations, it is not likely that mass is the only factor that regulates particle toxicity. Identification of the toxic component(s) of particulate matter that is responsible for the particle-associated adverse health effects has become an area of active investigation (10, 11). Urban aerosols represent a complex mixture of particles either emitted directly by pollution sources, generated by reactions that occur in the atmosphere, or produced by mechanical resuspension (12).

Specific particle components, such as metals, have been implicated in the pathogenesis of particle-induced pulmonary inflammation in studies using intratracheal instillation of ambient air particles (13) and in studies using residual oil fly ash (10, 14). Whether these findings can be generalized to humans exposed to real ambient particles is unclear as the process of collecting particles with filters can affect particle composition and other properties, as well as their deposition profile in the lung parenchyma. In addition, the concentration of particles administered in the instillation protocols is approximately an order of magnitude higher than those experienced under typical atmospheric conditions. Thus, studies investigating the inflammatory potential of particle constituents at levels close to those observed in urban air and using experimental approaches with minimal manipulation are needed for understanding particle toxicity.

The Harvard ambient particle concentrator (15) represents a suitable approach to conduct toxicity studies of atmospheric fine particles, 0.1 to 2.5 μm ($PM_{2.5}$). This device enriches the concentration of ambient particles by a factor of approximately 30 times, without substantially modifying their physicochemical characteristics (16). This work was designed (*1*) to determine whether short-term exposure to CAPs causes pulmonary inflammation in normal rats and rats with CB; (*2*) to identify the site within the lung parenchyma where CAPs-induced inflammation occurs; and (*3*) to characterize the component(s) of CAPs that is significantly associated with the development of the inflammatory reaction.

METHODS

Experimental Groups and Exposure Protocol

Male adult Sprague-Dawley rats (body weight between 200 to 250 g) were obtained from Harlan Laboratories (Indianapolis, IN) and were handled in accordance with National Institutes of Health guidelines

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for the care and use of laboratory animals. Six weeks before CAPs exposure, the animals were randomly divided into four groups: (*1*) air treated, filtered air exposed (air-sham); (*2*) sulfur dioxide (SO_2) treated (CB), filtered air exposed (CB-sham); (*3*) air treated, CAPs exposed (air-CAPs); and (*4*) SO_2 treated, CAPs exposed (CB-CAPs).

The model of CB was developed by exposing rats to high concentrations of SO_2 for 5 hours/day, 5 days/week, during 5 weeks (6, 17). The average SO_2 concentrations for all experiments was 276.4 ± 9.1 parts per million. Control animals were maintained in the same conditions but were exposed to SO_2-free filtered air. Chronic bronchitic and normal control rats were subsequently exposed to either filtered air or CAPs for 5 hours on each of 3 consecutive days (6). Filtered air or CAPs exposures were administered on six different occasions to allow for variations in particle concentration and composition. In each experiment, 10 to 12 animals of each subgroup were exposed. Table 1 outlines the experimental protocol.

Exposures were administered using the Harvard ambient particle concentrator as previously described (6, 15, 16, 18). Particle characterization included gravimetric particle mass determinations (6, 11, 16), sulfate by liquid chromatography (11, 19), elemental analysis by x-ray fluorescence (11, 20), elemental carbon and organic carbon by a thermal and optical reflectance method (21), and particle size using a micro-orifice impactor (18, 22). Ambient outdoor concentrations of ozone, nitrogen dioxide, SO_2, and carbon monoxide were obtained from monitoring stations near our laboratory. Twenty-four hours after the last day of exposure, rats were euthanized using an overdose of sodium pentobarbital to perform either BAL or histopathologic studies.

BAL was performed as described previously to determine cell numbers, viability, and differential counts (6). Within the acellular BAL supernatant, lactate dehydrogenase (23), β-*N*-acetyl glucosaminidase (24), and total BAL protein (25) were measured. Protein and enzymatic reagents were obtained from Sigma Chemical Co. (St. Louis, MO), and chemical reagents were obtained from Fisher Scientific Co. (Pittsburgh, PA). BAL fluid measurements were not done on the March 1997 experiment.

For histopathologic studies, lungs were excised and fixed by intratracheal instillation of 2.5% glutaraldehyde in 0.1 M potassium phosphate buffer at a constant pressure of 20 cm H_2O. After fixation, all lung lobes (except for the cardiac lobe) were cut horizontally into uniform 2-mm sections. A random section from each lobe was processed for histology and analysis by light microscopy. Histologic slides were coded, and the observer was unaware of the code (blinded) until the analysis was completed.

Morphometric methods were applied to quantify the magnitude of neutrophil presence in the lung tissue. For this purpose, the numerical density of neutrophils (Nn) was determined by counting the number of such cells present in the alveolar septum at a magnification of ×450. Nn was assessed using an unbiased counting procedure (26, 27) with the aid of a grid that delineates a square of 225 μm. Nn was corrected by the density of alveolar parenchyma in the area of observation by applying a system of 100 points over the same area where Nn was determined and counting the number of points hitting alveolar tissue. Nn and density of alveolar parenchyma were determined for 10 microscopic fields for each slide for two locations: the centriacinar region (five fields/slide), defined as those alveolar structures that open directly to airways, and the peripheral acinar region (five fields/slide), defined as distal alveoli without an evident relationship to airways. Thus, a total of 40 measurements were obtained for each animal. Measurements performed in each animal were averaged within each anatomical site to provide a single data point for each location and for each animal. The morphometric measurements were performed by a single observer, with a difference in reproducibility below 10%.

Statistical Analysis

Descriptive statistics were calculated for the CAPs mass and compositional data, BAL data, and the tissue assessments. For regression analyses, biological data were log transformed to satisfy model assumptions of normality and homoscedastic variance. For the differential cell counts, total cell counts, protein, lactate dehydrogenase, and β-*N*-acetyl glucosaminidase data, analysis of variance techniques were used for exposure as a binary term, whereas linear regression models were used for actual exposure concentrations. Both statistical techniques fit exposure and CB status to each BAL response measurement. Exposure measures included element component concentrations, which were chosen based on results from previous factor analyses of Boston ambient particle data (11). In addition, because non-negligible experiment-to-experiment variability was observed in the sham subjects, a random experiment term was added to the models to control for this variability (28). Thus, effect estimates reflect within-experiment differences or differences between responses to CAPs exposures as compared with sham (filtered air) exposures for the same experiment. Effect estimates for continuous elemental composition exposures reflect how these within-experiment differences vary relative to the amount of measured elemental exposure for that experiment. Models specifying differential exposure effects by CB status were fit to each biologic response.

In the morphologic studies, multiple densities were recorded on each animal (centriacinar and peripheral acinar regions). To account for the repeated measures and locational differences, multivariate analysis of variance and multivariate linear regressions (29) were fit using the multivariate response (centriacinar Nn, peripheral Nn) as the dependent response. Exposure, presence of CB, anatomical location, and random experiment effects were included as explanatory variables. Models specifying differential exposure effects by location and by CB status were also fit to the data.

For comparability across biologic responses and particle compositions, results from the analyses are reported as standardized regression coefficients (30). These quantities represent the change in standardized response for one unit standard deviation change in concentration. Statistical significance for all models was based on an α of 0.05. All statistical analyses were performed using PROC MIXED in SAS version 8 (31). Graphical diagnostics of model adequacy were performed using the S-Plus statistical package (Mathsoft, Inc., Seattle, WA) (32).

RESULTS

Exposure Data

Particle mass, sulfate, organic carbon, and elemental concentrations for all exposure days are presented in Table E1 (*see* online data supplement). Concentrated particle mass levels exhibited considerable variation (range = 73.50 to 733.00 $\mu g/m^3$). Particle composition, which was determined for 16 of the 18 exposure days, also exhibited substantial day to day variation. The mass median aerodynamic diameter average ± the average geometric standard deviation for all experimental days was 0.27 ± 2.3

TABLE 1. EXPERIMENTAL DESIGN

Date	Total Number of Animals	Number of Animals for BAL Studies				Number per Group Histology Studies	Three-Day Mean CAPs Mass ($\mu g/m^3$)	Concentration Factor
		Air-sham	Air-CAPs	CB-Sham	CB-CAPs			
March 1997	40	8	8	8	8	2	170.7	19.9
June 1997	40	8	8	8	8	2	481.0	29.9
September 1997	48	10	10	10	10	2	187.1	12.1
January 1998	47	8	8	8	8	3–4	126.1	18.2
March 1998	40	8	8	7	8	2–3	267.3	35.1
June 1998	44	7	8	8	8	2–4	300.7	38.2

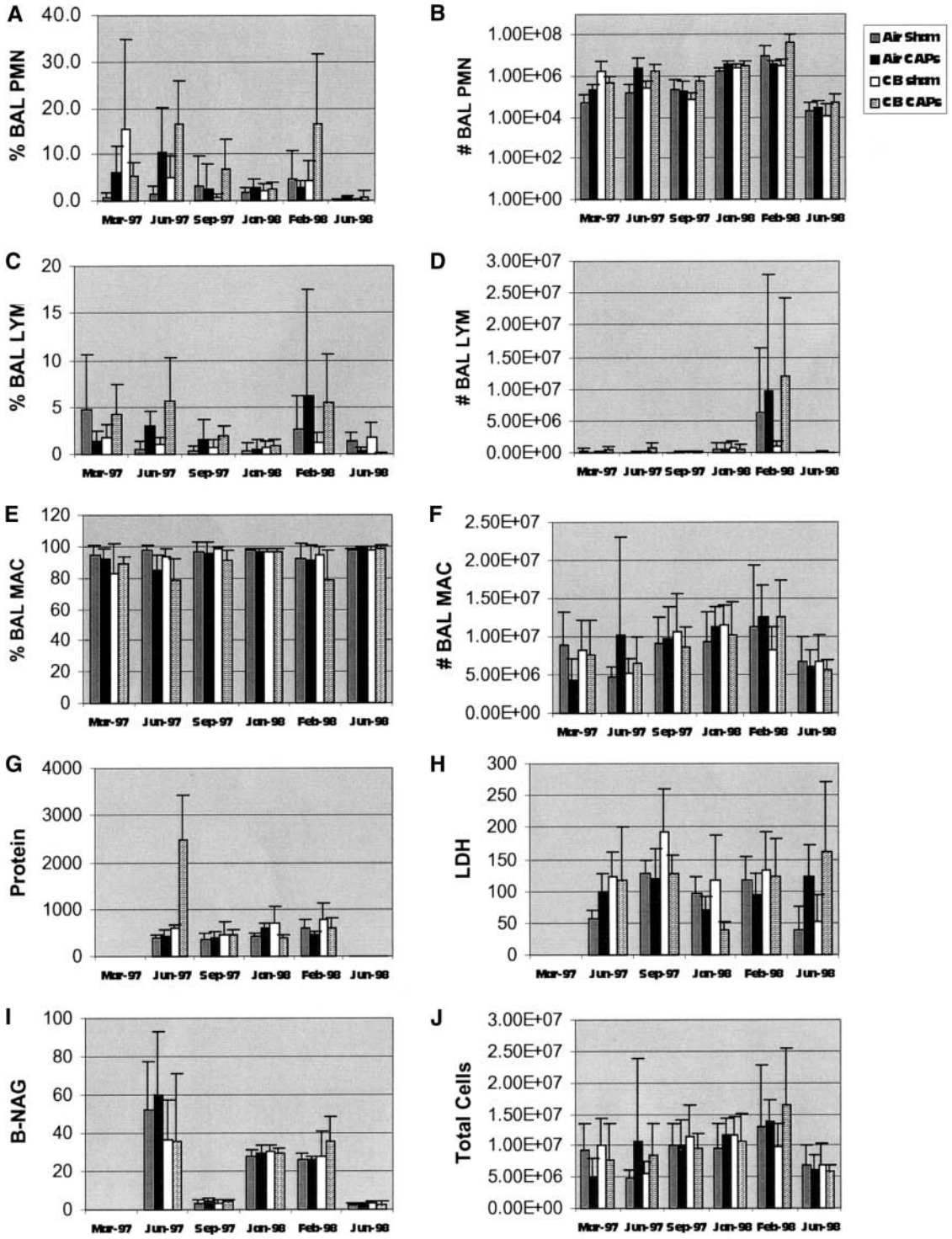

Figure 1. Mean ± SD for all BAL parameters for each treatment group in each experiment, including the percentage and numbers of neutrophils (*A* and *B*, respectively), the percentage and number of lymphocytes (*C* and *D*, respectively), the percentage and number of macrophages (*E* and *F*, respectively), protein (μg/ml) (*G*), lactic dehydrogenase (U/L) (*H*), β-*N*-acetyl glucosaminidase (U/L) (*I*), and total cells in the lavage (*J*). Variations among experiments and exposure groups may be observed.

μm. Temperature and relative humidity in the chamber for all experiments were 28.3 ± 1.6°C and 45.6 ± 6.6% for sham exposures and 28.9 ± 1.5°C and 47.7 ± 7.3% for CAPs exposures.

Acute Animal Responses

During exposure to either CAPs or filtered air, animals showed no signs of irritant inhalation or discomfort. Animals mostly slept or rested quietly throughout the exposures. No animals expired during any of these exposures.

BAL Data

Figure 1 presents all of the BAL data. Percentages of cell types, cell numbers, and BAL fluid assessments are illustrated. Inhalation exposure to CAPs for both the normal and chronic

bronchitic rats caused variable increases in the percentage and total number of polymorphonuclear neutrophils (PMNs) lavaged as compared with normal, sham-exposed controls. PMNs increased with CAPs exposure in four of six experiments in the normal rat groups and in five of six experiments in the chronic bronchitic rat groups. These changes were highly significant as shown in Figures 2A, 2B, and 2C when CAPs exposure was treated as a binary term. BAL lymphocyte percentages and counts also increased after CAPs exposure in four of six normal rat and five of six chronic bronchitic rat experiments, but these increases were not significant when exposure data were analyzed as a binary variable (CAPs versus Sham) (Figures 2A, 2B, and 2C). Macrophages showed little change, whereas total cell numbers showed considerable variability. Protein increased in three of five normal CAPs-exposed experiments and increased in two of five chronic bronchitic rat CAPs-exposed experiments. Lactate dehydrogenase increased in two of five normal rat CAPs-exposed experiments and in only one of five chronic bronchitic rat CAPs-exposed experiments. β-*N*-acetyl glucosaminidase increased slightly in three of five normal CAPs-exposed animal groups and only one of five CB-CAPs determinations.

The relationships between BAL cellular data and CAPs as a binary term or particle mass and component concentration for the second and third day of exposure regression variables are also shown in Figures 2A, 2B, and 2C. In Figure 2A, PMN levels increased significantly with particle exposures in both normal and CB rats when CAPs exposure was included as either a binary or continuous variable (CAPs mass). All of the components, except chlorine, had strong associations with BAL neutrophil measurements when both normal and chronic bronchitic animals were considered together. With the exception of silicon, which was not significantly associated for normal rats, particle components that were significantly associated with neutrophil increases overall remained significant when data were stratified by normal and CB groups. In all animals, increases in lymphocytes were not significantly related to particle mass as a binary term nor were they associated with any of the particle components in normal animals. The strongest association for all lymphocyte data was found for vanadium in the chronic bronchitic group.

Figures E1A, E1B, and E1C (*see* online data supplement) illustrate the relationship between BAL fluid measurements and CAPs as a binary term or 2nd- and 3rd-day particle mass and component concentrations as regression variables. Significant increases in protein levels were observed for lead, sulfate, elemental carbon, organic carbon, and silicon particle components in the analysis of both CAPs treatment groups. In analyses of the CB group alone, CAPs as a binary term, CAPs mass, and bromine were also significant in addition to those that

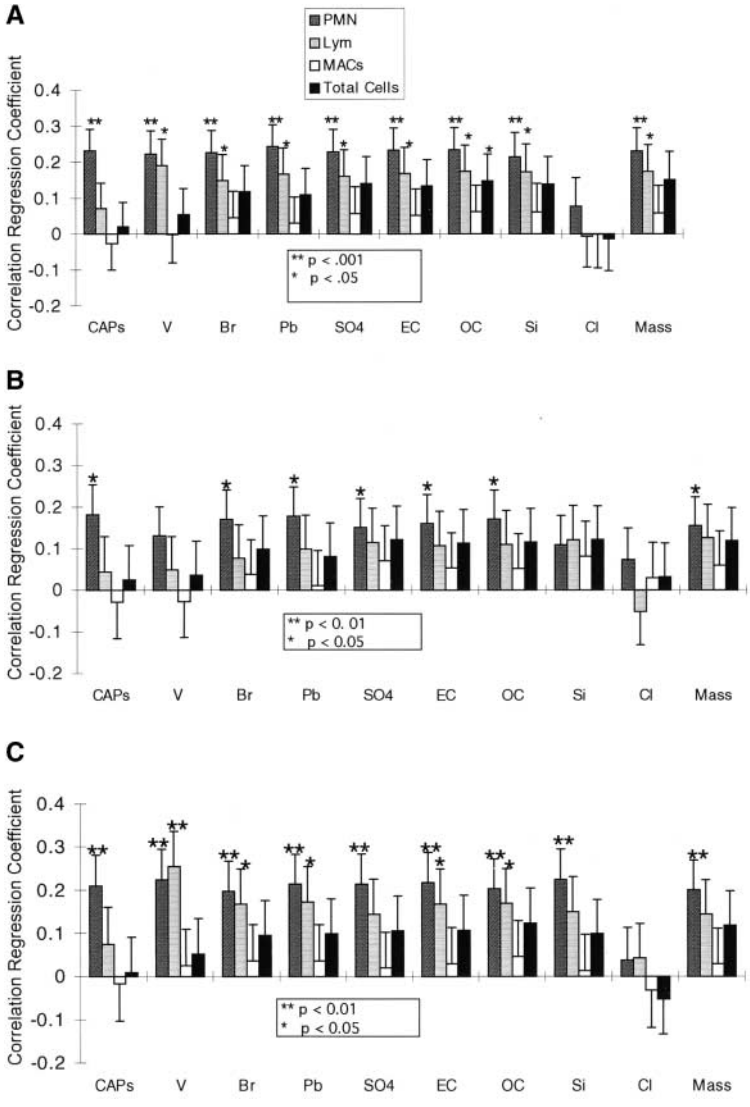

Figure 2. Standardized correlation coefficients for BAL cells versus CAPs as a binary term and CAPs mass and constituents using linear regression models. Standardized regression coefficients represent the change in response for one unit standard deviation change in concentration. Data are presented in *A* for normal and CB animals together and separately for normal animals in *B* and CB animals in *C*. Highly significant associations are observed between CAPs as a binary treatment, CAPs mass concentrations, and several components and BAL neutrophils (PMN). Weaker but significant associations are noted with some components and BAL lymphocytes (Lym). Macrophages (MACs) and total cells showed no significant differences. Br, bromine; Cl, chlorine; EC, elemental carbon; OC, organic carbon; Pb, lead; Si, silicon; SO4, sulfate; V, vanadium.

were significant in the total analysis. Lactate dehydrogenase and β-*N*-acetyl glucosaminidase showed few significant changes.

Histology

Variations in particle mass and composition were associated with variation in the morphologic changes in the lungs of CAPs-exposed animals. Although essentially no evidence of inflammation or histopathologic changes were observed in the lungs of normal rats exposed to filtered air, many animals exposed to CAPs developed an acute pulmonary inflammation, characterized by neutrophil recruitment, hyperplasia of the alveolar epithelium, and macrophage accumulation within the alveolar spaces. The histopathologic patterns observed in the normal rats exposed to either filtered air or CAPs are presented in Figures E2A–E2H (*see* online data supplement). In the photomicrographs depicted in Figures E2A and E2B, there is essentially no difference in the large airway morphology of animals exposed to either filtered air or CAPs. In Figure E2C, a normal airway–alveolar junction is illustrated without inflammation typical of sham (filtered air) exposures. In Figure E2D, epithelial hyperplasia of the terminal airway with interstitial neutrophils and increased numbers of macrophages is seen after 3 days of CAPs exposure. Illustrations in Figures E2E and E2F are higher magnifications of the junctional areas of Figures E2C and E2D. These show that there is no inflammation or other cellular changes in Figure E2E, but Figure E2F has increased visible neutrophils and macrophages. Photomicrographs illustrating minimal differences in the peripheral lungs of normal rats exposed to either clean air or CAPs are shown in Figures E2G and E2H. Chronic bronchitic rats exhibited airway inflammation, goblet cell hyperplasia, and constrictive bronchiolitis (*see* Figure E3 in the online data supplement). CAPs exposure in chronic bronchitic animals did not appear to exacerbate these lesions.

Figure E4A (*see* online data supplement) graphically presents the average Nn measured in the centriacinar and peripheral regions for the CAPs and filtered air sham exposure groups by experiment. Although variable in response, neutrophil density generally increased with CAPs as compared with filtered air exposures in both locations, with the response slightly greater in the centriacinar region. Figure E4B presents data for the animals with CB, which had considerable variability within treatment groups and experiments. Figure 3 graphically presents the descriptive statistics for Nn for the four exposure groups of all of the experiments combined. Significant differences in Nn were observed for central airway alveolar junctional location ($p = 0.036$) when data were stratified by CAPs exposure status. Significant locational differences, however, were not found for the particle components (data not shown). No significant correlation was found with ambient concentrations of ozone, SO_2, nitrogen dioxide, or carbon monoxide and Nn (data not shown).

Figure 4 illustrates the standardized correlation coefficients for Nn as this parameter relates to 2nd- and 3rd-day particle composition measurements in the normal and CB rats. In total analyses and the normal group, but not chronic bronchitic rats, inflammation was enhanced with CAPs exposures. Particle mass concentration was not significantly associated with increasing density of neutrophils in the tissue. The association between Nn in relationship to particle composition is also shown in Figure 4 using multivariate linear regression models for select particle components. Of the particle measures, CAPs exposure as a binary term, vanadium, and bromine were significant overall. In addition, in the normal animals, lead, chlorine, and elemental and organic carbon exhibited positive and significant associations with Nn. Sulfate, silicon, and particle mass were not significantly associated with tissue neutrophil density overall or in normal animals. There was no significant association with neutrophil density and any component in the CB group.

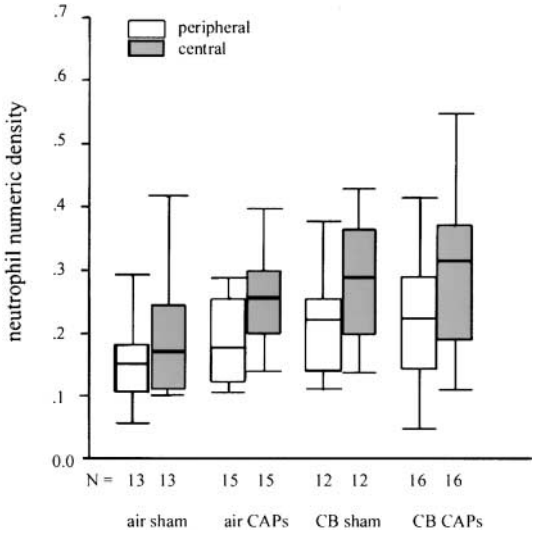

Figure 3. Neutrophil numerical density as box plots representing the values of neutrophil numerical density measured in the centriacinar and peripheral areas of the pulmonary acinus in the four experimental groups studied. Significant differences in Nn were observed for central airway alveolar junctional location (central) when data were stratified by CAPs exposure status ($p = 0.036$).

Based on the model analyses, significant associations between BAL parameters or the Nn and the particle constituents as listed in Figures 2, E1, and 4 indicate dose–response behavior. This dose–response relationship is illustrated graphically in Figure 5, in which exposure data for vanadium and box plots of neutrophil density are shown, reinforcing the hypothesis that the observed associations were causal.

DISCUSSION

Our findings suggest that short-term exposure to CAPs can induce pulmonary inflammation in rats. The magnitude of the inflammatory reaction to CAPs differed by experiment, with some of this interexperiment variability related to specific particle constituent concentrations, which were the most important predictors of pulmonary neutrophil accumulation, especially in the tissue assessments. Variability in exposure and response from experiment to experiment has been seen by others using CAPs (33, 34); however, those studies did not attempt to investigate the role of specific particle components.

Neutrophil increases in BAL were observed overall. When data were stratified by the disease status of the rats, stronger associations were observed in the chronic bronchitic animals (for example, the CAPs normal unstandardized coefficient was 2.411, $p = 0.0127$, whereas the CB unstandardized coefficient was 2.83, $p = 0.0040$; *see* online data supplement). The standardized coefficients of Figure 2 also reflect these differences. Similar findings were observed for the BAL protein data, for which the association was significant for all data and for the chronic bronchitic group. In contrast, findings from the morphologic analyses suggest that normal animals have a greater response to CAPs exposure as compared with chronic bronchitic animals. Significant overall associations were found for CAPs as a binary term, vanadium, and bromine. These associations were due to changes in the normal rats, with associations for the CB groups lacking significance.

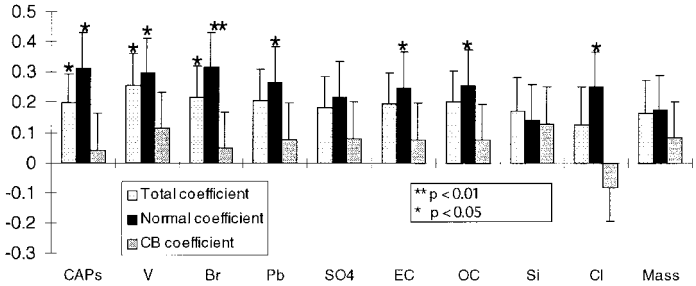

Figure 4. Standardized regression coefficients for CAPs and component factors in relationship to neutrophil density in lung tissue. Standardized regression coefficients represent the change in neutrophil density for one unit standard deviation change in concentration of CAPs mass or components. Data in the column labeled CAPs are based on CAPs as a binary term. Significant associations were observed with several components as well as CAPs as a binary term. (*See* Figure 3 for component legend.)

The apparent lack of association between morphometric data and CAPs exposure in the bronchitic rats may result from the difficulty in assessing morphologic changes in the chronic bronchitic animals. As shown in Figures E2 and E3, chronic bronchitic rats had more inflammatory changes compared with the normal animals as a result of their disease status, which might make morphologic detection of further change more difficult and may cause morphologic changes to be underestimated. In our study, for example, the Nn was corrected by the amount of alveolar tissue under analysis to avoid bias due to changes in the ratio between alveolar space/tissue in the different measurement areas. Because the chronic bronchitic animals experienced an evident thickening of alveolar walls (mainly in the centriacinar region) as a result of the chronic inflammation (Figure E3), it is possible that the magnitude of neutrophil recruitment was underestimated for these diseased animals. In addition, the timing of the postexposure morphologic measurements may have contributed to the observed differences between the normal and diseased animals. In both animal groups, morphologic assessments were made at the same time after exposure, which may or may not have been appropriate. Data from our study show, for example, that the chronic bronchitic animals had more free neutrophils in the airway and alveolar spaces, as assessed by BAL, as compared with the interstitium and vasculature, as assessed by morphology. These neutrophil differences may be related to "priming" (35, 36), where chronic bronchitic animals have increased chemokines (37) and thus an earlier neutrophillic response to CAPs exposure.

The inflammatory reaction observed in this study was not homogenous throughout the pulmonary parenchyma, with the reaction stronger in the centriacinar region. Our observations suggest that greater inflammation occurs in a small fraction of alveolar volume, specifically in the area located in the transition between the conducting airways and the peripheral gas exchange territories. The transition area from conducting airways to gas exchanging pulmonary parenchyma is the segment of the respiratory tract in which particles deposit most efficiently (38–40). In human subjects, this transition region is situated in a location of the respiratory tract that is unique in regard to both anatomic structure and fluid dynamics (27, 41). The generational total cross-sectional area of the airways starts to increase drastically around this region (41). The fluid dynamics also change with the Reynolds number approaching unity. Thus, fluid flow can be regarded as a viscous flow at the acinar entrance area.

The combination of structural alveolation and the transition of fluid flow to viscous flow may lead to chaotic gas flow in this region (42). This chaotic gas flow is characterized by large alveolar recirculation flows that rotate slowly with an associated stagnation saddle point near the opening of each rhythmically expanding and contracting alveolus. Furthermore, as predicted by theory, flow visualization experiments performed in excised rat acini suggested that substantial kinematic mixing may occur between the incoming tidal air (which may contain pollutant particles) and the alveolar residual gas (42–44). This mixing leads to increased particle trapping inside the alveoli and prolonged residence time for deposition in this region of the respiratory tract. Consequently, substantial particle deposition is expected to occur in this region, particularly near the entrance of the pulmonary acinus. These predictions are supported by recent experimental data in humans by Kim and coworkers (45–47), who found that the deposition of submicron particles peaked at around the anatomical dead space

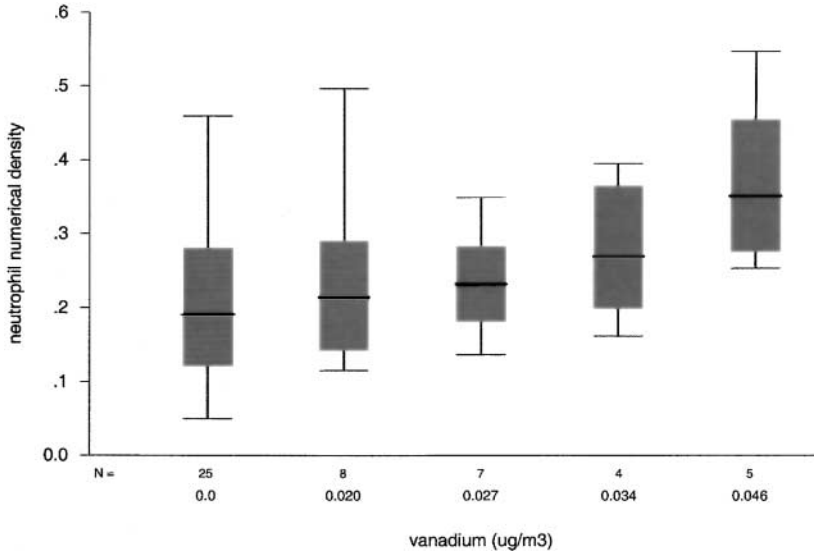

Figure 5. Box plot representing the variations of neutrophil density in the centriacinar region of the pulmonary parenchyma in normal animals at each level of exposure to vanadium (2-day average). This illustrates the dose–response relationship indicated by the correlation coefficients in Figure 4.

of approximately 200 ml (in humans), roughly corresponding to the entrance of the pulmonary acini. Also in support of this hypothesis, several autopsy studies of humans living in areas with high levels of ambient particles also found significant histopathologic abnormalities in the transition between terminal bronchioli and the alveolated region of the lungs (8, 9).

The relationship between our estimators of inflammation (Nn and PMNs in BAL) and CAPs exposure was significant only for the last 2 days of exposure. This is a time window that is consistent with that reported by epidemiologic studies in which the acute adverse health effects of particles have a short time lag (1–5). Particle mass as the single indicator of exposure did not have significant associations with pulmonary inflammation in the morphometric studies (*see* Figure 4). The association became stronger, however, when the composition of the particles was considered, suggesting that particle mass alone is not responsible for the observed effects.

To determine whether specific particle components were significantly associated with pulmonary inflammation, a series of modeling procedures was employed. Among the several parameters measured in our exposure studies, vanadium, bromine, lead, and organic carbon were those that exhibited a strong association with pulmonary inflammation. These results are consistent with those observed in canines exposed to CAPs (11). Interestingly, vanadium, bromine, lead, and to a lesser extent organic carbon originate from combustion sources (48). The associations obtained for these species were robust and were independent of several possible confounding events, such as presence of a pre-existing CB (induced by previous SO_2 exposure) and anatomical site within the lung. Moreover, the effects of significant particle components on pulmonary inflammation were dose dependent, indicating that the observed inflammatory response resulted from exposures to particles derived from combustion sources.

It is difficult from our study to identify how exposures to ambient particles initiate pulmonary inflammation. It has been shown that rats receiving intratracheal instillation of residual oil fly ash (a surrogate for ambient particles) developed acute pulmonary inflammation, which was dependent on the content of soluble transition metals (10). ROFA, which has a high content of vanadium, has been shown to disregulate phosphotyrosine metabolism and activate mitogen-activated protein kinase by pulmonary cells *in vivo* and *in vitro* (49, 50). Vanadium (in different valence states) was also shown to increase acutely cytokine expression by alveolar macrophages (51). Although fine particles increased transcriptional activation of nuclear factor-κβ–associated and/or regulated genes *in vitro* (52), approaches to define molecular mechanisms *in vivo* will require further studies.

It is important to note that the administered $PM_{2.5}$ exposures were higher (262 $\mu g/m^3$) than those observed in urban areas, especially in North American cities. These exposures, however, correspond to an equivalent cumulative 24-hour exposure of 55 $\mu g/m^3$, which is comparable to $PM_{2.5}$ concentrations observed in many large, urban centers in the developing world.

In conclusion, this work demonstrated that short-term exposures to $PM_{2.5}$ concentrated from Boston's atmosphere induce an inflammatory reaction in the lungs of rats. Such inflammation was observed in a time scale similar to those reported from acute epidemiologic studies. Quantitative histopathologic measurements demonstrated that greater densities of neutrophils are observed in the centriacinar region, an anatomical site characterized by relatively high fine particle deposition. The magnitude of pulmonary inflammation was associated with elements originating from combustion sources. These results support the concept that under controlled laboratory conditions, ambient fine particles are able to promote significant pulmonary injury that is associated with particle composition.

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