Inhalation of $PM_{2.5}$ Does Not Modulate Host Defense or Immune Parameters in Blood or Lung of Normal Human Subjects

Shirley D. Harder, Joleen M. Soukup, Andrew J. Ghio, Robert B. Devlin, and Susanne Becker

National Health and Environmental Effects Research Laboratory, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina, USA

We tested the hypothesis that exposure of healthy volunteers to concentrated ambient air particles (CAPS) between 0.1 and 2.5 µm in diameter is associated with modulation of human alveolar macrophage (AM) function, cytokine production, and immune phenotype in both blood and lung. Thirty-eight volunteers were exposed to either filtered air or CAPS from the immediate environment of the U.S. Environmental Protection Agency human studies facility in Chapel Hill, North Carolina, USA. Particle concentrations in the chamber during the exposures ranged from 23.1 to 311.1 µg/m^3. No symptoms were noted by volunteers after the exposure. Eighteen hours after exposure, analysis of cells obtained by bronchoalveolar lavage (BAL) showed a mild increase in neutrophils in both the bronchial (8.4 ± 2%) and alveolar fractions (4.2 ± 1.7%) in subjects exposed to the highest concentration of CAPS compared to neutrophils in the fluids of those exposed to filtered air (bronchial fraction 2.7 ± 0.6%; alveolar fraction 0.8 ± 0.3%). There was no change in the percentage of lymphocytes or AMs recovered in the lavage after inhalation of the highest particle levels (mean 207 µg/m^3). There was also no change in the proportion of lymphocytes in the BAL expressing CD3, CD4, CD8, CD19, nor activation markers CD25 or CD69. Particle inhalation did not affect the expression of CD11b, CD64, CD16, CD14, CD71 on AM, nor was there an effect on phagocytosis or oxidant generation following stimulation with zymosan A. IL-6 and IL-8 levels detected by enzyme-linked immunoabsorbent assay in the BAL were unrelated to inhaled particle levels. The distribution of lymphocyte subsets in blood obtained 18 hr after exposure to CAPS did not differ from that found before exposure. We conclude that ambient air particles are capable of inducing a mild inflammation in the lower respiratory tract but have no effect on immune phenotype or macrophage function under the conditions tested. *Key words*: air pollution particles, alveolar macrophages, bronchoalveolar lavage, cytokines, lymphocytes, oxidant production, phagocytosis. — *Environ Health Perspect* 109(suppl 4):599–604 (2001). *http://ehpnet1.niehs.nih.gov/docs/2001/suppl-4/599-604harder/abstract.html*

Elevated levels of air pollution particulate matter (PM) in the inhalable size range (< 10 µm in diameter) are associated with increased hospitalization for respiratory disease, including pneumonia and asthma (*1–3*). Most recently, the concentration of particles in the size range < 2.5 µm diameter ($PM_{2.5}$) has been suggested to be the most important determinant in pollution particle–induced health effects (*4,5*). The causative constituents of PM and the pathophysiologic mechanisms related to such health problems have not been determined. Part of this uncertainty regarding mechanism arises from the difficulty of exposing humans and animals to particles considered to be equivalent to those inhaled by populations included in the epidemiologic studies. Particles collected from specific emission sources such as residual oil fly ash and diesel, as well as water extracts of ambient air particles, have been demonstrated to cause an influx of neutrophils into the lung in both animals and humans (*6–8*). Furthermore, diesel particles have been shown to divert the immune response toward an allergic phenotype (*9,10*). *In vitro* studies with urban air particles have been shown to alter alveolar macrophage function in a manner promoting survival of microbes (*11–13*). However, this effect on macrophages resides in the coarse particle fraction (2.5–10 µm) rather than in the fine fraction (*14,15*).

The recent development of ambient particle concentrators has made it possible to perform controlled exposures of animals and humans by inhalation of real-world particles. In the present study we describe the effects of exposure of healthy individuals to concentrated $PM_{2.5}$ found in the air in Chapel Hill, North Carolina, on function and phenotype of immune cells in the lung and blood. The particles were concentrated 6- to 10-fold and volunteers were exposed to levels ranging from 23 µg/m^3 to 311 µg/m^3. These levels approximate the total levels of particles found in many metropolitan areas of the United States. Because increased incidence of asthma severity, allergy, and pneumonia can be associated with PM levels, we assessed various host defense parameters involved in regulating these diseases, including immune cell phenotype, alveolar macrophage (AM) function and inflammation.

Materials and Methods

Ambient Aerosol Exposure System

Particles between the sizes of 0.1 and 2.5 microns present in the Chapel Hill air were concentrated using a Harvard/EPA ambient fine particle concentrator (HAPC) consisting of three-stage virtual impactors. The principles by which this concentrator works have been previously described (*16,17*). A schematic of the concentrator and human exposure chamber is provided (Figure 1). The concentrator uses the inertial separator technique, thus concentrating particles only, not gases. Briefly, outside air is first drawn through an Anderson high-volume conventional impactor with a 2.5-µm cut-off size at a rate of 5,000 L/min. The exit flow from the Anderson impactor, which contains particles mainly < 2.5 µm in diameter, is drawn into the first stage of the concentrator in which five virtual impactor slits (1,000 L/min/slit) are arranged in parallel. The virtual impactor consists of two parts: The upper part is in the form of a rectangular nozzle through which airflow is accelerated, and the lower part is in the form of a sharp-edged slit that receives incoming particles. Each virtual impactor operates at a minor-to-total flow ratio of 0.2 so that 80% of airflow leaving the rectangular nozzle is deflected to the side stream (i.e., major flow) and 20% of the flow is extracted straight down into the receiving slit (i.e., minor flow). In this design, particles > 0.1 µm achieve sufficient momentum to cut across the deflecting major flow stream and enter the receiving slit, whereas particles < 0.1 µm follow the major stream and are exhausted. For this reason, particles smaller than 0.1 µm are not concentrated. Ideally, if all particles between 0.1 and 2.5 µm are

This article is based on a presentation at the Workshop on Inhaled Environmental/Occupational Irritants and Allergens: Mechanisms of Cardiovascular and Systemic Responses held 31 March to 2 April 2000 in Scottsdale, Arizona, USA.

Address correspondence to S. Becker, HSF, U.S. EPA, CB 7315, 104 Mason Farm Rd., Chapel Hill, NC 27599 USA. Telephone: (919) 966-0676. Fax: (919) 966-6271. E-mail: becker.susanne@epamail.epa.gov

The research described in this article has been supported by the U.S. Environmental Protection Agency. It has been subjected to agency review and has been approved for publication. Approval does not necessarily reflect the views of the Agency and no official endorsement should be inferred. Mention of trade names or commercial products does not constitute endorsement or recommendations for use.

The investigators greatly appreciate the invaluable contributions to this study by the medical staff M. Bassett, D. Levine, and S. Derrenbacker.

Received 22 December 2000; accepted 27 March 2001.

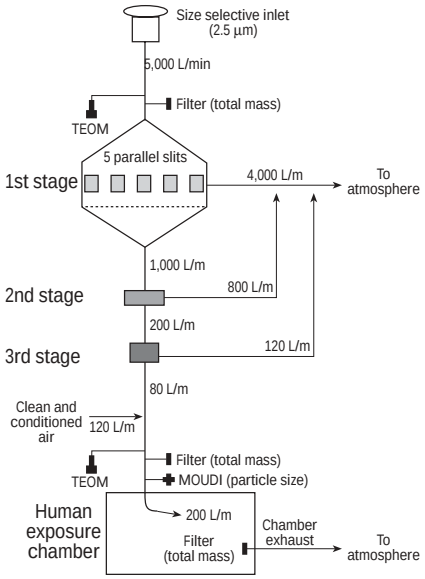

Figure 1. Schematic diagram of the Harvard/EPA ambient fine particle concentrator.

condensed into the minor flow, particle concentration in the minor flow will increase 5 times. In the present system, particles ranging in size from 0.1 to 2.5 µm are concentrated about 2.5- to 3-fold in the first stage, and a combined flow from five receiving slits (minor flow) is drawn into the second stage at the rate of 1,000 L/min. The second stage consists of a single virtual impactor identical to those in the first stage. Here, particles are concentrated 2.5- to 3-fold again and drawn into the third stage at the rate of 200 L/min. A single virtual impactor in the third stage operates at a minor-to-total flow ratio of 0.4 and concentrates particles about 2-fold at a flow rate of 80 L/min. Finally, the concentrated aerosols leaving the third stage are mixed with 120 L/min clean and conditioned air (20°C and 50% relative humidity), and the resulting conditioned aerosols are delivered into the exposure chamber at the rate of 200 L/min. The addition of the conditioned air dilutes the concentrated aerosols, but provides consistent temperature and humidity and allows sufficient airflow for subjects to exercise during exposure. In this study, particles were concentrated 6- to 10-fold at the inlet of the chamber.

A controlled exposure to air with no PM was required. Subsequently, filtered air (containing no metals, carbon, sulfates, or nitrates) was employed. Sham exposures were conducted using 200 L/min conditioned air and no air from the HAPC. Outside air was drawn in across beds of activated charcoal and potassium permanganate on alumina. After heating to 550°F to remove bound organics, the air was passed over cooling coils to a final temperature of 58°F. After passage through a series of HEPA filters, the air was introduced into the particle chamber.

The maximum concentration of aerosols to be delivered to the chamber varied depending on concentrations of naturally occurring $PM_{2.5}$ in the Chapel Hill air (which usually ranges from 5 to 30 µg/m^3). The exposure chamber is $4.0 \times 6.7 \times 7.5$ ft and constructed with aluminum panels and heavy-duty clear plexiglass for doors and windows. Because the air-pumping units are located downstream of the chamber and the HAPC, the chamber is operated under a slightly negative pressure (10–12 inches of water). Aerosols enter the chamber via a 6-inch diameter curved duct positioned on the top and middle of the chamber and exit via an exhaust duct positioned in the middle of one of the vertical walls (Figure 1). The subject sits between the inlet and exit duct with subject's head located less than 18 inches away from the inlet duct. A series of tests conducted in the present study have shown that the particle concentration at the subject's head position is at least 90% of that at the inlet duct.

Table 1. Physical characterization of particles.

	Quartile 1[a]	Quartile 2	Quartile 3	Quartile 4	Total CAPS
Number of subjects	8	10	10	10	30
$PM_{2.5}$ after concentrator (µg/m^3)		47.2 ± 5.3	107.4 ± 9.3	206.7 ± 19.2	120.4 ± 14.1
$PM_{2.5}$ before concentrator (µg/m^3)		9.9 ± 3.0	30.3 ± 3.8	37.6 ± 4.6	26.4 ± 3.0
Concentration factor	9.4 ± 2.3	4.1 ± 0.7	6.4 ± 1.0	6.5 ± 0.9	
MMAD (microns)	0.54 ± 0.06	0.67 ± 0.05	0.72 ± 0.03	0.65 ± 0.028	
GSD of MMAD (δ_g)	2.44 ± 0.16	2.39 ± 0.14	2.24 ± 0.084	2.35 ± 0.72	

[a]Clean air.

Particle Characterization

Air was sampled just prior to entering the HAPC and again just before entering the chamber from the inlet duct. Particles were collected on preweighed 47-mm Teflon filters (2-µm pore, Gelman Sciences, Ann Arbor, MI, USA) at a flow rate of 10 L/min for 2 hr during the exposure. Filters were weighed on an electrobalance (Mettler UMT2, Mettler Instruments, Columbus, OH, USA) in a temperature- (20°C) and humidity- (45%) controlled room. This balance can reliably weigh masses as low as 1 µg. The end net filter weight, sampling time, and flow rate were used to calculate the particle concentration in micrograms per cubic meter. Filters with sequestered PM (both before and after concentration) were analyzed for a number of components, including transition metals, elemental and organic carbon, sulfates, and nitrates. There were no appreciable differences in chemical composition of particles before and after concentration.

The particle size distribution was obtained using a micro-orifice uniform deposit impactor (MOUDI, MSP Corporation, Minneapolis, MN, USA), which is an eight-stage cascade impactor containing a series of micro-orifices that collect particles on preweighed 37-mm Teflon filters (2-µm pore, Gelman Sciences, Ann Arbor, MI, USA). Aerosols were sampled from the inlet duct at a flow rate of 30 L/min for 2 hr. The filter substrates from the impactor were weighed under a controlled environment following the same procedure described above for total filter samples. The weights from each stage were used to determine the mass median aerodynamic diameter (MMAD) and the geometric standard deviation (GSD). These data are reported in Table 1. In addition, ozone was measured inside the exposure chamber; concentrations did not exceed 0.05 ppm.

Study Population

Volunteers responding to a newspaper advertisement were prescreened over the telephone using the following criteria: age between 18 and 40 years of age; nonsmokers for at least 5 years prior to study; no history of allergies or respiratory diseases (food allergy, hay fever, dust allergies, rhinitis, asthma, chronic bronchitis, chronic obstructive pulmonary disease, tuberculosis, hemoptysis, or recurrent pneumonia); and not presently on any medication prescribed by a physician (except birth control pills). A urine pregnancy test was performed on all female subjects and a positive result excluded the subject from further participation in this study. The average age of the subject population was 26.2 ± 0.7 years and included 38 volunteers of which 36 were males and 2 were females.

Exposure to Concentrated Air Particles

Each volunteer had a single exposure to either filtered air or concentrated air particles (CAPS). Total exposure time was 2 hr. Subjects entered the exposure chamber and sat on a recumbent bicycle ergometer. Subjects exercised for 30 min of each hour. The schedule of exercise was 15 min on a cycle ergometer, 15 min rest, and this was repeated 4 times. Exercise intensity, i.e., cycle ergometer workload, was adjusted so subjects breathed at a ventilatory rate, normalized for body surface area, of 25 L/m^2/minute. In most subjects this will be about 50 L/min [i.e., a oxygen consumption (VO_2) of approximately 1.0 L/min]. A cycle ergometer work setting of 75–100 watts achieved such a

physiologic response for most subjects. During the 2-hr exposure, particle concentrations were monitored continuously at the inlet duct of the chamber by using a tapered element oscillating microbalance [(TEOM), Series 1400a, Rupprecht & Patashnick, Inc., Albany, NY, USA]. The TEOM was used to monitor a consistency or short-term excursion of exposure concentration. The average exposure concentrations were determined by filter samples as described above.

Bronchoscopy with Lavage

Using a standard protocol (*18*), the volunteers underwent bronchoscopy with lavage 18 hr after exposure to either filtered air or CAPS. Previous investigation in both animals and humans indicated that an inflammatory response resulted between 18 and 24 hr after exposure to particles (*6*,*7*). The fiberoptic bronchoscope was wedged into a segmental bronchus of the lingula. Following instillation and aspiration of a 20-mL bronchial sample (BL), three 50-mL aliquots of sterile saline were instilled and immediately aspirated to obtain the bronchoalveolar wash (BAL). The procedure was repeated on the right middle lobe. Samples were put on ice immediately after aspiration and centrifuged at 300×*g* for 10 min at 4°C. Cells were washed once with RPMI medium and viability determined via trypan blue exclusion. Viability exceeded 85% in all samples and there was no difference between air- and CAPS-exposed individuals. Cell numbers were determined using a hemacytometer. Cell differentials were performed on cytocentrifuged slides stained with a modified Wright Stain (Leukostat Solution, Fisher Scientific, Suwannee, GA, USA). At least 200 cells per slide were counted. There was no difference in recovery of BL or BAL fluid between air- and CAPS-exposed individuals, and all fluid recoveries were within 10% of one another. Consequently, soluble components were normalized per milliliter of fluid. Interleukin (IL)-8 and IL-6 levels were measured using enzyme-linked immunoabsorbent assay (ELISA) kits purchased from R&D Systems (Minneapolis, MN, USA).

Staining and Analysis of Cells in Lung and Blood

Venous blood was collected into heparinized vacutainers immediately before and 18 hr after the exposure. Heparinized whole blood or lavage fluid was mixed with fluorescein isothiocyanate (FITC) or phycoerythrin conjugated monoclonal antibodies (Coulter Corp., Miami, FL, USA) according to manufacturer's recommended procedures for direct staining. Blood was then lysed with fluorescent-activated cell sorter [(FACS); Becton Dickinson, San Jose, CA, USA] lysing solution, washed, and fixed in 0.5% paraformaldehyde. Stained cell preparations were held at 4°C until analyzed. Analysis was performed using a Becton Dickinson FACSort equipped with a 488-nm laser. Cell populations were identified and gated on the basis of forward and side scatter characteristics. Cell Quest software (Becton Dickinson, Version 3.1) for analysis of cell surface marker expression was employed.

Characteristics of antibodies selected for immunofluorescence staining of cell surface membrane markers are described in Table 2. Isotypic controls of immunoglobulin G subclasses corresponding to these antibodies were included for setting gates and 10,000 counts per sample were analyzed.

Phagocytosis

Saccharomyces cerevisiae zymosan A or *Staphylococcus aureus* bacteria (Bioparticles; Molecular Probes, Inc., Eugene, OR, USA) conjugated to FITC were prepared per manufacturer directions. AM (2×10^5) from exposed individuals were incubated with 10× bioparticles for 1 hr at 37°C/5% CO_2 before being placed on ice and immediately analyzed for bioparticle uptake by FACSort. Percent phagocytosis and mean fluorescence was analyzed by assessing the bioparticle uptake in the CAPS-exposed AM population compared to filtered air control AM.

Oxidant Generation

Oxidant generation was measured via chemiluminescence performed on a Berthold LB953 autolumat (Perkin Elmer, Norwalk, CT, USA). Human AM (10^5 cells in 100 µL RPMI without phenol red) or 100 µL blood diluted 1:10 into Hank's balanced salt solution (HBSS) (Sigma, St Louis, MO, USA) and 600 µL luminol reagent (0.15 mM luminol, 1.3 mM calcium chloride, 135 mM sodium chloride, 5 mM morpholinoethanesulfonic acid, 0.4 mM magnesium chloride, 0.5 mM magnesium sulfate, 0.8 mM sodium phosphate monobasic, and 5.5 mM D-glucose) (pH 7.2) (Sigma) were injected simultaneously into tubes containing 10× human serum opsonized zymosan A (Sigma). Resultant chemiluminescence was measured over a 30-min period. The data are expressed as integrated chemiluminescence counts.

Table 2. Surface markers investigated on lymphocytes.

Marker	Expression	Marker	Expression
CD45	All leukocytes	HLA-DR	Activated T lymphocytes
CD19	B lymphocytes	CD45RO	Memory lymphocytes
CD3	T lymphocytes	CD25	Activated T and B (IL-2)
CD4	T-helper cells	CD69	Activated T, B lymphocytes and macrophages
CD8	T-suppressor cells		
CD16	NK cells	CD56	NK cells

NK, natural killer.

Statistics

Data are expressed as mean values ± standard error. Differences between air- and CAPS-exposed groups were tested using the *t* test of independent means. For those variables that were significantly altered, the population was divided into quartiles and differences between groups were compared using one-way analysis of variance (ANOVA) (*19*). The post hoc test employed was Scheffe's test. Two-tailed tests of significance were employed. Significance was assumed at $p < 0.05$.

Results

Study Population and Exposure

The subject population included 38 volunteers (26.2 ± 0.7 years of age; 36 males and 2 females). There were eight exposures to filtered air (PM mass by measured filtered weights of 2.9 ± 1.9 µg/m^3) and 30 exposures to CAPS (PM mass of 120.5 ± 14.0 µg/m^3). There was a substantial range in CAPS exposures, reflecting the variation in particles outside the facility (Table 1), with individual exposures ranging from 23 to 311 µg/m^3. Considering time of exposure and ventilation rates, we estimate that individual lung exposures approximated a total dose of 1,200 µg on those days with the highest PM mass. The study population was divided into quartiles, with the eight individuals exposed to filtered air defining the first quartile (quartile 1) and the remaining 30 exposures arranged into groups of 10 with ascending PM mass (quartiles 2, 3, and 4). Differences between the quartiles in PM mass were significant (F = 41.2; $p < 0.0001$). Excluding air exposures, the concentration factor was 6.5 ± 0.9. There were differences (F = 3.6; p = 0.04) between the concentration factors with post hoc testing revealing significance only between the second and third quartiles. Measurement of iron, zinc, and sulfur by x-ray fluorescence verified concentration factors that approximated the value for total mass (8.5 ± 4.4, 10.8 ± 3.9, and 6.8 ± 1.4, respectively). The size distribution of exposure aerosols was approximately log normal, with the values of MMAD and GSD being 0.65 ± 0.03 and ~2.35, respectively. There was a slight increase in MMAD from 0.54 to 0.72 µm with an increase in mass concentration from quartile 2 to quartile 4.

Table 3. Bronchoalveolar lavage cells following CAPS exposure.

	Quartile 1 (air)	Quartile 2	Quartile 3	Quartile 4	Total CAPS
$PM_{2.5}$		47.2 ± 5.3	107.4 ± 9.3	206.7 ± 19.2	120.4 ± 14.1
Total count ($\times 10^6$)	15.9 ± 1.9	20.3 ± 3.0	23.0 ± 2.1	20.8 ± 2.0	21.4 ± 1.3*
Macrophages (%)	80.6 ± 2.9	82.4 ± 2.2	82.9 ± 1.3	75.4 ± 3.7	80.2 ± 1.6
Neutrophils (%)	0.8 ± 0.3	1.4 ± 0.4	2.0 ± 0.4	4.2 ± 1.7	2.5 ± 0.6*
Lymphocyte (%)	16.8 ± 2.4	13.1 ± 1.7	13.1 ± 1.3	19.0 ± 3.1	15.1 ± 1.3
Monocytes (%)	1.2 ± 0.3	0.9 ± 0.2	1.3 ± 0.3	2.2 ± 0.5	1.4 ± 0.2
Epithelial cells (%)	1.3 ± 0.3	0.8 ± 0.1	0.8 ± 0.1	1.4 ± 0.3	1.1 ± 0.1

*$p < 0.05$.

Table 4. Lymphocyte subpopulations in BAL following CAPS exposure.

	Percent of gated lymphocytes			
	Quartile 1 (air)[a]	Quartile 2	Quartile 3	Quartile 4
CD3	85 ± 3	78 ± 2	69 ± 6	80 ± 5
CD19	2 ± 0.5	2 ± 0.4	2 ± 0.3	2 ± 0.4
CD4	48 ± 5	45 ± 6	36 ± 5	51 ± 8
CD8	39 ± 5	33 ± 4	31 ± 6	33 ± 5
CD56	4 ± 1	6 ± 2	4 ± 1	5 ± 1
CD25	17 ± 1	18 ± 3	17 ± 2	16 ± 2

[a]Clean air.

Table 5. Expression of receptors on AMs from individuals exposed to CAPS.

	Percent of gated macrophages			
Receptor	Quartile 1	Quartile 2	Quartile 3	Quartile 4
CD11b	41 ± 6	43 ± 9	46 ± 9	44 ± 6
CD14	119 ± 20	97 ± 23	134 ± 23	133 ± 33
CD16	259 ± 70	192 ± 80	277 ± 117	434 ± 152
CD64	84 ± 12	114 ± 18	89 ± 20	121 ± 19

Table 6. Phagocytosis and oxidant generation by AMs following CAPs exposure.

	Percent of phagocytic cells			
	Quartile 1	Quartile 2	Quartile 3	Quartile 4
Zymosan	60 ± 4	62 ± 5	55 ± 5	58 ± 6
Streptococcus	88 ± 4	89 ± 3	89 ± 2	85 ± 5
Chemiluminescence (integrated cpm $\times 10^5$)	820 ± 130	1056 ± 195	833 ± 115	1478 ± 722

cpm, counts per minute.

Changes in Bronchoalveolar Lavage Fluid and Cells

Inflammation. Total cells found in BAL fluid were increased in those individuals exposed to CAPS (Table 3). Individuals exposed to air had $15.9 \pm 1.9 \times 10^6$ cells, whereas those exposed to CAPS had $21.4 \pm 1.3 \times 10^6$ cells ($p = 0.04$). The percentage of macrophages, lymphocytes, monocytes, and epithelial cells were not increased after CAPS exposure. However, the percentage of neutrophils significantly increased following particle exposure (2.5 ± 0.6 for CAPS and 0.8 ± 0.3 for air; $p = 0.016$). In addition, absolute numbers of neutrophils were increased in BAL fluid following CAPS exposure (0.56×10^6) as compared with air exposure (0.09×10^6) ($p = 0.0013$). Neutrophil influx appeared to be dependent on dose, with the greatest elevations occurring in the those subjects exposed to the highest concentration of particles ($F = 2.9$; $p = 0.05$).

Lymphocytes. BAL lymphocytes from individuals exposed to CAPS and filtered air were stained with antibodies recognizing mature T cells (CD3), T-helper cells (CD4), T-suppressor cells (CD8), B cells (CD19), NK cells (CD56), and lymphocyte activation marker, the IL-2 receptor CD25. The results of the flow cytometric analysis of percent of cells positive for each of the markers are shown in Table 4. The data are presented in quartiles of CAPS concentration in the exposure chamber. It was apparent that inhalation of particles had no effect on lymphocyte subsets obtained by lavage. The early lymphocyte activation marker CD69 (not shown) was also assessed with negative results (not shown).

Alveolar macrophages. The expression of cell-surface receptors involved in host defense against microbes complement receptor/β-integrin CD11b, the endotoxin receptor CD14, and Fc receptors CD16 and CD64 were analyzed on AM 18 hr following particle inhalation. As in the results with lymphocyte markers, the surface receptor data are presented as a function of PM mass expressed in quartiles. The data are shown in Table 5. There was no change in receptor expression even at the highest PM levels. Phagocytosis of both FITC-conjugated bacteria and yeast particles (Table 6) was performed on AM from air- or CAPS-exposed subjects. The range of uptake of bacterial particles was 85.5–92.5% in all quartiles whereas yeast particle uptake ranged from 50 to 67% (Table 6). CAPS did not affect phagocytosis of either bacteria or yeast by AM. Oxidant generation by AM exposed to air or CAPS was measured in a luminol assay using human serum opsonized yeast particle as the oxidant stimulant (Table 6, bottom). As with phagocytosis, there was no significant difference in the chemiluminescence response between CAPS and air exposure.

Cytokines. Concentrations of IL-6 and IL-8 in the BAL were analyzed by ELISA (Table 7). Interestingly, IL-8 levels were considerably lower in the most heavily exposed individuals (66.4 ± 21.7 pg/mL) compared with those exposed to air (288.8 ± 109.6 pg/mL), although these differences did not reach statistical significance.

Changes in Blood

Blood was obtained before and 18 hr post-exposure. Blood cell differentials and total cell counts per milliliter did not change following CAPS exposure (Table 8). Lymphocyte cell-surface markers CD3, CD4, CD8, CD19, and CD56 were analyzed pre- and 18 hr post-CAPS inhalation. The proportions of lymphocyte subsets did not change with CAPS exposure nor was there evidence of activation measured by CD69 expression on the leukocyte populations (Table 9).

Chemiluminescence generation by blood granulocytes and monocytes in response to opsonized zymosan stimulation is a sensitive method of assessing activation of these cells. In the subjects exposed to CAPS, no effect on this host defense parameter was found (Table 10).

Discussion

This study demonstrates that in young, healthy volunteers exposed to ambient air particles, a small increase in neutrophils can be found in the lower respiratory tract, but that no effect of particle inhalation could be found involving the cellular immune system. The highest concentration of $PM_{2.5}$ employed in this investigation, 311 μg/m^3 and a mean of 207 μg/m^3 in the highest exposure quartile, would be an uncommon level of fine particles to be encountered in this country. However, the total exposure for individuals living in environments with much lower $PM_{2.5}$ levels would still be greater than that to which these volunteers were exposed for 2 hr, as a resident of any urban area in the United States will be exposed to elevated particle levels all through the day. The influx of neutrophils into the lung of CAPS-exposed individuals was dose dependent, with those subjects exposed to the highest concentration of CAPS having the most neutrophils. The number of neutrophils present in BAL fluid was comparable to that found in healthy young volunteers exposed to low levels (0.10 ppm) of ozone for several hours (*20*). Neutrophils present in the BL of humans exposed to CAPS were also similar quantitatively to those found after human exposure to 300 μg/m^3 diesel exhaust for 1 hr (*7*). Although the latter study did not observe increased neutrophils in the alveolar fraction, this disparity may be explained by the differences in particle composition or source, timing of bronchoscopy (6 hr

Table 7. Soluble components in the BAL following CAPS exposure.

	Quartile 1 (air)	Quartile 2	Quartile 3	Quartile 4	Total CAPS
$PM_{2.5}$		47.2 ± 5.3	107.4 ± 9.3	206.7 ± 19.2	120.4 ± 14.1
IL-8 (pg/mL)	288.8 ± 109.6	182.4 ± 67.7	59.9 ± 14.6	66.4 ± 21.7	102.9 ± 25.2
IL-6 (pg/mL)	6.6 ± 1.5	6.8 ± 2.4	5.4 ± 1.1	5.4 ± 1.1	5.9 ± 0.9

Table 8. Blood parameters and CAPS exposure.

	Quartile 1		Quartile 2		Quartile 3		Quartile 4	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Neutrophils (/mm^3)	2.85 ± 0.23	2.54 ± 0.20	3.09 ± 0.41	2.83 ± 0.33	2.88 ± 0.47	2.59 ± 0.37	3.71 ± 4	3.57 ± 0.35
Lymphocytes (/mm^3)	1.69 ± 0.17	1.64 ±0 .16	1.67 ± 0.14	1.73 ± 0.12	1.68 ± 0.15	1.79 ± 0.20	1.78 ± 0.14	1.76 ± 0.12
Monocytes (/mm^3)	0.38 ± 0.03	0.38 ± 0.03	0.44 ± 0.04	0.48 ± 0.03	0.33 ± 0.03	0.34 ± 0.05	0.47 ± 0.06	0.34 ± 0.05
Platelets (/mm^3)	203 ± 11	208 ± 17	216 ± 18	209 ± 15	206 ± 12	199 ± 19	220 ± 9	215 ± 9
Ferritin (ng/mL)	45 ± 5	48 ± 8	86 ± 1	87 ± 12	80 ± 14	84 ± 21	70 ± 15	68 ± 17

Abbreviations: post, postexposure; pre, preexposure.

Table 9. Lymphocyte markers in blood following CAPS inhalation.

	Percent of gated lymphocytes							
	Quartile 1		Quartile 2		Quartile 3		Quartile 4	
Antigen	Pre	Post	Pre	Post	Pre	Post	Pre	Post
CD3	68 ± 4	66 ± 3	67 ± 2	70 ± 2	73 ± 1	75 ± 3	70 ± 3	71 ± 4
CD19	9 ± 1	9 ± 1	12 ± 1	12 ± 1	9 ± 1	10 ± 1	10 ± 1	10 ± 2
CD4	44 ± 5	43 ± 3	38 ± 6	43 ± 4	51 ± 3	52 ± 3	47 ± 4	53 ± 3
CD8	24 ± 3	23 ± 2	29 ± 2	27 ± 2	22 ± 2	23 ± 2	23 ± 2	18 ± 2
CD69	18 ± 2	17 ± 2	14 ± 2	16 ± 2	17 ± 2	9 ± 3	16 ± 3	14 ± 3

Table 10. Chemiluminescence response in blood to opsonized zymosan in blood following CAPS exposure.

	Integrated CPM × 10^5			
	Quartile 1	Quartile 2	Quartile 3	Quartile 4
Preexposure	120 ± 15	165 ± 30	195 ± 45	182 ± 35
Postexposure	102 ± 10	150 ± 33	145 ± 19	178 ± 36

following diesel exhaust exposure vs 18 hr following CAPS exposure), or discrepancies in the total particle dose delivered to the lung in the two studies.

Acute exposure to CAPS did not affect macrophage surface receptor expression or phagocytic function. This may not be surprising, as $PM_{2.5}$ did not affect AM receptor expression and function when these cells were stimulated *in vitro* with the fine PM fraction. Our previous studies have shown that particles in the coarse $PM_{2.5–10}$ fraction alter the phenotype and phagocytic capability of these cells. $PM_{2.5}$ assessed simultaneously had no effect on phagocytosis or oxidant generation (*13,15*). Similarly, host defenses of mice exposed to $PM_{2.5}$ and $PM_{2.5–10}$ were found to be altered by $PM_{2.5–10}$ but not $PM_{2.5}$ (*21*). Therefore, it is possible that $PM_{2.5}$ are more active in affecting mortality and health end points depending on cardiac function (*22*) than in altering host defenses. The possibility also exists that a different $PM_{2.5}$ exposure or sampling time may result in different findings. Future studies will deal with the *in vivo* role of coarse particles in affecting human lung host defense and immune function, in a similar study protocol as presented here.

From the lack of effects of CAPS on the makeup of lymphocyte population in the lung, it is concluded that short-term inhalation of $PM_{2.5}$ may not alter local immune capability. Furthermore, there was no change in the leukocyte composition in the blood. Some components likely to be present in CAPs have been shown to affect lymphocyte influx into the lung. In a recent study, soluble components of Utah Valley PM were segmentally instilled into healthy volunteers, and this resulted in an increase in CD4-positive lymphocytes in the lung, as well as an increase in activated CD25, CD69 positive cells (*23*). The effect could not be ascribed to total soluble metals in the PM. The proinflammatory and immune modulatory components in the Utah PM remain unidentified. The local concentration of the Utah PM extract following segmental instillation certainly was much higher than can be expected in the present study. CAPS of the $PM_{2.5}$ fraction also contain diesel soot which has been shown to induce B-cell activation and the production of immunoglobulin E (*10*). Phenotyping T-cell subsets and B cells would not reveal this stimulation, although one could expect an increase in cells with a stimulated phenotype, i.e., CD69 expression. The evidence for changes in immune function following acute particle inhalation is scarce. Salvi et al. (*7*) found a significant increase in CD4 and CD8 cells in the airways mucosa 6 hr following inhalation of diesel exhaust for 1 hr. B cells were also increased in the airways. Events following cigarette smoke inhalation may also reflect responses to pollution particles. In a study where cigarette smoke was acutely inhaled, a transient rise in leukocytes and neutrophil activation was found (*24,25*). In the blood following CAPS inhalation there was no evidence of bone marrow stimulation, as total leukocyte cell counts and cell differentials were unaffected. The blood neutrophils did not show activation in the chemiluminescence assay.

This study has demonstrated that inhalation of moderate levels of $PM_{2.5}$ has little acute effect on the human host defenses, including immune activation and lung macrophage function under the conditions tested. The study population was young and healthy, while effects on particles have been implicated in individuals with preexisting respiratory problems including asthma and serious lung infections. These individuals already are in a state of immune activation so that cellular inflammation in the respiratory tract with activated and inflammatory cells may be more susceptible to toxic insult than resting cells. Future studies will aim to enroll subjects with mild to moderate respiratory disease to resolve the question of possible immune dysfunction and will include additional associated biomarkers.

References and Notes

1. Dockery DW, Speizer FE, Stram DO, Ware JH, Spengler JD, Ferris BG. Effects of inhalable particles on respiratory health of children. Am Rev Respir Dis 139:587–594 (1989).
2. Bates DV, Sizto R. Relationships between air pollutant levels and hospital admissions in Southern Ontario. Can J Public Health 74:117–122 (1983).
3. Pope CA III. Respiratory disease associated with community air pollution and a steel mill, Utah Valley. Am J Public Health 79:623–628 (1989).
4. Schwartz J, Dockery DW, Neas LM. Is daily mortality associated specifically with fine particles? JAMA 46(10):927–939 (1996).
5. Schwartz J, Neas LM. Fine particles are more strongly associated than coarse particles with acute respiratory health effects in school children. Epidemiology 11:6–10 (2000).
6. Pritchard RJ, Ghio AJ, Lehmann JR, Winsett DW, Tepper JS, Park P, Gilmour MI, Dreher KL, Costa DL. Oxidant generation and lung injury after particulate air pollution exposure increase with the concentrations of associated metals. Inhal Toxicol 8:457–477 (1996).
7. Salvi S, Blomberg A, Rudell B, Kelly F, Sandstrom T, Holgate ST, Frew A. Acute inflammatory responses in the airways and peripheral blood after short-term exposure to diesel exhaust in healthy human volunteers. Am J Respir Crit Care Med 159:702–709 (1999).
8. Costa DL, Lehmann JR, Winsett D, McGee J, Ghio A. Pulmonary toxicity of Utah Valley PM: are empirical indices of adverse health effects coherent with the epidemiology [Abstract]? Am J Respir Crit Care Med 157(3):A880 (1998).
9. Casillas AM, Hiura T, Li N, Nel AE. Enhancement of allergic inflammation by diesel exhaust particles: permissive role of reactive oxygen species. Ann Allergy Asthma Immunol 83:624–629 (1999).
10. Takenaka H, Zhang K, Diaz-Sanchez D, Tsien A, Saxon A. Enhanced human IgE production results from exposure to the aromatic hydrocarbons from diesel dust direct effects on B-cell IgE production. J Allergy Clin Immunol 95:103–115 (1995).
11. Holian A, Hamilton RF Jr, Morandi MT, Brown SD, Li L. Urban particle-induced apoptosis and phenotype shifts in human alveolar macrophages. Environ Health Perspect 106(3):127–132 (1998).
12. Becker S, Soukup JM, Gilmour MI, Devlin RB. Stimulation of human and rat alveolar macrophages by urban air particulates:

effects on oxidant radical generation and cytokine production. Toxicol Appl Pharmacol 141:637–648 (1996).
13. Monn C, Becker S. Cytotoxicity and induction of proinflammatory cytokines from human monocytes exposed to fine ($PM_{2.5}$) and coarse particles ($PM_{10-2.5}$) in outdoor and indoor air. Toxicol Appl Pharmacol 15;155(3):245–252 (1999).
14. Soukup JM, Becker S. Decreased CD 11b expression, phagocytosis, and oxidative burst in urban particulate pollution-exposed human monocytes and alveolar macrophages. J Toxicol Environ Health 11;55(7):455–477 (1998).
15. Soukup JM, Becker S. Human alveolar macrophage responses to air pollution particulates are associated with insoluble components of coarse matter including particulate endotoxin. Toxicol Appl Pharmacol 171:20–26 (2001).
16. Sioutas C, Koutrakis P, Godleski JJ, Ferguson ST, Kim CS, Burton RM. Fine particle concentrators for inhalation exposure: effect of particle size and composition. J Aerosol Sci 28:1057–1071 (1997).
17. Sioutas C, Kourakis P, Ferguson ST. Development and evaluation of a prototype ambient particle concentrator for inhalation exposure studies. Inhal Toxicol 7:633–644 (1995).
18. Devlin RB, McDonnell WF, Mann R, Becker S, House DE, Schreinemachers D, Koren HS. Exposure of humans to ambient levels of ozone for 6.6 hours causes cellular and biochemical changes in the lung. Am J Respir Cell Mol Biol 4:72–81 (1991).
19. Ghio AJ, Bassett M, Chall AN, Levin DG, Bromberg PA. Bronchoscopy in healthy volunteers. J Bronchology 5:185–194 (1998).
20. Colon T. Statistics in Medicine. Boston:Little, Brown, & Co., 1974.
21. Gilmour MI. Effects of $PM_{2.5}$ and $PM_{2.5-10}$ samples and water-soluble extracts on pulmonary inflammation and streptococcal infection in mice [Abstract]. Am J Respir Crit Care Med 159(3):A26 (1999).
22. Devlin RB, Cascio W, Kehrl H, Ghio A. Changes in heart rate variability in young and elderly humans exposed to concentrated ambient air particles [Abstract]. Am J Respir Crit Care Med 16(3):A239 (2000).
23. Harder SD. Unpublished data.
24. Blain AD, Kirkpatrick U, Devine C, Nader S, McCollum CN. The influence of acute smoking on leucocytes, platelets and endothelium. Artherosclerosis 141:133–139 (1998).
25. Terashima T, Wiggs B, English D, Hogg JC, van Eeden SF. The effect of cigarette smoking on the bone marrow. Am J Respir Crit Care Med 155(3):1021–1026 (1997).