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EFFECTS OF POLLUTANT ATMOSPHERES ON SURFACE RECEPTORS OF PULMONARY MACROPHAGES

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The effects of two multicomponent pollutant atmospheres on the surface receptors (FcR) and phagocytic activity of rat pulmonary alveolar macrophages have been studied. FcR are crucial for the macrophages to become cytotoxic against target cells. The atmospheres were composed of pollutants that are prevalent in the South Coast Air Basin of southern California. Rats were exposed nose-only to a 7-component oxidant- and sulfate-containing atmosphere for 4 h/d for either 7 or 21 consecutive days. In another experiment rats were exposed 5 h/d for 5 consecutive days to another pollutant combination—acid droplets plus carbon-containing dilute diesel engine exhaust. In both experiments matched rats were exposed nose-only to purified air to be used as controls. Each of the atmospheres studied significantly reduced FcR activity for at least 3 d following the exposure, with the group of rats exposed to the 7-component atmosphere for 21 d exhibiting the most pronounced effect. Macrophages from rats exposed to the diesel exhaust plus acid atmosphere and the 7-component atmosphere for 7 d had significantly reduced phagocytic activity for at least 3 d postexposure, while the macrophages from rats exposed to the latter atmosphere for 21 d had phagocytic activity near control values. The decrease in phagocytosis and inhibition of FcR of macrophages suggests an impairment of macrophage function that probably renders the host vulnerable to bacterial and/or viral infections.

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INTRODUCTION

People living in areas heavily impacted by vehicular and stationary-source air emissions are exposed to a large variety of airborne particles and gases. Air pollution in the South Coast Air Basin of southern California consists of a complex mixture of chemical species, which include oxidant products such as ozone (O_3) and nitrogen dioxide (NO_2), acidic products such as nitric acid (HNO_3), sulfuric acid (H_2SO_4) and associated sulfates, insoluble particles (such as graphitic carbon), sulfur dioxide (SO_2), free radicals, and other species (Finlayson-Pitts and Pitts, 1986).

It is not possible to fully simulate in the laboratory the complex products of photochemical processes that take place in atmospheres containing emissions from petroleum combustion. Two surrogate atmospheres have, therefore, been developed and used in this laboratory for exposures of animals in the effort to assess the potential health effects of air pollutant combinations on the mammalian respiratory system. One atmosphere consists of a mixture of gases and particles based on the proportions of key pollutants observed at the air monitoring station at Lennox, Calif., an area that receives pollutants from stationary as well as mobile sources. The seven-component mixture contains O_3, NO_2, SO_2, soluble sulfate aerosols, trace metal ions (Fe^{3+} and Mn^{2+}), and insoluble iron oxide (Fe_2O_3) particles (as a surrogate for insoluble atmospheric particles). Acidic reaction products such as particulate bisulfate ion (HSO_4^-) and HNO_3 vapor formed by the interactions of the gases and particles that make up the atmosphere are also present in the exposure atmosphere. The other complex atmosphere under study concentrates on acid-coated particles formed from diesel exhaust aerosol in the presence of HNO_3 vapor (formed by O_3 + NO_2 in ambient air) and H_2SO_4 (formed when SO_2 is present in a photochemically polluted atmosphere). Diesel engine emissions account for about 60% of the total emission of elemental carbon in the California South Coast Air Basin (Cass et al., 1982). Elemental carbon in the fine-particle size range (aerodynamic diameter less than 10 μm) is emitted into this air basin at a rate of about 15 metric tons per day (Cass et al., 1982). Samples of acid mists and fogs have been collected in California's coastal region and found to have pH values as low as 2.2 (Brewer et al., 1983). In such samples ion analysis indicates that nitrate ion (NO_3^-) and sulfate ion (SO_4^{2-}) are present in ratios (NO_3^-/SO_4^{2-} = 2) that are consistent with the relative emission rates of nitrogen oxides (NO_x) and SO_2 (Waldman et al., 1982; Brewer et al., 1983).

Several biologic endpoints have been used in our laboratory to evaluate the effects of single and combined air pollutants (Phalen et al., 1980, 1986; Mannix et al., 1982; Mautz et al., 1985; Bhalla et al., 1986), including a measurement of macrophage mobility (McAllen et al., 1981). Macrophages are one of the important immunological elements that protect the host from infection, and probably from neoplastic disease (Sone,

1986). The roles of pulmonary macrophages in lung defense and several disease processes have been recently reviewed (Brain, 1986). The resident macrophages in the lung defend against both particulate environment pollutants and pathogenic microorganisms. They possess distinct receptors (FcR) on their surfaces for the Fc portion of immunoglobulin (IgG) which help in the recognition of foreign particles (Gaafar and Doyle, 1971). These receptors also mediate phagocytosis and lysis by the macrophages (Boltz-Nitulescu and Spiegelberg, 1982; Boltz-Nitulescu et al., 1981). The binding of these receptors with immune complexes facilitates the phagocytosis of IgG-coated particles (such as bacteria), stimulates both the secretion of reactive oxygen intermediates and the release of lysosomal hydrolases, and also mediates antibody-dependent cellular cytotoxicity (Johnston et al., 1985).

Hadley et al. (1977) have shown that inhaled toxic substances can impair FcR activity (ability to fix cytophilic antibody on the surface of macrophages). Inhalation exposure to nickel (Reichrtova et al., 1986) and in vitro exposure to lead (Jian et al., 1985) have been shown to cause an inhibitory effect on FcR activity. The purpose of our studies was to compare the effects of the two multicomponent atmospheres on the FcR activity of pulmonary macrophages, and to shed light on the sensitivity of this assay in detecting pollutant-related lung injury. In addition, the effects of these atmospheres on nonspecific phagocytic activity using polystyrene latex microspheres were also determined and compared with the effects on Fc receptor activity.

The exposure durations for these studies were chosen in order to model two distinct air pollutant exposure scenarios. A 5-day period of daily exposures of rats to dilute diesel engine exhaust and acids was selected in order to simulate an environmental exposure to a complex carbon-acid atmosphere during an extended air pollution episode. The 7 or 21 d of daily exposures to the 7-component atmosphere were intended to represent subchronic exposure to a complex air pollution mixture that contains both sulfate and oxidant and generates acids as well as nitrate radical (Platt et al., 1980). A pilot exposure to ozone was also performed in order to evaluate the endpoints following a single acute exposure to an atmosphere that has been demonstrated in our laboratory to affect respiratory tract permeability (Bhalla et al., 1986), alter upper and lower respiratory tract particle clearance rates (Kenoyer et al., 1981), and produce pulmonary lesions (Mautz et al., 1985).

MATERIALS AND METHODS

A flow diagram of the general procedures used in performing the study is shown in Fig. 1. The details of the methods used are described below.

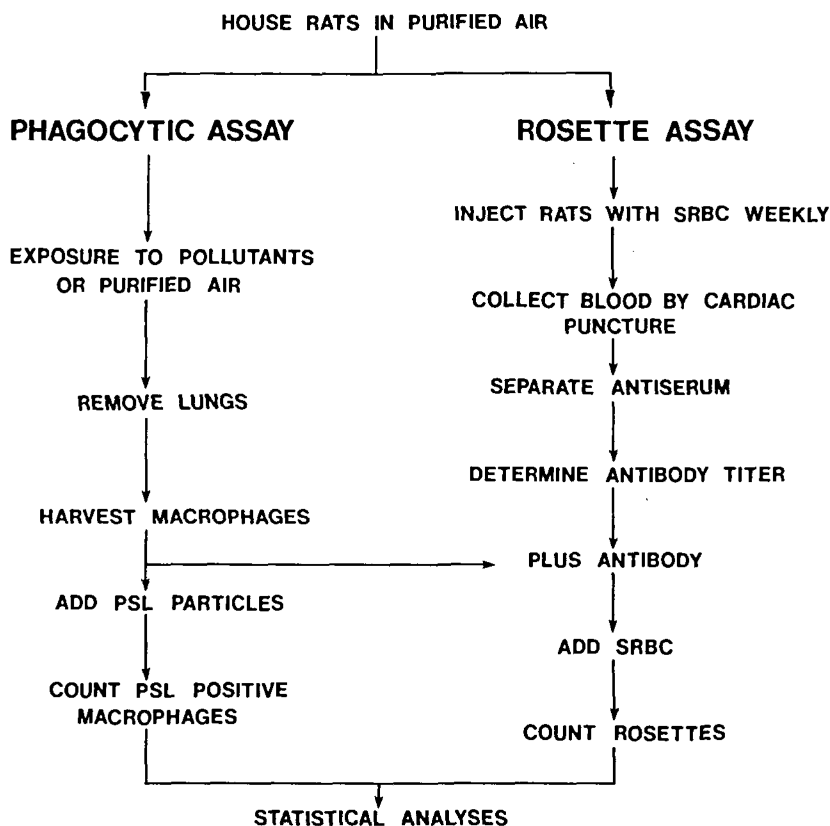


FIGURE 1. Flow diagram of the experimental procedures used in the study.

Animals

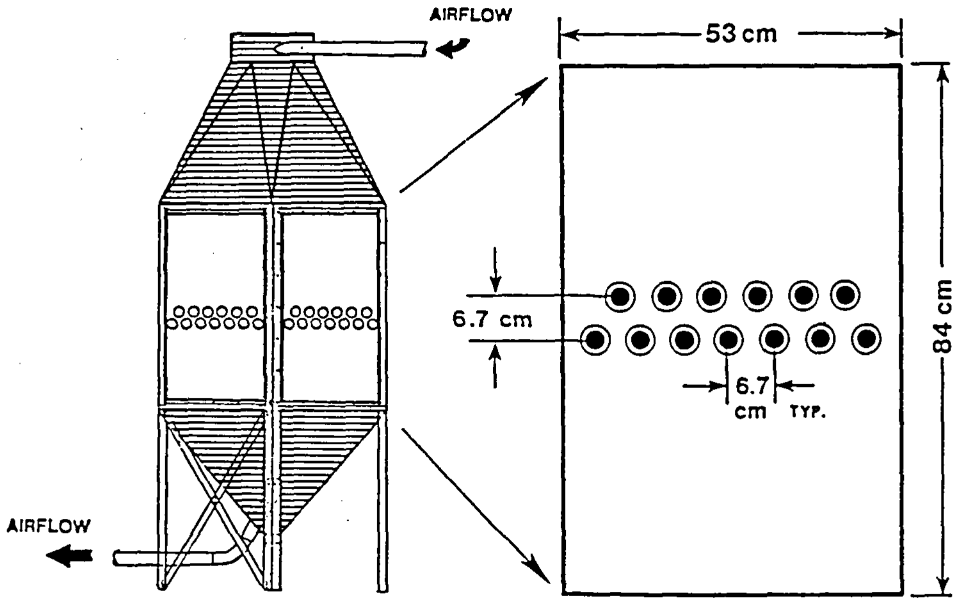
The animals used in this study were barrier-reared Sprague-Dawley rats (Hilltop Lab Animals, Scottdale, Pa.). Male rats weighing approximately 200 g were delivered in filtered shipping boxes in order to minimize prior exposure of the animals to particulate pollutants. The rats were housed in a particle- and gas-filtering laminar-flow caging system, in wire-bottom stainless steel cages for about 1 wk prior to the start of the experimental protocol. Sodium chloride (rock salt) placed in trays beneath the wire suppressed ammonia formation in the animal housing area. Serologic data provided by the animal supplier indicated that the rats were free of respiratory viral infection. Quality control histopathologic examinations were performed on 10% of the rats within 5 d after arrival to establish that the rats were free of lung disease. Between inhalation exposures and after completion of a set of exposures, rats were returned to the laminar-flow caging system.

Exposure System

Groups of rats were exposed to either purified air or a pollutant atmosphere using 1-m^3 stainless steel University of Rochester–type chambers modified for nose-only exposure (Fig. 2). For each pollutant exposure group a matched group of control rats was randomly selected from the same supply batch and exposed to purified air under identical conditions. Each rat was placed into a cylindrical plastic tube with an aluminum nosepiece tapered toward the forward end so that only the tip of the rat's nose protruded into the atmosphere. The exterior of the nosepiece had a cylindrical fitting by which it could be inserted and sealed into one of the nose-only ports in the wall of the chamber. Each port was supplied by a channel on the interior wall of the chamber, which directed the atmosphere downward past the nose of each rat to prevent rebreathing of that atmosphere by the exposed rat and to prevent sharing of that sample of atmosphere with another rat. This exposure system was designed in order to minimize stress, provide for the comfort of the rats, and prevent the neutralization of acids in the pollutant atmospheres by ammonia generated from the rats' excreta. The chambers were supplied with air that had passed through coarse particulate filters, gas scrubbers, a humidifier, and a high-efficiency particulate filter. When appropriate, pollutants were injected into the airstream just prior to the exposure chamber. Temperature and humidity were controlled; an exposure temperature of 22.2 ± 1.0(SD)°C was selected to ensure the comfort of the rats, and the relative humidity was 85 ± 2%.

Pollutant Atmosphere Generation Techniques

Ozone and 7-Component Atmospheres Ozone was generated by passing medical-grade oxygen through two corona-discharge ozonizers (Sander Ozonizer, type III, Osterberg, West Germany) operated in parallel to improve the stability of generated ozone. The ozone output of the generators was affected by small fluctuations in the laboratory AC line voltage, so a line voltage controller was used with the units. Nitrogen dioxide and sulfur dioxide were obtained from cylinders of research quality compressed gas (Matheson Gas Products, Cucamonga, Calif.). These gases were metered into the chamber air supply stream through separate ports located downstream of the O_3 injection port. Sulfate aerosols (about 0.3 μm mass median aerodynamic diameter) containing catalytic metal ions were generated by nebulizing a mixed sulfate aqueous solution (Ho et al., 1980; Kleinman et al., 1985). The sulfate compounds and their concentrations in the mixed solution were ammonium sulfate, 31.2 g/l; ferric sulfate, 27.4 g/l; and manganese(II) sulfate, 0.49 g/l. Insoluble iron oxide (Fe_2O_3) particles (surrogates for insoluble air pollutants) were produced by the nebulization of a dilute iron oxide colloidal solution (sol) in distilled water followed by drying and heat treatment. The sol



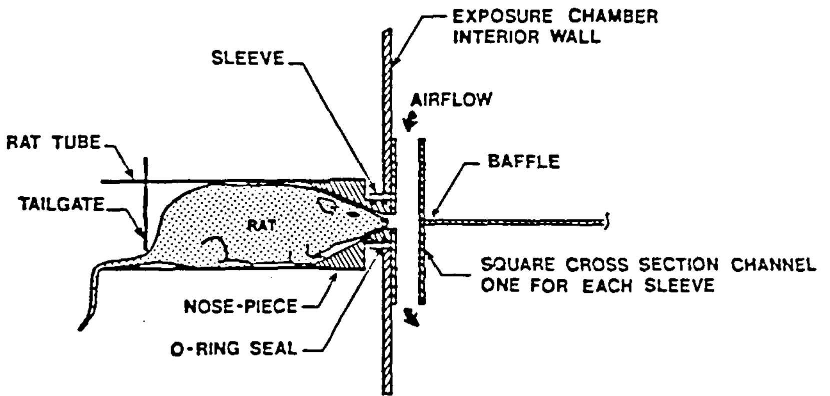


FIGURE 2. Nose-only system used for rat exposures. (a) Modification to Rochester chambers. (*b*) Sectional view.

was prepared by the deionization of a ferric nitrate solution, as described by Wales et al. (1980). The sol was aerosolized using a Collision compressed-air nebulizer. Following nebulization, the iron oxide aerosol was mixed with purified dilution air and then passed through a 450°C tube furnace to stabilize the structure of the particles and remove physically adsorbed water. The aerosol exiting the furnace consisted of insoluble, spherical-aggregate particles with a mass median aerodynamic diameter (MMAD) of about 0.3 μm and a geometric standard deviation (GSD) of 1.6–1.8. The iron oxide particles were brought to Boltzmann charge equilibrium by passage through an ^{85}Kr aerosol neutralizer, and then were introduced into the chamber air stream.

Diesel Soot and Acid Atmosphere The diesel soot and acid atmosphere consisted of a mixture of sulfuric acid-coated diesel soot aerosols and HNO_3 vapor. Diesel exhaust aerosols were obtained from an 11 hp, single-cylinder diesel engine, operating at constant speed (1800 rpm) and load (78% of maximum). The fuel used was Phillips number 2 diesel control fuel, lot G075. After passing through an exhaust system and muffler, a portion of the total exhaust was split off and immediately diluted 1:9 with purified air. Sulfur trioxide vapor (SO_3), obtained by bubbling a stream of ultradry air through fuming sulfuric acid, was then mixed with the dilute exhaust. The SO_3 reacted rapidly with some of the water vapor in the dilute exhaust to produce a sulfuric acid coating on the soot particles. After a short aging period the acid-coated soot aerosol was further diluted by mixing with the chamber air supply, resulting in an overall exhaust dilution ratio of 1:135 at the inhalation chamber. A more detailed description of the sulfuric acid-coated soot generation system has been published (Walters et al., 1988). Nitric acid vapor was generated by bubbling a metered flow rate of purified air through concentrated reagent-grade nitric acid immersed in a constant temperature bath. The HNO_3 vapor exiting the bubbler was diluted with purified air and filtered before entering a separate port and mixing with the chamber air-supply stream.

Atmosphere Characterization Techniques

The methods used in characterizing the ozone-alone and 7-component atmospheres have been described in greater detail by Kleinman et al. (1984). All gas monitoring was via samples obtained at the rat breathing zone using fluorocarbon sampling lines. Nitrogen dioxide and nitric oxide were monitored using a chemiluminescent monitor (model 952A, Beckman Instruments, Inc., Fullerton, Calif.). Sulfur dioxide was monitored using a pulsed fluorescence detector (series 43, Thermo Electron Corp., Hopkinton, Mass.). Ozone was measured by ultraviolet absorption (model 1003-AH, Dasibi Environmental Corp., Glendale, Calif.). The aerosol stability during exposures was determined using an infrared light-scattering aerosol mass monitor (model RAM-1, GCA Corporation,

Bedford, Mass.). Cascade impactor samples (Sierra model 210K eight-stage ambient impactor, Anderson Samplers, Atlanta, Ga.) were used to provide size-classified particles for chemical and gravimetric analyses. Aerosols were collected on Zeflour (Gelman Sciences, Inc., Ann Arbor, Mich.) or acid-washed Pallflex filters (Pallflex Products, Inc., Putnam, Conn.) for gravimetric and chemical analyses of atmospheric components and reaction products. Nylon backup filters were used to collect nitric acid vapor from the reaction of O_3 and NO_2. Upstream and backup filters were ultrasonically leached with distilled water and analyzed for nitrate and sulfate ions by ion chromatography; acidity was determined from pH measurement of the leachate solutions. For the 7-component atmosphere, total iron and manganese were determined by extracting filter samples in 5% HCl and measuring metal concentrations in the extracts using a flame-type atomic absorption spectrophotometer (model AA-475, Varian Associates, Sunnyvale, Calif.).

Exposure Protocols Three different exposure protocols were employed in conducting these studies: (a) exposure to 0.8 ppm O_3 for 4 h, (b) exposure to the dilute diesel engine exhaust and acids atmosphere (0.46 mg/m^3 diesel soot + 0.38 mg/m^3 HNO_3 + 0.18 mg/m^3 H_2SO_4) 5 h/d for 5 consecutive days, and (c) exposure to the 7-component atmosphere [0.30 ppm O_3 + 1.2 ppm NO_2 + 2.5 ppm SO_2 + 0.27 mg/m^3 $(NH_4)_2SO_4$ + 0.22 mg/m^3 $Fe_2(SO_4)_3$ + 0.004 mg/m^3 $MnSO_4$ with an insoluble aerosol of 0.15 mg/m^3 Fe_2O_3] 4 h/d for either 7 or 21 consecutive days. The latter two exposure lengths were chosen in order to check for the presence of an early effect (after 7 d of exposure) and to investigate the possibility of development of tolerance or enhancement of effect (after 21 d).

Preparation of Antiserum to Sheep Red Blood Cells Adult female rats were injected intraperitoneally with 5×10^8 sheep red blood cells (SRBC) (Mission Labs, Rosemead, Calif.) once a week for 4 wk. Ten days after the last injection, blood was collected by cardiac puncture and the sera were separated. The rats were given two booster doses (5×10^8 SRBC) a week apart and bled again 7 d after the last injection. The sera from each different collection period were pooled and the pooled serum was inactivated by heating at 57°C for 30 min. The serum was stored in 2-ml aliquots at -20°C for further use.

The anti-SRBC titer of the serum was determined against lysis of SRBC in the presence of rabbit complement. Aliquots of 0.2 ml of anti-SRBC serum of different solutions were added to 1×10^8 SRBC in 1 ml and incubated at 37°C for 30 min. The cells were centrifuged at $200 \times g$ for 30 min and resuspended in phosphate-buffered saline (PBS), 0.2 ml rabbit complement at 1:5 dilution (Microbiological Associates, Bethesda, Md.) was added, and the suspensions were incubated again for 30 min at 37°C. Normal saline, 2 ml, was added to each tube and the suspended SRBC were centrifuged at $200 \times g$ for 30 min. The supernatant was collected and the optical density of hemoglobin was measured at 430 nm.

Supernatant collected from SRBC in the absence of anti-SRBC serum was used as control. Nonspecific lysis of SRBC with water was used as 100% lysis. The anti-SRBC titer expressed in percent of lysis was calculated using the equation

$$\text{Specific lysis} = [(A - B) \times 100]/C$$

where A is the absorption of the supernatant with antibody, B is the absorption of the supernatant in absence of antibody, and C is the absorption of the supernatant with nonspecific lysis.

Determination of Cytophilic Antibody Titer of Anti-SRBC Serum The cytophilic antibody titer of the rat anti-SRBC serum collected was determined using the rosette assay technique as published earlier (Rao et al., 1979a; Weissler et al., 1986). Rats were deeply anesthetized with sodium pentobarbital (40 mg/kg body weight) and were sacrificed by exsanguination. The lungs were removed, minced into small pieces (approximately 1 mm or less in diameter) with iridectomy scissors, and placed in a petri dish containing Hanks balanced salt solution (HBSS) (Irvine Scientific Co., Santa Ana, Calif.) chilled over a bed of ice. The resident cells were obtained by stirring and squeezing the lung pieces in sterile HBSS. The cells were filtered through a nylon membrane (Nitex 51/52, Martin Supply Co., Baltimore, Md.) to separate them from large fragments. The cells were washed with HBSS and centrifuged at 200 × g for 10 min. The supernatant was discarded and the cell pellet was resuspended in 1 ml HBSS. The viability of the cell population was determined by trypan blue dye exclusion.

The concentration of viable cells was adjusted to 1×10^6 in 1 ml HBSS and 0.1 ml of the cell suspension was added to each well of a Lab-Tek chamber (V. W. R. Scientific, Norwalk, Calif.) containing 0.4 ml HBSS. The chambers were incubated for 1 h at 37°C with 5% CO_2. After the first incubation, nonadherent cells were removed by washing the chambers three times with HBSS. Two-tenths milliliter of anti-SRBC serum (various dilutions) in PBS was added to each chamber containing adherent cells and the cells were incubated for 30 min as above. After incubation the serum was removed and 0.1 ml SRBC (1×10^7 cells/ml) was added to each of the chambers along with 0.2 ml HBSS followed by incubation for 30 min at 37°C with 5% CO_2. Unbound SRBC were then washed away gently with HBSS, and 0.4 ml HBSS was added to each of the chambers for examination of the preparation with an inverted phase-contrast microscope (Nikon Diaphot). The number of macrophages that formed rosettes with SRBC was counted. A macrophage with three or more SRBC attached to its surface was counted as a rosette (Fig. 3). Cells incubated with normal rat serum instead of anti-SRBC serum served as negative controls. A minimum of 300 cells was counted from each of the rats. The percentage of macrophages that formed rosettes was calculated using

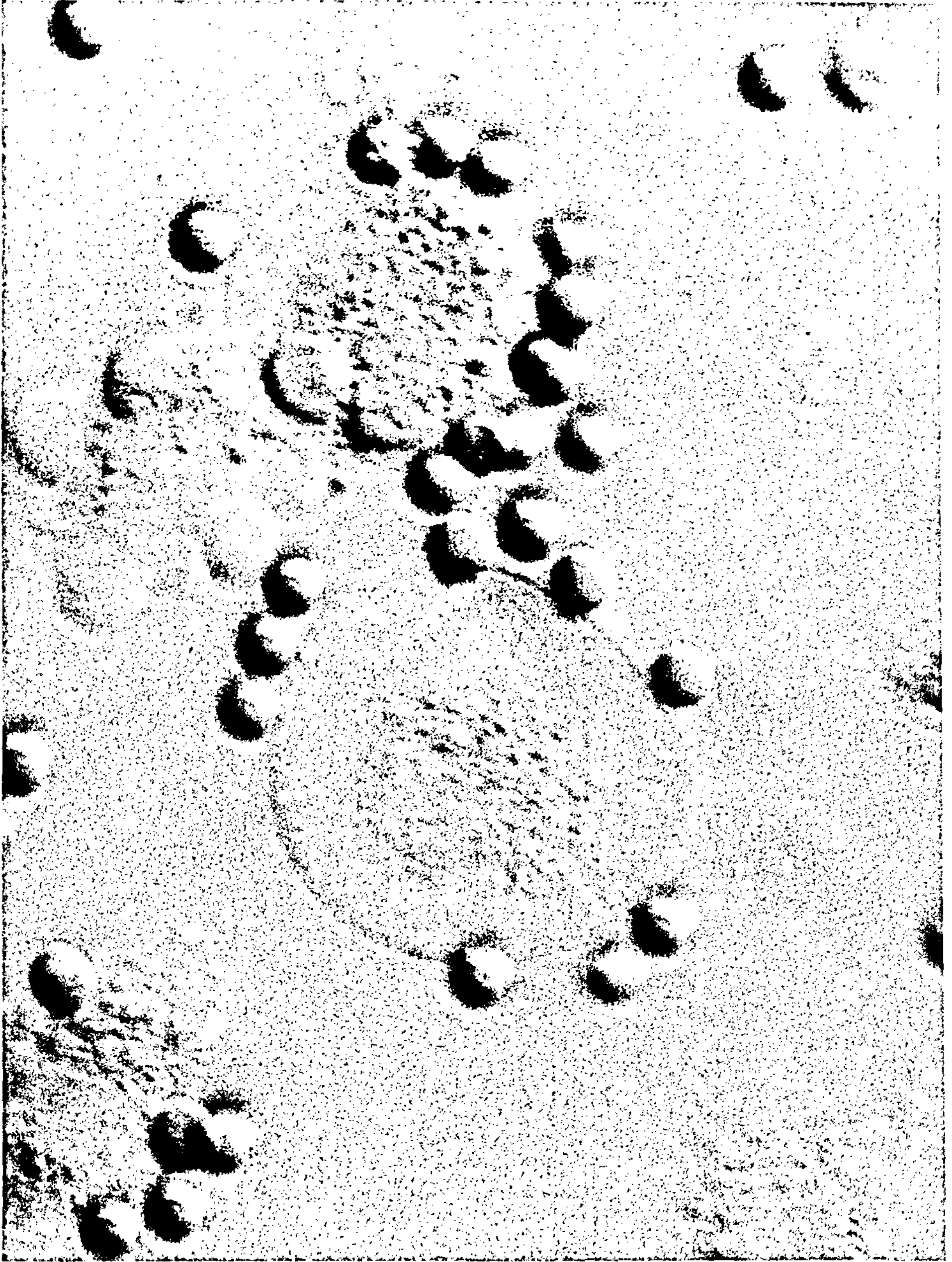


FIGURE 3. Photomicrograph of rat pulmonary macrophages forming rosettes with sheep red blood cells in presence of cytophilic antibodies to SRBC, ×400.

the equation

$$\text{Percent of rosettes formed} = [(X - Y) \times 100]/Z$$

where X represents number of macrophages forming rosettes with antibody, Y represents number of macrophages forming rosettes without antibody, and Z represents total number of macrophages counted.

In Vitro Assay for Cytophilic Antibody Receptors (FcR) on Macrophages

After exposure to pollutant atmospheres, each group of rats was sacrificed at a preselected time interval D_i (d 0, 2, and 4, and, when additional numbers of exposed rats could be made available, data were also taken on d 1, 3, or 7). Pulmonary macrophages from these groups of rats were obtained as described earlier. The same method that was explained previously was used to determine the percent of rosette forming macrophages using anti-SRBC serum (of 1:20 dilution, at which maximum percent of rosettes was formed as determined by rosette assay). The macrophages obtained from the rats exposed to purified air were used as controls.

Phagocytic Assay Using Polystyrene Latex Particles The nonspecific phagocytic activity of pulmonary macrophages was measured using polystyrene latex (PSL) particles (1.1 μm diameter, Duke Scientific Corporation, Palo Alto, Calif.). Briefly, 0.1 ml of the cell suspension was added to each of the Lab-Tek chambers containing 0.4 ml HBSS and incubated for 60 min at 37°C with 5% CO_2. Nonadherent cells were removed by washing the chambers three times with HBSS. Then 0.1 ml of PSL suspension in HBSS (volume/volume dilution 1:2000) was added along with 0.2 ml HBSS to each of the chambers and incubated for 90 min as above. The wells were washed to remove free PSL and the slide was observed with the inverted phase-contrast microscope. Macrophages that had engulfed PSL were counted. A minimum of 300 macrophages was counted from each rat, and the percent of phagocytic cells was determined.

Statistical Analysis

The data were statistically analyzed by one-way analysis of variance, Tukey multiple-comparison test with unequal sample sizes, and Student's t-tests. The level of significance was set at $p < 0.05$.

RESULTS

Rat anti-SRBC antiserum showed a good lytic activity (undiluted serum had 600 units/ml; 1 unit is the amount of antiserum that causes 50%

lysis of 1 × 10^8 SRBC in presence of complement) against sheep red blood cells. Maximum cytophilic activity, based on the percent of rosettes formed by lung macrophages, was observed with a 1:20 dilution of anti-SRBC serum. The effects of the exposures to ozone alone and to the two multicomponent atmospheres on pulmonary macrophage Fc receptor activity are shown in Table 1. All of the atmospheres studied significantly reduced ($p < 0.05$) rosette formation immediately after the end of the exposure (postexposure d 0). Following the ozone exposure this effect persisted on postexposure d 2 and was less marked, though statistically not significant, on d 4 (18.1 ± 3.9 versus 25.0 ± 6.2). A significant decrease in rosette formation was also observed with rats exposed to diesel exhaust plus acids on d 0 through d 2 (16.1 ± 1.8 versus 28.6 ± 5.9) as compared to clean-air-exposed control rats. Exposure to the 7-component atmosphere for 7 d caused effects through d 4 (18.0 ± 4.4 versus 26.6 ± 6.9) that were similar to the effects observed with the ozone exposure alone. Continued exposure to this 7-component atmosphere for 21 d demonstrated greater reduction in the percent of macrophages forming rosettes on postexposure d 0 (3.0 ± 2.0 versus 21.4 ± 3.6) than was observed on d 0 after 7 d of exposure. However, by postexposure d 7 the number of rosettes formed had returned to the control levels. A gradual increase in the percent of macrophages that formed

TABLE 1. *Effect of Pollutant Atmospheres on Fc Receptors of Pulmonary Macrophages*

	Percent of rosettes formed on day D_i after Pollutant Exposure[a]					
Exposure	Purified air controls	D_0	D_2	D_3	D_4	D_7
Ozone: 4 h[d]	25.0 ± 6.2 $n = 12$	8.2 ± 3.2[b] $n = 7$	8.4 ± 2.1[b] $n = 7$	18.9 ± 2.2[c] $n = 6$	18.1 ± 3.9[c] $n = 6$	—
Diesel + acids: 5 d[e]	28.6 ± 5.9 $n = 8$	16.8 ± 4.2[b] $n = 5$	16.1 ± 1.8[b] $n = 5$	19.1 ± 1.3 $n = 5$	—	—
7-Component atmosphere: 7 d[f]	26.6 ± 6.9 $n = 9$	10.3 ± 6.6[b] $n = 6$	12.9 ± 10.5[b] $n = 6$	—	18.0 ± 4.4[c] $n = 6$	—
7-Component atmosphere: 21 d[f]	21.4 ± 3.6 $n = 13$	3.0 ± 2.0[b] $n = 6$	4.1 ± 2.7[b] $n = 5$	—	16.1 ± 3.2[c] $n = 6$	19.2 ± 4.2[c] $n = 6$

[a]Values are the means ± SD.
[b]$p < 0.05$ versus control.
[c]$p < 0.05$ versus D_0.
[d]At 0.8 ppm ozone.
[e]At 0.46 mg/m^3 diesel soot + 0.38 mg/m^3 HNO_3 + 0.18 mg/m^3 H_2SO_4.
[f]At 0.3 ppm O_3 + 1.2 ppm NO_2 + 2.5 ppm SO_2 + 0.27 mg/m^3 $(NH_4)_2SO_4$ + 0.22 mg/m^3 $Fe_2(SO_4)_3$ + 0.004 mg/m^3 $MnSO_4$ + 0.15 mg/m^3 Fe_2O_3.

TABLE 2. Effect of Pollutant Atmospheres on Phagocytic Activity of the Macrophages

	Percent of macrophages with polystyrene latex particles on day D_i after pollutant exposure[a]				
Exposure	Purified air controls	D_0	D_2	D_3	D_4
Diesel + acids: 5 d[d]	66.1 ± 8.8 $n = 8$	41.1 ± 9.4[b] $n = 5$	53.3 ± 3.6[b] $n = 5$	50.7 ± 2.5[b] $n = 5$	—
7-Component atmosphere: 7 d[e]	78.9 ± 5.4 $n = 9$	57.5 ± 6.9[b] $n = 6$	68.6 ± 5.9[b,c] $n = 6$	—	73.4 ± 6.9[c] $n = 6$
7-Component atmosphere: 21 d[e]	75.2 ± 7.5 $n = 9$	75.0 ± 7.3 $n = 6$	72.4 ± 3.8 $n = 5$	—	72.8 ± 6.4 $n = 6$

[a]Values are the means ± SD.
[b]$p < 0.05$ versus control.
[c]$p < 0.05$ versus D_0.
[d,e]See footnotes e and f, respectively, of Table 1.

rosettes was observed from d 0 to d 2, 3, or 4 following exposure to ozone, diesel plus acids, and the 7-component atmosphere (Table 1).

The effects of the two multicomponent atmospheres on the nonspecific phagocytic activity of the macrophages are shown in Table 2. It was observed that there was a significant ($p < 0.05$) depression of phagocytic activity immediately after exposure to diesel plus acids, and the effect persisted through d 3 without any signs of recovery (50.7 ± 2.5 versus 66.1 ± 8.8). Similar effects were observed with the rats exposed for 7 d to the 7-component atmosphere on d 0 (57.5 ± 6.9 versus 78.9 ± 5.4) and also d 2. When studied on d 4, after the end of exposure no significant reduction in the phagocytic activity was observed (73.4 ± 6.9 versus 78.9 ± 5.4). In contrast, rats exposed for 21 d to the same 7-component atmosphere showed no impairment of the phagocytic activity (75.0 ± 7.3 versus 75.2 ± 7.5).

DISCUSSION

The rationale for these studies is based on the assumption that tests of immunological functions such as Fc receptor activity and phagocytic activity of pulmonary macrophages may serve as more sensitive methods to detect early damage to the bronchoalveolar region of the lung than has been the case for other endpoints (histopathology, particle clearance, and permeability evaluations) employed to detect the effects of various air pollutants.

Results in Relation to Other Studies

Acute and chronic lung lesions after exposure to O_3 occur in the centriacinar region as defined by Lum et al. (1983) and Barry et al. (1985). Epithelial responses after a single exposure, or after the onset of continuous O_3 exposure, are early death of alveolar type 1 (T_1) epithelial cells (2–12 h), and proliferation of alveolar type 2 (T_2) cells over 2 to 4 d. Influx of monocytes and macrophages begins in the first 12 h, with edema of centriacinar walls and increased free macrophages present by 24 h and maximal at 48 h (Evans, 1984; Mautz et al., 1985). In continued or repeated exposure for 7 d or longer, T_1 cells have been described as having increased thickness and numbers, T_2 cells have become hyperplastic, macrophages have doubled, and matrix components in the centriacinar walls have increased (Moore and Schwartz, 1981; Barry et al., 1985).

Histologic evaluation of rat lungs in groups of rats other than those used in the present study was performed as described by Mautz et al. (1985). A single 4-h exposure to 0.8 ppm O_3 produced an influx of free alveolar and intraseptal macrophages associated with interstitial edema at 48 h. This inflammatory reaction was no longer apparent by 8 d following the single exposure. The findings in the present experiment indicate a decrease in FcR ability to form rosettes through cytophilic antibody immediately after a 4-h exposure to 0.8 ppm O_3. Partial return toward normal was observed on d 3 and 4, a period during which histologic evidence of the influx of macrophages had passed a peak and had begun to decline. These comparative observations indicate that impairment of macrophage FcR function occurs early and persists during evolution of the acute histologic response of the rat lungs to ozone.

Studies with diesel exhaust and acids using the same concentrations and exposure regimen described here did not alter early (50 h, mucociliary) or late (30 d, alveolar) clearance of insoluble inhaled polystyrene latex microspheres studied as described by Phalen et al. (1980) and others (Kenoyer et al., 1981; Mannix et al., 1983). This atmosphere also failed to induce histologic or autoradiographic abnormalities in nasal or pulmonary airway epithelial or in the alveolar zone of exposed rat lungs (unpublished). Negative findings observed in the above methods imply the possibility that macrophage function might be a more sensitive indicator of the biologic effects of this atmosphere than the other endpoints currently in use.

The experiments reported here were part of a series of studies of experimental atmospheres designed to represent special features of photochemical air pollution. The 7-component atmosphere was designed as a "worst case" surrogate for an atmosphere based on ambient monitoring data. Exposure to this atmosphere was performed for 4 h daily for 21 d in an experiment conducted in two series: first, with components at

twice the concentration used in the present study, and second, at the concentrations reported here. In the first series (with higher concentrations of the 7-component atmosphere), histologic lesions characteristic of the transient inflammatory response to O_3 (Mautz et al., 1985) were no longer present when lungs were examined after 7 or 21 d of exposure. Nasal epithelial cell killing was present, however, as demonstrated by increased [^{3}H]thymidine labeling of epithelial cell nuclei in rats examined after 7 or 21 d of exposure (unpublished).

Permeability has been studied by instilling tracer molecules into the lumen of the trachea or of the bronchoalveolar zone of lungs of rats, followed by measurement of the transfer of tracers to blood (Bhalla et al., 1986). Exposure for 21 d to twice the concentrations of the 7-component atmosphere increased permeability in rats examined on d 7 and 21 of exposure, but the increase was not present in rats that recovered from exposure for 1 or more days (Bhalla et al., 1987). This finding suggested that permeability was increased even when histologic lesions in the lung were absent. Permeability was not increased in rats parallel to those used in the present experiment (unpublished). The finding that the 7-component atmosphere increased permeability of respiratory epithelia at twice the concentrations used here, but did not do so at the concentrations used in the present series of experiments, led us to report the present findings due to interest in the apparently greater sensitivity of measurements of macrophage function than that of other endpoints that had been used to study this important experimental atmosphere.

Significance of the Study

FcR competence was present in 21.4–28.6% of lung macrophages from control rats (Table 1), while phagocytic activity was observed in 66.1–78.9% of macrophages from controls (Table 2). This discrepancy may be factitious since the same cell that formed a rosette of SRBC was not examined for its capacity to engulf polystyrene latex microspheres. However, the identification of rosettes is potentially as exact an observation as the identification of engulfed rather than adherent PSL microspheres, so we are inclined to regard the discrepancy as real. The discrepancy is more likely to be understood on the grounds that the two functions depend on different mechanisms of cell–particle attachment: rosettes of SRBC form at sites on the macrophage that bear Fc receptors for anti-SRBC immunoglobulin G (IgG), while PSL microspheres attach at random without a requirement for FcR sites, and PSL engulfment is analogous to endocytic or other nonspecific incorporation of nonbiologic protein-free particles into a macrophage. For this reason, we cannot assume that the injury induced by exposure to the toxic atmospheres studied here necessarily involved the same sites on macrophages.

The decrease in antibody-mediated rosette formation of macro-

phages after exposure to different pollutant atmospheres suggests an inactivation of Fc receptors present on the cell membrane of the macrophages. Reichrtova et al. (1986) have demonstrated similar impairment of antibody-mediated rosette formation with pulmonary macrophages from rabbits on exposure to nickel downwind from a refinery dump site. The inhibition of Fc receptors on peritoneal macrophages was also reported with circulating immune complexes (Rao et al., 1979b). Rao et al. (1979b) also showed that the inhibition of Fc receptors on macrophages can be mediated through adoptive transfer of the suppressor inducer cells generated by soluble circulating immune complexes.

Mitchell and Mokyr (1972) have demonstrated earlier a reduction of acid phosphatase along with inhibition of Fc receptors of macrophages induced by immune complexes. In the present study, inhibition of FcR on pulmonary macrophages on exposure to diesel soot plus acids and 7-component atmosphere (7 and 21 d) may indicate a nonspecific suppression of acid phosphatase and lysozymes. Suppression of phagocytic activity of alveolar macrophages was reported on exposure to diesel emissions (Castranova et al., 1985). Such reduction was also observed in other systems (Rao and Mitchell, 1983). It is important to mention here that pulmonary macrophages, on exposure to various metals, exhibited different responses in their phagocytic activity. Lead showed a suppression of phagocytic activity, while cadmium failed to do so (Hilbert et al., 1986).

Pulmonary macrophages from rats exposed to the seven-component atmosphere for a short term (7 d) showed reduction in both rosette formation and phagocytic activity. On the contrary, the macrophages from rats exposed to the same atmosphere for longer periods (21 d) did not show a decrease in phagocytic activity. This could be due to recovery after initial damage caused by short-term exposure. To understand the mechanism(s) involved in such changes associated with short- and long-term exposures further studies at the subcellular level are necessary.

The recovery in the percent of macrophage rosettes with time after the end of exposure to pollutant atmospheres leads to a few speculations. One of them is that the Fc receptors destroyed or impaired by pollutant atmospheres are reactivated or regenerated with time. Second, there may be an influx of a fresh population of pulmonary macrophages generated from the precursors such as promonocytes that were not significantly affected by fresh or residual pollutants. In vitro investigations of pulmonary macrophages maintained in culture and studied at different time intervals may give some insight about the regeneration of Fc receptors. The changes in the receptor activity described here may play an important regulatory role in the immune response and in immunologic surveillance.

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