

Residual Oil Fly Ash Exposure Enhances Allergic Sensitization to House Dust Mite

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Epidemiological studies have shown an association between elevated levels of particulate matter air pollution and increased morbidity and hospital visits in asthmatics. Residual oil fly ash (ROFA) is a primary combustion particle containing sulfate and metals such as vanadium, nickel, and iron. In this study the effect of ROFA on sensitization to house dust mite (HDM) was examined in a Brown Norway rat model of pulmonary allergy. Rats were instilled via the trachea with 200 or 1000 μg ROFA 3 days prior to local sensitization with 10 μg HDM and were challenged with 10 μg HDM 14 days later. Immunological endpoints were examined at 2, 7, and 14 days after sensitization and at 2 and 7 days after challenge (16 and 21 days post-sensitization, respectively). Antigen-specific immunoglobulin E and associated immediate bronchoconstriction responses to antigen challenge were increased in the ROFA-treated groups compared with the HDM control group. Lymphocyte proliferation to antigen was enhanced at Days 7 and 21 in the bronchial lymphocytes of ROFA-treated groups. Bronchoalveolar lavage fluid (BALF) eosinophil numbers and lactate dehydrogenase were significantly increased in the 1000 μg ROFA group at Days 2 and 16, BALF total proteins were elevated at Days 2 and 7 in both ROFA-treated groups, and BALF interleukin (IL)-10 was elevated in the 1000 μg ROFA group at Day 2. These results suggest that ROFA has an adjuvant effect on sensitization to HDM. © 1999 Academic Press

Key Words: particulate matter; house dust mite; allergy; rat; lung; IgE; adjuvant; IL-10.

The prevalence of allergic asthma is increasing worldwide and currently affects about 13 million people in the United States (Mannino *et al.*, 1998). It is not clear to what extent

these increases reflect a rise in the number of sensitized individuals or improved diagnosis of the disease. Asthma is most prevalent in urban areas (Weiss *et al.*, 1992; Evans, 1992), where levels of air pollutants are highest. A variety of air pollutants have been shown to exacerbate allergic asthma. Sulfur dioxide causes bronchoconstriction and asthma-like symptoms (Balmes *et al.*, 1987), nitrogen dioxide from wood stoves has been associated with increased asthma morbidity (Ostro *et al.*, 1994), and ozone exposure elicits bronchial reactivity and pulmonary inflammation in asthmatics (Kreit *et al.*, 1989). Experimental animal models of human asthma have also shown that air pollutants exacerbate pulmonary allergy. Matsamura (1970a) first reported that guinea pigs exposed to high levels of O_3 , NO_2 , or SO_2 prior to ovalbumin sensitization had augmented allergic responses upon antigen challenge. NO_2 exposure has been shown to exacerbate pulmonary allergic responses to house dust mite (HDM) antigen in Brown Norway (BN) rats (Gilmour *et al.*, 1996), while diesel exhaust and diesel exhaust particles have an adjuvant effect in a murine model of ovalbumin allergy (Miyabara *et al.*, 1998; Takano *et al.*, 1997).

A major component of urban air pollution is particulate matter from combustion sources including fossil-fueled power plants (Natusch *et al.*, 1974). These particles, such as residual oil fly ash (ROFA), are a health concern because they typically have an aerodynamic diameter $<2.5 \mu\text{m}$ (PM_{2.5}) and can deposit deep in the respiratory tract (Salvaggio, 1994). Fossil fuel combustion PM_{2.5} may also contain relatively high levels of metals including Pb, V, Zn, and Cu, which may contribute to its toxicity (Natusch *et al.*, 1974). Recent epidemiological studies have shown a strong correlation between increases in particulate matter and increases in morbidity and mortality rates due to respiratory disease (Dockery *et al.*, 1993; Pope, 1989). Other studies have correlated increased emergency room visits from asthma attacks (Schwartz *et al.*, 1993; Lipsett *et al.*, 1997), and more frequent bronchodilator usage among asthmatics during PM excursions (Peters *et al.*, 1997).

Occupational exposure to ROFA has been shown to cause bronchiolitis obliterans (Boswell and McCunney, 1995), airway obstruction (Hauser *et al.*, 1995a), and upper airway

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inflammation (Hauser *et al.*, 1995b). Recent animal studies have shown that intratracheal instillation of ROFA in Sprague–Dawley rats resulted in acute lung injury (increased airway inflammation, lactate dehydrogenase [LDH], and total proteins in the lavage fluid) following exposure (Dreher *et al.*, 1997; Gavett *et al.*, 1997). ROFA instillation also caused upregulation of fibronectin mRNA expression and associated fibrotic lesions in the lungs of Sprague–Dawley rats (Su *et al.*, 1995). Since ROFA is a combustion source particle, it contributes to the fine ambient air PM burden (U.S. Environmental Protection Agency, 1994), and it is a reasonable particle source to investigate the effects of PM_{2.5} on allergic asthma. The purpose of the present study was to determine if intratracheal instillation of ROFA prior to pulmonary sensitization to HDM antigen could act as an adjuvant and enhance allergic immune responses.

MATERIALS AND METHODS

Animals. Inbred female BN rats (strain BN/Ss Nhsd) 8–10 weeks old and weighing 160–180 g were purchased from Harlan Sprague–Dawley, Inc. (Indianapolis, IN). All animals were housed in animal facilities with high-efficiency particulate air filters, were fed rat chow and water *ad libitum*, and were handled according to the Animal Care and Use Committee of our institution. Randomly selected animals, tested serologically upon arrival, and sentinels monitored throughout the study were free of Sendai virus, pneumonia virus, mouse hepatitis virus, and a variety of other rodent viruses, as well as *Mycoplasma sp.* Rats were also monitored and found to be free of ectoparasites and endoparasites.

Particulate source and characteristics. ROFA particles were collected on a Teflon-coated glass fiber filter by Southern Research Institute (Birmingham, AL) downstream of the cyclone of a power plant burning low sulfur number 6 residual oil at a temperature of 204°C (Hatch *et al.*, 1985). The collected ROFA particles had a mass median aerodynamic diameter (MMAD) of 1.95 μ m and contained mainly bioavailable transition metals such as vanadium, nickel, and iron (Dreher, *et al.*, 1997), and a number of sulfated compounds. Mount St. Helen's volcanic ash was collected on June 8, 1980; the MMAD of the particles was 2.2 μ m (Graham *et al.* 1985).

Antigen. Purified dust mite antigen from *Dermatophagoides farinae* was obtained from ground, whole-bodied mites after defatting, extraction in 0.125 M ammonium bicarbonate and dialysis against distilled water (Greer laboratories, Lenoir, NC). The resultant extract contained >75% of group I allergen, as assessed by the vendor.

Experimental design. Rats were divided into 5 groups ($n = 6$ –7 per group), lightly anesthetized by halothane inhalation (Aldrich Chemical Co., Milwaukee, WI), and instilled via the trachea with either 300 μ l of 0.90% sterile sodium chloride solution (Sigma Chemical Co., St. Louis, MO) or with 200 or 1000 μ g of ROFA suspended in 300 μ l sterile saline using a fiberoptic laryngoscope (Costa *et al.*, 1986). Three and 5 days after exposure to ROFA, rats were anesthetized by halothane inhalation and sensitized via the trachea with 300 μ l sterile saline containing 10 μ g HDM. Two weeks following the second sensitization, rats were anesthetized with halothane and challenged via the trachea with 10 μ g HDM in 300 μ l sterile saline. Endpoints were examined 2, 7, and 14 days after the second sensitization, and 2 and 7 days after allergen challenge.

Total IgE and HDM-specific IgE ELISA. Levels of total and HDM-specific IgE antibodies were determined in the serum of all rats by enzyme-linked immunosorbent assay (ELISA). To measure total IgE, 96-well polystyrene plates (Costar, Cambridge, MA) were coated with 2.5 μ g/ml mouse monoclonal antibody to rat IgE (Serotec, Ltd., Oxford, UK). For antigen

specific assays, plates were coated with 10 μ g/ml HDM. After overnight incubation at 4°C, plates were washed 5 times with deionized, pyrogen-free water containing 0.05% Tween-20 and were treated successively with (1) 200 μ l/well PBS + 1.0% BSA (blocking buffer), (2) 100 μ l/well rat serum diluted 1:5 in blocking buffer or rat IgE myeloma following a standard concentration curve ranging from 39 to 20,000 ng/ml (Harlan Bioproducts, Inc., Indianapolis, IN), (3) 300 ng/ml biotin-labeled mouse monoclonal antibody to rat IgE (Serotec, Ltd.), and (4) phosphatase-labeled streptavidin diluted 1:1,500 (Kirkegaard and Perry Laboratories, Gaithersburg, MD). Plates were washed five times with 0.05% Tween-20 and were incubated at 37°C between each step. *p*-Nitrophenyl phosphate disodium (Kirkegaard and Perry Laboratories) was added as a substrate for streptavidin, and plates were developed for 5 min at room temperature and read at 405 nm with a Thermomax ELISA plate reader (Molecular Devices, Menlo Park, CA).

Immediate bronchoconstriction responses to HDM. Two weeks after sensitization, animals treated as stated in experimental design along with additional control groups consisting of acidified Mount St. Helens (MSH) dust (60 μ g per rat in 300 μ l saline) + HDM sensitization, acidified MSH + sham (saline) sensitization, and 1000 μ g ROFA + sham sensitization were placed in a whole-body plethysmograph (Buxco Electronics, Troy, NY) equipped with a pneumotachograph and pressure transducer to monitor pulmonary-function responses as previously described (Dong *et al.*, 1998; Lambert *et al.*, 1998). The amount of MSH that was used as an inert particle control (60 μ g) was determined from the fraction of insoluble particles in ROFA (6%) based on a 1000 μ g dose (Dreher *et al.*, 1997). MSH–saline solutions were acidified to the pH of ROFA solutions (pH 2.6) using 0.63 mM H₂SO₄.

The index of bronchoconstriction that was measured was based on the wave shape of the box flow signal (Hamelmann *et al.*, 1997). The box flow signal reflected the difference between the animal's nasal flow and the flow due to chest expansion (thoracic flow). During bronchoconstriction, a difference in magnitude and phase appeared between the nasal flow and the thoracic flow. The time delay (expressed in arbitrary units of enhanced pause) between nasal and thoracic flows during response to challenge has been determined to be proportional to specific airway resistance (Pennock *et al.*, 1979).

In the experiments described here, baseline readings were measured for 10 min prior to HDM challenge in animals which received either 1000 μ g of ROFA prior to HDM sensitization, 1000 μ g of ROFA + sham-sensitization with saline, or rats which were HDM-sensitized only. Animals were then removed from the plethysmograph, anesthetized with halothane, intratracheally instilled with either 10 μ g HDM in 300 μ l saline or sham-challenged with saline (ROFA only animals) and placed in the plethysmograph for a further 20 min to determine immediate bronchoconstriction responses following challenge.

Lymphocyte proliferation assay. Lung-associated lymph nodes were removed from the right mainstem bronchus of each rat at all time points following sensitization. Lymph-node-cell suspensions were prepared using ground-glass homogenizers. After centrifugation at 1500 rpm, red blood cells were lysed with 0.89% ammonium chloride, and cells were washed 3 times by centrifugation (1500 RPM, 10 min) and resuspended in RPMI medium containing 10% fetal calf serum, 5% penicillin-streptomycin, and 10^{−5} M β -mercaptoethanol (Gibco BRL Life Technologies, Gaithersburg, MD). Viability was assessed by trypan blue dye exclusion, and cells were counted with a hemocytometer and resuspended to 3 $\times$ 10⁶ cells/ml in complete medium. Cells (100 μ l aliquots) were added in triplicate to 96-well flat-bottom tissue culture plates and incubated in the presence of complete medium or 10 μ g/ml HDM. After 3 days, cell cultures were pulsed with [³H]thymidine (0.5 μ Ci/well). Cells were harvested onto filter paper 18 h later using a Skatron Combi Cell Harvester (Skatron Instruments, Inc., Sterling, VA), and the incorporated [³H]thymidine was measured after the addition of scintillation fluid (Packard Instrument Co., Meriden, CT) using a Packard 2500-TR scintillation analyzer (Packard Instrument Co.). Lymphocyte responsiveness was expressed as the difference in radiotracer counts between cells incubated with HDM and those of the cells incubated with media alone.

Bronchoalveolar lavage. Rats were euthanized with an intraperitoneal injection of 200 mg/kg of pentobarbital (Abbott Laboratories, North Chicago,

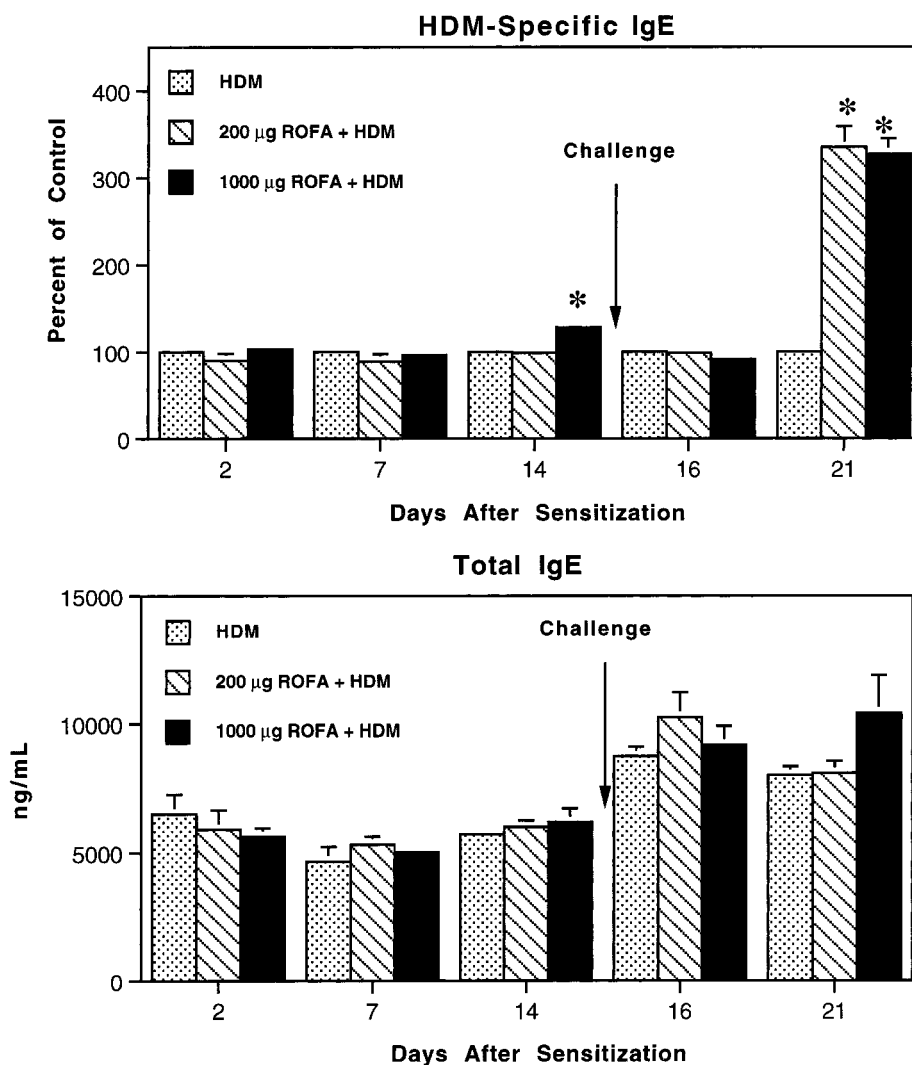


FIG. 1. Effect of ROFA on serum immunoglobulin (Ig) E 2, 7, 14, 16, and 21 days after sensitization with HDM. Total IgE in the serum is in nanograms/ml $\pm$ SEM and was determined from a standard curve of rat myeloma IgE. HDM specific IgE data were transformed to percentage of mean values of HDM control groups and are shown as means $\pm$ SEM. Data were adjusted for multiple comparisons after ANOVA was applied. *Significant difference at the level of 0.05 when each ROFA-treated group was compared to the HDM control group ($n = 6-7$).

IL) and bled by cardiac puncture. The trachea was exposed, cannulated, and tied off with suture. The lungs were lavaged 3 times with a single volume of warmed saline (35 ml/kg). Total cell counts were performed with a hemocytometer and cell viability was assessed by trypan blue dye exclusion. Cells from each sample (50,000) were centrifuged onto a glass slide using a Cytospin Model II (Shandon, Pittsburgh, PA) and stained with Diff-Quick (American Scientific Inc., Sewickley, PA) for identification of macrophages, eosinophils, lymphocytes, and neutrophils. At least 200 cells were counted for each specimen.

Total protein, IL-10, and LDH measurement in BALF. After slides were made for differential counts, BALF was centrifuged (1500 rpm, 10 min, 4°C) and an aliquot of the supernatant was stored at 4°C for biochemical analysis. Lactate dehydrogenase levels were determined using kit 228 (Sigma Chemical Co.), and total protein levels were determined using Coomassie Plus reagent (Pierce, Rockford, IL). Assays were adapted for automated analysis by a Cobas Fara II centrifugal spectrophotometer (Hoffman-La Roche, Branchburg, NJ). An aliquot of BALF was stored at -70°C for later analysis of IL-10 concen-

trations by ELISA using a rat Cytoscreen kit purchased from Biosource International (Camarillo, CA).

Statistical analysis. Immediate bronchoconstriction data were analyzed by comparing all groups using repeated-measures analysis of variance (ANOVA). Group differences were considered significant if the test statistic type I error was <0.05 .

Statistical analysis of ELISA, lymphocyte proliferation, BALF total protein, LDH, IL-10, and inflammation data was made by ANOVA. Pairwise comparisons following an overall significant ANOVA were performed using Duncan's multiple range comparison test. The level of significance was set at 0.05.

RESULTS

IgE ELISAs

Serum HDM-specific IgE was significantly elevated at Day 14 after sensitization in the 1000 µg ROFA-treated group and

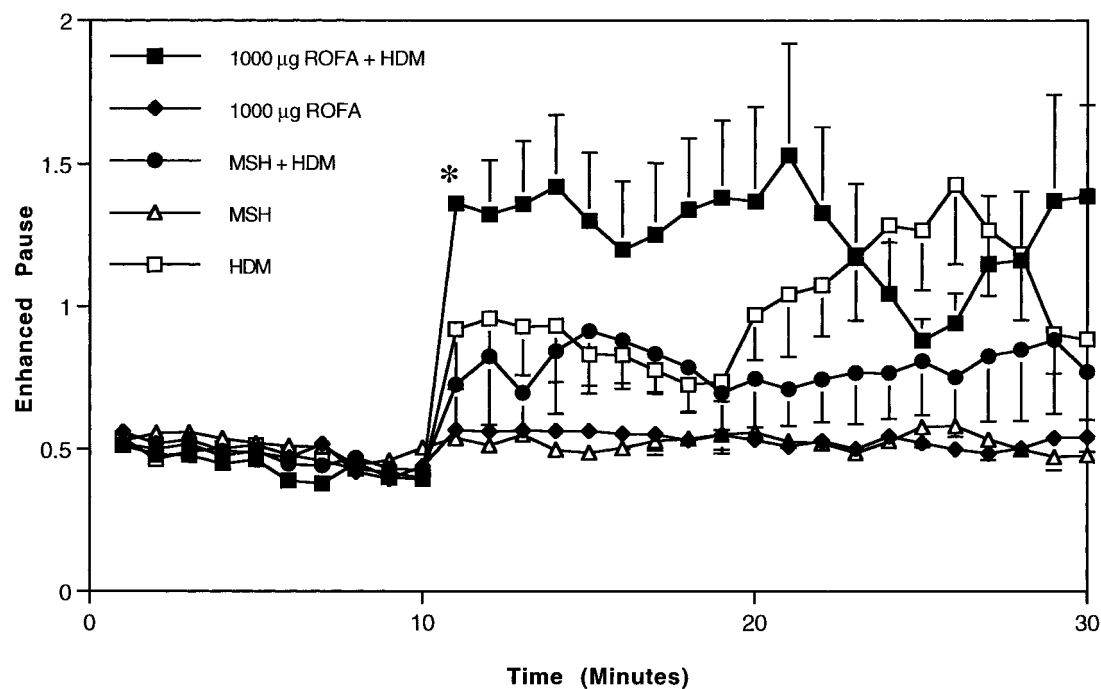


FIG. 2. Effect of ROFA on immediate bronchoconstriction responses to HDM challenge in sensitized rats. Baseline readings were obtained for 10 min for each rat prior to HDM challenge. Data are shown in arbitrary units of enhanced pause (mean $\pm$ SEM), which represents the time lag between inspiration and expiration and is proportional to specific airway resistance. *Significant different at the level of 0.05 for the first 12 min following antigen challenge ($n = 7-8$).

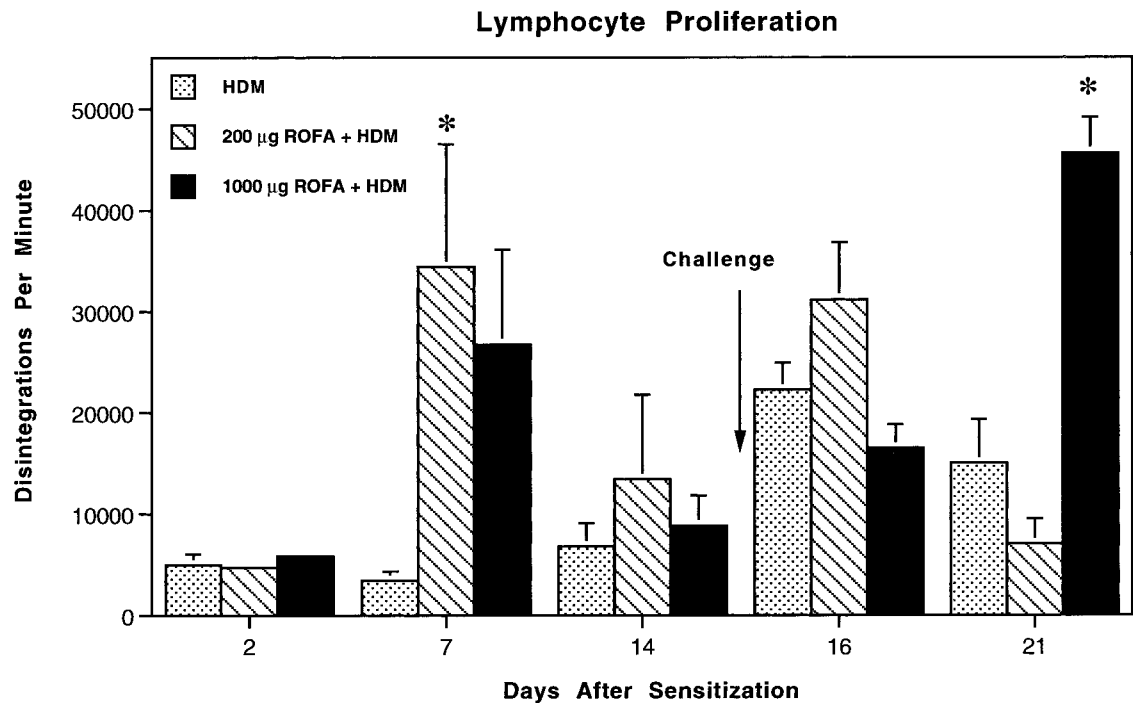


FIG. 3. Effect of ROFA on cell proliferation of lung-associated lymph node cells 2, 7, 14, 16, and 21 days after HDM sensitization. Lymphocytes (3×10^5) were incubated with 10 µg/ml HDM antigen for 4 days. Proliferative responses were assessed by [^{3} H]thymidine incorporation over the last 18 h of incubation. Data are presented as mean counts per minute $\pm$ SEM. Data were adjusted for multiple comparisons after ANOVA was applied. *Significant difference at the level of 0.05 when each ROFA-treated group was compared to the HDM control group ($n = 6-7$).

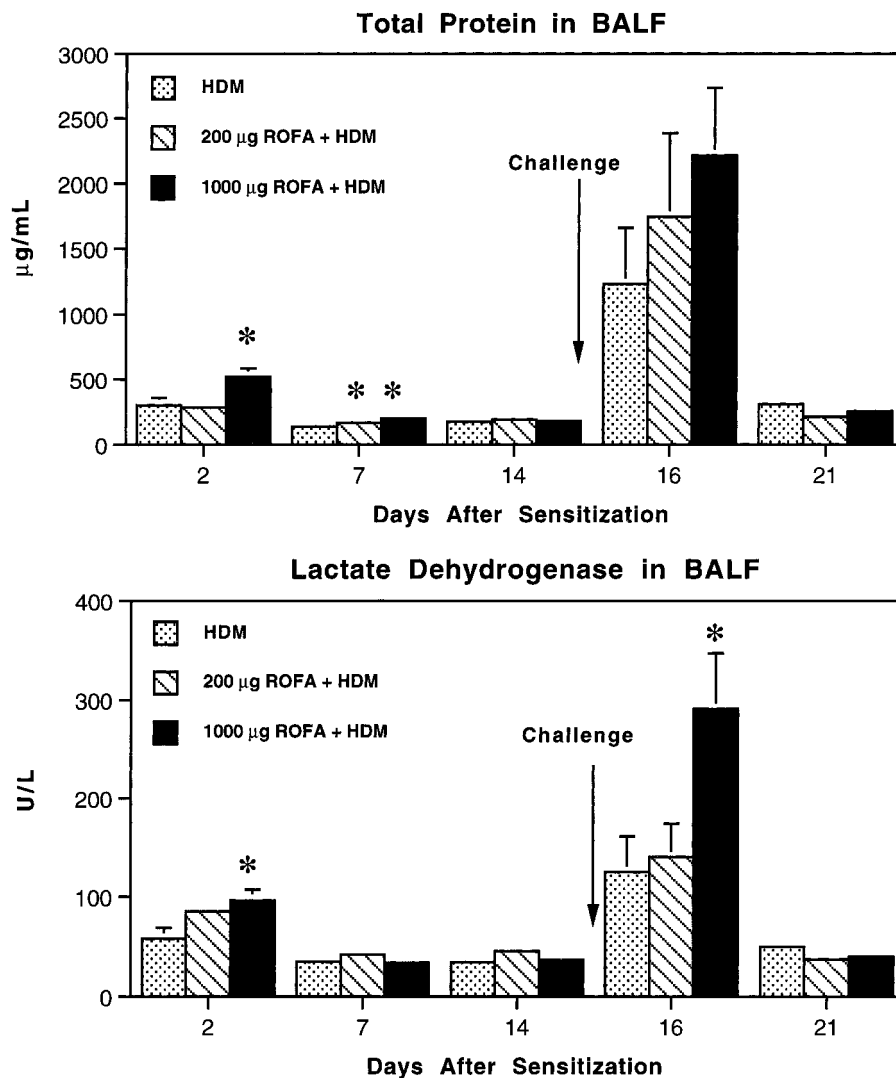


FIG. 4. Effect of ROFA on total protein and LDH in the BALF 2, 7, 14, 16, and 21 days after HDM sensitization. Lavage fluid was centrifuged at 1500 rpm for 10 min at 4°C prior to analysis. Data are presented as means $\pm$ SEM. Data were adjusted for multiple comparisons after ANOVA was applied. *Significant difference at the level of 0.05 when each ROFA-treated group was compared to the HDM control group ($n = 6-7$).

was significantly elevated in both the 200 and 1000 μ g ROFA-treated groups at Day 21 after sensitization (7 days after challenge) compared to the HDM-sensitized group (Fig. 1). Although there was a trend for increased levels of total IgE in the serum of ROFA-treated rats at Day 16 (2 days after challenge with HDM) and at Day 21 (7 days after challenge with HDM), none of the observed differences reached statistical significance compared to HDM control rats.

Immediate Bronchoconstriction

HDM-sensitized rats experienced an increased immediate response to antigen challenge compared to control animals (Fig. 2). This antigen-induced bronchospasm was further augmented in animals which received 1000 μ g ROFA prior to HDM sensitization. ROFA-treated animals had significantly

higher enhanced pauses for 12 min following challenge with HDM compared with all groups tested. For this 12 min time period an average enhanced pause of 1.3 was observed for rats that received 1000 μ g ROFA prior to HDM sensitization and 0.9 for animals that were sensitized with HDM without prior ROFA treatment. ROFA treatment alone did not elicit a significant immediate bronchoconstriction response (enhanced pause = 0.5). Similarly, inert particles (MSH) had no significant effect on the immediate response either alone or coupled with HDM sensitization. In the time period between 23 and 30 min (13–20 min after challenge), the immediate response of the HDM-sensitized ROFA-treated group went down, while the immediate response of the HDM sensitized group went up, but there was no significant difference in the mean enhanced pauses between these groups during this time.

Lymphocyte Proliferation

Lymphocyte proliferation to antigen was increased in cells from the bronchial lymph nodes of HDM-sensitized rats at Day 16 (2 days after challenge) compared with earlier timepoints (Fig. 3). Lymphocytes from animals in the 200 μ g ROFA group had significantly higher proliferation to antigen at an earlier timepoint (Day 7) than did those from the 1000 μ g ROFA or HDM-sensitized groups. Lymphocytes from animals instilled with 1000 μ g ROFA prior to HDM sensitization had increased proliferative responses to HDM at Day 21 after sensitization (7 days after challenge) compared with the 200 μ g ROFA or HDM control groups.

Total Protein, IL-10, and LDH in BALF

After challenge, HDM caused increased BALF protein as a measure of lung edema (Fig. 4). Although there was a trend for increased protein in the ROFA groups at this timepoint, it was not significant. Total protein concentrations were significantly elevated in the BALF of 1000 μ g ROFA-treated animals at Day 2, and in the BALF of both 200 and 1000 μ g ROFA-treated rats at Day 7 following sensitization compared with HDM controls. Lactate dehydrogenase (LDH), a measure of cell damage, was increased in HDM-sensitized animals 2 days after challenge (Day 16), and this increase was significantly greater in the 1000 μ g ROFA group. LDH was also significantly increased 2 days after sensitization in the 1000 μ g ROFA group compared to the 200 μ g ROFA and HDM-sensitized groups, which was probably a direct effect of ROFA-induced lung tissue damage. ELISA assay of BALF on Day 2 after sensitization showed significantly higher levels of the TH2 cytokine IL-10 in both ROFA groups compared with the HDM control group (Fig. 5).

Airway Inflammation

Macrophage numbers were significantly elevated in the BALF of rats treated with 1000 μ g ROFA at Day 14 compared to the 200 μ g ROFA and HDM control groups (Fig. 6). Instillation of 1000 μ g ROFA prior to sensitization to HDM resulted in a significant increase in lavagable eosinophils and lymphocytes at Day 2, and lymphocytes were significantly elevated in the BALF of HDM-sensitized animals at Day 7. Antigen challenge resulted in increased numbers of eosinophils in the lungs of HDM-sensitized animals at Day 16 (2 days after challenge), but eosinophil numbers were significantly greater in the 1000 μ g ROFA group compared to the HDM control group suggesting that prior ROFA exposure enhanced the allergic response to HDM challenge. Inflammation was resolved in all groups by Day 21.

DISCUSSION

Allergic (extrinsic) asthma is characterized by elevated serum IgE titers, airway eosinophilia, episodic reversible bron-

IL-10 in BALF 2 Days After Sensitization

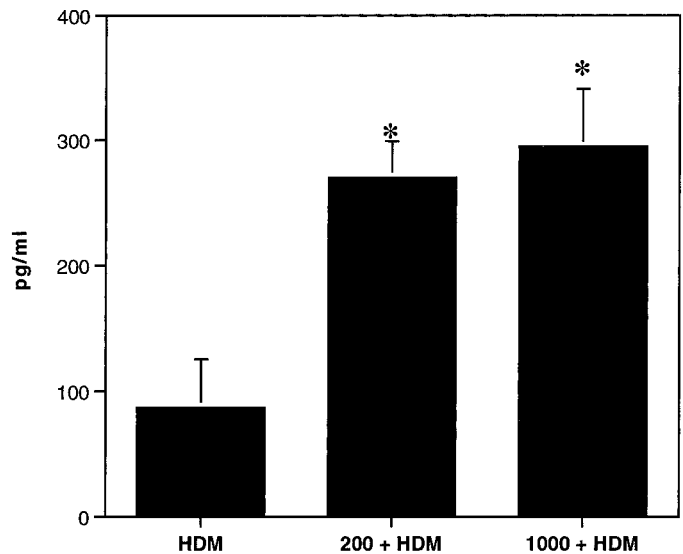


FIG. 5. Effect of ROFA on IL-10 expression in the BALF 2 days after HDM sensitization. Lavage fluid was centrifuged at 1500 rpm for 10 min and stored at -70°C prior to analysis. Data are presented as means $\pm$ SEM. Data were adjusted for multiple comparisons after ANOVA was applied. *Significant difference at the level of 0.05 when each ROFA-treated group was compared to the HDM control group ($n = 7$).

choconstriction, and airway hyperresponsiveness. While several epidemiological and clinical studies have demonstrated that air pollutant exposure can exacerbate some of these symptoms in asthmatics (Peden, 1997), it is not known whether the rate or degree of initial allergic sensitization may also be affected. Studies in animals thus far have shown that exposure to O_3 , NO_2 , SO_2 , and diesel particles (DEP) may increase allergic sensitization (Gilmour, 1995).

The results presented here confirm the adjuvant effect of another air pollutant (ROFA) and provide additional information on the kinetic relationship between immune function, airway responses, and immune-mediated pulmonary inflammation. Even though the instilled bolus of ROFA did not resemble a natural inhalation exposure, the total concentration delivered to the lung (1 mg) may be less than that inhaled in occupational settings over a work day (Hauser *et al.*, 1995a). Furthermore, additional studies from our laboratory have shown that an equivalent deposition of ROFA by inhalation or instillation routes evokes pulmonary inflammatory responses which are qualitatively and quantitatively similar (Costa *et al.*, 1998).

Allergic sensitization is known to be initiated by the processing of antigen in dendritic cells with subsequent presentation to CD4^+ and CD8^+ T lymphocytes in the draining lymph nodes (Lambrecht *et al.*, 1996). Immunomodulating cytokines are then produced which polarize T helper (CD4^+) cells to a Th1 or a Th2 phenotype, and provide signals for cellular activation (Corrigan and Kay, 1990; Abbas *et al.*, 1996). Interleukin (IL)-4 and IL-10 are the principle cytokines which

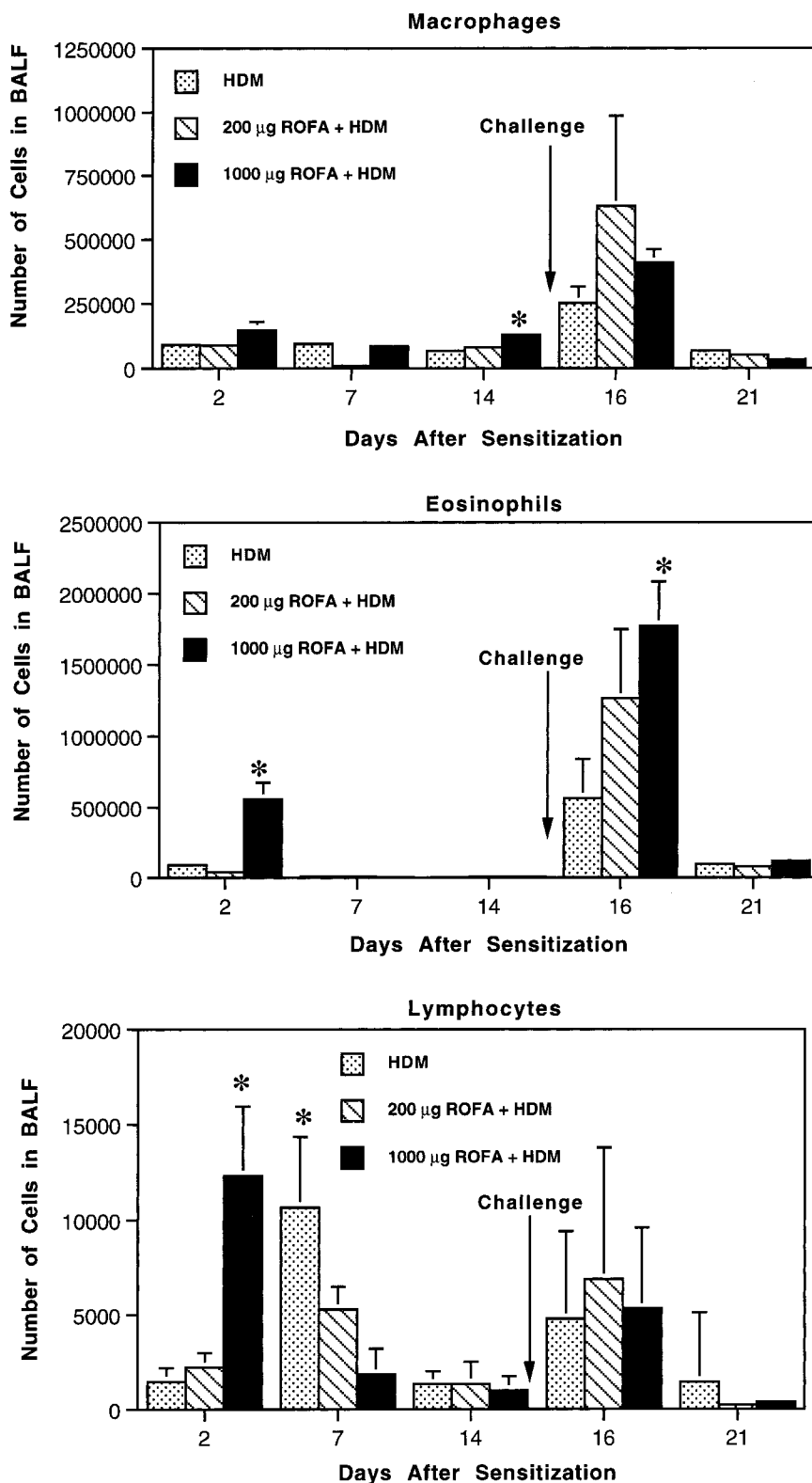


FIG. 6. Effect of ROFA on BALF inflammatory cell numbers 2, 7, 14, 16, and 21 days after HDM sensitization. Pulmonary cells obtained by bronchoalveolar lavage were centrifuged onto microscope slides and stained with Diff Quick. Differential counts were performed for each rat and data are presented as mean cell numbers $\pm$ SEM. Data were adjusted for multiple comparisons after ANOVA was applied. *Significant difference at the level of 0.05 when each ROFA-treated group was compared to the HDM control group ($n = 6-7$).

promote Th2 (IgE-mediated) responses and facilitate B cell class-switching to IgE production (Gascan *et al.*, 1991; Powrie *et al.*, 1993).

Previous studies in mice have shown that coexposure to DEP and ovalbumin antigen increased IL-4 production in pulmonary lymphocyte cultures and elevated levels of antigen-specific IgE antibody titers and airway eosinophilia after antigen challenge (Muranaka *et al.*, 1986; Takafuji *et al.*, 1987, Takano *et al.*, 1997; Miyabara *et al.*, 1998). In the experiments described here, rats exposed to ROFA resulted in increased IL-10 levels 2 days after sensitization with HDM compared to controls. Although we were unable to detect IL-4 in the BAL fluid of any sample, it has recently been proposed that IL-10 may be a more useful marker for Th2 responses in the rat and correlates better with IgE production (Van Loveren *et al.*, 1999). The temporal primary immune response which developed after sensitization (consisting of both IgE production and lymphocyte activity) was significantly enhanced by ROFA exposure and translated into a more intense allergen-induced bronchoconstriction and immune-mediated pulmonary inflammation. These data support the notion of a direct relationship between levels of immune function and the extent of allergic lung disease and confirm our previous findings where NO₂ gas acted as an immunologic adjuvant (Gilmour *et al.*, 1996). As in that study, the secondary boosting of immunity seen 7 days after antigen challenge was higher in the ROFA-exposed animals, suggesting that these animals would be more reactive to a subsequent allergen exposure.

Despite numerous reports of enhanced allergic immune responses in humans and animals exposed to combustion particles and oxidant gases, the mechanisms by which these effects occur are not clear. It is known that air pollutant exposure causes pulmonary inflammation and edema (Bascom *et al.*, 1996) and that damage to the bronchial epithelium may enhance translocation of antigen to the submucosa (Matsamura, 1970a,b). From these effects, it is conceivable that dendritic cells encounter more antigen which may result in increased accessory cell activity. Alternatively, pro-inflammatory mediators such as IL-6 and TNF- α are produced by bronchial epithelial cells during lung injury (Carter *et al.*, 1997) and may also play a role in promoting allergic immune responses (Vercelli *et al.*, 1989; Casale *et al.*, 1996; Rincon *et al.*, 1997). Finally, certain soluble components of ROFA may act directly on B lymphocytes since purified human B cells exposed *in vitro* to aromatic hydrocarbons from diesel exhaust secrete more IgE than control cultures (Takenaka *et al.*, 1995).

In summary we have demonstrated that exposure to ROFA, a component of fine ambient air PM, enhances allergic sensitization. One likely mechanism for ROFA's effect is its influence on pulmonary cells at the time of lymphocyte sensitization. To test this theory more fully, a comprehensive profile of pro-inflammatory and immunomodulatory cytokines is required to identify which mediators are associated with immune adjuvancy. Should differential cytokine expression prove to be

an important indicator of adjuvancy, it may provide a means to extrapolate from animal experiments to potential mechanisms in humans. While it is unethical to sensitize humans and study how PM exposure may affect this process, analysis of cytokine responses elicited in human lung cells by *in vitro* PM exposure is possible. Such studies may provide evidence that PM exposures may act as an adjuvant to enhance allergic sensitization in humans.

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