

EFFECT OF INHALED ULTRAFINE CARBON PARTICLES ON THE ALLERGIC AIRWAY RESPONSE IN RAGWEED-SENSITIZED DOGS

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Episodic increases in air pollution have been associated with the exacerbation of asthma symptoms. Ultrafine particles are a component of air pollution and may be involved in causing the adverse health effects associated with high air pollution. We evaluated the effects of ultrafine particle inhalation on immune and airway responses in a beagle dog model of allergic asthma. Six allergic (ragweed sensitive) and six nonallergic dogs were exposed to ultrafine carbon particles ($232.3 \pm 2.5 \mu\text{g}/\text{m}^3$, $35.2 \pm 0.3 \text{ nm}$) for 1 h, followed by a challenge with vehicle (water) as a negative control. Airway resistance was measured during particle exposure and after vehicle challenge. Immune responses 3 days before and after (1 h and 1, 4, 7, and 11 days) particle exposure were assessed by measuring total immunoglobulin E (IgE) and ragweed-specific IgE and IgG in serum and bronchoalveolar lavage fluid (BALF), and cell differentials in BALF. Each dog was exposed a second time to ultrafine carbon particles ($251.4 \pm 5.3 \mu\text{g}/\text{m}^3$, $34.9 \pm 0.5 \text{ nm}$) for 1 h followed by a challenge with ragweed and the same measurements. Airway resistance did not change during particle exposure in any of the dogs, and ragweed-induced airway reactivity was not altered by particle exposure. Total and ragweed-specific serum IgE and total IgE in BALF were higher in allergic dogs at all time points. Particle exposure did not affect antibody levels in serum or BALF in allergic dogs. Nonallergic dogs developed specific IgG in response to multiple inhalation exposures to ragweed, but this was not associated with particle exposure. Neutrophils were elevated in BALF for all groups 1 day after particle exposure. In conclusion, despite the induction of low level inflammation in the lungs of allergic and nonallergic dogs, exposure to ultrafine carbon particles did not alter airway reactivity or immune responses.

Epidemiological studies conducted at various sites around the world have shown an association between increased levels of ambient particulate matter (PM) and morbidity and mortality in individuals with preexisting respiratory or cardiac disease (Bascom et al., 1996; U.S. Environmental Protection Agency [EPA], 2001). Increases in PM are positively associated with exacerbation of asthma symptoms, increased asthma medication use, and increased hospital admissions for asthma (Delfino, 1998; Sunyer et al., 1997; Taggart et al., 1996; Thurston et al., 1997; Timonen & Pekkanen

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1997; Wong et al., 2001). Three studies focusing on the incidence of asthma attacks in the Netherlands, Denver, and Los Angeles indicated a weighted mean 3% increase in asthma attacks for each increase of $10 \mu\text{g}/\text{m}^3$ (Ostro et al., 1991; Pope et al., 1991; Whittemore & Korn, 1980). The causality of this association has not been established. Investigators have hypothesized that particle number, which is predominated by the ultrafine component of PM, may be associated with adverse health effects (Oberdörster et al., 1995; Peters et al., 1997; Utell & Frampton, 2000).

Although ultrafine particles (commonly defined as $<0.1 \mu\text{m}$ in diameter) comprise only 1% to 8% of the mass of ambient PM, they are present in very large numbers (Ebelt et al., 2001; Hughes et al., 1998; Peters et al., 1997). Currently, the composition and concentration of ultrafine particles in ambient air are not well characterized. Carbonaceous agglomerates from combustion sources are believed to comprise a large component of ultrafine particles in urban settings (Hildemann et al., 1994). There may also be a significant quantity of volatile organic and sulfur compounds adsorbed to the carbonaceous agglomerates or undergoing homogeneous nucleation to form other ultrafine particles (Kittelson, 1998; Kittelson & Abdul-Khalek, 1998). In addition, urban ultrafine particles may also contain metals such as, iron, platinum, and vanadium (Dye et al., 1997).

Animal studies indicate that at relatively high exposure levels and on an equal mass basis, ultrafine carbon and titanium dioxide particles have a greater toxic potential than accumulation-mode fine particles ($0.1\text{--}1.0 \mu\text{m}$ diameter) (Li et al., 1999; Oberdörster et al., 1990, 1992). However, ultrafine carbon or platinum particle exposures at levels approximately equivalent to the highest ultrafine PM levels recorded in ambient air have no significant effects on the lungs of young or old healthy mice and rats (Oberdörster et al., 2000). In contrast, the same study also showed that in some animal models with preexisting lung inflammation, ultrafine particle exposure induces a small additional inflammatory response. These data suggest that individuals with preexisting conditions may be at greater risk of adverse health outcomes from ultrafine particle exposure.

A recent study of adults with a history of asthma in Erfurt, Germany, found that the largest decreases in peak expiratory flow (PEF) were more strongly associated with ultrafine than with fine particle exposure levels (Peters et al., 1997). Assessment of adult asthmatics in Helsinki, Finland, indicated that ultrafine particle number concentrations tended to be inversely but nonsignificantly associated with forced vital capacity, forced expiratory volume in 1 s, and PEF (Penttinen et al., 2001). Additionally, assessment of asthmatic children in Kuopio, Finland, found that variations in PEF were no more associated with ultrafine particles than with PM_{10} or black smoke (Pekkanen et al., 1997). A potential weakness of these studies is that the different particle size fractions evaluated were highly intercorrelated. Thus, associations between reduced lung function and different particle size fractions may lose some of their statistical significance.

In the present study we sought to determine whether acute inhalation of ultrafine carbon particles will alter lung function in an animal model of allergic asthma. Beagle dogs sensitized to ragweed and exhibiting an allergic airway response to inhaled ragweed (Redman et al., 2001) were exposed to ultrafine carbon particles. Airway resistance and lung inflammation were assessed by evaluating measurements taken at baseline and following exposure.

METHODS

Animals

Beagles were born and maintained in the LRR dog colony. Dogs were housed in temperature controlled kennel buildings with access to indoor and outdoor runs. The dogs were fed 350 g dry dog food once per day (Wayne Mini Lab Dog Diet 8759; Teklad Premier Laboratory Diets, Madison, WI), and water was available at all times.

The procedure for sensitizing dogs to ragweed has been described previously (Redman et al., 2001). Briefly, beagle puppies were sensitized by intraperitoneal injection of ragweed extract (ragweed short, *Ambrosia artemisiifolia*, Bayer Corporation, Elkhart, IN) combined with aluminum hydroxide (Accurate Chemical and Scientific, Westbury, NY) within 24 h after birth and then weekly for 4 wk. The dogs received additional subcutaneous injections weekly during wk 5 through 8, and then biweekly during wk 10 through 22. Six ragweed-sensitized and six age-matched nonsensitized control dogs were selected for this study. Both sensitized and nonsensitized dogs were challenged by inhalation 3 times with ragweed 7–9 mo after birth; results of these challenges have been described previously (Redman et al., 2001). Approximately 10 mo after the last ragweed inhalation challenge, the same dogs (~18 mo of age) were used for the current study protocol (Figure 1).

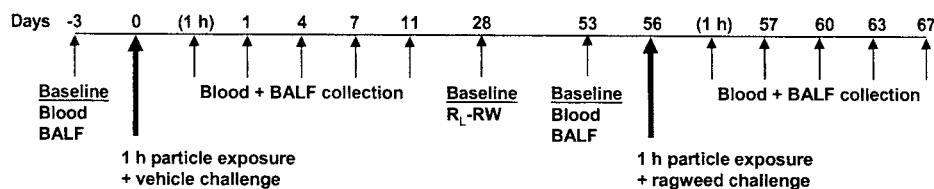


FIGURE 1. Schematic summary of exposure protocol used in the present study. Three days before the first ultrafine particle exposure blood and bronchoalveolar lavage fluid (BALF) was collected to determine baseline antibody and cell differential levels. Immediately before exposure to ultrafine particles baseline pulmonary resistance (R_L) was recorded (average over 5 min). Dogs were then challenged for 1 h to ultrafine particles and challenged with water (vehicle control) immediately thereafter. Additionally, Pulmonary resistance (R_L) was measured continuously during particle exposure and immediately after water challenge. Blood and BALF were collected 1 h and 1, 4, 7, and 11 days after the end of particle exposure. Approximately 1 month after the first particle exposure each ragweed (RW)-sensitized dog was challenged with several test doses of RW to determine a concentration of RW that caused a two- to threefold increase in R_L . Again, approximately 1 mo later the dogs were taken through the same procedures already described except that the dogs were challenged with RW.

All animal procedures were carried out in accordance with protocols approved by the LRRI Institutional Animal Care and Use Committee. LRRI is fully accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care.

Exposures

The dogs were sedated with acepromazine (Phoenix Pharmaceutical, St. Joseph, MO) subcutaneously (2 mg in 200 μ l). About 30 min later, the dogs were anesthetized with intravenous propofol (3.7 mg/kg induction and 2.0 mg/kg maintenance every 3 min; Abbott Laboratories, North Chicago, IL). An esophageal balloon catheter was positioned in the portion of the esophagus where fluctuations in esophageal pressure were maximal. The dogs were intubated with an endotracheal tube, placed in a 79 cm $\times$ 28 cm $\times$ 25 cm sealed Plexiglas box, and ventilated using a Harvard pump (Harvard Apparatus, Holliston, MA) to change box pressure. The endotracheal tube was connected to a port at one end of the box and the Harvard pump was connected at the other end. A reciprocating positive to negative change in box pressure sustained the dogs at a tidal volume of about 200–240 ml, a respiratory rate of 18 breaths/min, and an end-tidal CO₂ range of 4–5% (LB2, medical gas analyzer; Beckman Instruments, Palo Alto, CA). Dogs were then exposed to ultrafine particles for 1 h and immediately thereafter challenged with 5 breaths of vehicle (water) or ragweed via the endotracheal tube.

Ultrafine carbon particles were generated using an electric spark discharge generator with graphite electrodes (model CFG 1000, PALAS Co., Karlsruhe, Germany). Carbon particles were generated in an atmosphere of 99.999% argon to prevent oxidation during aerosol formation. HEPA-filtered diluting air was added to the particle stream to prevent coagulation after leaving the generator. Oxygen was added to the exposure atmosphere to compensate for the depletion of oxygen by addition of argon, resulting in a final oxygen concentration of 20.6%. Ultrafine carbon particles contained organic carbon comprising 40–70% of total carbon (McDonald et al., 2001). The number, mass, and size of the particles were determined using a size mobility particle sampler (SMPS, model 3080, TSI, Inc., St. Paul, MN). The first set of 1-h particle exposures (with subsequent vehicle challenge) resulted in an average particle exposure concentration of $232.3 \pm 2.5 \mu\text{g}/\text{m}^3$, a count median diameter (CMD) of $35.2 \pm 0.3 \text{ nm}$, and a geometric standard deviation (GSD) of 1.51. The second set of 1-h particle exposures (with subsequent ragweed challenge) resulted in an average particle exposure concentration of $251.4 \pm 5.3 \mu\text{g}/\text{m}^3$, a CMD of $34.9 \pm 0.5 \text{ nm}$, and a GSD of 1.52. The particle number concentration was $\sim 6 \times 10^6 \text{ particles}/\text{cm}^3$ for both exposures.

Ragweed (Bayer) or vehicle (water) aerosols were generated using a jet nebulizer (number 950, Hospitak, Lindenhurst, NJ) and delivered directly through the endotracheal tube. Dogs were given 5 breaths of the aerosolized ragweed or vehicle, standardized to an end-inspiratory transpulmonary pres-

sure of about 15 cm H₂O. Several ragweed test challenges were used to identify the concentration of ragweed that led to a two- to threefold increase in airway resistance for each ragweed-sensitized dog. Each nonsensitized dog received a test challenge using a 3-mg/ml concentration of ragweed to confirm its nonresponsiveness. These concentrations were used for the ragweed challenge following particle exposure.

Pulmonary Resistance

Resistance was measured during and immediately after particle exposure in response to five breaths of nebulized vehicle or ragweed. Transpulmonary pressure was measured by connecting the esophageal catheter to one side of a differential pressure transducer (model MP45, Validyne, Northridge, CA); the other side was connected to a port at the proximal end of the endotracheal tube. Respiratory flow was measured using a pneumotachograph (Fleisch number 1) connected to the endotracheal tube. Outputs from flow and transpulmonary pressure transducers were amplified with a Buxco amplifier (Buxco Electronics, Sharon, CT) as necessary and converted from analog to digital form using appropriate hardware (AT-MIO-16 card, National Instruments, Austin, TX). Custom-designed computer software (LabView 5.1, National Instruments) was used to facilitate integration of the flow signal to yield volume and to derive the total pulmonary resistance. Resistance was measured for 5 min immediately before particle exposure (baseline) and continuously during particle exposure. Pulmonary resistance was recorded for at least 5 min after the vehicle or ragweed challenge or until the peak resistance was clearly observed. Changes in pulmonary resistance are expressed as percent change from baseline.

Bronchoalveolar Lavage

Bronchoalveolar lavage fluid (BALF) was collected as previously described (Collie et al., 1997). BALF was collected at 1 h, day 1, day 4, day 7, and day 11 after the end of particle exposure (Figure 1) in order to assess the degree of inflammation in the lung over time. Briefly, the dogs were sedated with acepromazine (Phoenix Pharmaceutical) subcutaneously (2 mg in 200 μ l). Approximately 30 min later, the dogs were anesthetized with isoflurane. A fiberoptic bronchoscope (5.5 mm OD, model BF-4B2, Olympus, Lake Success, NY) was advanced into the right cardiac lobe and wedged in a segmental bronchus. The segment was lavaged by instillation and withdrawal of five 10-ml aliquots of normal saline (0.9% NaCl) solution. The process was repeated for the left cardiac lobe. For each time point, the same airway segment was lavaged. Cells were separated from BALF by centrifugation at 300 $\times$ g for 10 min at 4°C. The cell pellets were washed with RPMI 1640 (Hyclone, Logan, UT), resuspended in 5 ml of 10% fetal calf serum (Sigma, St. Louis, MO) in RPMI 1640, and counted using a cell counter (model ZBI, Coulter Electronics, Hialeah, FL). Cells were used to prepare cytocentrifuge slides for evaluation of cell differentials. Cytocentrifuge smears were stained using a

modified Wright—Giemsa stain (Diff-Quik, Baxter Healthcare, McGraw Park, IL). The numbers of eosinophils, neutrophils, macrophages, lymphocytes, and mast cells were determined according to standard morphologic criteria (Rebar et al., 1980). A final average was determined by combining numbers from the left and right cardiac lobes. BALF was aliquoted, frozen at -80°C , and assayed for total immunoglobulin (Ig) E and ragweed-specific IgE and IgG at a later time.

Blood Collection

Serum was obtained from whole blood drawn from the jugular vein. Blood was allowed to clot, and serum was collected following centrifugation at $1500 \times g$ for 15 min at 4°C . Serum was stored at -80°C until evaluation of total IgE and ragweed-specific IgE and IgG was carried out. Blood smears were prepared to determine cell differentials in blood. Smears were stained and counted as already described for BALF cells.

Immunoglobulin Analyses

Measurements of serum and BALF total IgE and ragweed-specific IgE and IgG levels were performed by enzyme-linked immunosorbent assay (ELISA) as previously described in detail (Redman et al., 2001). Briefly, for total IgE, 96-well microtiter plates were coated with 100 μl dog serum diluted 1:1000 in 0.1 M sodium carbonate buffer (pH 9.6) or 100 μl undiluted BALF and incubated overnight at 4°C . Plates were washed and blocked for 2 h at room temperature with 0.4% bovine serum albumin in phosphate-buffered saline (PBS). A monoclonal mouse anti-dog IgE (antibody D9, provided by Dr. Doug DeBoer, University of Wisconsin School of Veterinary Medicine, Madison) was then added to each well at a dilution of 1:2000 and incubated for 2 h at 37°C . After washing, 100 μl donkey anti-mouse IgG horseradish peroxidase-conjugated antibody (Jackson Laboratories, Westgrove, PA) was added to each well at a dilution of 1:1000 and incubated for 2 h at 37°C . Plates were washed and then peroxidase substrate (ABTS peroxidase substrate system, Kirkegaard and Perry Laboratories, Gaithersburg, MD) was added to each well and incubated for 30 to 60 min at 37°C . Color development was stopped by adding 5% sodium dodecyl sulfate in water to each well. Plates were read at an optical density (OD) of 405 nm on a microtiter plate reader (Molecular Devices, Sunnyvale, CA). Results are expressed in terms of the actual OD values measured units.

For measurement of ragweed-specific IgG and IgE, 96-well plates were coated with 100 μl of 5- $\mu\text{g}/\text{ml}$ ragweed extract in phosphate-buffered saline (PBS, pH 7.2) and incubated overnight at 4°C . Plates were washed with PBS-0.05% Tween-20 (Sigma) and blocked with 2.5% skim milk at room temperature for 1 h. Samples (100 μl per well) were diluted in 2.5% skim milk and incubated for 1 h at 37°C . After washing, 100 μl goat anti-dog IgG (Kirkegaard and Perry Laboratories), diluted 1:1000, or D9 mouse anti-dog IgE monoclonal antibody, diluted 1:2000 in 2.5% skim milk, was added to each

well, and the plates were incubated for 1 h at 37°C. Plates were washed and either 100 μ l rabbit anti-goat IgG conjugated to horseradish peroxidase (for detecting dog ragweed-specific IgG; Kirkegaard and Perry Laboratories) or donkey anti-mouse IgG conjugated to horseradish peroxidase (for detecting dog ragweed-specific IgE; Jackson Laboratories), diluted 1:2000 in 2.5% skim milk, was added to each well. Plates were then incubated for 1 h at 37°C and washed. Peroxidase substrate was added to each well and color development was detected as described earlier for total IgE.

Statistical Analyses

Data are expressed as mean $\pm$ SEM. All data sets were evaluated using repeated measures analysis of one-way analysis of variance with Tukey multiple comparison test to compare groups using GraphPad Prism version 3.0 software (GraphPad Prism Software, San Diego, CA). A value of $p < .05$ was considered significant.

RESULTS

Pulmonary Resistance

No changes in pulmonary resistance from baseline levels were recorded during particle exposure for either sensitized (allergic) or nonsensitized (nonallergic) dogs (Figure 2). Vehicle challenge served as a negative control and had

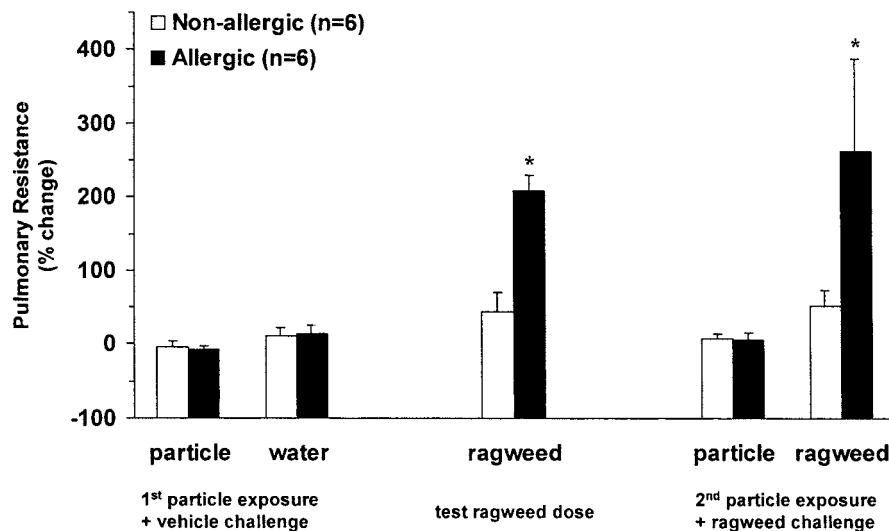


FIGURE 2. Measurement of pulmonary resistance during ultrafine particle exposure (particle) and water or ragweed challenge immediately thereafter. Data are presented as percent change from baseline (mean $\pm$ SE) where the baseline response is set as zero. Since there was no observable change in pulmonary resistance at any time during particle exposure, data are presented as the 1-h average. Asterisk indicates significant at $p < .05$ allergic versus nonallergic dogs.

no effect on pulmonary resistance. Baseline responses to ragweed challenge were carried out to identify a dose of ragweed that induced at least a 200% change in resistance for each allergic dog. These challenges confirmed that allergic dogs developed increased pulmonary resistance to ragweed challenge whereas nonallergic dogs did not. Preexposure to particles exposure did not alter ragweed-induced pulmonary resistance in allergic dogs. Baseline R_L levels in allergic and nonallergic animals were not significantly different.

Systemic Immune Response

Total and ragweed-specific IgE levels in the serum were significantly higher at all time points in allergic dogs than in nonallergic dogs (Figure 3, A and B). Ragweed-specific IgG levels in the serum were significantly higher in allergic dogs than in nonallergic dogs at all time points prior to ragweed challenge (Figure 3C). However, following ragweed challenge, nonallergic dogs developed ragweed-specific IgG levels nearly equivalent to allergic dogs. Ragweed exposure did not significantly increase total IgE or ragweed-specific IgE and IgG above initial baseline levels in allergic dogs. Particle exposure did not affect serum antibody levels at any time point in either allergic or nonallergic dogs. In addition, no differences in the number of or percentage of blood monocytes, lymphocytes, eosinophils, or neutrophils were observed at baseline or following any treatment in allergic or nonallergic dogs (data not shown).

Pulmonary Immune Response

Total IgE levels in BALF were significantly higher in allergic dogs than in nonallergic dogs (Figure 4A), similar to the findings in serum. In contrast to serum, ragweed-specific IgG levels in BALF from nonallergic dogs did not increase after ragweed challenge to the levels observed in allergic dogs (Figure 4B). Ultrafine particle exposure did not alter antibody levels in BALF. The levels of ragweed-specific IgE in BALF were below the level of detection for the ELISA assay.

The percentage of eosinophils in BALF from allergic dogs tended to be higher at baseline and all time points after treatment than those in nonallergic dogs, but the differences were not statistically significant (Table 1). Neither particle exposure nor subsequent vehicle or ragweed challenge significantly changed eosinophil numbers from baseline levels in allergic or nonallergic dogs. There was no significant difference in the number of neutrophils at baseline in the BALF of allergic and nonallergic dogs (Table 1). Neutrophil numbers were increased in allergic and nonallergic dogs 1 day following particle exposure. However, due to the large interanimal variability, statistical significance above baseline for day-1 neutrophils could only be achieved in the nonallergic dogs challenged with ragweed. In contrast, the percentage of neutrophils in BALF were significantly increased 1 day after particle exposure in all dogs (Table 1). No differences in the percentage of neutrophils were observed between vehicle- and ragweed-challenged dogs.

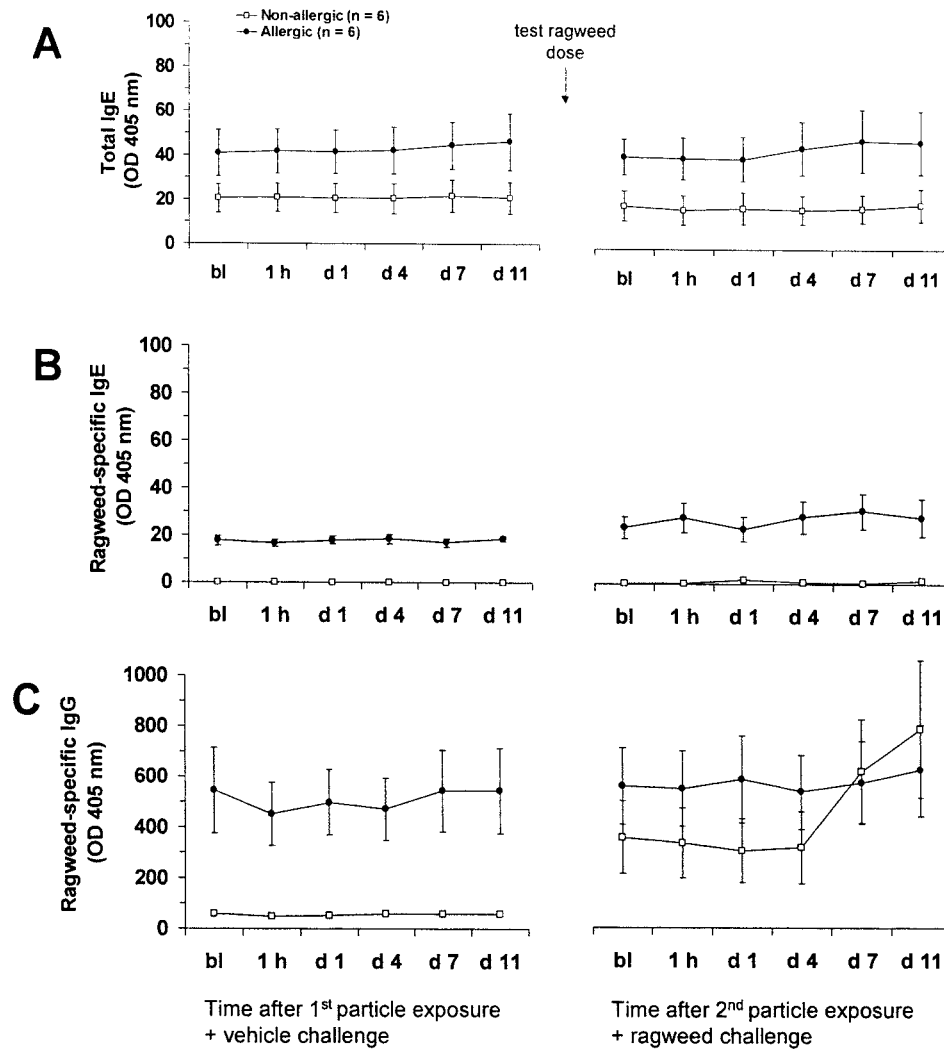


FIGURE 3. Levels of serum total IgE (A), ragweed-specific IgE (B), and ragweed-specific IgG (C) before and after particle exposure. Antibody levels were significantly ($p < .05$) higher in allergic versus non-allergic dogs at all time points except for ragweed-specific IgG following ragweed challenge.

DISCUSSION

We have shown previously that the dogs used in this study developed allergic sensitivity to ragweed and when challenged by inhalation to ragweed develop increased pulmonary resistance and allergic inflammation (Redman et al., 2001). In the present study, we show that a 1-h exposure to ultrafine carbon particles did not increase pulmonary resistance above baseline levels during exposure or immediately thereafter following challenge

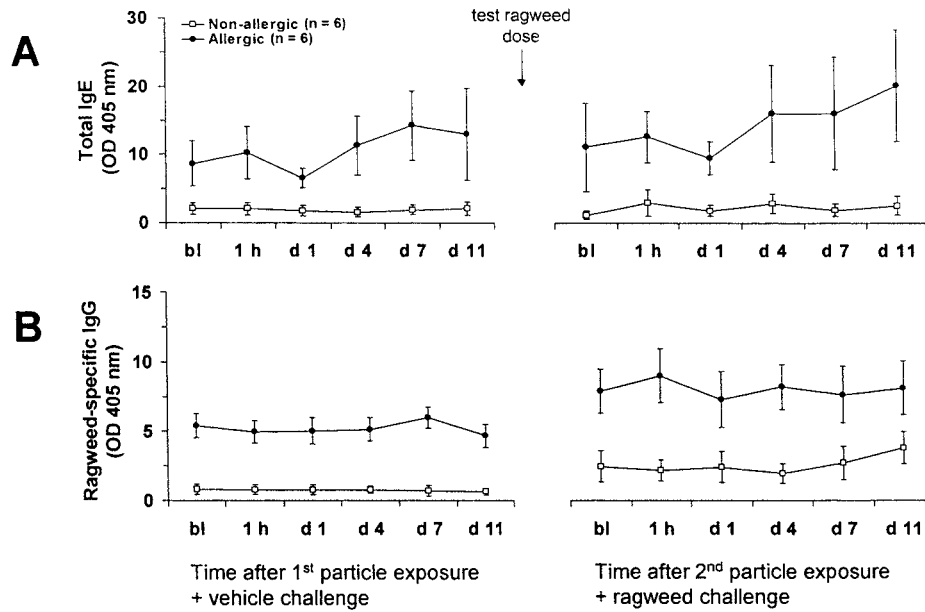


FIGURE 4. Levels of bronchoalveolar lavage fluid total IgE (A) and ragweed-specific IgG (B) before and after particle exposure. Antibody levels were significantly ($p < .05$) higher in allergic versus nonallergic dogs at all time points.

TABLE 1. Bronchoalveolar lavage cell findings following exposure

	Neutrophils (%)		Eosinophils (%)	
	Nonallergic	Allergic	Nonallergic	Allergic
Particle + vehicle				
Baseline	1.96 ± 0.12	5.77 ± 2.41	4.21 ± 1.55	9.65 ± 2.81
1 h	8.67 ± 5.91	10.89 ± 3.27	4.52 ± 1.54	13.63 ± 3.55
Day 1	27.08 ± 11.71 ^a	38.38 ± 8.36 ^b	2.83 ± 1.65	10.31 ± 3.52
Day 4	9.27 ± 4.25	8.25 ± 1.89	4.42 ± 2.28	18.15 ± 5.87
Day 7	4.85 ± 1.47	16.63 ± 7.80	3.63 ± 1.44	16.71 ± 8.89
Day 11	3.25 ± 0.70	4.58 ± 1.55	2.67 ± 1.33	21.31 ± 9.36
Particle + ragweed				
Baseline	7.50 ± 6.13	1.94 ± 0.48	7.40 ± 4.22	23.75 ± 6.85
1 h	5.35 ± 1.61	12.59 ± 5.9	4.21 ± 1.31	10.92 ± 2.74
Day 1	43.52 ± 7.30 ^c	39.04 ± 9.77 ^c	2.33 ± 0.72	15.04 ± 5.76
Day 4	10.53 ± 3.01	11.81 ± 2.10	6.36 ± 2.22	17.58 ± 5.4
Day 7	5.04 ± 0.89	5.21 ± 0.38	4.56 ± 1.23	17.27 ± 5.56
Day 11	6.92 ± 2.53	6.10 ± 0.78	6.27 ± 3.25	20.29 ± 6.61

^aSignificant at $p < .05$ vs. baseline.

^bSignificant at $p < .01$ vs. baseline.

^cSignificant at $p < .001$ vs. baseline.

with vehicle or ragweed in either allergic or nonallergic dogs. Although serum ragweed-specific IgG levels in nonallergic dogs were elevated, this was likely in response to ragweed exposure and not particle exposure. We have previously observed an increase in serum IgG levels in nonsensitized dogs or mice that have been repeatedly exposed to allergen (Barrett et al., 2002; Redman et al., 2001). The increase in IgG alone did not lead to allergic airway responses in either species. Allergic dogs maintained elevated antibody levels in comparison to nonallergic dogs, but the levels were not altered by particle or ragweed treatment. The only measurable difference in any of the endpoints was an increase in BALF neutrophils 1 day after particle exposure in both allergic and nonallergic dogs. However, the increase in neutrophils may have been due to the instillation-lavage procedures rather than the particle exposure. We have found that instillation of as little as 1.5 ml of saline into an airway segment will lead to a transient increase in BALF neutrophils in that segment 1 day after instillation in either allergic or nonallergic dogs (Rudolph et al., 2001). Thus, instillation of 5×10 ml saline washes for the 1-h lavage time point likely caused the transient increase in neutrophils measured one day later.

The lack of lung inflammation due to ultrafine carbon exposure in nonallergic dogs is not surprising in light of recent evidence from rodent studies. Exposure of healthy, young and old mice and rats to ultrafine carbon or platinum particles at approximately $100 \mu\text{g}/\text{m}^3$ for 6 h had little or no effect on

Lymphocytes (%)		Macrophages (%)		Total cells ($\times 10^6/\text{ml}$)	
Nonallergic	Allergic	Nonallergic	Allergic	Nonallergic	Allergic
8.67 $\pm$ 2.64	7.31 $\pm$ 0.51	84.77 $\pm$ 2.64	77.31 $\pm$ 5.46	2.97 $\pm$ 0.42	4.78 $\pm$ 1.89
6.44 $\pm$ 0.26	7.66 $\pm$ 0.99	79.60 $\pm$ 0.26	66.61 $\pm$ 4.93	4.07 $\pm$ 1.31	4.53 $\pm$ 1.25
5.77 $\pm$ 0.53	7.65 $\pm$ 0.82	64.00 $\pm$ 0.53	43.29 $\pm$ 7.40 ^a	7.35 $\pm$ 4.12	7.85 $\pm$ 3.17
11.27 $\pm$ 1.08	12.27 $\pm$ 0.71	74.42 $\pm$ 1.08	60.67 $\pm$ 5.35	3.48 $\pm$ 0.88	4.16 $\pm$ 1.33
8.65 $\pm$ 1.29	8.29 $\pm$ 0.62	82.15 $\pm$ 1.29	57.85 $\pm$ 8.15	3.07 $\pm$ 0.76	8.16 $\pm$ 2.93
8.35 $\pm$ 1.71	11.19 $\pm$ 1.28	85.02 $\pm$ 1.71	62.50 $\pm$ 8.64	3.40 $\pm$ 0.83	6.65 $\pm$ 4.06
5.31 $\pm$ 1.58	9.10 $\pm$ 1.20	79.52 $\pm$ 6.12	64.23 $\pm$ 7.51	2.96 $\pm$ 0.69	2.74 $\pm$ 0.60
6.38 $\pm$ 1.65	9.19 $\pm$ 1.33	82.92 $\pm$ 2.48	64.02 $\pm$ 5.17	3.73 $\pm$ 0.71	3.50 $\pm$ 0.52
6.52 $\pm$ 1.11	6.69 $\pm$ 1.36	47.02 $\pm$ 7.02 ^b	38.94 $\pm$ 7.73	5.98 $\pm$ 0.77	9.07 $\pm$ 4.84
10.08 $\pm$ 1.08	8.52 $\pm$ 1.34	73.36 $\pm$ 4.80	61.15 $\pm$ 4.62	2.73 $\pm$ 0.31	4.24 $\pm$ 0.27
8.98 $\pm$ 1.17	7.44 $\pm$ 1.28	80.60 $\pm$ 3.10	69.94 $\pm$ 6.47	2.09 $\pm$ 0.24	2.30 $\pm$ 0.51
9.54 $\pm$ 1.51	10.42 $\pm$ 1.21	77.00 $\pm$ 5.24	62.83 $\pm$ 5.66	2.64 $\pm$ 0.56	3.25 $\pm$ 0.31

markers of inflammation such as BALF neutrophils, cytokine levels, or reactive oxygen species production (Oberdörster et al., 2000). Studies using healthy animals in which adverse health effects were observed have been performed at higher ultrafine particle concentrations than found in ambient air or with environmentally irrelevant particles such as Teflon (Donaldson et al., 1998; Johnston et al., 2000; Oberdörster et al., 1990, 1992, 1995).

In contrast, some rat and mouse models with preexisting lung inflammation or disease have exhibited slightly enhanced lung inflammation following ultrafine particle exposure at lower particle concentrations. Inhaled ultrafine carbon particles ($100 \mu\text{g}/\text{m}^3$) induced a small inflammatory response (e.g., BALF neutrophils) over background lung inflammation in young rats preexposed to endotoxin, and ultrafine carbon and platinum particles induced a small inflammatory response in young and old elastase-treated mice (Oberdörster et al., 2000). Interestingly, ultrafine carbon particles had little or no effect on inflammation in old rats pretreated with endotoxin and old Tsk mice with or without endotoxin pretreatment (Oberdörster et al., 2000). In the present study, we did not observe enhanced lung inflammation due to ultrafine particle exposure in allergic dogs, even when they were challenged with ragweed.

The work of Oberdörster et al. (2000) suggests that an active inflammatory response in the lungs at the time of ultrafine particle exposure may be necessary in order to observe enhanced lung inflammation due to particle exposure. Thus, lack of a response to ultrafine particles in the allergic dogs in this study may have been due to the timing and dose of ragweed challenge. Although the dose of ragweed given to allergic dogs in this study induced bronchoconstriction, it did not induce inflammation in the lung. A higher dose of ragweed that induces significant lung eosinophilia and is given prior to ultrafine particle challenge may make the allergic dogs more susceptible to particle-induced effects. Studies in human asthmatics have shown that decreased PEF in association with ultrafine particle exposure has a maximal effect when a 5-day exposure average is used, compared to a 24-h average, which shows no effect (Penttinen et al., 2001; Peters et al., 1997). This suggests that repeated exposures to ultrafine particles over several days may also induce pulmonary inflammation and/or bronchoconstriction in allergic dogs.

An estimate of the human exposure concentration represented by that used in the present study ($\sim 250 \mu\text{g}/\text{m}^3$) can be made using the National Council on Radiation Protection (NCRP, 1997) human deposition model. That model predicts an alveolar deposition fraction for a human with a minute ventilation of 9.24 L/min is 0.30 for 35-nm particles. At an alveolar surface area of 102 m^2 (Stone et al., 1992) and a 1-h exposure to $10 \mu\text{g}/\text{m}^3$, the predicted deposited dose would be $\sim 16 \text{ ng}/\text{m}^2$ of alveolar surface in the human lung. Based on data from multiple studies, the alveolar deposition fraction of 35-nm particles is estimated at 0.25 in the dog lung (Schlesinger, 1985). At an alveolar surface area of 52 m^2 (Stone et al., 1992), 4.0 L/min minute ventilation, and similar exposure conditions, the deposited dose

would be $\sim 11 \text{ ng/m}^2$ alveolar surface in the dog lung. Thus, a dog exposure concentration of $250 \text{ }\mu\text{g/m}^3$ ($\sim 6 \times 10^6$ particles/ cm^3) of ultrafine particles may be approximately equivalent to a mean exposure concentration of about $170 \text{ }\mu\text{g/m}^3$ ($\sim 4 \times 10^6$ particles/ cm^3) for human exposures. Recent measurements indicate that ultrafine particle numbers in ambient air range from 1 to $5 \times 10^4/\text{cm}^3$ (Hughes et al., 1998; Pekkanen et al., 1997; Penttinen et al., 2001; Peters et al., 1997). Thus, particle exposure concentrations in the present study were approximately 100-fold higher than what humans might encounter in the ambient environment.

Ultrafine carbon particles were used in the present studies with the realization that ambient ultrafine particles are physically and chemically diverse. Thus, results of the present study may not represent effects, or lack thereof, of other types of ultrafine particles, although the ultrafine particles generated in this study contained about 40–70% organic carbon, which makes them probably more relevant to ambient ultrafine particles than pure elemental carbon particles (McDonald et al., 2001). In conclusion, the results of this study do not support the hypothesis that ultrafine carbon particle exposure exacerbates asthma symptoms. However, other allergen doses and timing, duration of particle exposure, and species of particles may produce different results.

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