Effects of ultrafine carbon particle inhalation on allergic inflammation of the lung

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Background: Epidemiologic studies show that exposure to particulate air pollution is associated with asthma exacerbation. Ultrafine particles (diameter <100 nm) may contribute to these adverse effects.

Objective: To investigate potential adjuvant activity of inhaled elemental carbon ultrafine particles (EC-UFPs) on allergic airway inflammation.

Methods: The effects of ultrafine particle inhalation on allergic airway inflammation was analyzed in ovalbumin-sensitized mice and nonsensitized controls. Particle exposure (526 μg/m^3, 24 hours) was performed 24, 96, or 168 hours before or 24 or 72 hours after ovalbumin aerosol challenge. Allergic inflammation was analyzed at different time points after allergen challenge by means of bronchoalveolar lavage cell count and cytokine/total protein assays, lung histology, and airway hyperresponsiveness.

Results: In sensitized mice, inhalation of ultrafine particles 24 hours before allergen challenge caused a significant increase of bronchoalveolar lavage inflammatory cell infiltrate, protein, IL-4, IL-5, and IL-13 compared with relevant controls. These adjuvant effects were dose- and time-dependent and were still present when particle exposure was performed 4 days before allergen challenge. The adjuvant effect of ultrafine particles was also documented by increased mucus production, peribronchiolar and perivascular inflammation, and enhanced airway hyperresponsiveness. In contrast, particle exposure in sensitized mice after allergen challenge caused only moderate effects, such as a delay of inflammatory infiltrate and a reduction of cytokines in bronchoalveolar lavage fluid.

Conclusion: Exposure to ultrafine carbon particles before allergen challenge exerts strong adjuvant effects on the manifestation of allergic airway inflammation. Allergen-sensitized individuals may therefore be more susceptible to detrimental health effects of ultrafine particles. (J Allergy Clin Immunol 2006;117:824-30.)

Key words: *Particulate matter, elemental carbon ultrafine particles, allergic inflammation*

Several epidemiologic and clinical studies have shown an association between increased ambient air particle concentration and adverse respiratory and cardiovascular health effects, leading to enhanced mortality rates as well as to exacerbations of respiratory morbidity.[1-5] Ultrafine particles (UFPs, less than 0.1 μm in aerodynamic diameter) may contribute to the health effects of particulate matter (PM) for several reasons. UFPs are characterized by a high number concentration, low mass concentration, and a big surface area.[6] Compared with larger particles, they have a higher deposition rate in the peripheral lung, can cross the pulmonary epithelium to reach the interstitium,[7] and have enhanced capability to produce reactive oxygen species.[8-10] UFP originate mainly from incomplete combustion processes by road traffic emission and industry. Although only few studies have measured the number concentration of UFPs in ambient air, there is some evidence that, over the last decades, the UFP number concentration is on the rise.[11]

Asthma is a disease characterized by periodic airflow limitation, airway inflammation, and airway hyperresponsiveness (AHR).[12] Evidence on the effects of particulate air pollution on asthma exacerbation and hospital admissions is increasing.[5,13-18] A panel study on subjects with asthma found that UFP number concentration correlated closely with alterations in lung function[19]; furthermore, variations in the concentration of fine particles (PM 2.5) and UFP correlated with use of asthma medications.[20] These studies suggest that people with allergic asthma are more susceptible to the short-term acute effects of fine and ultrafine particle exposures.[21] Solid experimental studies addressing this hypothesis, however, are still lacking.

Several studies in mice have analyzed the effects of fine particles on the allergic sensitization phase[22-28] and have demonstrated that PM influences cytokine production, enhances specific IgE response, and modulates immunoglobulin-isotype switching. In particular, carbon black, which resembles the carbonaceous core of diesel exhaust, has been shown to enhance allergic sensitization[24-26,28] and stimulate both T_H1 and T_H2 responses.[29]

In contrast, there are few experimental data available on the effects of fine particles and UFPs on the elicitation phase of the allergic response.[30-32] Elemental carbon (EC)-UFPs are relatively inert particles, yet they represent a major component of urban airborne PMs.[33] In the current

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Abbreviations used
AHR: Airway hyperresponsiveness
BAL: Bronchoalveolar lavage
BALF: Bronchoalveolar lavage fluid
EC: Elemental carbon
NS: Nonsensitized
OVA: Ovalbumin
Penh: Enhanced pause
PM: Particulate matter
S: Sensitized
UFP: Ultrafine particle

study, we thus investigated in sensitized mice the effects of EC-UFP inhalations at various time points before and after allergen challenge on manifestations of allergic airway inflammation, such as bronchoalveolar lavage (BAL) cellular infiltrate, local cytokine production, mucus hypersecretion, and pulmonary function after methacholine challenge.

METHODS

Animals and materials

Balb/c mice 5 to 7 weeks old (Charles River, Sulzfeld, Germany) were housed in individually ventilated cages (VentiRack, cage type CU-31; Biozone, Margate, United Kingdom) and received a standard pellet diet and water ad libitum. The study was conducted under federal guidelines for the use and care of laboratory animals and was approved by the Government of the District of Upper Bavaria and the Animal Care and Use Committee of the GSF Research Center. All chemicals were purchased from Sigma-Aldrich Chemie (Deisenhofen, Germany) unless otherwise specified.

LPS depletion and measurement

To minimize LPS contamination, the ovalbumin solution prepared for aerosol challenges was eluted over polymyxin B columns (Endotoxin Detoxi-Gel; Pierce Chemical Co, Rockfort, Ill) according to the manufacturer's instructions. LPS concentration after depletion was <0.002 EU/μg protein, as determined by limulus amebocyte lysate assay (Cambrex Bio Science, Apen, Germany). The amount of LPS deposited in the airways during ovalbumin challenge was calculated to be less than 10 pg/mouse.

Allergen sensitization/challenge protocol

Mice were sensitized by repetitive intraperitoneal injections of 1 μg ovalbumin (grade VI; Sigma-Aldrich Chemie)/alum (2.5 mg; Pierce Chemical Co) in PBS on days 0, 14, 28, 48, and 72. Blood samples were taken before and after sensitization. Ovalbumin-specific IgE and IgG_1 were measured in plasma samples by ELISA as described previously.[34] Ovalbumin/alum sensitized mice (day 80) were characterized by high titers of ovalbumin-specific IgE compared with nonsensitized controls (632.7 ± 92.4 vs <2 arbitrary units/mL) and ovalbumin-specific IgG_1 (1277.57 ± 167.2 vs <0.1 μg/mL). On day 86, the mice were aerosol-challenged for 20 minutes with 1% ovalbumin in PBS or with PBS alone delivered by Pari-Boy nebulizer (Pari, Starnberg, Germany).

EC-UFP production, characterization, and exposure

Elemental carbon ultrafine particles were generated by electric spark discharge (Palas model GFG1000; Karlsruhe, Germany) using agglomerated carbon particles as previously described.[35] EC-UFP size distributions were characterized by a count median diameter of 34.8 ± 0.5 nm with a geometric SD of 1.48 ± 0.09 and a volume median diameter of 53.1 ± 13.4 nm with a geometric SD of 1.46 ± 0.17. The UFPs were analyzed for organic carbon content using a standard thermo-optical desorption method.[36] When the chemical nature of the thermally desorbed gases was investigated with a gas chromatograph mass spectrometer, less than 2% of the desorbed mass was identified as organic.

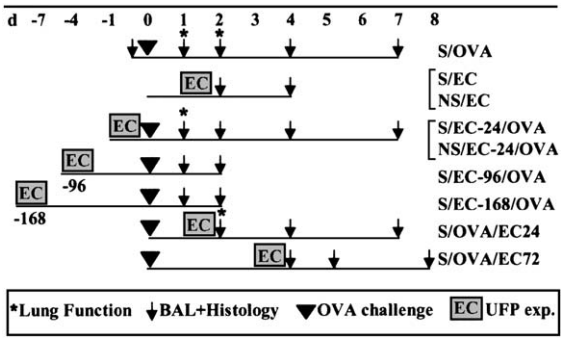

FIG 1. Experimental groups *(left)* and designation *(right). EC,* 24 hours exposure to elemental carbon UFP (526 μg/m^3); ▼, ovalbumin aerosol challenge; ↓, sacrifice, BAL, and histology; *, lung function tests. EC-24, EC-96, EC-168, EC24, and EC72 indicate time points when EC exposure was initiated in reference to ovalbumin challenge. *UFP exp.*, UFP exposure.

Animals were housed in 330-L exposure chambers with a horizontal displacement flow of 100 L/min (18.2 exchanges of the chamber air/h). EC-UFP exposures were performed for 24 hours with a mass concentration of 526 ± 47 μg/m^3, which corresponds to a particle number concentration of 9.3 ± 0.9 × 10^6/cm^3. For the dose-response study, particle mass concentrations of 332 and 119 μg/m^3 were obtained by bypassing the corresponding generator output flux via a venturi nozzle into the exhaust line.

Study design

A schematic representation of the study protocol is shown in Fig 1. BAL analysis was performed at 0, 24, 48, 96, or 168 hours after ovalbumin challenge (n = 4/time point), and lung function tests were performed 24 and 48 hours after ovalbumin challenge (n = 6/time point).

The effect of EC-UFP inhalation on allergic airway inflammation was analyzed by using particle exposure with 526 μg/m^3 EC-UFP for 24 hours, unless otherwise specified. Sensitized mice were exposed to EC-UFP 24 hours before ovalbumin challenge (S/EC-24/OVA group) and were analyzed 24, 48, 96, or 168 hours after challenge (n = 4/time point). Lung function tests were performed 24 hours after ovalbumin challenge (n = 6). To evaluate how long EC-UFP exposure has an effect on subsequent allergen-induced airway inflammation, sensitized mice were exposed to EC-UFP 96 hours (S/EC-96/OVA) or 168 hours (S/EC-168/OVA) before OVA challenge and were analyzed 24 and 48 hours after allergen challenge (n = 4/time point). To analyze the effect of EC-UFP exposure on an ongoing allergic inflammation, sensitized mice were first challenged with ovalbumin, subsequently (24 or 72 hours later) exposed to EC-UFP (S/OVA/EC24 and S/OVA/EC72), and analyzed at 3 later time points (48, 96, 168 hours for S/OVA/EC24; and 96, 120, 192 hours after ovalbumin challenge for S/OVA/EC72, n = 4/time point).

The following experimental groups served as controls: (1) ovalbumin-sensitized and ovalbumin-challenged mice exposed to filtered air (S/OVA group), (2) ovalbumin-sensitized mice exposed to

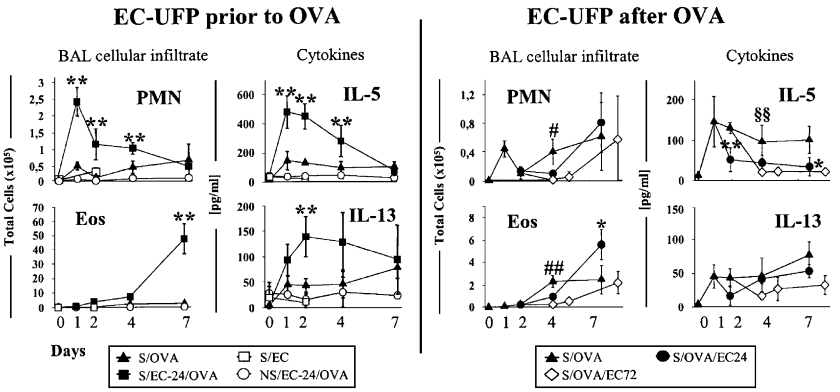

FIG 2. Bronchoalveolar lavage cells and cytokines. EC-UFP exposure 24 hours before ovalbumin challenge *(left)* in sensitized *(S/EC-24/OVA)* and nonsensitized mice *(NS/EC-24/OVA)*. Sensitized and challenged mice exposed to filtered air *(S/OVA)* and sensitized mice exposed to EC-UFP only *(S/EC)* served as controls. EC-UFP exposures 24 hours *(S/OVA/EC24)* or 72 hours *(S/OVA/EC72)* after ovalbumin challenge *(right)*. Mean ± SD (n = 4/group). **P* < .05, ***P* < .01 versus S/OVA; #*P* < .05, ##*P* < .01 versus S/OVA/EC24, S/OVA/EC72; §§*P* < .01 versus S/OVA/EC72. *PMN*, Polymorphonuclear granulocytes; *Eos*, eosinophils.

EC-UFP for 24 hours without subsequent ovalbumin challenge (S/EC group), (3) nonsensitized mice exposed to EC-UFP 24 hours before ovalbumin challenge (NS/EC-24/OVA), and (4) nonsensitized, nonchallenged mice exposed to EC-UFP (NS/EC). Lung function tests were performed in untreated animals (n = 12) as baseline control and 24 hours after EC-UFP inhalation in the NS/EC group (n = 6).

BAL analysis

Airways were lavaged 5 times with 0.8 mL PBS. Aliquots of cell-free BAL fluid were assayed in duplicate for total protein (Coomassie Protein Assay; Pierce Chemical Co) and for IL-4, IL-5, IL-13 and IFN-γ by 2 site ELISAs using antibodies from BD Biosciences (Heidelberg, Germany) and BioSource (IL-13; Camarillo, Calif) as suggested by the manufacturer. Viability and yield of BAL cells were quantified via trypan blue (Sigma-Aldrich Chemie) exclusion in a hemocytometer. Differential BAL cell count (400 cells/sample) was performed on cytospins (600 rpm for 10 minutes) fixed and stained with Diff-Quick (Dade Behring, Marburg, Germany).

Histological evaluation

After BAL, the lungs were removed and fixed in 10% buffered formalin. The left lobe and the right caudal lobe were used for histopathology. After paraffin embedding, 5-μm sections were stained with hematoxylin-eosin and periodic acid–Schiff. To confirm the presence of eosinophils in the inflammatory infiltrate, a specific eosinophil staining (Luna stain, as described by Mehlhop et al[37]) was used. Mucus hypersecretion and inflammatory cell infiltration were graded on a scale from 0 to 4 in a blind fashion as recently described.[38]

Lung function tests

Breathing patterns were assessed in unrestrained animals using whole-body plethysmography (Buxco Electronics, Sharon, Conn) as described by Drorbaugh and Fenn.[39] The enhanced pause (Penh), determined before and after methacholine exposure, was applied as an index of airway hyperresponsiveness.[40] After an adaptation period of 15 minutes, baseline values were measured for 5 minutes, followed by a 4-minute inhalation of PBS (negative control) and measurement of Penh during 3 minutes thereafter. Increasing doses of methacholine aerosol (M1-M4) were then delivered to the mice: 10 mg/mL methacholine nebulized for 1, 2, and 4 minutes (M1-M3), followed by 40 mg/mL methacholine for 2 minutes (M4). A data recording interval of 3 minutes was introduced after each methacholine level. The mean of the Penh values determined in the 2nd and 3rd minute was used for quantifying AHR.

Statistical analysis

Data are expressed as means ± SDs. For statistical evaluation, 1-way ANOVA with post hoc Scheffé comparisons was used. A P value < .05 was considered significant.

RESULTS

EC-UFP inhalation before allergen challenge augments allergic airway inflammation

In sensitized mice, EC-UFP inhalation 24 hours before ovalbumin challenge (S/EC-24/OVA) caused an extensive increase of BAL inflammatory infiltrate compared with sensitized and challenged mice (S/OVA) exposed to clean air (Fig 2). The enhanced response was characterized by an early boost of the neutrophil peak and increased numbers of eosinophils (as well as macrophages and lymphocytes; see this article's Table E1 in the Online Repository at www.jacionline.org) at later time points. In contrast, exposure of sensitized mice to EC-UFP 24 hours or 72 hours after allergen challenge showed only minor effects, mostly leading to a delayed onset of the allergic airway inflammation, characterized by a decrease of the cellular infiltrate as long as 96 hours after allergen challenge and a slight augmentation of cellular infiltrate at later time points (Fig 2; see this article's Table E1 in the Online Repository at www.jacionline.org). In the absence of ovalbumin challenge, sensitized mice did not develop a BAL inflammatory infiltrate (data not shown), nor did sensitized mice exposed to EC-UFP only (S/EC). Also, no inflammatory response was shown in exposures to EC-UFP 24 hours before allergen challenge in nonsensitized mice (NS/EC-24/OVA; Fig 2).

A similar pattern emerged when protein and cytokine concentrations were analyzed in BAL fluid (BALF). EC-UFP inhalation 24 hours before ovalbumin challenge (S/EC-24/OVA) caused a marked increase in IL-5 and

IL-13 over prolonged periods (Fig 2) compared with sensitized and challenged mice (S/OVA) that had not inhaled EC-UFP. Similar effects were observed when IL-4 and total protein levels were analyzed (see this article's Table E1 in the Online Repository at www.jacionline.org). In contrast, exposure to EC-UFP 24 and 72 hours after ovalbumin challenge led to minor downmodulation of BALF cytokines, which in the case of IL-5 reached statistical significance (Fig 2). No significant alterations in the IFN-γ concentration were observed in any of the groups (data not shown). No induction of cytokines and protein levels was observed in any of the control groups (S/EC and NS/EC-24/OVA; Fig 2).

Adjuvant effects of EC-UFP inhalation are dose- and time-dependent

Dose dependency. Sensitized mice were exposed to different concentrations of EC-UFP (119-526 μg/m^3) 24 hours before allergen challenge. Even at the lowest concentration tested (119 μg/m^3), EC-UFP inhalation induced a moderate increase in BAL inflammatory cells (S/EC119/OVA) compared with controls not exposed to EC-UFP (S/OVA; Fig 3, *A*). Increasing the EC-UFP exposure led to a dose-dependent increase of BAL cellular infiltrate (S/EC332/OVA and S/EC526/OVA). Similarly, EC-UFP effects on BALF cytokine and protein levels were found to be dose-dependent (data not shown).

Time dependency. Sensitized mice were exposed to EC-UFP 24 hours (S/EC-24/OVA), 96 hours (S/EC-96/OVA), or 168 hours (S/EC-168/OVA) before allergen challenge. Inhalation of EC-UFP 4 days before allergen challenge still had a significant adjuvant effect on the BAL cellular infiltrate (Fig 3, *B*), whereas inhalation 1 week before allergen challenge only induced minor changes that did not reach statistical significance.

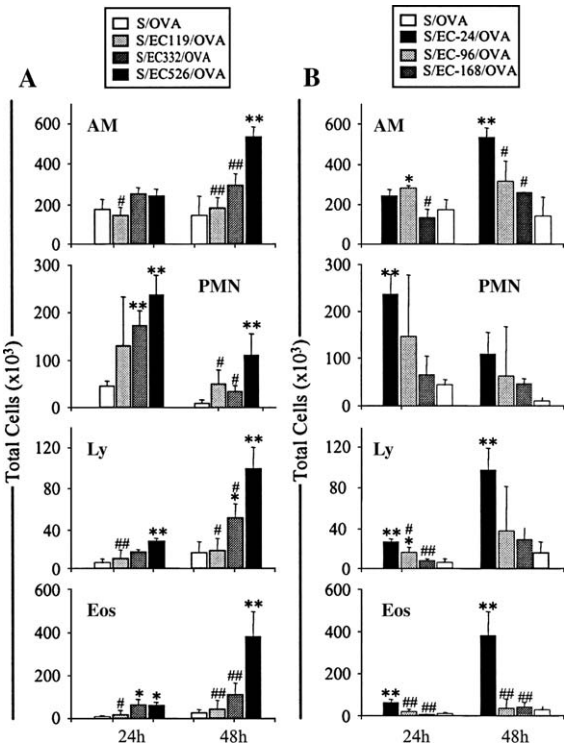

FIG 3. Dose and time response. **A,** EC-UFP exposures with decreasing concentrations (526, 332, or 119 μg/m^3) 24 hours before ovalbumin challenge. **B,** EC-UFP exposure (526 μg/m^3) performed 24 hours *(S/EC-24/OVA)*, 96 hours *(S/EC-96/OVA)*, or 168 hours *(S/EC-168/OVA)* before ovalbumin challenge. Sensitized and challenged mice exposed to filtered air served as controls *(S/OVA)*. Mean ± SD (n = 4/group). *P < .05, **P < .01 versus S/OVA; #P < .05, ##P < .01 versus S/EC-24/OVA. *AM,* Alveolar macrophages; *Ly,* lymphocytes; *PMN*, polymorphonuclear granulocytes; *Eos*, eosinophils.

EC-UFP before OVA challenge enhances lung allergic inflammatory infiltrate and mucus production

Elemental carbon ultrafine particle exposure in both sensitized and nonsensitized mice had minor effects on lung histopathology (Fig 4, *A, B*; see this article's Table E2 in the Online Repository at www.jacionline.org). In sensitized mice, ovalbumin challenge evoked a moderate inflammatory infiltrate and mucus production (S/OVA; Fig 4, *C*; see this article's Table E1 in the Online Repository at www.jacionline.org). EC-UFP exposure before ovalbumin challenge caused an increase in mucus production and in inflammatory infiltrate compared with all controls, as scored in Table E1 (see the Online Repository at www.jacionline.org) and shown in Fig 4, *D* (S/EC-24/OVA). EC-UFP after allergen challenge led to slightly lower inflammatory infiltrate scores (data not shown).

EC-UFP before ovalbumin challenge increases AHR

In ovalbumin-sensitized animals, lung function tests performed 24 hours after ovalbumin challenge showed an increase in Penh after increasing methacholine concentrations (S/OVA; Fig 5). EC-UFP inhalation 24 hours before ovalbumin challenge caused a significantly stronger increase in Penh (S/EC-24/OVA; P < .01 vs untreated, P < .05 vs S/OVA; Fig 5). Exposure to EC-UFP in the absence of allergen challenge (S/EC) caused a moderately increased baseline Penh, which shifted the first half of the curve upward (P < .01 vs untreated animals), but the slope of the curve, which represent the increase in Penh, was comparable with the one obtained in untreated animals. Additional lung function tests of ovalbumin-sensitized and challenged mice (S/OVA; n = 6) and ovalbumin-sensitized mice exposed to EC-UFP 24 hours after ovalbumin challenge (S/OVA/EC24; n = 6) were performed 48 hours after ovalbumin challenge and showed no significant alteration of mean Penh after increasing methacholine provocation (data not shown).

DISCUSSION

Epidemiologic and experimental studies suggest that anthropogenic air pollutants, in particular PMs, are

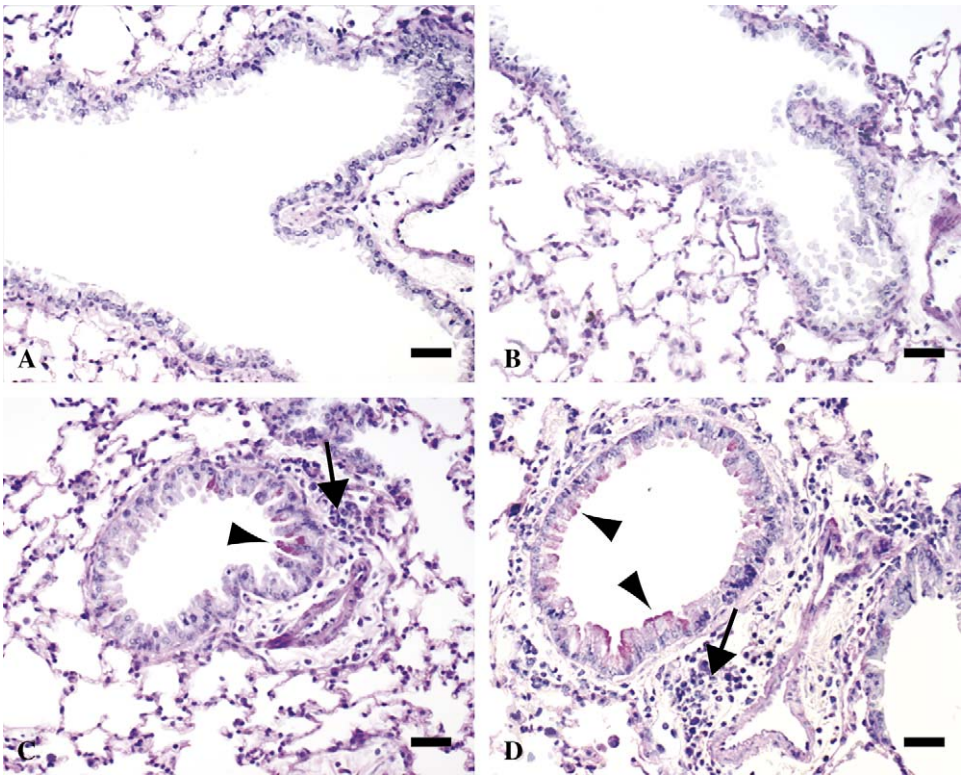

FIG 4. Lung histology 48 hours after ovalbumin challenge (periodic acid–Schiff staining). **A,** Nonsensitized mice exposed to EC-UFP 24 hours before ovalbumin challenge *(NS/EC-24/OVA)*. **B,** Sensitized mice exposed to EC-UFP *(S/EC)*. **C,** Sensitized and challenged mice exposed to filtered air *(S/OVA)*. **D,** Sensitized mice exposed to EC-UFP 24 hours before ovalbumin challenge *(S/EC-24/OVA)*. *Arrows,* Inflammatory infiltrate; *arrowheads,* mucus hypersecretion; *scale bar,* 30 μm.

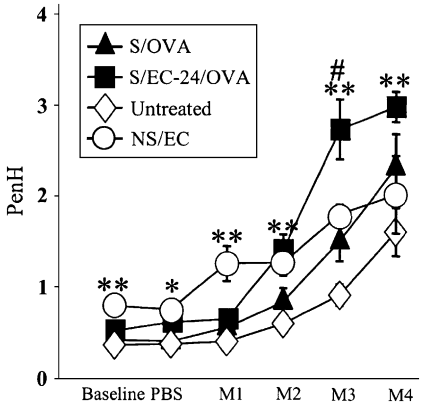

FIG 5. Airway hyperresponsiveness (represented by Penh) was measured by noninvasive body plethysmography 24 hours after ovalbumin challenge in untreated mice *(untreated)*, ovalbumin-sensitized and ovalbumin-challenged mice *(S/OVA)*, nonsensitized mice exposed to EC-UFP *(NS/EC)*, and ovalbumin-sensitized mice exposed to EC-UFP 24 hours before ovalbumin challenge *(S/EC-24/OVA)*. Mean ± SEM; n ≥ 6/group. **P* < .05, ***P* < .01 versus untreated; #*P* < .05 versus S/OVA.

important cofactors in the development of pulmonary health disorders. In the current study, we investigated the effects of EC-UFP inhalation on allergic responses in a mouse model of allergic airway inflammation. EC-UFP inhalation before allergen challenge caused a pronounced augmentation of the allergen-induced inflammatory response, as documented by increased inflammatory cell infiltrate, BALF cytokines, bronchial mucus production, and AHR. The adjuvant effects of EC-UFP inhalation were dose- and time-dependent and still present when EC-UFP exposure preceded allergen challenge for as long as 4 days. EC-UFPs, merely by virtue of their large surface area, can cause increased lung burden of reactive oxygen species.[8,10] We thus may speculate that free radicals produced by EC-UFP exposures can injure epithelial cells and promote the synthesis of proinflammatory mediators, which may in turn increase the permeation of allergens. Deposited UFPs are not readily phagocytized by alveolar macrophages, but tend to penetrate into the interstitium,[41] were they may contribute to the allergen-induced inflammatory process. In addition, at particle concentrations comparable with those we used, UFP agglomerates are formed, which can be phagocytized by alveolar macrophages.[42] Thus, modulation of macrophage function may be another level at which EC-UFPs exert their adjuvant activity on allergen-induced airway inflammation.[43]

Both epidemiologic and human experimental studies provide evidence for an adjuvant effect of PMs on the elicitation phase of the allergic response. Increases in PM

concentrations were shown to be associated with a decline in lung functions, higher frequency of hospital emergency visits, and increased *in vivo* nasal allergen-specific IgE and T_H2-type cytokines.[17,44-46] Experimental data on the effects of particles in the elicitation phase of the allergic response *in vivo* are scant.[30-32] Different approaches and different disease models have been used that are difficult to compare. Although some investigators analyzed the effect of aqueous extracts from environmental dust samples after intratracheal instillation,[31] others investigated the effects of concentrated air particle inhalation[30] in murine asthma models. Effects of UFP inhalation on the elicitation of allergic asthma have also been investigated in a canine model. In contrast with our findings, this study using ragweed-sensitized dogs did not observe any adjuvant activity of UFP inhalation on allergen-induced airway inflammation.[32] Differences in species, study design, and exposure conditions may account for this. In addition, the allergen dose used for challenge was not enough to induce significant eosinophilia.

We chose inhalation of EC-UFP because it represents the core of diesel exhaust particles and is a major component of urban airborne PM.[33] In addition, inhalation rather than intratracheal instillation represents the natural exposure condition. Furthermore, using a standardized source of particles characterized by a defined chemical composition eliminates the problems that can occur by using urban particulates such as the concentrated ambient particles, affected by day-to-day variations. To our knowledge, this is the first study that examines the effects of ambient relevant particles during a 7-day period after ovalbumin challenge. This enabled us to evaluate the kinetic of the inflammatory response.

In contrast with the strong effects of EC-UFP before ovalbumin challenge, EC-UFP exposures after ovalbumin challenge lead to a minor, bimodal modulation of the allergic inflammation, characterized by an initial decrease of the allergic inflammatory infiltrate as long as 4 days after ovalbumin challenge and a subsequent increase at later time points. The early decrease of inflammatory infiltrate was also shown in ovalbumin-sensitized mice exposed to concentrated air particles after ovalbumin challenge.[30] Unfortunately, no information about later time points is available in the literature. The reduction of total protein, IL-5, and IL-13 in the BALF after EC-UFP exposure prompted us to address the question of whether this effect could be caused by protein absorption by the particles. We therefore exposed *in vitro* BALF from S/OVA mice for 24 hours to increasing concentrations of EC-UFP (5, 50, 500 μg/mL) and analyzed total protein and IL-5 concentrations. Although no changes were seen at lower concentrations, incubation of BALF with 500 μg EC-UFP for 24 hours reduced the amount of total protein and IL-5 to half (data not shown). On the basis of a multiple path particle deposition model,[47] we estimated a total mass deposition of 24.2, 10.2, and 5.3 μg EC-UFP/day/mouse lung for the 3 exposure conditions (526, 332, and 119 $\mu g/m^3$ EC-UFP). This estimate, however, does not allow to calculate UFP concentrations in the local microenvironment, where UFP may reach levels at which relevant protein absorption may occur.

Ultrafine particles have been shown to increase in the past years.[11] Recent measurements indicate that UFP numbers in ambient air range from $2 \times 10^4/cm^3$ to $2 \times 10^5/cm^3$, with mass concentrations exceeding 50 $\mu g/m^3$ near major highways.[48,49] In a recent study that evaluated the on-road UFP concentration in Minnesota highways,[50] concentrations as high as 1×10^7 UFP/cm^3 were found, which means that people driving on a highway are directly exposed to such concentrations. To model peak UFP exposure conditions, we chose similar high number concentrations and demonstrated strong adjuvant activity on allergen-induced airway inflammation. In contrast, EC-UFP inhalation in nonsensitized control animals had negligible effects on pulmonary inflammation. This is in line with previous reports showing that carbon or platinum UFP inhalation had no effects in healthy mice, but only in compromised animals.[7] To the best of our knowledge, our study is the first to demonstrate that in sensitized animals, inhalation of relatively inert EC-UFP has strong adjuvant activity when inhaled before allergen challenge, whereas EC-UFP inhalation during an ongoing allergic inflammation does not. Thus the sequence of events seems to be critical and should be considered when studying *in vivo* effects of PM exposure. In addition, our study strongly supports the concept that allergic sensitization represents a susceptibility factor for the effects of UFP on allergen-induced allergic inflammation of the lung.

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