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The Upper Airway Response to Pollen Is Enhanced by Exposure to Combustion Particulates: A Pilot Human Experimental Challenge Study

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Although human experimental studies have shown that gaseous pollutants enhance the inflammatory response to allergens, human data on whether combustion particulates enhance the inflammatory response to allergen are limited. Therefore, we conducted a human experimental study to investigate whether combustion particulates enhance the inflammatory response to aeroallergens. "Enhancement" refers to a greater-than-additive response when combustion particulates are delivered with allergen, compared with the responses when particulates and allergen are delivered alone. Eight subjects, five atopic and three nonatopic, participated in three randomized exposure-challenge sessions at least 2 weeks apart (i.e., clean air followed by allergen, particles followed by no allergen, or particles followed by allergen). Each session consisted of nasal exposure to combustion particles (target concentration of 1.0 mg/m^3) or clean air for 1 hr, followed 3 hr later by challenge with whole pollen grains or placebo. Nasal lavage was performed immediately before particle or clean air exposure, immediately after exposure, and 4, 18 and 42 hr after pollen challenge. Cell counts, differentials, and measurement of cytokines were performed on each nasal lavage. In atopic but not in nonatopic subjects, when allergen was preceded by particulates, there was a significant enhancement immediately after pollen challenge in nasal lavage leukocytes and neutrophils (29.7×10^3 cells/mL and 25.4×10^3 cells/mL, respectively). This represents a 143% and 130% enhancement, respectively. The enhanced response for interleukin-4 was 3.23 pg/mL ($p = 0.06$), a 395% enhancement. In atopic subjects there was evidence of an enhanced response when particulates, as compared to clean air, preceded the allergen challenge. *Key words*: allergy, atopy, cytokines, inflammatory cells, particulates. *Environ Health Perspect* 111:472–477 (2003). doi:10.1289/ehp.5862 available via *http://dx.doi.org/* [Online 6 December 2002]

Epidemiologic studies suggest that particulate matter (PM) air pollution may enhance symptom severity and frequency in allergic asthmatics. There is also evidence that air pollution is associated with an increase in hospital emergency room visits for asthma (Lipsett et al. 1997; Schwartz et al. 1993). Although the epidemiologic evidence is consistent, the mechanisms by which air pollution increase asthmatic symptoms remains unclear.

Animal studies have shown enhanced allergic sensitization by combustion particles (Hamada et al. 2000; Lambert et al. 2000), as well as a greater-than-additive interaction between combustion particles and allergic response to ovalbumin (Gavett et al. 1999; Kobayashi et al. 2000). There is also evidence that exposure to ambient pollutants enhances the allergic response to allergen challenge in humans. Experimental human studies have shown that exposure to ozone (Jorres et al. 1996; Molfino et al. 1991; Peden et al. 1995) and nitrogen dioxide alone (Devalia et al. 1994; Strand et al. 1997, 1998; Tunnicliffe et al. 1994) or with sulfur dioxide (Rusznak et al. 1996) enhance the allergic response. In addition, there is also limited human evidence demonstrating that particulate exposure (e.g., diesel exhaust) may enhance the allergic response (Diaz-Sanchez et al. 1997; Takenaka et al. 1995).

To examine the relationship between combustion particulate exposure and enhanced inflammatory responses in humans, we conducted an experimental study with fuel-oil combustion particulates and pollen grains. To simulate real-life exposure, we developed a system whereby we could challenge the subject with aerosolized whole pollen grains rather than using allergen extracts as in earlier human experimental exposure studies.

We chose the upper airway as a model of allergic inflammation and nasal lavage (NL) as a noninvasive method for obtaining samples. The nasal cavity is an excellent model of allergic inflammation and provides an easily accessible site for study. There is sufficient evidence that NL can be successfully used to investigate the inflammatory response in the nasal cavity (Bascom et al. 1989; Castells and Schwartz 1988; Durham et al. 1992; Lebel et al. 1988; Skoner et al. 1990; Togias et al. 1988).

Methods

Subjects. The study included atopic subjects with and nonatopic subjects without self-reported seasonal allergic rhinitis. All subjects signed a consent form approved by the Harvard School of Public Health Human Subjects Committee. Subject exclusion criteria included asthma or a history of asthma, current (within the last year) smokers, immunotherapy (to any allergen/antigen) in the previous 3 years, nasal abnormalities such as nasal septal deviations or polyps, perennial rhinitis, or any acute or chronic condition or disease for 3 months before the study. All subjects were screened for and had normal pulmonary function.

Subjects with a respiratory tract infection were not studied until the acute event had clinically resolved and at least 3 weeks had elapsed. At least 4 weeks before and during the study session, subjects were asked not to use H_1 histamine-receptor antagonists, nasal beclomethasone (and other nasal steroids), nasal or inhaled cromoglycate, and oral or inhaled steroids. They refrained from using aspirin, nonsteroidal anti-inflammatory agents, and vitamin C and E supplements for 10 days before and during the study sessions. For 6 hr before the exposure session, subjects did not have caffeinated medications or beverages. Subjects were instructed not to engage in moderate to heavy physical exertion at least 4 hr before the exposure session and during the day of the exposure session.

Skin prick test. We conducted skin prick tests using six common inhalant allergens (*Dermatophagoides pteronyssinus*, mixed grasses, ragweed, birch tree, oak tree, and *Alternaria*) and positive and negative controls (histamine and saline, respectively). A positive test was recorded if the maximum wheal diameter was 3 mm larger than the saline control. Subjects were defined as atopic if one or more skin tests were positive. We used the results of the skin prick test to determine the aeroallergen used for the challenge.

Exposure-challenge protocol. All exposure challenge sessions were performed during the winter months and therefore out of season for pollens. To familiarize themselves with the breathing apparatus and the NL procedure, each subject participated in a training session at least 2 weeks before the first session. The order of the three sessions was single-blind, randomized, and each session was separated by at least 2 weeks. The three sessions consisted of *a*) residual oil fly ash particulate exposure followed by no allergen challenge, referred to as session A (S_A), *b*) clean air exposure followed by allergen challenge, referred to as session B (S_B), and *c*) particulate exposure followed by allergen challenge, referred to as session C (S_C).

On the day of each session, the subject reported to the laboratory between 0800 and 1000 hr for a 1-hr particulate or clean air exposure while seated at rest. After exposure, the subject was free to leave the lab but was instructed not to exercise or to leave the building. Three hours after the particulate exposure the subject was challenged with an allergen or a placebo.

Particulate exposure. A bulk source of residual oil fly ash (ROFA) from a Boston power plant was resuspended using a Wright Dust Feed Aerosol Generator (Model MK-II; L. Adams Ltd., London, UK). The ROFA was delivered using a disposable nasal mask (Respironics, Inc., Murrysville, PA). The air stream, containing particles, was divided into two streams: 4 L/min was passed through a Harvard Marple Impactor (Harvard Instruments, Boston, MA) to eliminate particles > 2.5 μm, and the rest of the air stream was passed through a 0.2-μm filter. The output from the Harvard impactor was isokinetically fed into the nasal mask. Particle size, composition, and airborne concentration were measured during the exposures. We determined concentration gravimetrically using 0.2-μm Millipore filters and by continuous monitoring with a real-time Aerosol Monitor (Model RAS-1; MIE Inc., Bedford, MA). The size distributions were determined with an Aerosizer (Model MACCII; API Inc., Amherst, MA).

Allergen (whole pollen grain) challenge. Pollen grains were aerosolized using a vibrating drum apparatus developed in our laboratory. The equipment consisted of an acoustic speaker to generate acoustic waves. On top of the speaker, a 15-inch long by 5-inch diameter Plexiglas tube was placed. Both ends of the Plexiglas tube were fitted with dental rubber dams to form a drumlike cover. The Plexiglas tube has 0.5-inch fittings on the bottom and top. The top fitting was connected to a pump pulling approximately 30 lpm of room air. The subject's breathing mask was connected between the Plexiglas tube and the pump.

The subject breathed through the mask for 30 sec during the aerosolization of the pollen. To aerosolize the pollen, a bolus of pollen was injected into the Plexiglas drum through the bottom fitting using a 1 cc tuberculin syringe. The syringe was filled with 0.5 cc pollen (dry allergen source material; Hollister Stier Laboratories LLC, Spokane, WA) for each allergen challenge. The subject was instructed to perform slow shallow inspirations through the nose during the pollen delivery phase. The no allergen (placebo) challenge session consisted of filling a 1 cc tuberculin syringe (identical to the type used for allergen challenge) with air and introducing the air into the allergen delivery drum. Because of the design of the challenge apparatus, the subjects could not see the syringe and were therefore blinded as to whether they were receiving allergen or placebo.

We monitored the pollen aerosol count using the aerosizer (Model LD; Amherst Process Instruments Inc., TSI, Amherst, MA), which measures particles in the size range of 0.12–200 μm. The atopic subjects were challenged with either ragweed or tree pollen based on whether they tested positive for them on the skin prick test, while the nonatopic subjects were challenged with red oak. We chose ragweed and tree pollen because they were reliably delivered using our whole-grain pollen aerosol technique.

NL protocol. At the beginning of each exposure-challenge session, two sequential NLs, 5 min apart, were performed. We used the second lavage as the baseline NL ($NL_{baseline}$). Nasal lavage was again performed immediately (NL_{IA}), 4 hr (NL_4), 18 hr (NL_{18}), and 42 hr (NL_{42}) after allergen challenge. The subject tilted his or her head backward while a soft-plastic catheter tip was inserted into one nostril. A 10-mL syringe, attached to the catheter, was used to instill 5 mL of warm (37°C), sterile, Dulbecco's phosphate-buffered saline (PBS) solution (Sigma Chemical Co., St. Louis, MO). The procedure was repeated on the other nostril. *N*-acetyl cysteine (Sigma) was added to the recovered NL fluid to a final concentration of 1 mM, and the sample was incubated for 15 min at 37°C. The *N*-acetyl cysteine was added to dissolve mucous. The sample was then centrifuged at 915 × *g* at 4°C for 10 min. The cell pellet was resuspended in PBS, and total leukocytes were determined by hemocytometer counts. We determined differential cell counts from modified Wright-Giemsa-stained cytocentrifuge preparations. Nasal lavage cell counts were expressed as cells per milliliter, and differential cell counts were expressed as percent cell type.

Immunoassays for proteins and cytokines. We measured cytokine levels in NL fluid using commercial ELISA kits with chemiluminescent detection (R&D Systems, Minneapolis, MN).

We measured interleukin (IL)-3, IL-4, IL-5, IL-8, IL-13, tumor necrosis factor (TNF)-α, monocyte chemotactic protein (MCP)-1, and granulocyte macrophage-colony stimulating factor (GM-CSF). Albumin was measured by nepholometery (BN100, Dade-Behring, Deerfield, MI) using a low albumin protocol recommended by the manufacturer. We measured total IgE, eosinophil cationic protein (ECP) and tryptase using a commercial immunofluorometric instrument (AutoCAP; Pharmacia and Upjohn, Kalamazoo, MI) and using kits supplied by the same company.

Symptom questionnaire. Immediately before exposure, immediately after, and 4 and 18 hr after allergen challenge the subject completed a questionnaire on nasal, ocular, and upper and lower respiratory symptoms.

Statistical analysis. An enhanced inflammatory response was defined as a greater-than-additive response when combustion particulates were delivered with allergen, as compared to the sum of the responses when particulates and allergen were delivered alone. More specifically, an enhanced response occurred when the inflammatory response in the particulate exposure-allergen challenge session (S_C) exceeded the sum of the responses in the sessions to particulates alone (S_A) and allergen alone (S_B). We defined the inflammatory response in each session as the difference between the baseline level of cells or cytokines and their level at time *t* after the allergen challenge. Time *t* was either immediately after, 4 hr, 18 hr, or 42 hr after allergen challenge. Mathematically the inflammatory response for person *i* at time *t* after exposure-challenge is $\Delta_{it} = (NL_{it} - NL_{ibaseline})$, where NL_{it} = cell count or cytokine level at time *t* for person *i*, $NL_{ibaseline}$ = cell count or cytokine level at baseline for person *i*. For each of the three sessions, for each person *i* at time *t* we calculated a Δ_{it}. The Δ_{it} for person *i* at time *t* for S_A is referred to as A_ Δ_{it}, for S_B as B_ Δ_{it}, and for S_C as C_ Δ_{it}. When C_ Δ_{it} exceeded the sum of A_ Δ_{it} and B_ Δ_{it}, a greater-than-additive response occurred in person *i* at time *t*. The magnitude of the enhanced response in person *i* at time *t* is referred to as EΔ_{it}, where E$\Delta_{it} = [C\_ \Delta_{it} - \{A\_ \Delta_{it} + B\_ \Delta_{it}\}]$. At each time *t*, a we used Wilcoxon signed-ranks test to determine whether the distribution of the EΔ_{it} values was zero (null hypothesis); if they were not different from zero, this was evidence that there was no enhancement of the inflammatory response. The alternative hypothesis was that the EΔ_{it} values have a positive central location; if the EΔ_{it} values have a positive central location, this was evidence that particulates enhanced the response to pollen. We used the Wilcoxon signed-ranks test because the EΔ_{it} values were not normally distributed. A one-sided test was used because *a priori* we

expected a positive enhancement. Because this was an exploratory analysis, *p*-values of ≤ 0.1 were considered suggestive of an enhanced response.

In addition to the formal statistical tests, we calculated an average enhanced response for each NL parameter at each time *t* by summing the $E\Delta_{it}$ values at each respective time *t* for all subjects and dividing by the number of subjects *i*. This is referred to as "average enhanced response" and represents the average magnitude of the enhanced response across all subjects (i.e., the magnitude of the response that is greater than that expected if the particulate and allergen responses were strictly additive). Since the Wilcoxon signed-ranks test assesses whether the ranks, and not the mean, of the Δ_{it} values are significantly different from zero, there may not necessarily be a direct relationship between the average enhanced response presented in the tables and the level of their statistical significance.

To further quantify the relative magnitude of the enhanced response, we calculated the percent enhancement for each nasal lavage parameter at each time *t* by dividing the average enhanced response by the average of the baseline levels of the respective nasal lavage parameter from sessions A, B, and C. For instance, if the average enhanced response is equal to the average baseline level, then a 100% enhanced response occurred. We used the average baseline level from the three sessions to calculate percent enhancement because baseline levels in healthy, nonexposed subjects vary temporally (Hauser et al. 1994). By averaging the baseline values across the three sessions, the estimate of a true baseline will be more precise.

A clean air/no allergen treatment arm was not included in the present study because statistically we could test for interaction by comparing whether the magnitude of the response to the two treatments together (i.e., particulates and allergen) exceeded the response to each treatment alone (i.e., particulates alone and allergen alone). We made the choice not to include a clean air/no allergen session *a priori* because of funding limits.

Results

Eight of the twelve subjects recruited completed the full protocol consisting of three randomized exposure-challenge sessions. One subject completed only two sessions, two subjects completed one session, and one subject withdrew before completing any sessions. Subjects did not complete all three sessions because of self-reported upper-respiratory-tract illnesses (i.e., flu) that precluded further testing during the study period.

Eight nonsmoking subjects, 23–39 years old, contributed data to the analysis. There were four male subjects (two were allergic to ragweed and two were nonatopic) and four female subjects (three were allergic to red oak and one was nonatopic); five subjects were atopic and three were nonatopic. Demographic data are presented in Table 1. Of the eight subjects, six did not report nasal, ocular, or respiratory symptoms after any of the exposure challenge protocols. Therefore, analysis of symptom data was uninformative.

The mean (SD) ROFA concentration delivered in the particulate exposure session was 0.96 (0.20) mg/m^3; the target concentration was 1.00 mg/m^3. The mass median diameter of the particles was 1.55 ± 0.44 μm with σ$_G$, a measure of particle size distribution, of 1.52 ± 0.12. The value of σ$_G$ indicates that a polydisperse particle distribution was generated. The average number of delivered pollen grains/cc air was 7.23 ± 11.32 for ragweed and 0.58 ± 0.62 pollen grains/cc air for red oak.

At all time points after allergen challenge, there was a greater-than-additive response for total leukocytes, neutrophils, and macrophages per milliliter when particulates and allergen were given sequentially as compared to when each was given alone (Table 2). We found few eosinophils in the NL fluid, precluding statistical analysis. The average enhanced response for total leukocytes and neutrophils per milliliter were maximal immediately after allergen challenge and decreased over time. Immediately after challenge, total leukocytes per milliliter were enhanced by 143%. For neutrophils and macrophages per milliliter, the response immediately after challenge was significantly enhanced by 130 and 211%, respectively.

In addition to the enhanced cellular response, there was a greater-than-additive response in IL-4 and IL-8 at all time points after allergen challenge when particulates and allergen were given sequentially as compared to when each was given alone (Table 2). The enhanced response for IL-4 was maximal immediately after challenge and decreased over time to a minimum at 42 hr after allergen challenge. Immediately after and 4 hr after allergen challenge, the IL-4 response was enhanced by 395 and 410%, respectively. In contrast, the IL-8 response remained enhanced throughout the postchallenge period. For IL-8, the response immediately after and 4 hr after allergen challenge was enhanced 130 and 132%, respectively.

Although the number of atopic and nonatopic subjects was small (five and three subjects, respectively), there were marked differences in inflammatory response based on atopic status. For IL-4, the enhanced response for the five atopic subjects (Table 3) was 5- to 10-fold higher than for the three nonatopics and demonstrated a similar decreasing pattern as that observed for all subjects as a group. In atopics, the IL-4 response for immediately after and 4 and 18 hr after allergen response was enhanced by 370, 363, and 204%, respectively. For the three nonatopics, the IL-4 responses for immediately after and 4 and 18 hr after allergen response were not significantly different from zero (0.38 pg/mL, 0.68 pg/mL, and –0.34 pg/mL, respectively).

For IL-8, the average enhanced response for atopics remained elevated at all time points after allergen challenge. In atopic subjects, the IL-8 response for immediately after and 4 and 18 hr after allergen response was enhanced by 84, 101, and 90%, respectively. Unexpectedly, in nonatopics, the IL-8 response immediately after allergen challenge was larger than in atopics, though it was not significant due to the small sample size. In nonatopic subjects, the IL-8 response for immediately after and 4 and 18 hr after allergen response was enhanced by 162, 153, and 90%, respectively.

The stratified analysis for the inflammatory cell counts also showed marked differences based on atopic status. The average enhanced response for atopics was significant for total leukocytes and neutrophils per milliliter immediately after challenge and was of borderline significance 4 and 18 hr after allergen challenge (Table 3). Leukocytes per milliliter were enhanced by 291, 216, and 313%, respectively, and neutrophils per milliliter were enhanced by 285, 193, and 323%, respectively. Interestingly, total leukocytes and neutrophils per milliliter did not decrease over time as observed for all subjects as a group, but remained elevated or increased at 42 hr after allergen. Macrophages per milliliter were enhanced by 237, 129, and 234% immediately after and 4 and 18 hr after allergen challenge, respectively. For the nonatopic subjects, at all time points the average enhanced response for inflammatory cells were not statistically different from zero and were of smaller magnitude than for the atopic subjects (data not shown).

We did not see an enhancement in IL-3, TNF-α, or total IgE in the group of subjects as a whole or within strata of atopics and nonatopics. The average enhanced responses were not significantly different from zero (data not shown). Furthermore, because most of the NLs had nondetectable levels of tryptase, ECP, albumin, IL-5, IL-13, MCP-1, and GM-CSF, we were unable to perform meaningful statistical analyses on these markers.

Discussion

The present study provides evidence of a greater-than-additive interaction between particulate exposure and allergen challenge. Specifically, a 1-hr residual oil fly ash particulate exposure 3 hr before challenge with

pollen enhanced the nasal inflammatory response. The enhanced nasal response consisted of an increase in inflammatory cells (total leukocytes, neutrophils, and macrophages increased, but eosinophils did not) and in cytokines IL-4 and IL-8. The inflammatory cell influx and cytokine response differed by atopic status. Atopics had an enhanced IL-4 and inflammatory cell response, but nonatopics did not. However, for IL-8 there was an enhanced response in both atopics and nonatopics, though it was larger in nonatopics. The cellular and cytokine responses showed different time courses. The enhanced inflammatory cell response was present immediately after allergen challenge and remained present for 42 hr after challenge, whereas the IL-4 response was maximal immediately after allergen challenge but diminished by 18 hr after allergen. Although the subjects in our study did not report clinical symptoms, the observed enhanced inflammatory response may have clinical relevance because the ROFA and

pollen exposures were brief, and individuals are chronically exposed to allergens and to airborne particulates in their everyday lives.

IL-4, produced by T cells and mast cells, contributes to allergic inflammation (Brown and Hural 1997; Ryan 1997) by a variety of mechanisms. Among other functions, it induces IgE production by B-lymphocytes, promotes differentiation of immature lymphocytes, and inhibits Th1 responses (Brown and Hural 1997; Ryan 1997). The increased levels of soluble IL-4 may reflect mast cell activation by particulate exposure and may represent one mechanism by which particulates enhance the allergic response. IL-8 is a chemokine that recruits neutrophils (Mukaida et al. 1998; Teran et al. 1996, 1997) and is produced by a number of cell types including macrophages and epithelial cells. The increased levels measured in the present study most likely contributed to the observed enhanced neutrophil response.

In the present study, because only the number of neutrophils, but not eosinophils, were enhanced, this suggests that the enhanced

cellular response may be an irritant response rather than an allergic response. In addition, only IL-4 (aTh2-type cytokine) was elevated, and no other Th2-type cytokines or other parameters associated with allergic airway responses were enhanced. Therefore, the enhanced response observed may represent an enhanced irritant response rather than an allergic response.

One of the strengths of the present study was that several factors were controlled in the design. Because exposure-challenge sessions were randomized, at least 2 weeks apart, and performed at the same time of day within a subject, carryover effects and diurnal variability were minimized. Furthermore, although we did not measure the subjects' exposures to ambient pollutants, the magnitude of this exposure relative to the particulate exposure was low. In addition, since ambient exposure to ragweed or red oak pollen during the winter months in Boston, Massachusetts, is unlikely, confounding by ambient allergens is also unlikely.

By design we chose to elicit only a minimal allergic response because it would be difficult to detect enhancement of a full immediate response. If we had elicited a maximal or near-maximal immediate response, any enhancement by particulate exposure would have been obscured. In addition, a minimal response was expected because the subjects were tested out of allergy season and were not primed at the time of challenge because it had been several months since they were last exposed to these allergens. Therefore, as expected, most subjects did not report upper-respiratory-tract symptoms in response to allergen. Further evidence of a minimal allergic response was that we were unable to detect changes in tryptase, ECP, and eosinophils. This confirmed that the size of our dose was appropriately small because it did not elicit a full, immediate-phase response. If a full, immediate-phase response was elicited by the allergen alone, our power to detect an enhancement of this response by particulates would be minimal.

We chose to study red oak and ragweed pollen because they are two important airborne allergens causing allergic rhinitis and asthma. Although the symptom response was minimal in the present study, particulate air pollution may contribute significantly to increased symptoms in individuals with seasonal allergies because in the ambient environment individuals are chronically exposed to these allergens and to airborne particulates. The chronicity of exposure may result in magnified responses as compared to the brief 1-hr exposure to particulates in this study.

We used ROFA, a component of ambient particulates, rather than collecting ambient PM_{10} (PM ≤ 10 μm in diameter). We made

Table 1. Study subject demographics.

Subject	Age	Sex	Atopic status	Allergens with positive skin prick test
1	28	Female	Atopic	Red oak, grass mix, mite[a]
2	39	Male	Atopic	Ragweed[b]
3	38	Male	Nonatopic	N/A
4	27	Male	Nonatopic	N/A
5	30	Female	Nonatopic	N/A
6	23	Male	Atopic	Ragweed[b]
7	30	Female	Atopic	Red oak, mite[a]
8	32	Female	Atopic	Red oak, grass mix, mite,[a] ragweed,[b] birch[c]

[a]*Dermatophagoides pteronsyssinus.* [b]Mixture of short and giant ragweed. [c]White birch.

Table 2. Average enhanced response for nasal lavage parameters immediately after (IA) and 4, 18, and 42 hr after allergen challenge (all subjects, $n = 8$).[a]

NL parameter	Average enhanced response at time t				
	Baseline[b]	IA	4 hr	18 hr	42 hr
Inflammatory cells					
Leukocytes/mL	15,200	21,700*	14,900	19,200	1,900
Neutrophils/mL	13,900	18,000*	11,700	17,200	900
Macrophages/mL	900	1,900**	1,600**	1,800	600
Cytokines					
IL-4 (pg/mL)	0.55	2.2**	2.2**	0.99	0.81
IL-8 (pg/mL)	220	422**	427**	294	458*

[a]See text for explanation of average enhanced response. [b]Average baseline value from sessions A, B, and C. *$p < 0.1$; **$p < 0.05$ using Wilcoxon signed-ranks test.

Table 3. Average enhanced response for nasal lavage parameters immediately after (IA) and 4, 18, and 42 hr after allergen challenge (atopic subjects, $n = 5$).[a]

NL parameter	Average enhanced response at time t				
	Baseline[b]	IA	4 hr	18 hr	42 hr
Inflammatory cells					
Leukocytes/mL	10,200	29,700**	22,000*	32,000*	44,100
Neutrophils/mL	8,900	25,400**	17,100	28,700**	42,300
Macrophages/mL	900	2,200	1,200	2,200	−100
Cytokines					
IL-4 (pg/mL)	0.88	3.23*	3.18*	1.79*	1.63
IL-8 (pg/mL)	213	179*	215	191	172

[a]See text for explanation of average enhanced response. [b]Average baseline value from sessions A, B, and C. *$p < 0.1$; **$p < 0.05$ using Wilcoxon signed-ranks test.

this choice because we needed a source of particles that was sufficiently large to allow us to use the same particle source for all exposure sessions (16 particulate exposure sessions). The use of ROFA allowed us to standardize the composition of particles delivered across sessions within subjects and across sessions between subjects. We chose a high target concentration of ROFA (1 mg/m^3) because the subjects were only exposed to ROFA for 1 hr at rest.

One of the limitations of the present study was the small sample size, which limited the power of the study to detect statistically significant associations. Although there were only 8 subjects, the data consisted of a total of 24 exposure-challenge sessions, 3 per subject. Subjects were used as their own control, increasing study power given a limited number of subjects, and minimizing confounding by time-invariant individual characteristics. Because this was an experimental crossover study, we were able to tightly control the experimental conditions, minimizing random variability that can contribute to diluted measures of association. This improved our ability to detect an enhanced response after particulate exposure as compared to clean air exposure. However, despite the advantages of using an experimental crossover study, the small sample size did limit our ability to report the enhanced responses as being statistically significant, even though they were of moderate magnitude, generally greater than a 100% enhancement. We had less power to determine whether the enhanced allergic response differed by atopic status. However, despite the small sample size and limited power, we found a difference in response based on atopic status. Although our data are suggestive of a particulate enhanced response and a modification of response based on atopic status, these results need to be confirmed because they are based on a limited number of subjects.

Because of difficulties with aerosolization and standardization, the placebo challenge did not consist of particles of similar size and consistency to pollen. However, self-reports indicate that the subjects were not aware of whether they were receiving pollen or placebo challenge. More important, because subjects were used as their own controls, the use of room air as a placebo could not account for the observed enhanced responses. In addition, we did not have a clean air/no-allergen challenge session (i.e., a treatment arm without particulates and without allergen). A clean air/no-allergen challenge session was not necessary to detect enhancement. It was statistically appropriate to compare the session with particulates and allergen together with the sessions with allergen alone and with particulates alone.

Despite the difficulties we encountered in aerosolizing whole pollen grains, we chose to use whole pollen grains because they more closely approximate real-life exposure than does allergen extract. The major difficulty in aerosolizing whole pollen grains resulted from the large size of the pollen grains (i.e., > 10 mm) and their stickiness. These characteristics produced random between-session variability, which was larger for ragweed than for red oak. The random between-session variability would tend to bias results toward the null hypothesis, making it more difficult to detect enhanced responses.

To date, most of the human studies investigating whether ambient pollution enhances the allergic response have used gaseous pollutants. In general, these studies have found an enhanced allergic response of asthmatics to allergen after exposure to ozone, nitrogen dioxide, and a combination of nitrogen dioxide and sulfur dioxide (Jorres et al. 1996; Rusznak et al. 1996; Strand et al. 1998; Tunnicliffe et al. 2001). Tunnicliffe and colleagues (2001) studied the effect of a 1-hr exposure to nitrogen dioxide (100 or 400 ppb) on airway responses to inhaled allergen in eight asthmatic subjects. They found significant differences between air and 400 ppb NO_2 on the early asthmatic response (maximum percent change in forced expiratory volume in 1 sec [FEV_1] during the first 2 hr after challenge) and the late asthmatic response (maximum percent change in FEV_1) to inhaled allergen. Strand and co-workers (1997) studied 18 subjects with asthma and allergy to pollen. Subjects were exposed to low-level NO_2 (490 mg/m^3) for 30 min, followed 4 hr later by allergen inhalation challenge with standardized and freeze-dried allergen extracts. They found an enhancement of the asthmatic reaction during the late phase by NO_2 exposure, and peak expiratory flow after allergen challenge was on average 6.6% lower after NO_2 as compared to air exposure. However, they did not find changes in serum eosinophil numbers nor eosinophil cationic protein levels. As in our study, they delivered the pollutant several hours before allergen challenge because of earlier work suggesting a delayed response to the pollutant.

There are limited experimental human studies investigating whether particulates enhance the allergic response. Takenaka and co-workers (1995) found that an extract of polyaromatic hydrocarbons from diesel exhaust particulates enhanced human IgE production from B-cells derived from both nonallergic and allergic individuals. Diaz-Sanchez and co-workers (1997) investigated combined diesel exhaust particulate and ragweed allergen challenge on nasal humoral immune responses in 13 ragweed-sensitive subjects. Subjects were challenged with the short ragweed allergen *Amb a* I. As compared with challenge with ragweed alone, challenge with diesel exhaust particulates and ragweed induced higher ragweed-specific IgE, but not total IgE, in nasal washes 1 and 4 days after challenge. In addition, challenge with diesel and ragweed resulted in decreased or no change in mRNA expression for Th 1-type cytokines (interferon-γ and IL-2), but elevated mRNA expression for Th 2-type cytokines (IL-4, -5, -6, -10, -13). Subjects did not report a significant increase in symptom severity scores after challenge with diesel exhaust particulates and ragweed allergen, as compared to challenge with ragweed alone.

In a recent study on 10 atopic asthmatic adults, particulate sulfuric acid exposure potentiated early asthmatic response to grass pollen allergen (Tunnicliffe et al. 2001). Subjects were exposed to sulfuric acid particulates at concentrations of 0.1 and 1.0 mg/m^3 for 1 hr. Fourteen hours after exposure subjects underwent a fixed-dose allergen challenge with grass pollen allergen. The early asthmatic response was defined as the maximum percentage change in FEV_1 during the first 2 hr after allergen challenge. The difference between the 1 mg/m^3 sulfuric acid exposure and air was –4.3% [95% confidence interval (CI), –1.2 to –7.4), and the 0.1 mg/m^3 sulfuric acid exposure and air difference was –2.6% (95% CI, 0.0 to –5.3).

There are several potential mechanisms by which particulates may produce an enhanced allergic response. It is possible that the ROFA-induced inflammation (Hauser et al. 1995) leads to vasodilation and greater absorption of the allergen and therefore a greater allergic response. Another possible mechanism is that the ROFA could induce an increase in nasal cytokine production, which leads to an enhanced allergen response. Evidence for this possible mechanism comes from a study by Diaz-Sanchez and co-workers (1996) on enhanced nasal cytokine production in humans after *in vivo* nasal exposure to 0.3 mg of diesel exhaust particles in 200 μl saline. They found an increase in cytokine mRNAs for IL-2, IL-4, IL-5, IL-6, IL-10, and IL-13 and interferon-γ after challenge with diesel particulates. The nonspecific increase in cytokine expression was not simply the result of an increase in T-cells recovered in lavage fluid. The enhanced cytokine expression by diesel exhaust exposure may contribute to enhanced local IgE production. Evidence supporting this comes from earlier studies by the same research group that showed enhanced human IgE production both *in vivo* and of purified B-cells after exposure to the aromatic hydrocarbons from diesel exhaust (Diaz-Sanchez et al. 1994; Takenaka et al. 1995). The increased IgE production may increase the likelihood of allergic symptoms. In summary, the diesel exhaust

studies suggest a possible mechanism that may be relevant to ROFA.

In the present study, there was evidence of a greater-than-additive interaction between particulate exposure and allergen challenge. The particulates used were ROFA, a component of air pollution. The ROFA used in the present study consisted of a complex mixture that contained transition metals, including vanadium, nickel, and iron. It is unclear whether the enhanced response we observed was specific to ROFA or whether it would be caused by any particle, such as inert particulates. Further research is needed to investigate which constituents of the ROFA are responsible for the enhanced allergic response.

REFERENCES

Bascom R, Pipkorn U, Proud D, Dunnette S, Gleich GJ, Lichtenstein LM, et al. 1989. Major basic protein and eosinophil-derived neurotoxin concentrations in nasal lavage fluid after allergen challenge: effect of systemic corticosteroids and relationship to eosinophil influx. J Allergy Clin Immunol 84:338–346.

Brown MA, Hural J. 1997. Functions of IL-4 and control of its expression. Crit Rev Immunol 17:1–32.

Castells M, Schwartz LB. 1988. Tryptase levels in nasal-lavage fluid as an indication of the immediate allergic response. J Allergy Clin Immunol 82:348–355.

Devalia JL, Rusznak C, Herdman MJ, Trigg CJ, Tarraf H, Davies RJ. 1994. Effect of nitrogen dioxide and sulphur dioxide on airway response of mild asthmatic patients to allergen inhalation. Lancet 344:1668–1671.

Diaz-Sanchez D, Dotson AR, Takenaka H, Saxon A. 1994. Diesel exhaust particles induce local IgE production *in vivo* and alter the pattern of IgE messenger RNA isoforms. J Clin Invest 94:1417–1425.

Diaz-Sanchez D, Tsien A, Casillas A, Dotson AR, Saxon A. 1996. Enhanced nasal cytokine production in human beings after *in vivo* challenge with diesel exhaust particles. J Allergy Clin Immunol 98:114–123.

Diaz-Sanchez D, Tsien A, Fleming J, Saxon A. 1997. Combined diesel exhaust particulate and ragweed allergen challenge markedly enhances human *in vivo* nasal ragweed-specific IgE and skews cytokine production to a T helper cell 2-type pattern. J Immunol 158:2406–2413.

Durham SR, Ying S, Varney VA, Jacobson MR, Sudderick RM, Mackay IS, et al. 1992. Cytokine messenger RNA expression for IL-3, IL-4, IL-5 and granulocyte/macrophage colony-stimulating factor in the nasal mucosa after local allergen provocation: relationship to tissue eosinophilia. J Immunol 148:2390–2394.

Gavett SH, Madison SL, Stevens MA, Costa DL. 1999. Residual oil fly ash amplifies allergic cytokines, airway responsiveness, and inflammation in mice. Am J Respir Crit Care Med 160:1897–1904.

Hamada K, Goldsmith CA, Goldman A, Kobzik L. 2000. Resistance of very young mice to inhaled allergen sensitization is overcome by coexposure to an air-pollutant aerosol. Am J Respir Crit Care Med 161:1285–1293.

Hauser R, Elreedy S, Hoppin JA, Christiani DC. 1995. The upper airway response in workers exposed to fuel oil ash: nasal lavage analysis. Occup Environ Med 52:353–358.

Hauser R, Garcia-Closas M, Kelsey KT, Christiani DC. 1994. Variability of nasal lavage polymorphonuclear leukocyte counts in unexposed subjects: its potential utility for epidemiology. Arch Environ Health 49:267–272.

Jorres R, Nowak D, Magnussen H. 1996. The effect of ozone exposure on allergen responsiveness in subjects with asthma or rhinitis. Am J Respir Crit Care Med 153:56–64.

Kobayashi T. 2000. Exposure to diesel exhaust aggravates nasal allergic reaction in guinea pigs. Am J Respir Crit Care Med 162:352–356.

Lambert AL, Dong W, Selgrade MJK, Gilmour MI. 2000. Enhanced allergic sensitization by residual oil fly ash particles is mediated by soluble metal constituents. Toxicol Appl Pharmacol 165:84–93.

Lebel B, Bousquet J, Morel A, Chanal I, Godard P, Michel FB. 1988. Correlation between symptoms and the threshold for release of mediators in nasal secretions during nasal challenge with grass pollen grains. J Allergy Clin Immunol 82:869–877.

Lipsett MS, Hurley S, Ostro B. 1997. Air pollution and emergency room visits for asthma in Santa Clara County, California. Environ Health Perspect 105:216–222.

Molfino NA, Wright SC, Katz I, Tarlo S, Silverman F, McClean PA, et al. 1991. Effect of low concentrations of ozone on inhaled allergen responses in asthmatic subjects. Lancet 338:199–203.

Mukaida N, Harada A, Matsushima, K. 1998. Interleukin-8 (IL-8) and monocyte chemotactic and activating factor (MCAF/MCP-1): chemokines essentially involved in inflammatory and immune reactions. Cytokine Growth Factor Rev 9:9–23.

Peden DB, Woodrow Setzer R Jr, Devlin RB. 1995. Ozone exposure has both a priming effect on allergen-induced responses and an intrinsic inflammatory action in the nasal airways of perennially allergic asthmatics. Am J Respir Crit Care Med 151:1336–1445.

Rusznak C, Devalia JL, Davies RJ. 1996. Airway response of asthmatic subjects to inhaled allergen after exposure to pollutants. Thorax 51:1105–1108.

Ryan JJ. 1997. Interleukin 4 and its receptor: essential mediators of the allergic response. J Allergy Clin Immunol 99:1–5.

Schwartz J, Slater T, Larson T, Pierson WE, Koenig JQ. 1993. Particulate air pollution and hospital emergency room visits for asthma in Seattle. Am Rev Respir Dis 147:826–831.

Skoner DP, Lee L, Doyle WJ, Boehm S, Fireman P. 1990. Nasal physiology and inflammatory mediators during natural pollen exposure. Ann Allergy 65:206–210.

Strand V, Rak S, Svartengren M, Bylin G. 1997. Nitrogen dioxide exposure enhances asthmatic reaction to inhaled allergen in subjects with asthma. Am J Respir Crit Care Med 155:881–887.

Strand V, Svartengren M, Rak S, Barck C, Bylin G. 1998. Repeated exposure to an ambient level of NO_2 enhances asthmatic response to a nonsymptomatic allergen dose. Eur Respir J 12:6–12.

Takenaka H, Zhang K, Diaz-Sanchez D, Tsein A, Saxon A. 1995. Enhanced human IgE production results from exposure to the aromatic hydrocarbons from diesel exhaust: direct effects on B-cell IgE production. J Allergy Clin Immunol 95:103–115.

Teran LM, Carroll MP, Frew AJ, Redington AE, Davies DE, Lindley I, et al. 1996. Leukocyte recruitment after local endobronchial allergen challenge in asthma. Am J Respir Crit Care Med 154:469–476.

Teran LM, Johnston SL, Schroder J-M, Church MK, Holgate ST. 1997. Role of nasal interleukin-8 in neutrophil recruitment and activation in children with virus-induced asthma. Am J Respir Crit Care Med 155:1362–1366.

Togias A, Nacleiro RM, Proud D, Pipkorn U, Bascom R, Iliopoulos O, et al. 1988. Studies on the allergic and nonallergic nasal inflammation. J Allergy Clin Immunol 81:782–790.

Tunnicliffe WS, Burge PS, Ayres JG. 1994. Effect of domestic concentrations of nitrogen dioxide on airway responses to inhaled allergen in asthmatic patients. Lancet 3344:1733–1736.

Tunnicliffe WS, Evans DE, Mark D, Harrison RM, Ayres JG. 2001. The effect of exposure to sulphuric acid on the early asthmatic response to inhaled grass pollen allergen. Eur Respir J 18:640–646.