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

Russ Hauser, Timothy M. Rice, G.G. Krishna Murthy, Matt P. Wand, Daniel Lewis, Toni Bledsoe, and Joseph Paulauskis

1Department of Environmental Health and 2Department of Biostatistics, Harvard School of Public Health, Boston, Massachusetts, USA; 3National Institute for Occupational Safety and Health, Morgantown, West Virginia, USA

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 (Lipsitt 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. 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. 1994; Strand et al. 1997) 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 allergenic 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 atopics with and without nasal polyps and with extremes of asthma severity 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/agent) 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 H1 histamine-receptor antagonists, nasal beclomethasone (and other nasal steroids), nasal or inhaled cromoglicate, 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 caffinated 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 atopics 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.

Address correspondence to R. Hauser, Department of Environmental Health, Occupational Health Program, Harvard School of Public Health, Building I, Room 1405, 665 Huntington Avenue, Boston, Massachusetts 02115 USA. Telephone: (617) 432–3526. Fax: (617) 432–5441. E-mail: rhauser@hohp.harvard.edu

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**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 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_{4h}), 4 hr (NL_{18h}), 18 hr (NL_{42h}), 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 from modified Wright-Giemsa-stained cytocentrifuge preparations 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 nephelometry (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 

$$
\Delta_{it} = (NL_{it} - NL_{baseline}),
$$

where $NL_{it}$ = cell count or cytokine level at time $t$ for person $i$. $NL_{baseline}$ = 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 $\Delta_{it}$. The $\Delta_{it}$ for person $i$ at time $t$ for $S_A$ is referred to as $A_{it}$, for $S_B$ as $B_{it}$, and for $S_C$ as $C_{it}$. When $C_{it}$ exceeded the sum of $A_{it}$ and $B_{it}$, a greater-than-additive response occurred in person $i$ at time $t$. The magnitude of the enhanced response in person $i$ at time $t$ was determined by $E_{it}$, which $E_{it} = [C_{it} - (A_{it} + B_{it})]$. At each time $t$, a we used Wilcoxon signed-ranks test to determine whether the distribution of the $E_{it}$ 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 $E_{it}$ values have a positive central location; if the $E_{it}$ 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 $E_{it}$ 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 \( \leq 0.1 \) 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 \( \Delta t \) 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 \( \Delta t \) 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 \textit{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 \( \sigma_G \), a measure of particle size distribution, of 1.52 ± 0.12. The value of \( \sigma_G \) 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 182%, 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 atotics 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 atotics, 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 atotics 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-\( \alpha \), or total IgE in the group of subjects as a whole or within strata of atotics and nonatotics. 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 (a Th2-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.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age</th>
<th>Sex</th>
<th>Atopic status</th>
<th>Allergens with positive skin prick test</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>28</td>
<td>Female</td>
<td>Atopic</td>
<td>Red oak, grass mix, mite*</td>
</tr>
<tr>
<td>2</td>
<td>39</td>
<td>Male</td>
<td>Atopic</td>
<td>Ragweed*</td>
</tr>
<tr>
<td>3</td>
<td>38</td>
<td>Male</td>
<td>Nonatopic</td>
<td>N/A</td>
</tr>
<tr>
<td>4</td>
<td>27</td>
<td>Male</td>
<td>Nonatopic</td>
<td>N/A</td>
</tr>
<tr>
<td>5</td>
<td>30</td>
<td>Female</td>
<td>Nonatopic</td>
<td>N/A</td>
</tr>
<tr>
<td>6</td>
<td>28</td>
<td>Male</td>
<td>Atopic</td>
<td>Ragweed*</td>
</tr>
<tr>
<td>7</td>
<td>30</td>
<td>Female</td>
<td>Atopic</td>
<td>Red oak, mite*</td>
</tr>
<tr>
<td>8</td>
<td>32</td>
<td>Female</td>
<td>Atopic</td>
<td>Red oak, grass mix, mite, *</td>
</tr>
</tbody>
</table>


### 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).

<table>
<thead>
<tr>
<th>NL parameter</th>
<th>Baseline</th>
<th>IA</th>
<th>4 hr</th>
<th>18 hr</th>
<th>42 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukocytes/mL</td>
<td>15,200</td>
<td>21,700*</td>
<td>14,900</td>
<td>19,200</td>
<td>1,900</td>
</tr>
<tr>
<td>Neutrophils/mL</td>
<td>13,900</td>
<td>18,000*</td>
<td>11,700</td>
<td>17,200</td>
<td>900</td>
</tr>
<tr>
<td>Macrophages/mL</td>
<td>900</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-4 (pg/mL)</td>
<td>0.55</td>
<td>2.2**</td>
<td>2.2**</td>
<td>0.99</td>
<td>0.81</td>
</tr>
<tr>
<td>IL-8 (pg/mL)</td>
<td>220</td>
<td>422**</td>
<td>427**</td>
<td>294</td>
<td>458*</td>
</tr>
</tbody>
</table>

*See text for explanation of average enhanced response. **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).

<table>
<thead>
<tr>
<th>NL parameter</th>
<th>Baseline</th>
<th>IA</th>
<th>4 hr</th>
<th>18 hr</th>
<th>42 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukocytes/mL</td>
<td>10,200</td>
<td>29,700**</td>
<td>22,000*</td>
<td>32,000*</td>
<td>44,100</td>
</tr>
<tr>
<td>Neutrophils/mL</td>
<td>8,900</td>
<td>25,400**</td>
<td>17,100</td>
<td>28,700**</td>
<td>42,300</td>
</tr>
<tr>
<td>Macrophages/mL</td>
<td>900</td>
<td>2,200</td>
<td>1,200</td>
<td>2,200</td>
<td>–100</td>
</tr>
<tr>
<td>IL-4 (pg/mL)</td>
<td>0.88</td>
<td>3.23*</td>
<td>3.18*</td>
<td>1.79*</td>
<td>1.63</td>
</tr>
<tr>
<td>IL-8 (pg/mL)</td>
<td>213</td>
<td>179*</td>
<td>215</td>
<td>191</td>
<td>172</td>
</tr>
</tbody>
</table>

*See text for explanation of average enhanced response. **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/m³) 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 particular 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 pollen 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; Tunncliffe et al. 2001). Tunncliffe 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 NO₂ on the early asthmatic response (maximum percent change in forced expiratory volume in 1 sec [FEV₁] during the first 2 hr after challenge) and the late asthmatic response (maximum percent change in FEV₁) to inhaled allergen. Strand and co-workers (1997) studied 18 subjects with asthma and allergy to pollen. Subjects were exposed to low-level NO₂ (490 mg/m³) for 30 min, followed by 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 NO₂ exposure, and peak expiratory flow after allergen challenge was on average 6.6% lower after NO₂ 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 studying whether particularites 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 1. 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 alone, 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 (Tunncliffe et al. 2001). Subjects were exposed to sulfuric acid particulates at concentrations of 0.1 and 1.0 mg/m³ 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 FEV₁ during the first 2 hr after allergen challenge. The difference between the 1 mg/m³ sulfuric acid exposure and air was −4.3% [95% confidence interval (CI), −1.2 to −7.4], and the 0.1 mg/m³ 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 allergic 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


