ENHANCED ALLERGIC SENSITIZATION IN ANIMALS EXPOSED TO PARTICULATE AIR POLLUTION

M. I. Gilmour, M. J. K. Selgrade
Experimental Toxicology Division, National Health and Environmental Effects Research Laboratory, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina, USA

A. L. Lambert
Curriculum in Toxicology, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA

Epidemiological studies have found an association between elevated levels of particulate matter (PM) air pollution and increased medication use and hospital visits by asthmatics. While it is known that asthmatics are generally more sensitive to airborne contaminants such as sulfur dioxide and tobacco smoke, it is difficult to test which components of air pollution may also contribute to the induction of pulmonary allergy (sensitization) because of the risk in creating disease. Recent studies in mice and rats, however, have demonstrated that pulmonary exposure to combustion particles such as diesel and residual oil fly ash (ROFA) can exacerbate immunological sensitization (in the form of immunoglobulin E antibody and lymphocyte reactivity) to experimental and natural allergens. Subsequent allergen challenge in these animals results in a greater allergen-induced bronchoconstriction, elevated numbers of eosinophils in the lung, and enhanced airway responsiveness to cholinergic agents compared to what occurs in similarly immunized animals pretreated with vehicle or “inert” particles. Although the mechanisms for these effects are not known, it has been demonstrated that the adjuvant effects of diesel and ROFA can be reproduced with hydrocarbons and soluble transition metals from diesel and ROFA, respectively. In addition, analysis of mediator expression and release over the sensitization phase has revealed that PM exposure can enhance production of Th2 cytokines such as interleukin-5 (IL-5) and the proinflammatory cytokine tumor necrosis factor-alpha (TNF-α). These experimental systems demonstrate the potential of particulate air pollutants to enhance allergic sensitization and can be further used to elucidate the mechanism for these effects.

The prevalence of allergic asthma is increasing in Western societies and currently affects about 17 million people in the United States (CDC, 1998). Although the reasons for this upward trend are not known, it is generally agreed that environmental exposures and changes in lifestyle are involved in both disease pathogenesis and disease incidence. Increased sensitivity to allergens

The authors are grateful to Dr. S. H. Gavett for careful review of the manuscript. This article is not subject to U.S. copyright laws.

The research described in this article has been reviewed by the National Health and Environmental Effects Research Laboratory. Approval does not signify that the contents necessarily reflect the views and policies of the agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

Address correspondence to Dr. M. Ian Gilmour, Mail Drop 92, U.S. Environmental Protection Agency, Research Triangle Park, NC 27711, USA. E-mail: gilmour.ian@epa.gov
has been reported following viral infection (Welliver & Duffy, 1993), exposure to second-hand cigarette smoke (Martinez et al., 1992), and in obese children (Luder et al., 1998). In addition, the introduction of “tight housing” with lower ventilation rates and more time spent indoors may contribute to the induction of asthma by increasing exposure to indoor allergens (Institute of Medicine, 1993). Air pollution is also a potential risk factor for asthma. Several epidemiological studies have demonstrated that during periods of high air pollution, hospital admissions and medication use for asthma rise (Peters et al., 1997; Lipsett et al., 1997). In contrast to the clear association between poor air quality and exacerbation of asthma, the role that air pollutants play in the actual induction of asthma (allergic sensitization) is controversial. Comparative studies between East and West Germany before unification showed that asthma was more prevalent in the West, while industrial air pollution and levels of bronchitis were higher in the East (von Mutius et al., 1992). It has been argued, however, that intrinsic differences in lifestyle between Eastern and Western Blocs (e.g., occupancy rates, diet, rate of infection, antibiotic use, and immunization practices) may have confounded the direct comparison of asthma incidence and its association with air pollution levels (Bjorksten, 1997). Further analyses of respiratory allergies between polluted and “clean” counties in East Germany have since shown a strong association between sensitization and levels of air pollution (Heinrich et al., 1999). Also, several studies in Europe and Japan have reported an increased frequency of allergic sensitization in individuals exposed to higher levels of vehicle exhaust while living in urban areas or close to highways (Devalia et al., 1996).

While the epidemiological evidence suggests an interaction between exposure to certain air pollutants and allergic sensitization, controlled clinical studies cannot address this issue without the risk of sensitizing human subjects. The role of air pollutants as immunologic adjuvants in already sensitized individuals has been examined, however, and diesel exhaust particles (DEP), at least, may potentiate certain specific immune responses. In a study of allergic rhinitics and mild asthmatics, Diaz-Sanchez et al. (1994) found that intranasal exposure to 0.3 mg DEP significantly enhanced immunoglobulin (Ig) E and IgG4 antibody production, but had no effect on other IgG subclasses, IgA, or IgM antibody. They also reported that IgE- but not IgA-forming cells were increased in the nasal wash after DEP challenge, and mRNA coding for a variety of epsilon heavy chain variants was increased. The same group (Takenaka et al., 1995) later stimulated peripheral blood mononuclear cells from healthy human donors with interleukin-4 (IL-4) and anti-CD40 antibody along with organic extracts from diesel exhaust particles, and found that IgE production was increased compared to stimulated controls incubated with the organic extract vehicle. These studies demonstrated that DEP might act as an immunological adjuvant to boost IgE production in both naïve and antigen-sensitized cells.

Experimental animal models of human asthma have also shown that air pollutants exacerbate pulmonary allergy. Matsamura (1970) first reported that guinea pigs exposed to high levels of O₃, NO₂, or SO₂ prior to ovalbumin
sensitization had augmented allergic responses upon antigen challenge. Several studies have since reproduced these effects at lower concentrations of pollutant in a variety of different species (reviewed in Gilmour, 1995). With respect to particles, experiments have shown that diesel exhaust particles behave as adjuvants (reviewed in Nel et al., 1998). Muranaka et al. (1986) first demonstrated that IgE antibody production was increased in mice immunized intraperitoneally with ovalbumin mixed with diesel exhaust particles compared to mice that received antigen alone. They subsequently administered antigen and DEP into the nostrils of mice (Takafuji et al., 1987) and found that the inclusion of the DEP in the immunization mixture dramatically enhanced levels of antigen-specific IgE compared to antigen exposure alone. Using a similar experimental design, Fujimaki et al. (1994) compared lymphocyte proliferation and IL-4 production in the mediastinal lymph nodes (MLN) of mice immunized with ovalbumin via the trachea with or without addition of DEP particles. They found that DEP enhanced lymphoproliferative responses to antigen to 4–17 times control levels and that this effect was associated with increased IL-4 production and IgE antibody in MLN culture supernatants and sera, respectively.

Another major component of urban air pollution is particulate matter from combustion sources including fossil-fueled power plants (Natusch et al., 1974). Residual oil fly ash (ROFA) is a product of oil combustion by power plants and contains sulfates and soluble transition metals, namely iron (Fe), vanadium (V), and nickel (Ni) (Hatch et al., 1985; Dreher et al., 1997). These metals have well-documented human health effects. Vanadium is associated with boilermaker’s bronchitis (Levy et al., 1984); nickel is a known contact sensitizer (Goebler et al., 1993; Motolese et al., 1992) and inducer of allergic asthma (Novey et al., 1983); and iron inhalation causes siderosis, a nonfibrotic lung disease (Nemery, 1990). Recent animal studies have shown that these soluble metals mediate ROFA’s pulmonary toxicity in rats (Kodavanti et al., 1997; Dreher et al., 1997). Our objective was to determine whether pulmonary exposure to ROFA could act as an adjuvant for allergic sensitization in rats in a fashion similar to that seen in mice exposed to diesel particles. In addition, since soluble transition metals are the injurious components in ROFA, we investigated whether these metals were responsible for enhanced allergic sensitization seen with ROFA exposure, and which cytokines were associated with these effects.

**APPROACH**

The rat model of pulmonary allergy to house dust mite and subsequent investigations of particle-enhanced immune function have been described in detail elsewhere (Lambert et al., 1998, 1999, 2000). In these experiments, 10- to 12-wk-old female Brown Norway rats were anesthetized with halothane and instilled via a tracheal cannula with up to 1 mg ROFA; Mount St. Helens Ash (MSH) as an inert particle; or equivalent concentrations of each metal sulfate (NiSO$_4$, 105.12 µg; FeSO$_4$, 58.49 µg; and VSO$_4$, 98.2 µg per rat) found in 1 mg
of a preparation of ROFA (described in detail by Dreher et al., 1997). Three and 5 days later the rats were sensitized to house dust mite (HDM) allergen with two intratracheal instillations of 10 μg purified Der f1 antigen (Figure 1). After 2, 7, and 14 days, animals were euthanized and antigen-specific antibody and lymphoproliferative responses were measured in the serum and bronchial lymph nodes, respectively. Total RNA was extracted from the left lung lobe for the measurement of mRNA expression of an array of immune cytokines by reverse-transcription polymerase chain reaction (RT-PCR). Additional animals were challenged with house dust mite allergen 14 days after sensitization and airway responses, eosinophil numbers and biochemical markers of lung injury in lung fluid and immune parameters in lymph nodes and serum were monitored immediately and at 2 and 7 days after challenge.

**RESULTS AND DISCUSSION**

The ROFA or metal sulfate exposure caused significant pulmonary inflammation in the form of increased protein levels and influx of monocytes and
granulocytes, compared to instillation with acidified saline or inert Mount St. Helens particles (MSH). These effects were evident 2 days after allergic sensitization but subsided to baseline levels by 7 days. The development of immune responses to house dust mite (in the form of specific antibody and lymphoproliferative responses) was increased in the ROFA-treated animals during the sensitization phase, and this enhancement was magnified after a subsequent antigen challenge (Table 1). In addition, immediate airway responses to antigen challenge were significantly increased in the ROFA-exposed animals, and pulmonary eosinophilia measured 2 days after challenge was more severe.

Instillation of the various metal sulfates produced similar adjuvant effects to those seen with ROFA, although certain metals appeared to affect different endpoints. Nickel strongly enhanced the production of specific IgE to HDM and associated immediate airway responses after antigen challenge. Pretreatment with iron, on the other hand, did not affect antibody responses, but resulted in increased pulmonary inflammation after the antigen challenge. Instillation of the metal mixture resulted in effects similar to that seen with ROFA except the increased pulmonary eosinophilia was not significantly different from controls.

In an effort to explain the increased immune responses and subsequent lung injury after pretreatment with ROFA or its associated metals, molecular and protein analyses of proinflammatory and Th2 cytokines were performed on pulmonary lymph nodes and lung tissue soon after allergen sensitization, and again after the challenge procedure. The mRNA expression of each of the cytokines listed in Table 2 was upregulated after allergen sensitization, although ROFA or metal treatment did not initially influence this effect. In contrast, TNF-α protein was increased in the ROFA treated animals 2 days after sensitization, and by 7 days, IL-5 cytokine mRNA expression was also increased in these animals. After antigen challenge, the ROFA- and metal-treated animals all displayed higher mRNA and protein levels for most of the cytokines compared to sensitized and challenged animals treated with MSH (Table 2).

<table>
<thead>
<tr>
<th>Exposure</th>
<th>IgE</th>
<th>Immediate response</th>
<th>Lymphocyte proliferation to antigen</th>
<th>Eosinophils</th>
</tr>
</thead>
<tbody>
<tr>
<td>ROFA</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Ni</td>
<td>+</td>
<td>=</td>
<td>=</td>
<td>=</td>
</tr>
<tr>
<td>Fe(II)</td>
<td>=</td>
<td>+</td>
<td>=</td>
<td>+</td>
</tr>
<tr>
<td>V</td>
<td>+</td>
<td>=</td>
<td>=</td>
<td>=</td>
</tr>
<tr>
<td>Ni + Fe(II) + V</td>
<td>+</td>
<td>=</td>
<td>=</td>
<td>=</td>
</tr>
<tr>
<td>Saline/MSH</td>
<td>=</td>
<td>=</td>
<td>=</td>
<td>=</td>
</tr>
</tbody>
</table>

Note. +, Greater levels of immune function and immediate airway responses than those observed with dust mite alone (=). MSH is Mount St. Helens ash as an inert control particle.
TABLE 2. Adjuvant Effects of ROFA or Its Constituent Metals on Immune Cytokine Levels in Rats Sensitized to House Dust Mite

<table>
<thead>
<tr>
<th>Exposure</th>
<th>IL-4</th>
<th>IL-5</th>
<th>IL-6</th>
<th>IL-10</th>
<th>IL-13</th>
<th>TNF-α</th>
</tr>
</thead>
<tbody>
<tr>
<td>ROFA</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Ni</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Fe(II)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>V</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Ni + Fe(II) + V</td>
<td>=</td>
<td>=</td>
<td>ND</td>
<td>ND</td>
<td>=</td>
<td>ND</td>
</tr>
<tr>
<td>MSH/saline</td>
<td>=</td>
<td>=</td>
<td>ND</td>
<td>ND</td>
<td>=</td>
<td>ND</td>
</tr>
</tbody>
</table>

Note. +, Greater levels of mRNA cytokine message or cytokine protein compared to levels in house dust mite control animals. MSH is Mount St. Helens ash as an inert particle control. ND, not detected.

Our results suggest that the ROFA or metal treatment prior to allergic sensitization affected the initial priming process such that after a subsequent allergen challenge, the secondary immune responses and subsequent lung injury were significantly increased. Although the mechanisms for these effects are not known, we speculate that pulmonary inflammation may play a role in this adjuvancy. Proinflammatory mediators such as IL-6 and TNF-α are produced by bronchial epithelial cells and macrophages after particulate exposure (Becker et al., 1996; Carter et al., 1997), and may promote allergic immune responses. IL-6 stimulates the polarization of naïve T cells (Th0 cells) to Th2 cells via induction of IL-4 gene expression (Rincon et al., 1997) and influences IgE synthesis (Vercelli et al., 1989). TNF-α stimulates B lymphocyte activity (Kehrl et al., 1987) and is increased in the lungs of asthmatics (Casale et al., 1996), where it facilitates extravasation of leukocytes into the tissue via upregulation of vascular cell adhesion molecule 1 (VCAM-1) (Klein et al., 1989; Moser et al., 1992). In addition to the activation processes just described, pulmonary inflammation also features edema from epithelial damage, which may result in a loss of barrier function and enhanced translocation of inhaled antigen to the submucosa (Matsamura, 1970).

Whether the mechanism for particulate-enhanced allergic responses in rodents lies with generalized pulmonary inflammation, changes in epithelial permeability, or other alterations in immunological priming, more research is needed to determine which components of ambient air pollutants produce these adjuvant effects and at what concentrations. Although several broad epidemiological surveys have not found an association between ambient air pollution levels and the incidence of human asthma in the United States, the relationship between personal pollutant exposure and the rate or degree of allergic sensitization is not known. Thus, before these animal studies are discounted because of perceived species differences or experimental design, a better understanding of asthma incidence and of individual exposure to both indoor and outdoor contaminants is needed. Furthermore, the extent of acute or chronic lung injury following exposure to high concentrations of vehicu-
lar emissions and other air pollutants in urban environments has not been adequately characterized. While there is sure to be variability in individual responsiveness as occurs with, for example, ozone exposure (McDonnell et al., 1985), individuals genetically predisposed to develop diseases such as asthma may also be more susceptible to other forms of respiratory insult, which could enhance the process of allergic sensitization.

REFERENCES


