Air Pollutant-Enhanced Respiratory Disease in Experimental Animals

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Studies in animals have shown that a wide range of airborne particulates including cigarette smoke, acid aerosols, metals, organic compounds, and combustion products can interfere with the normal defense processes of the lung to enhance susceptibility to respiratory infection or exacerbate allergic diseases. Such detrimental effects are less easy to quantify in humans because of the difficulties in obtaining comprehensive exposure history and health status in large populations and because of the inherent dangers of inducing disease in clinical studies. In this article we describe examples of how air pollutants affect lung disease in experimental animal systems. This information can be used to predict the health risk of simple and complex exposures and to lend insight into the mechanisms of air pollution toxicity. *Key words*: air pollution, bacteria, combustion, eosinophil, immunity, infection, lung, lymphocyte, macrophage, phagocyte allergy. — *Environ Health Perspect* 109(suppl 4):619–622 (2001).
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Numerous epidemiologic studies have found that hospital admissions for pneumonia, asthma, and bronchitis increase during air pollution episodes (*1–3*). Although the specific mechanisms for these increases are not known, it is generally accepted that pollutant exposure can exacerbate preexisting respiratory illnesses and may possibly also enhance the development of new disease. Although early reports focused on the effects of pollutant exposure on pulmonary infection, the increased incidence of asthma in recent decades has prompted investigations into how toxicant exposure affects development of allergic responses and immune-mediated lung disease. In this article we illustrate how exposure to air pollutants from combustion sources can exacerbate infectious and allergic lung disease in rodents and discuss potential mechanisms for these effects.

The interaction of particles and microbial pathogens has been most extensively studied with the streptococcus infectivity model. Coffin and Blommer (*4*) first showed that mice exposed to irradiated automobile exhaust were more susceptible to a subsequent pulmonary infection with *Streptococcus zooepidemicus*. Later, it was also established that the same ranking of toxicity for a number of metal salts occurred whether the animals were exposed by inhalation or were instilled with an estimated dose of material based upon the concentration of aerosol exposure and measured pulmonary deposition (*5*). Subsequent work then showed that instillation of 100 µg of bentonite, oil fly ash, metal salts (CdO, ZnO, $NaAsO_2$, $SnCl_2$, and $CoNO_3$), and ambient air particles collected in Germany enhanced mortality to infection by more than 50% (*6*), whereas other particulates characterized as having intermediate potency (< 50% excess mortality) included an ambient air particle sample from Washington DC, three coal fly ash samples, powdered latex, BeO, and Fe_2O_3. Low-potency particles, which did not enhance mortality significantly, included samples of coal fly ash, an ambient air sample from St. Louis, Missouri, and volcanic ash from Mount St. Helens, Washington. In other published studies, animals exposed by inhalation to particulates such as carbon black (*7*), coal dust (*8*), and titanium dioxide (*9*) had decreased intrapulmonary killing of bacteria, further demonstrating that particulate inhalation affects the bactericidal activity and/or clearance mechanisms of the respiratory tract.

In contrast to reduced immune function, which results in increased susceptibility to infection, elevated immune responses to allergens (hypersensitivity) occurs in a significant percentage of the population to produce a variety of allergic disease, including asthma (*10*). Initial studies in animals exposed to particulates showed that diesel exhaust particles (DEPs) have an adjuvant effect on allergic immune responses. Muranaka et al. (*11*) first reported that IgE antibody production in mice immunized intraperitoneally with ovalbumin mixed with DEPs increased compared to that in mice given antigen alone. They subsequently administered antigen and DEPs into the nostrils of mice and found that production of immune cytokines, lymphocyte activity in pulmonary lymph node, and levels of antigen-specific IgE were enhanced by DEPs (*12,13*). Further studies have shown that the organic (hydrocarbon) component of DEPs may cause these adjuvant effects (*14*) and that similar effects can occur with other combustion materials such as oil fly ash (*15*).

Increased interest in the health effects of particulate air pollution and recent debate over regulatory standards in the United States and Europe have prompted additional research into which types and concentrations of pollutants affect respiratory health. Below we summarize two studies from our laboratory that illustrate particulate matter (PM)-enhanced infectious and allergic lung disease in rodents. We then discuss potential mechanisms for these effects and offer future directions to correlate animal studies with potential health effects in humans.

Approach

Infectivity Study

The streptococcus infectivity model has been used extensively to study the effect of airborne pollutants on host defenses in mice (*16*). The purpose of this present study was to compare the effect of inhaled woodsmoke and oil furnace emissions on susceptibility to infection. The oil emissions from No. 2 fuel oil were generated by a Williamson model 1167-15 residential oil furnace (Williamson-Thermoflo, Milwaukee, WI, USA), operating on a 10-min on, 20-min off cycle. The woodsmoke was generated from split oak cordwood (moisture content 20–25%) and burned in a Lopi "Answer" noncatalytic stove (Travis Industries, Inc., Kirkland, WA, USA) at a rate of 2–3 kg per hour. New wood was added every 2 hr. In each case the emissions were diluted with HEPA filtered air and piped to a 0.3 m^3 Rochester inhalation chamber. Emissions were monitored continuously for gas and particle concentrations in the inlet pipe to the exposure chamber (Table 1). Five-week-old CD1 mice (Charles River, Raleigh, NC, USA) were exposed for 4 hr to diluted woodsmoke (1:20)

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The research described in this article has been reviewed by the National Health and Environmental Effects Research Laboratory, U.S. Environmental Protection Agency, and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the agency. The animal studies described below were reviewed and accepted by our institutional animal welfare committee.

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Table 1. Summary of woodsmoke and oil furnace emission characteristics.

Parameter	Oil furnace emission	Wood smoke emission
Carbon dioxide (ppm)	7,291 ± 439.7	5,734 ± 58.7
Carbon monoxide (ppm)	2 ± 1.5	200 ± 29.3
Total hydrocarbons (ppm)	11.1 ± 0.88	ND
Oxides of nitrogen (ppm)	4.4 ± 0.35	2.2 ± 0.1
Chamber temperature (°F)	73 ± 0.7	71 ± 0.37
Relative humidity (%)	ND	56 ± 1.9
Chamber flow (cfm)	9	10.7
Total suspended particles (mg/m^3)	0.54 ± 0.255	1.9 ± 0.32

ND, not done.

Table 2. Effect of woodsmoke or oil emission exposure on mortality to streptococcal infection in mice.

	Oil furnace		Woodstove	
	Air control	Exposed	Air control	Exposed
Mortality	6	0	0	21
Number dead/total	1 of 18	0 of 18	0 of 38	8 of 38

or diluted oil furnace emissions (1:5) at airborne concentrations of 1.93 and 0.54 mg/m^3, respectively (Table 1). Air-exposed and emission-exposed mice were then infected by aerosol with *Streptococcus zooepidemicus* and mortality was monitored over a 20-day period. Under these conditions the oil furnace emissions did not significantly enhance susceptibility to infection (Table 2). Exposure to wood stove emissions, however, resulted in increased mortality to infection (8 of 28) compared to air-exposed controls (0 of 28). Our current studies assess host immune responses following exposure to these emissions and determine the physicochemical components responsible for the observed effects.

Allergy Study

In a model of immune-mediated pulmonary hypersensitivity developed in our laboratory, Brown Norway (Charles River) rats immunized with house dust mite (HDM) allergen produce high levels of specific IgG and IgE antibody compared with those of controls (*17*). Subsequent intratracheal antigen challenge causes a rapid bronchoconstriction, an eosinophilic inflammatory response, and increased mucus-producing goblet cells in the airways. At the peak of inflammation (1–2 days postchallenge), the rats have increased airway responses to acethylcholine, indicating enhanced airway responsiveness.

In this experiment we examined whether intratracheal instillation of 1 mg residual oil fly ash (ROFA) 3 days before local sensitization or 3 days before allergen challenge could alter antibody levels, immune-mediated inflammation, and allergen-induced airway hyperresponsiveness. Ten- to 12-week-old female Brown Norway rats were anesthetized with halothane and instilled via a tracheal cannula with 1 mg of ROFA or acidified saline. One day later the rats were sensitized with an intratracheal instillation of 17 µg purified HDM allergen. Two weeks later the animals were challenged with the antigen and after a further 24 hr, airway hyperresponsiveness, eosinophil numbers, and immune parameters in lymph nodes and serum were assessed.

ROFA instillation 3 days prior to sensitization resulted in increased levels of IgG and IgE antibody (Figure 1) but no differences in immune-mediated inflammation or airway hyperresponsiveness 1 day postantigen challenge (Figures 2, 3). ROFA treatment 3 days prior to challenge did not affect antibody levels (Figure 1) but enhanced pulmonary eosinophil numbers (Figure 2) and airway hyperresponsiveness (Figure 3) compared to saline-instilled allergic animals or nonimmunized rats instilled with ROFA. Exposure to ROFA prior to both sensitization and challenge procedures resulted in significant increases in all the immune, inflammatory, and physiologic parameters,

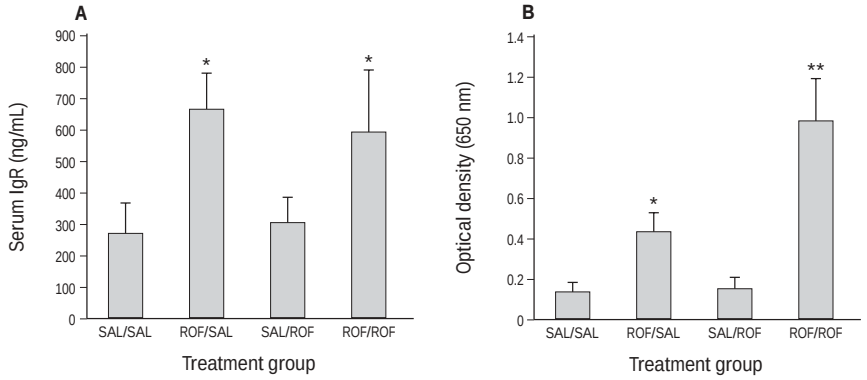

Figure 1. Antigen-specific IgG and total IgE levels in the serum of rats exposed to oil fly ash 3 days before allergic sensitization (ROF/SAL) with HDM, 3 days before allergen challenge (SAL/ROF), or 3 days before both sensitization and challenge (ROF/ROF). SAL, saline; ROF, ROFA. All measurements reflect data obtained 2 days after antigen challenge. Single asterisk (*) and double asterisk (**) indicate significantly different ($p < 0.05$) from saline controls (SAL/SAL) and between all other treatments, respectively; $n = 4–6$ rats.

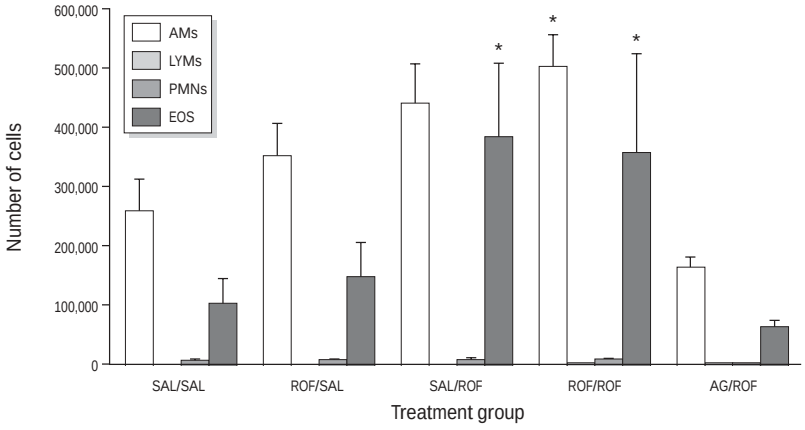

Figure 2. Number of pulmonary inflammatory cells [alveolar macrophages (AMs), lymphocytes (LYM), PMNs, and eosinophils (EOS)] in the bronchoalveolar lavage fluid of rats exposed to 1 mg of ROFA 3 days before allergic sensitization (ROF/SAL) with HDM, 3 days before allergen challenge (SAL/ROF), or 3 days before both sensitization and challenge (ROF/ROF). All measurements reflect data obtained 2 days after antigen challenge. *Significantly different ($p < 0.05$) from saline controls (SAL/SAL). AG/ROF represents unsensitized animals instilled with ROFA and challenged with HDM; $n = 4–6$ rats.

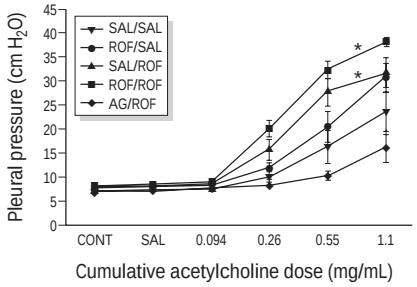

Figure 3. Pleural pressure measurements following stepwise increases of intravenous acetylcholine in rats exposed to ROFA 3 days before allergic sensitization (ROF/SAL) with HDM, 3 days before allergen challenge (SAL/ROF), or 3 days before both sensitization and challenge (ROF/ROF). All measurements reflect data obtained 2 days after antigen challenge. *Significantly different ($p < 0.05$) from saline controls (SAL/SAL). AG/ROF represents unsensitized animals instilled with ROFA and challenged with HDM ($n = 4–6$ rats).

compared to saline-instilled controls, which received HDM or ROFA alone.

Discussion

The experiments described here illustrate that exposure to PM can enhance the severity of infectious and allergic lung disease in rodents. We have previously shown that ozone-enhanced susceptibility to the streptococci is directly related to the phagocytic activity of the alveolar macrophages (*16,18*). Although we did not assess phagocytosis after the emission exposures described above, other studies have demonstrated that exposure to particulates such as carbon black (*7*), smoke (*19*), lead oxide (*20*), titanium dioxide (*9*), and road dust (*21*) reduce macrophage phagocytosis and impair pulmonary clearance of inhaled bacteria. In addition, a number of *in vitro* studies using both animal and human cells have also reported that PM or its toxic constituents reduces alveolar macrophage function. For example, exposure to acrolein or benzofuran adsorbed onto carbon black lowered rat alveolar macrophage phagocytosis (*22*), and human peripheral blood monocytes had decreased phagocytic activity after incubation with a particulate air sample collected from an industrial area in Germany (*23*). Possible mechanisms for reduced macrophage phagocytosis include intracellular overloading of particulate, direct toxicity of internalized or extracellular particles, and the co-production of suppressive mediators such as prostaglandins and corticosteroids.

Although there is less information about how particle exposure affects the acquired immune system, it is known that a proportion of urban air particulates is derived from combustion of fossil fuels and industrial discharges and may contain metals, solvents, aromatic hydrocarbons, and other chemicals that can modulate specific immune function. Of the metals, cadmium, vanadium, chromium, lead, and nickel have been most extensively studied and shown to decrease antibody formation, antigen processing, and lymphocyte proliferation in experimental animals (*24,25*). Organic compounds that show immunotoxic properties—benzene, trichloroethylene, dioxins, phenols, organotonins, and diester phorbol compounds—are also found in the atmosphere in varying concentrations and have been shown experimentally to alter immune function (*26*).

Taken together, these studies show that exposure to airborne particulates and especially to those containing toxic chemicals can affect immune function. This could reduce the development of protective immunity during initial infection, slow the clonal expansion of memory cells in response to re-infection, or impair vaccine effectiveness. Additional research is warranted to determine the concentrations and types of particles responsible for these effects.

With respect to allergic lung disease, several epidemiologic and experimental studies offer compelling evidence that airborne particulates can exacerbate asthma. Most asthmatics display nonspecific bronchial hyperresponsiveness to a wide range of inhaled substances including cigarette smoke, hypertonic saline, and cold air. These challenges are not antigenic in nature but rather behave as irritants in provoking inflammation and/or bronchoconstriction. Similar effects have been seen in allergic animals exposed to ROFA as illustrated here and in previous reports (*27,28*). This general increase in airway responsiveness and lung injury through dual exposure to allergen and PM may be an additive effect of two proinflammatory stimuli, although synergistic relationships in which both stimuli are required to elicit a pulmonary response are also possible.

In addition to exacerbating existing disease, there is also evidence that exposure to combustion materials may act as an adjuvant to enhance initial sensitization to allergen. In the experiments described here, and in our previous studies (*29,30*), ROFA exposure prior to sensitization resulted in increased IgE antibody and severity of allergic lung disease. We have since demonstrated that these effects can be mimicked by the administration of the pluri-potent cytokine tumor necrosis factor-α (*31*), suggesting that the mechanism for the adjuvant effects of combustion materials may be related to inflammatory processes at the site of immunization. Further experiments are needed to determine the factors that cause this effect and under what circumstances the immune response is directed toward an allergic (Th2) or a protective (Th1) response.

In conclusion, there are many examples in the literature that report increased incidence and severity of respiratory disease in experimental animals following exposure to particulate and/or gaseous air pollutants. Although these studies provide biologic plausibility for the epidemiologic findings and offer clues for the mechanisms of these effects, additional effort is required in a number of directions. Toxicologic examination of PM samples from various sources and geographical areas will provide a physicochemical basis to contrast and compare health effects from various regions. Exposure studies that either harness or create realistic pollution episodes are also needed to study the effect of both acute and chronic PM exposure on healthy and, perhaps more importantly, diseased animals to better model real life situations. Finally, results from such research can be extrapolated from health effects in animals to predicting risk in exposed populations.

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