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Project Summary

Determining Effect of Pollutants on the Immune System

A. Zarkower, J. Davis, F. Ferguson, and D Strickler

The purpose of this project was to determine the effects of fly-ash inhalation on the ability of animals to resist infections, neoplastic growth, and the development of hypersensitive responses. Mice were exposed to fly ash from two sources, carbon black, and filtered air only. Following various exposure periods (days to months), the immunologic competence of lymphcytes, neutrophiles, and macrophages was assessed.

Fly-ash inhalation resulted in a decreased response in the spleens and mediastinal lymph nodes to *Escherichia coli* antigen given by aerosol. This suppression was much less severe than that following inhalation of carbon black and silica quartz. Fly ash had little effect on the ability of T and B lymphocytes to respond to mitogens PHA (phytohemagglutin (PHA) and lipopolysaccharide) and to be stimulated for cytolytic response against tumor cells. Both the *in vitro* response of the (BCG)-BACILLE CALETTE GUERIN-sensitized micre to purified protein derivative of tuberculin and *in vivo* delayed-type hypersensitive reactions were increased.

Exposure to fly ash had the following more pronounced effects on macrophages: a decrease in the number of pulmonary macrophages capable of phagocytosis; a decrease in antibody-dependent cell-mediated cytolysis (ADCC), in contrast to enhancement of ADCC after intratracheal injection of fly ash and silica and inhalation of silica; an increase in cytotoxie activity against H50 tumor cells after intratracheal injection of fly ash and a decrease after inhalation of fly ash; and decreased ability to activate T cell mitogenesis after fly-ash inhalation.

This Project Summary was developed by EPA's Health Effects Research Laboratory, Research Triangle Park, NC, to announce key findings of the research project that is fully documented in a separate report of the same title (see Project Report ordering information at back).

Introduction

The lung is the primary exposure site for gases and for particles smaller than 2 μm in diameter After inhalation, toxic substances may interact with surface proteins and cells or may enter the body and be carried to such organs as the lymphoid tissues, liver, and kidneys, which are the principal organs of clearance, detoxification, and excretion. Although exposure to airborne substances can also result in absorption by the gastrointestinal tract and the skin, the lung accepts the greatest burden.

Inhalation of toxic substances can cause disease either by direct damage to lung tissues or by affecting other organ systems. Direct lung damage often follows exposure to high concentrations of toxic minerals (silica, asbestos) or gases (ozone, nitrogen dioxide). Substances that can have indirect effects include lead, which can affect a

variety of tissues (gastrointestinal tract, nervous system, and blood); cadmium, which can cause kidney damage and osteomalacia; and mercury, which can affect the central nervous system. Gases and organic and inorganic particulates have a variety of effects on immunological responses in the body, including suppression or enhancement of antibody formation, cellular immunoresponses, and phagocytic activities of macrophages. Decreased antibody responses may increase susceptibility to infectious diseases; increased stimulation of specific types of antibodies may increase resistance to infectious diseases, but may also lead to immunopathological conditions such as immediate hypersensitivity and immune complex diseases. Decreased cellular immune responses may decrease resistance to certain infectious agents and certain neoplastic processes, while increased cellular immunocompetence may manifest itself as delayed-type hypersensitivity. Changes in neutrophile and macrophage functions also can affect resistance to both infectious and neoplastic diseases

In the near future, expanded use of coal for power generation and as stock for liquid and gaseous fuels is expected to yield a number of products (e g., SO_2, NO_2, hydrocarbons, and particulates) that could react with biological systems. Fly ash will be a large component of the se coal-derived air pollutants, and it is composed of many elements that can cause cell damage. Since inhaled particulates will come into intimate contact with cells of the immune system (macrophages and lymphocytes), changes in immunological responses may occur, leading ultimately to decreased ability to cope with infectious agents and neoplastic cells. This project was designed to test the effects of fly-ash inhalation on cells involved in the immune response and their functions.

Exposure Facilities

Female BALB/c mice were exposed to fly ash (and in some cases, carbon) in specially designed chambers. Compressed air was pulsed into a dustbed, and the particles entered an air stream passing through the dust-generator cavity. This air stream was mixed with a stream of filtered air before it entered the exposure chamber. Control chambers received air streams from the same sources, though without the particles. After varying lengths of time, the animals were removed from the chambers, and a variety of tests was done to determine the immunologic competence of lymphocytes, neutrophiles, and macrophages.

Results

In the first series of experiments, mice were exposed to carbon and fly ash (from the Pennsylvania State University (PSU) power plant) for 7, 21, 35, and 56 days. The total concentrations of the generated particulates and the proportions in the respirable size range (<2.1 μm) are given in Table 1. Inhalation of both fly ash and carbon decreased the numbers of antibody-forming cells in the spleen following stimulation of the mice with *Escherichia coli* antigen aerosols. The effect of carbon was much greater than that of fly ash. Antibody-forming activity in the mediastinal lymph nodes was enhanced after 7 days of exposure, followed by suppression after 21 days and recovery after 35 and 56 days. Carbon and fly ash both caused hypertrophy of the lymph nodes, with an increase in the number of lymphocytes at all times after exposure. Serum agglutinating activity also decreased.

Little change occurred in the lymphocyte responses to T and B cell mitogens. The recognition activity of the cells was decreased after a 21-day exposure, as was lymphocyte response to Con A. No significant change was found in the cytolytic capacity of T cells after any period of exposure.

A nine-month exposure to fly ash at somewhat higher concentrations resulted in a highly significant decrease in splenic antibody-forming cell response in mice exposed to *E. coli* lipopolysaccharide antigen by either aerosol or intratracheal injection. Plaque-forming cell responses in the mediastinal lymph nodes were lower in both cases, though not significantly.

In a second series of experiments, mice were exposed to carbon and fly ash provided by the U.S Environmental Protection Agency (EPA), as well as that from the PSU power plant. The total concentrations of the particulates and the proportions in the respirable size range are given in Table 2.

The number of antibody-forming cells in the spleens decreased after 7 and 24 days of exposure to fly ash, and decreased serum antibody activity was found after 7, 21, and 56 days of exposure. Carbon dust caused a very pronounced and progressive reduction in the number of antibody-forming cells in the spleens after all times of ex-

Table 1. *Particulate Exposure Concentrations Expressed as μg/m³ of Air*

		Mean Fly Ash ± Standard Error		*Mean Carbon Black ± Standard Error*	
Experiment	*Exposure Period*	*Total*	*< 2.1 μm*	*Total*	*< 2.1 μm*
Responses to antigenic stimulations in vivo	*7 days (7/28-8/4/77)*	*2667 ± 1417*	*655 ± 348*	*4736 ± 1683*	*947 ± 555*
	21 days (7/28-8/18/77)	*3357 ± 798*	*959 ± 236*	*4987 ± 1334*	*1387 ± 522*
	35 days (7/28-9/2/77)	*3220 ± 512*	*1009 ± 153*	*4805 ± 855*	*1561 ± 349*
	56 days (7/28-9/22/77)	*2658 ± 463*	*860 ± 140*	*4615 ± 700*	*1472 ± 269*
Responses to mitogens in vitro and recognitive activity of T cells	*7 days (8/8-8/15/77)*	*4976*	*1433*	*2461*	*859*
	21 days (8/8-8/30/77)	*3686 ± 647*	*1214 ± 110*	*4879 ± 1644*	*1815 ± 560*
	35 days (8/8-9/13/77)	*3281 ± 440*	*1130 ± 85*	*4301 ± 1049*	*1679 ± 363*
	56 days (8/14-10/11/77)	*2277 ± 350*	*840 ± 135*	*4956 ± 762*	*1642 ± 296*
Cytolytic activity of stimulated T cells	*7 days (9/2-9/9/77)*	*2417*	*909*	*2148*	*924*
	21 days (9/2-9/23/77)	*1535 ± 563*	*562 ± 230*	*4237 ± 1458*	*1291 ± 485*
	35 days (9/2-10/7/77)	*1732 ± 444*	*643 ± 182*	*4448 ± 1053*	*1220 ± 350*
	56 days (8/19-10/14/77)	*2137 ± 380*	*798 ± 151*	*4445 ± 670*	*1449 ± 267*

Table 2. *Concentration of Particulates Expressed as μg/m³ of Air*

Experiment	Exposure Period	Mean Fly Ash ± Standard Error		Mean Carbon Black ± Standard Error	
		Total	< 2.1 μm	Total	< 2.1 μm
In vivo responses to antigenic stimulations	7 days (11/16-11/23/77)	2232 ± 256	933 ± 77	4492 ± 1917	1538 ± 1029
	21 days (11/18-12/9/77)	2039 ± 343	785 ± 139	4707 ± 1192	1472 ± 386
	21 days (11/23-12/14/77)	2143 ± 286	764 ± 130	3608 ± 355	1104 ± 125
	35 days (12/8/77-1/12/78)	2059 ± 289	741 ± 86	2511 ± 346	857 ± 157
		*1628 ± 375	433 ± 77		
	56 days (12/15/77-2/9/78)	1502 ± 285	543 ± 100	2934 ± 459	841 ± 125
		*2634 ± 430	615 ± 98		
	148 days (12/22/77-5/19/78)	1459 ± 140	535 ± 50	3509 ± 312	1178 ± 119
		*1831 ± 221	461 ± 50		
In vitro responses to mitogens, recognitive activity of T cells and phagocytosis by macrophages	7 days (12/8-12/15/77)	2262 ± 298	794 ± 104	3180 ± 550	932 ± 161
		*1159 ± 228	366 ± 72		
	21 days (11/15-12/6/77)	2042 ± 343	809 ± 136	5399 ± 1372	1407 ± 429
	35 days (11/16-12/21/77)	2115 ± 235	804 ± 90	4027 ± 910	1248 ± 292
	56 days (1/10-3/7/78)	1334 ± 237	502 ± 86	2984 ± 512	885 ± 139
		*2674 ± 97	623 ± 97		
	148 days (12/13/77-5/9/78)	1297 ± 131	509 ± 48	3520 ± 342	1162 ± 130
		1879 ± 240	465 ± 55		
Cytolytic activity of stimulated T cells	7 days (1/13/77-1/20/78)	1905 ± 387	661 ± 134	3758 ± 1933	996 ± 512
		*3129 ± 1281	691 ± 283		
	35 days (11/29/77-1/3/78)	2113 ± 384	703 ± 117	3172 ± 526	909 ± 142
		*2819 ± 839	676 ± 194		
	56 days (1/11-3/8/78)	1134 ± 237	502 ± 86	2984 ± 512	885 ± 139
		*2674 ± 429	623 ± 96		
	154 days (12/20-5/23/78)	1459 ± 140	535 ± 50	3509 ± 312	1178 ± 119
		*1831 ± 221	461 ± 50		

*Exposure data for fly ash supplied by EPA.

posure, with almost complete suppression after 148 days of exposure. The effect of fly ash on the mediastinal lymph nodes varied from an increase in the number of antibody-forming cells after 7 and 21 days of exposure to no effect or a small decrease of response after 56 days. The cellular immune reactions were not affected, except for suppression of cytolytic activity by PSU fly ash after 35 days. Both fly ash and carbon caused a progressive decrease in phagocytic activity by lung-derived macrophages from 21 to 146 days of exposure.

A third series of experiments examined the effects of fly ash on various functions of the alveolar macrophage. Exposure of mice to PSU fly ash at concentrations of 742 μg/m³ of air of particles <2.1 μm for up to four weeks decreased the proportions of macrophages capable of phagocytosis and the proportion of very active macrophages (seven or more bacteria phagocytized).

For practical reasons, golden hamsters were used instead of mice in studies of antibody-dependent cell-mediated cytotoxicity by alveolar macrophages. Intratracheal injection of 2 mg silica into golden hamsters significantly enhanced the ADCC 1, 7, 14, 42, and 70 days after injection; intratracheal injection of 2 mg fly ash resulted in enhancement at 14, 42, and 70 days. Inhalation exposure of golden hamsters to respirable-sized silica at an average concentration of 3102 μg/m³ of air enhanced ADCC function slightly after 7 days and significantly after 14, 42, and 70 days. Inhalation of fly ash (5886 μg/m³) did not change ADCC after 7 and 14 days, but caused significant suppression after 42 and 70 days.

Intratracheal injection of BCG-primed hamsters with 2 mg silica activated alveolar macrophages to become tumoricidal against hamster H50 tumor cells, while injection of silica or BCG priming alone did not. Injection of fly ash activated alveolar macrophages to become cytotoxic against H50 tumor cells to an even greater degree than silica. Inhalation of fly ash for six weeks after BCG activation resulted in decreased pulmonary derived macrophage mediated cytotoxicity.

Addition of normal alveolar macrophages to spleen cell cultures enhances T-cell response to phytohemagglutin. This enhancement of mitogenesis was significantly less with the addition of alveolar cells from mice exposed to fly ash of respirable size (761 μg/m³ of air) for four weeks. After 70 days of exposure to fly ash (1494 μg/m³), macrophages from golden hamsters had a significantly increased enhancing effect on T lymphocytes' response to phytohemagglutin.

In a fourth series of experiments, mice were exposed to fly ash (1494 μg/m³, of respirable size) for 164 days and were sensitized with BCG four weeks before removal from exposure chambers. The spleen-derived lymphocytes of fly-ash-exposed animals were slightly more responsive to purified protein derivative of tuberculin than were those of controls. A parallel experiment measuring *in vivo* pulmonary sensitization (the delayed-type hypersensitive response) gave similar results. Further studies would be required to confirm these observations and to determine whether longer exposures would potentiate cellular immune sensitization and whether such a cellular reaction would be protective or lead to tissue damage.

For the fifth series of studies, mice were maintained in exposure chambers for one, two, three, and four weeks and exposed to PSU fly ash at 2577 ± 318 μg/m³ of air, with 25.72 ± 3.24% of the

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particles $< 2.1\ \mu m$ in diameter and $68.36 \pm 5.56\% < 4.7\ \mu m$. Differences in viability of macrophages from the fly-ash-exposed and control mice, as determined with trypan blue absorption, were not statistically significant. Inhalation exposure of mice to fly ash for two, three, and four weeks significantly reduced the number of alveolar macrophages that phagocytized *Staphylococcus aureus* in *in vitro* culture.

The elements present in respirable-sized fly ash particles used in this project were determined by energy dispersive X-ray analysis (EDXA). This type of analysis in both the scanning and transmission electron microscopy modes was used to determine the elements present in alveolar macrophages from mice exposed and not exposed to fly ash.

Examination of control alveolar macrophages in the transmission mode revealed so much variation in ultrastructure that characteristic alveolar macrophages could not be ascertained. Variations were observed in the amounts of mitochondria, endoplasmic reticulum, phagosomes, lysosomes, secondary lysosomes, and myelin figures and in the shape of nuclei. Scanning electron microscopy also revealed much variation among control cells, including differences in cell shape and size, membrane ruffling, and cytoplasmic projections. All of the ultrastructural and morphological differences among control cells were also seen among cells exposed to fly ash, for all exposure periods.

The tissues of mice exposed to fly ash for 6 weeks (short-term) and 31 weeks (long-term) were histologically examined. Pigmented alveolar macrophages were observed in the lungs after both short- and long-term exposures. The black non-birefringent pigment resembled anthracotic pigment; particles that were birefringent resembled silica. Pigment was more randomly dispersed in the lung after short-term than after long-term exposure. In the long-term-exposed lungs, pigment was more prominent and tended to localize in alveoli immediately adjacent to terminal bronchioles. Uptake of pigment by mediastinal lymph nodes was more intense in long-term-exposed mice. Other reticuloendothelial tissues (i.e., Peyer's patches, mesenteric lymph nodes, spleen, and liver) did not show evidence of fly-ash-pigment uptake. Lymphoid accumulation associated with fly-ash-pigment deposition was more prominent in the long-term-exposed mice. For both exposure times, lymphoid accumulation is interpreted as a reaction to the presence of fly ash pigment.

Conclusions

Fly ash in relatively high concentrations had little effect on antimicrobial and antitumor activities of lymphocytes and macrophages. Inflammatory changes suggestive of early pneumoconiosis were seen in the lungs after 217 days of exposure to fly ash.

A. Zarkower, J. Davis, F. Ferguson, and D. Strickler are with the Pennsylvia State University, University Park, PA 16802.
***Judith A. Graham** is the EPA Project Officer (see below).*
The complete report, entitled "Determining Effect of Pollutants on the Immune System," (Order No. PB 81-171 829; Cost: $9.50, subject to change) will be available only from:
National Technical Information Service
5285 Port Royal Road
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Telephone: 703-487-4650
The EPA Project Officer can be contacted at:
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U.S. Environmental Protection Agency
Research Triangle Park, NC 27711

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