Journal of Toxicology and Environmental Health, Part A Current Issues

ISSN: 0098-4108 (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/uteh19

Effects of coal dust and diesel exhaust on immune competence in rats

M. Sharon Mentnech , Daniel M. Lewis , Stephen A. Olenchock , Judith C. Mull , Wayne A. Koller & Trent R. Lewis

To cite this article: M. Sharon Mentnech , Daniel M. Lewis , Stephen A. Olenchock , Judith C. Mull , Wayne A. Koller & Trent R. Lewis (1984) Effects of coal dust and diesel exhaust on immune competence in rats, Journal of Toxicology and Environmental Health, Part A Current Issues, 13:1, 31-41, DOI: 10.1080/15287398409530479

To link to this article: https://doi.org/10.1080/15287398409530479

EFFECTS OF COAL DUST AND DIESEL EXHAUST ON IMMUNE COMPETENCE IN RATS

M. Sharon Mentnech, Daniel M. Lewis, Stephen A. Olenchock, Judith C. Mull, Wayne A. Koller

Division of Respiratory Disease Studies,
National Institute for Occupational Health and Safety,
Morgantown, West Virginia

Trent R. Lewis

Experimental Toxicology Branch,
Division of Biomedical and Behavioral Sciences,
National Institute for Occupational Health and Safety,
Cincinnati, Ohio

The effects on the immune system of rats that had been exposed to a 2-mg/m^3 dose of either respirable coal dust, diesel exhaust fumes and particulates, or the combination of these were studied. Animals that were housed similarly but exposed only to filtered air served as controls. After 12 and 24 mo of exposure, the rats were tested for immunocompetency by enumerating antibody-producing cells in the spleen 4 d after immunization with sheep erythrocytes and by monitoring the proliferative response of splenic T-lymphocytes to the mitogens concanavalin A and phytohemagglutinin. The results of this study indicate that no major alterations occurred in the immunologic functions measured as a result of exposure to either coal dust, diesel exhaust fumes and particulates, or their combination.

INTRODUCTION

Respirable coal dust can cause mild to severe pulmonary disease where the pathological damage is manifested in nonreversible fibrotic lesions causing progressive decrement in pulmonary function with an increased pulmonary dust burden (Morgan, 1980; Parkes, 1982). The severity of the disease, however, is not totally related to dust burden, because disease progression can continue after removal of individuals from occupational exposure (Seaton, 1983; Shennan et al., 1981). Investigations of coal workers' pneumoconiosis, especially in the forms of simple and complicated pneumoconiosis (progressive massive fibrosis), have suggested the

The authors thank Beverly J. Wilhelm for her secretarial assistance.

Mention of company names or products does not constitute endorsement by the National Institute for Occupational Safety and Health.

Requests for reprints should be sent to M. Sharon Mentnech, Immunology Section, DHHS, CDC, NIOSH, 944 Chestnut Ridge Road, Morgantown, West Virginia 26505.

Journal of Toxicology and Environmental Health, 13:31–41, 1984

possibility of immunological involvement either as a direct result of coal dust exposure or because of a preexisting immunologic condition that predisposes an individual to progressive development of pneumoconiosis. In this regard, several nonspecific aberrant immunologic phenomena have been described: increased prevalence of rheumatoid factor (Soutar et al., 1974); high prevalence of antinuclear antibodies (Lippmen et al., 1973; Rom et al., 1983); lung-associated antibodies (Burrell, 1972); antiimmunoglobulin G antibodies (Mentnech et al., 1980); weak association with histocompatibility antigens (Heise et al., 1979); altered T-cell function (Robertson et al., 1983); as well as hypergammagobulinemia and hyperproteinemia (Hahon et al., 1980).

For economical and efficiency reasons, diesel-fueled equipment is rapidly being introduced into underground coal mines. Renewed interest in the physiological processing of coal dust has been prompted because of the unknown health effects that would result from breathing both diesel exhaust and coal dust. The known potential health hazards of diesel exhaust includes: noxious gases (CO, NO, NO_x, SO_x); volatilized organic acids; aldehyde and aliphatic hydrocarbons (i.e., benz[a]pyrene, formaldehyde, etc.); and diesel particulates (Hinners et al., 1980). Several components have been demonstrated to be biologically reactive: NO_2 has been shown to alter the quality of immune response in lung tissue (Hillman et al., 1983), as have CO (Snella and Rylander, 1979) and SO_x (Fenters et al., 1979). The mutagenic and carcinogenic capabilities of diesel exhaust are still under investigation, but the production of numerous hydrocarbons, some with histories of mutagenicity and carcinogenic potential, has generated concern about possible health effects (Guerrero et al., 1980; Orthoefer et al., 1980). The diesel exhaust particulate is generally a submicron-size particle with a carbonaceous core whose surfaces adsorb both hydrocarbons and noxious gases. These particles can achieve deep pulmonary penetration with 15–30% deposition, and the particles can be engulfed by alveolar macrophages (Vostal, 1980).

Epidemiological studies of coal miners utilizing diesel equipment in underground mines have indicated an increase in respiratory symptoms (Reger et al., 1982). Numerous animal exposure studies have been done with coal dust, but fewer studies have used diesel exhaust or the combination of coal dust and diesel exhaust. To date, the main effects of exposure to diesel exhaust (i.e., 12 mo or less) are the production of minor pathological changes and some reduced pulmonary ventilatory responses (Wiester et al., 1980).

Animal exposures to both coal dust and diesel exhaust have produced mixed pathological pictures, indicative of each part separately. In the coal-dust exposures, histological examination revealed typical pulmonary effects of coal-dust-laden macrophages with some focal lesions containing polymorphonuclear lymphocytes. Diesel-exhaust exposure alone resulted in a generalized reaction resembling the response to a pulmonary irritation:

increased ratio of lung to body weight, some fine-particular accummulation within macrophages, and slight focal thickening of interstitium (Wiester et al., 1980; Karagianes et al., 1981; Busch et al., 1981; Pepelko et al., 1982; Green et al., 1983).

The present study was undertaken to determine if long-term (24 mo) inhalation exposure to coal dust, diesel exhaust, and/or the combination of both would result in detectable immunological consequences. For this purpose B-cell and T-cell responses were chosen. The resulting information would give an indication of any major shifts in immunological response in humoral (B-cell) and cellular immunity (T-cell).

MATERIALS AND METHODS

Coal Dust

The coal was selected from the eastern bituminous Pittsburgh coal seam at the 600-ft level (183 m) below the surface at the Blacksville Number 1 Mine, West Virginia. Freshly cut coal fragments were collected at the face and prepared for the exposure chamber by fracturing the sample to a level where 40% by weight was within the respirable range ($< 7\ \mu m$) and sealing under nitrogen until introduction into the exposure chambers by a Wright dust feeder (Green et al., 1983).

Diesel Exhaust

Diesel emissions were generated by a four-cylinder, four-cycle Caterpillar diesel engine fueled with no. 2 diesel fuel containing less than 0.5% sulfur and equipped with a water exhaust scrubber.

Animals

Specific-pathogen-free (SPF) Fisher-344 male rats were used for this study. The animals were preimmunized against *Sendai* virus and quarantined for 2 wk before introduction into the inhalation protocol. Once exposure was initiated, they continued for 7 h/d, 5 d/wk, for 12 or 24 mo. After each exposure period, animals were randomly selected from each exposure group for immunocompetency testing. Representative animals from each exposure group and from each time period were assayed serologically for *Sendai* virus and *Mycoplasma* antibodies. Chamber environments were maintained at a temperature of $21 \pm 1°C$ and relative humidity of $50 \pm 10\%$.

Exposure

The animals were separated into four groups: (1) respirable coal dust exposure of 2 mg/m^3; (2) respirable diesel particulate of 2 mg/m^3; (3) respirable diesel particulate of 1 mg/m^3 plus coal particulate of 1 mg/m^3; and (4) controls, which received filtered fresh air. Gravimetric

measurements of the respirable particulate were monitored daily, and concentration measurements of carbon dioxide, carbon monoxide, nitrogen monoxide, nitrogen dioxide, sulfur dioxide, and ammonia were assayed and were maintained within permissible exposure limits for airborne contaminants in the mines. Diesel exhaust and/or coal dust was diluted with filtered air to achieve the appropriate concentration of coal dust and diesel exhaust. The exact description of the chambers, the precise protocol of exposure, and the results of the environmental monitoring are described elsewhere (Hinners et al., 1980; Green et al., 1983). After the appropriate exposure periods, the animals were transported to Morgantown, West Virginia, in barrier-filtered transport cages and, upon arrival, were placed in a controlled access area with filtered-air housing until utilized. The animals were allowed to acclimatize for at least 1 d before the first antigen challenges were begun, and testing was completed within 30 d.

Hematology

A Coulter Particle Counter Z_{B1} (Coulter Electronics, Inc., Hialeah, Fla.) with a 100-μm aperture was used for leukocyte counts and reference samples were used for standardization. Peripheral blood smears were fixed in acetone-free methanol and stained with Wright's stain (Camco Quick Stain; American Scientific, McGraw Park, Ill.). A cell-count differential was done by standard method of the microscopic examination of 100 leukocytes.

Antigen Administration

The hemolytic plaque assay was performed using a modification of methods previously described (Dresser, 1978; Jerne et al., 1974). Sheep erythrocytes were collected in 200 units of preservative-free sodium heparin/20 ml whole blood. Within 1 h, the cells were suspended in equal volumes of Alsever's solution (GIBCO, Grand Island, N.Y.) and stored at 4°C until used. Before administration, the erythrocytes were washed 3 times with sterile Dulbecco's phosphate buffered saline (PBS; GIBCO) and resuspended to 20% v/v in PBS. At 4 d before the plaque assay, the animals were injected intraperitoneally with 40 mg ketamine hydrochloride. Under anesthesia, 0.5 ml of 20% sheep erythrocytes was injected intravenously via the penial vein.

Spleen-Cell Separation

The animals were anesthetized with sodium pentobarbital, and the spleens were removed aseptically and placed in cold Hanks balanced salt solution (HBSS; GIBCO). After initial mincing, the splenic material was forced through a Bellco Cellector Sieve (80 mesh) (Bellco Glassware, Vineland, N.J.), and the resulting cell suspension was layered on commercial ficoll-hypaque and centrifuged at 400 X g for 20 min. Cells at the interface were removed, washed three times with HBSS, counted with a

Coulter Particle Counter Model Z_{BI}, and resuspended in HBSS at a concentration of 5×10^6 spleen cells/ml.

Plaque Formation Assay

Two hundred microliters of the spleen cell suspension were added to 4 ml of 1% w/v agarose in HBSS, preheated to 42°C, which contained 200 μl of 2% sheep erythrocyte suspension. The final solution was then plated on petri dishes containing 5 ml of 1.2% w/v agarose. The plates were incubated for 90 min with 5% CO_2 at 37°C under 95% humidity. After incubation, the plates were flooded with 10% fresh guinea pig complement (GIBCO) in HBSS and incubated for an additional 30 min. After final incubation, the plaques were counted with indirect light on a colony counter. Each animal was assayed utilizing 5 replicate samples at 3 different dilutions of spleen cells (1×10^6, 1×10^5, and 5×10^5 cells/ml).

Lymphocyte Blast Transformation Assay

The lymphocyte blast transformation (LBT) assay was performed as described by Waithe and Hirschhorn (1978). Samples of the spleen-cell suspensions were washed and resuspended in RPMI-1640 media (GIBCO) containing 4 m*M* Hepes buffer, 5 m*M* glutamine, 10% fetal calf serum, 100 units penicillin/ml, and 100 μg streptomycin/ml (complete medium). The cell density was adjusted to 2×10^6 cells/ml, and 100 μl of this suspension was placed in a 96-well culture plate. To each well, 100 μl of an appropriate dilution of mitogen was added. The mitogens and concentrations used were: concanavalin A (Con A; Pharmacia Fine Chemicals, Piscataway, N.J.) at 5, 1.0, and 0.5 μg/ml, and phytohemagglutinin (PHA; Wellcome Reagents Ltd., Beckenham, England) at dilutions of 1/125, 1/250, 1/500, and 1/1000. The mitogens were diluted in complete medium just before use from concentrated stock solutions that were stored at −70°C. The same lots of Con A or PHA were used throughout these experiments. After 96 h of cultures at 37°C under 5% CO_2, 1.0 μCi of [^{3}H]thymidine, (specific activity 2 Ci/m*M*; New England Nuclear, Boston, Mass.) was added to each well, and then the plates were reincubated for an additional 18 h before harvesting with a multiple channel automatic cell harvester (Brandle, Rockville, Md.). Radioactivity of each sample was determined using a Beckman LS-9000 liquid scintillation counter (Beckman Instruments, Fullerton, Calif.). Each dilution of mitogen was tested in replicates of four, and the mean counts per minute (cpm) for each dilution of mitogen was determined. Results are expressed as a stimulation index, which was calculated by dividing the mean cpm with mitogen by the mean cpm of cultures lacking mitogen. Results presented in this report are the maximal stimulation index obtained for each animal for each mitogen. In general, the 1.0-μg/ml concentration of Con A and 1/500 dilution of PHA produced the maximal responses.

Statistics

The plaque counts from each set of 5 assays/animal were corrected to reflect plaques per 10^6 spleen cells, and the mean, standard deviation, and standard error of the mean for each animal were compared using Student's *t*-test for two means. Comparisons included: each of the 4 groups compared among themselves at the 12-mo time period and the 24-mo time period; filtered air exposure versus diesel exhaust; filtered air exposure versus coal dust; filtered air exposure versus coal dust/diesel exhaust; diesel exhaust versus coal dust; diesel exhaust versus coal dust/diesel exhaust; and coal dust versus coal dust/diesel exhaust. Additionally, the 12-mo animal groups were compared to the 24-mo animal groups, and the combined 12 and 24-mo groups were analyzed among the groups as listed.

RESULTS

Results of the hemolytic plaque assay are presented in Table 1. The data presented form the tabulation of 4–5 plaque determinations per individual spleen and represent the number of plaques observed per 10^6 recoverable spleen cells. At 12 mo, 53 animals were sampled, and at 24 mo, 41 animals. There were no statistically significant differences at the $p = 0.05$ level when any combination at the four experimental groups was compared by Student's *t*-test for two means. In addition, comparisons between the 12- and 24-mo groups were not significantly different, nor was any difference noted when the 12-mo and 24-mo groups were combined for larger group sizes and reevaluated.

The numbers of experimental animals in each group were originally selected to be the same. However, the 12- and 24-mo animal groups had non-group-associable premature deaths. In particular, the 24-mo exposure group had a 15.6% occurrence rate of leukemia. This attack rate was also non-group-related but reflects the observed frequency for leukemia in Fisher-344 rats (Goodman et al., 1979; Ward and Reynods, 1983). Our

TABLE 1. Summary Hemolytic Plaque Assay

Exposure groups	Exposure duration		
	12 months	24 months	Combined 12 + 24 months
Filtered air	116 ± 38[a] (16)[b]	281 ± 103 (20)	208 ± 16 (36)
Diesel exhaust	213 ± 61 (12)	155 ± 25 (17)	180 ± 29 (29)
Coal dust	108 ± 29 (14)	129 ± 26 (17)	120 ± 31 (20)
Diesel exhaust and coal dust	184 ± 71 (11)	172 ± 55 (17)	177 ± 43 (29)

[a]Mean hemolytic plaques/10^6 spleen cell, ± standard error.
[b]Number of animals in group.

TABLE 2. Maximum Lymphocyte Stimulation Index for Exposures at 24 Months

Exposure group	Phytohemagglutinin (kidney bean extract)	Concanavalin A (jack bean extract)
Filtered air	4.56 ± 0.73[a] (21)[b]	3.90 ± 0.58 (21)
Diesel exhaust	4.45 ± 0.71 (22)	4.59 ± 1.02 (22)
Coal dust	4.13 ± 0.58 (19)	4.10 ± 0.80 (19)
Diesel exhaust and coal dust	5.20 ±1.71 (18)	5.28 ± 2.27 (18)

[a]Mean standard error of the stimulation index.
[b]Number animals per group.

definition for exclusion from analysis was based on splenomegaly, elevated peripheral blood leukocyte count, and/or the appearance of blast cells in the peripheral blood. There were 15 animals that met at least 2 of these 3 criteria. These animals had an average spleen weight of 9.28 g and an average of 19% peripheral blast cells found in differential staining. The mean plaque response of these animals was $22.6/10^6$ recovered spleen cells.

The lymphocyte blast transformation (LBT) assay was performed only on animals that had been exposed for 24 mo. The results of this assay are summarized in Table 2 and were compared as described previously. There were no statistically significant differences in the responses to mitogens by the spleen cells from animals in the four exposure groups. Those animals that received the combined exposure of coal dust and diesel exhaust tended to have slightly higher responses, but the difference was not statistically significant at the $p = 0.05$ level.

There were a few animals whose spleen cells showed no response to the mitogen. We arbitrarily defined a positive response to mitogen as a stimulation index of 2.0 or greater. Using this criterion, we compared the incidence of nonresponders in the four exposure groups. By chi-square analysis, the difference in incidence of nonresponders was not statistically significant between the four groups. In general, those animals whose spleen cells failed to respond in the LBT assay had evidence of some systemic disease as defined by our exclusion criteria.

The *Mycoplasma* testing survey indicated that some animals developed a titer over the time period, but these results were also non-group-related and represented only a 10% conversion rate in the entire colony. In any case, this would have little effect on our sampling or would effect all groups equally.

DISCUSSION

The hemolytic plaque assay is used to detect primary antigen processing capabilities and the subsequent abilities to produce immunoglobulin

(Jerne et al., 1974). More specifically, in this study, the assay was used as a measure of B-cell functioning by monitoring splenic lymphocyte capability to process antigen and produce short-term, primary response, complement-fixing immunoglobulin M. This approach was adopted because, in coal workers' pneumoconiosis, nonspecific immunological reactions related to B-cell function such as rheumatoid factor, antinuclear antibody, and hypergammaglobulinemia have been described. Diesel exhaust, by nature, contains noxious gases and hydrocarbons, some of which can influence B-cell immune response. In addition, diesel exhaust contains carbon-core particulates that adsorb gases and hydrocarbons and achieve deep lung penetration, which results in lung damage by permanent deposition and serous fluid leaching (Kutz et al., 1980; Vostal, 1980; Eskew et al., 1982; Hilliam et al., 1983). Human epidemiology and animal experimentation support the concept of detectable B-cell alterations; however, the studies presented in this report did not show conclusive evidence of immunologic alterations after low-level, long-term inhalation exposure to diesel exhaust, coal dust, or the combination of both. There does appear to be a trend for the filtered-air group to have more immunoglobulin M-producing cells than the other groups. This trend is especially notable when the combined 12- and 24-mo animals are compared under exposure to coal dust, which yielded a mean of 120 plaques/10^6 spleen cells, while the air group had a mean of 208 plaques. While not statistically different at the $p = 0.05$ level, it does suggest an immunosuppressive effect as has been described by Eskew et al. (1982) as occurring in rats exposed to airborne fly ash; by Burns et al. (1980) in chronic silica exposure; and by Fenters et al. (1979) with sulfur acid mist with carbon particles. Kutz et al. (1980) indicated that various hydrocarbons could affect the immune response. Our study's lack of detection of these differences could be related to minimal number, the low level of exposure, or the duration of the experimental protocol. In addition, age-related effects in the 24-mo animals cannot be discounted. Although animals with overt evidence of leukemia were removed, the remaining animals at 24 mo usually showed evidence of testicular atrophy or other signs of aging associated with 30-mo-old Fisher-344 rats (Goodman et al., 1979; Ward and Reynolds, 1983).

The lymphocyte blast transformation (LBT) is a relatively simple procedure used to measure indirectly the functional capability of the cellular immune system (Luster et al., 1982). A decrease in the proliferative response of lymphocytes to the mitogens used would indicate impaired cellular immune competence and indicate an immunotoxic effect due to the exposures. The assay, as performed in this study, was intended to measure T-cell (thymus-derived lymphocyte) activity (Cunningham et al., 1976). This cell type is known to have immunoregulatory functions as well as effector functions in immunity to intracellular parasites (Luster et al., 1982). Thus, reduced T-cell function could lead to a variety of pathologic states such as increased susceptibility to infection and auto-

immune diseases. Because immunologic abnormalities have been postulated to occur in coal workers' pneumoconiosis, we considered these assays to be an important adjunct to the plaque assay. The combination of these two assays should have resulted in the detection of any major immunotoxic effects of either coal dust, diesel exhaust, or their combination.

The results showed no significant differences in the LBT response using spleen cells from rats from the four different exposure chambers. We elected to present the data in terms of stimulation index, because this tended to reduce the day-to-day variation in the results. The data were analyzed separately with respect to the net thymidine incorporated, and the same conclusions were obtained—i.e., no statistically significant differences existed among the four groups. However, regardless of how the data were analyzed, the animals used in this study tended to give relatively poor responses to the mitogens. This poor response may be age-related, and other investigators have observed similar age-related reductions in LTB responses (Cunningham et al., 1976). Just prior to performing this study, we performed the LBT assay using the spleen cells from eight normal young adult Fisher-344 rats (all less than 6 mo of age). This was done to test all of the reagents and to provide some baseline values for the LBT using rat spleen cells. For these 8 animals the mean maximal stimulation index for PHA was 48.79, and for Con A, 89.71. These values are markedly higher than we obtained with any of the 24-mo-exposed animals and probably reflect the age difference between the study animals and these 8 younger animals. Thus, the major effect on the cellular immune system seen in this study appears to be age-related declines in all groups, which may be compounded by systemic disease but which do not appear to be related to the exposure conditions.

In summary, immunocompetency testing on rats that had been exposed to diesel exhaust, coal dust, or both for 12 or 24 mo failed to demonstrate any immunotoxic effects. Because of the limited number of animals tested after 12 mo of exposure, and the age-related changes seen in the 24-mo-exposed animals, it is possible that subtle alterations in immune function may have gone undetected. The results obtained with the assays utilized in this study indicate that neither humoral immunity (as assessed by enumerating antibody producing cells) nor cellular immunity (as assessed by the lymphocyte blast transformation assay) were markedly affected by these exposures.

REFERENCES

Burns, C. A., Zarkower, A., and Ferguson, F. G. 1980. Murine immunological and histological changes in response to chronic silica exposure. *Environ. Res.* 21:298-307.

Burrell, R. 1972. Immunological aspects of coal worker's pneumoconiosis. *Ann. N.Y. Acad. Sci.* 200:94-105.

Busch, R. H., Filipy, R. E., Karagianes, M. T., and Palmer, R. F. 1981. Pathological changes associated with experimental exposure of rats to coal dust. *Environ. Res.* 24:53-60.

Cunningham, B. A., Sela, B., Yahara, I., and Edelman, G. M. 1976. Structure and activities of lymphocyte mitogens. In *Mitogens in Immunobiology*, eds. J. J. Oppenheim and D. L. Rosenstreich, pp. 13-30. New York: Academic.

Dresser, D. W. 1978. Assays for immunoglobulin secreting cells. In *Handbook of Experimental Immunology*, ed. D. M. Weir, pp. 28.1-28.4. Oxford: Blackwell Scientific.

Eskew, M. L., Zarkower, A., Scheuchenzuber, W. J., and Graham, J. A. 1982. Effects of fly ash inhalation on murine immune function: Effects on systemic response. *Environ. Res.* 28:375-385.

Fenters, J. D., Bradof, J. N., Aranyi, C., Ketels, K., Ehrlich, R., and Gardner, D. E. 1979. Health effects of long-term inhalation of sulfuric acid mist-carbon particle mixtures. *Environ. Res.* 19:244-257.

Goodman, D. G., Ward, J. M., Squire, R. A., Chu, K. C., and Linhart, M. S. 1979. Neoplastic and nonneoplastic lesions in aging F-344 rats. *Toxicol. Appl. Pharmacol.* 48:237-248.

Green, F. H. Y., Boyd, R. L., Danner-Rabovsky, J., Fisher, M. J., Moorman, W. J., Ong, T. M., Tucker, J., Vallyathan, V., Whong, W. Z., Zoldak, J., and Lewis, T. 1983. Inhalation studies of diesel exhaust and coal dusts in rats. *Scand. J. Work Environ. Health* 9:181-188.

Guerrero, R. R., Rounds, D. E., and Orthoefer, J. 1980. Sister chromatid exchange analysis of Syrian hamster lung cells treated *in vivo* with diesel exhaust particulates. In *Health Effects Diesel Engine Emissions: Proceedings of an International Symposium*, eds. W. E. Pepelko, R. M. Danner, and N. A. Clarke, vol. 2, pp. 951-969. Cincinnati: Health Effects Research Laboratory.

Hahon, N., Morgan, W. K. C., and Petersen, M. 1980. Serum immunoglobulin levels in coal workers' pneumoconiosis. *Ann. Occup. Hyg.* 23:165-174.

Heise, E. R., Mentnech, M. S., Olenchock, S. A., Kutz, S. A., Morgan, W. K. C., Merchant, J. A., and Major, P. C. 1979. HLA-A1 and coalworkers' pneumoconiosis. *Am. Rev. Respir. Dis.* 119:903-908.

Hillam, R. P., Bice, D. E., Hahn, F. F., and Schnizlein, C. T. 1983. Effects of acute nitrogen dioxide exposure on immunity after lung immunization. *Environ. Res.* 31:201-211.

Hinners, R. G., Burhart, J. K., Malanchuk, M., and Wagner, W. D. 1980. Facilities for diesel exhaust studies. In *Health Effects of Diesel Engine Emissions: Proceedings of an International Symposium*, eds. W. E. Pepelko, R. M. Danner, and N. A. Clarke, vol. 2, pp. 681-697. Cincinnati: Health Effects Research Laboratory.

Jerne, N. K., Henry, C., Nordin, A. A., Fuji, H., Koros, A. M. C., and Lefkovits, I. 1974. Plaque forming cells: Methodology and theory. *Transplant Rev.* 18:130-191.

Karagianes, M. T., Palmer, R. F., and Busch, R. H. 1981. Effects of inhaled diesel emissions and coal dust in rats. *Am. Ind. Hyg. Assoc. J.* 42:282-391.

Kutz, S. A., Hinsdill, R. D., and Weltman, D. J. 1980. Evaluation of chemicals for immunomodulatory effects using an *in vitro* antibody-producing assay. *Environ. Res.* 22:368-376.

Lippman, M., Eckert, H. L., Hahon, N., and Morgan, W. K. C. 1973. Circulating antinuclear and rheumatoid factors in coal miners. *Ann. Intern. Med.*, 79:807-811.

Luster, M. I., Dean, J. H., and Moore, J. A. 1982. Evaluation of immune functions in toxicology. In *Methods of Toxicology*, ed. N. Hayes, pp. 516-586. New York: Raven Press.

Mentnech, M. S., Pearson, D. J., Elliott, J. A., Taylor, G., and Major, P. C. 1980. Correlation of Gm allotype with the incidence of anti-IgG antibody in coal workers' pneumoconiosis. *Clin. Immunol. Immunopathol.* 17:274-279.

Morgan, W. K. C. 1980. Pathophysiology of coal workers' respiratory disease. In *Health Implications of New Energy Technologies*, eds. W. N. Rom and V. E. Archer, pp. 233-238. Ann Arbor, Mich.: Ann Arbor Science.

Orthoefer, J. G., Moore, W., Kraemer, D., Truman, F., Crocker, W., and Yang, Y. Y. 1980. Carcinogenicity of diesel exhaust as tested in stain "A" mice. In *Health Effects of Diesel Engine Emissions: Proceeding of an International Symposium*, eds. W. E. Pepelko, R. M. Danner, and N. A. Clarke, vol. 2, pp. 1048-1072. Cincinnati: Health Effects Research Laboratory.

Parkes, W. R. 1982. Pneumoconiosis due to coal and carbon. In *Occupational Lung Disorders*, pp. 175–232. Boston: Butterworths.

Pepelko, W. E. 1982. Effects of 28 days exposure to diesel engine emissions in rats. *Environ. Res.* 27:16–23.

Reger, R., Hancock, J., Hankinson, J., Hearl, F., and Merchant, J. 1982. Coal miners exposed to diesel exhaust emissions. *Am. Occup. Hyg.* 26:799–815.

Robertson, M. D., Boyd, J. E., Fernie, J. M., and Davis, J. M. G. 1983. Some immunological studies on coalworkers with and without pneumoconiosis. *Am. J. Ind. Med.* 4:467–476.

Rom, W. N., Turner, W. G., Kanner, R. E., Renzetti, A. D., Peebles, C., Tan, E., and Olsen, D. M. 1983. Antinuclear antibodies in Utah coal miners. *Chest* 3:515–519.

Seaton, A. 1983. Coal and the lung. *Thorax* 38:241–243.

Shennan, D. H., Washington, J. S., Thomas, D. J., Dick, J. A., Kaplan, Y. S., and Bennett, J. G. 1981. Factors predisposing to the development of progressive massive fibrosis in coal miners. *Br. J. Ind. Med.* 38:321–326.

Snella, M.-C., and Rylander, R. 1979. Alteration in local and systemic immune capacity after exposure to bursts of CO. *Environ. Res.* 20:74–79.

Soutar, C. A., Turner-Warwick, M., and Parkes, W. R. 1974. Circulating antinuclear antibody and rheumatoid factor in coal pneumoconiosis. *Br. Med. J.* 3:145–152.

Vostal, J. J. 1980. Health aspects of diesel exhaust particulate emissions. *Bull. N.Y. Acad. Med.* 56:914–934.

Waithe, W. I., and Hirschhorn, K. 1978. Lymphocyte response to activators. In *Handbook of Experimental Immunology*, ed. D. M. Wier, pp. 26.1–26.10. Oxford: Blackwell Scientific.

Ward, J. M., and Reynolds, C. W. 1983. Large grandular lymphocyte leukemia. *Am. J. Pathol.* 111:1–10.

Wiester, M. J., Iltis, R., and Moore, W. 1980. Altered function and histology in guinea pigs after inhalation of diesel exhaust. *Environ. Res.* 22:285–297.

Received June 11, 1983
Accepted September 24, 1983