Lymphocytic Alveolitis in a Crematorium Worker*

Thomas L. Schauble, M.D.; and Elizabeth A. Rich, M.D.

An asymptomatic cremator was found incidentally to have lymphocytic alveolitis by bronchoalveolar lavage, and the basis for this finding was investigated. No known causes of lymphocyte alveolitis including sarcoidosis, hypersensitivity pneumonitis, berylliosis, tuberculosis, or fungal diseases of the lung were found. By exclusion, therefore, exposure to formaldehyde and/or to compounds in the residual ash likely were etiologic in the development of the lymphocytic alveolitis.

(Chest 1994; 105: 617-19)

The initial event in many of the chronic interstitial lung diseases of both known and unknown etiology is believed to be an inflammatory alveolitis. It is upon this background of inflammatory cells that alveolar damage occurs, leading to the progressive derangement of both structure and function associated with the interstitial pneumonitides. Normally, the cells in alveolar regions are comprised of approximately 90 percent macrophages, and 6 to 10 percent lymphocytes; neutrophils and eosinophils are not seen. Alveolitis refers to an alteration in this normal cell pattern and as assessed by bronchoalveolar lavage (BAL) can be categorized as lymphocytic, neutrophilic, or eosinophilic.1,2 We report a case of lymphocytic alveolitis discovered serendipitously in a "healthy" volunteer research subject whose occupation was that of a crematorium worker.

CASE REPORT

A 36-year-old white man was recruited through a newspaper advertisement for "healthy, nonsmokers to participate in lung research." Venipuncture and BAL were performed after signed consent approved by the Investigational Review Board of University Hospitals of Cleveland. On our routine questionnaire, the subject denied current or remote tobacco use, respiratory tract infection in the preceding 6 weeks, asthma, knowledge of prior PPD testing, known allergies, or risk behaviors for infection with human immunodeficiency virus. He had no history of significant medical illnesses and was taking no prescription or over-the-counter medications. His vital signs, pulmonary, and cardiac examinations were normal.

Bronchoalveolar lavage was performed by instillation of 240 ml of sterile normal saline solution in 30-ml aliquots into each of two different subsegments of the right middle lobe. Approximately 80 percent of the BAL fluid was recovered. The cells were separated from the supernatant after centrifugation at 350 × g for 10 min and counted on a hemacytometer. The total number of cells recovered was 13 × 10⁶. The total numbers of cells obtained by BAL from healthy nonsmoking subjects in our laboratory (approximately 100 per year) has ranged from 7 to 25 × 10⁵ cells, with a mean of approximately 17 × 10⁵. Differential counts of three separate Wright's stained cytocentrifuge preparations revealed 58 ± 9 percent macrophages (mean ± SD; normal range 90 to 95 percent), 41 ± 6 percent lymphocytes (normal 7 to 10 percent), < 2 percent neutrophils (normal < 2 percent), and no eosinophils (normal 0). Simplified myeloperoxidase staining using benzidine dihydrochloride revealed that the cells which appeared morphologically to be lymphocytes were not small, immature macrophages because these cells did not stain positively as would be expected for monocytes recently migrated into alveolar spaces. Stains for nonspecific esterase which is found in mature macrophages showed that the total percentage of mature macrophages was 60 ± 4 percent consistent with the Wright's stain.

We had only once observed an abnormal pattern from approximately 100 BALs per year for several years performed on healthy subjects for research purposes. That subject too was asymptomatic and had a lymphocytic alveolitis; upon further investigation, he was found to have a hypersensitivity pneumonitis-type disorder which could be attributed to heavy exposure to birds at work. On our advice, that subject modified his work habits. Therefore, since we believed that perhaps this second subject presenting with a lymphocytic alveolitis might have a treatable or modifiable disorder that was early in its course, the patient was contacted and returned for a complete evaluation. He denied shortness of breath, dyspnea on exertion, cough, hemoptysis, or chest pain. He also denied fever, arthralgias, rashes, weakness or fatigue, night sweats, or weight loss. He was a lifetime nonsmoker and was not exposed to cigarette or marijuana smoke passively. He denied taking either over-the-counter or prescription drugs. He had no pets and no significant exposure to animals. He had no recent significant travel history; he lived in Cleveland, Ohio, having moved there from Kentucky 14 years previously. He denied hobbies entailing significant exposures to dusts, fumes, vapors, animal droppings, or danders. He denied knowledge of prior PPD testing or exposure to tuberculosis. Family history was negative for significant respiratory illnesses.

The subject has been employed in a human crematorium for the preceding 6 years. His duties consisted of placement of the body and casket in the retort, and raking and sweeping the ashes the following day after cooling. He claimed that he had worn a protective mask periodically especially the previous year of the 6 years that he had worked there, but noted the presence of a very high dust content in the air after a cremation. As part of his duties, he swept the residual ashes which liberates even more dust. The caskets used for cremations are primarily carbon steel which is over 98 percent iron and less than 2 percent carbon, manganese, phosphorous and silicon. A small number of the caskets are bronze and are made from 99.5 percent pure copper and zinc. The subject also noted sweeping large numbers of bone fragments remaining after the cremation process as well as dental fillings and hip prostheses which were not decomposed.

Pulmonary function tests revealed a normal cardiac silhouette. The lungs were free of active infiltrates or effusions. There was a partially calcified subcarinal lymph node consistent with remote granulomatous disease. Pulmonary function testing revealed no abnormalities of spirometry, lung volumes, or diffusion capacity. Room air arterial blood gases revealed mild hypoxemia for age with a PaO₂ of 83 mm Hg, but he did not desaturate with 6 min of exercise (maximal heart rate > 85 percent predicted).

Laboratory examination revealed an hematocrit value of 38 percent (normal 40 to 53) and hemoglobin concentration of 12.0 g/dl (normal 13.9 to 16.9), with a MCV of 82 fl, MCH, 25.6 pg; ferritin, 10 µg/L (normal 15 to 300); serum iron, 73 µg/dl (normal 60 to 170); and a TIBC of 366 µg/dl (normal 225 to 425). Free erythrocyte protoporphyrin was 20.3 µg/dl (normal 1.0 to 7.10) which excluded lead poisoning. Stool guaiac was negative. White blood cell count was 6,600/mm³ with 67 percent neutrophils, 18 percent lymphocytes, and 14 percent monocytes. Special serologies for antinuclear antibodies and rheumatoid factor were negative. Angiotensin converting enzyme level was 40 U/L (normal 9 to 52). Quantitative fungal antibody titers were negative. Five unit PPD Mantoux skin test was negative at 48 h with positive mumps, Candida, and trichophyton controls. A serum sample was screened for precipitins and complement-fixing antibodies to antigens associated with hypersensitivity pneumonitis and was negative. The filters from the subject's protective mask were also sent for an
extended screen and culture. The extended hypersensitivity screen was negative, but the cultures of the filters were positive for a few colonies of the molds Cladosporium, Chrysosporium, and Fusarium. When the subject's serum was specifically checked for antibodies to these molds, however, none was found.

The subject wished further workup of his alveolitis. Therefore, a repeat bronchoscopy with BAL and transbronchial biopsies was performed after signed consent. A period of approximately 6 months had elapsed between the two lavages. He had continued to work at the crematorium with the same exposures although he had spent a few hours per day working in the office rather than all day in the crematorium. The total number of cells obtained by BAL at the second bronchoscopy was 40 × 10⁶ cells. Differential cell count of the BAL fluid revealed 78 ± 5 percent alveolar macrophages (AM), 21 ± 4 percent lymphocytes, and < 2 percent neutrophils. Thus, the total number of cells was increased over normal and the percentage of alveolar lymphocytes was still increased more than twofold over normal, although the percentage had decreased as compared to the first bronchoscopy. By FACS analysis, the alveolar lymphocytes were > 50 percent CD4+ and no CD8+ cells were observed. These data indicated that the subject did not have a typical hypersensitivity pneumonitis which is more often characterized by a CD8 lymphocyte predominance. Because the patient had an iron deficiency anemia, the diagnosis of pulmonary hemosiderosis was considered. A Prussian blue stain of the cells obtained by BAL, however, was negative. Histologic analysis of the transbronchial biopsy specimens revealed a submucosal infiltrate composed predominately of small lymphocytes and also marked thickening of the basement membrane (Fig 1). There was no evidence of granuloma formation, connective tissue, neoplasm, or microorganisms. The histologic picture of basement membrane thickening and lymphocyte infiltration was thought by the pathologist to be possibly consistent with asthma. A methacholine challenge test was performed which was negative.

Because of the very high dust burden in the air of the retort after raking the residual ashes, the possibility of a berylliosis-like syndrome was entertained. The inhalation of beryllium can result in a delayed hypersensitivity reaction eventuating in a lymphocytic alveolitis and subsequent granulomatous interstitial lung disease, and the question was raised concerning the possibility of a similar mechanism in this subject. The diagnosis of berylliosis is best made by utilizing the lymphocyte blastogenesis (transformation) assay. In the same manner that T lymphocytes of patients with beryllium-induced lung disease demonstrated increased blastogenesis when stimulated with beryllium salts, we stimulated the subject's T cells with various elements felt to be found in high concentration in the postcremation ashes in the retort, to determine if any of these substances elicited lymphocyte transformation. The following stimuli were used at 1, 10, and 100µg/ml to stimulate circulating T lymphocytes: phytohemagglutinin (PHA) (a mitogen used as a positive control); beryllium oxide (negative control); calcium and phosphate (bone remains); lead, copper, zinc, elemental iron (casket materials); silver and mercury (dental fillings); and titanium (artificial hip prostheses). Peripheral blood mononuclear cells were added to flat-bottomed 96 well plates at 10⁶ cells per well in RPMI 1640 culture medium (Whittaker Bioproducts, Walkersville, Md) plus 10 percent pooled human serum. Incorporation of ³H-thymidine into DNA was measured after 3 days in culture for phytohemagglutinin (mitogen) and 5 days in culture for the other potential antigenic stimuli. Phytohemagglutinin was the only stimulus that activated the T cells from either healthy subjects (n = 3) or from this subject with lymphocytic alveolitis: incorporation of ³H-thymidine into DNA (assay for blastogenesis or DNA synthesis) for PHA was > 90,000 counts per minute for both the control subjects and the patient. There was no difference in the response of the blood lymphocytes from control subjects and the patient and specific responses were < 1,500 counts per minute. To test for responses of alveolar lymphocytes to selected stimuli (PHA, beryllium, calcium, and phosphorus), BAL lymphocytes from control subjects and the patient were separated at AM by adherence of AM to plastic for 2 h and subsequent passage of the plastic nonadherent cells over nylon wool columns. Blood monocytes (10⁶ cells) were added as antigen-presenting cells to the lymphocytes (10⁶ cells). The BAL T lymphocytes responded only to PHA (33 to 40,000 counts per minute). As with the blood lymphocytes, there were no specific responses of alveolar lymphocytes to the other stimuli (< 1,500 counts per minute).

Not having identified an occupationally related causative agent in the subject's environment, a specialist from a technical environmental consulting agency (Tenco, Inc., Cincinnati, Ohio) was enlisted for an opinion. It was noted that the room in which the cremations were performed was poorly ventilated and that formaldehyde fumes permeated the air. A RAST test for formaldehyde (Clinical Immunology and Allergy, Liberty, Mo) therefore was performed that was negative. The subject had no evidence of contact dermatitis and patch tests to both formaldehyde and phenol (both used in embalming) were negative. A negative patch test for contact dermatitis to formaldehyde, however, would not preclude that the process in the lung was related to this compound. The subject was advised to wear a specially fitted face mask during any activity at the crematorium outside of the office.

**DISCUSSION**

Lymphocytic alveolitis is found in sarcoidosis, hypersensitivity pneumonitis, berylliosis, tuberculosis, and fungal infections of the lung. Each of these disorders was ruled out in this subject. The etiology of this subject's lymphocytic alveolitis remains unclear, but is likely related to his occupation as a cremator. The total number of cells was increased at the second bronchoscopy, and the percentage of alveolar lymphocytes was still increased although less so than at the first bronchoscopy. Although the amount of time spent in the crematorium proper (by increased office duties) had been decreased between the two bronchoscopies, the subject had not avoided the exposures at the crematorium. Therefore, we cannot account for the differences in the percentage of lymphocytes obtained at the two procedures, but have demonstrated that with continued exposure, the increase in the percentage of alveolar lymphocytes was maintained. Additionally, the absolute number of alveolar lymphocytes and macrophages was increased upon further exposure.

**Figure 1.** Transbronchial biopsy of crematorium worker. The arrow points to the thickened basement membrane of the epithelium of a distal bronchus. Patches of lymphocytes beneath the epithelium are the present (hematoxylin-eosin, original magnification × 320).
The transbronchial biopsy (performed at the second bronchoscopy) showed patches of lymphocytic infiltrate beneath the bronchial epithelium and marked basement membrane thickening of the distal bronchial walls. The pathologic basis for these nonspecific findings was unclear, but such findings conceivably may be seen in asthma or other chronic obstructive lung diseases. Our subject, however, had no symptoms of asthma, no chronic cough, and no history of smoking, and had a negative methacholine challenge test.

Because of diagnosis of any of the known causes of a lymphocytic alveolitis were found to be unlikely causes in this subject, it is conceivable that some element in the residual ash (or the ash itself) may have been the offending agent. The elements/compounds used in the assay of lymphocyte blastogenesis were those likely to be highest in the ash or residual materials including calcium, phosphate, lead, copper, zinc, elemental iron, silver, mercury and titanium. The absence of specific responses to these agents by lymphocytes from this subject could be secondary to lack of standardization and previous experience with the lymphocyte blastogenesis assay using these elements. The concentrations utilized, however, bracketed those used by Rossman et al in studies showing that lymphocytes from patients with berylliosis specifically respond to beryllium in vitro.

It is possible that fumes generated from bodies embalmed with formaldehyde or phenol caused the findings in this subject. Formaldehyde is associated with pulmonary abnormalities including irritation of the airways and asthma. No convincing evidence has been generated, however, that formaldehyde causes permanent abnormalities in lung function. Formaldehyde is highly water soluble and is not believed to reach the lower airways because it is absorbed in the mucous membranes of the upper airways. Interestingly, it has been suggested that a prerequisite for impairment in lung function after inspiration of formaldehyde is the presence of dust in the inhaled air that would allow the formaldehyde to reach the lower airways. A crematorium with both residual dusts and formaldehyde as part of the atmosphere would fulfill this prerequisite.

Although the data are inconclusive regarding the precise offending material(s), we suggest that this subject's lymphocytic alveolitis may be secondary to exposure to human crematorium ashes or residual materials coupled with formaldehyde fumes and suggest that this occupation be considered as potentially contributory to the development of adverse pulmonary consequences. Currently, precautions against bloodborne pathogens apply to crematoria as elsewhere. With respect to chemical exposures, the Occupational Health and Safety Administration (OSHA) regulates crematoria for nuisance dust only (time weighted average > 15 mg/m³; unless the particle size is less than 10 microns in which case, the time weighted average is 5 mg/m³). Under OSHA standards, nuisance dust would not be exceeded in crematoria because of the intermittent nature of dust generated. Wearing a facial mask is not mandatory; we submit, however, that even in the unlikely event of developing pulmonary disease masks should be worn in crematoria. The Federal Environmental Protection Agency is currently proposing to further regulate crematoria which would encompass new standards for the incineration of medical waste. Several medical issues have been raised concerning cremations. A mercury zinc battery in a pacemaker left in a body exploded with force enough to damage the cremation chamber during cremation raising the possibility not only of injury but also of release of toxic fumes or infectious material from the corpse. Mercury poisoning and exposure to radioactivity have also been raised as potential health hazards of cremation. According to the Cremation Association of North America, 380,000 or 17.2 percent of deaths in the United States were followed by cremation in 1990 which is an increase from 4.4 percent in 1970. On the West Coast of the United States, approximately 40 percent of deaths now are followed by cremation. Therefore, as more and more cremations become the mode, it is possible that cremation-associated lung dysfunction may become apparent, particularly if preventive precautions are not taken.

ACKNOWLEDGMENTS: We thank Mary Malotke, Tencon, Inc., Cincinnati, Ohio, for her inspection of the crematorium and for her comments and insights into the possible nature of the environmental exposure of our subject. We also thank Dr. Craig Elments, Dermatology, Cleveland, Ohio, for his clinical evaluation of our subject's skin and for interpreting the patch tests, and Dr. J. N. Fink at the Wood VA Medical Center in Milwaukee, Wisc, for screening for precipitins and complement-fixing antibodies to antigens.

REFERENCES