

Immunofluorescent studies in patients with farmer's lung

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Pulmonary tissues from patients with farmer's lung have been studied with the use of fluorescein-labeled globulins from patients with this disease and with fluorescein-labeled antisera specific for IgG, IgA, IgM and C3 complement. Immunoglobulins of all three classes were found in the plasma cells and lymphocytes. The walls of the bronchioles appeared rich in antigen, staining well with the fluorescein-labeled globulins isolated from patients with the disease. Fixed C3 complement was present in the histiocytes, suggesting the antecedent presence of antigen-antibody complexes. No histologic or immunologic evidence of vasculitis could be found. These findings suggest further investigation of the hypothesis that at least part of the pathogenesis of farmer's lung may involve a cytotoxic type II reaction. In this event, antigen adsorbed to the cells reacts with antibody in the presence of complement, causing cellular destruction. The presence or absence of a delayed component is still uncertain.

Farmer's lung continues to be one of the most important environmental agricultural diseases in this country. The immunologic process and its relationship to the pathophysiology of the class of diseases characterized by hypersensitivity pneumonitis remain in question. Historically, farmer's lung was the classic example of this group, but in more recent times there have been reports of similar diseases such as maple bark disease,¹ mushroom grower's disease,² pigeon breeder's disease,³ bagassosis,⁴ sequoiosis,⁵ and others. More recently, patients with a similar syndrome have been described who have been exposed to air conditioners in which thermophilic actinomyces were growing.⁶ Two other lately described diseases which may also fall into this category were found in coffee bean workers⁷ and furriers.⁸ The immunologic events that take place in these diseases are little known. Some light has been shed on the problem since both Pepys and associates⁹ and Kobayashi and co-workers¹⁰ demonstrated precipitins first to moldy hay and then to extracts of the thermophilic actinomyces, principally *Micropolyspora faeni* and *Thermoactinomyces vulgaris*. Inhalation tests have also been utilized to reproduce the classic symptoms of farmer's lung.¹¹

In 1969, Gray, Wenzel, and Emanuel¹² described a procedure for the immunofluorescent staining of *M. faeni* utilizing the gamma globulins isolated from patients with farmer's lung labeled with fluorescein isothiocyanate. A search

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Supported in part by Grant No. UI CD-00398 from the National Institutes of Health.

Presented at the Annual Meeting of the American Academy of Allergy, February 23, 1971, Chicago, Ill.

Received for publication May 11, 1971.

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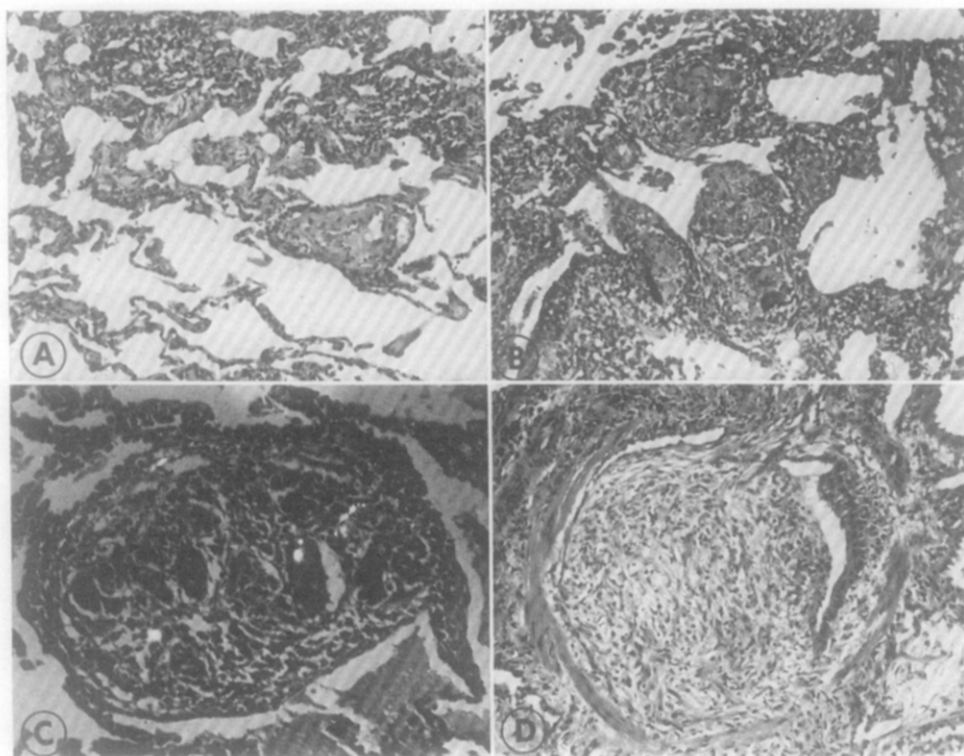


FIG. 1. **A**, Diffuse interstitial pneumonitis with chronic inflammatory cell infiltrate in the alveolar walls. (Original magnification X40.) **B**, Granuloma formation with Langhans-type giant cells, epithelioid cells and lymphocytes. (Original magnification X40.) **C**, Foreign body giant cells with doubly refractile material. (Original magnification X40.) **D**, Bronchiolitis obliterans with fibroblastic masses containing a mixture of chronic inflammatory and histiocytic cells. (Original magnification X40.)

for the organisms in the lung parenchyma led to the detection of the antigen in the bronchioles and the immunoglobulins and C3 complement to be described in this report.

MATERIALS AND METHODS

Farmer's lung serum (FLS) was obtained from patients with active farmer's lung in whom the disease was confirmed by history, lung biopsy, and precipitin tests. Gamma globulins were isolated from the FLS on DEAE cellulose¹³ and labeled with fluorescein isothiocyanate using the technique of Rinderknecht.¹⁴ After separation of the labeled gamma globulins on Sephadex G-25, the solution was ultrafiltered to restore it to its original volume.

Fluorescein-labeled antisera to IgG, IgA, IgM, and C3 complement and antihuman globulin were obtained from Hyland Laboratories. The antisera were checked by immunoelectrophoresis and found to produce a single line with samples of the purified protein and whole human serum. The C3 complement line was typically gull-wing in form.

Lung tissue was obtained at open thoracotomy from 4 patients with farmer's lung, 2 with malignant disease, and one with histoplasmosis. Frozen sections were cut immediately on a freezing microtome. Routine sections were also prepared and were stained with the conventional H and E method.

The frozen sections were fixed briefly in acetone, stained with the antisera for 30 minutes and then washed in buffered saline for a period of 30 minutes with three changes of buffer.

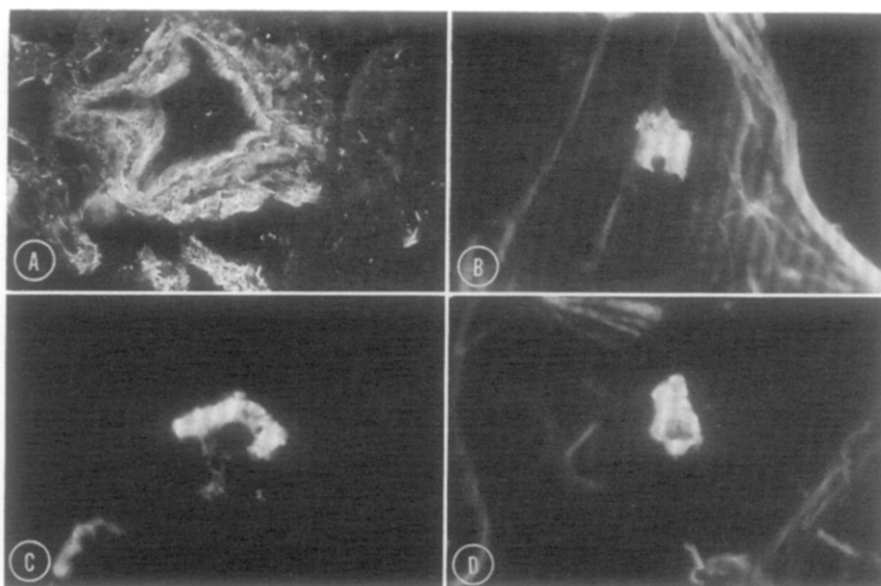


FIG. 2. **A**, Bronchiole stained with fluorescein-labeled gamma globulin from a patient with farmer's lung. (Original magnification X1,200.) **B**, Plasma cell stained with fluorescein-labeled anti-IgG. **C**, Large lymphocyte or plasma cell stained with fluorescein-labeled anti-IgA. (Original magnification X1,200.) **D**, Plasma cell or large lymphocyte stained with fluorescein-labeled anti-IgM. The fibrinous material is elastic membrane which exhibits autofluorescence. (Original magnification X1,200.)

The preparations were observed with a Leitz microscope equipped with an ultra dark field condenser. The filter was a BG 12 with an orange barrier filter in conjunction with BG 38 heat filter. Photographs were taken with a Leica camera using qAf Anscochrome 500 film. An exposure time of 2 to 3 minutes was used depending on the intensity of the fluorescing cells.

RESULTS

Histologic study of the lung revealed findings typical of farmer's lung. The most consistent finding was a diffuse interstitial pneumonitis (Fig. 1, A). It consisted of an infiltrate of chronic inflammatory cells in the alveolar walls. The predominant cell was the lymphocyte, but there were also a large number of plasma cells and a variety of mononuclear and histiocytic forms. Granuloma formation with Langhans-type giant cells, epithelioid cells, and lymphocytes was seen in about one third of the patients (Fig. 1, B). Foreign body giant cells which contained doubly refractile material also could be seen (Fig. 1, C). Another important histologic feature was the presence of organizing endobronchial exudates, bronchiolitis obliterans (Fig. 1, D). There were characteristic fibroblastic masses with a mixture of chronic inflammatory and histiocytic cells. Fibrosis could be seen with special stains.

When the sections were stained with fluorescein-labeled gamma globulins obtained from FLS, the entire wall of the bronchioles stained brilliantly (Fig. 2, A), providing evidence that antigens of *M. faeni* were contained there in high concentration. This was seen in 2 patients acutely ill with farmer's lung,

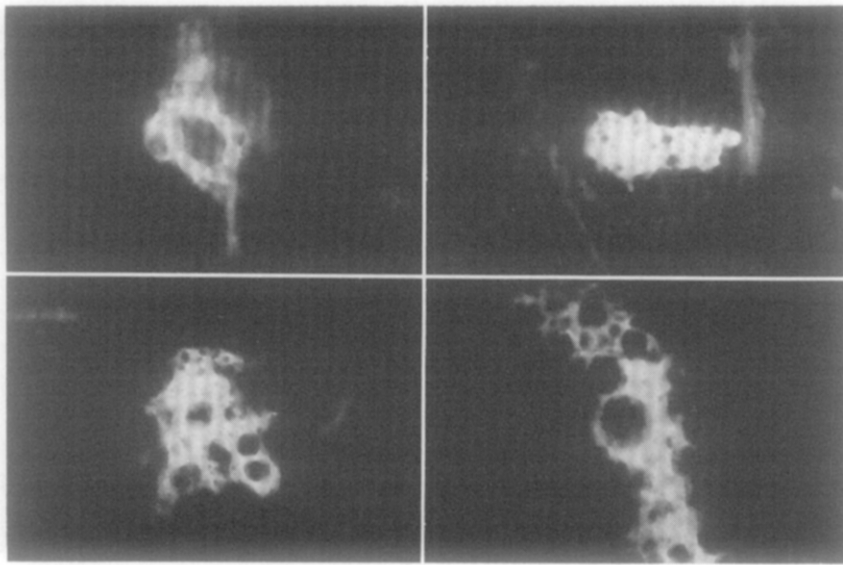


FIG. 3. Large histiocytic-like cells stained with anti-C3 complement. (Original magnification $\times 1,200$.)

while 2 others with more chronic disease were negative. No bronchiolar staining was seen with nonspecific labeled IgG. The immunoglobulins IgG, IgA, and IgM were found in plasma cells and lymphocytes widely scattered throughout the diseased lung (Fig. 2, B, C, and D). All of the patients showed great numbers of fluorescing histiocytic-like cells (Fig. 3) in sections stained with anti-C3 complement. These findings suggest that there may be antigen-antibody complexes with fixed complement in the lungs of patients with farmer's lung.

Immunofluorescent study of the lung sections in the 2 patients with malignant disease and the patient with histoplasmosis revealed a scattering of plasma cells and lymphocytes containing IgG. The large histiocytes containing C3 complement were not found in these sections. No complement-containing cells were found in areas of normal lung parenchyma in these same patients.

DISCUSSION

Although these studies are preliminary, they definitely demonstrate *M. faeni* antigen and immunoglobins IgG, IgA, and IgM and C3 complement in the lungs of patients with farmer's lung. Fluorescent techniques have also been employed to demonstrate the cells of *Cryptococcus neoformans* in tissues of patients affected with cryptococcosis.¹⁵ The polysaccharide antigen was also demonstrated in the tubules and interstitial tissues of the kidneys, although intact organisms were not found.¹⁵ These polysaccharides have also been demonstrated in the bronchial epithelium, in alveolar exudates, and within macrophages participating in the granulomatous reaction.¹⁶ This is very similar to the observations reported in this paper. This is the first report, however, of this phenomenon in patients with farmer's lung.

The above findings suggest that at least part of the immune events in

farmer's lung involve the third component of complement. Immediate or type I sensitivity does not appear to play a role in the disease. Attempts at demonstrating an immediate skin reactivity have not been successful, nor have we or others observed asthmatic-like wheezing or slowing of the expiratory flow rate.^{17, 18} Pepys¹⁹ has suggested that farmer's lung is an Arthus (Type III) reaction, but his findings are not consistent with our own observations. None of our lung biopsies demonstrated necrotizing inflammatory lesions of the venules nor have polymorphonuclear cells been found in the numbers seen in an Arthus reaction. A more plausible explanation appears to be a type II or cytotoxic reaction, in which absorption of an antigen or hapten (drug) renders the tissue cells temporarily susceptible to cytotoxic antibody and complement.²⁰ An important consideration is the observation of Gell and Coombs²⁰ that bacterial polysaccharides are often very adept at fixing passively onto cells. Our preliminary studies show that the principal component of the farmer's lung antigen is a polysaccharide. Reaction of antibody with the cell-antigen complex might then bring on cellular destruction.

The problem of a delayed component in farmer's lung remains unresolved. The histologic picture of farmer's lung, in which a number of histiocytic cells and macrophages are found, certainly would suggest a specific cellular immunity associated with delayed allergy. Preliminary studies in this laboratory indicate that migration inhibitory factor is produced in patients with farmer's lung. Studies of the delayed component and further delineation of the relationship between the immune process and the granulomatous reaction should more clearly define the pathologic process in farmer's lung.

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