Immunofluorescence Identification of Thermopolyspora polyspora, the Causative Agent of Farmer’s Lung

ROBERT L. GRAY, FREDERICK J. WENZEL, AND DEAN A. EMANUEL
Marshfield Clinic Foundation for Medical Research and Education and the Marshfield Clinic, Marshfield, Wisconsin 54449

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Farmer’s lung is a serious disabling pulmonary disease found in agricultural workers. The disease is believed to be a hypersensitivity to the thermophilic actinomycetes, principally Thermopolyspora polyspora. This organism is difficult to stain with the usual bacteriological stains and thus far has not been demonstrated in the lung tissue by microscopic methods. In this paper, it is demonstrated that the fluorescent-antibody technique is a simple method for the positive identification of T. polyspora. The technique can also be used as a rapid screening test for the detection of antibodies to T. polyspora in the patient’s serum. In addition, it opens up the possibility of the identification of T. polyspora in the lung tissue of patients with farmer’s lung and makes available a means for the study of the immunological reaction in the lung parenchyma. No false positive or cross-reactions with Thermoactinomycetes vulgaris or Streptomyces griseus could be demonstrated.

Farmer’s lung is a serious disabling chest disease found among agricultural workers throughout the world. Since the first report of the disease by Campbell in 1931 (1), many investigators have studied the disease and its etiology. Moldy hay was first suggested as a cause of farmer’s lung by Pepys in 1962 (8). In 1963, he demonstrated that Thermopolyspora polyspora was the most potent source of farmer’s lung antigen (7). Other investigators (3) also demonstrated the relationship of T. polyspora to the disease. In 1964, Wenzel et al. (10) reported the isolation of T. polyspora by cultures from a lung biopsy of a patient with active farmer’s lung. However, no staining technique has been found capable of identifying these organisms in lung tissue. This paper is a report of the initial phase in the development of an immunofluorescent technique for the demonstration and identification of the organism.

MATERIALS AND METHODS

Antigen. T. polyspora previously isolated from a patient with farmer’s lung was grown on nutrient agar at 55 C for 1 week. The identity of the organism was confirmed by J. Lacey. Control organisms, Thermoactinomycetes vulgaris and Streptomyces griseus, were grown in a similar manner.

Antibody. Serum from a patient with farmer’s lung confirmed by lung biopsy was used. This serum formed precipitin lines with an extract of T. polyspora in the agar gel diffusion test (11).

Anti-human serum globulin conjugated with fluorescein isothiocyanate (Colorado Serum Co., Denver, Colo.) was used to demonstrate the antigen-antibody complex.

The buffer for slide washing was FA (Difco) phosphate (0.2 m)-buffered saline (0.15 m) with a pH of 7.2; it was used specifically for fluorescent-antibody (FA) techniques.

FA test. Cultures of T. polyspora, T. vulgaris, and S. griseus were grown on nutrient agar at 55 C for 1 week. Smears were made on previously cleaned slides, with buffered 0.85% saline solution to form the suspension. After drying, the smears were fixed in absolute methanol for 15 min and air-dried.

A drop of the positive (by precipitin test) and a drop of the negative control sera were spread carefully over the smears with glass rods. The slides were then placed in a moist chamber for 30 min at room temperature. After rinsing the slides in buffered saline for 30 min, they were air-dried.

Then the smears were overlaid with human anti-serum globulin conjugated with fluorescein isothiocyanate, allowed to stand for 30 min, and again washed for 30 min with buffered saline. The slides were allowed to dry completely and then cover slips were applied with Permount.

Microscopic examination. The preparations were observed with a Leitz fluorescent microscope equipped with an ultradark field condensor. The objective used
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was planapochromate X170/0.17 A 1.10 oil, with iris. The eyepieces were periplan 10X. The primary and secondary filters used were, respectively, BG 38 and BG 12.

Photographic equipment. Photographs were taken with a Leica camera; we used Kodak Tri-X film at an exposure time of 15 sec under regular lighting conditions.

RESULTS

Controls were run with the unknowns each time the FA test was done. The control serum was obtained from individuals who had no contact with farming and whose sera did not form precipitin lines to T. polyspora in the double diffusion agar gel test.

Positive results could not be found when T. vulgaris or S. griseus were tested with the positive or control sera.

A microscopic view of T. polyspora stained with alkaline crystal violet shows that the spores are globose, approximately 1 μm in diameter, and that they are borne in lateral chains on simple mycelium (Fig. 1).

Figure 2 shows the fluorescing spores of T. polyspora stained by the above method. Figure 3 is the negative control.

DISCUSSION

Evidence as to how farmer's lung is produced by T. polyspora is obscure; however, following suggestions by Germuth (5) and Crowle (4), one might postulate that the disease process in the lung is caused by reaction to an antigen-antibody complex. Initial sensitization takes place in the lung with the formation of precipitating antibodies.

Previously developed evidence has demonstrated that a patient's serum usually contains circulating antibodies during the illness and that they persist for various periods of time after the acute phase of the disease has terminated; thus, the diagnosis can be established or verified by serological means. The agar gel diffusion test is the most sensitive indicator of the disease at this time. However, there is still the problem of positive identification of the organism found in the lung biopsy material. Although the organism can be stained in cultures by use of alkaline crystal violet, this method has not demonstrated the organism in lung tissue.

Since its development by Coon and Kaplan (2), the FA technique has been recognized as a
sensitive and potentially specific method for identification. However, the use of the FA test as a diagnostic tool has been seriously neglected. In fact, as far as it is known, there have been no reports describing the use of the FA technique in connection with farmer's lung disease.

The immunofluorescence technique has been adopted in this laboratory in order to develop a method for both establishing the identity of *T. polyspora* and providing a means of studying the antigen-antibody reaction in the lung parenchyma. The method appears to be specific in that fluorescence could not be obtained with either *T. vulgaris* or *S. griseus*, and control sera did not elicit a positive reaction with *T. polyspora*. This specificity is important because *T. vulgaris* has also been isolated from the lung tissue of a patient with farmer's lung (9). Studies such as those outlined in this paper are also being undertaken to establish the identity of *T. vulgaris*.

Histological examination of the lung tissue from patients with farmer's lung shows an infiltrate composed of a variety of cells. Much useful information can be derived from the identification of the cell type involved in the disease process. The disease clinically and histologically appears to be of the delayed type; however, recent evidence has suggested that an Arthus phenomenon may be involved (6).

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LITERATURE CITED

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Volume 17, no. 3, page 454–456. It has been brought to our attention that the preferred name for *Thermopolyspora polyspora* is *Micropolyspora faeni* (Cross et al., J. Gen. Microbiol. 50:351–359, 1968).