Complement Fixation Test as an Aid in the Diagnosis of Farmer’s Lung

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Received for publication 16 December 1974

Complement-fixing antibodies to *Micropolyspora faeni* were measured in farmers and controls. Farmers with known or suspected farmer's lung showed significantly higher titers than control groups. Variations in titer were measured and correlated with the clinical disease.

Farmer's lung is a hypersensitivity pneumonitis initiated most frequently by inhaling *Micropolyspora faeni* (11). Some investigators have suggested that the disease process involves a type III immune response to a specific etiologic agent (7), while others have suggested a type II (9) or type IV (6) response or even a combination of reactions. This classification of hypersensitivity reactions has been reviewed by Coombs and Gell (3). The diagnosis of hypersensitivity pneumonitis is made largely on the presence of clinical symptoms after exposure to moldy forage containing one or more thermophilic actinomycetes or other organic material, along with radiologic and/or histologic patterns compatible with interstitial pneumonitis. The presence of precipitating antibodies detected by immunodiffusion is used to confirm the diagnosis, but the absence of these antibodies cannot be used to exclude the disease. The inability to detect these antibodies may be related to the lack of identification of the specific etiological agent (11) or conceivably to the lack of sensitivity of the technique.

This report describes the use of the complement fixation (CF) test in the diagnosis of hypersensitivity pneumonitis due to *Micropolyspora faeni*. The relative sensitivity of this test was compared with that of immunodiffusion and an assessment was made of the potential application of the CF test as a prognostic tool for following patients with farmer's lung.

Three groups of subjects were used. Group I consisted of 60 farmers seen at the Marshfield Clinic with clinical symptoms and/or histologic or radiologic evidence of hypersensitivity pneumonitis. The criteria used for diagnosis included a history of exposure to moldy forage, cough, dyspnea, rales, and/or chest pain after exposure. In a few instances, histologic examination of pulmonary biopsy material showed changes consistent with farmer's lung. Group II was composed of 30 farmers seeking medical care for reasons other than pulmonary disease. At the time of testing, these patients did not meet the criteria mentioned above. No attempt was made to match this group as to age or sex with group I. Group III was a normal control population from whom 24 serum samples were collected during premartial physical examinations. The method for the preparation of the *M. faeni* antigen has previously been described (5). Briefly, the extract is a concentrated lyophilized culture filtrate produced by the double-dialysis procedure (4). The antigen used in the CF procedure was the same as that used in the immunodiffusion tests. The method of determining the presence of precipitating antibodies has recently been described (5). The method outlined by the Laboratory Branch Complement Fixation Procedure (LBCF) (3) was followed, using the *M. faeni* antigen. Soluble *M. faeni* antigen dilutions were determined by the block titration procedure by titering against a heat-inactivated rabbit anti-*M. faeni* serum or against a human serum known to contain precipitins against *M. faeni* extracts. Complement controls of 5.0, 2.5, and 1.25 50% hemolytic complement (CH₅₀) units were prepared for each antigen and antiserum dilution. The dilution of antigen used in the test system was the smallest amount of antigen reacting with the highest dilution of antiserum showing 30% hemolysis. Dilutions of antigen showing anti-complementary activity with 5, 2.5, and 1.25 CH₅₀ units were not used in the test system.

The test was performed as follows. The optimal antigen concentration in 0.2 ml was incubated with the dilutions of antisera (0.2 ml) for 15 min at room temperature, followed by the addition of 5 CH₅₀ units in a volume of 0.4 ml. The mixture was incubated overnight at 4 C or alternatively by modifying the LBCF with incu-
bation at 37°C for 30 to 60 min. After incubation, 0.2 ml of sensitized sheep erythrocytes (equal volumes of optimally diluted hemolysin and 2.8% sheep erythrocyte suspension mixed and incubated for 15 min at room temperature) was added. The final mixture was incubated for 30 min at 37°C. Tubes were centrifuged at 5 min at 2,000 rpm. Percent hemolysis was visually compared with hemoglobin standards prepared fresh daily. Each test included the following controls: rabbit anti-\textit{M. faeni} and/or human serum containing precipitins to \textit{M. faeni}; \textit{M. faeni} antigen in the absence of antibody; anti-\textit{M. faeni} antibody in the absence of antigen, and the hemolytic system control. The results are expressed as that dilution of antibody which when reacted with standardized antigen in the presence of 5 CH₅₀ units of complement resulted in lysis of 50% of sensitized sheep erythrocytes.

The sera from the three groups of patients were tested for precipitating and complement-fixing antibodies (Table 1). Of the 60 sera tested from patients in group I, 22 showed the presence of precipitating antibodies and 35 showed a positive CF titer. All of the sera positive by immunodiffusion were also positive by CF. When 35 positive sera were tested by the 50% end point CF technique, 19 showed a titer of 8 or greater. Of the 13 sera showing a positive CF but negative precipitins to \textit{M. faeni}, all had a titer of 4 or less. Five of six patients of group II and three of three patients of group III also had titers of \textit{M. faeni} of 4 or less. The 50% end point CF was also used to retrospectively follow the levels of antibody in three patients from group I (Fig. 1). The titers in these patients correlated well with the clinical history. One patient (patient 3) who has had no recurrence of the disease has demonstrated a continuous decrease in levels of antibody over a 9-month period. The other two patients have shown similar declines in antibody levels followed by an increase in titer corresponding to a recurrence of active disease.

A number of techniques have been developed to detect antibodies directed against the thermophilic actinomycetes believed to be responsible for one type of hypersensitivity pneumonitis. Three of these techniques, variations of the immunodiffusion test, have recently been evaluated for clinical testing (5). However, all three tests suffer from the inability to readily quantitate the response. The application of the passive hemagglutination test for the detection of antibodies directed against \textit{M. faeni} has been reported (10). Recently, the indirect fluorescent antibody test has been used to detect antibodies to \textit{M. faeni} (1). The authors found a close correlation between evidence of sensitization to \textit{M. faeni} and the presence of clinical symptoms in the patient. The CF test in farmer's lung was compared with the immunoelectrophoresis test (7). These authors describe the lack of usefulness of the test, particularly in mild cases. The results presented here suggest that the CF test may be used for the detection of antibodies to \textit{M. faeni}. All of the patients in group I with precipitins also showed a CF titer. Thirteen of these patients had no detectable precipitins to \textit{M. faeni} but did have precipitins to one or more of the other thermophilic actinomycetes. Of these 13 patients, 6 had no detectable CF antibodies and the remainder had a CF titer to \textit{M. faeni} less than 4. The presence of precipitating antibodies to organisms other than \textit{M. faeni} does not appear to significantly alter the results of the CF test to \textit{M. faeni}.

Of the three patients in whom titers of CF antibodies were followed over the course of the

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\begin{tabular}{|c|c|c|c|c|c|c|c|}
\hline
Group & PPT & CF & \multicolumn{5}{|c|}{CF titer} \\
& & & <4 & 4 & 8 & 16 & 32 & 64 & \geq 128 \\
\hline
I (60) & 22 & 1 & 3 & 3 & 4 & 4 & 3 & 6 & 6 \\
II (30) & 6 & 1 & 3 & 1 & 3 & 4 & 4 & 3 & 6 \\
III (24) & 6 & 1 & 3 & 1 & 3 & 4 & 4 & 3 & 6 \\
\hline
\end{tabular}
\caption{Serological response to \textit{Micropolyspora faeni}}
\end{table}

* I, Farmers with suspected clinical disease; II, farmers with no current pulmonary disease; III, normal controls. Numbers in parentheses indicate number of farmers studied.
* PPT, Positive precipitating test.
* Number of patients showing positive response.

\begin{figure}
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\includegraphics[width=\textwidth]{figure1.png}
\caption{Fifty percent end point titers of three patients from group I, plotted against time.}
\end{figure}
illness, two showed increases in titers corresponding to recurrences of the pneumonitis. The third patient has remained well since the first episode of clinical disease and his CF antibody titer has continuously decreased. These results, although preliminary, suggest that further investigation may demonstrate the value of the CF test in following the course of the illness.

LITERATURE CITED