

Detection of *Aspergillus fumigatus* Serum Precipitins by Counterimmunoelectrophoresis

THOMAS H. DEE

Section of Infectious Diseases, Department of Medicine, The Medical College of Wisconsin,
Milwaukee County General Hospital, Milwaukee, Wisconsin 53226

Received for publication 18 July 1975

Detection of serum precipitins to *Aspergillus fumigatus* by counterimmunoelectrophoresis is compared with the immunodiffusion technique. Eight of nine (89%) sera from patients with proven *A. fumigatus* infection were positive by both methods. No serum from subjects with other systemic mycoses, bacterial infections, or healthy controls had detectable precipitins. The highest serum precipitin titers were found in sera of patients with the mycetomal and invasive forms of the disease. Detection of *A. fumigatus* serum precipitins by counterimmunoelectrophoresis compares favorably with immunodiffusion and has the advantage of significantly reducing the time required for results.

A number of serological tests have been evaluated as an aid in the diagnosis of aspergillus infections. These include complement fixation, immunodiffusion, latex agglutination, immunoelectrophoresis, and immunofluorescence (3, 4, 13, 14, 16, 17). Of these procedures, immunodiffusion is the simplest and most widely performed test for *Aspergillus* antibodies. Though very sensitive and specific, this method may take 72 to 96 h for precipitin bands to be discernible (6).

The present report describes the detection of *A. fumigatus* serum precipitins by counterimmunoelectrophoresis (CIE) compared with immunodiffusion (ID) in regards to specificity and sensitivity.

MATERIALS AND METHODS

Patient population. Serum was obtained from nine patients with proven *A. fumigatus* infection (group I), nine patients with mycotic infection other than *A. fumigatus* (group II), six cases of significant bacterial infection (group III), and seven normal controls (group IV).

The patients with *A. fumigatus* infection included six cases of pulmonary aspergilloma (mycetoma) and one case each of invasive pulmonary aspergillosis, nonendocarditis intravascular infection, and bronchiectasis with positive sputum culture. Diagnosis of *A. fumigatus* infection was based on isolation of the organism from sputum, biopsy, or surgical specimens, characteristic radiographic picture, and clinical presentation. All patients had isolation of *A. fumigatus*. One patient had *A. flavus* isolated in addition to *A. fumigatus*.

The nine cases of other systemic mycotic infections included three cases of significant candidiasis and one case each of mucor endocarditis, cryptococcal meningitis, coccidioidal meningitis, *Torulop-*

sis glabrata fungemia, rhodotorula fungemia, and pulmonary blastomycosis. Diagnosis in each case was made by isolation of the organism, appropriate serological studies, and clinical manifestations.

The six cases of bacterial etiology included infection with pneumococcus, *Staphylococcus aureus*, *Streptococcus viridans*, *Proteus mirabilis*, *Escherichia coli*, and *Mycobacterium tuberculosis*. All cases except *M. tuberculosis* had at least two consecutive sets of blood cultures positive for the same organism. The patient with *M. tuberculosis* infection had positive sputum cultures and characteristic chest X-ray findings.

The seven normal controls were medical personnel with no history of recent bacterial or fungal infection.

Precipitin detection. ID was done in a manner previously described (9). A plastic mold was placed on a projector cover slide (3.25 by 4 inches; approximately 8 by 10 cm) (Eastman Kodak Co., Rochester, N. Y.). The slide was flooded with 14 ml of 1% Noble agar (Difco Laboratories, Detroit, Mich.) in 0.075 M barbital buffer (pH 8.6). After the agar had been allowed to solidify for 24 h at 4 C, wells 6 mm in diameter and 6 mm apart were cut in a circle and filled with sera from the patients. The center well was filled with *A. fumigatus* antigen. The plates were incubated in a moist chamber and observed for presence and number of precipitin bands for 1 week.

The antigen used was prepared from a culture filtrate of *A. fumigatus* strain 507. The organism was grown as a surface culture in Czapek-Dox medium at 37 C for 2 months. The filtrate was dialyzed against distilled water, drawn through a membrane filter (45- μ m pore size, Millipore Corp.), and lyophilized. The antigenic extract was reconstituted with 0.9% NaCl solution to 10 mg/ml. The undiluted antigen was used in the ID studies. The use of undiluted antigen was found to give optimal results in regards to rapidity of reaction, definition of precipitin bands, and number of precipitin bands produced with a proven case serum.

CIE was performed by a method previously described (9). A plastic mold was placed on a projector slide cover glass (3.25 by 4 inches; approximately 8 by 10 cm) and flooded with 14 ml of 1% agarose (Marine Colloids, Inc., Rockland, Me.), prepared in 0.075 barbital buffer (pH 8.6). After the agar had been allowed to solidify for 24 h at 4 C, parallel rows of wells, 3 mm in diameter and 3 mm apart, were cut. Serum from a patient was placed in the anodal (+) and serial dilutions of antigen in the cathodal (-) wells. Each serum was tested against serum twofold dilutions of prepared antigen from 1:20 and 1:1,280. This range of antigen dilutions was used to minimize false negative readings due to antigen excess or precipitation at or in the antibody well. Antigen dilutions of less than 1:20 were not used for this reason. Electrophoresis was performed for 90 min at 25 mA with 475 V. Plates were read immediately thereafter and then placed in a saline wash overnight and read the next morning. Each positive serum was subjected to serial twofold dilutions and run against the aforementioned antigen dilutions to determine the precipitin titer. The precipitin titer was the last dilution of serum at which precipitins could be detected with the antigen dilutions employed. An example of a positive serum by CIE is shown in Fig. 1.

RESULTS

The results of serum precipitin detection by each method are summarized in Table 1. In group I, eight of nine patients (89%) had *A. fumigatus* serum precipitins detected by both CIE and ID. One patient with pulmonary aspergilloma was negative by both techniques. No patients in groups II, III, or IV had detect-

able precipitins by either method. It should be mentioned that six of eight positive sera had detectable precipitin lines immediately after CIE. These were from patients with mycetomal and invasive pulmonary diseases. The appearance of discernible precipitin lines by ID required 24 to 72 h for all positive sera.

The serum precipitin titers by CIE and number of precipitin bands by ID are summarized for each positive serum in Table 2. The highest precipitin titers were found in patients with pulmonary involvement; the highest titer (1:128) occurred with the invasive form. The greatest number of precipitin bands was found in the mycetomal and invasive form of the disease.

DISCUSSION

Because of the difficulty occasionally encountered in obtaining cultural and/or histologic materials for making the diagnosis of aspergillosis, a number of serological tests were studied to this end. The most frequently used and simplest to perform has been ID.

In 1959, Pepys et al. (16) using ID demonstrated the presence of serum precipitins to *A. fumigatus* in patients with this organism isolated in their sputum. Further investigation by Longbottom and Pepys (14) demonstrated *A. fumigatus* serum precipitins by ID in 98% of patients with aspergilloma. They found no detectable precipitins in healthy controls. Campbell and Clayton (5) detected precipitins in 92% of patients with aspergilloma and 70% of patients with allergic aspergillosis. No healthy

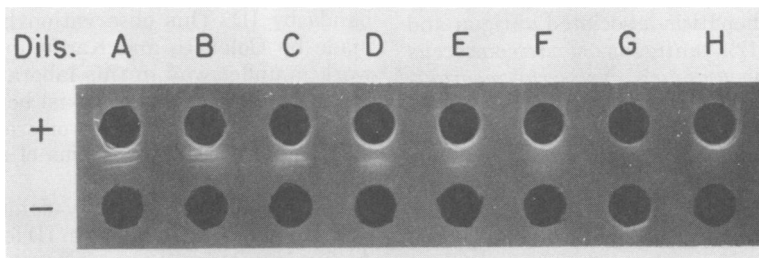


FIG. 1. Demonstration of *A. fumigatus* serum precipitins by counterimmunoelectrophoresis. Serum was placed in anodal (+) wells and serial dilutions of *A. fumigatus* antigen were placed in the cathodal (-) wells. The following antigen dilutions (Dils.) were employed: A, 1:20; B, 1:40; C, 1:80; D, 1:160; E, 1:320; F, 1:640; G, 1:1280; H, saline.

TABLE 1. Detection of *A. fumigatus* serum precipitins by CIE and ID

Group	Clinical state	CIE			ID		
		No. tested	No. positive	Positive (%)	No. tested	No. positive	Positive (%)
I	<i>A. fumigatus</i> infection	9	8	89	9	8	89
II	Other mycotic infection	9	0	0	9	0	0
III	Bacterial infection	6	0	0	6	0	0
IV	Normal controls	7	0	0	7	0	0

TABLE 2. Comparison of precipitin titers by CIE and precipitin bands by ID

Patient	Disease	Counterimmunoelectrophoresis (titer)	Immunodiffusion precipitin bands (no.)
1	Invasive pulmonary aspergillosis	1:128	6
2	Aspergilloma	1:16	3
3	Aspergilloma	1:8	4
4	Aspergilloma	1:16	3
5	Aspergilloma	1:8	2
6	Aspergilloma	1:16	2
7	Intravascular infection	1:2	2
8	Bronchiectasis/positive sputum	1:2	1

controls were studied. Recently, Coleman and Kaufman (6), evaluating the sensitivity and specificity of ID, found *A. fumigatus* serum precipitins in 91% of patients with aspergilloma, 88% of patients with invasive aspergillosis, and 50% of allergic bronchopulmonary aspergillosis. No precipitins were detected in any patients with other systemic mycoses, bacterial or neoplastic diseases, or healthy controls.

CIE has undergone extensive evaluation during the past 5 years as a means for rapidly detecting antigens of or precipitating antibodies to viral, bacterial, and fungal agents. These include hepatitis-associated antigen and antibody (1, 12), antigens of *Streptococcus (Diplococcus) pneumoniae*, *Neisseria meningitidis*, *Haemophilus influenzae* type b (7, 8, 10), and antibodies to influenza A₂ (2), staphylococcal teichoic acid (15), and candida (9). The technique is simple to perform, requires equipment and reagents which are readily available and affordable to even the smallest of hospitals, and provides rapid results.

In this study, all patients with *A. fumigatus* serum precipitins detected by ID were positive by CIE also. One patient was negative by both techniques. The overall rate of precipitin detection was 89%, which compares favorably with rates of detection expressed by other authors. Serum precipitins were not detected by either method in any patients with other systemic mycoses, bacterial infection, or healthy controls (groups II, III, and IV). Again, this is in agreement with other published reports using ID and further attests to the specificity of serum precipitin detection in *A. fumigatus* infections (5, 6, 14).

These results are seemingly in variance with the recently published report by Flaherty et al. (11). In their study, CIE correlated poorly with immunodiffusion techniques used for detection of *A. fumigatus* serum precipitins in hypersensitivity pneumonitis. A number of factors may account for this apparent contradiction. First, comparison of the CIE techniques used in this study and Flaherty's report reveal differences which may be of significance. These include differences in buffer molarity, the amount and time of application of voltage and amperage, and my use of an overnight saline wash. Furthermore, serial dilutions of antigen were employed with each of the test sera in an effort to avoid a prozone phenomenon. There is no mention of this procedure being used in the report by Flaherty et al. Secondly, whereas Flaherty's group investigated patients with hypersensitivity pneumonitis, the present study involves a different patient population. These factors may have contributed to the better correlation of CIE with ID in the present study.

CIE precipitin titers were determined on all positive sera. Although the number of patients studied is too small to draw any firm conclusions or to submit to statistical analysis, the data suggest that patients with the mycetomal and invasive pulmonary forms of the disease have higher precipitin titers. Indeed, the highest titer was found in the one case of invasive aspergillosis. Likewise, this group of patients with mycetomal or invasive forms appears to have a greater number of precipitin bands by ID. This observation has also been made by Coleman and Kaufman (6). Further work is under way in this laboratory to determine if a correlation does exist between precipitin titers by CIE, number of precipitin bands by ID, and the clinical forms of *A. fumigatus* infection.

The results of this study demonstrate that CIE compares favorably with ID for detection of *A. fumigatus* serum precipitins in regards to specificity and sensitivity. Although the number of patients so studied is small, no discordance was found between the techniques and no false positive results were found in the other clinical states studied. CIE has the added advantage of significantly reducing the time required for precipitin detection, a factor which can be of clinical importance in evaluating the critically ill patient.

ACKNOWLEDGMENTS

I wish to thank Jordan Fink and Thanong Santives for permission to study their patients, and V. Kurup and Gertrude Schribner for supplying the *A. fumigatus* antigen.

LITERATURE CITED

1. Alter, H. J., P. V. Holland, and R. H. Purcell. 1971. Counterimmunoelectrophoresis for detection of hepatitis associated antigen. Methodology and comparison with gel diffusion and complement fixation. *J. Lab. Clin. Med.* 77:1000-1010.
2. Berlin, B. S., and S. Pirojboot. 1972. A rapid method of demonstration of precipitating antibody against influenza virus by counterimmunoelectrophoresis. *J. Infect. Dis.* 126:345-347.
3. Biguet, J., J. Fruit, A. Vernes, and A. Capron. 1970. La réaction de fixation du complément et l'immuno-électrophorèse appliquées au diagnostic immunologique de l'aspergillose pulmonaire. *Rev. Immunol.* 34:193-204.
4. Brønnestraum, R., and T. Hallberg. 1965. Precipitins against an antigen extract of *Aspergillus fumigatus* in patients with aspergillosis and other pulmonary diseases. *Acta Med. Scand.* 177:385-392.
5. Campbell, M. J., and Y. M. Clayton. 1964. Bronchopulmonary aspergillosis. *Am. Rev. Resp. Dis.* 89:186-196.
6. Coleman, R. M., and L. Kaufman. 1972. Use of the immunodiffusion test in the serodiagnosis of aspergillosis. *Appl. Microbiol.* 23:301-308.
7. Coonrod, J. D., and M. W. Rytel. 1972. Determination of aetiology of bacterial meningitis by counterimmunoelectrophoresis. *Lancet* 1:1154-1157.
8. Coonrod, J. D., and M. W. Rytel. 1973. Detection of type-specific pneumococcal antigens by counterimmunoelectrophoresis. I. Methodology and immunologic properties of pneumococcal antigens. *J. Lab. Clin. Med.* 81:770-777.
9. Dee, T. H., and M. W. Rytel. 1975. Clinical application of counterimmunoelectrophoresis in detection of candida serum precipitins. *J. Lab. Clin. Med.* 85:161-166.
10. Dorff, G. J., J. D. Coonrod, and M. W. Rytel. 1971. Detection by immunoelectrophoresis of antigen in sera of patients with pneumococcal bacteremia. *Lancet* 1:578-579.
11. Flaherty, D. K., J. Barboriak, D. Emanuel, J. Fink, J. Marx, V. Moore, C. E. Reed, and R. Roberts. 1974. Multilaboratory comparison of three immunodiffusion methods used for the detection of precipitating antibodies in hypersensitivity pneumonitis. *J. Lab. Clin. Med.* 84:298-306.
12. Gocke, D. J., and C. Howe. 1970. Rapid detection of Australia antigen by counterimmunoelectrophoresis. *J. Immunol.* 104:1031-1032.
13. Hipp, S., D. S. Berns, V. Tompkins, and H. R. Buckley. 1970. Latex slide agglutination test for aspergillus antibodies. *Sabouraudia* 8:237-241.
14. Longbottom, J. L., and J. Pepys. 1964. Pulmonary aspergillosis: diagnostic and immunologic significance of antigens and C substance in *Aspergillus fumigatus*. *J. Pathol. Bacteriol.* 88:141-151.
15. Nagel, J. G., C. U. Tuazon, T. A. Cardella, and J. N. Sheagren. 1975. Teichoic acid serologic diagnosis of staphylococcal endocarditis: use of gel diffusion and counterimmunoelectrophoretic methods. *Ann. Intern. Med.* 82:13-17.
16. Pepys, J., R. W. Riddell, K. M. Citron, Y. M. Clayton, and E. I. Short. 1959. Clinical and immunologic significance of *Aspergillus fumigatus* in the sputum. *Am. Rev. Resp. Dis.* 80:167-180.
17. Warnock, D. W. 1974. Indirect immunofluorescence test for the detection of *Aspergillus fumigatus* antibodies. *J. Clin. Pathol.* 27:911-912.