JOURNAL OF CLINICAL MICROBIOLOGY, July 1990, p. 1575–1579
0095-1137/90/071575-05$02.00/0

Vol. 28, No. 7

Use of Immunoblotting To Detect *Aspergillus fumigatus* Antigen in Sera and Urines of Rats with Experimental Invasive Aspergillosis

BESSIE YU, YOSHIHITO NIKI, AND DONALD ARMSTRONG*

Infectious Disease Service, Department of Medicine, Memorial Sloan-Kettering Cancer Center, New York, New York 10021

Received 21 December 1989/Accepted 10 April 1990

Immunoblotting was used to detect *Aspergillus fumigatus* antigen in sera and urines of immunosuppressed rats experimentally infected with *A. fumigatus*. Organisms were administered by both intravenous and intratracheal injections. Intravenously infected rats developed disseminated aspergillosis, but intratracheally infected rats developed pulmonary disease only. Fungal cultures of blood and urine samples from infected rats were negative. In the urines of intravenously infected rats, antigen was detected 24 to 48 h after infection; in the urines of intratracheally infected animals, antigen was detected on days 4 to 5 after infection. Antigen in serum was detected later than antigen in urine was. Following sodium dodecyl sulfate-polyacrylamide gel electrophoresis and immunoblotting of serum and urine samples, the most strongly reacting antigenic materials were found in the 88-, 40-, 27-, and 20-kilodalton regions. These dominant antigens appeared to be the same as those of control antigens prepared from *A. fumigatus* grown in vitro. Rabbit antiserum to *Aspergillus* filtrate antigen was found to be more immunoreactive than antiserum to mycelial or conidial antigen. No mycelium-specific antigens were detected.

Invasive aspergillosis is a major cause of morbidity and mortality in severely immunocompromised patients (3, 12, 24). Untreated invasive aspergillosis is uniformly fatal. Although early diagnosis and treatment may reduce the mortality rate (1, 7, 17), diagnosis is extremely difficult, especially in the early stage of the disease, because blood or respiratory specimens rarely yield positive cultures (24).

Various techniques have been used to detect *Aspergillus* antibody for the diagnosis of invasive aspergillosis (7, 10–12, 17). However, severely immunocompromised patients often fail to develop antibodies; hence, the detection of *Aspergillus* antigen offers a more reliable method to diagnose and manage invasive disease. Reports have included both enzyme-linked immunosorbent assay (ELISA) (4, 6, 13, 16, 23) and radioimmunoassay (6, 19–22) techniques to detect *Aspergillus* antigens from the sera or urines of both human and animal models. The sensitivities of these methods have varied, especially in cases of pulmonary aspergillosis (16).

In the present study we used two different animal aspergillosis models, pulmonary aspergillosis (18) and disseminated aspergillosis, and attempted to detect circulating and excreted *Aspergillus* antigens by immunoblotting.

MATERIALS AND METHODS

Animal model of invasive aspergillosis. Male Sprague-Dawley rats (weight, 125 to 150 g; Charles River Breeding Laboratories, Inc., Wilmington, Mass.) were immunosuppressed by subcutaneous injection of cortisone acetate (100 mg/kg) three times per week throughout the experiment. The animals were given a low-protein diet (8% protein; ICN Biochemicals, Cleveland, Ohio) and tetracycline in their drinking water (250 mg dissolved in 750 ml of water). Animals were infected 3 days after the third cortisone acetate injection.

An isolate of *Aspergillus fumigatus* (H11-20) from a rat dying of pulmonary aspergillosis while on steroids for the production of *Pneumocystis carinii* pneumonia was used in this study (18). The organism, which was maintained in silica gel at 4°C (0.5 g of silica gel H [Sigma Chemical Co., St. Louis, Mo.], 0.5 ml of sterile skim milk), was grown on Sabouraud dextrose agar (4% dextrose; BBL Microbiology Systems, Cockeysville, Md.) for 5 days at 30°C. Conidia were scraped into a 0.02% Tween 80 solution, vortexed to disperse clumps, washed two times in sterile saline, and counted in a hemacytometer. Viability of the conidia was assessed by plating them onto Sabouraud dextrose agar.

A total of 14 rats were used for each experiment. Three groups of four rats each were infected as follows. Group A rats were each given 10^6 conidia by intratracheal (i.t.) injection. This was accomplished by injecting the conidial suspension directly into the trachea by tracheostomy while the rats were under ethrane (enflurane; Anaquest, Madison, Wis.) anesthesia. Ethrane was administered by inhalation. Group B rats were each given 10^6 conidia via the jugular vein. Group C rats were each given 10^7 conidia via the jugular vein. A fourth control group (group D), consisting of two rats, received cortisone acetate only. This experiment was repeated once.

Urine and blood samples were collected before the initiation of cortisone acetate injections, on the day prior to infection, and daily until death. While the rats were under anesthesia (20 mg of ketamine [Parke, Davis & Co., Morris Plains, N.J.] per kg, 2.5 mg of xylazine [Mobay Corp., Shawnee, Kans.] per kg), urines were collected by the clean-catch method with a test tube and blood was collected from the tail vein. At the time of death or when the animals were very ill, organs (lung, liver, spleen, and kidney) were collected, minced, homogenized (Brinkman Instruments, Inc., Westbury, N.Y.), and cultured for fungi on Sabouraud dextrose agar. Control rats were sacrificed at the end of 2 weeks, and their organs were cultured as described above.

Antigen preparation. All antigens were prepared from 5-day-old cultures of *A. fumigatus* H11-20 grown on Sabouraud dextrose agar at 30°C.

(i) Filtrate antigen. *A. fumigatus* conidia were seeded into 250 ml of Czapek Dox broth (Difco Laboratories, Detroit,

* Corresponding author.

Mich.) in stationary 1,000-ml flasks and grown for 6 to 8 weeks at room temperature under normal laboratory fluorescent lighting. The supernatant (approximately 200 ml per flask) from these cultures was filtered through several layers of gauze and glass wool and then through a 45-μm-pore-size filter and dialyzed overnight at 4°C against three changes of distilled water using a membrane with a molecular weight cutoff of 6,000 to 8,000 (Spectrapor 1; Spectrum Medical Industries, Los Angeles, Calif.). The filtrate was then concentrated approximately 20 times by submerging the dialysis tubing containing the antigen in polyethylene glycol 20,000 G (Fisher Scientific Co., Pittsburgh, Pa.). A 1:10 dilution of this antigen was used for rabbit immunization and for Western blotting (immunoblotting).

(ii) Mycelial antigen. *A. fumigatus* conidia were seeded in 250 ml of Czapek Dox broth in 1,000-ml flasks and shaken at 30°C for 3 days under normal laboratory lighting. Each 250 ml of culture yielded 10 to 12 ml of packed mycelium. The mycelium was collected by filtration through several layers of gauze, squeezed dry, washed two times with sterile saline, and suspended as a 30% (vol/vol) suspension in distilled water. The suspension was homogenized (Brinkman homogenizer) and then sonicated at 100 W (model 185C sonifier; Heat Systems, Plainview, N.Y.) for 1 h with 15-min bursts and constant cooling on ice. About 99% of the mycelia were fragmented, as ascertained by microscopic examination. The suspension was centrifuged at 20,000 × *g* for 1 h, and the supernatant was concentrated 20 times against polyethylene glycol 20,000 G and used as antigen for rabbit immunization and Western blotting (immunoblotting).

(iii) Conidial antigen. Conidia were collected by scraping the surface growth from 5-day-old cultures of *A. fumigatus* grown on Sabouraud dextrose agar. The conidia were suspended in 0.02% Tween 80 solution, vortexed, washed two times in sterile saline, and suspended as a 5% (vol/vol) live suspension in saline for rabbit immunization.

The protein content of the filtrate and mycelial antigens was estimated by using a protein assay kit (Bio-Rad Laboratories, Richmond, Calif.), and the carbohydrate content of both antigens was estimated by the phenol sulfuric acid method of Dubois et al. (5). The filtrate antigen had a protein concentration of 550 μg/ml and a carbohydrate content of 440 μg/ml. The mycelial antigen had a protein content of 510 μg/ml and a carbohydrate content of 480 μg/ml. The conidial suspension was used for rabbit immunization only, and its protein and carbohydrate contents were not assayed. All antigens were stored at −70°C until they were used. Conidia remained viable, and germination did not occur at this temperature.

Antiserum production. Three groups of New Zealand White rabbits were immunized, respectively, with (i) filtrate antigen, (ii) mycelial antigen, and (iii) conidial antigen. Each antigen (1 ml) was mixed with 1 ml of Freund adjuvant for each injection. First injections were given with Freund complete adjuvant, and subsequent injections were given weekly in Freund incomplete adjuvant for a period of 3 weeks and then monthly for a period of 4 months. Rabbits were bled for testing 7 days after each monthly injection, and sera were tested for reactivity against filtrate and mycelial antigens by immunoblotting.

Immunoblotting. Each rat urine or serum sample was diluted 1:4 or 1:5, respectively, in sample buffer (2 ml of 0.5 M Tris hydrochloride, 2 ml of 10% sodium dodecyl sulfate, 2 ml of glycerol, 0.2 ml of 2-mercaptoethanol) and boiled for 5 min. Each *Aspergillus* antigen was diluted 1:5 in sample buffer and similarly treated. Negative controls consisted of normal rat urine or serum samples, and positive controls were the same urine and serum samples mixed with a 1:40 dilution of *Aspergillus* filtrate antigen. Prestained molecular weight markers (phosphorylase *b*, 110,000; bovine serum albumin, 84,000; ovalbumin, 47,000; carbonic anhydrase, 33,000; soybean trypsin inhibitor, 24,000; and lysozyme, 16,000; Bio-Rad Laboratories) were run with each gel. Pyronin Y (Bio-Rad Laboratories) was used as the tracking dye.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis and protein blotting were performed by the method of Damato et al. (2) by using a Mini Protean II cell (Bio-Rad Laboratories) and Mini Trans-Blot apparatus (Bio-Rad Laboratories). A 10% resolving (lower) and a 3.5% separating (upper) gel were used. The electrophoresis chambers were filled with a 1:4 dilution of running buffer (24 g of TRIS, 115.1 g of glycine, 8 g of sodium dodecyl sulfate, made up to 2 liters with distilled H_2O). For each chamber, electrophoresis was carried out at 15 mA for 10 min or until the tracking dye began to enter the resolving gel. The current was then increased to 20 mA until the dye reached the bottom of the gel plates (40 to 50 min). Transblot onto nitrocellulose paper (pore size, 0.45 μm; Bio-Rad Laboratories) was carried out for 30 min at 70 V and then for 2.5 h at 100 V in transblot buffer (12 g of TRIS; 57.6 g of glycine; 800 ml of methanol, made up to 4 liters with distilled H_2O). The nitrocellulose sheets were soaked overnight at 4°C in blotto buffer (100 ml of phosphate-buffered saline [PBS; pH 7.4], 5 g of nonfat dry milk [Carnation]), rinsed in PBS with 0.5% Tween 20, blotted dry, and cut into strips of 2 to 3 mm. Before the strips were cut, a straight line was drawn on the nitrocellulose sheet along the upper edge of the gel slab to facilitate perfect alignment of the strips after immunoblotting. The strips were incubated at 37°C for 30 min in blotto buffer with 4% goat serum. Primary antibody (rabbit antiserum to *A. fumigatus* antigen or normal rabbit serum control, 1:100) was added to each strip and incubated for 3.5 h at 37°C. After three 10-min washes with PBS–0.5% Tween 20, the strips were incubated with biotinylated anti-rabbit immunoglobulin G (1:1,000; Vector Laboratories, Burlingame, Calif.) for 30 min at 37°C. After washing again with PBS–0.5% Tween 20, horseradish peroxidase avidin D (1:1,000; Vector Laboratories) was added and incubation was resumed for 30 min at 37°C. Color was then developed with freshly prepared substrate buffer (10 ml of PBS [pH 7.4], 2 ml of substrate stock solution containing 0.3 g of 4-chloro-1-naphthol in 100 ml of methanol, 0.004 ml of 30% H_2O_2).

Treatment with protease. Protease attached to cross-linked agarose beads (P4531; Sigma) was used to treat *A. fumigatus* filtrate and mycelial antigens (4). Each antigen (0.5 ml) was mixed with 150 mg of protease agarose and incubated at 37°C overnight. Control antigen samples were tested without protease. A positive control consisted of 100 μg of bovine serum albumin (Sigma) per ml of 0.1 M disodium EDTA (pH 7.2), which was incubated with protease under identical conditions. The mixtures were centrifuged at 1,400 × *g* for 15 min, and the supernatants were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The gels were stained with Coomassie brilliant blue R250 (0.1 g of Coomassie brilliant blue R250, 40 ml of methanol, 10 ml of glacial acetic acid, diluted up to 100 ml with distilled H_2O) for 40 min and destained (40 ml of methanol, 10 ml of glacial acetic acid, diluted up to 100 ml with distilled H_2O) for 3 h. The supernatants from the treated *A. fumigatus* antigens were also used for immunoblotting against rabbit antiserum to *A. fumigatus* filtrate antigen.

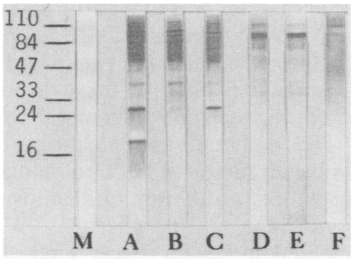

FIG. 1. Immunoblots of *A. fumigatus* filtrate and mycelial antigens. Blots were developed with rabbit antisera to *A. fumigatus* antigens. Lanes A through C, filtrate antigen reacted with antiserum to filtrate antigen, mycelial antigen, and conidial antigen, respectively; lanes D through F, mycelial antigen reacted with antiserum to filtrate antigen, mycelial antigen, and conidial antigen, respectively; lane M, molecular mass standards for proteins (in kilodaltons).

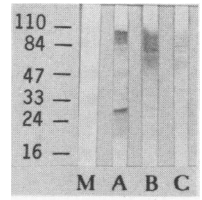

FIG. 2. Immunoblots of a urine sample from an i.v.-infected rat developed with rabbit antisera to *A. fumigatus* antigens. Lanes: A, infected urine reacted with filtrate antiserum; B, infected urine reacted with mycelial antiserum; C, infected urine reacted with conidial antiserum; M, molecular mass standards for proteins (in kilodaltons).

RESULTS

Animal model of invasive aspergillosis. Preliminary experiments were performed to determine the lethality of the inoculum that was used. The i.t.-infected rats injected with 10^6 conidia died between 7 and 14 days after infection. Intravenously (i.v.) infected rats given 10^6 conidia died 5 to 6 days after infection. In rats given 10^7 conidia i.v., death occurred 4 to 5 days after infection.

Cultures of urine and blood samples from all groups of animals were negative for fungi. In the i.t.-infected rats, lung cultures were positive for *A. fumigatus*; liver, spleen, and kidney cultures were negative. In i.v.-infected animals, all the organs cultured (lung, liver, spleen, and kidney) were positive for *A. fumigatus*. Multiple mycotic lesions were evident in all organs. In i.t.-infected rats, lungs lesions were more numerous than those in the i.v.-infected group were. Cultures of the lesions uniformly grew *A. fumigatus*. Organs from the control animals were all negative for fungi.

Sensitivity of the assay. The control *A. fumigatus* filtrate antigen had a protein concentration of 550 μg/ml. This antigen could be detected up to 1:5,000 by Western blotting (immunoblotting) against rabbit antiserum to filtrate antigen. The limit of antigen detection for this antigen was, therefore, 110 ng of protein per ml. The mycelial antigen, which had a protein content of 510 μg/ml, was detectable up to 1:4,500 by Western blotting against rabbit antiserum to filtrate antigen. The limit of antigen detection for this antigen was 113 ng of protein. The conidial antigen suspension was used for rabbit immunization only, and its protein content was not tested.

Reactivity of rabbit antibody. The rabbit antiserum to filtrate antigen reacted more strongly against both filtrate and mycelial antigens (Fig. 1). When antiserum to either mycelial or conidial antigen was reacted against the filtrate antigen, the 20-kilodalton (kDa) band could not be detected. When any one of the three antisera (filtrate, mycelial, or conidial) was reacted against mycelia antigen, the 27- and 20-kDa bands could not be detected. The filtrate antiserum also formed stronger bands in immunoblots of urine samples of infected rats (Fig. 2), with the strongest band being in the 27-kDa region. The band in the 40-kDa region was not seen when antisera to mycelial or conidial antigens were reacted with the same urine samples.

Antigenuria and antigenemia. In the i.v.-infected rats given 10^6 conidia, antigenuria was detected 48 h after infection. In those given 10^7 conidia, antigenuria was detected 24 h after infection; bands were seen in the 88-, 33-, and 27-kDa regions. After 48 and 72 h the bands became stronger. The bands in the 40- and 20-kDa regions were first detected 72 h postinfection. The strongest reacting band in immunoblots of the rat urine samples was in the 27-kDa region (Fig. 3).

In i.t.-infected rats, antigenuria was first detected 4 days postinfection. The antigen bands in this group also became progressively stronger. Weak bands in the 88- to 110-kDa region were first seen on day 4 postinfection. By day 8 postinfection, bands were seen in the 88-, 40-, 33-, 27-, and 20-kDa regions, with the 27-kDa band being the strongest (Fig. 4). Bands in immunoblots of the control *Aspergillus* antigen were also seen in the same regions.

Antigenemia was detected 1 to 2 days later than antigenuria. The bands from the serum samples migrated farther toward the anode (Fig. 5). The positions of the bands in immunoblots of serum samples from infected rats were seen in the same regions as those of serum samples from normal rats spiked with *A. fumigatus* antigen.

Treatment with protease. After treatment of the *A. fumigatus* antigens and a bovine serum albumin control specimen with protease agarose, bands were no longer seen by Coomassie brilliant blue R250 staining, and *Aspergillus* antigen bands could no longer be detected by Western blotting (immunoblotting).

DISCUSSION

The use of immunoblotting to detect various antigens in urine and serum samples has been reported previously. *Toxoplasma* antigens were detected in the sera of mice that were acutely infected with *Toxoplasma gondii* (8). Antigens of *Borrelia burgdorferi*, the agent of Lyme disease, were detected in urine and serum samples of infected mice and

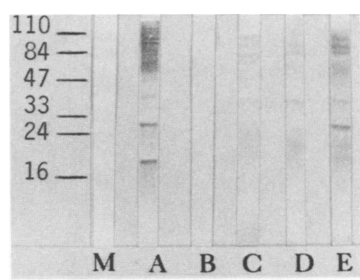

FIG. 3. Immunoblots of urine samples from i.v.-infected rats. Rats received 10^7 *A. fumigatus* conidia. Blots were developed with rabbit antiserum to *A. fumigatus* filtrate antigen. Lanes: A, normal rat urine spiked with a 1:20 dilution of *A. fumigatus* filtrate antigen; B, preinfected rat urine; C, rat urine 24 h postinfection; D, rat urine 48 h postinfection; E, rat urine 72 h postinfection; M, molecular mass standards for proteins (in kilodaltons).

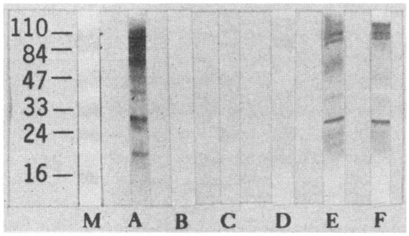

FIG. 4. Immunoblots of urine samples from i.t.-infected rats. Rats received 10^6 *A. fumigatus* conidia. Blots were developed with rabbit antiserum to *A. fumigatus* filtrate antigen. Lanes: A, normal rat urine spiked with a 1:40 dilution of *A. fumigatus* filtrate antigen; B, rat urine preinfection; C, rat urine 4 days postinfection; D, rat urine 6 days postinfection; E, rat urine 8 days postinfection; F, rat urine 12 days postinfection; M, molecular mass standards for proteins (in kilodaltons).

humans (9). *Aspergillus fumigatus* antigen was found in serum samples of experimentally infected rabbits (14).

Most studies in animals with experimental aspergillosis use the i.v. route of infection (13–15). The i.t. model was previously used in this laboratory in amphotericin B aerosol studies (18). In the i.t. model, a progressive bronchopulmonary aspergillosis develops in all infected animals, as proven by smears of lung homogenates and histological examination, and most of the animals die after 7 days. This model of infection mimics the usual route of infection in human disease.

Antigenemia and antigenuria developed in both i.v.- and i.t.-infected rats and were affected by the number of conidia injected. Antigen was detected 1 to 2 days earlier in rats given the higher inoculum. Over time, the mortality rate also increased when a larger inoculum was injected.

Disseminated disease developed in i.v.-infected rats, but i.t.-infected rats developed pulmonary aspergillosis only. In both groups, daily cultures of blood and urine samples were negative for *A. fumigatus*. It is evident, therefore, that a kidney infection is not required for antigenuria to occur.

Others have detected *A. fumigatus* antigen bands of various molecular weights by immunoblotting (11, 14). One assay (11) detected nine components of the *A. fumigatus* antigen that ranged in molecular mass from 33 to 88 kDa. Another group (4) detected an 80-kDa band in the serum of experimentally infected rabbits. In our studies, we also detected a 40-kDa band and two higher-molecular-mass bands in the 80- to 90-kDa regions. Additional bands, which were not reported by others, were detected in the regions of 27 and 20 kDa. The strongest band seen in immunoblots of

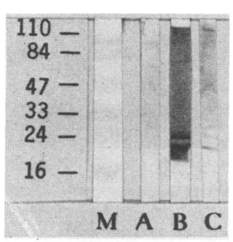

FIG. 5. Immunoblot of a serum sample from an infected rat reacted with rabbit antiserum to *A. fumigatus* filtrate antigen. Lanes: A, rat serum preinfection; B, rat serum spiked with a 1:40 dilution of filtrate antigen; C, serum from an infected rat (10^6 conidia i.v., day 5); M, molecular mass standards for proteins (in kilodaltons).

antigen and urine samples was that in the 27-kDa region. The lower-molecular-mass band in the 20-kDa region was seen in immunoblots of urine samples later during the disease process. The clinical significance of these bands needs to be ascertained by testing specimens from patients with proven aspergillosis. The numbers and positions of the antigen bands were similar in rats with both pulmonary and disseminated disease; hence, a differential diagnosis could not be made by this criterion.

Antigen appeared in serum 1 day later than antigen in urine. This may be due to limitations of the detection system or that antigen-antibody complexes may not have been dissociated since sera were not treated with acid. Antigen could also have been trapped because of the high concentration of serum albumin and globulins. Bands in the serum migrated more toward the anode, probably because of the high albumin concentration in serum. Devising a method to treat serum without dissociating the antigen could increase the sensitivity of the assay. An inhibition ELISA for galactomannan has also detected antigen in urine before it detected antigen in serum (6); antigen was found in the serum of only 2 of 12 patients, but antigen was detected in urine of 7 of 13 patients.

Although hyphal invasion is usually seen in humans and animals with aspergillosis, our studies showed no evidence of mycelium-specific antigens. Rabbit antiserum to filtrate antigen detected more antigen bands in urines from infected rats than did antiserum to mycelial or conidial antigens. Mycelial antigen, however, induced in vivo could be antigenically different from that found in vitro.

Excreted *Aspergillus* carbohydrate antigen was detected early after infection by other workers (6). In our model, the time of onset of antigenuria was dependent on the dose of *Aspergillus* injected, although in i.t.-infected rats, antigenuria was detected 3 days later than it was in i.v.-infected rats. This was probably due to a slower disease progression and lower antigen load in i.t.-infected animals since their disease was confined to the lungs. This could also present a problem in the early diagnosis of aspergillosis in human patients.

The immunoblot technique used in these studies was highly reproducible. By using the Bio-Rad Mini Protean apparatus, the gel electrophoresis time could be shortened from 4 to 5 h to only 1 h. Urine and serum samples could be used immediately after collection and needed no pretreatment to dissociate antigen-antibody complexes as in other test systems, such as ELISA or radioimmunoassay. The 3.5-h incubation time with primary antibody produced bands with the same numbers and intensities as those obtained by overnight incubation; this shortened the immunosorbent assay time from 20 h to only 7 h.

The antigens detected in our study appeared to be protein in nature, as they were sensitive to protease treatment and were stained by Coomassie brilliant blue R250, a protein stain. Other investigators, (4, 13, 16), using the inhibition ELISA, were able to detect very small amounts of carbohydrate antigen (10 ng). The limit of *Aspergillus* antigen detection in our system was 110 ng of protein. Whether protein or galactomannan antigens are more significant in cases of aspergillosis needs to be investigated further. The sensitivity of the Western blot (immunoblot) for *Aspergillus* protein antigens needs to be improved. Dominant antigen fractions should be used to produce antibodies, and a more rapid test, such as the dot immunosorbent assay or an ELISA, could then be used to obtain more sensitive and specific results.

ACKNOWLEDGMENTS

We thank Theresa M. Cunningham and Dawn Conover from the Animal Health Service of Sloan-Kettering Institute for excellent technical assistance.

LITERATURE CITED

1. **Aisner, J., S. C. Schimpff, and P. H. Wiernik.** 1977. Treatment of invasive aspergillosis: relation of early diagnosis and treatment to response. Ann. Intern. Med. **86:**539–543.
2. **Damato, J. J., H. Kim, D. R. Fipps, N. Wylie, and D. S. Burke.** 1988. High resolution HIV Western blot methodology using Bio Rad Mini Protein II test system. Lab. Med. **19:**753–757.
3. **DeGregorio, M. W., W. M. F. Lee, C. A. Linker, R. A. Jacobs, and C. A. Ries.** 1982. Fungal infections in patients with acute leukemia. Am. J. Med. **73:**543–548.
4. **deRepentigny, L., M. Boushira, L. Ste-Marie, and G. Bosisio.** 1987. Detection of galactomannan antigenemia by enzyme immunoassay in experimental invasive aspergillosis. J. Clin. Microbiol. **25:**863–867.
5. **Dubois, M., K. A Gilles, J. K. Hamilton, P. A. Rebers, and F. Smith.** 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. **28:**350–356.
6. **Dupont, B., M. Huber, S. J. Kim, and J. E. Bennett.** 1987. Galactomannan antigenemia and antigenuria in aspergillosis: studies in patients and experimentally infected rabbits. J. Infect. Dis. **155:**1–11.
7. **Fisher, B. D., D. Armstrong, B. Yu, and J. W. M. Gold.** 1981. Invasive aspergillosis: progress in early diagnosis and treatment. Am. J. Med. **71:**571–577.
8. **Huskinson, J., P. Stepick-Biek, and J. Remington.** 1989. Detection of antigens in urine during acute toxoplasmosis. J. Clin. Microbiol. **27:**1099–1101.
9. **Hyde, F. W., R. C. Johnson, T. J. White, and C. E. Shelburne.** 1989. Detection of antigens in urine of mice and humans infected with *Borrelia burgdorferi*, etiologic agent of Lyme disease. J. Clin. Microbiol. **27:**58–61.
10. **Marier, R., W. Smith, M. Jansen, and V. T. Andriole.** 1979. A solid phase radioimmunoassay for the measurement of antibody to *Aspergillus* in invasive aspergillosis. J. Infect. Dis. **140:**771–779.
11. **Matthews, R., J. P. Burnie, A. Fox, and S. Tabaqchli.** 1985. Immunoblot analysis of serological responses in invasive aspergillosis. J. Clin. Pathol. **38:**1300–1303.
12. **Meyer, R. D., L. S. Young, D. Armstrong, and B. Yu.** 1973. Aspergillosis complicating neoplastic disease. Am. J. Med. **54:**6–15.
13. **Patterson, T. F., P. Miniter, J. L. Ryan, and V. T. Andriole.** 1988. Effect of immunosuppression and amphotericin B on *Aspergillus* antigenemia in an experimental model. J. Infect. Dis. **158:**415–422.
14. **Philips, P., and G. Radigan.** 1989. Antigenemia in a rabbit model of invasive aspergillosis. J. Infect. Dis. **6:**1147–1150.
15. **Reiss, E., and P. F. Lehmann.** 1979. Galactomannan antigenemia in invasive aspergillosis. Infect. Immun. **25:**357–365.
16. **Sabetta, J. R., P. Miniter, and V. T. Andriole.** 1985. The diagnosis of invasive aspergillosis by an enzyme-linked immunosorbent assay for circulating antigen. J. Infect. Dis. **152:**946–953.
17. **Schaefer, J. C., B. Yu, and D. Armstrong.** 1976. An aspergillosis immunodiffusion test in the early diagnosis of aspergillosis in adult leukemia patients. Am. Rev. Respir. Dis. **113:**325–329.
18. **Schmitt, H. J., E. M. Bernard, M. Hauser, and D. Armstrong.** 1988. Aerosol amphotericin B is effective for prophylaxis and therapy in a rat model of pulmonary aspergillosis. Antimicrob. Agents Chemother. **32:**1676–1679.
19. **Shaffer, P. J., G. Medoff, and G. S. Kobayashi.** 1979. Demonstration of antigenemia by radioimmunoassay in rabbits experimentally infected with *Aspergillus*. J. Infect. Dis. **139:**313–319.
20. **Talbot, G. H., M. H. Weiner, S. L. Gerson, M. Provencher, and S. Hurwitz.** 1987. Serodiagnosis of invasive aspergillosis in patients with hematologic malignancy: validation of the *Aspergillus fumigatus* antigen radioimmunoassay. J. Infect. Dis. **155:**12–27.
21. **Weiner, M. H.** 1980. Antigenemia detected by radioimmunoassay in systemic aspergillosis. Ann. Intern. Med. **92:**793–796.
22. **Weiner, M. H., and M. Coast-Stephen.** 1980. Immunodiagnosis of systemic aspergillosis. I. Antigenemia detected by radioimmunoassay in experimental infection. J. Lab. Clin. Med. **93:**111–119.
23. **Wilson, E. V., H. M. Hearn, and D. W. R. Mackenzie.** 1987. Evaluation of a test to detect circulating *Aspergillus fumigatus* antigen in a survey of immunocompromised patients with proven or suspected invasive disease. J. Med. Vet. Mycol. **25:**365–374.
24. **Young, R. C., J. E. Bennett, C. L. Vogel, P. P. Carbone, and V. T. DeVita.** 1970. Aspergillosis. The spectrum of disease in 98 patients. Medicine (Baltimore) **49:**147–173.