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Detection of a *Trichosporon beigelii* Antigen Cross-Reactive with *Cryptococcus neoformans* Capsular Polysaccharide in Serum from a Patient with Disseminated *Trichosporon* Infection

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Latex beads coated with anti-*Cryptococcus neoformans* antibody were agglutinated by serum from a bone marrow transplant recipient having a disseminated infection caused by *Trichosporon beigelii*. The cryptococcal latex agglutination titer in the serum of the patient rose to 1:2,560 by the time of his death. Necropsy confirmed the disseminated *Trichosporon* infection and absence of *C. neoformans*. Cell wall extracts of the isolate of the patient and two additional strains of *T. beigelii* agglutinated anti-*Cryptococcus*-coated latex beads. The antigen in the serum of the patient and in the extracts responsible for the agglutination was not destroyed by proteolytic enzymes or heat. A single antigen reactive with rabbit anti-*Trichosporon* serum could be identified in the serum of the patient and the cell wall extracts by rocket immunoelectrophoresis and crossed immunoelectrophoresis. Rocket immunoelectrophoresis and indirect fluorescent-antibody staining demonstrated that anti-*Trichosporon* antibody recognized the capsular polysaccharide of *C. neoformans*.

Trichosporon beigelii (formerly *Trichosporon cutaneum*) is a fungus characterized by the formation of blastoconidia and mycelium which fragments into arthroconidia (2, 9, 11, 19). It is a widely distributed inhabitant of the environment and may cause white piedra, a superficial infection of the hair shaft. During the past several years, reports of disseminated infection in immunocompromised patients have been published. Most of these cases have involved patients with hematological malignancies undergoing chemotherapy resulting in severe granulocytopenia. Invasive disease of the respiratory and gastrointestinal tract with subsequent dissemination has been documented (4, 7, 13, 20). Histological examination of infected tissues show sparsely branched, radiating hyphae measuring 3 to 5 μm in diameter. *Trichosporon* sp. could be confused with *Candida albicans* and *Aspergillus* in tissue; this has led to development of a fluorescent-antibody technique for demonstrating *T. beigelii* in tissue (M. A. Gordon, Abstr. Annu. Meet. Am. Soc. Microbiol. 1983, F82, p. 396).

T. beigelii shares with members of the genus *Cryptococcus* the ability to synthesize starch and hydrolyze urea. In 1963, Seeliger and Schroter (16) prepared rabbit antisera against *T. beigelii* and whole-cell extracts of *Cryptococcus neoformans* and *T. beigelii*. They demonstrated cross-reactivity between antigens of these two fungi.

We recently encountered a patient who underwent bone marrow transplantation and subsequently developed a fatal disseminated *T. beigelii* infection. Cultures taken during life and necropsy demonstrated that a combined infection due to *Trichosporon* sp. and *Cryptococcus* sp. was not present in this patient. Retrospective analysis of serial serum specimens from this patient showed that his serum agglutinated anti-*Cryptococcus*-coated latex beads (AC-latex beads) 11 days before *T. beigelii* could be recovered from his blood. Data were obtained indicating that a heat-stable cell wall antigen of *T. beigelii* was responsible for agglutinating AC-latex beads.

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MATERIALS AND METHODS

Sera from the infected patient. Sera were obtained from a 33-year-old male with aplastic anemia who was admitted to the University of Wisconsin Hospital for bone marrow transplantation on 14 May 1983. Sera were obtained on the day of admission (7 days before transplantation) and on days 3, 10, 14, 20, 30, 37, 45, and 51 after his initial bone marrow transplant. He received total body irradiation and cyclophosphamide treatment as preparation for transplantation. He then received unrelated, haploid-matched, mixed-lymphocyte-reactive donor marrow which had been treated with anti-T-cell monoclonal antibody and complement. The patient became febrile the day of transplantation and was treated with several antimicrobial agents for the remainder of his course. Blood cultures were taken every 48 h. Intravenous amphotericin B was begun empirically on day 12 after transplantation. *T. beigelii* was recovered from cultures of sputum, stool, and blood on posttransplant days 31, 35, and 48, respectively. The organism was recovered from standard blood culture bottles (Bactec 6B; Becton Dickinson, Towson, Md.). Six blood cultures on subsequent days also grew the organism. Respiratory failure began on day 42. A second marrow transplantation was attempted 49 days after the first. The patient expired 60 days after the first transplantation. Necropsy showed gross lesions consistent with *Trichosporon* infection in lungs, liver, spleen, kidneys, and brain. Gomori methenamine silver stain of tissue sections confirmed infection of trachea, lungs, stomach, heart, liver, spleen, kidneys, brain, adrenal glands, and multiple lymph nodes.

Organisms and serological reagents. The *T. beigelii* isolated from the blood of the patient (strain UW) was identified by standard techniques. It had typical morphological features; did not ferment sugars; was urease positive; and assimilated glucose, galactose, sucrose, maltose, and lactose. Two strains of *T. beigelii* (ATCC 4155 and ATCC 34148) were obtained from the American Type Culture Collection, Rockville, Md. A fresh clinical isolate of *Cryptococcus neoformans* was used for some studies.

Organisms used to prepare antigens were grown in Sabouraud glucose broth at 25°C for 3 days and harvested by centrifugation. Cells of strains UW, ATCC 4155, and ATCC 34148 were disrupted by passage through a French press at 700 to 1,060 kg/cm^2. Cell walls were separated from the supernatant cytoplasmic extract by centrifugation at 1,600 × *g*. The cytoplasmic extracts were dialyzed against distilled water and lyophilized. Cell walls were washed three times in acetone and air dried. To obtain cell wall extracts (CWE), the cell walls were then suspended in 0.05 M sodium citrate buffer (pH 7.0) and sealed in Parr bombs. The Parr bombs were placed in an oven at 130°C for 2 h. After cooling, the Parr bombs were opened and their contents were centrifuged at 1,600 × *g*. The supernatant was saved, and the sediment was resuspended in citrate buffer and autoclaved for 30 min. The sediment was centrifuged again, and the supernatants were pooled, dialyzed against distilled water, and lyophilized. Protein content of the CWE was determined by the Lowry method with crystalline bovine albumin as a standard (10); nitrogen content of the CWE ranged from 1.0 to 1.5%.

A dithiothreitol (DTT) extraction procedure, used previously to solubilize surface antigens of *Candida albicans* (12), was used to obtain an extract of strain UW. The extract was dialyzed against phosphate-buffered saline (pH 7.4) and concentrated by dialysis against 10% polyethylene glycol. The extract was stored at −20°C.

Capsular polysaccharide preparations from *C. neoformans* (serotypes A, B, and D) were donated by John Bennett, National Institutes of Health, Bethesda, Md. Methods of obtaining these polysaccharides have been published (1).

For immunization, strain UW whole organisms grown and harvested as above were killed with 1% Formalin, washed, and adjusted to a 2% suspension. Anti-*Trichosporon* serum was obtained from two rabbits by injecting 1 ml of this vaccine subcutaneously thrice weekly for 2 weeks and then once monthly four times. Animals were bled every 2 weeks. Sera that produced strong precipitin reactions against the DTT extract were pooled. For some experiments, a portion of this serum pool was absorbed with Formalin-killed strain UW. Serum was absorbed by mixing approximately 10^9 strain UW whole organisms per ml of serum in a capped tube. The tube was rotated for 24 h and spun at 600 × *g* for 10 min. All sera were stored at −70°C.

For cryptococcal latex agglutination tests, a commercially available test kit (IM Inc, American Microscan, Lexington, Ky., catalog number 34248, lot 183) was used as directed by the manufacturer. Agglutination reactions (0 to 4+) and titers were determined as described in the directions included in the kit. Agglutination titer of the serum obtained at necropsy was verified by the Wisconsin State Laboratory of Hygiene with standard reagents obtained from the Centers for Disease Control, Atlanta, Ga. All sera from the patient were tested before and after boiling. Some serum specimens were treated with proteolytic enzymes to see whether this diminished their ability to produce agglutination of AC-latex beads. The method described by Stockman and Roberts (18) was used to treat the specimens. For each specimen digested with proteolytic enzyme, individual portions were treated with four proteolytic enzymes, namely, protease K, protease type V from *Streptomyces griseus* (pronase), protease type VII (subtilisin BPN'), and protease type I from pancreas (catalog numbers P-0390, P-5005, P-5255, and P-4630 from Sigma Chemical Co., St. Louis, Mo.).

Crossed immunoelectrophoresis, crossed-line immunoelectrophoresis, and rocket immunoelectrophoresis were performed by previously described techniques (17). Indirect fluorescent-antibody staining of various fungi was done by applying 10-μl portions of cells suspended in phosphate-buffered saline to wells (diameter, 5 mm) on Teflon-coated microscope slides (Cel-Line Associates, Inc., Newfield, N.J.). Slides were air dried and fixed with acetone. *Trichosporon* hyphae were homogenized with a hand-held tissue grinder before application to slide wells. Target cells were adjusted to give 5 to 10 single-celled hyphal fragments or blastoconidia per ×480 field. Anti-*Trichosporon* sera were diluted in phosphate-buffered saline containing 0.05% Tween 20. Fluorescein-conjugated goat anti-rabbit immunoglobulin G (Cappel Laboratories, Cochranville, Pa.) was diluted 1:320 in the same buffer supplemented with 0.001% Evans blue. Slides were exposed to dilutions of each test serum for 15 min, washed again, and dried in a stream of air. A drop of glycerol mounting fluid (pH 9) (8) was added to each slide before the cover slip was added. Slides were examined under a Ploem optical system with a Leitz microscope (17).

RESULTS

Reactions with AC-latex beads produced by a 1:2 dilution of representative sera are shown in the upper row of Fig. 1 (left to right: 7 days before transplantation and days 37, 45, and 51 posttransplantation). None of these sera produced agglutination of latex beads coated with normal rabbit immunoglobulin. Clearly, sera obtained from the patient on day 37 posttransplantation and thereafter specifically agglutinated AC-latex beads. Agglutination titers were as follows: day 30, 2+ reaction, undilute; day 37, 1:2; day 45, 1:4; day 51, 1:256; necropsy, 1:2,560. Titers of the latter three sera were unchanged by boiling. Titers of serum samples from day 51 were 1:256 to 1:512 after treatment with proteolytic enzymes. The titer of the necropsy serum was unchanged by treatment with proteolytic enzymes. Samples of CWE, DTT extract, and cytoplasmic extract obtained from strain UW all agglutinated AC-latex beads. When these antigens were mixed with anti-*Trichosporon* serum, agglutination of AC-latex beads was blocked. Anti-*Trichosporon* serum absorbed with *Trichosporon* sp. was unable to block agglutination of AC-latex beads by these antigens. These data indicated that for each antigen preparation, agglutination of the AC-latex beads was due to at least one antigen that was reactive with both anti-*Trichosporon* and anti-*Cryptococcus* antibody.

The sera of the patient and strain UW CWE contained a heat-stable antigen reactive with AC-latex beads. Therefore, we felt that the antigen in patient sera that agglutinated AC-latex beads would be found in the strain UW CWE. However, for cryptococcal latex agglutination to be positive when used to assay sera from any patient with severe *Trichosporon* infection, multiple strains of *Trichosporon* sp. would have to produce an antigen reactive with AC-latex beads. Therefore, we prepared CWE from two culture collection strains of *T. beigelii*. We found readily discernible agglutination of AC-latex beads by solutions containing >0.5 μg of strain UW CWE per ml, >1 μg of ATCC 34148 CWE per ml, or >10 μg of ATCC 4155 CWE per ml. Agglutination of AC-latex beads produced by 10 μg of strain UW CWE per ml, 10 μg of ATCC 34148 CWE per ml, 10 μg of ATCC 4155 CWE per ml, and 100 μg of ATCC 4155 CWE per ml are shown in the lower row of Fig. 1 (left to right). The same antigens failed to agglutinate normal globulin-coated latex. Although we tested CWE of only two culture collection strains that contained an antigen reactive with AC-latex beads, it seems likely that the antigen responsible for agglu-

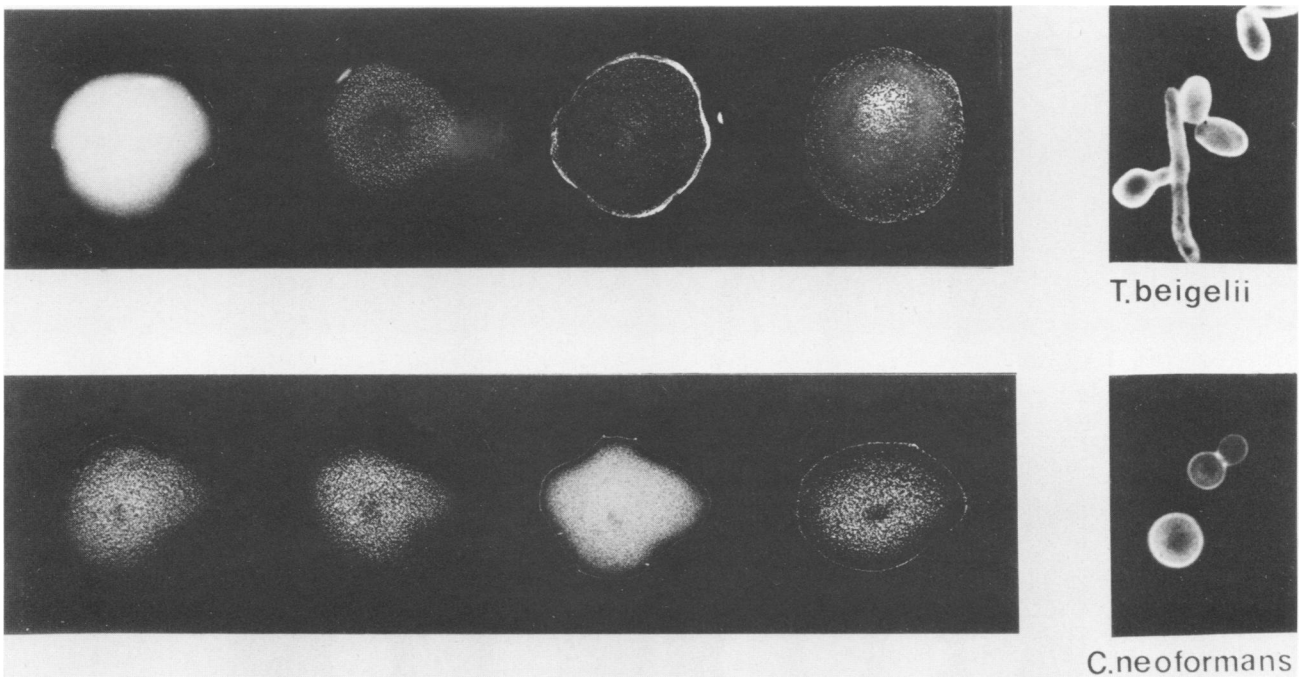

FIG. 1. Latex agglutination reactions and indirect fluorescent-antibody staining. Results of latex agglutination experiments are shown. Upper row shows reactions of AC-latex beads with serum from the patient over the course of infection. Lower row shows reactions of AC-latex beads with *T. beigelii* antigen extracts (see details in the text). Inserts show indirect fluorescent-antibody staining of *C. neoformans* and *T. beigelii* by anti-*Trichosporon* serum (original magnification, ×800).

tination of AC-latex beads could be widely expressed by *T. beigelii* strains.

Studies were done to further characterize the CWE of these three *T. beigelii* strains. First, crossed immunoelectrophoresis against rabbit anti-*Trichosporon* serum was performed to compare the DTT extract and the CWE of the UW strain. At least 12 antigens were detected in the DTT extract (Fig. 2A). Thus, the antiserum detected a number of antigens extracted from the surface of the organism by DTT. By contrast, only one antigen was identified in the CWE (Fig. 2B). This antigen produced a rather characteristic, broad precipitin arc. Crossed-line immunoelectrophoresis (not shown) demonstrated that this antigen was present in the DTT extract. A precipitin arc could be detected by rocket immunoelectrophoresis in the necropsy serum of the patient which fused with the arc produced by the strain UW CWE that had been placed in an adjacent well (Fig. 3A). This served to identify the antigen in the CWE as the one responsible for agglutination of AC-latex beads. Rocket immunoelectrophoresis also demonstrated that CWE of the

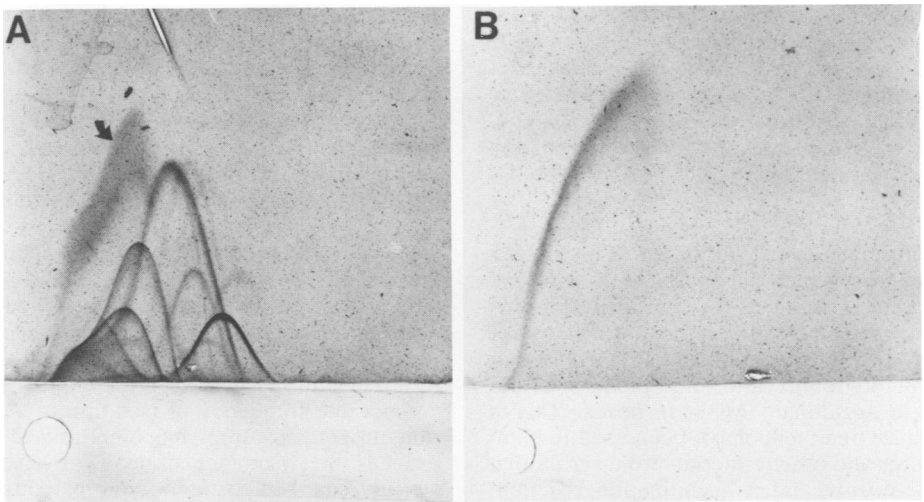

FIG. 2. Analysis of antigen extracts of *T. beigelii* UW by crossed immunoelectrophoresis. Wells contained 30 μl of DTT extract (A) or 120 μg of CWE (B). Both plates contained 18.2 μl of rabbit anti-*Trichosporon* serum per cm^2. First-dimension electrophoresis (anode at right of each plate) was performed at 10 V/cm for 30 min. Second-dimension electrophoresis (anode at the top of each plate) was done at 2 V/cm for 18 h. Arrow in (A) indicates precipitin arc corresponding to that in (B).

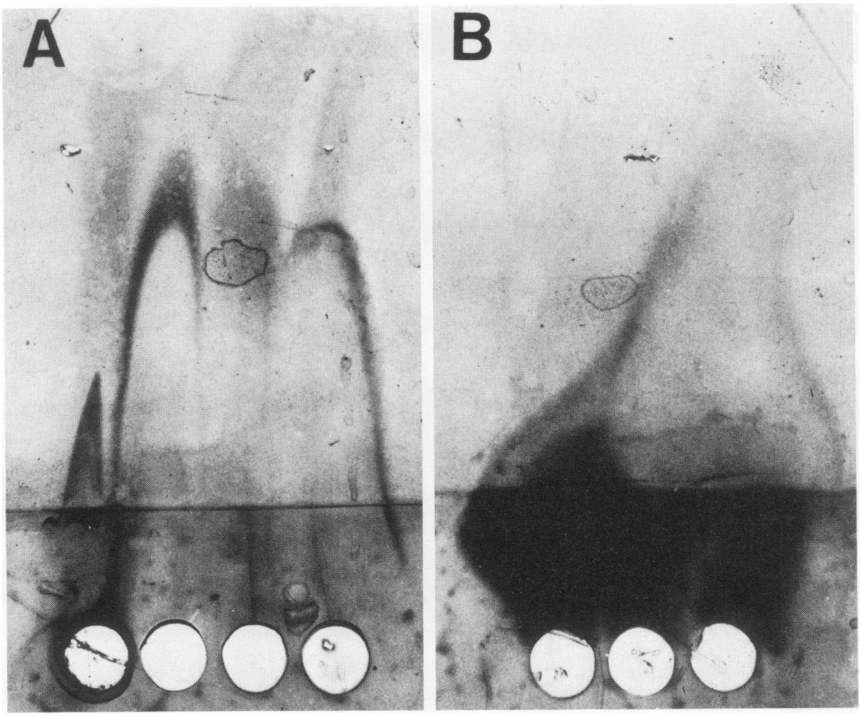

FIG. 3. Rocket immunoelectrophoresis of *T. beigelii* and *C. neoformans* antigens. (A) Wells contained (left to right) 30 μl of serum obtained from the patient at necropsy, 120 μg of CWE of *T. beigelii* UW, 120 μg of *T. beigelii* ATCC 34148, and 120 μg of *T. beigelii* ATCC 4155. (B) Wells contained (left to right) 240 μg of capsular polysaccharide of *C. neoformans* serotype D, B, and A. The indicator gel of each plate contained 22.2 μl of rabbit anti-*Trichosporon* serum per cm^2. Electrophoresis was at 2 V/cm for 18 h (anode at top of each plate).

culture collection strains contained an antigen which cross-reacted with one in the strain UW CWE. Rocket immunoelectrophoresis of each capsular polysaccharide of *C. neoformans* produced a precipitin arc that migrated into the indicator gel and a dense precipitin arc that migrated poorly from the sample well. When rocket immunoelectrophoresis was done with the necropsy serum of the patient placed next to cryptococcal polysaccharides (data not shown), the lower edges of the arc produced by the serum of the patient were lost in the dense, poorly migrating arcs of the cryptococcal antigens. Indirect fluorescent-antibody staining also demonstrated cross-reactivity between surface components of *T. beigelii* and *C. neoformans* (Fig. 1). Anti-*Trichosporon* serum stained both *T. beigelii* UW and *C. neoformans* to a titer of 1:2,560. When the antiserum was absorbed with *T. beigelii*, no staining of either organism was seen at any dilution tested.

DISCUSSION

Our results indicate that *T. beigelii* possesses an antigen during invasive infection which is in common with the capsular polysaccharide antigen of *C. neoformans*. This antigen can produce a false-positive cryptococcal latex agglutination test. Treatment of sera containing the *Trichosporon* antigen with proteolytic enzymes and boiling does not decrease its ability to agglutinate AC-latex beads. This is important because such treatments have been used to eliminate rheumatoid factor and other materials from serum that can produce a false-positive latex agglutination reaction. Since the antigen responsible for agglutinating AC-latex beads can be obtained from cell wall fragments of *T. beigelii* and can withstand proteolytic enzymes and boiling, we believe that it is a polysaccharide. This is interesting when one considers that Gorin and Spencer have demonstrated that *T. beigelii* releases a polysaccharide containing mannose, arabinose, and xylose into its surrounding medium (5). The main component of the cryptococcal capsular polysaccharide is a glucuronoxylomannan (3, 12). All of these observations suggest that the antigen of *T. beigelii* is similar to the capsular polysaccharide of *C. neoformans* and may be a similar polysaccharide. Further analysis of the antigen will be required to define the structure of its antigenic determinants. Although there are physiological similarities between *T. beigelii* and *C. neoformans*, the former does not appear to possess a capsule. *T. beigelii* grown on malt extract medium or a chemically defined medium does not show evidence of a capsule by either light (9) or electron (19) microscopy.

Our preliminary studies with CWE of three *T. beigelii* strains suggest that there is an antigen that may be shared among strains of this species. CWE of ATCC 4155 was less potent than that of ATCC 34148 or strain UW in agglutinating AC-latex beads. Because ATCC 4155 and ATCC 34148 were isolated in 1921 and 1976, respectively, it is possible that multiple passages of ATCC 4155 have decreased expression of the cell wall antigen cross-reactive with *C. neoformans* polysaccharide. A minimum concentration of 0.5 to 1 μg of CWE per ml of strain UW or ATCC 34148 was needed to produce discernible agglutination of AC-latex beads.

Since the frequency of isolation of *T. beigelii* from sputum, urine, and stool in compromised hosts significantly exceeds the incidence of invasive disease (6, 14, 15), a sensitive method for identifying patients with invasive disease would be useful. If the incidence of *Trichosporon* infection in such patients increases, one would like to have a test for a *Trichosporon* antigen that would be specific. Our observations indicate one should be cautious in interpreting

a positive cryptococcal latex agglutination test produced by serum from an immunocompromised patient who has had *T. beigelii* cultured from blood or a tissue biopsy.

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