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Immunoblot Analysis of the Serological Response in Invasive *Trichosporon beigelii* and *Blastoschizomyces capitatus* Infections

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The serological response to *Trichosporon* sp. was examined by the immunoblot technique. Antibodies to a range of antigens (200 to 16 kilodaltons) were detectable in three patients with invasive *Trichosporon beigelii* or *Blastoschizomyces capitatus* infections and 10 uninfected controls. High levels of preexisting antibodies may contribute to the rarity of systemic infections.

Systemic infection with *Trichosporon beigelii* (formerly *Trichosporon cutaneum*) or *Blastoschizomyces capitatus* (formerly *Trichosporon capitatum*) is rare but increasingly recognized. Of the 25 cases reported, 13 have occurred in patients with acute leukemia (2, 5–7, 10, 17, 18, 21, 26). Others were associated with multiple myeloma (7, 19), aplastic anemia (15, 25), renal transplantation (19), chronic active hepatitis (9), bronchial carcinoma (24), endocarditis (1, 3, 13), and intracapsular lens extraction (20). *T. beigelii* caused 18, *B. capitatus* caused 6, and an unknown *Trichosporon* species caused 1 of these cases. Overall mortality was 78%. Such infection has not been associated with diseases characterized by impaired mononuclear cell-mediated immunity, such as Hodgkin's disease, suggesting deficits in delayed hypersensitivity is not a major underlying susceptibility factor.

Trichosporon species are normal inhabitants of the soil, and *T. beigelii* may cause white piedra, a superficial infection of the hair shaft. Of 3,340 clinical yeast isolates at the Memorial Sloan-Kettering Cancer Center, New York, N.Y., only 13 (0.4%) were *T. beigelii*, compared with 2,289 (68.5%) *Candida albicans* (8). Rivera and Cangir noted a recent increase in the frequency of *T. beigelii* from throat and urine cultures, but only one of their severely immunocompromised patients developed clinical disease (18). When 353 severely immunocompromised patients at the Johns Hopkins Oncology Center, Baltimore, Md., were screened for *Trichosporon* species over a 37-month period, 13 patients (3.7%) had positive stool, skin, or urine cultures, but only 3 of them developed systemic disease (7). It therefore appears that immunosuppressed patients colonized with *Trichosporon* species can often successfully resist the development of invasive disease. Antibody may have some protective value. The immunoblotting technique was used to analyze the serological response to *Trichosporon* species in 3 patients with systemic infections and 10 controls, 6 of whom were severely immunocompromised leukemic patients.

The two patients who died from systemic *B. capitatus* infections, as reported elsewhere (2), were undergoing induction chemotherapy for acute myeloblastic leukemia when *B. capitatus* was isolated from blood cultures. At autopsy, histological examination showed involvement of the spleen, esophagus, and kidneys of both patients. *B. capitatus* was cultured from the spleen and kidney of one patient and from the spleen, kidney, lung, and liver of the other. The third patient was a 60-year-old female undergoing cytotoxic chemotherapy for acute myeloid leukemia who developed fever unresponsive to antibiotics and a scattered erythematous macular rash. Blood cultures grew *T. beigelii*. The pyrexia resolved with amphotericin B which was discontinued after 300 mg due to deterioration of renal function. There was a concomitant resolution of her neutropenia. None of these patients was colonized by *C. albicans*. Four sera from each patient were examined in the same run so that any changes observed did not reflect changes in the analytical method, and the blots were repeated when possible. There was insufficient serum to prepare blots against *B. capitatus* and *T. beigelii*, and the latter was examined because it is a more common infection (8).

Control sera were obtained from six other leukemic patients and four postoperative surgical patients treated for gram-negative septicemias. Concomitantly, all six leukemic patients had oral *C. albicans* infections, confirmed by culture, but none of them had systemic candidosis according to clinical, cultural, and serological criteria (14). When screened, none of the leukemic or surgical controls grew *B. capitatus* or *T. beigelii* from oral and perineal swabs, nor did samples from the surgical patients grow *C. albicans*.

T. beigelii NCPF 3077 was grown on Sabouraud glucose agar at 30°C for 3 days, harvested, washed three times in distilled water, and disrupted in a Mickle homogenizer for 30 min. After being centrifuged at 12,000 × *g* for 10 min, the supernatant was assayed for protein by the method of Lowry et al. (12). *C. albicans* NCTC 3153 was prepared similarly. Immunoblotting was performed as previously described (4, 14). Briefly, 50 μg of *T. beigelii* or *C. albicans* was applied to a 10% slab gel, and sodium dodecyl sulfate-polyacrylamide gel electrophoresis was performed in a discontinuous buffer system (11). A current (90 V, 350 mA) was applied for 1.5 h for transfer from gel to nitrocellulose membrane in a transblotting chamber (Bio-Rad Laboratories, Richmond, Calif.), essentially as described by Towbin et al. (23), with as buffer 25 mM Tris per liter–192 mM glycine per liter in 20% methanol (pH 8.3). After overnight incubation at 4°C in 3% bovine serum albumin (Sigma Chemical Co., St. Louis, Mo.), nitrocellulose strips were incubated with a 1:10 dilution of the patient's serum at room temperature for 2 h. Sera were diluted in 3% bovine serum albumin–0.05% Tween 20

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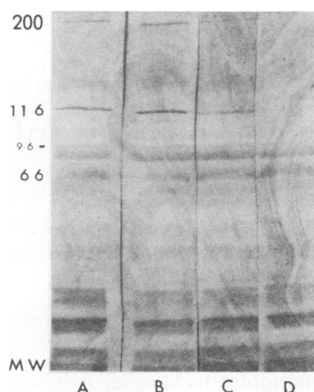

FIG. 1. Immunoblots of the IgM response in a fatal case of *B. capitatus* septicemia; sera were collected on 6 April (A), 8 April (B), 16 April (C), and 20 April (D), the day the patient died. MW, Molecular weight (× 10^3).

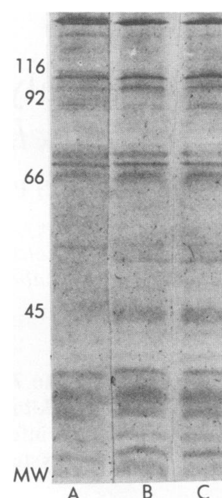

FIG. 2. Immunoblots of the IgG response in the patient who survived systemic *T. beigelii* infection; sera were collected on 10 October (A), 20 October (B), and 12 November (C). MW, Molecular weight (× 10^3).

in buffered saline. Strips were washed and then incubated for 1 h at room temperature with alkaline phosphatase-conjugated goat anti-human immunoglobulin M (IgM) or IgG (Sigma), at a 1:1,000 dilution. After washing, they were incubated in equal volumes of naphthol AS-MX phosphate (Sigma; 0.4 mg/ml in distilled water) and Fast Red TR Salt (Sigma; 6 mg/ml in 0.2 M Tris per liter [pH 8.2]) as described by O'Connor and Ashman (16). Antigen-antibody binding, identified by the deposition of red stain of various intensities, was measured by reflectance densitometry (Chromoscan 3; Joyce Loebl) as discussed by Towbin and Gordon (22).

A total of 25 different antigen bands, 200 to 16 kilodaltons (kDa), were detected by patient and control sera. In the patients with fatal *B. capitatus* infections, IgG to bands of 200 and 116 kDa faded as the infection progressed, as did IgM to bands 200, 116, and 96 kDa in one of these patients (Fig. 1; Table 1). In the patient who survived systemic *T. beigelii*, the major change was the rise in IgG to the 45-kDa band (Fig. 2; Table 1). Antibody to this band occurred in four controls but in neither of the fatal *B. capitatus* infections. Other bands were not associated with marked changes in antibody levels during the systemic infections. All bands recognized by the patient sera were also recognized by two or more controls, in whom antibody levels were constant (five paired sera were examined). Antibodies were mainly of the IgG class. Bands of 92, 84, 39, 36, 35, and 32 kDa were all recognized by at least seven controls. Bands of 176, 164, and 140 kDa were detected by sera of the patients, who were leukemic, and all the leukemic controls, but not by the sera of nonleukemic controls. Some bands (45, 30, 28, and 16 kDa) were recognized by sera from the patient with *T. beigelii* and not by sera from those with *B. capitatus*,

TABLE 1. Densitometry of the antibody response to four *Trichosporon* antigenic bands present in patients with systemic infection and controls with leukemia or gram-negative septicemia

Patient and serum no.	Densitometry trace ht (mm) for the following antibody to the indicated antigen band (kDa):							
	IgM				IgG			
	200	116	96	45	200	116	96	45
Patient 1 (*B. capitatus*)								
1	25	58	14		97	36	34	
2	20	51	11		92	31	29	
3		30	10		41	28	16	
4		4	6		34	25	12	
Patient 2 (*B. capitatus*)								
1	9	8			69	20	12	
2	9	8			54	17	13	
3	10	9			36	13	13	
4	11	9			5	10	14	
Patient 3 (*T. beigelii*)								
1				10	83	62	30	9
2				10	84	63	32	18
3				10	83	65	31	31
4				10	85	66	33	45
Controls (n = 10)[a]	1 (3)	5 (10)	11 (23)		85 (46)	67 (58)	14 (12)	29 (22)

[a] Values are means (standard deviation).

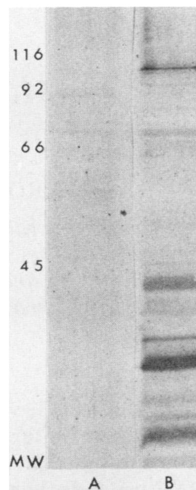

FIG. 3. Immunoblots of the IgG response in a control patient with gram-negative septicemia against *C. albicans* (A) and *T. beigelii* (B). MW, Molecular weight ($\times 10^3$).

whereas others (176, 66, 48, 43, and 40 kDa) were recognized by both of the latter but not the former.

All the controls had large amounts of antibodies to *T. beigelii* but little if any to *C. albicans* (Fig. 3), despite the fact that none of them was colonized with *T. beigelii* but six were colonized with *C. albicans*. The presence of antibody to *Trichosporon* spp. may reflect prior exposure to this yeast. Unlike *C. albicans*, it is common in the environment but infrequently isolated from man (7, 8). Alternatively, the presence of antibody may have been due to cross-reaction with some other organism. Recently, cross-reactivity was described between antibody to *T. beigelii* and another basidiomycetous yeast, *Cryptococcus neoformans* (15). Whatever the origin of this preexisting antibody, it may be protective. Because it is present even in severely immunocompromised patients, it may be one of the reasons for the rarity of invasive *Trichosporon* infections compared with systemic candidosis. Unfortunately, it will also make serodiagnostic tests based on antibody detection for this infection difficult to develop.

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