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Demonstration of a Cell Wall Antigen Cross-Reacting with Cryptococcal Polysaccharide in Experimental Disseminated Trichosporonosis

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Patients with disseminated infections caused by *Trichosporon beigelii* have a circulating antigen that cross-reacts with the polysaccharide capsule of *Cryptococcus neoformans*. We studied the localization of this antigen by immunoelectron microscopy in a rabbit model of experimental disseminated trichosporonosis. Deparaffinized lung sections were examined by using a murine monoclonal anti-cryptococcal polysaccharide antibody and colloidal gold particles coated with goat antibody to murine immunoglobulin G. Antigen that cross-reacted with the monoclonal antibody was observed in the *T. beigelii* cell wall and in a fibrillar matrix extending from the cell wall.

Trichosporon beigelii is a saprobic yeast that is widely distributed in nature and that may colonize skin and mucosal surfaces in humans (5, 12). It is associated with hypersensitivity pneumonitis (14), superficial infections such as onychomycosis (3), and a nodular infection of the hair shaft called white piedra (15). Increasingly, *T. beigelii* has been recognized as an agent of disseminated infections involving the lungs, skin, kidneys, eyes, liver, heart, and brain in immunosuppressed individuals. Disseminated trichosporonosis occurs most frequently in granulocytopenic patients and is generally fatal, despite aggressive antifungal therapy, unless the bone marrow recovers (5, 17). When isolated from blood, tissue biopsy specimens, or normally sterile body fluids from an immunocompromised patient, *T. beigelii* should be regarded as clinically significant until proven otherwise. A positive serum cryptococcal latex agglutination reaction may help to substantiate the diagnosis of disseminated trichosporonosis (1, 8, 9).

The presence of antigenic similarity between the cell wall of *T. beigelii* and the *Cryptococcus neoformans* capsular polysaccharide may not be coincidental, because they are both doliporous basidiomycetes (7, 11). Seeliger and Schroter (13) first recognized the presence of shared antigens between *T. beigelii* and *C. neoformans*. McManus and colleagues (8) later reported two patients with disseminated trichosporonosis, both of whom had positive serum tests for cryptococcal polysaccharide antigen by latex agglutination. Furthermore, they described an extractable, heat-stable cell wall antigen of *T. beigelii* that agglutinated latex beads coated with antibody to *C. neoformans* (9). Kobayashi and colleagues (6) developed an immunohistochemical method for identifying *T. beigelii* in tissue sections by immunoperoxidase staining. They noted that unabsorbed antiserum to *T. beigelii* was weakly cross-reactive with *C. neoformans* and that absorption with *C. neoformans* had no significant effect on the strong reactivity with *T. beigelii*. However, antiserum absorbed with *C. neoformans* failed to cross-react. They concluded that the major antigen associated with *T. beigelii* is not in common with the capsular polysaccharide antigen of *C. neoformans*. Histological sections of lung from a patient with disseminated trichosporonosis indicated that the cross-reactive antigen was situated in the cell wall and extracellular matrix of *T. beigelii* (10). Since little is known, however, about the production and localization of this cross-reactive antigen in vivo, we therefore sought to determine by immunoelectron microscopy the localization of this antigen in an experimental model of disseminated trichosporonosis. We also investigated whether *T. beigelii* infection in this experimental model would produce the extracellular matrix similar to that found in human infections and whether cross-reactive antigen was present in the matrix in vivo.

T. beigelii TCM-NCI was isolated from the blood of a granulocytopenic patient with disseminated, refractory trichosporonosis. The patient's serum reacted with cryptococcal capsular polysaccharide by latex agglutination at a titer of 1:256. The identity of the isolate was confirmed by standard microbiological techniques (2). Cultures of the isolate were maintained at −70°C in a skim milk suspension and at 4°C in sterile saline. The inoculum was grown in the Emmon modification of Sabouraud glucose broth (Media Department, National Institutes of Health, Bethesda, Md.) in a gyratory water bath overnight at 37°C for 18 h, centrifuged, and washed; and the concentration of the inoculum was adjusted with a hemacytometer and confirmed by quantitative cultures of 10-fold serial dilutions. Microscopically, the organisms appeared predominantly as blastoconidia and arthroconidia. The inoculum was administered as a 10^9-CFU suspension in 10 ml of sterile normal saline.

Eleven pathogen-free female New Zealand White rabbits (weight, 2.0 to 3.0 kg; Hazleton, Rockville, Md.) were used in this study. Silastic central venous catheters were inserted

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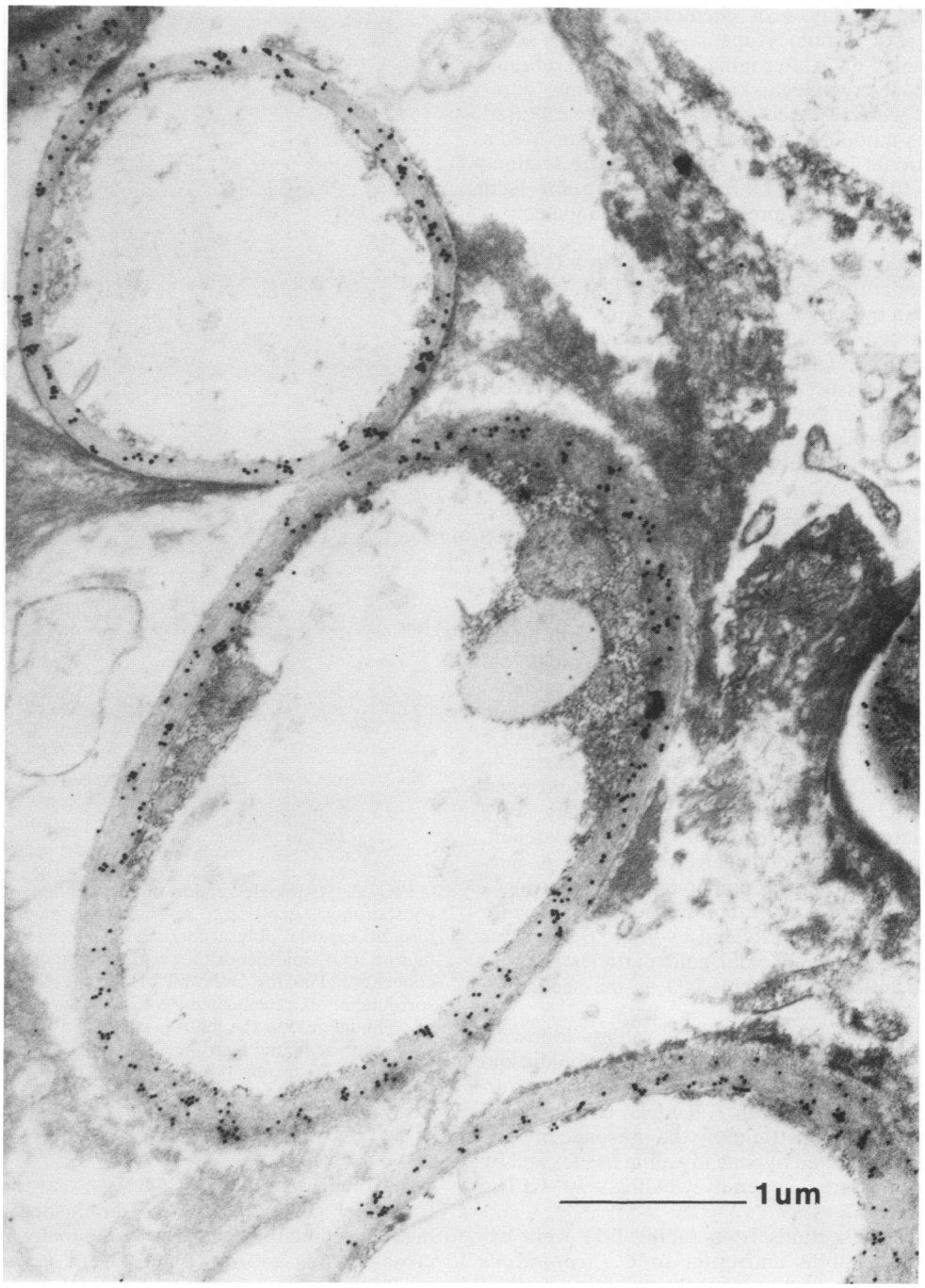

FIG. 1. Histologic section of rabbit lung demonstrating blastoconidial forms of *T. beigelii* after exposure to polyclonal rabbit antiserum to *C. neoformans* capsular polysaccharide and overlaid with colloidal gold particles coated with goat antibody to rabbit IgG. There is significant deposition of colloidal gold in the cell wall. The extracellular fibrillar matrix is expressed more densely at the polar ends of the organisms in this specimen, and at these points colloidal gold deposition in the matrix is demonstrated. Photo, electron micrograph; magnification, ×25,900.

for continued nontraumatic venous access, as described previously (16). Granulocytopenia (<500/μl) was produced by administration of cytosine arabinoside (kindly provided by The Upjohn Co., Kalamazoo, Mich.) as follows: 400 mg/m^2/day intravenously (i.v.) on days 1 to 5 (induction) and 400 mg/m^2/day i.v. every other 2 days (maintenance). Granulocytopenia was maintained for 1 to 3 weeks. Total granulocyte counts were monitored daily. Methylprednisolone (kindly provided by The Upjohn Co.) was administered at 2 mg/kg/day i.v. during induction. Ceftazidime (Glaxo, Research Triangle Park, N.C.) at 150 mg/kg/day i.v. and vancomycin (Eli Lilly, Indianapolis, Ind.) at 15 mg/kg/day

i.v. were initiated on day 4 of chemotherapy and were continued throughout granulocytopenia. Rabbits were sacrificed between 1 and 5 days after inoculation. The cerebrum, cerebellum, lung, liver, spleen, kidney, quadriceps, vitreous, and choroid were resected and quantitatively cultured onto the Emmons-modified Sabouraud glucose agar (Media Department, National Institutes of Health). Tissue sections were placed in 10% neutral buffered Formalin for light microscopy, and others were prepared for immunoelectron microscopy.

Histological specimens were fixed overnight at 4°C in 2% glutaraldehyde–sodium cacodylate buffer (0.2 M, pH 7.4) and then washed in buffer solution, postfixed in 2% osmium tetroxide, dehydrated in ethanol-propylene oxide, and embedded in Spurr (Pella, Tustin, Calif). Sections of paraffin-embedded necropsy tissue specimens were deparaffinized, washed in buffer, dehydrated, and embedded in Spurr. Thin sections were prepared on nickel grids (Pella) that were etched with 3% H_2O_2 for 10 min, washed with filtered Tris hydrochloride buffer (0.02 M, pH 7.2), and immersed in drops of filtered 10% chicken egg albumin (Sigma, St. Louis, Mo.) for 10 min. The grids were then incubated with a 1:100 dilution of murine monoclonal antiserum to *Cryptococcus* capsular polysaccharide (kindly provided by Thomas R. Kozel) for 48 h at 4°C. Control grids were incubated with buffer. After incubation, the grids were washed with buffer and immersed for 1 h in a solution containing colloidal gold particles (diameter, 20 nm; Janssen Life Science Products, Piscataway, N.J.), which were coated with goat antibody to murine immunoglobulin G (IgG) that were diluted 1:20 in 1% albumin–Tris hydrochloride. The grids were then washed in filtered buffer and deionized water, blotted dry, and post-stained with uranyl acetate and lead citrate; they were examined and micrographed by using a Zeiss (Oberkochen, Federal Republic of Germany) electron microscope. Sections were also examined in a similar manner by using a 1:100,000 dilution of polyclonal rabbit antiserum to *Cryptococcus* (titer, 1:1056; kindly provided by J. E. Bennett) as the primary antibody and colloidal gold particles (20 nm) coated with goat antibody to rabbit IgG as the secondary antibody.

Tissue sections from granulocytopenic rabbits following inoculation with 10^9 CFU i.v. of *T. beigelii* arthroconidia and blastoconidia were examined by light microscopy to determine the presence of infection with *T. beigelii*. Disseminated trichosporonosis was demonstrated by the presence of blastoconidia, arthroconidia, and hyphae invading tissues and by quantitative tissue cultures, generally yielding $\geq 10^4$ CFU/g of each tissue sampled.

Deparaffinized lung sections from rabbit T-11 were exposed to polyclonal rabbit antiserum to *C. neoformans* capsular polysaccharide and were subsequently overlaid with colloidal gold particles coated with goat antibody to rabbit IgG. Figure 1 demonstrates blastoconidial forms expressing the fibrillar extracellular matrix extending from the cell wall. Deposition of the colloidal gold particles is generally confined to the cell wall and the extracellular matrix. Because we used rabbit tissue in the experimental model, we wanted to be certain that some of the gold labeling was not due to the binding of rabbit IgG to epitopes that were not related to the cross-reactive antigen of *T. beigelii*. A murine monoclonal antibody to *C. neoformans* capsular polysaccharide was obtained, and Fig. 2 demonstrates the localization and relative concentration of the cross-reactive antigen. Once again, the gold labeling was concentrated in the cell wall of *T. beigelii* and extended into the extracellular matrix projecting from the cell wall. Note that the relative concentration of gold labeling was diminished, representing the higher specificity of the murine monoclonal antibody for the cross-reacting antigen. Figure 3 is a negative control demonstrating no deposition of colloidal gold in either the cell wall or the extracellular matrix.

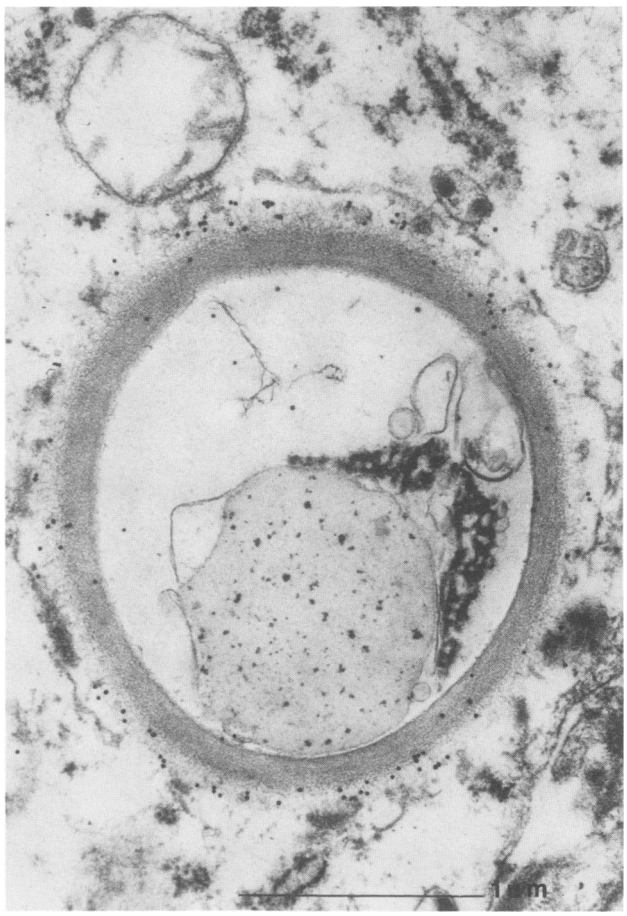

FIG. 2. Histologic section of rabbit lung with disseminated trichosporonosis exposed to murine monoclonal antibody to *C. neoformans* capsular polysaccharide and overlaid with goat antibody to murine IgG. Gold deposition representing cross-reactive antigen is concentrated in the cell wall of *T. beigelii* and extends into the surrounding extracellular matrix. The more prominent localization of colloidal gold in the matrix, compared with that in Fig. 1, most likely represents the high specificity of the monoclonal antibody for cryptococcal capsular polysaccharide. Photo, electron micrograph; magnification, ×32,700.

Our results demonstrate that the extracellular matrix produced by *T. beigelii* in invasive human disease can be reproduced in an experimental model of disseminated trichosporonosis. The cell wall and extracellular matrix produced by invasive strains of *T. beigelii* contain an antigen that cross-reacts with *C. neoformans* capsular polysaccharide, as shown by colloidal gold labeling detected by immunoelectron microscopy. One advantage of using a monoclonal antibody for this study is the increased specificity it has for specific epitopes of the capsular polysaccharide compared with the specificity of polyclonal antisera. The gold labeling of cross-reacting antigen was less intense and required a higher concentration of the primary antibody for detection of

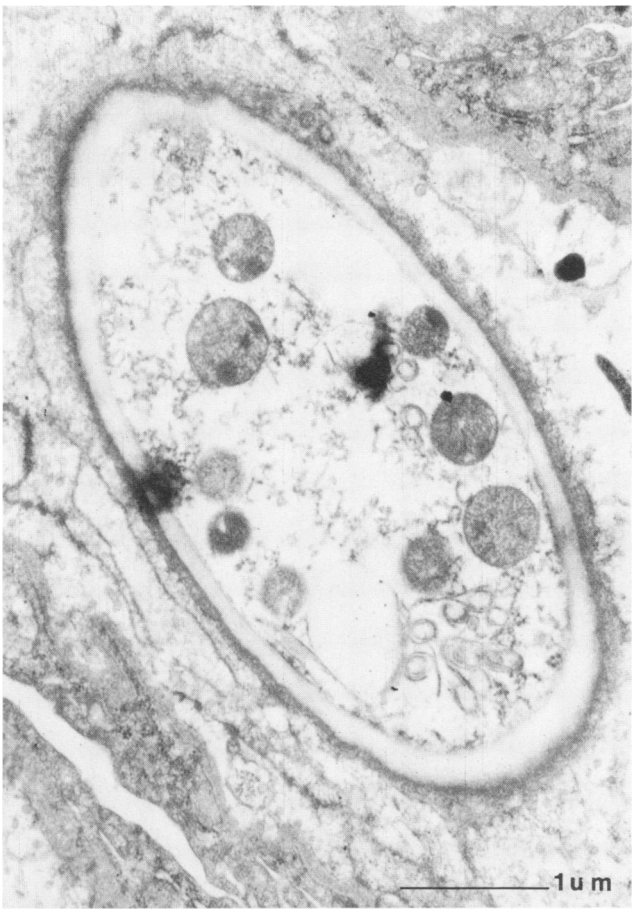

FIG. 3. Histologic section of rabbit lung with disseminated trichosporonosis, negative control, demonstrating no deposition of colloidal gold in either the cell wall or the extracellular matrix. Photo, electron micrograph; magnification, ×20,200.

the cross-reacting antigen than was required when polyclonal sera were used. This supports the findings of Kobayashi et al. (6) that only a minor component of the *T. beigelii* cell wall antigen is shared with *C. neoformans*. This postulate is further supported on a biochemical basis. Gorin and Spencer (4) detected a polysaccharide from *T. beigelii* containing xylose, arabinose, and mannose that was released into its surrounding medium. Perhaps it is the mannan shared by these two organisms that is the cross-reactive epitope. Further studies with monoclonal antibodies against various components of the cell wall of *T. beigelii* may more clearly define the nature of this cross-reactive antigen.

The extracellular matrix produced by invasive *T. beigelii* is derived in part from the cell wall. We believe that when invasive disease occurs with *T. beigelii*, this extracellular matrix is elaborated, probably directly into the bloodstream, allowing it to be detected by the latex agglutination test for cryptococcal capsular polysaccharide. In a previous study (10) (data not shown), we were unable to detect the presence of the extracellular matrix by electron microscopy when *T. beigelii* was grown on solid medium. Formation of the extracellular matrix may be induced by the presence of mammalian tissue. That host tissue proteins may contribute in part to the formation of the matrix cannot be excluded. Our finding that the production of this extracellular matrix and detection of a cross-reactive antigen were present in a second isolate of *T. beigelii* implicated in disseminated human mycosis is supported by the analysis of cell wall extracts performed previously by McManus et al. (9). Although the potency of agglutination with anti-*C. neoformans*-coated latex beads varied among the strains, they found that cell wall extracts from three isolates of *T. beigelii* shared this cross-reactive antigen. One of the isolates was pathogenic, and the other two were reference isolates.

In summary, results of our studies suggest that pathogenic isolates of *T. beigelii* produce a fibrillar extracellular matrix during invasive disease, and this can be reproduced in an experimental model of disseminated trichosporonosis. This extracellular matrix is composed of elements of the cell wall. The cell wall and extracellular matrix contain an antigen that is similar to cryptococcal capsular polysaccharide and that can be detected by immunoelectron microscopy. The specific antigen shared by *C. neoformans* and *T. beigelii* remains to be determined.

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REFERENCES

1. **Campbell, C. K., A. L. Payne, A. J. Teall, A. Brownell, and D. W. R. Mackenzie.** 1985. Cryptococcal latex antigen test positive in a patient with Trichosporon beigelii. Lancet **ii**:43–44.
2. **Cooper, B. H., and M. Silva-Hunter.** 1985. Yeasts of medical importance, p. 526–541. *In* E. H. Lennette, A. Balows, W. J. Hausler, Jr., and H. J. Shadomy (ed.), Manual of clinical microbiology, 4th ed. American Society for Microbiology, Washington, D.C.
3. **Fusaro, R. M., and N. G. Miller.** 1984. Onychomycosis caused by *Trichosporon beigelii* in the United States. J. Am. Acad. Dermatol. **11**:747–749.
4. **Gorin, P. A. J., and J. F. T. Spencer.** 1967. A novel pentosylmannan arising from the yeast *Trichosporon cutaneum*. Can. J. Chem. **45**:1543–1549.
5. **Haupt, H. M., W. G. Merz, W. E. Beschorner, W. P. Vaughan, and R. Saral.** 1983. Colonization and infection with *Trichosporon* species in the immunocompromised host. J. Infect. Dis. **147**:199–203.
6. **Kobayashi, M., S. Kotani, M. Fujishita, H. Taguchi, T. Moriki, H. Enzan, and I. Miyoshi.** 1988. Immunohistochemical identification of *Trichosporon beigelii* in histologic section by immunoperoxidase method. Am. J. Clin. Pathol. **89**:100–105.
7. **Kreger-van Rij, N. J. W., and M. Veenhuis.** 1971. Septal spores in *Trichosporon cutaneum*. Sabouraudia **9**:36–38.
8. **McManus, E. J., M. J. Bozdech, and J. M. Jones.** 1985. Role of the latex agglutination test for cryptococcal antigen in diagnosing disseminated infection with *Trichosporon beigelii*. J. Infect. Dis. **151**:1167–1169.
9. **McManus, E. J., and J. M. Jones.** 1985. Detection of a *Trichosporon beigelii* antigen cross-reactive with *Cryptococcus neoformans* capsular polysaccharide in serum from a patient with disseminated *Trichosporon* infection. J. Clin. Microbiol. **21**: 681–685.
10. **Melcher, G. P., M. G. Rinaldi, C. L. Frey, and D. J. Drutz.** 1988. Demonstration, by immunoelectronmicroscopy, of a cell wall antigen in *Trichosporon beigelii* that cross-reacts with *Cryptococcus neoformans* capsular polysaccharide. J. Infect. Dis. **158**:901–902.
11. **Oblack, D. L., and J. C. Rhodes.** 1983. Use of the rapid diazonium blue B test to differentiate clinically important species of *Trichosporon* and *Geotrichum*. Sabouraudia **21**:243–245.

12. **Rose, H. D., and V. P. Karup.** 1977. Colonization of hospitalized patients with yeast-like organisms. Sabouraudia **15**:251–256.
13. **Seeliger, H. P. R., and R. Schroter.** 1963. A serological study on the antigenic relationship of the form genus *Trichosporon*. Sabouraudia **2**:248–263.
14. **Shimazu, K., M. Ando, T. Sakata, K. Yoshida, and S. Araki.** 1984. Hypersensitivity pneumonitis induced by *Trichosporon cutaneum*. Am. Rev. Respir. Dis. **130**:407–411.
15. **Steinman, H. K., and R. B. Pappenfort.** 1984. White piedra—a case report and review of the literature. Clin. Exp. Dermatol. **9**:591–598.
16. **Walsh, T. J., J. Bacher, and P. A. Pizzo.** 1988. Chronic silastic central venous catheterization for induction, maintenance, and support of persistent granulocytopenia in rabbits. Lab. Anim. Sci. **38**:467–471.
17. **Walsh, T. J., K. R. Newman, M. Moody, R. C. Wharton, and J. C. Wade.** 1986. Trichosporonosis in patients with neoplastic disease. Medicine **65**:268–279.