JOURNAL OF CLINICAL MICROBIOLOGY, Nov. 2003, p. 5333–5336 Vol. 41, No. 11
0095-1137/03/$08.00+0 DOI: 10.1128/JCM.41.11.5333–5336.2003

Allergic Fungal Sinusitis Associated with *Trichoderma longibrachiatum*

Patrick Tang,[1] Subhash Mohan,[1] Lynne Sigler,[2] Ian Witterick,[3] Richard Summerbell,[4] Iivi Campbell,[1] and Tony Mazzulli[1]*

Department of Microbiology, Toronto Medical Laboratories and Mount Sinai Hospital,[1] and Department of Otolaryngology, Mount Sinai Hospital,[3] Toronto, Ontario, and University of Alberta Microfungus Collection and Herbarium, Edmonton, Alberta,[2] Canada, and Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands[4]

Received 18 March 2003/Returned for modification 18 July 2003/Accepted 20 August 2003

We describe allergic fungal sinusitis caused by *Trichoderma longibrachiatum* in a patient with a history of atopy and asthma. A Gram stain of a sinus biopsy specimen was initially thought to contain yeast cells, but when *Trichoderma* was recovered in culture, these cells were subsequently recognized as chlamydospores. The patient was successfully managed with a combination of sinus lavage, oral corticosteroids, itraconazole, and allergen immunotherapy. This case also points out that careful scrutiny of direct smears is required to ensure that fungal structures are not misinterpreted.

CASE REPORT

A 52-year-old woman was diagnosed with chronic sinusitis in 1997 after complaining of nasal congestion, sinus pressure, and headaches. She had a history of atopy and asthma since childhood. Since 1997, she required low-dose oral corticosteroids for the asthma. A CT scan of the sinuses done in September 1997 showed pansinusitis without evidence of polyps. Both osteomeatal units were opacified, and mucosal thickening was seen. She had eosinophilia and her serum immunoglobulin E (IgE) level was elevated at 2,227 μg/liter. Endoscopy revealed edema of the mucosal membranes in both middle meatii with thick mucus. No polyps or masses were visualized.

Because of frequent acute episodes of sinusitis requiring therapy with various antibiotics, it was felt that she would benefit from sinus surgery. She underwent bilateral endoscopic ethmoidectomy and antrostomy in December 1998 with significant relief of her nasal symptoms. However, her symptoms returned in May 1999 and she was sent for allergy testing. Through skin testing, she was found to be allergic to various environmental agents but not to molds. At this time, her blood count was normal with no eosinophilia. However, her serum IgE level was still elevated (2,190 μg/liter). She required a bilateral endoscopic maxillotomy and revision ethmoidectomy in January 2000. The tissue showed edema with chronic mucosal inflammation. Inflammatory polyps were also removed from the left ethmoid sinus. Fungal elements were not observed in periodic acid-Schiff stains of the tissue. She had transient relief of her symptoms until April 2000 when her nasal congestion returned.

The presence of inflammatory polyps and mucosal changes in her sinuses was consistent with an allergic etiology, so she was reinvestigated for allergies in April 2000. Repeat skin testing now showed a reaction to *Aspergillus* spp. and several other common molds. Allergic fungal sinusitis (AFS) was suspected and she was started on nebulized itraconazole and a leukotriene receptor antagonist. These were discontinued in June 2001, as she had no improvement.

Bilateral endoscopic antral lavage was done in January 2002. A large amount of tenacious yellow mucus was removed from both maxillary sinuses. Biopsy specimens were taken from the left and right antrum for direct microscopy and culture. A fast-growing mold was isolated in pure culture and identified as a *Trichoderma* species. Given the high propensity for relapse of AFS, a trial of allergen immunotherapy and oral itraconazole was also initiated with some improvement. The patient's symptoms are presently well controlled with low-dose corticosteroids and intranasal itraconazole. Her sinuses remain free of any inflammatory polyps or mucosal changes.

AFS is the most common form of fungal sinusitis. Patients afflicted with AFS are immunocompetent but often have a history of atopy and asthma exacerbated by their sinusitis (26). AFS is analogous to allergic bronchopulmonary aspergillosis in that the fungus acts as an allergen to elicit an intense immune response (1). There is no invasion of the mucosa, granuloma formation, or tissue necrosis.

The diagnosis of AFS is often missed in cases of unexplained chronic sinusitis. Approximately 6 to 7% of chronic rhinosinusitis cases requiring surgery are caused by AFS (13, 28). The primary etiologic agents described in AFS are dematiaceous fungi, especially *Bipolaris spicifera* or *Curvularia lunata* in some geographic regions (20, 26), *Aspergillus* species (8, 13), and the basidiomycete *Schizophyllum commune* (12, 30, 31). Presumably, the disease begins with colonization of the paranasal sinuses by spores of an airborne fungus. In a susceptible patient, this leads to a fungal-specific IgE and IgG response (8, 9). The result is sinus obstruction due to buildup of allergic mucin and hypertrophy of the sinus mucosa, often with polyp formation (19).

Treatment for this disease usually involves surgical debridement to remove the hypertrophic tissue and mucinous concretions (1). Nasal and oral corticosteroids are often used to modulate the immune response (27). In refractory cases, a trial of systemic antifungal therapy may be warranted (5, 19, 30, 34).

* Corresponding author. Mailing address: Department of Microbiology, Mount Sinai Hospital, 600 University Ave., Toronto, Ontario, Canada M5G 1X5. Phone: (416) 586-4695. Fax: (416) 586-8746. E-mail: tmazzulli@mtsinai.on.ca.

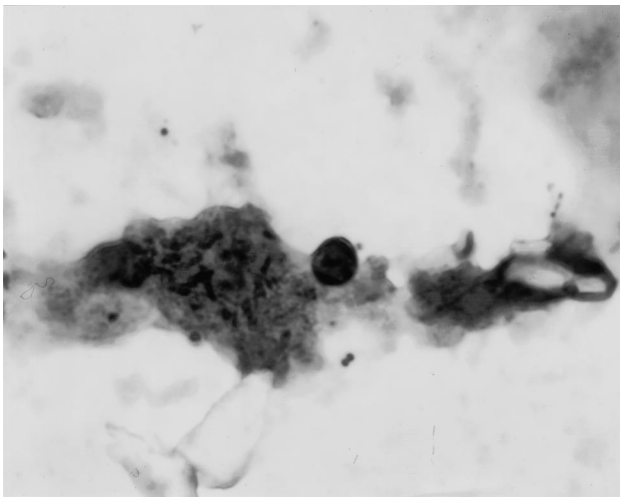

FIG. 1. Gram stain of antral biopsy specimen showing globose cell initially thought to represent a nonbudding yeast.

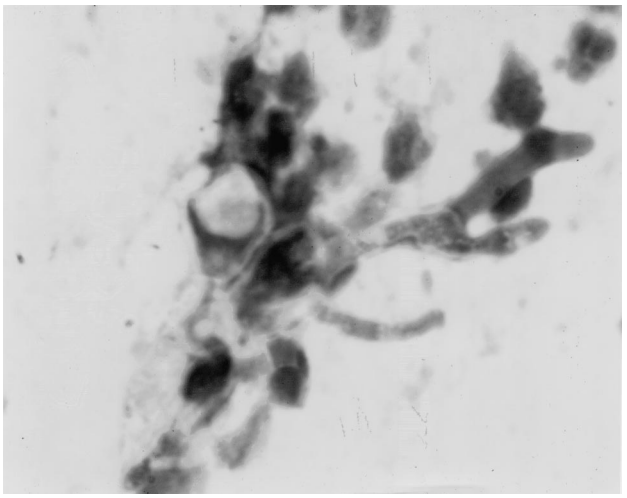

FIG. 2. Gram stain of antral biopsy specimen revealing septate hyphae.

The role of fungal-specific allergen immunotherapy is promising but still remains under evaluation (18). However, this is a chronic disease with frequent recurrences and none of these therapies has been highly effective.

While *Trichoderma* species are usually regarded as saprophytes, there are rare reports of serious infections in immunocompromised hosts. It has become apparent that most reports of infection implicate the thermotolerant species *T. longibrachiatum*, as determined through a better understanding of species concepts and by molecular reevaluation of published case isolates (3, 7, 14, 25). Furukawa et al. (6) described acute invasive sinusitis secondary to *T. longibrachiatum* in an immunocompromised patient, and Kuhls et al. (14) briefly mentioned a case of maxillary sinus infection. We have described here a case of colonization of the paranasal sinuses by *T. longibrachiatum* in a patient with AFS.

Microbiology. The left- and right-antrum biopsy specimens from our patient were inoculated on sheep's blood, MacConkey, and chocolate agar media for bacteria and on inhibitory mold agar, esculin base medium, and special blood agar for fungi. Gram stains of the specimens revealed polymorphonuclear cells, bacteria, and round cells initially reported as yeast cells (Fig. 1). Septate hyphae and round to oval yeast-like cells were seen with calcofluor staining.

Within 48 h, a thin, spreading, fragile, flaky, and granular mold began to appear on all culture media. The fungus was bright yellow on blood agar and chocolate agar but uncolored on MacConkey agar. In a scotch-tape lactophenol aniline blue (LPAB) preparation, structures consistent with *Trichoderma* species were observed. With the recovery of a mold, the Gram stain was reexamined and the presence of oval to round nonbudding cells was confirmed. Septate hyphae were also found in different fields (Fig. 2).

The fungus was subcultured on potato dextrose agar (PDA). Several days later, a tuft of dense white cottony mycelium, touching the lid of the petri dish, appeared above the existing yellowish-green growth, suggesting the possible presence of a second fungus. A LPAB preparation of the cottony growth revealed oval structures (Fig. 3) resembling the conidia of *Scedosporium apiospermum*. However, a subculture on Littman oxgall agar yielded a fungus with the characteristic *Trichoderma* morphology and the oval structures were later identified as chlamydospores.

Cultures from the biopsy specimens were referred to the University of Alberta Microfungus Collection and Herbarium (Edmonton, Canada) where they were retained as UAMH 10147 and 10147A. The fungus was identified as *T. longibrachiatum* based on the following features: a radius of 60 mm on PDA after 65 h at 35°C (radius of 55 mm at 30°C); a yellow diffusing pigment absent at 35°C but present at 30°C; phialides that were mostly solitary, cylindrical, and tapered at the neck; and conidia that were smooth and ellipsoidal and measuring 3.5 to 5 μm long and 2.5 to 3 μm wide on PDA. Chlamydospores were common, appearing either terminal or oval to globose or intercalary and cylindrical to barrel shaped and measuring 6 to 10 μm long and 3 to 5 μm wide. These features agree with criteria described by Samuels et al. (25), which

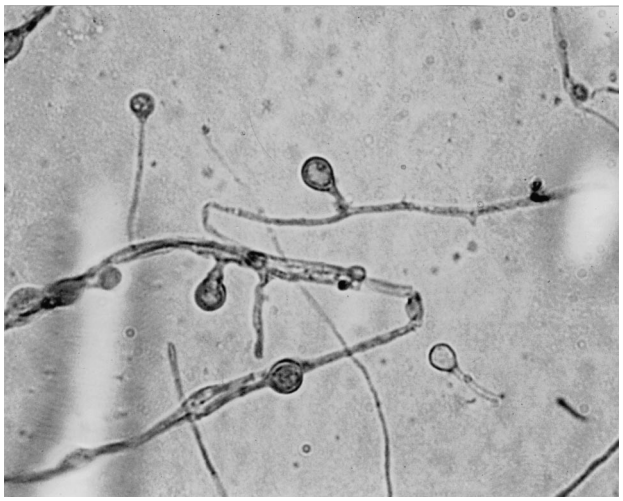

FIG. 3. LPAB preparation showing chlamydospores of *T. longibrachiatum*.

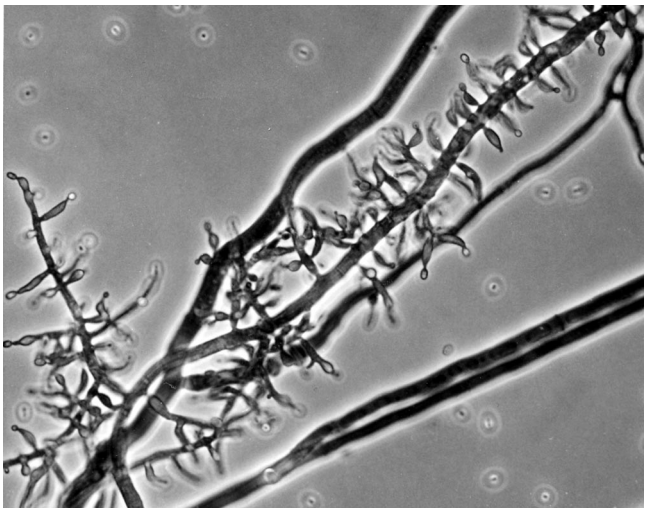

FIG. 4. Slide culture preparation showing the cylindrical phialides and conidia of *T. longibrachiatum*.

include fast-growing yellowish-green colonies on PDA with a radius greater than 35 mm at 40°C in 65 h; a yellow diffusing pigment present at 30°C but absent at 40°C; and hyphae sparingly branched and forming phialides that are mostly solitary, not typically in whorls. Phialides are usually cylindrical and gradually tapered with smooth, oblong to ellipsoidal conidia. Chlamydospores are commonly present. The typical microscopic features of *T. longibrachiatum* are illustrated in Fig. 4 and in recent publications (3, 24). Identity of the case isolate (UAMH 10047) was confirmed by sequencing of the nuclear ribosomal internal transcribed spacer region, which revealed 100% homology with GenBank sequences of *T. longibrachiatum*.

Discussion. *T. longibrachiatum* is a member of the *Trichoderma* section *longibrachiatum* and is the main human pathogenic species in the genus (3, 14, 25, 32). Members of this section are connected to teleomorphs in the *Hypocrea schweinitzii* complex though no teleomorph is known for *T. longibrachiatum* (25). The close relative *T. citrinoviride* (teleomorph *H. schweinitzii*) has been linked to one report of infection (14). Both species have worldwide distributions, but neither has been isolated from Australasia (25). They occur in soil, wood, decaying vegetation, or other cellulose-containing substances. *Trichoderma* species may also be cultured from materials in water-damaged buildings (14). A Danish study determined that almost half of the *Trichoderma* isolates obtained from building materials represented *T. longibrachiatum* and *T. citrinoviride* (17).

T. longibrachiatum is an uncommon cause of invasive, sometimes fatal infection in immunocompromised patients (3, 32). Sites involved include brain (7, 21, 24, 29), skin (21), and peritoneum (33). A recent case of otitis externa in an otherwise healthy 12-year-old boy was resolved following treatment with topical nystatin (10). Acute invasive sinusitis secondary to *T. longibrachiatum* was reported in a patient who received a liver and small-bowel transplant (6). In that case, the infection was successfully treated with surgical debridement followed by administration of amphotericin B and oral itraconazole. Previous reports on *T. longibrachiatum* infections have documented in vitro resistance to various antifungals including itraconazole and amphotericin B (6, 21, 24, 29). The relationship between in vitro MIC for various antifungals and in vivo response has not been established for *Trichoderma* species. The previously described cases of *Trichoderma* disease have been in profoundly immunocompromised patients, and the poor outcomes were likely independent of treatment with a susceptible or nonsusceptible drug. We elected to use oral itraconazole in our patient as previous studies have shown it to be the oral antifungal agent with the lowest MIC against *T. longibrachiatum*. High MICs are not necessarily predictive of outcome, as in cases of *Acremonium* (now *Fusarium*) *falciforme* infection which respond to itraconazole even though the in vitro MIC was measured as 32 μg/ml (3). However, given the possibility of in vivo resistance, we cannot attribute the patient's recovery to the use of oral itraconazole.

AFS has been associated with several different fungi but not previously with *T. longibrachiatum*. The etiology of *T. longibrachiatum* in our patient's case was confirmed by the presence of characteristic fungal elements in the surgical specimen and by growth of the fungus in pure culture. No other pathogens were identified. Direct microscopic visualization of the fungal elements in the sinus biopsy sample suggests that the fungus was indeed growing in the sinus rather than existing as a dormant conidium. The Gram stain was useful in identifying the fungal elements even though the round to oval nonbudding cells were initially misinterpreted as yeast cells. Retrospective examination revealed that these cells were actually chlamydospores, which are a notable microscopic feature of *T. longibrachiatum* growing in culture. Rare septate hyphae were also observed near the chlamydospores.

The exact pathophysiology of AFS is still unknown. It is thought that certain individuals are more susceptible to nasal sinus colonization with fungus due to changes in the osteomeatal complex or composition of the mucin (1). In the atopic patient, a reaction ensues leading to the development of AFS, whereas in a nonreactive individual, the formation of a fungus ball or "sinus mycetoma" may occur. AFS remains an underdiagnosed condition due to not only a lack of awareness among physicians but also the inability to demonstrate the presence of fungi in many suspected cases. The current diagnostic criteria for AFS are (i) chronic rhinosinusitis; (ii) the presence of allergic mucin; and (iii) the presence of fungi within that mucin, confirmed by histology, culture, or both (4, 20, 23). Establishing a causal relationship between the fungal culture results and the clinical presentation of AFS can be difficult, since many of the fungi isolated are ones that are more commonly considered as contaminants. Sometimes, more than one fungus may be grown. Clinical correlation is often necessary for interpretation of direct smears and fungal cultures from such patients.

The role of individual fungi, including *Trichoderma* species, in causing AFS remains to be elucidated (2, 22). Although exposure to *T. citrinoviride* in damp buildings has been associated with an increased risk of developing adult asthma, as determined by increased concentrations of IgG antibody levels, the findings do not correlate with a causal relationship (11). *T. viride* has been shown to produce volatile trichothecene compounds which can induce histamine release from human mast

cells (15). In addition to these mycotoxins, fungal-specific IgE may also contribute to the inflammatory response (16). Further work in identifying the agents of AFS may help in elucidating the common mechanisms of this disease.

L. Sigler thanks the Natural Sciences and Engineering Research Council of Canada for financial support and C. F. C. Gibas for sequencing the case isolate.

REFERENCES

1. **Bent, J. P., III, and F. A. Kuhn.** 1994. Diagnosis of allergic fungal sinusitis. Otolaryngol. Head Neck Surg. **111:**580–588.
2. **Chrzanowski, R. R., N. T. Rupp, F. A. Kuhn, A. E. Phillips, and W. K. Dolen.** 1997. Allergenic fungi in allergic fungal sinusitis. Ann. Allergy Asthma Immunol. **79:**431–435.
3. **deHoog, G. S., J. Guarro, J. Gene, and M. J. Figueras.** 2000. Atlas of clinical fungi. Centraalbureau voor Schimmelcultures, Baarn, The Netherlands.
4. **deShazo, R. D., and R. E. Swain.** 1995. Diagnostic criteria for allergic fungal sinusitis. J. Allergy Clin. Immunol. **96:**24–35.
5. **Frenkel, L., T. L. Kuhls, K. Nitta, M. Clancy, D. H. Howard, P. Ward, and J. D. Cherry.** 1987. Recurrent *Bipolaris* sinusitis following surgical and antifungal therapy. Pediatr. Infect. Dis. J. **6:**1130–1132.
6. **Furukawa, H., S. Kusne, D. A. Sutton, R. Manez, R. Carrau, L. Nichols, K. Abu-Elmagd, D. Skedros, S. Todo, and M. G. Rinaldi.** 1998. Acute invasive sinusitis due to *Trichoderma longibrachiatum* in a liver and small bowel transplant recipient. Clin. Infect. Dis. **26:**487–489.
7. **Gautheret, A., F. Dromer, J. H. Bourhis, and A. Andremont.** 1995. *Trichoderma pseudokoningii* as a cause of fatal infection in a bone marrow transplant recipient. Clin. Infect. Dis. **20:**1063–1064.
8. **Goldstein, M. F., P. C. Atkins, F. C. Cogen, M. J. Kornstein, R. S. Levine, and B. Zweiman.** 1985. Allergic *Aspergillus* sinusitis. J. Allergy Clin. Immunol. **76:**515–524.
9. **Gourley, D. S., B. A. Whisman, N. L. Jorgensen, M. E. Martin, and M. J. Reid.** 1990. Allergic *Bipolaris* sinusitis: clinical and immunopathologic characteristics. J. Allergy Clin. Immunol. **85:**583–591.
10. **Hennequin, C., T. Chouaki, J. C. Pichon, V. Strunski, and C. Raccurt.** 2000. Otitis externa due to *Trichoderma longibrachiatum*. Eur. J. Clin. Microbiol. Infect. Dis. **19:**641–642.
11. **Jaakkola, M. S., S. Laitinen, R. Piipari, J. Uitti, H. Nordman, A. M. Haapala, and J. J. Jaakkola.** 2002. Immunoglobulin G antibodies against indoor dampness-related microbes and adult-onset asthma: a population-based incident case-control study. Clin. Exp. Immunol. **129:**107–112.
12. **Kamei, K., H. Unno, J. Ito, K. Nishimura, and M. Miyaji.** 1999. Analysis of the cases in which *Schizophyllum commune* was isolated. Nippon Ishinkin Gakkai Zasshi **40:**175–181.
13. **Katzenstein, A. L., S. R. Sale, and P. A. Greenberger.** 1983. Allergic *Aspergillus* sinusitis: a newly recognized form of sinusitis. J. Allergy Clin. Immunol. **72:**89–93.
14. **Kuhls, K., E. Lieckfeldt, T. Borner, and E. Gueho.** 1999. Molecular reidentification of human pathogenic *Trichoderma* isolates as *Trichoderma longibrachiatum* and *Trichoderma citrinoviride*. Med. Mycol. **37:**25–33.
15. **Larsen, F. O., P. Clementsen, M. Hansen, N. Maltbaek, T. Ostenfeldt-Larsen, K. F. Nielsen, S. Gravesen, P. S. Skov, and S. Norn.** 1998. Volatile organic compounds from the indoor mould *Trichoderma viride* cause histamine release from human bronchoalveolar cells. Inflamm. Res. **47:**S5–S6.
16. **Larsen, F. O., H. W. Meyer, N. Ebbehoj, F. Gyntelberg, D. Sherson, B. Netterstrom, S. Gravesen, and S. Norn.** 1997. Are fungi-specific IgE found in staff suffering from nonallergic sick building syndrome? Inflamm. Res. **46:** S79–S80.
17. **Lubeck, M., S. K. Poulsen, P. S. Lubeck, D. F. Jensen, and U. Thrane.** 2000. Identification of *Trichoderma* strains from building materials by ITS1 ribotyping, UP-PCR fingerprinting and UP-PCR cross hybridization. FEMS Microbiol. Lett. **185:**129–134.
18. **Mabry, R. L., S. C. Manning, and C. S. Mabry.** 1997. Immunotherapy in the treatment of allergic fungal sinusitis. Otolaryngol. Head Neck Surg. **116:**31–35.
19. **Marple, B. F.** 2001. Allergic fungal rhinosinusitis: current theories and management strategies. Laryngoscope **111:**1006–1019.
20. **McClay, J. E., B. Marple, L. Kapadia, M. J. Biavati, B. Nussenbaum, M. Newcomer, S. Manning, T. Booth, and N. Schwade.** 2002. Clinical presentation of allergic fungal sinusitis in children. Laryngoscope **112:**565–569.
21. **Munoz, F. M., G. J. Demmler, W. R. Travis, A. K. Ogden, S. N. Rossmann, and M. G. Rinaldi.** 1997. *Trichoderma longibrachiatum* infection in a pediatric patient with aplastic anemia. J. Clin. Microbiol. **35:**499–503.
22. **Ponikau, J. U., D. A. Sherris, E. B. Kern, H. A. Homburger, E. Frigas, T. A. Gaffey, and G. D. Roberts.** 1999. The diagnosis and incidence of allergic fungal sinusitis. Mayo Clin. Proc. **74:**877–884.
23. **Ramadan, H. H., and H. A. Quraishi.** 1997. Allergic mucin sinusitis without fungus. Am. J. Rhinol. **11:**145–147.
24. **Richter, S., M. G. Cormican, M. A. Pfaller, C. K. Lee, R. Gingrich, M. G. Rinaldi, and D. A. Sutton.** 1999. Fatal disseminated *Trichoderma longibrachiatum* infection in an adult bone marrow transplant patient: species identification and review of the literature. J. Clin. Microbiol. **37:**1154–1160.
25. **Samuels, G. J., O. Petrini, K. Kuhls, E. Lieckfeldt, and C. P. Kubicek.** 1998. The *Hypocrea schweinitzii* complex and *Trichoderma* sect. *Longibrachiatum*. Stud. Mycol. **41:**1–54.
26. **Schubert, M. S., and D. W. Goetz.** 1998. Evaluation and treatment of allergic fungal sinusitis. I. Demographics and diagnosis. J. Allergy Clin. Immunol. **102:**387–394.
27. **Schubert, M. S., and D. W. Goetz.** 1998. Evaluation and treatment of allergic fungal sinusitis. II. Treatment and follow-up. J. Allergy Clin. Immunol. **102:**395–402.
28. **Schwietz, L. A., and D. S. Gourley.** 1992. Allergic fungal sinusitis. Allergy Proc. **13:**3–6.
29. **Seguin, P., B. Degeilh, I. Grulois, A. Gacouin, S. Maugendre, T. Dufour, B. Dupont, and C. Camus.** 1995. Successful treatment of a brain abscess due to *Trichoderma longibrachiatum* after surgical resection. Eur. J. Clin. Microbiol. Infect. Dis. **14:**445–448.
30. **Sigler, L., S. Estrada, N. A. Montealegre, E. Jaramillo, M. Arango, C. De Bedout, and A. Restrepo.** 1997. Maxillary sinusitis caused by *Schizophyllum commune* and experience with treatment. J. Med. Vet. Mycol. **35:**365–370.
31. **Sigler, L.** 2003. Miscellaneous opportunistic fungi: *Microascaceae* and other ascomycetes, hyphomycetes, coelomycetes and basidiomycetes, p. 637–676. *In* D. H. Howard (ed.), Pathogenic fungi in humans and animals. Marcel Dekker, New York, N.Y.
32. **Summerbell, R. C.** 2003. *Aspergillus*, *Fusarium*, *Sporothrix*, *Piedraia*, and their relatives, p. 237–498. *In* D. H. Howard (ed.), Pathogenic fungi in humans and animals. Marcel Dekker, New York, N.Y.
33. **Tanis, B. C., P. H. van der, M. L. van Ogtrop, R. E. Kibbelaar, and P. C. Chang.** 1995. Fatal fungal peritonitis by *Trichoderma longibrachiatum* complicating peritoneal dialysis. Nephrol. Dial. Transplant. **10:**114–116.
34. **Washburn, R. G., D. W. Kennedy, M. G. Begley, D. K. Henderson, and J. E. Bennett.** 1988. Chronic fungal sinusitis in apparently normal hosts. Medicine **67:**231–247.