A 49-year-old woman underwent bilateral lung transplantation for advanced idiopathic pulmonary fibrosis. During the postoperative period she received immunosuppressive medications as well as corticosteroids. *Aspergillus fumigatus* grew from a sputum sample, and she was treated with nebulized amphotericin. She was discharged on tacrolimus and prednisone. After initially doing well, she required re-hospitalization for treatment of cytomegalovirus and *Pseudomonas aeruginosa* pneumonia. She was treated with ganciclovir and cephalosporin and, after a 2-week hospitalization, was discharged. Seven months after transplantation she developed progressive sinusitis, treated with antibiotics and sinus debridement surgery. *Aspergillus* organisms were recovered and, at the periphery of the tangled masses of *Aspergillus* hyphae, numerous amebic cysts were also identified, which were morphologically consistent with *Acanthamoeba* spp. Subsequent electron microscopy and immunofluorescent staining confirmed this impression. She was initially treated with intravenous amphotericin, later changed to voriconazole and caspofungin. Debridement of the sinuses 3 weeks later revealed fungal hyphae but no amebae. Infections with *Acanthamoeba* have rarely been reported in lung transplantation but have been recognized in bone-marrow and renal transplant patients, and have been lethal in many cases, particularly in patients with immunosuppression due to human immunodeficiency virus infection. More recently, aggressive antimicrobial therapy has resulted in successful outcomes, as discussed herein.

*Acanthamoebae* are ubiquitous free-living protists with a wide distribution in nature. They are perhaps best known as agents responsible for a persistent keratitis in contact lens wearers (1), and have also been widely reported as opportunistic infections in individuals with the acquired immune deficiency syndrome (AIDS) (2, 3). Occasional cases of granulomatous amebic encephalitis have been seen in immunocompetent patients, typically with fatal outcomes (3). We report here a case of *Acanthamoeba* rhinosinusitis, diagnosed by light and electron microscopy and confirmed by immunofluorescence microscopy, in a patient with...
bilateral lung transplantation. We also provide a brief survey of the literature on Acanthamoeba infections, with special attention to organ transplant recipients. Increased awareness of this entity and aggressive antibiotic therapies have resulted in better outcomes for this potentially fatal infection.

Case report

The patient was a 49-year-old woman who underwent bilateral lung transplantation for end-stage lung disease secondary to pulmonary fibrosis. She had been treated preoperatively with Cytoxan (cyclophosphamide) and was mildly neutropenic at the time of surgery. She underwent bilateral lung transplantation and postoperatively was treated with tacrolimus, mycophenolate mofetil, and prednisone for immunosuppression. While her initial postoperative course was fairly uneventful, she did have one sputum culture positive for Aspergillus fumigatus, and she was given amphotericin by nebulizer in addition to her immunosuppressive medications.

Five weeks after discharge she was again admitted to the hospital for 10 days with shortness of breath. She was believed to have cytomegalovirus (CMV) pneumonitis, and Pseudomonas aeruginosa was cultured from bronchoalveolar lavage fluid. These were treated with ganciclovir and cefepime, respectively, with good response.

Approximately 7 months after transplantation she was re-admitted for evaluation of headaches, sinusitis, and fever. Computed tomographic scan of the sinuses showed opacification and she underwent sinus debridement. Tissues were submitted for routine histologic processing, which by light microscopy yielded abundant hyphae with acute-angle branching consistent with Aspergillus sp. and necrotic tissue. At the periphery of the necrosis, numerous cysts were seen, subsequently identified as Acanthamoeba sp. (Fig. 1). The amebic cysts were thin- to thick-walled structures from 12 to 15 μm in diameter, and showed what appeared to be a round nucleus, granular chromatin, and occasional cysts showed a centrally located nucleolus (Fig. 2). Rare amebae consistent with trophozoites were seen (Fig. 2, inset).

Immunostains developed by our laboratory (4) confirmed the Aspergillus while the ameba showed no staining. The cysts were variably stained by Gomori’s methenamine silver stain and were also weakly stained with periodic acid Schiff stain. Fungal cultures were positive for A. fumigatus. Tissue was obtained from the paraffin blocks and re-processed for electron microscopy. Diligent search yielded a few organisms available for examination, all cysts. Some of these demonstrated a dense cyst wall and virtually no remaining cytoplasm. Others showed a three-part wall, with a thick inner layer, a distinct outer layer, and a relatively thin amorphous layer in between (Fig. 3). None of the cysts available for study had well-preserved nuclei or nucleoli. A few electron-dense bodies were seen in the cytoplasm. Immunofluorescent studies performed on tissue biopsy slides at the Centers for Disease Control and Prevention in Atlanta were positive for Acanthamoeba spp.

Subsequently, she was treated with increased dosages of amphotericin, voriconazole, and caspofungin. A sinus debridement 3 weeks later again revealed Aspergillus but no amebae were found, and her condition gradually improved. Although Aspergillus could be identified in a sinus debridement 4 weeks later, no amebae have been identified in any specimens after the initial episode.

Discussion

Infections continue to be the leading cause of death in the year following lung transplantation. As with other transplants, the drugs used in im-
munosuppression to prevent organ rejection adversely affect humoral and cell-mediated immune responses. The commonest infectious agents tend to be bacterial, followed by viral infections, particularly CMV, and fungal infections. These infections can represent both diagnostic and therapeutic challenges, and early diagnosis with prompt treatment has a significant impact on survival (5). In immunocompetent individuals, amebic keratitis has been a major concern to ophthalmologists, occurring mainly in patients with trauma, contact lens wearers, or corneal transplantation (1). However, *Acanthamoeba* are increasingly being recognized as opportunistic infections in immunosuppressed patients, including solid organ transplants, bone marrow transplants, and individuals infected with the human immunodeficiency virus (HIV) (6–8). Several cases of *Acanthamoeba* infections in renal transplant patients have been reported, including both localized skin lesions and systemic disease.

To our knowledge, this report presents the third reported case of *Acanthamoeba* infection in a lung transplant patient. Van Hamme et al. (9) reported a fatal case of acanthamoebiasis from Belgium in a lung transplant patient, which was initially diagnosed from cutaneous lesions but later became systemic. Oliva et al. (10) reported a case of widely disseminated acanthamoebiasis in a single lung transplant patient, successfully treated with combination antibiotic therapy. Our patient presented with *Acanthamoeba* rhinosinusitis, and the infection was controlled by surgical debridement and combination antimicrobial therapy.

*Fig. 3.* Electron micrograph of *Acanthamoeba* cyst, showing inner endocyst layer and outer ectocyst layer (TEM, original magnification × 14,500).

*Acanthamoeba* are widely distributed in the environment and have been isolated from soil, sand, ponds and streams, tap water, seawater, physiotherapy pools, and other environmental sources. Their cysts resist dehydration, biocides, chlorination, antibiotics, and low temperatures. Mazur et al. (11) showed that cysts retained viable amebae after 24 years of storage at 4°C. Wang and Feldman (12) have shown that *Acanthamoeba* can be recovered occasionally from throat swabs in otherwise healthy individuals. Thus, there is ample opportunity for exposure to *Acanthamoeba* in an ordinary household environment. Our patient had previously visited hot tubs but not for a 2-year interval before lung transplantation.

Several investigators have studied the immune response to *Acanthamoeba*. While both antibody and complement are important in promoting recognition of amebas by phagocytic cells such as neutrophils and macrophages, killing of the amebas is dependent on lymphokine/monokine priming of the neutrophils (13, 14). Thus, the immunosuppressive effects of cyclosporine and tacrolimus, inhibiting T-lymphocyte signal transduction and IL-2 transcription, may form the basis for human infection by *Acanthamoeba* in highly immunosuppressed transplant patients.

A variety of therapeutic regimens have been used in the treatment of *Acanthamoeba* infections. Teknos et al. (15) used a combination of surgical debridement, gentamicin nasal rinses, and itraconazole for one of their HIV patients with a mixed *Aspergillus/Acanthamoeba* rhinosinusitis, and debridement followed by intravenous pentamidine and topical ketoconazole, followed by oral itraconazole in another patient with skin and nasal *Acanthamoeba*. Oliva et al. (10) used pentamidine, 5-fluorocytosine, and topical chlorhexidine gluconate/ketoconazole cream with eventual resolution of disseminated acanthamoebiasis in their lung transplant patient. Successful treatment regimens have been few; these are summarized by Marciano-Cabral and Cabral (13). However, in their experience the diagnosis was often made late and multidrug toxicity complicated the disease course.

In summary, we report a third case of *Acanthamoeba* infection in a lung transplant patient. While one of the previously reported cases was ultimately fatal, this and another case eventually cleared the infection. Infections, along with bronchiolitis, continue to be the major causes of mortality during the first 5 years after lung transplantation. Early diagnosis and aggressive therapy seem to have played a role in the positive outcomes for these patients, thus highlighting the need for recognition of the distinctive morphology of *Acanthamoeba* infections.

### References


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