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NOTES

First Reported Case of *Aspergillus granulosus* Infection in a Cardiac Transplant Patient

MOHAMAD G. FAKIH,[1]* GERTRUDE E. BARDEN,[2] CLARE A. OAKES,[1] AND CHARLES S. BERENSON[1]

Department of Medicine and Microbiology Laboratory, Department of Veterans Affairs Medical Center, State University of New York at Buffalo, Buffalo, New York,[1] and Microbiology Laboratory, Department of Veterans Affairs Medical Center, West Haven, Connecticut[2]

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We report a case of disseminated infection with *Aspergillus granulosus* in a cardiac transplant recipient on immunosuppressive therapy. This is the first reported case in which this organism has been described as a pathogen. This organism bears morphological features different from those of more common *Aspergillus* species and should be considered a potential pathogen in immunocompromised patients.

Invasive *Aspergillus* infections are most frequently caused by *A. fumigatus* and *A. flavus* (2, 16, 21) and are often fatal in organ transplant patients and immunosuppressed hosts. We report a case of disseminated *A. granulosus* infection in a cardiac transplant patient.

Case report. A 45-year-old cardiac transplant recipient was admitted to the Buffalo Veterans Affairs Medical Center with progressive congestive heart failure 6 months posttransplantation. An endomyocardial biopsy showed no evidence of rejection, and he was maintained on azathioprine, prednisone, and cyclosporin A. A physical examination showed him to be a middle-aged, cushingoid, white male. His vital signs were normal. His lungs were clear to auscultation. The remainder of the examination was unremarkable.

Laboratory values included a leukocyte count of 5,000/mm^3, with 89% polymorphonuclear leukocytes; serum chemistries and liver enzymes were unremarkable. Urinalysis was normal. A chest X ray on admission showed fine nodular infiltrates in both lower-lung fields. Sinus films showed no abnormalities. The sputum Gram stain and culture showed mixed bacteria but no evidence of *Nocardia* or fungal organisms. Computerized tomography of his chest further defined new bilateral nodular pulmonary densities, including a 2-cm-diameter left basilar nodule and three smaller right basilar nodules. Bronchoalveolar lavage showed no evidence of bacterial pathogens, including *Legionella* and *Nocardia* organisms, acid-fast bacilli, or fungi. Needle aspiration of the left basilar lung nodule was technically unsuccessful. Because of severe congestive heart failure, an open-lung biopsy was initially deferred but was strongly reconsidered in view of the inability of other procedures to yield a diagnosis. On the 25th day of hospitalization, the patient developed distinct, separate 5-mm-diameter violaceous skin nodules on his right arm and right leg which were biopsied. Hematoxylin-eosin staining of both skin biopsies revealed necrotizing, suppurative, and granulomatous inflammation. Gomori methenamine silver staining revealed branching, invasive, hyphal elements. Periodic acid-Schiff, mucicarmine, acid-fast, and Gram stains were noncontributory.

* Corresponding author. Mailing address: Department of Veterans Affairs Medical Center, Infectious Diseases-151, 3495 Bailey Ave., Buffalo, NY 14215. Phone: (716) 862-3304. Fax: (716) 862-3419.

Separate cultures on Sabouraud agar of biopsy samples from each site revealed pure growth of 15 to 20 colonies of the same fungal organism. No other organisms grew on either culture. Each isolate initially produced multiple velvety colonies with an off-white color that showed reverse tan-to-brown pigmentation as the growth matured. Microscopically, the most prominent feature seen with lactophenol cotton blue stain was clusters of spherical to subspherical hülle cells (Fig. 1). Conidial heads were relatively sparse, exhibiting two rows of phialides. Conidiophores were tan and smooth walled, with thin elongated vesicles. Subcultures on Sabouraud agar at 25°C had areas of light slate-green color but were mostly tan to brown. Subcultures on Czapek-Dox agar exhibited macroscopic and microscopic appearances similar to those on Sabouraud agar, with variable amounts of slate-green pigmentation. Areas without green pigmentation were off-white, becoming tan centrally. Some tan pigment was seen on the reverse of the colony. Definitive identification of this organism as *A. granulosus* and susceptibility testing was performed at the Mycology Reference Laboratory, Audie L. Murphy Memorial Veteran's Hospital, San Antonio, Tex. Susceptibility testing by the dilution macrobroth method showed in vitro 24-h MICs and minimum lethal concentrations of <0.14 and 0.29 mg/ml for amphotericin B and of 0.07 and 1.25 mg/ml for itraconazole, respectively. In vitro resistance to fluconazole was found (MIC, >80 mg/ml) (15).

Amphotericin B was well tolerated at 0.5 mg/kg/day intravenously. After 480 mg (total) over 4 weeks had been given, a repeat computerized-tomography scan of his chest showed partial clearing of pulmonary nodules. Despite initial overall clinical improvement, the patient's congestive heart failure progressed. He developed intractable ventricular fibrillation and expired 8 weeks after admission. A postmortem examination demonstrated resolving pneumonia with necrotic foci which contained residual invasive hyphal elements. Pathologic evaluation suggested that these fungal elements were morphologically identical to those found during earlier skin biopsies. Unfortunately, postmortem lung samples were mishandled in such a way that fungal cultures of lung tissue could not be performed.

Discussion. Invasive *Aspergillus* infections occur most commonly in patients with impaired cell-mediated immunity and

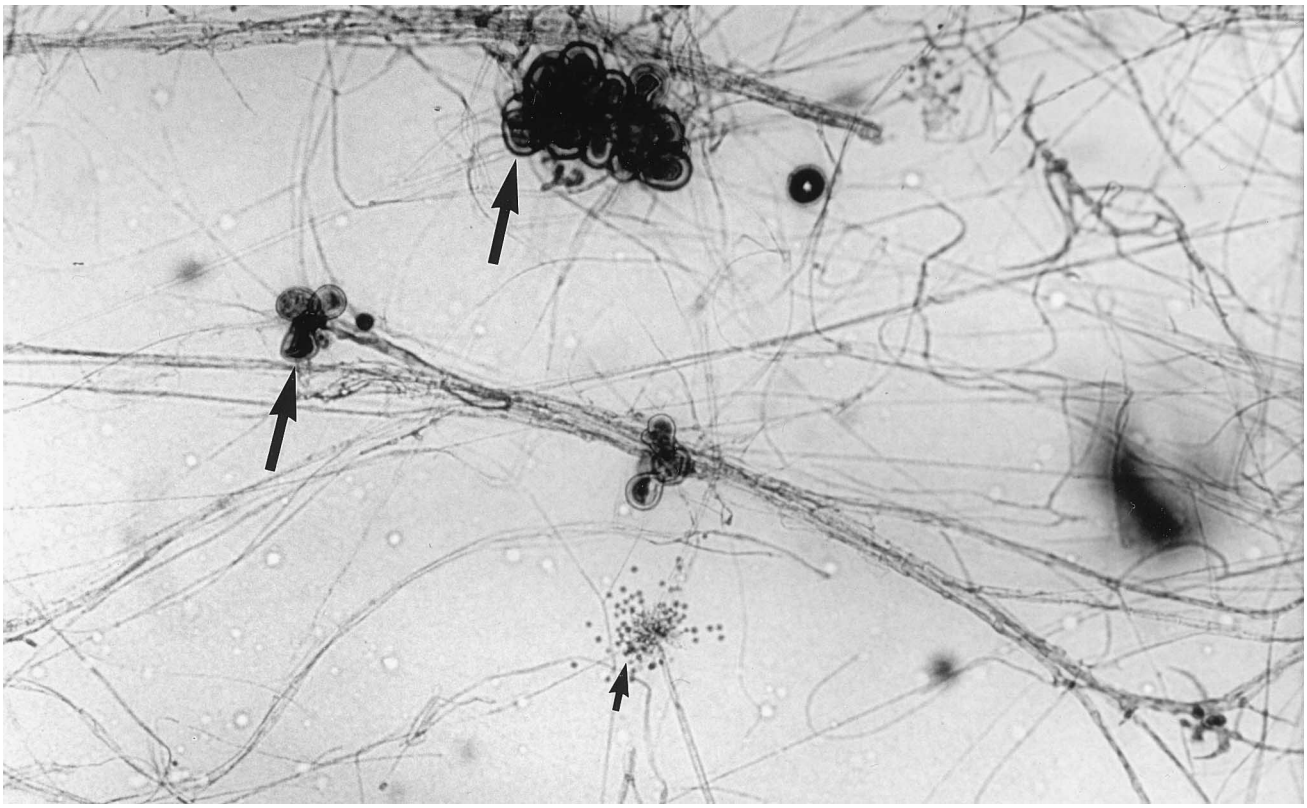

FIG. 1. Lactophenol cotton blue preparation of culture sample. Globose to subglobose hülle cells are seen in clusters (large arrows) with relatively sparse conidia (small arrow).

neutropenia. Competent neutrophils (5, 18) and macrophages (19) are required to inhibit hyphal growth and kill *Aspergillus* conidia. Chronic corticosteroids and immunosuppressive agents used with organ transplantation impair macrophage function and are associated with increased incidence of invasive aspergillosis (8, 11, 19). Invasive pulmonary aspergillosis, the most common *Aspergillus* infection of immunocompromised patients, often disseminates. Patients commonly present with fever, pleuritic chest pain, hemoptysis, and bilateral pulmonary infiltrates (2, 21). Weiland et al. found that 86% of renal transplant patients with positive sputum cultures and clinical symptoms had invasive aspergillosis (21). However, the absence of *Aspergillus* organisms from sputum culture, even when obtained by bronchoalveolar lavage, cannot be used as a reliable indicator to rule out invasive aspergillosis (21). The difficulties associated with making a timely diagnosis are highlighted by reports that show that postmortem diagnosis of systemic aspergillosis is fairly common (23). Therefore, the most reliable and specific means of diagnosing invasive aspergillosis remains transthoracic (7, 13, 22) or open-lung biopsy.

While primary cutaneous *Aspergillus* infection has been reported (1, 6, 12), most cutaneous *Aspergillus* infections are due to hematogenous seeding from a pulmonary source or direct extension from deeper infected tissue (20). Cutaneous lesions are seen in about 5% of patients with disseminated *Aspergillus* infections (2, 20). Skin lesions start as erythematous or violaceous papules, which progress to necrotic ulcers with black eschars (3).

A. granulosus is classified in the *A. versicolor* group, a large heterogeneous group which contains 16 other *Aspergillus* species. The species of this group typically exhibit conidial heads in some shade of green with phialides in two series (14). Hülle cells, a prominent and interesting microscopic characteristic of our isolate but not commonly seen with clinical isolates of *Aspergillus* species, are specialized cells of unknown function. Other *Aspergillus* groups that have members which produce hülle cells are the *A. nidulans*, *A. ornatus*, *A. ustus*, and *A. flavipes* groups. Globose to subglobose hülle cells, similar to those produced by *A. granulosus* and some other members of the *A. versicolor* group, may also be seen in the *A. nidulans* and *A. ornatus* groups (14, 17). The *A. ustus* and *A. flavipes* groups produce elongate hülle cells that look quite different from the hülle cells typical of members of the *A. versicolor*, *A. nidulans*, and *A. ornatus* groups. When an isolate has hülle cells, an identification of the *Aspergillus* group to which the organism belongs can usually be made. *A. raperi*, the only species in the *A. ornatus* group which produces hülle cells, can be distinguished from all the other *Aspergillus* species which produce hülle cells by its uniseriate phialides. Unlike *A. versicolor* species, all species in the *A. nidulans* group which produce hülle cells, except *A. subsessilis*, produce them either (i) in association with cleistothecia, specialized spherical structures which contain asci and ascospores (sexual structures), or (ii) as continuous crusts in asexual species. *A. subsessilis* is an asexual *A. nidulans* group species which produces abundant hülle cells that are not in continuous crusts. It can be relatively easily recognized by its very short conidiophores, among other characteristics. *A. versicolor* group species are all asexual, and their hülle cells, when produced, are not in continuous crusts.

A. granulosus can be distinguished from other members of the *A. versicolor* group by the pale gray-green to light blue-

green color of the areas of the colony where conidial head production has occurred, lack of any compact hyphal masses or sclerotia, production of globose to subglobose hülle cells in small colorless clusters, and tan to light-brown conidiophores. No other species shares all of these characteristics. Other characteristics, such as conidial size, conidiophore length and diameter, and hülle cell size can also be studied to confirm an identification.

This is the first reported case of proven infection of any type due to *A. granulosus*. Our patient appears to have had disseminated disease due to this organism, originating from a pulmonary source, with cutaneous manifestations. This is supported by the finding of pure growth of multiple colonies of the same organism on cultures of two geographically distant lesions, with no growth of any other organisms, a finding that is best explained by hematogenous spread (3, 20). Furthermore, pathological examination of both lesions demonstrated numerous invasive hyphal elements and no other organisms. Of interest, both tissue sections displayed acute-angle branching of fungal forms with an unusual, almost moniliform appearance. This type of morphology in tissue sections has previously been reported for other *Aspergillus* species (4, 9). Kwon-Chung and Bennett have described five different types of *Aspergillus* hyphal morphology in tissue and reported that bulbous swellings and constrictions in *Aspergillus* hyphae in areas of marked inflammation are not rare (10). Thus, relying on a classic appearance of dichotomously branching septate hyphae for identification of this and other *Aspergillus* species in tissue may lend inaccuracy to the diagnosis. One should consider *A. granulosus* and other clinically uncommon *Aspergillus* species as causes of life-threatening, disseminated fungal infection in organ transplant recipients, especially when an isolate has the unusual morphologic features described. Definitive identification may require sending the organism to a reference laboratory.

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