Chromoblastomycosis Caused by *Exophiala spinifera*

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We report the second case of chromoblastomycosis caused by *Exophiala spinifera*; this is the first known case in the United States. Examination of biopsied tissue showed thick-walled, internally septated, chestnut brown muriform cells (sclerotic bodies) within multinucleated giant cells present in the dermis that were characteristic of chromoblastomycosis. The individual cells within the muriform cells disarticulated from the outer wall of the parent cell and from each other to form endothidia within the outer walls of the parent cells. After fracture of the outer walls, the endothidia were released. This unique process of endothidial formation in vivo for the propagation of muriform cells was observed for the first time. Initial treatment with itraconazole and 5-fluorocytosine followed by treatment with itraconazole and heat resulted in marked improvement in the patient’s lesions. This infection reiterates the fact that the dematiaceous fungus *E. spinifera*, a well-known etiologic agent of phaeohyphomycosis, can cause more than one type of infection and supports earlier observations that chromoblastomycosis and phaeohyphomycosis represent extremes of a continuum of infections.

The fungal elements of dematiaceous pathogens in tissue exhibit wide variation. They may be hyaline to phaeoid or produce dark granules or chestnut brown, thick-walled, internally septated muriform cells (sclerotic bodies); they may form septate, branched hyphal elements, large thick-walled cells, yeastlike cells, or a combination of these variants. The presence of one of these fungal forms in tissue often becomes the diagnostic hallmark of a disease. Diseases caused by dematiaceous fungi include eumycotic mycetoma, chromoblastomycosis, and phaeohyphomycosis. The formation of granules in tissue by the causal fungus is diagnostic of mycetoma. Similarly, the production of sclerotic bodics is the diagnostic hallmark of chromoblastomycosis. Phaeohyphomycosis, on the other hand, encompasses a heterogenous group of infections that range from superficial, cutaneous, and subcutaneous to systemic and encompass a number of any of these forms [1].

In recent years, however, well-known etiologic agents of chromoblastomycosis, such as *Fonsecaea pedrosoi* and *Phialophora verrucosa*, have been described as causal agents of phaeohyphomycosis [2]. In addition, a fungus such as *Exophiala jeaneselmei*, that has traditionally been known to be an etiologic agent of eumycotic mycetoma can cause phaeohyphomycosis [2] and chromoblastomycosis [3]. Recently, another well-known etiologic agent of phaeohyphomycosis (namely, *Exophiala spinifera* [4]) was described as causing chromoblastomycosis [5]. We report the first known case of chromoblastomycosis caused by *E. spinifera* in the United States in which hitherto undescribed morphological forms of the fungus in tissue were observed.

**Case Report**

A 62-year-old man who had been a landscaper in the past was seen on 25 February 1993 for treatment of chromoblastomycosis. In 1988 he had had a sudden onset of diffuse arthralgias, and a diagnosis of rheumatoid arthritis was made. After a number of unsuccessful therapeutic regimens, treatment with azathioprine and prednisone was started. Attempts to taper his prednisone dosage to <16 mg/d were unsuccessful because of recurrent pain. His clinical course was complicated by cryptococcal meningitis in 1989; this infection was treated successfully with amphotericin B and 5-fluorocytosine.

In August 1991 a verrucous lesion developed on the index finger of the right hand. The patient was a heavy equipment operator, certainly with much potential exposure to fungi; however, because of rheumatoid arthritis, he had not worked outdoors since 1988. He underwent cryosurgery, but the lesion did not resolve. Histologic examination of biopsied tissue in March 1992 showed marked hyperkeratosis, parakeratosis, and irregular epidermal acanthosis with intraepidermal pustule formation. There were numerous multinucleated giant cells con-
Figure 1. Boggy, nodular lesions studded with tiny pustules on the right forearm of a man with chromoblastomycosis caused by *Exophiala spinifera*.

Several months later new lesions developed over the right elbow and right forearm. In late 1992 and early 1993, he was treated with ketoconazole for a 6-week period followed by fluconazole for another 6 weeks; there was no effect on the lesions. He was first seen by one of us (A.A.H.) in February 1993; his medications at that time included prednisone (16 mg/d) and azathioprine (135 mg/d).

Physical examination was most remarkable for an elongated, boggy, nodular mass on the right anteromedial forearm with a number of tiny pustules studded over its surface (figure 1); in addition, he had a cushingoid appearance. There was much induration over the mass, but no warmth or erythema. In addition, the posterior aspect of his forearm had a firm indurated nodule. There was a well-healed scar on the right finger at the base of the metacarpal joint. There was no adenopathy. Biopsy of an erythematous papule on the right lateral antecubital fossa and the nodular mass on the right anteromedial forearm showed moderate-to-marked pseudoepitheliomatous hyperplasia. There was a dense, mixed, dermal infiltrate that consisted of lymphocytes, histiocytes, occasional neutrophils, and foreign body–type giant cells. Foci of necrosis were also evident within areas of dense inflammation. There were frequent brown, round-to-oval, thick-walled muriiform cells (sclerotic bodies) within the dermis, often within multinucleated giant cells, that ranged from 5 to 12 μm in diameter. As the muriiform cells matured and enlarged in size within the outer walls of the mother cells, the individual cells disarticulated from each other within the outer walls of the mother cells and formed dematiaceous sporangium-like cells containing endoconidia (figure 2). The sporangia measured 12–20 μm in diameter. When the outer sporangial walls ruptured, individual endoconidia were released (figure 3).

Culture of a portion of the biopsied tissue yielded shiny, pasty, black colonies after 5–7 days of incubation at both 25°C and 37°C. Microscopic examination of India ink wet mounts showed a large number of encapsulated budding cells as were described by Mackinnon et al. [6]. After 2 weeks of incubation at 25°C, pasty yeastlike colonies developed fine, suedelike, aerial mycelium. After 2 weeks of incubation at 25°C, slide cultures on potato dextrose agar yielded septate, branched, subhyaline–to–pale brown hyphae with several lateral, erect, spinelike, septate conidiophores that were much darker than the vegetative hyphae and bore conidiogenous cells at the tips that were integrated with annelation, cylindrical to lageniform, and smooth. The hyaline, one-cell conidia were subglobose to ellipsoidal and measured 2–3 × 1.5–3.0 μm. They aggregated in masses and tended to slide down the conidiophores or along the hyphae (figure 4). On the basis of encapsulated yeastlike early growth, distinctive spinelike conidiophores, and robust, distinctive, snoutlike, annellated tips of the conidiogenous cells,
Chromoblastomycosis Due to Exophiala spinijera

The isolate was identified as E. spinijera (Centers for Disease Control and Prevention B-5383). Immediately after the biopsied tissue was obtained, the patient was treated with itraconazole (200 mg/d) and 5-fluorocytosine (150 mg/[kg·d]). In addition, the patient wrapped his forearm in a heating pad on an intermediate setting each night. While this therapeutic regimen was administered, his lesions markedly improved. The patient was lost to follow-up from April 1993 until July 1993, during which time treatment with itraconazole and 5-fluorocytosine was discontinued. When the patient was seen again in July 1993, recurrence of multiple tender subcutaneous nodules on the right forearm was noted. Treatment with itraconazole alone (400 mg/d) was started with initial improvement in his condition; however, within 4 months, recurrence and progression of subcutaneous nodules had occurred. 5-Fluorocytosine was again added to his therapeutic regimen, and the appearance of his forearm improved. However, the nodules did not entirely resolve; they persisted in a linear distribution along the right forearm. Attempts to reduce the prednisone dosage were unsuccessful. Surgical “debulking” was accomplished in June 1994, but within several months after the procedure, he had recurrence of multiple subcutaneous nodules despite continuation of therapy with itraconazole and 5-fluorocytosine. At the present time his prednisone dosage is being very slowly reduced, and he has persistent bulky subcutaneous nodules on the right forearm, with occasional spontaneous drainage of purulent material in which E. spinijera continues to grow.

The initial isolate (CDC B-5383) and the isolate recovered in 1994 after treatment with itraconazole (CDC B-5580) were referred to the Mycology Reference Laboratory at The University of Texas Medical Branch in Galveston, Texas, for in vitro sensitivity studies. MICs of amphotericin B and itraconazole for B-5383 and B-5580 were determined following the procedures recommended by the National Committee for Clinical Laboratory Standards [7]. The MIC of amphotericin B was found to be 0.25 μg/mL. The MIC of itraconazole for the pretreatment isolate (B-5383) was 2.0 μg/mL, while the MIC for the posttreatment isolate (B-5580) increased twofold to 4.0 μg/mL.

Discussion

In spite of the wide geographic distribution of E. spinijera (namely, Asia, Australia, North America, Central America, and South America), only 12 human infections (10 cases of phaeohyphomycosis and 2 cases of chromoblastomycosis) have been described in the literature (table 1). E. spinijera was also described as the causal agent of subcutaneous phaeohyphomycosis in two domestic short-hair cats from Australia [18]. In most cases of phaeohyphomycosis caused by E. spinijera, the tissue reactions were manifested by hyperkeratosis, acanthosis, and pseudopitheliomatous hyperplasia. The dermis and the soft tissue often showed intense supplicative and granulomatous inflammatory reactions characterized by the formation of microabscesses. The phaeoid hyphal elements occurred singly or in unorganized clusters as budding cells, thick-walled cells, branched, septate hyphae, moniliform hyphae, or a combination of any of these forms. The cutaneous epithelial hyperplasia and inflammation were similar to that seen in cutaneous blastomycosis, cutaneous sporotrichosis, and chromoblastomycosis.

Clinical recovery occurred in four of the 10 cases of phaeohyphomycosis caused by E. spinijera (table 1). Early diagnosis and complete surgical resection of lesions resulted in cure in case 1 [8]. Despite prolonged treatment with amphotericin B and ketoconazole, the infection in a 6-year-old boy from El Salvador (case 3) continues to be progressive [10]. In cases 2 and 10 where the correct diagnosis was not made or was delayed, the outcome was fatal [9, 17]. Treatment with amphotericin B and ketoconazole with or without 5-fluorocytosine was associated with initial clinical improvement in lesions, but relapses were common. A patient with severe rheumatoid arthritis who was treated with prednisone and had a nodular lesion on the dorsal aspect of the ring finger (case 8) was successfully treated by surgical excision of the nodular lesion followed by oral itraconazole (50 mg daily for 6 weeks then 100 mg daily for an additional 5 weeks) [15]. The initial treatment with 200 mg of ketoconazole twice a day for 3 months was not associated with clinical improvement. The E. spinijera isolate was susceptible to itraconazole (MIC, <0.018 μg/mL).

The present case represents the second known human case of chromoblastomycosis caused by E. spinijera, a well-known causal agent of phaeohyphomycosis. The Mexican patient with chromoblastomycosis (case 11) [5] was treated with oral itraconazole (200 mg/d) for a period of 1 year; the size of the lesion decreased by ~50%. Subsequently, apparent resolution occurred with two treatments with liquid nitrogen, leaving only an achromic atrophic scar at the site of infection 6 months after the last treatment.
Table 1. Clinical features of human cases of phaeohyphomycosis and chromoblastomycosis caused by *Exophiala spinifera*.

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Sex/age (y)/country</th>
<th>Underlying condition</th>
<th>Site of infection</th>
<th>Treatment</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 [8] 1968</td>
<td>F/72/USA</td>
<td>Tuberculosis</td>
<td>Nasal granuloma</td>
<td>Surgical removal</td>
<td>Recovered</td>
</tr>
<tr>
<td>2 [9] 1958</td>
<td>M/7/India</td>
<td>?</td>
<td>Bilateral granulomatous crusted lesions on face, trunk, and joints of extremities</td>
<td>Pen, Sm, INH, Nys</td>
<td>Died</td>
</tr>
<tr>
<td>7 [14] 1987</td>
<td>M/34/USA</td>
<td>Kidney transplant</td>
<td>Nontender subcutaneous nodule on right arm</td>
<td>AmB, Ket</td>
<td>?</td>
</tr>
<tr>
<td>8 [15] 1989</td>
<td>F/62/USA</td>
<td>Rheumatoid arthritis; treatment with Prd</td>
<td>Painless soft-tissue nodule on the fourth finger of left hand</td>
<td>Ket, Itr</td>
<td>Recovered</td>
</tr>
<tr>
<td>9 [16] 1993</td>
<td>M/13/Pakistan</td>
<td>?</td>
<td>Multiple crusted swellings and abscesses over the body</td>
<td>AmB, 5-FC</td>
<td>Clinical improvement</td>
</tr>
<tr>
<td>10 [17] 1994</td>
<td>M/12/Brazil</td>
<td>?</td>
<td>Subcutaneous abscesses over the right side of the face, left elbow, fingers, and toes</td>
<td>AmB</td>
<td>Died</td>
</tr>
<tr>
<td>12* [PR] 1992</td>
<td>M/62/USA</td>
<td>Rheumatoid arthritis; treatment with Prd and Aza</td>
<td>Verrucous lesions on the right elbow and forearm</td>
<td>Ket, 5-FC, Itr, heat</td>
<td>Clinical improvement</td>
</tr>
</tbody>
</table>

NOTE. AmB = amphotericin B; Aza = azathioprine; 5-FC = 5-fluorocytosine; INH = isoniazid; Itr = itraconazole; Ket = ketoconazole; Nys = nystatin; Pen = penicillin; PR = present report; Prd = prednisone; Sm = streptomycin; ? = unknown.

In the present case, however, some unique features in the process of propagation of muriform cells of *E. spinifera* in tissue were manifested. The thick-walled muriform cells were divided internally by septation in different planes. These thallic fungal cells propagated in host tissue by planate division, which is characteristic of chromoblastomycosis. The individual cells within the outer wall of the parent cell disarticulated from the outer wall and from each other to form endoconidia; these endoconidia were released after fracture of the outer wall. In vitro formation of endoconidia resulting from disarticulation of meristematic cells by *Botryomyces caespitosus* and *Wangiella dermatitidis* (two agents of phaeohyphomycosis) was described by de Hoog and Rubio [19] and Matsumoto et al. [20], respectively. However, *B. caespitosus* propagates in tissue by the process of budding [21]. *W. dermatitidis* multiplies in tissue by producing yeastlike cells and hyphal elements typical of phaeohyphomycosis.

Itraconazole treatment may be effective for some patients with phaeohyphomycosis and chromoblastomycosis. According to Sharkcy et al. [22], itraconazole appears to be the drug of choice for the treatment of patients with phaeohyphomycosis, including those refractory to other antifungal agents. However, although the rate of response to itraconazole therapy was encouraging in the study by Sharkcy et al., only one of six evaluable patients with an underlying immunodeficiency was apparently cured. In the present case initial treatment with itraconazole and 5-fluorocytosine followed by treatment with itraconazole alone and heat was associated with initial improvement in the lesions, but on the basis of clinical recurrence and progression of lesions while therapy was being administered and a small rise in MIC, *E. spinifera* became resistant. At the present time it must be concluded that a fully satisfactory regimen, either medical or surgical, for the treatment of extensive chromoblastomycosis in immunocompromised hosts does not exist.

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References


