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CASE REPORT

Pulmonary Colonization by *Chrysosporium zonatum* Associated with Allergic Inflammation in an Immunocompetent Subject

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We report a case of noninvasive pulmonary disease due to *Chrysosporium zonatum* in an immunocompetent male. The fungus colonized an existing tuberculous cavity and was isolated from transbronchial lavage fluid and from a percutaneous aspiration specimen. The disease was accompanied by the unusual feature of an allergic reaction. The fungus ball was successfully treated by intracavitary administration of amphotericin B. *C. zonatum* is the anamorph of the heterothallic ascomycete *Uncinocarpus orissi*, and the identity of the case isolate was verified by formation of ascospores in mating tests with reference isolates.

CASE REPORT

A 72-year-old man initially visited another hospital complaining of a productive cough and fever in December of 1995. He had experienced pulmonary tuberculosis 10 years earlier. Previous chest X-rays revealed thin wall cavities in the right upper lobe (RUL), but otherwise he was in good health. Changes in the chest X-rays, i.e., thickened cavity walls with airspace consolidation in the RUL, suggested the presence of a bacterial infection. However, bacteriological cultures of blood and sputum specimens were negative. The patient failed to respond to empirical antibiotic therapy with clindamycin and imipenem and developed dyspnea. At admission to Saga Medical School Hospital on 25 January 1996, chest X-rays and computed tomography (CT) revealed enlarged cavities, in one of which an air-fluid level was noted, in the RUL. Bilateral mixed airspace and interstitial infiltrations, distributed mainly in the right lower lobe (RLL), were also observed. An increase in eosinophils was noted in peripheral blood (around 900/μl) and in bronchoalveolar lavage (BAL) fluid from the RLL (14%). Histopathological examination of transbronchial lung specimens from the RLL indicated interstitial infiltration of numerous lymphoplasmacytoid cells and smaller numbers of neutrophils and eosinophils. No organisms were detected by periodic acid-Schiff and Ziehl-Neelsen staining. As his clinical symptoms and pulmonary infiltrations worsened, peripheral eosinophils were elevated to 2,600/μl, suggesting involvement of an allergic process. Corticosteroid therapy was started on 20 February. Consequently, the symptoms subsided and the diffuse infiltrations and air-fluid level in the cavity diminished. A follow-up CT scan revealed a homogeneous round mass suggestive of a fungus ball in one of the cavities (Fig. 1). While receiving oral prednisolone at 10 mg/day, the patient developed a mild fever and an intermittent productive cough. Because the clinical course resembled allergic bronchopulmonary aspergillosis, oral itraconazole (ITRA), 100 mg/day, was added on 22 March.

Specimens were collected by BAL on 19 March and CT-guided percutaneous needle aspiration on 10 April and sent for microbiological analysis. Microscopic observations of gram- and Giemsa-stained specimens were negative for hyphae. The samples were cultured on sheep blood agar at 35°C for 2 days and Sabouraud dextrose agar (Difco Laboratories, Detroit, Mich.) at 30°C for 7 days. On Sabouraud dextrose agar plates, both specimens grew an apparently identical white mould that was later identified as *Chrysosporium zonatum*. Despite ITRA therapy for 8 weeks, chest X-rays revealed enlargement of the fungus ball. Therapy was switched to amphotericin B (AMB) on 22 May and given by continuous intracavitary instillation since once-a-day administration had caused a severe cough. The drug was delivered through an indwelling percutaneous catheter at a rate of 9.8 mg/day for 4 weeks and at 19.6 mg/day for 6 weeks. During AMB treatment, the corticosteroid was discontinued without recurrence of the symptoms or peripheral eosinophilia. A chest CT scan on 22 July revealed that the fungus ball had diminished remarkably. No pulmonary infiltration was noted. Therapy with AMB was discontinued on 12 August. He has remained free of symptoms, and a follow-up chest X-ray taken in January 2001 showed no evidence of recurrence.

To determine whether the patient was sensitized to *C. zonatum*, a skin test using crude extract prepared from the case isolate was performed in January 1997. Briefly, the fungus was cultured in BBL Mycophil Broth (Becton Dickinson Microbiology Systems, Cockeysville, Md.). A conidial suspension was prepared in phosphate-buffered saline and sonicated for 15

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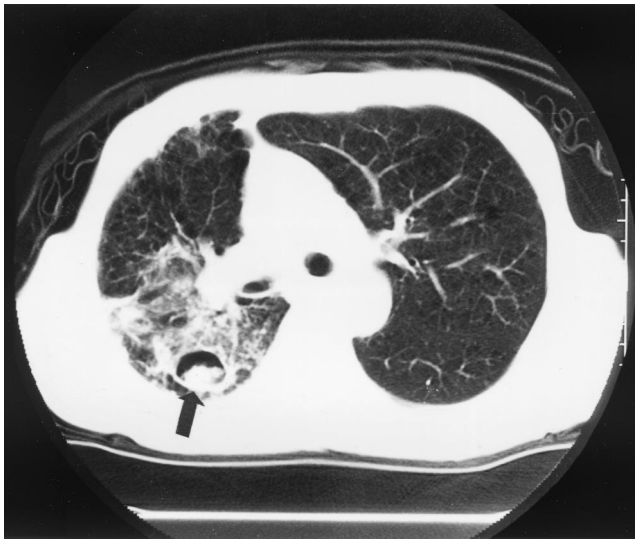

FIG. 1. CT after corticosteroid treatment. A fungus ball (arrow) is visible in a cavity in the RUL, while the infiltration in the lung fields has diminished.

FIG. 2. Colony of *C. zonatum* (case isolate) on potato dextrose agar after 21 days at 28°C. Original magnification, ×1.0.

min. The suspension was centrifuged at 100,000 × *g* for 30 min at 4°C. The supernatant was then collected as an antigen extract and sterilized with a 0.22-μm-pore-size microfilter. When the patient was subcutaneously injected with 100 μl of the antigen extract diluted to 1:10,000, he was positive for both immediate- and delayed-type reactions. Four volunteer controls, including two members of the patient's family, were negative.

Mycology. The BAL isolate was sent to the University of Alberta Microfungus Collection and Herbarium (UAMH), where it was assigned accession number UAMH 9068. Colonies on potato dextrose agar (Difco) at 28°C were fast growing, reaching a diameter of 3 cm in 7 days (8.5 cm in 21 days), and were flat, powdery, and initially yellowish white, becoming buff, with the reverse uncolored (Fig. 2). Growth at 37°C was similar but slightly faster (4 cm in 7 days). The isolate was resistant to cycloheximide, as judged by its equivalent growth on Mycosel medium (BBL). The microscopic morphology was examined in slide culture preparations. Conidia (aleurioconidia) were formed at the ends of short stalks that arise at an acute angle and are often slightly curved (Fig. 3). Conidia also formed on the sides of the hyphae, i.e., sessile or, less commonly, in an intercalary position (arthroconidia). Aleurioconidia were club shaped to broadly obovoid (egg shaped), were slightly warty, and measured 5 to 7 μm long and 3 to 4 μm wide; arthroconidia measured 7 to 11 μm long and 2 to 3 μm wide. The identification was confirmed by mating the case isolate with reference strains of *Uncinocarpus orissi*, which was identified previously as the teleomorph of *C. zonatum* (12). It was determined as a plus mating type based on its formation of ascomata and ascospores when paired with a minus mating type strain (UAMH 8936).

Discussion. Recently, *C. zonatum* has been identified as the cause of disseminated infection accompanied by chronic granulomatous disease (CGD) in a Greek patient (9) and of pulmonary colonization secondary to prior tuberculosis in two Japanese patients, one of whom is described here (L. Sigler, E. Roilides, E. Bibashi, K. Naitoh, S. Hayashi, K. Nishimura, K. Kamei, A. Kojima, S. Matsubara, Y. Nakahara, N. Flaris, and H. Katsifa, Abstr. 98th Gen. Meet. Am. Soc. Microbiol., abstr. F-91, 1998). Although the clinical findings of our patient resemble those of invasive fungal disease, it is more likely that the inflammation arose from an allergic reaction to the colonizing fungus. This is supported by several findings. (i) Histological examination showed interstitial pneumonia in the RLL but no evidence of fungal invasion. (ii) Eosinophils were increased in both the peripheral blood and BAL fluid. (iii) Symptoms and the infiltrations on the chest X-rays improved promptly after corticosteroid administration. (iv) The patient was sensitized to extracts of *C. zonatum*, showing both immediate- and delayed-type reactions. The mode of allergic inflam-

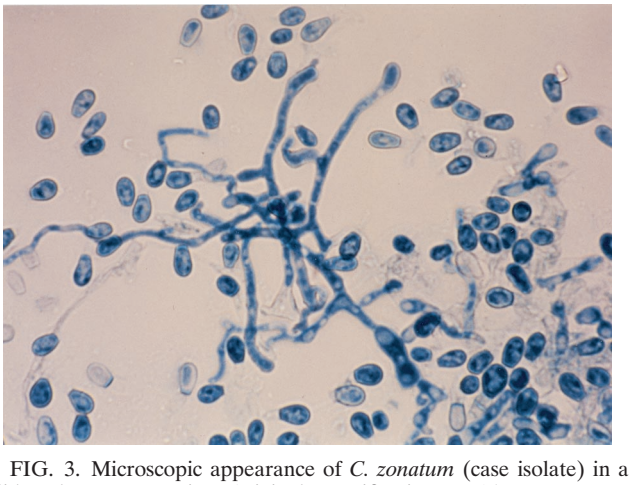

FIG. 3. Microscopic appearance of *C. zonatum* (case isolate) in a slide culture preparation. Original magnification, ×645.

mation, i.e., absence of asthma, presence of interstitial pneumonia, and association with a fungus ball, was somewhat different from that typically seen in aspergillosis (5). However, these characteristics do not seem to be unique to *C. zonatum* since a few reports have documented similar unusual conditions with *Aspergillus* species (6, 7, 10, 15).

When a patient with a fungus ball is symptomatic but not a candidate for surgery, intravenous administration or direct instillation of AMB has been tried (1) but the effectiveness of this treatment has not been established. It is noteworthy that our patient was treated successfully, suggesting that continuous instillation of AMB into a cavity may be more effective than intermittent administration because of prolonged exposure of the fungus to the drug. Also, compared with *Aspergillus* species (2, 4), *C. zonatum* is more susceptible to AMB. Previously published drug susceptibility data on *C. zonatum*, including our isolate, indicate that the MIC of AMB is less than 0.25 μg/ml (9). Although the relationship between in vitro drug susceptibility results and the in vivo outcome is under intense research (3, 8), our experience, together with the results from the CGD patient with *C. zonatum* invasive infection (9), suggests that, in a clinical situation, *C. zonatum* responds to AMB but not to ITRA. ITRA MICs for three human isolates were 0.56 to 2 μg/ml, with the MIC for our patient's isolate (UAMH 9068) being the lowest one (9). Therapy with ITRA was associated with exacerbation of disease in the patient with underlying CGD (9). Similar findings were observed in the present patient.

Members of the genus *Chrysosporium* are common soil saprobes, many of which are connected to sexual states (teleomorphs) in the ascomycete order *Onygenales* (11). Although *Chrysosporium* species are occasionally isolated from respiratory or cutaneous specimens, most are not considered clinically significant (11, 13). There are rare reports of *Chrysosporium* species causing invasive disease or sinusitis in immunocompromised and immunocompetent hosts. A problem with most of these reports is that the etiologic agent is not described in sufficient detail to determine which species was involved (see references 11 and 13 for further discussions). *C. zonatum* is recognized by its fast growth at 37°C and by colonies that darken to buff. First described in 1989 in Kuwait, this species is now known to occur in North America, Europe, Asia, and the Middle East (12). Mating studies revealed that the species is the anamorph of an ascomycete that has been named *U. orissi* (*Onygenaceae*) (12); however, the placement of the teleomorph in the genus *Uncinocarpus* was disputed in a later study (14). Further investigation is needed to clarify the relationship between *U. orissi* and *U. reesii*, the type species of the genus *Uncinocarpus*.

When a *Chrysosporium* species grows from samples collected by the percutaneous or transbronchial route, the result should be interpreted carefully. In our patient, a biopsy of the RUL was not performed due to a high risk of bleeding. Since we obtained the same fungus in both a percutaneous aspiration specimen and BAL fluid, we concluded that *C. zonatum* was responsible for the disease.

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