Misleading Manifestations of *Coccidioides immitis* In Vivo

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We describe a case of coccidioidomycosis in which several unusual morphologic forms of *Coccidioides immitis* occurred in biopsy tissue from the right lower lung of a patient. To our knowledge, this is the first case where so many diverse morphologic forms were manifested in a single patient in the absence of typical endospore-luting spherules. Immature spherules demonstrating segmentation mimicked morula forms of *Prototheca* spp. Certain elements resembled budding cells of *Blastomyces dermatitidis*. These consisted of juxtaposed immature spherules without endospores, a germinating endospore, or thick-walled hyphal cells. Branched, septate hyphae and moniliform hyphae consisting of chains of thick-walled arthroconidia or immature spherules were also present. Complement fixation and immunodiffusion tests performed on the patient’s serum were negative for *C. immitis*, *B. dermatitidis*, and *Histoplasma capsulatum* antibodies. Fluorescent-antibody studies were carried out with a specific *C. immitis* conjugate. All of the diverse fungal tissue elements stained positive with a moderate to strong (2 to 3+) intensity.

*Coccidioides immitis*, a dimorphic fungus, manifests wide morphologic variation in its mycelial as well as its parasitic form. In its mycelial form, fast-growing colonies may vary in texture from cottony to velvety, powdery, or granular with smooth, folded, or zonate surfaces. Even though the majority of isolates produce dull white colonies in culture, atypical variants producing gray, lavender, buff, lemon yellow, or brown colonies have been described in the literature (4, 5, 7).

In tissue, *C. immitis* typically forms spherules containing endospores. Infrequently, it produces uncharacteristic hyphae forming arthroconidia in cavitory lesions or air spaces in the lungs or in pleural spaces (2, 3, 14–17, 20). In rare cases, appressed immature or atypical spherules resembling budding cells of *Blastomyces dermatitidis* have been reported (9, 16). We describe a human case in which several unusual morphologic forms of *C. immitis* occurred concomitantly in a lung tissue specimen. Multiple diverse morphologic forms, any of which could lead to an incorrect diagnosis, are described herein.

**Case report.** The patient, an 80-year-old male, was admitted on 11 November 1994 for a workup on a cavitory lesion (2 by 2 cm) in the right lower lung. The patient was seen by one of us (G.V.) because histologic examination of his resected lung was positive for fungal elements. He had lived in San Francisco, Calif., for 1.5 years, and during that time he had visited Arizona, New Mexico, and the Rio Grande valley, areas known to be endemic for coccidioidomycosis. Hospital records indicated that other travel occurred at unspecified times to Michigan, northeastern Mexico, Philippines, Utah, and Washington, D.C. A bronchoscopy and a needle biopsy examination were nondiagnostic. Resection of the right lower lobe of the lung was done to rule out and treat possible cancer. No attempt was made to culture the biopsied tissue. Histologic examination of the biopsied tissue revealed what were believed to be broad-based, yeast-like cells and, in some areas, delicate, septate hyphal elements. A tentative diagnosis of blastomycosis was made. Treatment with itraconazole (400 mg/day) was initiated.

The patient’s serum and sections of formalin-fixed, paraffin-embedded lung tissue were submitted to the Mycoses Immunodiagnostic Laboratory of the Centers for Disease Control and Prevention for serologic and histologic studies. Tissue sections were stained with hematoxylin and eosin and Gomori-methenamine silver (GMS). Complement fixation and immunodiffusion tests were performed for *B. dermatitidis*, *C. immitis*, and *Histoplasma capsulatum* antibodies and interpreted in accordance with protocols described earlier (8). None of these antibody tests were positive. Although blastomycosis and histoplasmosis were included in the differential diagnosis, the presence of branching septate and moniliform hyphae and morula-like bodies in the biopsied tissue was not consistent with the in vivo morphological manifestations of *B. dermatitidis* and *H. capsulatum*. These findings together with the patient’s travel history suggested a possible diagnosis of coccidioidomycosis. Accordingly, our initial approach was to stain the tissue with a *C. immitis*-specific conjugate.

**Immunofluorescence staining.** Deparaffinized tissue sections were treated with 1% trypsin for 45 min (11). Specific fluorescein isothiocyanate-conjugated rabbit *C. immitis* antiglobulins were prepared, and direct staining was performed in accordance with the method of Kaplan and Clifford (6). Tissues treated with the labeled *C. immitis* antiglobulins were examined with a Leitz Ortholux II incident-light fluorescence microscope. A specimen was considered positive if the fungal elements stained weakly (1+) or with a greater intensity.

Sections stained with GMS showed fungal elements exhibiting a wide variety of morphologic forms, none of which were pathognomonic for any particular fungal pathogen. Some spherule-like elements showed multiple cleavages in different planes that resembled the morula forms of *Prototheca* spp. (Fig. 1). Others occurred as oval to spherical cells either singly, in pairs, or in groups of three to five, or sometimes in small chains (Fig. 1). Typical mature spherules with endospores were not observed. Fungal cells forming germ tubes (Fig. 2) and appressed hyphal cells with thick walls mimicked the broad-based budding cells of *B. dermatitidis* (Fig. 3). There were septate, hyphal elements of various lengths present. Some of the hyphal cells that were aligned in a row became thick-walled, resembling moniliform hyphae (Fig. 4). In other areas, septate,
branched hyphal elements of various lengths were also observed (Fig. 5). A hematoxylin-and-eosin-stained slide revealed the fungal elements to be hyaline.

Immunofluorescence studies of biopsy tissue with a C. immitis-specific conjugate revealed the fungal elements to be those of C. immitis. The conjugate stained the young spherules, hyphal cells, and moniliform hyphal elements with a 2 to 3+ intensity.

Atypical forms of C. immitis in tissues from humans have been recognized in several studies (2, 3, 12, 14, 15, 17, 19, 20). Immature spherules frequently cannot be distinguished from budding cells of B. dermatitidis, H. capsulatum var. duboisii, or Prototheca spp. On occasion, one or two diverse forms, such as hyphal elements with or without arthroconidia or appressed cells resembling budding cells of B. dermatitidis, occur, and in most instances they are accompanied by the typical endosporulating spherules. In the present study, exhaustive microscopic examination of numerous fields of the biopsied tissue sections revealed only the existence of the four aforementioned diverse morphologic forms of C. immitis (morula-like, broad-based yeast-like, moniliform, and true hyphae). The observation of cells resembling the morulas of Prototheca spp. was an unusual one for us. However, such structures were observed for an earlier case of coccidioidomycosis (12). Any of the four atypical forms could be confused with the tissue forms of other pathogenic fungi. Histologic diagnosis was complicated because none of these diverse forms were found in association with typical
elements of *C. immitis*. Diagnosis of coccidioidomycosis was achieved by identifying the polymorphic forms by using a *C. immitis* fluorescent-antibody reagent. The value of using multiple methods for establishing a diagnosis cannot be overemphasized. In this study, culture was not attempted and serologic tests were negative. Negative serologic tests are not unusual with sera from patients with coccidioidal cavitary disease. Approximately 40% of patients with coccidioidal pulmonary cavitation may be negative for coccidioidomycosis by serologic tests (18). However, a higher percentage of such patients might be serologically diagnosed by immunodiffusion testing of concentrated sera (13). Histologic results were equivocal, and the use of a specific fluorescent-antibody reagent established the diagnosis.

Culture remains the most reliable means to establish a definitive diagnosis. Even though no attempts were made to culture the tissue in our case, we stress the need for surgeons and operating room assistants to conserve tissue for culture.

With the greater frequency in international travel, the incidence of coccidioidomycosis is increasing in areas where this infection was not previously seen. Cases were recently identified in India and Japan, where 1 and 14 cases of coccidioidomycosis, respectively, were documented in the literature (1, 10). Of the 14 patients, 12 had a history of travel to areas where coccidioidomycosis is endemic. In two Japanese patients, there was no history of travel to the areas of endemicity, but both patients worked in cotton mills and handled raw cotton imported from areas of endemicity, which exposed them to the infectious arthroconidia of *C. immitis*. These facts emphasize not only the importance of noting the patient’s history of travel to areas of endemicity but also any contact with materials imported from areas of endemicity that can serve as a source of *C. immitis*. Equally important is the need for laboratorians to be aware of the diverse manifestations of *C. immitis* in tissue which can lead to misdiagnoses.

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REFERENCES