Cross-Reactivity in *Histoplasma capsulatum* variety *capsulatum* Antigen Assays of Urine Samples from Patients with Endemic Mycoses

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We evaluated cross-reactivity in the antigen assay used for the diagnosis of histoplasmosis by testing urine samples from patients with disseminated fungal infections. The mycoses chosen for this study were selected on the basis of the observation that during clinical testing, cross-reactions may occur between *Histoplasma capsulatum* var. *capsulatum, Paracoccidioides brasiliensis, Blastomyces dermatitidis, Coccidioides immitis,* and *Penicillium marneffei.* We detected antigen in 12 of 19 patients with blastomycosis, 8 of 9 with paracoccidioidomycosis, in 17 of 18 with *P. marneffei* infection, and in one with disseminated *H. capsulatum* var. *duboisii* infection. Cross-reactions were not observed in the assays for six patients with disseminated coccidioidomycosis. Cross-reactivity between the agents of other endemic mycoses should be considered in interpreting a positive *H. capsulatum* var. *capsulatum* antigen assay. Antigen detection may provide a rapid, provisional diagnosis for patients with serious infections caused by one of these organisms.

Antigen detection is useful for the rapid diagnosis of histoplasmosis. A polysaccharide antigen is detected in urine samples from most patients with disseminated histoplasmosis or extensive, acute pulmonary histoplasmosis. The *Histoplasma capsulatum* antigen assay was shown to be highly specific in earlier studies of patients infected with bacterial or selected fungal pathogens. Cross-reactions were not demonstrated for patients with serious infections caused by *Candida, Aspergillus,* or *Cryptococcus* species [1–3]. Cross-reactions were noted in the urine of a patient with blastomycosis [2] and the CSF of a patient with coccidioidal meningitis [4]. However, the cross-reaction for the patient with coccidioidomycosis was only marginally positive and was not reproducible in subsequent studies.

Cross-reactions have subsequently been recognized in sporadic cases of blastomycosis and paracoccidioidomycosis but not in cases of coccidioidomycosis, cryptococcosis, candidiasis, or aspergillosis.

To more fully evaluate cross-reactivity between pathogens that have previously been identified with use of the *H. capsulatum* var. *capsulatum* antigen assay, we tested urine samples from additional patients infected with *Paracoccidioides brasiliensis, Blastomyces dermatitidis, Coccidioides immitis,* and *Penicillium marneffei.*

Materials and Methods

Antigen assay. The solid-phase enzyme immunoassay used for this experiment was a modification of a previously described assay [2], for which biotinylated antibody to *H. capsulatum* var. *capsulatum* was used in place of radiolabeled antibody to detect antigen in urine samples from infected patients. Results of >50% above the mean of two control urine samples from healthy laboratory workers were regarded as positive and assigned a value by dividing the mean optical density of the test sample by 1.5 times the mean of the normal control samples. Results of at least 1.0 units were considered to be positive.

Patient specimens. Urine specimens were collected from patients with disseminated infections or extensive pulmonary infections caused by *H. capsulatum* var. *duboisii* ($n = 1$), *P. brasiliensis* ($n = 9$), *B. dermatitidis* ($n = 19$), *C. immitis* ($n = 6$), or *P. marneffei* ($n = 18$). (A diagnosis of disseminated disease was made if there were clinical or laboratory findings of involvement at extrapulmonary sites.) Samples were stored at −70°C until the day of testing, and the tests were performed in a single assay.

Results

Cross-reactivity was noted in samples from 63.2% of patients (12 of 19) with blastomycosis, including four of six with isolated pulmonary infections; in samples from 88.9% of patients (8 of 9) with paracoccidioidomycosis, including four of five

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with isolated pulmonary involvement; in samples from 94.4% of patients (17 of 18) with disseminated *P. marneffei* infection; and in a sample from one patient with disseminated African histoplasmosis. The antigen level appeared to be lower in the patients with pulmonary blastomycosis than in those with extrapulmonary blastomycosis (figure 1). However, the antigen levels were similar in patients with isolated pulmonary paracoccidioidomycosis and in those with disseminated infection (figure 1).

Cross-reactions did not occur in the urine samples from six patients with disseminated coccidioidomycosis. The amount of antigen in the urine samples from these patients was similar to that in urine samples from patients with histoplasmosis, based on a comparison of these samples with high-positive and low-positive urine samples from patients with antigenuria, which were run in each assay as standard controls.

Discussion

Our results establish that cross-reactions occur in the *H. capsulatum* var. *capsulatum* antigen assay for patients with paracoccidioidomycosis, blastomycosis, African histoplasmosis, and *P. marneffei* infections. Although assay of CSF yielded a borderline positive result (1.0 units) for one of 11 patients with coccidioidal meningitis in an earlier study [4], we did not detect cross-reactions in the urine specimens from any of the six patients with coccidioidomycosis in the present study. Although the number of patients in the present study was small, the borderline significance of the CSF antigen result, the negative results for the six patients with coccidioidomycosis, and the lack of any positive results attributable to coccidioidomycosis during routine testing led us to conclude that cross-reactions are unlikely to occur with *C. immitis.*

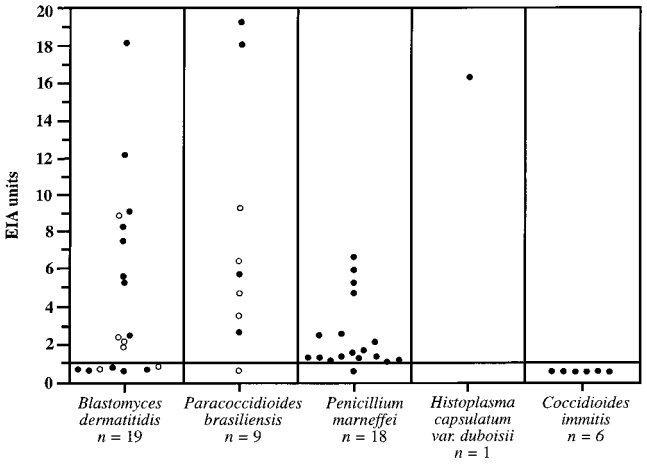

Figure 1. Detection of *H. capsulatum* var. *capsulatum* antigen in urine samples from patients with disseminated (●) or pulmonary (○) blastomycosis, paracoccidioidomycosis, *Penicillium marneffei* infection, African histoplasmosis, or coccidioidomycosis. Results are expressed as enzyme immunoassay (EIA) units, and results of 1 unit or higher, shown above the horizontal line, are positive.

Earlier studies did not show cross-reactions in urine specimens from patients with aspergillosis ($n = 8$), candidiasis ($n = 18$), or cryptococcosis ($n = 7$) [1–4]. Furthermore, cross-reactivity between these fungi has not been demonstrated in clinical testing. However, cryptococcal and *Histoplasma* antigens have been detected in a few patients with concurrent cryptococcosis and histoplasmosis.

The specific epitopes responsible for the immunologic recognition of *H. capsulatum* var. *capsulatum* antigen in patients with histoplasmosis have not been adequately evaluated. The antigen remains immunologically active after boiling but is destroyed by treatment with periodate, and it binds to concanavalin A [1].

The amounts of urine available from the patients described in the present report were insufficient for performing experiments to characterize the cross-reacting antigens found in patients with other endemic mycoses. Earlier studies of the antibody response in rabbits immunized with *H. capsulatum* var. *capsulatum* antigen demonstrated a cross-reactive antigen in extracts of *H. capsulatum* var. *duboisii, B. dermatitidis,* and *P. brasiliensis* [5]; that antigen was not characterized biochemically. Cross-reactions between these fungi also have been demonstrated in tests for antibody in sera from infected patients [6]. The basis for cross-reactivity with *P. marnefeii* has not been studied. Together, these studies indicate that these fungi share antigens that both stimulate the production of cross-reactive antibodies and can be detected in the body fluids of patients with extensive infections.

Detection of a cross-reactive or shared antigen in patients with African histoplasmosis, blastomycosis, paracoccidioidomycosis, or penicilliosis marneffei may be useful for the diagnosis of these infections. However, since we specifically selected specimens from patients with extensive pulmonary or disseminated disease, our results do not allow determination of the sensitivity of antigen detection for diagnosing these mycoses. Larger studies that include patients with other manifestations are needed to fully define the sensitivity of antigen detection for the diagnosis of these diseases.

The epidemiological and clinical differences between these mycoses are helpful in the differential diagnosis; however, if a patient presents with a history of travel to an area where a specific mycosis is endemic, the clinician should also consider this when making the differential diagnosis. Blastomycosis and histoplasmosis both occur in large regions of the Ohio River and Mississippi River valleys but often can be distinguished clinically or serologically before mycological confirmation is obtained. Isolation of the pathogen or observation of characteristic findings in tissue specimens confirms the diagnosis.

Paracoccidioidomycosis is endemic in Latin America, but both histoplasmosis and paracoccidioidomycosis may occur in

some areas of the region. *P. marneffei* is endemic in Southeast Asia and China, but histoplasmosis also occurs in some parts of Southeast Asia. *H. capsulatum* var. *duboisii* is endemic in central Africa.

Since the decision to initiate treatment and the selection of antifungal agents are similar for patients with any of these mycoses, the cross-reactivity of the *Histoplasma* antigen assay does not reduce its value for rapid diagnosis. Antifungal treatment is indicated for patients with disseminated or extensive pulmonary infections; amphotericin B or itraconazole is the drug of choice, and selection of the specific agent is based on the severity of the clinical manifestations [7].

In conclusion, a cross-reactive or shared antigen may be detected in urine samples from patients with disseminated infections caused by *H. capsulatum* var. *capsulatum, H. capsulatum* var. *duboisii, P. brasiliensis, B. dermatitidis,* and *P. marneffei.* Antigen detection may be useful for the rapid diagnosis of disseminated infection caused by one of these organisms; however, the sensitivity of this test in these situations requires further evaluation.

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