Early Treatment With Fluconazole May Abrogate the Development of IgG Antibodies in Coccidioidomycosis

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Background. We have observed a number of patients who fail to develop coccidioidal complement fixing (CF) antibody (immunoglobulin [IgG]) after the initiation of early antifungal therapy. Although this is the first description of this phenomenon in mycology, a precedent for the abrogation of the immune response has been observed in other conditions, including primary syphilis and primary Lyme disease.

Methods. We conducted a retrospective case-control study to determine any patient-specific risk factors associated with this observation. Additionally, in vitro analysis of the coccidioidal CF (IgG) antigen (Cts1) was performed after Coccidioides was grown under escalating fluconazole concentrations.

Results. Seventeen patients persistently positive for coccidioidal IgM antibodies without developing an IgG response (cases) were compared with 64 consecutive patients who did develop coccidioidal CF (IgG) antibodies (controls). Early treatment with antifungals (within 2 weeks of symptom onset) was associated with an abrogation of IgG antibody production ($P < .001$). With immunodiffusion testing, control serum demonstrated a lack of IgG seroreactivity when Coccidioides posadasii grown in the presence of escalating fluconazole doses (0.5–128 μg/mL) was used as the antigen; however, control serum remained seroreactive for the presence of IgM. The coccidioidal IgG antigen (Cts1) was shown to be diminished when cultures were grown in the presence of fluconazole, lending further in vitro plausibility to our findings.

Conclusions. The abrogation of an IgG response in patients treated early in the course of coccidioidal infection may complicate serodiagnosis and epidemiologic studies, and further study to determine the potential clinical implications should be performed.
METHODS

A retrospective case-control study was designed to test whether a higher incidence of early antifungal therapy (receipt of antifungal therapy <14 days after symptom onset) was seen in patients who failed to develop coccidioidal complement fixing (CF) antibodies (IgG) than in patients who either did not receive antifungal therapy or received antifungal therapy ≥14 days after symptom onset. This study was approved by the Institutional Review Board of the University of California, Davis.

Cases were identified by a review of all patients noted to be persistently positive for IgM antibodies (>90 days) from the Coccidioidomycosis Serology Laboratory database between 2003 and 2010. Patients were excluded from evaluation if they developed IgG antibody at any time or if insufficient clinical information was available for chart abstraction. For the control cohort, we included consecutive patients with a known diagnosis of coccidioidomycosis who were evaluated between 2009 and 2010 at the University of California, Davis Medical Center. Patients were excluded from either group if they did not meet previously established criteria for the diagnosis of proven or probable coccidioidomycosis [3].

Chart abstraction was performed to identify demographic variables, including age, sex, and ethnicity; date of symptom onset; antifungal agents prescribed; type of coccidioidal infection; and any comorbid conditions that may have accounted for a failure to manifest an appropriate immunologic response. Data analysis was performed using R software (version 2.10). Differences in demographic characteristics were evaluated using Student’s t test for continuous variables and Fisher’s exact test for categorical variables. Differences were considered statistically significant at P < .05.

Spherule-endospore (SE) cultures were prepared as described elsewhere using the C. posadasii strain Silveira [8]. Cultures were grown in the absence of fluconazole (Pfizer) (growth control) and with increasing concentrations of fluconazole, ranging from 0.5 to 128 μg/mL. Culture filtrates were collected at 72 hours. Serologic testing for the presence of antigens reactive with IgM and IgG antibodies by immunodiffusion was performed as described elsewhere, using previously heated antigen to detect IgM antibody reactivity and unheated antigen to detect the antigen (chitinase Cts1) reactive with IgG [4].

Reverse-transcription polymerase chain reaction analysis of Cts1 messenger RNA (mRNA) expression was performed as described elsewhere [8], except the following primers were used for chitinase (Cts1) mRNA expression: Cts1 forward, 5'-GTGGTC ATAACCCGCAAGAT-3'; Cts1 reverse, 5'-CGGTGAAGCAA-TAGTGAGCA-3'. Chitinase enzyme activity was measured in solution using a colorimetric method with the chromogenic substrate, p-nitrophenyl β-D-N,N′,N′′-triacetetylchitotriose (Sigma-Aldrich), as described elsewhere [8].

RESULTS

Table 1 shows the characteristics of the 17 patients who were persistently positive for IgM antibodies without developing an IgG response (case patients). Nine male and 8 female patients, ranging in age from 11 to 70 years (mean, 44.1 years), made up the case group. Sixty-four consecutive patients who developed IgG antibodies served as controls. Five patients had proven...


**Figure 1.** Immunodiffusion testing for presence of coccidioidal antibody. Serologic testing using control serum (CS) known to be reactive to both coccidioidal precipitin (hSpAg) (immunoglobulin [IgM] antigen) (line of identity indicated by white arrows), and coccidioidal complement fixation antigen (CF Ag) (IgG antigen) (line of identity indicated by black arrows). Results demonstrate a reaction of control serum with both control wells (hSpAg and CF Ag). Control serum also demonstrates seroreactivity in all other wells, including the growth control well (Coccidioides grown in the absence of fluconazole ("No flu") (A, C) and wells containing Coccidioides posadasii grown in the presence of escalating fluconazole concentrations (from 0.5 to 128 |g/mL). Reaction was confirmed as IgM after dissolution following the addition of dithiothreitol.


disease (meningitis in 2, pulmonary disease in 3), and the remaining 12 (all pulmonary) had probable disease according to criteria established by the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group [3]; this conclusion was also supported by the improvement of all patients after initiation of antifungal therapy. Early treatment with antifungals (within 2 weeks of symptom onset) was seen in the majority of patients (15 of 17), and concurrent corticosteroid therapy was seen in 2 of 17 (both also received antifungals). The 2 patients who did not receive early antifungal therapy received fluconazole 3 or 4 weeks after symptom onset. Six patients underwent repeated serum testing until precipitin (IgM) antibody was no longer detectable (mean time to resolution of IgM antibody, 470 days; range, 130–1353 days). The duration of follow-up available ranged from 112 to 1998 days (mean, 602 days).

Development of IgG antibodies was significantly associated with initiation of antifungal therapy within 14 days of the appearance of symptoms (P < .001). None of the 15 patients who received antifungal therapy within 14 days of symptoms developed IgG antibody (4 of these 15 patients were culture positive). Of the 66 patients who received antifungal therapy (controls and 2 case patients) after 14 days, all but 2 developed the IgG antibody (1 of the controls was culture positive and receiving chronic prednisone therapy).

We additionally found no association between development of IgG antibody and patient ethnicity (P = .343), nor was the development of IgG antibodies associated with the type of coccidioidal infection (P = .121). However, in almost all cases, the infection was a primary pulmonary infection (75 of 81 patients) with cavitary pneumonia, disseminated disease, or meningitis in the remaining 6 patients.

As mentioned elsewhere, it had been speculated that the growth of C. posadasii in the SE phase in the presence of fluconazole might inhibit the production of chitinase (Cts1), the antigen responsible for IgG seropositivity. Figure 1 shows the seroreactivity of control serum known to be positive for both coccidioidal precipitin (IgM) and CF (IgG) antibodies. The serum is shown to be reactive to both previously heated (60°C) coccidioidin (heated spherulin antigen [hSpAg]), indicating the presence of IgM antibody (the "inner" line), and unheated antigen, indicating the presence of IgG antibody. A reaction consistent with IgG was also observed against all Coccidioides preparations, including those grown in the absence of fluconazole (controls in Figures 1A and 1C) and those grown under escalating fluconazole concentrations. A reaction consistent with IgG was observed in filtrates of cultures grown in the absence of fluconazole, and questionable reactions were observed in filtrates from very low concentrations of fluconazole. The presence of IgM was confirmed after dissolution of lines of identity with the addition of dithiothreitol.

Figure 2 illustrates the reactivity of control serum known to be positive only for CF (IgG) antibodies with culture filtrates. The serum is shown to be reactive only to CF antigen and growth controls. The failure of these lines to dissolve after the addition of dithiothreitol confirms their identity as IgG.

*Coccidioides* SE phase Cts1 mRNA expression declined inversely with fluconazole exposure, whereas ribosomal RNA expression remained unchanged in the presence of escalating fluconazole concentrations (Figure 3). These findings suggest that the observed decline in Cts1 mRNA is not secondary to organism-wide decrease in protein synthesis or fungal death, but rather that the reduction may be limited to genes such as *CTS1*. These results are further substantiated by the reduction in endochitinase activity when a *Coccidioides* SE phase isolate is exposed to increasing fluconazole concentrations (Figure 4).

**DISCUSSION**

Infection with *Coccidioides* spp. is associated with the development of T- and B-cell immune responses [9]. However, in
some patients with coccidioidal infection failed to mount an IgG response when early antifungal therapy was prescribed. Although this phenomenon appears uncommon, it may complicate serodiagnosis and epidemiologic studies examining the incidence and prevalence of this reportable disease. A precedent for the abrogation of an IgG antibody response after early treatment has been described after antimicrobial therapy in primary syphilis and primary Lyme disease [6, 10]. It is thought that the rapid bactericidal effects of antimicrobial therapy reduce the antigenemia to a degree that immunologic recognition is not well established and T and B cells thus do not interact effectively. Antimicrobial therapy may thus preclude the full development of the IgG response but not the IgM antibody response [10], as was observed in our case-control study reported here.

The major antigen for coccidioidal IgM appears to be a glycopeptide, and the major antigen for coccidioidal complement fixation IgG is chitinase Cts1 [11]. In the presence of antifungal therapy, the amount of exposed chitinase may be reduced at a critical early stage in the immune response before a sustained B-cell response has developed. Our in vitro investigation has confirmed antigenic differences in filtrates of cultures grown in the presence of fluconazole, as observed by the immunodiffusion results and total Cts1 mRNA expression. Similarly, recent work indicates that another triazole, voriconazole, suppresses expression of the chitinase genes CHT1, CHT2, and CHT3 in Candida albicans [12].

It is unlikely that IgG antibodies are protective against the development of coccidioidal infection in view of both relapses and progression of disease despite high complement fixation (IgG) titers. Although in the past the control of coccidiodomycosis was primarily thought to be T-cell–dependent, more recent evidence has suggested a role of B cells in protecting against Coccidioides spp. In fact, vaccination against coccidiodomycosis is not fully protective in B-cell–deficient mice [13].

A recent observational study found that patients who received antifungals experienced a higher complication rate (including the development of disseminated disease) after withdrawal of antifungal therapy [14]. In line with our data, this observation could be attributed to diminished humoral immunity after antifungal therapy, but the interval between symptoms and receipt of antifungal therapy was not discussed in the other study. Additionally, a lack of randomization in that study limits the ability to draw inferences on the decision to treat or not to treat primary pulmonary coccidioidomycosis. The clinical implications of a failure to develop IgG antibodies thus remain uncertain. Patients treated within 14 days of symptom onset may have been more symptomatic than those who were not. However, in the absence of prospective trials and assignment of symptom scores at the time of initial clinical presentation, this variable cannot be definitively evaluated.

In conclusion, we have observed a statistically significant abrogation of the IgG antibody response in some patients with coccidioidal infection when early antifungal therapy was prescribed. Although this phenomenon appears uncommon, it may complicate serodiagnosis and epidemiologic studies examining the incidence and prevalence of this reportable disease. Antimicrobial therapy may thus preclude the full development of the IgG response but not the IgM antibody response, as was observed in our case-control study reported here.
invasive coccidioidomycosis when early antifungal therapy (<14 days after symptom onset) is initiated. Although this phenomenon is uncommon, it may complicate serodiagnosis and the epidemiology regarding this reportable disease. Furthermore, the potential clinical impact of this phenomenon will need to be analyzed in future studies. It remains to be determined whether abrogation of an IgG antibody response reflects a significant lessening of disease burden after early antifungal therapy, or merely a reduction in antibody response, and clinical follow-up of the case patients is needed.

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