Immune Reconstitution Cryptococcosis after Initiation of Successful Highly Active Antiretroviral Therapy

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Five of 10 patients who commenced successful highly active antiretroviral therapy (HAART) for infection with human immunodeficiency virus type 1 (HIV-1) concurrent with or soon after a diagnosis of cryptococcal infection experienced clinical events characterized by sterile inflammation. Two patients developed aseptic meningitis with elevated intracranial pressure, 1 developed intrathoracic lymphaedonopathy with hypercalcemia, 1 developed cavitary pneumonia at the site of a cryptococcal nodule, and 1 developed a supraclavicular abscess. These events occurred 2–11 months after initiation of HAART. For 3 patients, biopsy demonstrated findings atypical for acquired immunodeficiency syndrome–associated cryptococcosis. Results of fungal cultures were negative for all 5 patients, and cryptococcal antigen levels had declined markedly in 4 patients. The timing and clinical features of and biopsy findings for these cases of cryptococcosis suggest the existence of a paradoxical reaction to Cryptococcus infection that occurs in the context of HIV immune restoration.

Immune reconstitution syndromes in patients infected with HIV result from an exaggerated inflammatory response to an opportunistic pathogen during immune restoration. This is analogous to the paradoxical reaction described in patients with tuberculosis in the pre-AIDS era, a reaction that was characterized by worsening signs and symptoms of tuberculosis but which was associated with negative culture results and was ascribed to improved immune responsiveness. In HIV-infected patients, the immune reconstitution syndromes most commonly described have been responses to tuberculosis [1] or infection with Mycobacterium avium [2] or cytomegalovirus [3]. The occurrence of an exaggerated inflammatory response to Cryptococcus neoformans in patients with AIDS who initiate HAART after a previous diagnosis of cryptococcal infection is less commonly recognized. We observed unusual culture-negative manifestations of cryptococcal infection in 4 patients who responded favorably to antiretroviral therapy that was initiated soon after a diagnosis of cryptococcal infection, which prompted a systematic investigation into the frequency and nature of this occurrence.

PATIENTS AND METHODS

At Jacobi Medical Center, a city hospital in Bronx, New York, the records of all patients with a positive result of a serological test for cryptococcal antigen between January 1998 and September 2001 were reviewed using the hospital’s computerized database. Cryptococcal infection was defined by a positive culture result or by the presence of fever and a clinical syndrome compatible with cryptococcal infection in association with a positive result of a serological test for cryptococcal antigen. Patients with cryptococcal infection who subsequently initiated HAART and who achieved an undetectable HIV-1 load or a >3-log decrease in virus load...
were identified. Such patients could be considered at risk for immune reconstitution cryptococcosis. The hospital’s computerized database and the HIV clinic records were reviewed for the occurrence of clinical events after initiation of HAART. The research was performed in accordance with the ethical standards of the institutional review boards of the Albert Einstein College of Medicine and Jacobi Medical Center.

RESULTS

Ten patients were identified who were at risk for immune reconstitution cryptococcosis. Nine patients had culture-positive cryptococcal infection, and 1 patient received a diagnosis of presumed pulmonary cryptococcosis on the basis of the findings of fever and pulmonary nodules and a positive result of a serological test for cryptococcal antigen. Before initiation of HAART, HIV-1 loads ranged from 45,000 to >750,000 copies/mL (as determined by RT–PCR); post-HAART virus loads were undetectable (<400 or <50 copies/mL) in 9 patients and were reduced by >3 log in 1 patient. Five patients experienced a clinical course compatible with immune reconstitution cryptococcosis while receiving HAART and fluconazole. Three developed focal granulomatous inflammation; in these patients, organisms morphologically compatible with \textit{C. neoformans} were revealed by histological examination; however, no fungus grew in cultures of biopsy specimens after 28 days of incubation. One patient developed hilar/mediastinal lymphadenopathy 2 months after initiation of HAART; 1 patient developed a supraclavicular mass 11 months after initiation, and 1 patient developed cavitating pneumonia 2 months after initiation. The granulomatous hilar inflammation was associated with marked hypercalcemia. Two other patients, who had cryptococcal meningitis before HAART initiation, developed a syndrome of meningismus, elevated intracranial pressure (ICP), sterile CSF cultures, and markedly reduced titers of CSF cryptococcal antigen (compared with those during the prior meningitis), which occurred 4 and 10 months, respectively, after initiation of HAART. Five patients did not experience events suggestive of relapsing cryptococcosis when successful HAART was initiated after the development of cryptococcal meningitis. Details of the clinical course of immune reconstitution cryptococcosis in the 5 patients are presented in the following case reports (table 1).

\textbf{Patient 1.} A 31-year-old man presented with AIDS nephropathy in November 1998. His CD4 count was 25 cells/mm\textsuperscript{3}, and his HIV-1 load was 556,000 copies/mL. In January 1999, the patient presented with cryptococcal meningitis characterized by confusion, ataxia, and elevated ICP. After receiving amphotericin B for 1 month (total dose, 1050 mg), HAART and fluconazole therapy were initiated. By March 1999, his CD4 count was 66 cells/mm\textsuperscript{3} and his virus load was <400 copies/mL. Starting in February 1999, chest radiography revealed right hilar and paratracheal lymphadenopathy. Despite the development of marked intrathoracic lymphadenopathy (figure 1), the patient was afebrile and felt healthy. His serum cryptococcal antigen titer declined from 1:1024 to 1:128. Coincident with the enlargement of the intrathoracic lymph nodes, the patient developed persistent hypercalcemia associated with a low level of intact parathyroid hormone (iPTH) and a relatively elevated 1,25-dihydroxy vitamin D (calcium level, 12.9 mg/dL; iPTH level, <9 pg/mL; and 1,25-dihydroxy vitamin D level, 35 pg/mL); these findings were suggestive of the pattern of hypercalcemia associated with granulomatous inflammation. A mediastinal lymph node biopsy performed in May 1999 revealed granulomata with extensive

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<td>10</td>
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\textbf{NOTE.} ABC, abacavir; AZT, zidovudine; cryp Ag, cryptococcal antigen; d4T, stavudine; EFV, efavirenz; ICP, intracranial pressure; RTV, ritonavir; SOV, soft gel saquinavir; 3TC, lamivudine.
\textsuperscript{a} Interval between initial cryptococcal infection and the initiation of HAART.
\textsuperscript{b} Interval between the initiation of HAART and the onset of immune reconstitution syndrome.
Figure 1. Chest radiograph (obtained 10 May 1999) of patient 1 reveals marked intrathoracic lymphadenopathy, which occurred coincident with hypercalcemia, an increase in CD4 count, and a decrease in HIV-1 load.

Figure 2. Chest radiograph (obtained 30 May 1999) of patient 2 reveals an extensive infiltrate with cavitation, which occurred coincident with a large decrease in HIV-1 load.

necrotic areas, the centers of which contained organisms morphologically compatible with *C. neoformans*. The result of staining for acid-fast bacilli (AFB) was negative. Cultures of the biopsy specimen were negative for fungi, bacteria, and mycobacteria. Lymphadenopathy eventually regressed (by August 1999) without any change in therapy.

**Patient 2.** A 65-year-old man who experienced fevers, fatigue, exercise intolerance, and 2 spiculated densities (revealed on a lung CT scan) tested positive for HIV in March 1999. His CD4 count was 39 cells/mm³, and his HIV-1 load was 534,200 copies/mL. Mycobacterial cultures of blood, sputum, and bone marrow samples revealed nothing abnormal. Blood cultures also revealed nothing abnormal, and neither did a blood culture performed in a fungal isolator tube. The patient received a clinical diagnosis of pulmonary cryptococcosis on the basis of a serum cryptococcal antigen titer of 1:128 and the presence of fever and the observed lung densities. The patient began receiving treatment with fluconazole (400 mg/day) and HAART. In May 1999, he developed fever and chest pain, and an infiltrate was observed at the site of one of the original nodules. Results of multiple blood cultures were negative. Although the patient continued to receive therapy with ceftriaxone and, later, with ticarcillin-clavulanate, in addition to trimethoprim-sulfamethoxazole (daily), azithromycin (weekly), HAART, and fluconazole, the infiltrate progressed and cavitated (figure 2). Chest CT revealed a thick-walled cavity with a surrounding interstitial infiltrate and a nodular lesion with early cavitation at the sites of the original 2 spiculated nodules. In June 1999, the patient's CD4 count was 57 cells/mm³, the virus load was >750,000 copies/mL, and the serum cryptococcal antigen titer was 1:256; the patient underwent biopsy of the extensive cavitary infiltrate. Histological analysis of biopsy specimens revealed extensive fibrosis between ill-defined epithelioid granulomas that contained organisms morphologically compatible with *C. neoformans*. Results of AFB staining of the specimens were negative, although *Mycobacterium avium* complex (MAC) grew in culture. The result of a fungal culture was also negative.

**Patient 3.** In April 1999, a 58-year-old man tested positive for HIV coincident with nephrotic-range proteinuria. His CD4 count was 102 cells/mm³, and his virus load was >1750,000 copies/mL. Shortly after diagnosis, the patient required hemodialysis for presumed end-stage renal disease ascribed to AIDS nephropathy. HAART was initiated, and, 1 week later, the patient developed fever. *C. neoformans* grew in cultures of blood and CSF. Cryptococcal antigen titers were 1:256 in serum and 1:2 in CSF. The patient received amphotericin B for 2 weeks (total dose, 390 mg), and then therapy was switched to oral fluconazole (200 mg/day, adjusted for renal failure). Eleven months after initiation of HAART, when the patient's CD4 count was 231 cells/mm³ and the virus load was <50 copies/mL, the patient developed a prominent, painless right supraventricular mass without associated fever or constitutional symptoms. Analysis of needle aspirate samples showed acute and chronic inflammation as well as neutrophils, granuloma formation, and organisms morphologically compatible with *C. neoformans*. Despite treatment with amphotericin B (in place of fluconazole), the mass persisted unchanged. Partial surgical

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excision revealed an abscess that contained organisms morphologically compatible with *C. neoformans* and abundant neutrophils, fibrin, and necrotic debris surrounded by sheets of histiocytes and giant cells. The result of AFB staining was negative. Results of cultures for fungi, mycobacteria, and bacteria were negative. There were no local sequelae after surgery, and there was no recurrence of disease as of May 2002; the patient continued to receive fluconazole.

**Patient 4.** A 27-year-old man presented in September 2000 with cryptococcal meningitis characterized by elevated ICP (>570 mm H₂O) and a CSF cryptococcal antigen titer of 1:4096. In addition,*C. neoformans* also grew in cultures of blood and of a lung biopsy specimen obtained during the evaluation of the interstitial pulmonary infiltrates. The lung biopsy revealed organisms morphologically compatible with *C. neoformans*. The patient’s CD4 count was 10 cells/mm³, and the virus load was 45,496 copies/mL. The patient received amphotericin B (total dose, 720 mg) with flucytosine for 17 days and underwent several lumbar punctures (for relief of headache), after which he was discharged from the hospital to receive fluconazole (400 mg/day). HAART was initiated in November 2000. In March 2001, coincident with a CD4 count of 223 cells/mm³ and a virus load of <50 copies/mL, the patient developed headache with nausea and vomiting but without fever, such that he was unable to work for 2 weeks. Lumbar puncture revealed CSF inflammation, with a WBC count of 17 cells/mm³, a normal glucose level, a cryptococcal antigen titer of 1:32, and an elevated CSF opening pressure (OP) of 340 mm H₂O. Results of CSF cultures for fungi, bacteria, mycobacteria, were negative; the result of a CSF India ink preparation was also negative. The patient’s headache resolved after serial lumbar punctures and 2 weeks of amphotericin B therapy. The patient returned to work and remained asymptomatic while receiving HAART and fluconazole, until he returned to his native country (Mexico) in October 2001.

**Patient 5.** This patient was identified in retrospect. In August 1999, a 40-year-old man presented with cryptococcal meningitis characterized by an elevated CSF OP of 330 mm H₂O, a CSF cryptococcal antigen titer of 1:1024, a positive CSF India ink preparation result, and a CSF culture positive for *C. neoformans*. The patient’s CD4 count was 47 cells/mm³, and his HIV-1 load was 224,000 copies/mL. The patient received amphotericin B (total dose, 900 mg) and a daily dose of flucytosine (6 g/day) for 14 days, after which he initiated HAART and oral fluconazole (400 mg/day). In June 2000, when his CD4 count was 306 cells/mm³ and his virus load was <50 copies/mL, the patient developed severe headache and vomiting without fever. A blood sample obtained at a visit to the HIV clinic revealed a trough fluconazole level of 9.8 μg/mL (therapeutic range, 2–7 μg/mL). A CSF sample revealed a WBC count of 149 cells/mm³, a normal glucose level, a cryptococcal antigen titer of 1:1, and a CSF OP of 390 mm H₂O. Results of mycobacterial, fungal, and bacterial cultures of CSF samples were negative, and the result of a CSF India ink preparation was also negative. The patient was treated with amphotericin B and underwent serial lumbar punctures during a 3-week period. The symptoms and CSF pleocytosis eventually resolved. The patient had no additional symptoms as of June 2001, and he continued to receive oral fluconazole.

Five other patients who began HAART after cryptococcal meningitis was diagnosed did not experience clinical events compatible with a relapse of cryptococcosis. Three started HAART at the same time as or soon after diagnosis of cryptococcal meningitis, and 2 others initiated HAART >1 year after cryptococcal meningitis was diagnosed.

**DISCUSSION**

The occurrence of sterile inflammation in association with histologic evidence of *Cryptococcus* infection (in 3 patients) or inflammation of sterile CSF (in 2 patients) in 5 of 10 patients who favorably responded to HAART initiated after a diagnosis of cryptococcal infection appears to demonstrate the frequent occurrence of an immune reconstitution phenomenon due to *Cryptococcus* infection. Alternatively, inadequate adherence to antifungal suppressive therapy by these patients might have led to a relapse of cryptococcal infection—albeit an infection characterized by unusual manifestations and negative culture results. The conditions of all the patients eventually improved with continuation of HAART and antifungal therapy and without resorting to therapy with anti-inflammatory agents, such as corticosteroids. Arguably, patient adherence to antifungal therapy might have been better once the patients associated their recurrent symptoms with recrudescent cryptococcal infection. Thus, the possibility that inadequately controlled fungal infection played a role in the genesis of the clinical manifestations cannot be excluded.

Three of the 5 patients who developed recurrent manifestations of cryptococcosis had negative culture results by the time HAART was initiated; 1 patient began receiving HAART coincident with diagnosis of cryptococcemia, and 1 patient never had positive culture results. Of the 5 patients who did not develop recurrent manifestations of cryptococcal infection after HAART, 1 had commenced HAART coincident with CSF cultures that were persistently positive for *C. neoformans*; 4 others began HAART after negative CSF culture results were obtained. Inadequate treatment of the initial infection does not seem to explain the subsequent occurrence of the immune reconstitution syndrome.

The timing, clinical manifestations, and pathologic characteristics of these cases of cryptococcosis suggest that the clinical events were due to the immunologic changes associated with
successful HAART. The events occurred coincident with large declines in the HIV-1 load but not necessarily coincident with substantial increases in the CD4 count, a finding similar to that for patients with paradoxical worsening of tuberculosis, as described by Masahiro et al. [1].

Cryptococcosis in patients with AIDS is usually histopathologically associated with abundant yeast cell proliferation with a histiocytic response but without associated lymphocytes, neutrophils, or necrosis [4]. The presentations of patients 1 and 3 were unusual because of the associated necrosis; the presentation of patient 2 was unusual because of the extensive fibrosis; and that of patient 3 was unusual because of the marked neutrophilic inflammation.

Hilar and mediastinal lymphadenopathy can occur in the context of pulmonary cryptococcosis at presentation [5, 6]. However, the development of progressive hilar lymphadenopathy with hypercalcemia, despite antifungal treatment (as occurred in patient 1) has not been described previously, to our knowledge. One case study reported pulmonary cryptococcosis and coccidiodomycosis in which hypercalcemia developed while the patient was receiving antifungal treatment but only after antiretroviral therapy had been initiated [7]. Similarly, cavitary cryptococcal pneumonia occurs as a presenting manifestation of Cryptococcus infection, but it is infrequent [5, 6]. In patient 2, presumed pulmonary cryptococcal nodules progressed to histologically diagnosed cryptococcal cavitary infiltrates, despite antifungal therapy. Although MAC infection is frequently implicated in immune reconstitution events, the frequent inconsequential occurrence of MAC in sputum and the histologic findings for patient 2 argue against MAC as the etiologic agent of the cavitary pneumonia, despite the positive culture result. Focal masses due to C. neoformans, such as those that developed in patient 3, are described infrequently in case reports; typically, they are described as a presenting manifestation of cryptococcal infection.

HIV-associated aseptic meningitis occurs but is not typically associated with increased ICP. Of 14 patients with chronic HIV infection and unexplained CSF pleocytosis, only 1 had a CSF OP of >250 mm H2O [8]. The occurrence of a postcryptococcal aseptic meningitis syndrome with an elevated ICP after the initiation of HAART has been described in a case report [9].

A number of case reports have described unusual manifestations of C. neoformans infection occurring in patients receiving successful HAART. Blanche [10] described 2 patients who had previously had cryptococcal meningitis and developed culture-negative cryptococcal cervical lymphadenitis months after initiation of HAART. Lanzafame et al. [11] described 2 patients who developed fever, chest pain, and mediastinal lymphadenitis with histologic evidence of C. neoformans infection 6 months after the initiation of HAART. Manfredi et al. [12] described 2 patients who had variable degrees of immune reconstitution and multiple relapses of cryptococcal infection. The patient with better immune reconstitution developed multiple culture-negative lymph node lesions and subcutaneous abscesses with histologic evidence of C. neoformans infection but did not have fever or constitutional symptoms. Woods et al. [13] described 2 patients who developed cryptococcal meningitis soon after initiation of HAART, and they surmised that HAART provoked inflammatory symptoms of a preexisting but clinically silent cryptococcal infection.

Whether these unusual manifestations of Cryptococcus infection can be expected to occur frequently in patients who begin receiving HAART soon after Cryptococcus infection has been diagnosed remains to be determined. More-intensive antifungal therapy before HAART or a delay in initiating HAART after cryptococcosis has been diagnosed conceivably could prevent subsequent inflammatory reactions to C. neoformans. Further observations in patients who initiate HAART after cryptococcal infection has been diagnosed ought to define the frequency and spectrum of immune reconstitution cryptococcosis. The development of focal inflammatory lesions in patients after an initial episode of cryptococcal infection should prompt consideration of recent cryptococcal disease as a possible diagnosis even for patients who are taking antifungal prophylaxis and who are responding to HAART.

References