Paradoxical Inflammatory Reaction during Treatment of Cryptococcus neoformans var. gattii Meningitis in an HIV-Seronegative Woman

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A human immunodeficiency virus (HIV)–seronegative woman was admitted to the hospital with postpartum onset Cryptococcus neoformans var. gattii meningitis and markedly increased intracranial pressure. A poor initial response to antifungal therapy was followed, 2 months after hospital admission, by severe acute meningeal and cerebral inflammation and clearance of yeast cells from cerebrospinal fluid. This first reported case of paradoxical inflammatory reaction to C. neoformans illustrates important aspects of the host-pathogen interaction and highlights possible effects of immunomodulatory therapies.

Experimental studies indicate that capsular polysaccharides of Cryptococcus neoformans var. neoformans (CNVN) induce an immunosuppressive cytokine milieu [1]. This may constitute an important virulence factor, especially for patients who are already immunocompromised due to HIV infection or use of immunosuppressive drugs [2, 3]. There are recent reports of inflammatory reactions in HIV-seropositive patients with CNVN infection occurring as a result of immune restoration following effective antiretroviral therapy [4]. The immunomodulatory effects of Cryptococcus neoformans var. gattii (CNVG) have not been as clearly defined. CNVG is a geographically restricted fungal pathogen endemic in northern and central Australia and Papua New Guinea that usually causes disease in individuals with no overt immunodeficiency. The only consistently reported risk factors for CNVG infection are exposure to the eucalypt reser-

voirs of the organism and, in Australia, indigenous ethnic origin [5]. Here, we describe an HIV-seronegative patient with CNVG meningitis who developed severe CNS inflammation coincident with clearance of cryptococcal capsule from CSF 2 months after starting antifungal therapy. We believe that the clinical course can be explained by a paradoxical inflammatory reaction after reversal of CNVG-induced immunosuppression. This case raises important questions regarding the pathogenesis and therapy for cryptococcal meningitis.

Case report. An 18-year-old aboriginal woman from a remote desert community in central Australia was admitted to a regional hospital with general malaise and increasing headaches. Five days earlier, she had delivered a healthy full-term infant. Physical examination was performed at hospital admission and revealed a temperature of 37.8°C and mild neck stiffness. Lumbar puncture yielded clear CSF containing 6 mononuclear cells/μL. Numerous encapsulated yeasts were seen on india ink stains. She was initially treated with amphotericin B (0.5 mg/kg q.d.) plus oral flucytosine (25 mg/kg q6h). CNVG was subsequently identified in CSF cultures using standard laboratory methods. Susceptibility testing performed at a mycology reference laboratory (at Women’s & Children’s Hospital; Adelaide, Australia) revealed that the isolate was susceptible to amphotericin B (MIC, 0.125 μg/mL) and 5-fluorocytosine (MIC, 1.0 μg/mL) and had intermediate susceptibility to fluconazole (MIC, 16 μg/mL) and itraconazole (MIC, 0.25 μg/mL).

Seventeen days after presentation, the patient developed signs of increasing intracranial hypertension, including a reduced state of consciousness, severe bilateral papilledema (with retinal hemorrhage), and bilateral VI cranial nerve palsies. The patient was transferred to our hospital (Flinders Medical Centre; Bedford Park, South Australia) for further treatment. MRI of the brain performed at hospital admission showed non–contrast-enhancing lesions in the basal ganglia consistent with gelatinous pseudocysts due to cryptococcal infection but showed no meningeal enhancement (figure 1A and 1B). Lumbar puncture revealed a CSF opening pressure of >70 cm H2O, mild pleocytosis (2 polymorphonuclear cells/μL and 11 mononuclear cells/μL), and normal CSF glucose and protein concentrations. Numerous encapsulated yeasts were seen on india ink stains, and there was a scanty growth of CNVG. The results of tests for both serum and CSF cryptococcal antigen were negative at baseline and for all follow-up CSF samples, suggesting an unusual capsular composition. A prozone phenomenon was ex-
Figure 1. T1-weighted gadolinium-enhanced MRI scans of the brain performed at the time of hospital admission showing non-contrast-enhancing lesions in the basal ganglia in keeping with cryptococcal disease (A), with no meningeal enhancement (B). T1-weighted MRI scans of the brain performed on day 41 of hospitalization, ~8 weeks after commencement of antifungal therapy, show intense contrast enhancement of the basal ganglia lesions (C) and meninges (D).

cluded by predilution of samples and the use of pronase. The result of an HIV antibody test was negative.

Treatment with intravenous amphotericin B (0.7 mg/kg q.d.) was continued, along with oral flucytosine (50 mg/kg q.i.d.). Control of increased intracranial pressure was difficult, even with daily lumbar punctures and CSF drainages. A lumbar-peritoneal shunt was inserted and resulted in some improvement in the patient’s neurological condition. The shunt was removed on day 35 after admission to our hospital.

On day 39 of hospitalization, there was recurrent fever and worsening meningism, with deterioration of conscious state. Lumbar puncture revealed a CSF opening pressure of 41 cm H₂O and mild pleocytosis (6 polymorphonuclear cells/μL and 28 mononuclear cells/μL). Moderate numbers of encapsulated yeasts were seen, but culture remained negative for CNVG. The patient was treated empirically for possible shunt-related bacterial meningitis with cefepime and vancomycin. Cultures of CSF, blood, and urine samples obtained before administration of antibiotics were sterile, and the results of broad-range PCR (using primers for conserved sequences of bacterial ribosomal DNA) were negative. Template DNA for PCR was prepared from CSF specimens as described by Jaton et al. [6], and PCR was performed using...
previously published broad-range bacterial 16S rRNA gene primers and PCR conditions [7]. PCR products were stored at 4°C until analysis by 1% agarose gel electrophoresis and ethidium bromide staining under UV illumination.

A second MRI brain scan performed on day 41 of hospitalization revealed the development of intense contrast enhancement in the basal ganglia and the leptomeninges, particularly around the cerebellar folia (figure 1C and 1D). On day 46 of hospitalization, follow-up CSF examination revealed marked pleocytosis (400 polymorphonuclear cells/μL and 250 mononuclear cells/μL) and numerous encapsulated yeasts on India ink stain, but there was no fungal or bacterial growth. The CSF inflammatory infiltrate increased additionally to a peak level of 1550 WBCs/μL (74% polymorphonuclear cells) on day 53 and subsequently subsided (table 1). This was associated with clearance of encapsulated yeasts from CSF by day 56 of hospitalization and resolution of fever and meningism. Although the CSF pleocytosis was associated with the presence of significant numbers of erythrocytes (table 1), contamination by peripheral blood (in which the usual ratio of RBCs to WBCs is close to 1000) could account for only a small part of the neutrophil infiltrate observed.

The patient was transferred back to her local hospital, where her subsequent clinical course included the development of progressive bilateral optic atrophy and VI cranial nerve palsies, despite continued antifungal therapy and negative results of CSF India ink stains and cultures. She received a total dose of amphotericin B of 2.25 g over a period of 8 weeks, and therapy was subsequently switched to high-dose oral fluconazole to enable the patient to return to her community. Placement of a ventriculoperitoneal shunt was eventually required to control her persistently increased intracranial pressure, but at the most recent medical review, the patient was found to have had severe bilateral visual impairment, which is likely to be permanent.

### Methods

Aliquots of CSF were stored at −20°C until the day of analysis of cytokine concentrations. IL-10, IL-8, and TNF-α concentrations were measured by ELISA using samples obtained on days 3, 17, 39, 46, and 56 of hospitalization. Assays were performed in triplicate; mean cytokine concentrations are reported. The lower limits of detection of the assays for IL-10, IL-8, and TNF-α were 2 pg/mL, 20 pg/mL, and 10 pg/mL, respectively. IL-10 and IL-8 were undetectable in otherwise normal CSF specimens obtained from 2 control patients in whom a lumbar puncture had been performed to exclude subarachnoid hemorrhage. A low TNF-α concentration (69 pg/mL) was detectable in one control patient and undetectable in the second. The evolution of CSF cytokine concentrations in our patient is summarized in table 1.

### Discussion

We believe that this case provides an in vivo illustration of the immunomodulatory effects of *C. neoformans* infection. We observed prolonged cryptococcal persistence in CSF despite receipt of active antifungal therapy, followed by a severe paradoxical inflammatory reaction and microbial clearance. The cryptococcal capsule is an important virulence factor that directly inhibits phagocytosis and may induce a state of relative immunosuppression through the effects of such components as glucuronoxylomannan. The major capsular component of CNVN serotype A, for example, induces human PBMCs to secrete IL-10 [8], which suppresses the production of pro-inflammatory cytokines, such as TNF-α [9]. High CSF IL-10 concentrations, similar to those seen early in the course of our patient’s illness, have been found in immunosuppressed patients with CNVN meningitis [2, 3] and may inhibit the development of an effective host immune response. In murine models of cryptococcal infection, protective effects of Th1 type antigen-specific lymphocytes can be subverted by a shift to a Th2 cytokine response, including high levels of IL-10 production [1].

Polymorphonuclear cells are important effector cells that can

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**Table 1.** Cell counts and cytokine concentrations in consecutive CSF samples collected after admission to our hospital.

<table>
<thead>
<tr>
<th>Day of hospitalization</th>
<th>Cryptococcus numbers on India ink stain</th>
<th>Blood cell count, cells/μL</th>
<th>TNF-α level, pg/mL</th>
<th>IL-8 level, pg/mL</th>
<th>IL-10 level, pg/mL</th>
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<tbody>
<tr>
<td></td>
<td>Total WBC</td>
<td>RBC</td>
<td>PMN</td>
<td>MNC</td>
<td>IL-10</td>
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<td>3</td>
<td>+</td>
<td>13</td>
<td>29</td>
<td>2</td>
<td>11</td>
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<tr>
<td>17</td>
<td>+</td>
<td>72</td>
<td>108</td>
<td>21</td>
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<td>5</td>
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<td>46</td>
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<td>53</td>
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<td>1550</td>
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<td>1150</td>
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<tr>
<td>56</td>
<td>+/-</td>
<td>440</td>
<td>900</td>
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<td>-</td>
<td>180</td>
<td>150</td>
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</table>

**NOTE.** Cytokine values are shown as mean concentrations of triplicate measures. A scant growth of *Cryptococcus neoformans* was obtained from cultured CSF samples obtained on day 3 of hospitalization; subsequently, there was no growth from any sample. MNC, mononuclear cell; ND, not done; PMN, polymorphonuclear cell; UD, undetectable; −, absent; +/-, scanty; +, present; ++, abundant.
directly kill cryptococci [10]. The major endogenous neutrophil chemotactic factor IL-8 is produced by human microglial cells in vitro after exposure to glucuronoxylomannan from CNVN serotype A [11]. We found high CSF concentrations of IL-8 with minimal polymorphonuclear cell pleocytosis early in the patient’s course, an observation that has previously been reported in HIV-infected patients with cryptococcal meningitis [2]. Glucuronoxylomannan from CNVPN serotype A can inhibit polymorphonuclear cell migration by binding or inducing shedding of key polymorphonuclear cell adhesion molecules involved in the normal chemotactic response to inflammatory stimuli [1, 12]. There is evidence that CNVG capsular components may impair neutrophil migration to an even greater extent [13], which would be expected to enhance cryptococcal persistence. In the patient we describe, cryptococcal antigen was undetectable in CSF and serum, a phenomenon which has been observed in up to 5% of patients in published studies of cryptococcal disease [4]. The lack of detectable antigen implies a variant capsular structure, the pathogenic significance of which is unclear in this case.

After nearly 2 months of antifungal therapy, our patient developed clinical and radiological signs of severe meningeal and cerebral inflammation (figure 1C and 1D), without evidence of bacterial infection. Although some of the clinical deterioration may have been attributable to shunt removal 4 days earlier, the clinical presentation suggested a paradoxical inflammatory reaction similar to those described in association with human mycobacterial infections. The MRI changes of intense contrast enhancement similar to those described in association with human mycobacterial infections. The MRI changes of intense contrast enhancement similar to those described in association with human mycobacterial infections. The MRI changes of intense contrast enhancement similar to those described in association with human mycobacterial infections. The MRI changes of intense contrast enhancement similar to those described in association with human mycobacterial infections. The MRI changes of intense contrast enhancement similar to those described in association with human mycobacterial infections. The MRI changes of intense contrast enhancement similar to those described in association with human mycobacterial infections. The MRI changes of intense contrast enhancement similar to those described in association with human mycobacterial infections. The MRI changes of intense contrast enhancement similar to those described in association with human mycobacterial infections. The MRI changes of intense contrast enhancement similar to those described in association with human mycobacterial infections.

in vitro after exposure to glucuronoxylomannan from CNVN has been uncovered [16] or in whom severe inflammatory reactions have occurred [5] after receipt of antiretroviral therapy and immune reconstitution. To our knowledge, similar inflammatory reactions have not previously been reported in HIV-seronegative patients. The occurrence of paradoxical inflammatory reactions, such as those described here during the course of treatment of cryptococcal meningitis, may be an underrecognized complication that could contribute significantly to development of neurological sequelae. It is possible that the use of immune-stimulating cytokines as adjunctive therapy for cryptococcal disease, as has been proposed for some patients [17], could increase the risk of such reactions. Recognition of these reactions as a shift in immune responsiveness rather than as treatment failure is important to avoid inappropriate changes in antimicrobial therapy [15]. Short-term treatment with corticosteroids or other anti-inflammatory agents may warrant consideration in severe cases.

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Conflict of interest. All authors: no conflict.

References

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