An Immune Reconstitution Syndrome–Like Illness Associated with *Cryptococcus neoformans* Infection in Organ Transplant Recipients


**Background.** We describe an immune reconstitution syndrome (IRS)–like entity in the course of evolution of *Cryptococcus neoformans* infection in organ transplant recipients.

**Methods.** The study population comprised a cohort of 83 consecutive organ transplant recipients with cryptococcosis who were observed for a median of 2 years in an international, multicenter study.

**Results.** In 4 (4.8%) of the 83 patients, an IRS-like entity was observed a median of 5.5 weeks after the initiation of appropriate antifungal therapy. Worsening of clinical manifestations was documented, despite cultures being negative for *C. neoformans*. These patients were significantly more likely to have received tacrolimus, mycophenolate mofetil, and prednisone as the regimen of immunosuppressive therapy than were all other patients (*P* = .007). The proposed basis of this phenomenon is reversal of a predominantly Th2 response at the onset of infection to a Th1 proinflammatory response as a result of receipt of effective antifungal therapy and a reduction in or cessation of immunosuppressive therapy.

**Conclusions.** This study demonstrated that an IRS-like entity occurs in organ transplant recipients with *C. neoformans* infection. Furthermore, this entity may be misconstrued as a failure of therapy. Immunomodulatory agents may have a role as adjunctive therapy in such cases.

*Cryptococcus neoformans* infection has been documented in 1%–5% of transplant recipients [1–3]. Mortality rates among organ transplant recipients with cryptococcosis are in the range of 20%–42% [3]. In a study involving transplant recipients with cryptococcosis, we identified a subset of patients in whom worsening of symptoms or signs occurred after an initial response, despite ongoing receipt of appropriate antifungal therapy and cultures that were negative for *C. neoformans*. Description of this phenomenon exists in the literature on HIV infection, in which immune restoration after effective antiretroviral therapy has been associated with an inflammatory response and exacerbation of clinical manifestations [4–11]. This syndrome, known as “immune reconstitution syndrome” (IRS), is believed to result from enhanced—but partially reconstituted—pathogen-specific, cell-mediated immunity and induction of proinflammatory cytokines, leading to an exaggerated inflammatory reaction [5, 6, 11, 12]. We describe an IRS-like entity in transplant recipients with *C. neoformans* infection and propose its pathophysiologic basis.
METHODS

The patients were 83 organ transplant recipients with cryptococcosis who were identified at 22 transplantation centers in 5 countries. *C. neoformans* infection was defined as a positive result of a culture of a clinical specimen, histopathologic or cytopathologic examination of needle aspiration or biopsy specimens showing yeast cells, or a finding of cryptococcal antigen in blood or CSF specimens obtained from a patient with compatible clinical presentation [13]. Disseminated infection was defined as CNS infection or fungemia or ≥2 sites of involvement. The median duration of follow-up for the patients was 2 years (range, 30 days to 5 years).

Patients with an IRS-like entity were defined on the basis of criteria proposed for IRS in HIV-infected patients: (1) reappearance or worsening of previous manifestations after an initial response or appearance of new manifestations consistent with an infectious and/or inflammatory process, despite receipt of appropriate therapy; and (2) symptoms could not be explained by a newly acquired infection, by the expected clinical course of a previously recognized infectious agent, or by the adverse effects of therapy [14, 15].

Statistical analysis was performed using Prophet Statistics, version 6.0 (AbTech). Fisher’s exact test was used to compare categorical variables, and the Mann-Whitney *U* test was used to compare times to onset of infection.

RESULTS

Case Reports

Four organ transplant recipients had an IRS-like entity. **Patient 1.** A 65-year-old man underwent renal transplantation for adult polycystic kidney disease. Three months after transplantation, myositis in the right forearm developed; culture of muscle biopsy and blood specimens yielded *C. neoformans*. The serum cryptococcal antigen titer was 1:1024. The immunosuppressive therapy that he was receiving at the time comprised tacrolimus, MMF, and prednisone (20 mg q.d.). At the time of diagnosis, the dosage of MMF was gradually reduced and treatment with MF was discontinued, the prednisone dosage was reduced to 15 mg every other day, and the tacrolimus dosage was reduced from 7 mg b.i.d. to 2–3 mg b.i.d. ABLC was administered for 21 days, and ABLC therapy was then switched to fluconazole (400 mg daily). Three months later, the patient presented with worsening myonecrosis. The serum antigen titer, which had become negative, was now 1:256. Cultures of muscle tissue and blood specimens were repeatedly negative for *C. neoformans*. Despite receipt of treatment with liposomal amphotericin B (LAMB), the patient’s symptoms persisted, ultimately leading to amputation of the right forearm. The patient continues to receive fluconazole (200 mg q.d.) and was healthy as of 26 months after infection.

**Patient 2.** A 63-year-old male patient underwent renal transplantation for adult polycystic kidney disease. Three months after transplantation, myositis in the right forearm developed; culture of muscle biopsy and blood specimens yielded *C. neoformans*. The serum cryptococcal antigen titer was 1:2048. CSF analysis revealed 9 WBCs and a cryptococcal antigen titer of 1:2048. MMF therapy was discontinued, and treatment with ABLC was initiated, and 5-flucytosine therapy was then switched to fluconazole (400 mg daily). Three months later, the patient presented with worsening myonecrosis. The serum antigen titer, which had become negative, was now 1:256. Cultures of muscle tissue and blood specimens were repeatedly negative for *C. neoformans*. Despite receipt of treatment with liposomal amphotericin B (LAMB), the patient’s symptoms persisted, ultimately leading to amputation of the right forearm. The patient continues to receive fluconazole (200 mg q.d.) and was healthy as of 26 months after infection.

**Patient 3.** A 63-year-old man underwent renal transplantation for hypertensive nephropathy. His immunosuppressive regimen consisted of tacrolimus, MMF, and prednisone (10 mg q.d.). Twelve months after transplantation, nodular pulmonary lesions were documented. Bronchoalveolar lavage, CSF, and blood cultures yielded *C. neoformans*. The serum cryptococcal antigen titer was 1:2048. CSF analysis revealed 9 WBCs and a cryptococcal antigen titer of 1:1024. MMF therapy was discontinued, and the prednisone dosage was decreased. The dosage of tacrolimus was reduced from 4 mg b.i.d. to 2 mg b.i.d., and it was then reduced to 0.5 mg b.i.d. 10 days later. ABLC and 5-flucytosine were administered for 6 weeks and were then switched to fluconazole (200 mg po q.d.). He had been receiving fluconazole for only 2 days when he began experiencing fever, headache, nausea, and vomiting. CSF analysis now revealed 107 WBCs. However, all culture results were negative. LAMB therapy was initiated, and 5-flucytosine therapy was resumed. A protracted illness ensued, during which the allograft was lost; the patient died 5 months after his initial infection. Cryptococcal antigen titers ranged between 1:256 and 1:1024.
Table 1. Clinical and demographic characteristics of transplant recipients with and without an immune reconstitution syndrome (IRS)-like entity.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Patients with an IRS-like entity (n = 4)</th>
<th>Patients without an IRS-like entity (n = 79)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type of transplant</td>
<td></td>
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<tr>
<td>Kidney</td>
<td>2 (50)</td>
<td>40 (50)</td>
</tr>
<tr>
<td>Liver</td>
<td>1 (25)</td>
<td>21 (26)</td>
</tr>
<tr>
<td>Heart</td>
<td>0 (0)</td>
<td>8 (10)</td>
</tr>
<tr>
<td>Lung</td>
<td>0 (0)</td>
<td>6 (8)</td>
</tr>
<tr>
<td>Kidney-pancreas</td>
<td>1 (25)</td>
<td>2 (3)</td>
</tr>
<tr>
<td>Small bowel</td>
<td>0 (0)</td>
<td>2 (3)</td>
</tr>
<tr>
<td>Time to onset of Cryptococcus neoformans infection after transplantation, median (range)</td>
<td>10.5 mo. (3–29 mo.)</td>
<td>25 mo. (1 mo.–15 y)</td>
</tr>
<tr>
<td>Immunosuppressive regimen of tacrolimus, MMF, and prednisone</td>
<td>4 (100)</td>
<td>21 (27)</td>
</tr>
<tr>
<td>Allograft rejection</td>
<td>2 (50)</td>
<td>20 (25)</td>
</tr>
<tr>
<td>CMV infection</td>
<td>0 (0)</td>
<td>22 (28)</td>
</tr>
<tr>
<td>Initial site or type of involvement</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pulmonary</td>
<td>2 (50)</td>
<td>42 (53)</td>
</tr>
<tr>
<td>Skin or soft tissue</td>
<td>2 (50)</td>
<td>20 (25)</td>
</tr>
<tr>
<td>CNS</td>
<td>2 (50)</td>
<td>41 (53)</td>
</tr>
<tr>
<td>Disseminated infectiona</td>
<td>4 (100)</td>
<td>49 (62)</td>
</tr>
<tr>
<td>Initial antifungal therapy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lipid formulation of AmBb</td>
<td>3 (75)</td>
<td>36 (46)</td>
</tr>
<tr>
<td>AmBc</td>
<td>1 (25)</td>
<td>17 (22)</td>
</tr>
<tr>
<td>Fluconazole</td>
<td>0 (0)</td>
<td>23 (29)</td>
</tr>
<tr>
<td>Other</td>
<td>0 (0)</td>
<td>2 (3)</td>
</tr>
<tr>
<td>Survival 12 months after infection</td>
<td>3 (75)</td>
<td>54 (77)c</td>
</tr>
</tbody>
</table>

NOTE. Data are no. (%) of patients, unless otherwise indicated. AmB, amphotericin B deoxycholate; CMV, cytomegalovirus; MMF, mycophenolate mofetil; mo., month(s); y, year(s).

a Disseminated infection was defined as CNS infection and/or fungemia with ≥2 sites of involvement.
b Two of 3 patients in the IRS-like entity group and 16 of 36 in the non–IRS-like entity group also received flucytosine.
c One of 4 patients in the IRS-like entity group and 4 of 17 in the non–IRS-like entity group also received flucytosine with AmB.
d Outcome is shown for 70 patients who had reached 12 months of follow-up.

Patient 4. A 34-year-old woman underwent kidney-pancreas transplantation for diabetic nephropathy. The patient presented with headaches 29 months after transplantation. Her immunosuppressive regimen at the time consisted of tacrolimus, MMF, and prednisone (7.5 mg q.d.). A CT scan of the chest revealed bilateral pulmonary nodules and left lower lobe consolidation. CSF samples yielded *C. neoformans* on culture, CSF analysis revealed 26 WBCs, and the antigen titer was 1:408. The serum cryptococcal antigen titer was 1:2048. LAMB and flucytosine were prescribed, and therapy was continued for 24 days. The serum creatinine level increased from a baseline level of 1.2–1.5 mg/dL to 2.5–3.1 mg/dL during this period. MMF therapy (500 mg q.i.d.) was discontinued briefly and then resumed at a dosage of 500 mg b.i.d. The prednisone dose was tapered, and the tacrolimus dose was modified such that, by day 7, the patient was receiving 2.5 mg of prednisone q.d. and 1 mg of tacrolimus b.i.d. CSF culture results were negative 13 days after initiation of antifungal therapy, and the CSF antigen titer decreased to 1:64.

Thirty-days after employment of the initial antifungal therapy regimen, while she was receiving fluconazole (400 mg q.d.), the patient presented with worsening neurological symptoms and obtundation. CSF analysis revealed 60 WBCs, the CSF antigen titer was 1:128, and the serum titer was 1:8000.
Figure 1. Proposed basis for immune reconstitution syndrome (IRS)-like entity in organ transplant recipients. *Cryptococcus neoformans* infection and receipt of immunosuppressive agents leads to a predominantly Th2 response. Receipt of antifungal therapy and reduction of immunosuppressive agents causes reversal of Th2 to a Th1 cytokine profile and a proinflammatory response that may have led to IRS.

ink stains and cultures of CSF specimens yielded negative results. LAMB therapy was resumed and continued for 12 weeks. The patient was alive as of 3 years after infection.

**Results**

Overall, 4 (100%) of the 4 patients with an IRS-like entity were receiving tacrolimus, MMF, and prednisone as the immunosuppressive treatment regimen, compared with 21 (26.6%) of 79 other patients (*P* = 0.007). Case patients and all other patients did not differ significantly with regard to other variables assessed (table 1).

**DISCUSSION**

Host defense against cryptococcal infections is critically regulated by cell-mediated immunity, of which T helper (Th) CD4⁺ cells are the major effector cells [16, 17]. A Th1 response (characterized by proinflammatory cytokines—for example, IFN-γ) and TNF-α are protective against *C. neoformans*, whereas a Th2 response with the induction of IL-10 is associated with disease progression [16, 18–20]. Capsular polysaccharide of *C. neoformans* has been shown to stimulate IL-10 production and inhibit TNF-α and IL-1β secretion by human monocytes [21]. High IL-10 levels have been observed during cryptococcal infection [22]. Receipt of antifungal therapy was associated with a rebound in Th1 inflammatory response that led to exacerbation of clinical manifestations, but that facilitated the eradication of infection [23]. In HIV-infected patients with cryptoccocosis, the levels of soluble TNF receptor II, an anti-inflammatory cytokine, decreased in response to antifungal therapy [24]. In 2 patients with idiopathic CD4⁺ lymphopenia, defective production of IFN-γ and TNF-α—but not of IL-10—was documented [25]. One of these 2 patients with disease progression (despite receipt of antifungal treatment) was administered recombinant IFN-γ, which resulted in restoration of immunological parameters and a sustained clinical recovery [25].

IRS occurred in 8.3% of the HIV-infected patients with *C. neoformans* infection and correlated with more-profound immunosuppression (i.e., a lower CD4⁺ cell count at the onset of infection) [26]. An IRS-like entity was observed in 4.8% of the organ transplant recipients with cryptoccocosis who we describe. Patients who received a combination of tacrolimus, MMF, and prednisone were more likely to develop this syndrome than were all other patients. Immunosuppressive agents, such as tacrolimus, suppress the production of cytokines stimulated by Th1 cells (e.g., IL-2 and IFN-γ), compared with those produced by Th2 cells [27]. Tacrolimus causes significantly greater suppression of production of IFN-γ than cyclosporine A [27]. Corticosteroids, although less potent inhibitors, are also associated with a decrease in IL-2, IL-12, and IFN-γ levels and an increase in IL-10 levels [28, 29]. After a reduction in or
cessation of immunosuppressive therapy, a relative increase in Th1 response may have been greater in transplant patients receiving more-potent immunosuppression and could have accounted for the occurrence of IRS exclusively in these patients.

Of note is the development of lymphadenitis and granulomas in the lymph node and lung in patient 1. Such granulomas have been described in HIV-infected patients with IRS due to not only C. neoformans, but to other antigens as well [7, 30]. Granulomatous responses are strongly associated with containment and resolution of infection in humans as well as in laboratory animals [17, 31]. Although cryptococci may be visualized histopathologically, a consistent finding in IRS lesions in HIV-infected patients has been cultures that are negative for C. neoformans [7, 9, 10]. C. neoformans was not cultured in the 4 cases we describe. However, an increase in the cryptococcal antigen titer was observed, the basis for which is not known. Because tacrolimus has antifungal activity in vitro against C. neoformans, it is plausible that a reduction in the tacrolimus dose may have, in fact, led to the worsening of infection and an increase in the antigen titer [32, 33].

In summary, we have described an IRS-like entity during the evolution of cryptococcal infection in organ transplant recipients. Features suggestive of an inflammatory or an immune response were observed (i.e., granula formation, CSF inflammation, and enhancing CNS lesions in the absence of cultures positive for C. neoformans). There was no other infection or alternative diagnosis to account for these manifestations. The proposed biological basis for this entity (figure 1) is based largely on animal studies and should be validated by data from studies of transplant recipients.

Our study has implications relevant for the management of cryptococcosis in transplant recipients. An exacerbation of symptoms and signs due to evolving host response in IRS may be misconstrued as failure of therapy or as a “relapse.” The inflammatory reaction is deemed to be beneficial in the eradication of infection [34]. However, the induction of endogenous response may be suboptimal. This suggests a role for immunomodulatory therapies targeted toward augmenting cellular immunity, such as neutralization of suppressive cytokines, enhancement of Th1 responses with IFN-γ, and transfer of adoptive cellular immunotherapy, as has been attempted for Aspergillus infection [34, 35]. An overzealous or heightened inflammatory response, on the other hand, may be life-threatening [5] and could potentially be worsened by the exogenous administration of proinflammatory cytokines, such as IFN-γ. A potential concern with IFN-γ also is the risk of exacerbation of allograft rejection. One might speculate whether the administration of IFN-γ guided by the measurement of serum levels may be a logical approach for achieving the desired balance and optimal outcome.

CRYPTOCOCCAL COLLABORATIVE TRANSPLANT STUDY GROUP

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