Resolution of Rhinocerebral Zygomycosis Associated with Adjuvant Administration of Granulocyte-Macrophage Colony-Stimulating Factor

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We successfully treated 3 consecutive patients who had non-neutropenic rhinocerebral zygomycosis, by use of subcutaneous granulocyte-macrophage colony-stimulating factor therapy combined with traditional surgical and medical treatment. All patients are currently free of disease. Granulocyte-macrophage colony-stimulating factor should be considered as adjuvant therapy for rhinocerebral zygomycosis; however, optimum dose and length of therapy are unknown.

Rhinocerebral zygomycosis (also known as “mucormycosis”) is an infection that is caused by fungi of the class Zygomycetes, order Mucorales, which includes the genera Rhizopus, Mucor, and Absidia. These organisms are found in the soil and are important in the decay of organic material. In recent years, they have become more frequent in number and have become an important cause of morbidity and mortality as a result of the increasing number of immunocompromised patients.

Risk factors for zygomycosis include severe neutropenia, diabetes mellitus (with or without ketoacidosis), malnutrition, organ transplantation, and therapy with corticosteroids or deferoxamine. Acidosis and hyperglycemia provide an ideal environment for the growth of Zygomycetes, because such metabolic conditions inhibit the affinity and effectiveness of macrophages and thereby alter host defense mechanisms. Symptoms of the rhinocerebral form of zygomycosis include headache; rhinorrhea; and black, necrotic, intranasal or introral masses. Progression of the disease leads to orbital cellulitis, cavernous sinus syndrome, and even CNS involvement.

In tissue, Zygomycetes are thin-walled, nonseptated hyphae with branching at right angles (figure 1). Rhinocerebral zygomycosis is identified by fungal invasion along the elastic lamina of the blood vessels with subsequent thrombosis and tissue necrosis. Even when aggressive therapy with amphotericin B (AmB), hyperbaric oxygen, and aggressive surgical debridement is used, the mortality rate among patients without neutropenia is >50% for those with rhinocerebral mucormycosis and >90% for those with disseminated infections. The disease is usually fatal in patients with neutropenia; however, granulocyte colony-stimulating factor (G-CSF) recently has been reported to be useful as adjuvant therapy with AmB [1–4]. New therapeutic agents and novel approaches are needed to treat these fungal infections more effectively.

Granulocyte-macrophage colony-stimulating factor (GM-CSF) is a hematopoietic growth factor that stimulates proliferation and differentiation of hematopoietic progenitor cells. This human recombinant protein has been produced in 3 different cell types, including Saccharomyces cerevisiae, Escherichia coli, and mammalian cells. Sargramostim (Leukine; Immunex), which is derived from yeast, is the only form of synthetic recombinant human GM-CSF that is commercially available in the United States. It increases the production of granulocytes, macrophages, dendritic cells, Langerhans’ cells, eosinophils, and...
megakaryocytes. It also acts synergistically with erythropoietin to induce proliferation of RBC precursors.

GM-CSF has been approved by the US Food and Drug Administration for the following indications: (1) to accelerate the time to neutrophil recovery and to reduce severe and life-threatening infections when given after induction chemotherapy to patients with acute myelogenous leukemia who are older than 55 years; (2) to mobilize peripheral blood stem cells and to further accelerate myeloid reconstitution after transplantation of peripheral blood progenitor cells; (3) to promote hematopoietic reconstitution after autologous or allogeneic bone marrow transplantation in patients with acute lymphocytic leukemia, non-Hodgkin’s lymphoma, or Hodgkin’s disease; and (4) to promote myeloid recovery in cases of failure or delay of engraftment after autologous and allogeneic bone marrow transplantation.

GM-CSF also enhances the antimicrobial function of mature neutrophils and monocytes against bacterial and fungal targets. Evidence of antifungal activity has been shown in vitro by enhancement of the phagocytic activity of neutrophils against Candida albicans [5] and Torulopsis glabrata [6]. Enhancement of the antifungal activity of monocytes has been shown in vitro against C. albicans [7], Cryptococcus neoformans [8], Histoplasma capsulatum [9], and Aspergillus fumigatus [10]. GM-CSF has also been shown to reverse the steroid-induced inhibition of fungicidal activity of macrophages against A. fumigatus [11, 12]. This antifungal activity has also been demonstrated in animal models. Administration of GM-CSF to malnourished mice who previously had been inoculated with C. albicans has resulted in improved survival [13]. GM-CSF therapy has also reduced lung injury and mortality in rats who have neutropenia and otherwise lethal candidiasis [14].

In a randomized, placebo-controlled clinical trial that involved elderly patients who were undergoing chemotherapy for acute myeloid leukemia, GM-CSF reduced the incidence of death associated with fungal infections from 19.1% to 1.9% (P = .006) [15]. The exact mechanism by which GM-CSF exerts antifungal activity has not been defined, but it has been widely studied.

To our knowledge, no cases of neutropenia- or nonneutropenia-associated rhinocerebral zygomycosis that have been treated with GM-CSF have been reported. At least 8 patients (6 patients with neutropenia and 2 with diabetes) who had rhinocerebral as well as disseminated disease have been successfully treated with a combination of surgery, AmB, and G-CSF [1–4]. Resolution of neutropenia was an important factor in patient survival in these reports. Therefore, we hypothesized that enhancement of the neutrophil function and stimulation of the monocyte-macrophage component of the immune system against Zygomycetes would improve the chance of survival for patients with rhinocerebral zygomycosis. We describe 3 consecutive patients with rhinocerebral zygomycosis who received GM-CSF from 1994 through 1997.

Patient 1. A 51-year-old woman who had a history of diabetes and a 2-week history of sinusitis presented with pain on the left side of the face, periorbital swelling, erythema, and blurred vision of 3 days’ duration. Findings on physical examination indicated that the left orbit was swollen and erythematous (figure 2, left). The patient was unable to move her left eye; her left pupil was dilated and unresponsive to light, and a black nasal discharge was noticed. For 3 months, she had been treated with a combination of iv, oral, and inhaled steroids for asthmatic bronchitis. Her WBC count at the time of admission to the hospital was 23,600 cells/mm³. Intranasal ethmoidectomy with medial maxillectomy was performed on 28 September 1994. AmB therapy was administered in a dosage of 0.7 mg/kg/day. Cultures of samples obtained from the maxillary and ethmoid sinuses yielded Rhizopus species. Despite receiving aggressive treatment, the patient was febrile and continued to complain of increasing left-side orbital discomfort.

On 27 October 1994, a CT scan showed extensive bony sequestrum of the left maxilla and palate with obvious worsening of the disease. Therefore, the patient immediately underwent left orbital exenteration, left ethmoidectomy, partial maxillectomy, and bilateral sphenoidectomy. Culture of a tissue biopsy specimen revealed invasive mycelia, which again yielded Rhizopus species. At this time, GM-CSF was added to the treatment in an attempt to stimulate the patient’s own host defenses. Her WBC count was followed closely and was between 8000 cells/mm³ and 10,000 cells/mm³ before the institution of GM-CSF. An increase in the WBC count to 24,000 cells/mm³ was seen 48 h after administration of the first dose, and it remained in the range of 11,000–17,000 cells/mm³ thereafter. The patient had clinical improvement, and follow-up MRI showed resolution of the disease. She received a total of 4500 μg of sc GM-CSF during a period of 19 days and a total dose of 3 g of AmB. She continues to do well 4 years after discharge from the hospital (figure 2, right).

Patient 2. A 65-year-old man with a medical history of type 2 diabetes mellitus, asthmatic bronchitis that required therapy with steroids, and chronic sinusitis presented with a 2-week history of increasing right-side maxillary pain. He underwent a Caldwell-Luc procedure on 21 November 1995 and was found to have osteomyelitis of the right posterior maxilla. Histopathological examination of the maxillary bone showed necrosis and fungal hyphae that were morphologically compatible with zygomycosis. He began receiving AmB, 1 mg/kg/day. However, 1 week after initiation of treatment, a follow-up CT scan of the sinuses showed progression of the disease within the right maxillary sinus (figure 3).

The patient underwent extensive debridement of the necrotic areas and right medial maxillectomy (figure 4). His creatinine
level increased to 3.3 mg/mL, and treatment with AmB lipid complex (ABLC), 5 mg/kg/day, was initiated along with sc GM-CSF, 425 µg/day. His initial WBC count was 6600 cells/mm³, and 48 h after initiation of GM-CSF therapy, it swiftly increased to 11,900–13,800 cells/mm³ but then quickly returned to 6000–7000 cells/mm³. The patient had clinical improvement and recovery of renal function.

On 29 December 1995, a CT scan of the sinuses showed only mild mucosal thickening in the left maxillary sinus but no evidence of osteomyelitis. A biopsy specimen of the right maxillary sinus revealed chronic sinusitis, and no evidence of fungi was identified on fungal stains. Treatment with ABLC and GM-CSF was discontinued. The patient is doing well 3 years after discharge.

Patient 3. On 12 June 1996, a 52-year-old woman with insulin-dependent diabetes mellitus in ketoacidosis presented with right eye pain of 24 hours’ duration. Her initial WBC count was 28,900 cells/mm³. A CT scan of the orbits and sinuses revealed pansinusitis with the right maxillary sinus completely opaque and with no evidence of bone involvement. The patient underwent right anterior and posterior ethmoidectomy plus removal of the mucous membranes from the right ethmoid and maxillary sinuses. Histopathological examination demonstrated extensive necrosis and large, nonseptated fungal hyphae with intravascular forms that were consistent with zygomycosis. She began receiving ABLC, 5 mg/kg/day, and sc GM-CSF, 250 µg/day. Her WBC count remained mostly elevated (13,000–20,000 cells/mm³) while she received treatment with GM-CSF.

On 26 June 1996, it was noted that the patient continued to...
Figure 4. Patient 2. Top, Close-up view of the maxillary sinus showing the necrotic material. Bottom, Excised necrotic material.

have periorbital edema and low-grade fever. An MRI scan revealed osteomyelitis of the inferior aspect of the right orbit. She underwent inferior orbitotomy with excision of multiple areas of necrotic tissue from the inferior orbit. Histopathological examination showed necrosis and inflammation that were again consistent with zygomycosis.

The patient’s condition improved, as did the inflammatory process, as shown by a follow-up MRI scan of the orbit and sinuses. On 20 November 1996, the patient was asymptomatic, and biopsy specimens of the right maxillary sinus and orbit revealed no fungal elements; therefore, treatment with ABLC and GM-CSF was discontinued. The patient had received a total dose of 45,000 μg of GM-CSF and 53 g of ABLC. She showed no evidence of recurrent infection after 2 years of follow-up.

Results. The age range of the patients was 51–65 years. The patients presented with facial pain, and the sinuses were the main site of involvement. On the basis of histopathological evidence and culture (patient 1) of specimens collected from the sinuses, all patients were given a diagnosis of infection due to Zygomycetes. All patients were treated with prolonged administration of sc GM-CSF; aggressive surgical debridement; and AmB, ABLC, or both (table 1). All the patients survived.

GM-CSF, instead of G-CSF, was selected to investigate the hypothesis that enhancement of the intracellular fungal killing activity of the monocyte-macrophage component of the immune system by use of GM-CSF would lead to improved survival, especially among these 3 patients, who did not have neutropenia at the time of presentation. The dose of GM-CSF that was used was based on the US Food and Drug Administration–approved dosage for its current indications. A patient was considered cured only after all clinical, radiologic, and laboratory evidence of infection had disappeared.

GM-CSF therapy caused no significant side effects in any of our patients. The polymorphonuclear leukocyte count did not exceed 20,000 cells/mm³. We did not observe any adverse events, such as dyspnea, malaise, nausea, fever, rash, sinus tachycardia, headache, or chills. Some abnormalities were seen in evaluations of serum chemistry. However, all patients were seriously ill, and other factors, including AmB, could have contributed to these abnormalities.

Discussion. Rhinocerebral zygomycosis is a life-threatening disease that requires prompt intervention. Such intervention involves multispecialty care that includes consultations with experts in the fields of otorhinolaryngology, infectious diseases, ophthalmology, and even neurosurgery when brain involvement is encountered.

Table 1. Summary of the clinical characteristics, treatment, and outcome for 3 patients with nonneutropenic rhinocerebral zygomycosis.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age, years</th>
<th>Sex</th>
<th>Underlying conditions</th>
<th>Steroid therapy</th>
<th>Diagnosis</th>
<th>Treatment</th>
<th>Outcome, status at follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>51</td>
<td>F</td>
<td>Bronchial asthma, diabetes</td>
<td>Yes</td>
<td>Histopathological, culture</td>
<td>Surgery; AmB, 3 g; GM-CSF, 4500 μg</td>
<td>Cure, no recurrence at 4 years</td>
</tr>
<tr>
<td>2</td>
<td>65</td>
<td>M</td>
<td>Bronchial asthma, diabetes</td>
<td>Yes</td>
<td>Histopathological</td>
<td>Surgery; ABLC, 22 g; GM-CSF, 6000 μg</td>
<td>Cure, no recurrence at 3 years</td>
</tr>
<tr>
<td>3</td>
<td>52</td>
<td>F</td>
<td>Diabetes, ketoacidosis</td>
<td>No</td>
<td>Histopathological</td>
<td>Surgery; ABLC, 53 g; GM-CSF, 45,000 μg</td>
<td>Cure, no recurrence at 2 years</td>
</tr>
</tbody>
</table>

NOTE. ABLC, amphotericin B lipid complex (Abelcet; Liposome); AmB, amphotericin B; GM-CSF, granulocyte-macrophage colony-stimulating factor (Sargramostim [Leukine]; Immunex).
It is important to identify risk factors for rhinocerebral zygomycosis in these patients. Two of the patients who we studied had bronchial asthma as well as glucocorticoid use (both systemic and inhaled glucocorticoids). Three patients had diabetes mellitus. These risk factors have been widely cited in the literature, and the presence of such conditions/factors in patients should make the physician suspect this diagnosis. Diagnosis of rhinocerebral zygomycosis is usually based on clinical findings and histopathological evidence, because the organism that is involved is not easily grown in culture.

Treatment should consist of extensive and prompt surgical intervention as well as close monitoring, because many patients (including the patients described in the present report) require multiple surgical interventions. Drug therapy should consist of either AmB administered in high dosages of 1–1.2 mg/kg/day or administration of one of the new lipid formulations of AmB in a dosage of 5–7.5 mg/kg/day.

We believe that GM-CSF should also be considered as adjuvant treatment for patients with rhinocerebral zygomycosis. Our patients tolerated up to 450 µg of GM-CSF per day and a maximum cumulative dose of 45,000 µg without difficulty. At the present time, on the basis of unpublished data from Immunex, we would suggest sc administration of GM-CSF, 250 µg given 3 times per week, for patients without neutropenia.

With the increase in the number of immunosuppressed patients, we should expect to see more patients who have zygomycosis. Treatment of the 3 patients described in the present study illustrates a novel therapeutic approach to a difficult-to-treat and usually fatal disease. To our knowledge, this is the first report of patients with rhinocerebral zygomycosis that has been treated with adjuvant GM-CSF. The optimal dose and length of therapy are unknown. More studies are needed to elucidate the full potential of GM-CSF therapy and to corroborate our findings in patients with life-threatening zygomycosis, but these initial results are encouraging.

References


