Cytomegalovirus Ventriculoencephalitis in a Bone Marrow Transplant Recipient Receiving Antiviral Maintenance: Clinical and Molecular Evidence of Drug Resistance

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We describe a case of CMV ventriculoencephalitis in a severely immunocompromised bone marrow transplant recipient who was receiving combination therapy with ganciclovir and foscarnet for treatment of viremia and retinitis. Analysis of sequential viral isolates recovered from the patient’s cerebrospinal fluid suggested that disease developed because of the presence of viral resistance and, possibly, low tissue penetration of antiviral agents.

Cytomegalovirus (CMV) ventriculoencephalitis, which often occurs during therapy for CMV retinitis, has been well described in patients with advanced HIV infection, but it is rarely recognized in other patient groups [1]. In this report, we describe a patient who had prolonged T cell immunodeficiency after undergoing bone marrow transplantation (BMT) and who developed CMV ventriculoencephalitis while taking maintenance ganciclovir and foscarnet treatment for CMV viremia and retinitis.

A 6-year-old Peruvian girl with acute lymphoblastic leukemia in third remission received a T cell–depleted combined bone marrow transplant and peripheral blood stem cell transplant from her 4:6 human leukocyte antigen (HLA)–matched mother after cytoreduction with total body irradiation, thiotepa, and cyclophosphamide. Antithymocyte globulin and corticosteroids were administered after the transplant for the prevention of graft rejection. Both patient and donor were CMV seropositive. The early course after transplantation was complicated by CMV viremia before prophylaxis was initiated. Standard therapy with IV ganciclovir (5 mg/kg given every 12 h) and γ-globulin (400 mg/kg given every other day) alternating with CMV hyperimmune globulin was not sufficient to clear the persistent refractory viremia until foscarnet (60 mg/kg q8h) was added. Thereafter, the patient was maintained on once-daily doses of ganciclovir (5 mg/kg) and foscarnet (90 mg/kg) and biweekly doses of CMV hyperimmune globulin.

CMV retinitis was diagnosed and observed periodically by the ophthalmology service. Six months after receiving the transplant, the patient developed graft-versus-host disease of the skin; this required treatment with steroids and daclizumab. She responded to immunosuppressive therapy but continued to have severe immunosuppression, with an absolute CD4 cell count of <10 cells/μL, as measured at 2, 3, and 6 months after undergoing transplantation.

The patient was admitted to the hospital 203 days after transplantation with a fever. Treatment with broad-spectrum antibiotics was started for pneumonia and acute maxillary sinusitis. Recurrence of CMV viremia necessitated a change from maintenance induction dosing of ganciclovir and foscarnet. CMV hyperimmune globulin was administered every other day. The patient had persistent fever.

On day 17 of hospitalization, the patient developed a generalized tonic-clonic seizure after several days of frequent headaches. A CT scan of her head did not reveal hemorrhage, mass lesions, or mass effect. Lumbar puncture revealed a RBC count of 160 cells/mm³ and a WBC count of 308 cells/mm³, with 97% neutrophils; chemistry testing revealed a glucose level of 97 mg/dL (serum glucose level, 111 mg/dL) and a protein level of 46 mg/dL (table 1). Gram stain of the CSF showed 2+ neutrophils and no organisms. The results of an India ink preparation were negative, and culture did not yield bacteria or fungi. The results of a PCR test for Toxoplasma gondii (MRL Reference Laboratory) were negative. Specimens of CSF tested strongly positive for CMV by early antigen with shell vials of MRC-5 cells (Barlts) and by use of the CMV Immunofluorescence Assay (Chemicon International). In addition, the 65-kDa lower matrix phosphoprotein (pp65) was detected in CSF leukocytes using the CMV pp65 Antigenemia Immunofluorescence Assay (Chemicon International). CSF specimens tested positive for CMV DNA by PCR (ViroMed Laboratories). MRI
of the brain revealed a communicating hydrocephalus with abnormal periventricular enhancement.

Findings from the CSF testing and brain MRI strongly suggested CMV ventriculomechanitis. On day 19 of hospitalization, antiviral therapy was maximized to include granulocyte-macrophage colony-stimulating factor and cidofovir (5 mg/kg once per week for 2 weeks, then 5 mg/kg every 2 weeks) with concomitant oral probenecid and hydration. Blood samples were obtained, and skin biopsies were performed for the establishment of a CMV-specific cytotoxic T cell line. Peak and trough levels of ganciclovir in plasma (MRL Reference Laboratory) were 9.8 μg/mL (normal range, 4–11 μg/mL) and <0.1 μg/mL (normal range, 0.4–0.6 μg/mL), respectively. A random CSF ganciclovir level (MRL Reference Laboratory) that was drawn at a separate time was 1.5 μg/mL. Measurement of foscarnet and cidofovir levels was not readily available.

The patient’s headaches resolved. Serial lumbar punctures performed on days 31, 39, and 49 of hospitalization revealed decreasing WBC counts, and CMV shell vial assays became negative (table 1). While the patient was receiving intensified combined antiviral and other therapy, she developed worsening creatinine clearance, hypertension, and pancytopenia. Gastrointestinal bleeding and a left renal abscess caused by progressive hydrocephalus. Because of her worsening condition, her family decided to avoid further aggressive diagnostic and therapeutic interventions. On day 55 of hospitalization, the patient died of progressive multiorgan dysfunction and circulatory collapse. Autopsy was declined.

To improve our understanding of the pathogenesis of CMV ventriculomechanitis in this patient and the apparent virologic response to triple antiviral therapy, drug susceptibility analysis was performed. Three sequential CMV isolates from the patient’s CSF recovered on days 17, 19, and 23 of hospitalization were analyzed for their susceptibility to ganciclovir, foscarnet, and cidofovir by use of a standard cell culture plaque reduction assay in normal human dermal fibroblasts [2]. In comparison with the reference laboratory strain AD169, all 3 isolates showed decreased susceptibility to ganciclovir and cidofovir but unchanged susceptibility to foscarnet (table 2).

The genes UL54 encoding for DNA polymerase and UL97 encoding for phosphotransferase, both of which may harbor CMV drug resistance–associated mutations [3], were sequenced. The alignment of DNA sequences from the 3 sequential isolates revealed an identical UL54 and UL97 genotype, indicating no modification in CMV genotype during the short course of therapy. One amino acid change (D605E) was identified in UL97 when compared with the consensus sequence of the AD169 strain. This sequence polymorphism has been previously detected in several drug-sensitive CMV isolates [4]. Several amino acid substitutions were found in UL54, with D413E being associated with the decreased drug susceptibility. This mutation has been previously identified in a CMV clinical isolate with decreased susceptibility to ganciclovir and cidofovir [5]. The other UL54 substitutions (P608L, S655L, N685S, A688V, A885T, and N898D) have been found in CMV isolates that exhibited full drug susceptibility and are considered interstrain variations [6, 7]. In addition, a previously unrecognized 6–amino acid insertion (LTAPGV) between codons 1100 and 1106 of the AD169 strain was identified, conferring decreased susceptibility to ganciclovir and cidofovir but unchanged susceptibility to foscarnet.

Table 1. Analysis of serial lumbar punctures.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Day of hospitalization</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>17</td>
</tr>
<tr>
<td>RBC count, cells/mm³</td>
<td>160</td>
</tr>
<tr>
<td>WBC count, cells/mm³</td>
<td>308</td>
</tr>
<tr>
<td>Neutrophils, %</td>
<td>97</td>
</tr>
<tr>
<td>Glucose level, mg/dL</td>
<td>97</td>
</tr>
<tr>
<td>Protein level, mg/dL</td>
<td>46</td>
</tr>
<tr>
<td>CMV shell vial assay</td>
<td>+</td>
</tr>
<tr>
<td>CMV pp65 antigen</td>
<td>+</td>
</tr>
<tr>
<td>CMV PCR</td>
<td>+</td>
</tr>
</tbody>
</table>

**NOTE.** CMV, cytomegalovirus.

Table 2. Drug susceptibility of 3 sequential cytomegalovirus isolates that were recovered from the patient’s CSF specimens and cultured.

<table>
<thead>
<tr>
<th>Virus isolate</th>
<th>Drug susceptibility, IC₅₀, mean μM ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ganciclovir</td>
</tr>
<tr>
<td>Reference AD169</td>
<td>5.7 ± 1.1</td>
</tr>
<tr>
<td>Day of hospitalization</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>44 ± 12 (7.7)</td>
</tr>
<tr>
<td>19</td>
<td>30 ± 8 (5.2)</td>
</tr>
<tr>
<td>23</td>
<td>31 ± 9 (5.4)</td>
</tr>
</tbody>
</table>

**NOTE.** Data in parentheses are fold-change in drug susceptibility relative to the reference AD169 strain. IC₅₀, concentration that inhibits 50%.
and 1101 was identified at the C-terminus of the UL54 product. This region has been postulated to interact with the UL44 accessory protein without being directly involved in the DNA polymerization process [8]. Hence, the primary association of this UL54 insertion with the observed drug-resistance phenotype is not expected.

When CMV encephalitis occurs in immunocompromised adults, it is diagnosed most frequently (85%) in patients with advanced HIV infection and less frequently (12%) in patients who are immunocompromised because of some other cause (usually solid-organ transplantation) [1]. There are 2 distinct clinical and neuropathologic syndromes: diffuse micronodular encephalitis and ventriculoencephalitis. The latter syndrome occurs more frequently in patients with CD4 cell counts of <100 cells/mm³. Venticuloencephalitis is distinguished by an acute onset of lethargy, disorientation, cranial nerve palsies, and nystagmus. Ependymal enhancement and enlarged ventricles are seen using neuroimaging, and the CSF specimens show pleocytosis. CMV probably invades the CNS through the CSF via the ependymal cells lining the ventricles, and it causes destruction of the periventricular matter and cranial nerves. The prognosis is extremely poor [1, 9–11]. There seems to be a strong association between CMV retinitis and encephalitis, although the temporal sequence of eye and brain involvement remains unclear [10].

To our knowledge, CMV ventriculoencephalitis has not been reported in recipients of bone marrow transplants. The main clinical manifestations of CMV disease after BMT are interstitial pneumonitis, esophagitis, and enteritis. Retinitis and encephalitis are extremely rare. The reason for the low incidence of retinal and CNS disease in patients who undergo BMT is unknown [12].

CMV ventriculoencephalitis often develops in patients who have HIV infection, despite treatment for CMV-associated retinitis [9, 11, 13, 14]. Several reasons have been postulated for this. One possibility is a selection of drug-resistant variants of CMV during prolonged antiviral therapy. The vast majority of ganciclovir-resistant viruses have been described in patients with AIDS, with a significantly lower number reported for patients who have hematologic malignancy or who have undergone BMT [3, 5]. The frequency of ganciclovir-resistance development in patients with AIDS was found to be as high as 27% after 9 months of ganciclovir therapy [15]. Genotypically, the majority of ganciclovir-resistant isolates contain ≥1 characteristic mutations in the UL97 phosphotransferase gene [3]. In addition, a population of ganciclovir-resistant CMV strains isolated from patients with AIDS or hematologic malignancy has been described; these strains express specific mutations in the UL54 DNA polymerase gene alone or, more often, in combination with UL97 mutations. All of these patients had CMV end-organ disease, and all had treatment with ganciclovir, sequential therapy with ganciclovir followed by foscarnet, or a combination of ganciclovir and foscarnet [5, 6]. UL54 mutations do not arise as readily as do UL97 mutations, but when they do, it is usually after prolonged ganciclovir therapy [6]. Most ganciclovir-resistant UL54 mutant isolates exhibit cross-resistance to cidofovir [2, 6]. Consistent with these observations, the virus detected in our patient was shown to have decreased susceptibility to ganciclovir and cidofovir because of a mutation in the UL54 gene.

Immediately before and after the diagnosis of ventriculoencephalitis, isolates recovered from our patient’s blood samples remained culture negative, with very low blood CMV antigenemia, indicating a selective viral replication and a ganciclovir-resistance development in the brain. However, all CSF isolates remained susceptible to foscarnet, indicating that the observed ganciclovir resistance is not sufficient to explain the active viral replication in the CNS. Inefficient penetration of ganciclovir and foscarnet into the brain may not be sufficient to suppress viral replication completely and may lead to viral resistance and progression of disease [1, 10, 13, 14]. Varying concentrations of ganciclovir and foscarnet in CSF have been demonstrated in adult patients [16, 17]. Pharmacokinetic profiles in children are not known.

In our patient, after the development of ventriculoencephalitis, cidofovir was added to existing therapy of ganciclovir and foscarnet. Despite harboring a virus that was resistant to ganciclovir and cidofovir, the patient responded to therapy within 2–3 weeks. A combination of these 3 drugs might have acted synergistically. In vitro studies have shown a synergistic anti-CMV effect of cidofovir when it is given with either ganciclovir or foscarnet [18, 19].

An increased risk of drug-related nephrotoxicity may represent some limitation for the concomitant use of cidofovir with foscarnet [19]. For our patient, the complications associated with intensified antiviral as well as other therapy (e.g., amphotericin B and aminoglycoside) were reflected in her worsening renal failure and decreasing peripheral blood counts. Worsening hydrocephalus may have reflected the natural consequence of CMV encephalitis, despite virologic response to antiviral therapy.

We investigated the possibility of delivering antiviral medications intrathecally to increase concentrations in brain tissue, but this was not attempted, because there seemed to be negligible published experience with this route. In addition, immunotherapy with CMV-specific cytotoxic T cells has been described to be a safe and effective means of reconstituting cellular immunity against CMV [20]. An expansion of CMV-specific cytotoxic T cells was attempted, but an adequate number of cells was not available in time to benefit this patient.

In conclusion, our experience suggests that CMV ventriculoencephalitis can also be seen in immunocompromised pa-
tients after they undergo BMT. The disease can develop despite anti-CMV prophylaxis and routine preemptive therapy because of the emergence of resistance and, possibly, low penetration of the drugs into the CNS. Irrespective of the observed drug resistance we defined by virologic and molecular studies, combination antiviral therapy may still be helpful in providing a clinical response. However, such side effects as renal and bone marrow toxicity may pose limitations. Therefore, further studies need to be conducted to assess the efficacy of combination antiviral therapy for the treatment of CMV ventriculoencephalitis. Immune therapy through the use of novel strategies, such as CMV-specific cytotoxic T cells, may help in a durable clinical and virologic response.

**Acknowledgments**

We gratefully acknowledge Dr. Rosemary Soave, who provided editorial assistance. We also acknowledge Mr. Jeffrey Stiles, who performed the CMV lower matrix phosphoprotein assay on blood and CSF samples.

**References**