Recurrences of Cytomegalovirus Retinitis in a Human Immunodeficiency Virus–Infected Patient, Despite Potent Antiretroviral Therapy and Apparent Immune Reconstitution

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We describe a 42-year-old man with human immunodeficiency virus infection who developed multiple recurrences of cytomegalovirus (CMV) retinitis despite receiving highly active antiretroviral therapy and having apparent immune reconstitution as evidenced by CD4+ T lymphocyte counts of >200 cells/mm3. Laboratory investigation during one recurrence of retinitis confirmed that there was active CMV replication in the plasma and vitreous fluid. In addition, lymphoproliferative responses to CMV antigens were absent despite evidence of reactivity to Candida antigen and pokeweed mitogen. The clinical significance of this case and of other recently reported cases is discussed.

The use of potent combination antiretroviral therapy has led to a remarkable reduction in the morbidity and mortality rates associated with cytomegalovirus (CMV) disease in patients with HIV infection. Observations have included a decreased incidence of new cases of CMV disease [1, 2] and a longer interval of time between relapses of CMV infection [3]. Preliminary data also suggest that maintenance treatment for CMV disease can be safely discontinued for a substantial proportion of individuals with significant immune recovery who are receiving potent antiretroviral therapy [4]. In this report, we describe a patient with HIV infection who developed recurrent CMV retinitis despite having immunologic improvement and receiving antiretroviral therapy. Additional investigation revealed that specific immunity to CMV had not been restored.

CASE REPORT

A 42-year-old man initially had HIV infection diagnosed in 1985. He had a progressive decline in the CD4+ T lymphocyte count during the next 10 years. Complications of HIV infection during this period were relatively minor and included thrush and recurrent perianal herpes. Initial antiretroviral treatments included sequential zidovudine monotherapy, didanosine monotherapy, and combination therapy with zidovudine and zalcitabine, all of which were relatively standard at the time. In April 1996, he began treatment with stavudine, lamivudine, and indinavir. However, he had gastrointestinal side effects and difficulties with adherence. In June 1996, he developed CMV retinitis of the left eye, which responded to induction and maintenance therapy with parenteral ganciclovir. In September 1996, the nadir of his CD4+ T cell count (48 cells/mm3) was
reached.

In February 1997, he developed bilateral zone 1 CMV retinitis. Ganciclovir therapy was discontinued. He could not tolerate foscarnet therapy; he was treated with cidofovir, and improvement was noted. Antiretroviral therapy was continued, and the patient had improved adherence. In May 1997, his CD4\(^+\) T cell count rose to 307 cells/mm\(^3\) with a plasma HIV RNA level of 21,124 copies/mL. As shown in figure 1, this and all subsequent CD4\(^+\) T cell counts were >200 cells/mm\(^3\). In June 1997, cidofovir treatment was discontinued because of iritis and hypotony, and treatment with parenteral ganciclovir was started again. In March 1998, his treatment was changed to oral ganciclovir.

In June 1998, new zone 3 retinitis was noted in the right eye. Treatment with parenteral ganciclovir was started again. A ganciclovir ocular implant was placed in July 1998. The patient again responded to these treatments, which were changed to oral ganciclovir. By December 1998, his CD4\(^+\) T cell count had risen to 443 cells/mm\(^3\), and his plasma HIV RNA level was 4398 copies/mL. However, CMV retinitis developed in his left eye. At that time, PCR testing was positive for CMV DNA in both the plasma (22,000 copies/mL) and the vitreous fluid (14,800 copies/mL).

The results of rapid CMV cultures of blood and urine samples were negative. Genotypic analysis of CMV isolated from peripheral blood samples revealed changes associated with resistance to ganciclovir (UL97: L595S and H69Y; UL54: S655L, N685S, A885T, and N898D), although genotypic analysis of CMV isolated from vitreous fluid samples revealed no resistance mutations. Following 3 weeks of induction therapy with ganciclovir, retinitis decreased, and PCR testing of plasma was negative for CMV (<500 copies/mL). A ganciclovir ocular implant was placed in the left eye. However, CMV retinitis subsequently developed in his right eye in February and July 1999; both episodes were successfully treated with induction doses of parenteral ganciclovir.

Figure 1 illustrates the relationship among CD4\(^+\) T lymphocyte counts, plasma HIV type 1 RNA levels, and episodes of CMV retinitis over time. In light of the ongoing activity of CMV retinitis despite apparent immune reconstitution, additional laboratory study was undertaken.

**METHODS**

**Virological studies.** CMV cultures of blood and vitreous fluid samples were performed by use of the rapid shell vial technique [5]. Qualitative CMV PCR testing was performed as described elsewhere [6, 7], by means of a single amplification method followed by Southern blot detection of a CMV fragment located in the EcoRI fragment D region. Quantitative PCR analysis was performed using a competitive method as described elsewhere [6]. For CMV genotyping, the 1.1-kb and 3.7-kb regions of UL97 and UL54, respectively, were amplified, by means of PCR analysis, from DNA extracts from clinical specimens and were sequenced using an automated ABI sequencer (Applied Biosystems) [8].

**Immunologic studies.** Lymphoproliferative assays were performed as described elsewhere [9, 10]. The responder cell frequency assay measures the frequency of CMV-specific memory CD4 cells by adding a limiting dilution step to the lymphoproliferative assay. In brief, 24 replicate cultures that contain 100,000, 50,000, 25,000, 12,500, and 6250 PBMC per well are stimulated with CMV and mock-infected control antigens for 8 days. On the last day of culture, cells are pulsed with \(^3\)H-thymidine for 6 h and are harvested, and the incorporated radioactivity is measured in a scintillation counter. Responder wells were defined as those whose counts per minute exceed the mean counts per minute + 3 SDs for the control cultures at the same cell concentration. The percentage of nonresponder wells is plotted on a log scale against the number of cells per well plotted on a linear scale, and the responder cell frequency is interpolated at the nonresponder well frequency of 37%.

The assay for inducible cytokines was performed with separated PBMC. Supernatants from CMV-stimulated cultures of \(2 \times 10^6\) PBMC per milliliter in growth medium were harvested on day 3 for detection of IL-2 and on day 6 for detection of IFN-\(\gamma\) and IL-10, respectively; at peak supernatant detection of each cytokine (A. Weinberg, unpublished data). Cytokine levels were measured by ELISA (Pierce Endogen), according to the manufacturer’s instructions. The analytic sensitivities of the IL-2, IFN-\(\gamma\), and IL-10 assays were 6 \(\mu\)g/mL, 2 \(\mu\)g/mL, and 5 \(\mu\)g/mL, respectively.

**RESULTS**

Results of the immunologic studies performed for this patient at the time of his relapses of CMV infection in December 1998...
and February 1999 are shown in table 1. Lymphoproliferative responses to Candida antigen were detectable 2 of the 3 times that they were measured. Lymphoproliferative responses to pokeweed mitogen were detectable all 3 times that they were measured. However, there were no detectable lymphoproliferative or cytokine responses to CMV antigens at any of the 4 times that they were measured.

**DISCUSSION**

In contrast to most clinical and previously reported experiences, this report describes multiple recurrences of CMV retinitis in an HIV-infected patient, despite dramatic increases in the CD4+ T lymphocyte count after several years of potent antiretroviral therapy. Additional investigation documented an absence of CMV-specific immunity. In contrast, the patient did not have any other opportunistic infections and had adequate proliferative responses to Candida antigen on 2 of 3 occasions. Of note, negative results of the lymphoproliferative assay for Candida occurred around the time of recurrence of CMV retinitis. The genotypic patterns of the CMV strains amplified from blood and vitreous fluid specimens are consistent with previously reported patterns [11].

The UL97 mutations found in the isolate recovered from peripheral blood specimens obtained from this patient confer resistance to ganciclovir and frequently develop under selection pressure, such as that triggered by the use of oral ganciclovir treatment in this case. In contrast, the vitreous strain did not have the same UL97 mutations, which might be explained by the low level of penetration by ganciclovir in ocular fluids (levels insufficient, in this case, for resistance to emerge in CMV strains). However, these results could also be explained by amplification bias during the sequencing process. Specifically, if mixed viral populations are present in blood and vitreous fluid, the dominant genotypic pattern may obscure minor components of the virus population. Despite the detected resistance, all recurrences of retinitis responded well to the ganciclovir implant and/or parenteral ganciclovir, with the exception of one course of treatment with cidofovir.

The extent of immune reconstitution in HIV-infected patients with advanced immunosuppression has been the object of several studies. Findings have shown staggered improvement of immune function, including an increase in memory and naïve CD4+ T cell subsets, an increase in naïve and a decrease in activated CD8+ T cells, and improvement of CD4+ T cell reactivity to recall antigens [12–14]. Although in some cases normalization of the CD4+ Vβ repertoire has been described [15], in most cases, both the CD4+ and CD8+ Vβ repertoires remain abnormal [16, 17], which suggests that immune restoration may not be complete.

Although the incidence of end-organ disease caused by CMV among HIV-infected patients has dramatically decreased since the introduction of potent antiretroviral therapy, CMV retinitis has occurred in some patients despite high CD4+ T cell counts [18, 19]. Two forms of CMV-associated ocular inflammation have been described in HIV-infected patients receiving potent antiretroviral therapy. The first form has the typical appearance of CMV retinitis and occurs most frequently during the first few months of antiretroviral therapy. Its occurrence has been ascribed to the evolution of retinitis lesions present before the initiation of therapy. The second form, immune-mediated vitritis, has been ascribed to a reconstituted vigorous immune response to CMV antigens localized in the eye [20, 21].

Our case does not fit either of these patterns. Our patient developed recurrent CMV retinitis after receiving effective antiretroviral therapy for several years, and the clinical picture was consistent with reactivation of CMV retinitis as opposed to immune-mediated vitritis. This patient failed to mount a

### Table 1. Results of immunologic studies of an HIV-infected patient with recurrent cytomegalovirus (CMV) retinitis despite receiving potent antiretroviral therapy and having apparent immune reconstitution.

<table>
<thead>
<tr>
<th>Date</th>
<th>LPA, SI</th>
<th>RCF assay</th>
<th>Inducible cytokine</th>
<th>Results of LPA for</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Th1 (IL-2, IFN-γ)</td>
<td>Th2 (IL-10)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>PWM, SI</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
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<td>None</td>
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<td>&lt;1</td>
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</tr>
</tbody>
</table>

**NOTE.** LPA, lymphoproliferative assay; NA, not available; PBMC, peripheral blood mononuclear cells; PWM, pokeweed mitogen; RCF, responder cell frequency; SI, stimulation index; Th1, T helper type 1; Th2, T helper type 2.

* Positive responses are indicated by SIs of >4, >6, and >3 for CMV, PWM, and Candida antigen, respectively.

* Results of the RCF assay are expressed as the number of responders per 10^5 PBMC.

* Positive responses.
CMV-specific immune response after several episodes of reactivated CMV infection, despite having CD4+ T cell counts that were consistently >200 cells/mm³. Whether the persistence of active HIV replication, as indicated by repeatedly detectable plasma levels of HIV RNA, played a role in the lack of CMV-specific immune reconstitution is unknown.

Recently, 2 additional reports that involved 3 patients described the recurrence of CMV retinitis in patients with relatively high CD4+ T lymphocyte counts who were receiving antiretroviral therapy [22, 23]. In each of these 3 cases, laboratory investigation also revealed the absence of CMV-specific immunity. These 3 cases and our case have several clinical implications. First, they suggest that the decision to discontinue treatment of patients with CMV infection who have responded to antiretroviral therapy with increasing CD4 treatment of patients with CMV infection who have responded to antiretroviral therapy with increasing CD4 counts may occasionally be complicated by recurrent CMV disease. How often this complication occurs is currently unclear, although it does not appear to be common enough to alter current guidelines on the discontinuation of treatment of CMV disease for patients who have responded to antiretroviral therapy with immune reconstitution [24]. Second, recurrent CMV disease in these 4 patients was associated with the absence of CMV-specific cell-mediated immunity. A recent study of HIV-infected persons with CMV disease indicated that some patients do recover specific immunity to CMV as a result of highly effective antiretroviral therapy [25]. In contrast, failure to recover lymphocyte proliferative responses to CMV antigens has been associated with progression of CMV retinitis after discontinuation of maintenance therapy for CMV infection in patients receiving potent antiretroviral therapy [26].

These findings suggest that a test for CMV-specific immunity, such as determination of lymphoproliferative responses to CMV antigens, might be useful in the identification of those individuals whose treatment can be discontinued versus those who should continue receiving treatment despite other evidence of immunologic improvement. Finally, these cases underscore the importance, whenever possible, of initiating antiretroviral therapy before the development of immunodeficiency and opportunistic infections.

References


