

## Long-Lasting Remission of Cytomegalovirus Retinitis without Maintenance Therapy in Human Immunodeficiency Virus–Infected Patients

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Seven AIDS patients who were receiving suppressive therapy for previously diagnosed cytomegalovirus (CMV) retinitis were offered treatment with protease inhibitors (PIs). Secondary prophylaxis for CMV was discontinued after 3 months of therapy with PIs if patients had  $>150$  CD4 cells/mm<sup>3</sup> and a human immunodeficiency virus (HIV) load of  $<200$  copies/mL and if they were negative for CMV as determined by qualitative CMV polymerase chain reaction (PCR). Ophthalmologic exams were done periodically. After a median follow-up of 9 months (range, 9–12), no new episodes of CMV retinitis were observed. CD4 cell counts were  $>150$  cells/mm<sup>3</sup> in all cases, HIV loads were  $<200$  copies/mL, and results for qualitative CMV PCRs remained negative. These observations suggest that for selected patients with healed CMV retinitis who have immunologic and virologic evidence of a clinical response to potent combination antiretroviral therapy, temporary discontinuation of a chronic anti-CMV suppressive therapy may not result in further retinal necrosis. However, the long-term immunologic benefit of PIs and hence the safety of prolonged withdrawal of anti-CMV therapy is unknown.

Cytomegalovirus (CMV) is a common opportunistic infection among AIDS patients, with its incidence increasing with the severity of immunodeficiency. At least 20% of patients with CD4 lymphocyte counts of  $<100$  cells/mm<sup>3</sup> will develop CMV-related disease over a 2-year period [1]. Retinitis is the most common manifestation of CMV disease in human immunodeficiency virus (HIV)–infected patients, and intravenous or oral maintenance therapy is required to avoid or delay progression to retinal destruction and complete vision loss [1]. However, maintenance therapy is associated with a poor quality of life; morbidity, mainly related to catheter complications; and a high prevalence of adverse side effects [2, 3].

Data from observational studies suggest that newer methods for detection of CMV viremia, such as polymerase chain reaction (PCR) and the detection of pp56 antigenemia in peripheral blood polymorphonuclear leukocytes, could have a role for the early diagnosis of CMV disease [4, 5]. Although it has not been validated in prospective trials, CMV PCR appears to be the best technique for selecting HIV-positive patients suitable for primary prophylactic treatment (or preemptive therapy) and also for monitoring maintenance therapy with oral or intravenous ganciclovir and foscarnet [6–8].

Several clinical end-point studies with protease inhibitors (PIs) demonstrated a significant delay in the short-term devel-

opment of further clinical events and death in a group of patients with advanced HIV infection [9, 10]. In addition, other studies [11] have shown the immunologic and virologic benefit of antiretroviral therapy (ARVT) that includes PIs. However, important issues about the functional impact of the increase in CD4 cell counts are now being investigated. Connors et al. [12] have demonstrated that there is a decline in naive CD4<sup>+</sup> T cells during the course of HIV infection and that highly active antiretroviral therapy (HAART) cannot always restore the number of naive CD4<sup>+</sup> T cells even when a dramatic increase in the absolute CD4<sup>+</sup> T cell count is achieved. On the other hand, Autran et al. [13] have shown that immunologic reconstitution may be achieved after prolonged viral suppression. Whitcup et al. [14] reported a series of 4 patients who were followed a median of 6 months (range, 4–12) without recurrence of CMV after initiating ARVT with PIs.

The improved knowledge about the natural history of CMV disease and about the immunologic and virologic events following ARVT that includes PIs prompted us to discontinue secondary prophylaxis for CMV retinitis after having initiated ARVT combined with PIs in 7 patients with advanced AIDS.

### Patients and Methods

A cohort of 16 AIDS patients on CMV secondary prophylaxis because of previously diagnosed CMV retinitis were offered HAART. Three of the 16 patients could not adhere to the new therapeutic regimen. After 3 months of follow-up, 3 of the remaining 13 patients did not achieve an immunologic (CD4 cell count  $>150$ /mm<sup>3</sup>) or virologic (plasma HIV-1 RNA levels  $<200$  copies/mL) response. Three of the remaining 10 patients who fulfilled the HAART response criteria and who had negative qualitative plasma CMV PCR results refused to discontinue secondary prophylaxis.

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All patients gave written informed consent to allow us to interrupt maintenance therapy.

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This study focusses on the remaining 7 patients who consented to discontinue secondary prophylaxis. The patients included 6 men and 1 woman with a median age of 33 years (range, 30–43). Their risk behaviors for HIV infection were as follows: 5 were homosexual, 1 had been an injection drug user, and 1 was a blood recipient. All 7 patients presented a first AIDS-defining event prior to the diagnosis of CMV retinitis, with a median interval of 28 months between the two events. The characteristics of the 7 patients at the time of the diagnosis of CMV retinitis and during maintenance therapy are summarized in table 1. All of the patients had prior ARVT experience with reverse transcriptase inhibitors (nucleoside analogues), and all of them continued their ARVT regimen until the introduction of PIs.

During the maintenance period, catheter-related bacteremia involving *Enterobacter* species and *Bacillus cereus* was observed; however, the infections resolved with appropriate antimicrobial therapy. Three patients presented with neutropenia that required periodic administration of granulocyte colony-stimulating factor. Patient number 3 developed biopsy-confirmed CMV colitis 3 months after the diagnosis of CMV retinitis. No new AIDS-defining events were diagnosed during the maintenance period prior to discontinuation of secondary prophylaxis for CMV. After the initial diagnosis, all patients received induction and maintenance therapy with ganciclovir (induction therapy: 5 mg/kg twice daily for 2–3 weeks; maintenance therapy: 6 mg/kg daily, 5 days a week) or foscarnet (induction therapy: 100 mg/kg twice daily for 2–3 weeks; maintenance therapy: 120 mg/kg daily, 5 days a week). After the induction phase was completed, ophthalmologic evaluations were conducted every 4 weeks during the maintenance therapy.

Once PIs were made available for compassionate use in Spain, they were added to the ARVT regimen for each of the 7 patients. All but 1 patient had been receiving a nucleoside analogue combination since the diagnosis of CMV retinitis (median of 8 months; range, 3–12). Because most of the patients had been treated with combination therapy that included zidovudine plus zalcitabine, didanosine, or lamivudine (3TC), we offered treatment with the fol-

lowing combinations: 3TC-stavudine (d4T)-ritonavir (4 patients), 3TC-d4T-ritonavir-saquinavir (2 patients), and 3TC-d4T-indinavir (1 patient).

At the onset of CMV retinitis and every 3 months after the change in ARVT, we determined the HIV load for each patient by use of a reverse transcription-PCR (Amplicor Roche, Madrid) with a level of detection of 200 copies/mL. In addition, qualitative plasma CMV PCR was done as described [5] at the diagnosis of CMV retinitis, once the patient achieved a CD4 cell count >150/mm<sup>3</sup> after changing ARVT, and at 3, 6, and 9 months after discontinuation of secondary prophylaxis.

Secondary prophylaxis was stopped once the patient had a CD4 cell count >150/mm<sup>3</sup>, an undetectable plasma HIV load, and a negative plasma PCR for CMV. Secondary prophylaxis was planned to be reintroduced if CD4 cell counts declined to <150/mm<sup>3</sup> during follow-up. All patients achieved CD4 cell counts of >150 cells/mm<sup>3</sup> 3 months after the initiation of PIs. After secondary prophylaxis was discontinued, ophthalmologic evaluations were done weekly for the first month and monthly thereafter.

**Results**

Patients were followed for 9–12 months (median, 9) after the secondary prophylaxis was stopped, during which time, no new episodes of CMV retinitis and no new AIDS-defining events were recorded. Table 2 shows the most relevant laboratory data for each patient after CMV maintenance therapy was discontinued. Between the diagnosis of CMV retinitis and after 9 months of discontinued CMV prophylaxis, the median increase in CD4 cell counts was 245 cells/mm<sup>3</sup> (range, 124–975). All patients had a CD4 cell count of >180 cells/mm<sup>3</sup> 3 months after starting treatment with PIs. At 6 months, all patients had CD4 cell counts of >230/mm<sup>3</sup>, and at 9 months, all patients had >150 cells/mm<sup>3</sup>. At all time points during follow-up, all patients had a CD4 cell percent >10%, an HIV virus load of <200 copies/mL, and a negative qualitative CMV PCR.

**Table 1.** Characteristics of 7 AIDS patients at the time of CMV retinitis diagnosis and during the CMV maintenance therapy period.

Patient no.	Interval since AIDS diagnosis (months; median, 28)	Time on ARVT (months; median, 30)	CD4 cell count (cells/mm <sup>3</sup> ; median, 35)	HIV virus load (copies/mL; median, 125,920)	Qualitative plasma CMV PCR	Induction and maintenance therapy	Time on anti-CMV therapy (months; median, 11)	No. of relapses during maintenance therapy (median, 1)	Other complications
1	28	30	35	120,450	+	Ganciclovir	11	1	Anemia 1 episode of catheter-related bacteremia
2	12	27	60	110,000	+	Ganciclovir	3	—	2 episodes of catheter-related bacteremia, CMV colitis
3	16	28	28	340,320	+	Foscarnet	14	3	—
4	28	44	80	67,000	+	Ganciclovir	6	—	—
5	6	69	80	150,000	+	Ganciclovir	14	2	Neutropenia
6	38	12	20	125,920	+	Ganciclovir	11	1	Neutropenia
7	55	30	5	269,560	+	Ganciclovir	16	1	Neutropenia

NOTE. ARVT = antiretroviral therapy; PCR = polymerase chain reaction.

**Table 2.** CD4 cell counts for 7 AIDS patients after the discontinuance of CMV maintenance therapy.

Patient, HAART regimen	No. of CD4 cells/mm <sup>3</sup> (%)		
	At time CMV prophylaxis stopped* (median = 230)	9 months after CMV prophylaxis stopped (median = 300)	No. of months of follow-up (median = 9)
1, d4T + 3TC + IDV	320 (11%)	1010 (22%)	12
2, d4T + 3TC + RTV	190 (10%)	326 (15%)	9
3, d4T + 3TC + RTV	233 (12%)	152 (15%)	12
4, d4T + 3TC + RTV	524 (15%)	330 (15%)	9
5, d4T + 3TC + RTV	260 (14%)	300 (16%)	11
6, d4T + 3TC + RTV + SQV	183 (11%)	247 (13%)	9
7, d4T + 3TC + RTV + SQV	230 (13%)	250 (13%)	9

NOTE. At both time points, all patients had HIV loads of <200 copies/mL and negative results for qualitative polymerase chain reaction. During follow-up, no new episodes of CMV or new AIDS-defining events occurred. HAART = highly active antiretroviral therapy, d4T = stavudine, 3TC = lamivudine, IDV = indinavir, RTV = zidovudine, SQV = saquinavir.

\* 3 months after initiation of treatment with protease inhibitors.

## Discussion

CMV involvement of the retina appears to occur mainly in persons with a CD4 cell count of <100 cells/mm<sup>3</sup> [1]. However a recent report [15] suggests that CMV retinitis may appear shortly (<2 months) after the introduction of PIs even if the patient has a CD4 cell count of >150 cells/mm<sup>3</sup>.

Our data differ from those of Jacobson et al. [15]; however, this is probably due to the fact that our patients had CD4 cell counts of >150 cells/mm<sup>3</sup> longer than the other patients, and they might also have recovered their immunologic repertoire in a more consistent manner. Regarding the restoration of the immune system, Connors et al. [12] have examined the changes in CD4 T cell surface marker phenotype and antigen receptor following HIV ARVT. They found that ARVT induced increases in CD4 cell counts that led to only minor changes in previously disrupted repertoires. According to Connors et al. [12], CMV can be regarded as a latently infecting agent in HIV-infected patients. For this reason, the frequency of memory T cells with the appropriate receptor exceeds the critical number necessary to provide protection as long as CD4 cell counts remain >100 cells/mm<sup>3</sup>. Due to this high precursor frequency, we can hypothesize that after a sustained increase in total CD4 cell counts, an improvement in the repertoire needed for CMV prevention could occur.

Our observations suggest that for selected patients with healed CMV retinitis who have immunologic and virologic evidence of a clinical response to HAART, temporary discontinuation of chronic, CMV-suppressive therapy may not result in further retinal necrosis. However, the long-term immunologic benefit of PIs and hence the safety of prolonged withdrawal of anti-CMV therapy is unknown.

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## Major Expansions of Select CD8<sup>+</sup> Subsets in Acute Epstein-Barr Virus Infection: Comparison with Chronic Human Immunodeficiency Virus Disease

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CD8<sup>+</sup> lymphocyte phenotypes were characterized during acute Epstein-Barr virus (EBV) infection, and a comparison was made to previous studies of human immunodeficiency virus (HIV). This was of interest because CD8<sup>+</sup> cells contribute to immunologic control of both infections, but the usual outcome of EBV infection is benign, whereas untreated HIV infection is fatal. During acute EBV infection, CD8<sup>+</sup> cells expressed elevated levels of the activation antigens CD38 and HLA-DR, similar to that during chronic HIV infection. Within 16 weeks, when EBV latency is established, CD8<sup>+</sup> cell activation had resolved. In contrast, activation persists in HIV infection. Expression of CD38 and HLA-DR on CD8<sup>+</sup> cells could be a marker for ongoing viral replication in both infections. Other CD8<sup>+</sup> cell alterations observed in this study of acute EBV infection included increases in both CD62L<sup>-</sup> and CD62L<sup>+</sup> CD8<sup>+</sup> cells and unique kinetics in the expansion of the CD57<sup>+</sup>CD8<sup>+</sup> cell subset.

Epstein-Barr virus (EBV), a human  $\gamma$ -type herpesvirus, is commonly recognized as the agent that causes acute infectious mononucleosis (AIM) [1]. Initially, EBV replicates in pharyngeal epithelium but within a few weeks establishes a latent infection in B cells. The CD8<sup>+</sup> cell immune response, including CD8<sup>+</sup> lymphocytosis during AIM, is believed to be responsible for controlling acute EBV infection and maintaining viral latency. Despite the presence of cytotoxic T lymphocytes (CTL), a low level of EBV replication in pharyngeal epithelium can be intermittently detected years after AIM resolves.

In contrast to EBV, which rapidly establishes a viable host-pathogen relationship in most people, human immunodeficiency virus (HIV) replicates continuously in the lymphoid system of the infected host [2]. Replication occurs at such high levels that it ultimately leads to deterioration of the immune system despite the maintenance for years of anti-HIV-specific CTL. Like acute EBV infection, chronic HIV infection is ac-

companied by CD8<sup>+</sup> cell lymphocytosis. We and others have described selective expansions of certain CD8<sup>+</sup> subsets in chronic HIV infection [3]. Of particular importance, expansions of CD8<sup>+</sup> cells with elevated expression of the CD38 activation antigen provide strong prognostic value for predicting progression of HIV infection to AIDS and death [4]. In the current study, we examined the temporal pattern of CD8<sup>+</sup> cell alterations in EBV infection and the similarities and differences between the CD8<sup>+</sup> subsets that contribute to EBV- and HIV-induced CD8<sup>+</sup> lymphocytosis.

### Materials and Methods

*Study populations.* Thirteen acute EBV mononucleosis patients (12, 18–22 years old; 1, 37 years old; 6 men and 7 women) were followed at the UCLA Student Health Service. All had atypical lymphocytosis at presentation. Acute EBV mononucleosis was confirmed by quantitative immunofluorescent antibody test for the titers of specific antibodies against EBV antigens [1] done at SmithKline Beecham Clinical Laboratories. Symptoms of acute mononucleosis had resolved completely in all subjects by 16 weeks. Most patients contributed blood at 2, 4, 6, and 8 weeks, and all contributed at 0 and 16 weeks.

Data on the HIV-infected cohort ( $n = 98$ ) and the heterosexual control group ( $n = 50$ , men;  $32 \pm 7$  years old) have been published previously [3], and relevant data are summarized in table 1 for comparisons with EBV infection. The HIV-infected homosexual men were AIDS-free participants in the Los Angeles Multicenter AIDS Cohort Study (MACS) and had been infected for 1 to an estimated 8 years; their average absolute CD4<sup>+</sup> cell number was  $\sim 500/\text{mm}^3$ .

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All subjects provided informed consent, and guidelines for the US Department of Health and Human Services were followed in the conduct of this research.

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