CONCISE COMMUNICATION

Loss of Cytomegalovirus-Specific CD4+ T Cell Responses in Human Immunodeficiency Virus Type 1–Infected Patients with High CD4+ T Cell Counts and Recurrent Retinitis

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Clinical histories are reported for 2 patients treated with highly active antiretroviral therapy (HAART) who experienced multiple relapses of cytomegalovirus (CMV) retinitis, despite suppression of human immunodeficiency virus type 1 (HIV-1) viremia and improvement in CD4+ T cell counts (to >400 cells/μL). CMV-specific CD4+ T cell immune reconstitution was measured directly, using cytokine flow cytometry, which revealed persistent deficits in CMV-specific CD4+ T cell responses in both patients. CMV-specific T cells constituted 0.14% and 0.05% of the total CD4+ T cell count in these patients, which is significantly lower than the percentages for 34 control subjects (0.6%–46%; CD4+ T cell count range, 7–1039 cells/μL; P = .019). Deficits in pathogen-specific immune responses may persist in some individuals, despite suppression of HIV-1 replication and substantial increases in circulating CD4+ T cells after HAART, and such deficits may be associated with significant morbidity from opportunistic infections.

The immunodeficiency of human immunodeficiency virus type 1 (HIV-1) disease is proximally related to loss of CD4+ cells. After treatment with highly active antiretroviral therapy (HAART), viral replication is suppressed, CD4+ T cell counts increase, and the attendant risks of immunodeficiency are minimized [1]. Indeed, although opportunistic infections (OIs), such as cytomegalovirus (CMV) retinitis, once were observed frequently in HIV-1–infected individuals with CD4+ T cell counts <50 cells/μL, the incidence of such infections has declined following the widespread use of HAART [2]. It is possible, however, that immunodeficiency may persist even after the CD4+ T cell count has been restored. T cell production, for example, may proceed from intrathymic or extrathymic routes (or both). If the former, a complete T cell receptor (TCR) repertoire could be generated anew; if the latter, the repertoire that is produced might be reflective of that which exists at the time of treatment. Because HIV-1–infected adults differ with respect to functional thymic reserves and pretreatment CD4+ T cell nadirs [3], it follows that a diverse TCR repertoire will be generated in some, but not all, patients after the initiation of HAART.

Antigen-specific CD4+ T cell responses can be quantitated by cytokine flow cytometry (CFC), to determine the frequency of cells that produce effector cytokines (e.g., tumor necrosis factor [TNF]-α) after stimulation with viral antigens [4]. We demonstrated elsewhere that decreased CMV-specific CD4+ T cell responses were seen in patients with active retinitis, but the response levels could be restored in most individuals after therapy with ganciclovir and HAART [5]. We also demonstrated that HIV-1–infected patients with weak CMV-specific CD4+ T cell responses, as measured by CFC, are unlikely to maintain strong CMV-specific CD8+ cytotoxic T lymphocyte (CTL) responses [6]. These results suggest that decreased frequencies of virus-
specific CD4+ T cells in humans are associated with loss of CTL, as they are in animal models of chronic viral infection [7]. We report here 2 cases of HIV-1–infected individuals who had substantial increases in CD4+ T cell counts after the initiation of HAART but who also experienced multiple relapses of CMV retinitis. CMV-specific CD4+ T cell responses were measured by CFC, to determine whether deficits in CMV-specific CD4+ T cell responses persisted despite HAART.

**Patients and Methods**

**Case histories.** Patient number 1 is a 52-year-old man who was diagnosed with HIV infection in 1987. He was treated with successive single- or dual-nucleoside regimens beginning in January 1991, with a gradually declining CD4+ T cell count. He developed immune thrombocytopenic purpura and cutaneous Kaposi’s sarcoma (KS), which responded to systemic chemotherapy. By late 1994, his nadir CD4+ T cell count was 225 cells/μL (30%). In June 1995, he developed left temporal CMV retinitis; at that time, his plasma HIV load was 127,000 copies/mL, and his CD4+ T cell count was 344 cells/μL (40%).

Patient 1 was treated initially with successive courses of intravenous (iv) ganciclovir followed by cidofovir (initiated on a research protocol because of incomplete response to ganciclovir) and then was switched to iv foscarnet maintenance therapy. After the initiation of HAART (saquinavir, stavudine, and lamivudine) in March 1996, his CD4+ T cell count increased progressively to 570 cells/mL (38%) by December 1996. His HIV load initially fell below the detectable limit (<400 copies/mL) and was 626 copies/mL by December 1996. An attempt to taper maintenance foscarnet resulted in retinitis reactivation in January 1997, which resolved by March 1997 after intravitreal ganciclovir injection, followed by intracocular ganciclovir slow-release insert; maintenance foscarnet was continued. After his CD4+ T cell count rose to 989 cells/μL (43%), another attempt to taper maintenance therapy failed in February 1998. Since May 1998, his retinitis has remained inactive on continued foscarnet. In June 1999, his plasma HIV load was 715 copies/mL, with a CD4+ T cell count of 840 cells/μL (42%). Patient 1 has had no other OIs or HIV-associated complications, including recurrence of KS.

Patient number 2 is a 35-year-old man who was diagnosed with HIV-1 infection in May 1991. Beginning in February 1992, he also received single- or dual-nucleoside regimens. His CD4+ T cell count declined gradually, and ultimately he developed several AIDS-defining illnesses, including *Pneumocystis carinii* pneumonia (July 1995), pulmonary and duodenal KS (October 1995), which responded to systemic chemotherapy, and CMV colitis (January 1996), when his nadir CD4+ T cell count was 12 cells/μL (2%). Despite a limited course of iv ganciclovir, patient 2 developed CMV retinitis in his left eye (May 1996), which was followed quickly by disease in his right eye, at a time when his plasma HIV load was 18,000 copies/mL. He responded to foscarnet induction and maintenance therapy.

In August 1996, patient 2 initiated HAART (indinavir and lamivudine followed by the addition of zidovudine in October 1996) and experienced a prompt and sustained increase in total and percentage CD4+ T cell count. He was switched from foscarnet to cidofovir therapy in February 1997, to reduce the frequency of iv infusions. His HIV load became undetectable (<400 copies/mL) by December 1997, at which time nevirapine was substituted for zidovudine. He continued taking cidofovir and, later, received oral ganciclovir maintenance therapy. Nonetheless, patient 2 relapsed in January 1998 and was treated again with foscarnet. Eventually, maintenance therapy was switched to the investigational agent oral valganciclovir. Second (August 1998) and third (January 1999) recurrences were treated successfully with iv foscarnet and intravitreal plus iv foscarnet, respectively. After a fourth recurrence, he continues to be maintained on periodic bilateral intravitreal foscarnet. His CD4+ T cell count remains stable in the 400–700 cells/μL range (22%–33%), and his HIV load has remained undetectable since December 1997. He has been free of other opportunistic diseases, including recurrent *P. carinii* pneumonia and KS.

Patient 2 also developed multiple episodes of immune recovery vitritis complicated by cystoid macular edema, the first of which occurred 3 months after he started HAART. Each episode was treated successfully with pericentral or oral corticosteroids (or both). Three of his 4 episodes of CMV retinitis occurred during periods of corticosteroid therapy.

**Control subjects.** CMV-specific T cell responses for 18 of the 34 HIV-1–infected control subjects were described elsewhere [5]. The 2 patients who had relapses of CMV retinitis, despite successful HAART, were recruited for this study on the basis of their clinical histories. The control group consisted of HIV-1–infected subjects consecutively enrolled in cross-sectional and longitudinal studies of CMV-specific immune function.

**Measurement of CD4+ functional responses.** CMV-specific CD4+ T cell frequencies were characterized, as described elsewhere [5], after stimulation of peripheral blood mononuclear cells (PBMC) with a CMV lysate, a superantigen, or matched control antigens in 96-well microtiter plates for 6 h. The last 5 h of this stimulation took place after the addition of brefeldin A, to prevent export of intracellular cytokines. CD4+CD69+ T cells then were analyzed for the presence of intracellular TNF-α, using a FACSCaliber flow cytometer equipped with CellQuest software (both from Becton-Dickinson). Data were analyzed and presented, using CellQuest or FlowJo (Treestar Software) software. Positive control samples included PBMC from an HIV−–infected, CMV-seropositive individual. Negative controls included isotype control antibody–stained cells and cells stimulated with other viral antigens (e.g., HIV− or measles).

Frequencies of CMV-specific CD4+ T cells were calculated after analyzing 100,000 events, and they were adjusted by values obtained after stimulation with CMV control antigen (±0.1% for both experimental patients). Each reported result was averaged from 2 separate analyses. In our prior long-term (>1 year) longitudinal studies of HIV− and HIV-uninfected subjects, interassay CFC variability generally was <20% [5, 6].

**Results**

Patients 1 and 2 had gradual declines in total CD4+ and CD8+ T cell counts over several years. At the time points indicated by dashed arrows in figure 1A, both patients presented with CMV retinitis and with CD4+ T cell nadirs of 225 cells/...
Figure 1. Clinical time lines and funduscopic findings for 2 human immunodeficiency virus (HIV)-1–infected patients with recurrent cytomegalovirus (CMV) retinitis. A. Longitudinal total CD4⁺ (●) and CD8⁺ (○) T cell counts related to initial and recurrent episodes of CMV retinitis. Dashed arrows indicate initial episodes of retinitis for each patient; solid arrows indicate recurrences of retinitis requiring further anti-CMV therapy. Bars indicate the duration of highly active antiretroviral therapy (HAART), including nelfinavir for patient 1 and indinavir for patient 2. B. Left temporal fundus of patient 1. Arrowheads indicate a yellow-white retinal infiltrate typical of CMV retinitis. This photograph was taken 2 months after retinitis was diagnosed and intravenous ganciclovir therapy had been initiated. C. Retinal findings for patient 2. Arrowheads indicate yellow-white infiltrates in the right temporal fundus. This was his first relapse in the right eye (his third overall) and was controlled only after intravitreal foscarnet injections.
Figure 2. Cytomegalovirus (CMV)–specific CD4+ T cell responses in a human immunodeficiency virus (HIV)–1–infected control subject and in HIV-1–infected patients with recurrent retinitis. Peripheral blood mononuclear cells were stimulated with CMV antigens and were stained for an activation marker (CD69) and for intracellular tumor necrosis factor (TNF-α). CMV-specific CD4+ T cell frequencies (percentage shown in each plot) were determined by CD69+/TNF-α+ cells. A, CMV-specific response (2.18%) in a CMV-seropositive, HIV-1–infected control subject with a CD4+/T cell count of 438 cells/μL is typical of responses in individuals without a history of CMV retinitis [5]. B, Response (0.14%) in patient 1 during a period of retinitis quiescence in August 1998. C, Response (0.05%) of patient 2 at the time retinitis recurred in his right eye in January 1999.

We demonstrated elsewhere that, irrespective of CMV disease status, declines in the total CD4+ T cell count were only minimally associated with decreased CMV-specific T cell responses ($r^2 = .13$) [5]. To determine whether the inability to respond to CMV reflected global CD4+ T cell anergy or dysfunction, we stimulated cells with the superantigen staphylococcal enterotoxin B. T cells from patient 1 responded normally (19.88% of peripheral CD4+ T cells), whereas responses in patient 2 were diminished (2.86%), relative to those previously observed in patients without a history of CMV disease [5], and were consistent with broader immune dysfunction but not generalized CD4+ T cell anergy.

Discussion

Traditionally, surveillance and prophylaxis for OIs in HIV-1–infected subjects have been based on the CD4+ T cell count. Although HAART clearly facilitates the recovery of antigen-specific CD4+ T cell responses [5], it is known that CMV retinitis may occur in individuals who are receiving HAART and who have CD4+ T cell counts above those previously associated with clinical risk for this OI [8]. Such events may reflect a quantitative increase in T cells unaccompanied by a qualitatively diverse TCR repertoire [9].

Here, we described 2 individuals who experienced responses to HAART with evidence of HIV-1 virologic suppression and sustained increases in total (>500 cells/μL) and percentage (>18%) CD4+ T cell counts. Nevertheless, both patients had multiple relapses of CMV retinitis and, in both, we detected a deficit in CMV-specific CD4+ T cell responses. These cases stand in marked contrast to the reconstitution of CMV-specific
responses that we observed in most subjects with quiescent retinitis after anti-CMV treatment and HAART (n = 6; median CMV-specific response, 1.78%) [5] and to the lack of relapses recently observed in 14 subjects (with CD4⁺ T cell counts increased to ≥150 cells/µL) after a median of 16.4 months after the initiation of HAART and the cessation of anti-CMV therapy [10].

It is possible that the functional T cell deficits observed in such patients may have been precipitated or augmented by concomitant therapeutic interventions. Both patients, for example, received cytotoxic chemotherapy for KS before initiating HAART. In patient 1, this may have contributed to the development of CMV retinitis at a time when the CD4⁺ T cell count was as high as 344 cells/µL and in the context of a relatively modest CD4⁺ T cell nadir of 225 cells/µL. In addition, 3 of 4 relapses in patient 2 were associated temporally with corticosteroid therapy of immune recovery vitritis [11]. These treatments may have contributed to deficits in the functional TCR repertoire.

Recent studies demonstrated a decreasing incidence of OIs in HIV-1–infected subjects in the United States and Europe [2], and retrospective and prospective studies showed that prophylaxis for P. carinii pneumonia may be safely discontinued in most individuals with improved CD4⁺ T cell counts after HAART [12, 13]. The clinical histories and corollary laboratory data presented here suggest that restoration of an adequately diverse CD4⁺ TCR repertoire does not occur in all HAART-responsive subjects.

Mathematical models of increases in CD4⁺ T cell counts and direct measures of T cell turnover in HIV-1–infected subjects [14] have suggested that increases in CD4⁺ T cell counts may result from redistribution of T cells from lymph nodes, as well as from de novo T cell input into the peripheral pool. Thymus-derived input of naive T cells into the peripheral pool was predicted by radiographic studies of HIV-1–infected subjects [3] and was confirmed by the demonstration that the thymus continues to function throughout adulthood [15]. For HIV-1–infected individuals who maintain or increase T cell synthesis after HAART, “holes” in the immunologic repertoire might be repaired by input of new antigen-specific T cells into a deficient peripheral pool. In other individuals, such as those discussed here, persistent deficits in immunologic function may lead to ongoing risk and morbidity from OIs. For such individuals, CFC-based measures may prove to be useful in the evaluation of factors regulating the reconstitution of antigen-specific CD4⁺ T cell function. Validation of CFC-based function assessment in prospective clinical studies of HIV-1–infected subjects may determine the utility of this assay to guide strategies for OI prophylaxis and therapy after HAART.

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References