Cytomegalovirus (CMV) Retinitis Immune Restoration Disease Occurs during Highly Active Antiretroviral Therapy–Induced Restoration of CMV-Specific Immune Responses within a Predominant Th2 Cytokine Environment

Shelley F. Stone,1,3 Patricia Price,1,3 Mei-Ling Tay-Kearney,2 and Martyn A. French1,3

Plasma levels of cytomegalovirus (CMV)–specific immunoglobulin G (IgG), soluble (s) CD30, sCD26 (dipeptidyl peptidase IV [DPP IV]) enzyme activity, and tumor necrosis factor receptor–I (TNFR-I) were assessed in human immunodeficiency virus (HIV)–infected patients who experienced CMV retinitis (CMVR) as an immune restoration disease (IRD) during their first 6 months of highly active antiretroviral therapy (HAART) and in CMV-seropositive, HIV-infected patients with similar baseline CD4+ T cell counts who had uneventful immune reconstitution. Patients who experienced CMVR IRD had a significant increase in CMV-specific IgG during their first 12 months of HAART, indicating restored CMV-specific immune responses. They also had significantly higher levels of sCD30 both before HAART and for up to 12 months after start of treatment. sCD30 levels remained elevated during 48 months of HAART, suggesting persistence of a predominant Th2 cytokine environment. Levels of sCD26 (DPP IV) enzyme activity and TNFR-I did not differ significantly between the 2 groups at any time point.

Before highly active antiretroviral therapy (HAART) became available, cytomegalovirus (CMV) retinitis (CMVR) occurred mainly in human immunodeficiency virus (HIV)–infected patients with <50 CD4+ T cells/μL and was characterized by necrotizing retinitis with minimal intraocular inflammation. HAART reduces the incidence of CMVR and restores CMV-specific immune responses in some patients [1, 2]. Consequently, anti-CMV therapy can often be withdrawn without clinical relapse in patients with a good virological response to HAART and CD4+ T cell counts >50 cells/μL [1, 2].

However, we and others have reported CMV-associated retinitis in HIV-infected patients responding to HAART [3–5]. Other intraocular inflammatory diseases in patients receiving HAART include uveitis, cystic macular edema, vitritis, and papillitis [6, 7]. Each condition is characterized by vitreous inflammation in the eyes of persons with previous CMV retinitis and increasing CD4+ T cell counts. Therefore, these conditions may be immune restoration diseases (IRDs), a term that encompasses inflammatory diseases or atypical presentations of opportunistic infections in the context of immune reconstitution during HAART [3].

We have reported that patients who experienced human herpesvirus–associated IRDs (including patients with CMVR IRD) have increased plasma levels of bioavailable interleukin-6 both prior to HAART and for up to 4 years during therapy [8]. Furthermore, in a cross-sectional study from our laboratory, HIV-infected patients who experienced CMVR IRD after starting HAART had higher serum levels of soluble (s) CD30 than did patients with similar nadir CD4+ T cell counts and no history of IRD [9]. CD30 is a member of the tumor necrosis factor receptor (TNFR) superfamily and is expressed on T cells producing predominantly Th2 cytokines [10]. We also demonstrated no difference in sCD26 (dipeptidyl peptidase IV [DPP IV]) enzyme activity between patients with CMVR IRD and those without an IRD [9]. sCD26 (DPP IV) enzyme activity may mark a Th1 cytokine environment [11] or generalized T cell activation [9]. Therefore, these findings suggested that CMVR IRD after initiation of HAART may be a consequence of an immunopathological response against CMV that is characterized by a predominantly Th2 cytokine environment.

Here we describe a longitudinal study of plasma levels of CMV-specific IgG, sCD30, sCD26 (DPP IV) enzyme activity, and TNFR-I in patients receiving HAART...
48-month periods after start of HAART from CMV-seropositive, HIV-infected patients who experienced or did not experience CMVR IRD.

**Patients and Methods**

Patients. A retrospective study of 179 HIV-infected patients starting HAART at Royal Perth Hospital (Perth, Australia) or associated private clinics identified 6 patients who experienced a “relapse” of CMVR after therapy had begun (hereafter, “CMVR IRD patients”) [3]. One patient was excluded from this study because no pre-HAART plasma samples were available. Each patient experienced at least a 3-fold increase in CD4+ T cell counts and a 1 log10 decrease in HIV load during the first 6 months of HAART. CMVR was identified by routine ophthalmological examination (by M.-L.T.-K.) in the first 6 months of HAART. The median time from start of HAART to presentation with CMVR was 1.1 months (by M.-L.T.-K.) in the first 6 months of HAART. The median time from start of HAART to presentation with CMVR was 1.1 months (range, 0.4–5.9 months). All patients had experienced CMVR prior to starting HAART and had baseline CD4+ T cell counts < 50 cells/μL. Two of the 5 patients with CMVR IRD also experienced presumptive herpes simplex virus–associated encephalitis or myelitis during their first 6 months of HAART.

Five age-, sex-, and race-matched HIV-infected patients with no evidence of CMVR, as determined by routine ophthalmological examination (by M.-L.T.-K.) in the first 6 months of HAART, were selected retrospectively from the HIV database of the Department of Clinical Immunology and Biochemical Genetics, Royal Perth Hospital, as control subjects (hereafter referred to as “non-IRD patients”). All non-IRD patients were CMV seropositive, started HAART having a CD4+ T cell count < 50 cells/μL, and experienced the same level of immune reconstitution as the CMVR IRD patients. Two non-IRD patients had CMVR before they started HAART, 1 had CMV colitis, and 2 had no previous history of CMV disease. One non-IRD patient did not have an ophthalmological examination during the first 6 months of HAART, but this patient did not have a history of CMVR prior to beginning the therapy and has not presented with decreased visual acuity since starting HAART.

All 7 patients with CMVR prior to HAART were receiving intravenous or intravitreal ganciclovir or foscarnet as secondary prophylaxis both before and after starting HAART. All patients who experienced CMVR IRD were receiving anti-CMV therapy at the time of the IRD. All patients ceased prophylactic anti-CMV therapy when their CD4+ T cell count became > 100 cells/μL and their HIV load < 400 copies/mL. One CMVR IRD patient also experienced presumptive immune recovery vitritis after 24 months of HAART, which resulted in retinal detachment. No other CMVR IRD patient or non-IRD patient has presented with CMVR since starting HAART.

All CMVR IRD patients received HAART regimens that included lamivudine, stavudine, and indinavir. One patient was also taking didanosine. Three non-IRD patients also started HAART with lamivudine, stavudine, and indinavir. One patient received zidovudine rather than stavudine, and another received saquinavir and ritonavir in place of indinavir. No drug was significantly associated with CMVR IRD.

All clinical data from the database were confirmed by review of medical records. Plasma samples had been collected from all patients before the start of HAART and at 6-, 12-, 24-, and 48-month intervals while undergoing HAART and were stored at –20°C until assayed.

**Table 1.** Plasma levels of cytomegalovirus (CMV)–specific IgG, soluble (s) CD30, sCD26 (dipeptidyl peptidase IV [DPP IV]) enzyme activity, and tumor necrosis factor receptor (TNFR)–I and CD4+ and CD8+ T cell counts in human immunodeficiency virus–infected patients with CMV retinitis (CMVR) immune restoration disease (IRD) and those without IRD before and after start of highly active antiretroviral therapy (HAART).

<table>
<thead>
<tr>
<th>Time after start of HAART (in months), patient group</th>
<th>CMV-specific IgG, units/mL</th>
<th>sCD30, units/mL</th>
<th>sCD26 (DPP IV) enzyme activity, units/mL</th>
<th>TNFR-I, ng/mL</th>
<th>CD4+ T cell count, cells/μL</th>
<th>CD8+ T cell count, cells/μL</th>
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</thead>
<tbody>
<tr>
<td>Baseline (before HAART)</td>
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<tr>
<td>CMVR IRD</td>
<td>213 (151–414)</td>
<td>419 (308–1796)a</td>
<td>204 (84–395)</td>
<td>2.6 (0.6–3.5)</td>
<td>4 (0–35)</td>
<td>224 (188–536)</td>
</tr>
<tr>
<td>Non-IRD</td>
<td>93 (47–1110)</td>
<td>218 (114–250)</td>
<td>139 (95–390)</td>
<td>2.0 (1.5–3.9)</td>
<td>11 (0–20)</td>
<td>348 (242–441)</td>
</tr>
<tr>
<td>0–6</td>
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<tr>
<td>CMVR IRD</td>
<td>329 (133–933)</td>
<td>527 (256–810)a</td>
<td>142 (78–234)</td>
<td>2.0 (1.5–3.7)</td>
<td>63 (13–116)b</td>
<td>349 (200–702)c</td>
</tr>
<tr>
<td>Non-IRD</td>
<td>102 (40–1695)</td>
<td>175 (60–306)</td>
<td>153 (91–371)</td>
<td>1.4 (0.9–4.1)</td>
<td>45 (0–102)b</td>
<td>850 (364–1001)d</td>
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<tr>
<td>6–12</td>
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<tr>
<td>CMVR IRD</td>
<td>930 (398–1155)d</td>
<td>508 (378–540)b</td>
<td>158 (95–222)</td>
<td>3.0 (1.4–4.1)</td>
<td>104 (24–180)d</td>
<td>832 (182–220)</td>
</tr>
<tr>
<td>Non-IRD</td>
<td>330 (143–1800)</td>
<td>281 (66–459)</td>
<td>167 (151–299)</td>
<td>2.1 (0.9–2.5)</td>
<td>160 (110–250)d</td>
<td>1100 (520–1888)d</td>
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<tr>
<td>12–24</td>
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<tr>
<td>CMVR IRD</td>
<td>845 (810–1275)d</td>
<td>792 (326–3400)c</td>
<td>164 (103–266)</td>
<td>3.4 (1.9–5.8)</td>
<td>100 (32–210)d</td>
<td>680 (192–1302)a</td>
</tr>
<tr>
<td>Non-IRD</td>
<td>760 (293–6570)b</td>
<td>322 (78–1080)</td>
<td>175 (134–425)</td>
<td>1.3 (0.8–3.5)</td>
<td>306 (238–480)d</td>
<td>1600 (896–1972)d</td>
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<tr>
<td>24–48</td>
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<tr>
<td>CMVR IRD</td>
<td>600 (520–758)d</td>
<td>891 (423–1201)b</td>
<td>149 (96–158)</td>
<td>2.1 (1.6–9.0)</td>
<td>221 (23–528)d</td>
<td>1200 (94–1258)</td>
</tr>
<tr>
<td>Non-IRD</td>
<td>353 (210–5828)</td>
<td>444 (57–927)</td>
<td>164 (144–696)</td>
<td>2.1 (0.8–3.1)</td>
<td>289 (250–924)d</td>
<td>1502 (800–1822)d</td>
</tr>
</tbody>
</table>

**NOTE.** Data are median (range). All statistical comparisons were determined by Wilcoxon rank sum test.

a CMVR IRD group significantly different from non-IRD group (P < .05).

b Marginally higher than baseline (.05 < P < .1).

c CMVR IRD group marginally different from non-IRD group (.05 < P < .1).

d Significantly higher than baseline (P < .05).
Quantitation of CMV-specific IgG. Half-volume 96-well plates were coated overnight at 4°C with a predetermined optimal concentration of whole CMV sonicate (CMV strain AD169 grown in human fibroblasts; Department of Microbiology, Royal Perth Hospital). Plates were blocked with 10% fetal calf serum (FCS)/PBS for 2 h. Samples and standards were diluted in 10% FCS/PBS and incubated for 2 h. Pooled serum from HIV-seropositive, CMV-seropositive individuals was used as a standard and assigned an arbitrary value of 1000 units/mL of CMV-specific IgG. Anti–human IgG peroxidase conjugate (BioSource International) was added for 2 h at room temperature before addition of 3,3',5,5'-tetramethylbenzidine (TMB) substrate. The reaction was stopped with 2 M H₂SO₄, and the absorbance was read at 450 nm. The limit of detection and coefficient of variance were 10 units/mL and 15%, respectively.

Quantitation of sCD30. sCD30 levels were assayed by ELISA (Bender MedSystems) [9]. The limit of detection and coefficient of variance were 6.25 units/mL and 13%, respectively.

Quantitation of sCD26 (DPP IV) enzyme activity. sCD26 (DPP IV) enzyme activity was measured by an enzyme capture assay [9]. The limit of detection and coefficient of variance were 6.25 units/mL and 10%, respectively.

Quantitation of TNFR-I. Half-volume (50 μL) 96-well plates were coated overnight at 4°C with anti–TNFR-I antibody (R&D Systems). Plates were blocked with 5% bovine serum albumin (BSA)/PBS for 2 h. Samples and standards were then added for 2 h at room temperature. Biotinylated anti–TNFR-I detection antibody (R&D Systems) was added for 1 h followed by a streptavidin-peroxidase conjugate for 2 h and TMB substrate for 15 min. The limit of detection and coefficient of variance were 0.05 ng/mL and 15%, respectively.

Results

To analyze changes in levels of CMV-specific IgG, sCD30, sCD26 (DPP IV) enzyme activity, and TNFR-I and CD4⁺ and CD8⁺ T cell counts, we averaged individual patient results obtained during each 6- or 12-month interval after start of treatment (1–3 values/interval). The average values varied by ±20%. For each patient group, the median and range of the average values are given in table 1. Individual results for levels of CMV-specific IgG and sCD30 and CD4⁺ and CD8⁺ T cell counts are shown in figure 1.

Patients who experienced CMVR IRD had increased plasma levels of CMV-specific IgG and sCD30 but not sCD26 (DPP IV) enzyme activity or TNFR-I. Plasma levels of CMV-specific IgG were generally higher in CMVR IRD patients than in non-IRD patients.
patients, but these differences did not reach significance (table 1 and figure 1A). CMVR IRD patients experienced a rise in levels of CMV-specific IgG after starting HAART, with levels significantly higher than baseline after 6–12, 12–24, and 24–48 months \((P = .032, .008, \text{ and } .008, \text{ respectively})\). In contrast, non-IRD patients experienced only a marginal increase in levels of CMV-specific IgG above baseline after 12–24 months of HAART \((P = .095)\).

CMVR IRD patients had significantly higher plasma levels of sCD30 than did non-IRD patients before HAART \((P = .008)\) and after 0–6 and 6–12 months of HAART (both \(P = .016\)), with levels remaining high after 48 months (table 1 and figure 1B). Plasma levels of sCD30 were marginally higher than baseline in CMVR IRD patients after 24–48 months of HAART \((P = .09)\).

Plasma levels of sCD26 (DPP IV) enzyme activity did not differ significantly between the groups during any time interval (table 1). TNFR-I levels were marginally elevated in CMVR IRD patients after 12–24 months of HAART only \((P = .09)\).

**CMVR IRD patients experienced a significant increase in CD4\(^+\) but not CD8\(^+\) T cell counts during the first 6 months of HAART.** Before HAART, both groups had similar CD4\(^+\) and CD8\(^+\) T cell counts (table 1 and figure 1C and 1D). CD4\(^+\) T cell counts from CMVR IRD patients were significantly higher than baseline after 6–12, 12–24, and 24–48 months of HAART \((P = .016 \text{ for each time period})\). In contrast, CMVR IRD patients did not experience a significant increase in CD8\(^+\) T cell counts above baseline levels over any time interval. Non-IRD patients had significant elevation in CD4\(^+\) and CD8\(^+\) T cell counts after 6–12, 12–24, and 24–48 months of HAART \((P = .008 \text{ for each time period})\).

**Discussion**

This study showed that immunodeficient HIV-infected patients who experienced CMVR IRD during HAART have evidence of restored CMV-specific IgG antibody responses and predominantly Th2 cytokine environments, marked by elevated sCD30 levels.

The ability of HAART to restore CMV-specific immune responses varies between patients and within individual patients over time. Detection of positive CMV-specific lymphoproliferation and cytokine production has been associated with higher CD4\(^+\) counts and absence of CMV disease. However, 60% of such patients had negative CMV responses at \(\geq 1\) time point [12]. A single patient with multiple recurrences of CMVR reactivation, despite HAART and apparent immune reconstitution (as evidenced by CD4\(^+\) T cell counts \(> 200 \text{ cells/\mu L}\), showed negative lymphoproliferation responses to CMV despite positive responses to *Candida* and pokeweed mitogen [13]. However, in vitro assays, such as lymphoproliferation assays, may underestimate the ability of HIV-infected patients to mount CMV-specific immune responses [14]. For this reason, we assessed longitudinal plasma levels of CMV-specific IgG. All 5 CMVR IRD patients in this study experienced a significant elevation in levels of CMV-specific IgG during their first 12 months of HAART, which is parallel to a significant increase in CD4\(^+\) T cell counts. This demonstrates HAART-induced restoration of a CMV-specific immune response in these patients.

Before HAART was introduced, CMVR was restricted to late-stage HIV disease (AIDS), when patients have a predominantly Th2 cytokine profile. In our study, patients who experienced CMVR IRD had significantly higher plasma levels of sCD30 both before and for their first 12 months of HAART, compared with non-IRD patients. sCD30 levels remained elevated after 48 months of HAART, and these observed levels in our CMVR IRD patients may result from an inability to reverse the HIV-induced immune damage that results in the predominance of Th2 cytokine responses.

In our study, patients with a history of CMVR IRD had delayed recovery of their CD8\(^+\) T cell counts, compared with non-IRD patients (table 1 and figure 1D). Studies of mice and humans have shown that CD8\(^+\) lymphocyte responses are protective against CMV disease. Progressive loss of the CD3\(^+\)CD8\(^+\) cell subset may increase risk of CMVR in untreated HIV-infected patients [15]. If lower numbers of peripheral CD8\(^+\) T cells reflect lower numbers in the local inflammatory infiltrate, CD8\(^+\) T cells may play an important regulatory or suppressive role in preventing the development of CMVR IRD. However, a role for CD8\(^+\) T cells in the pathogenesis of CMVR IRD requires investigation in larger patient cohorts. IRDs are an increasingly important problem in the post-HAART era of the HIV-infection pandemic. Determining patients at risk of IRD before starting HAART should now be a priority. This study provides evidence that CMVR IRD correlates with restoration of a CMV-specific immune response and identifies sCD30 as a potential clinical marker for patients at risk of developing CMVR IRD.

**Acknowledgments**

We thank the staff of the Department of Clinical Immunology and Biochemical Genetics, Royal Perth Hospital, for collection and storage of plasma samples. We also thank Niamh Keane and Silvia Lee, for technical assistance, and each patient for participation in this project.

**References**


