The Association of Human Cytomegalovirus with Biomarkers of Inflammation and Immune Activation in HIV-1-Infected Women

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Abstract

Three groups of cytomegalovirus (CMV)-seropositive women (total $n=164$) were selected from the Chicago Women's Interagency HIV-1 Study to investigate the association between CMV coinfection and immune activation: (1) HIV-1 viremic, (2) HIV-1 aviremic, and (3) HIV-1 uninfected. Quantitative measures of CMV serum IgG, CMV DNA, and serum biomarkers interleukin (IL)-6, soluble CD163 (sCD163), soluble CD14 (sCD14), and interferon gamma-induced protein (IP10) were obtained. Levels of CMV IgG and the serum biomarkers were significantly higher in the HIV-1 viremic group compared to the aviremic and uninfected groups ($p<0.001$). No significant associations with CMV IgG levels were found for HIV-uninfected women. When each of the HIV-infected groups was analyzed, sCD14 levels in the viremic women were significantly associated with CMV IgG levels with $p<0.02$ when adjusted for age, CD4 count, and HIV viral load. There was also a modest association ($p=0.036$) with IL-6 from plasma and cervical vaginal lavage specimens both unadjusted and adjusted for CD4 count and HIV viral load. The association of CMV IgG level with sCD14 implicates the monocyte as a potential site for interaction of the two viruses, which eventually may lead to non-AIDS-defining pathological conditions.

Introduction

HUMAN CYTOMEGALOVIRUS (CMV), a member of the Herpesvirus family, produces infections in immunocompetent persons that are usually asymptomatic. It is mainly in the context of immunosuppression such as HIV/AIDS that CMV infection results in serious sight-threatening or life-threatening disease.^{1,2} In the era before combination antiretroviral therapy (cART) became available, CMV coinfection produced significant end-organ disease as HIV-1-infected patients progressed to AIDS.³ With the introduction of cART the incidence of symptomatic CMV disease in these patients has been greatly reduced, and non-AIDS-defining conditions have become the major cause of morbidity and mortality associated with HIV-1 infection.^{4,5}

CMV like HIV-1 establishes lifelong latency, and recent studies have suggested that periodic asymptomatic CMV reactivations in HIV-1-coinfected persons over a lifetime may contribute to non-AIDS-defining morbidity including cardiovascular disease, neurocognitive impairment, cancer, and renal disease.^{5–11} Despite undetectable levels of HIV-1 and CMV in plasma, these patients may continue to have increased levels of biomarkers of inflammation and immune activation such as

interleukin (IL)-6, interferon gamma-induced protein (IP10), as well as markers of monocyte activation soluble CD163 (sCD163) and soluble CD14 (sCD14). All of these biomarkers have been associated with a greater risk of cardiovascular disease and all-cause mortality as reported in several studies.^{5,12–21} Thus, it appears that the inflammatory response to CMV reactivation and replication could be linked to endothelial cell damage that in turn could lead to subclinical vascular changes and atherosclerosis.^{6,8,22–24}

Although there have been multiple studies of CMV coinfection in HIV-infected patients, the results have not always been consistent, which may be attributed in part to differences in study populations and types of specimens analyzed. The genome copies of CMV DNA in plasma from CMV-seropositive patients who are HIV-1 negative or who have undetectable plasma HIV-1 RNA are almost always below the lower limit of assay detection, which makes CMV reactivation as measured by DNA difficult to assess in asymptomatic patients who have CD4⁺ T cell counts above 200 cells/mm³, even when they have measurable HIV-1 viral loads. Therefore, plasma specimens obtained for routine medical care are not reliable for use in studies of CMV DNA as a quantitative marker of virus reactivation. However, specimens from other

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compartments such as semen, saliva, or cervicovaginal lavage (CVL) may have detectable CMV DNA when the CMV plasma DNA remains negative.²⁵

Several studies in HIV-1-infected men have used semen specimens to establish an association between CMV shedding and both HIV-1 shedding and increased levels of HIV-1 proviral DNA.^{26–30} Others have reported detectable levels of CMV DNA in CVL, saliva, and peripheral blood mononuclear cells (PBMCs).^{25,31,32} However, immunological markers of CMV infection in blood specimens have shown significant associations with biomarkers of inflammation and non-AIDS-defining events. Therefore, quantitative measures of the immune response to CMV have been used to establish associations with biomarkers of inflammation and disease conditions in HIV-1 patients. CMV-specific T cell counts and quantitative CMV IgG levels have been used most often.^{7,8} CMV-specific T cell responses were found to be strongly associated with carotid intima-media thickness in HIV-infected patients compared to HIV-uninfected controls.²⁴ CMV-specific CD8⁺ T cell counts have been reported to remain elevated after successful cART, which may lead to immunosenescence and age-related morbidity.⁸ Other studies have reported CMV IgG associated with cardiovascular and cerebrovascular disease in HIV-1-suppressed patients.^{7,9,21} However, others have not found any association between CMV IgG and non-AIDS-defining conditions in cART-suppressed patients.^{16,33} Therefore the pathogenesis of CMV coinfection in cART-treated HIV-infected patients remains unclear.

The goal of this exploratory study was to investigate the impact of the inflammatory effects associated with CMV/HIV coinfection in a well-characterized cohort of women, because many prior studies have examined the effects of these two viruses in predominantly male cohorts.^{7,16,21,33,34} The study was designed to quantitate and compare levels of CMV IgG with levels of markers of inflammation and immune activation across three groups of women: (1) HIV seronegative, (2) HIV seropositive/viremic, and (3) HIV seropositive/aviremic. We sought to test whether CMV IgG levels were associated with the levels of HIV viremia and the levels of these markers and whether the observed associations remained after adjustment for known sociodemographic and clinical confounders.

Materials and Methods

Selection of subjects

All women were enrolled in the Chicago site of the Women's Interagency HIV-1 Study (WIHS), an ongoing multisite prospective cohort study of women with and at risk for HIV-1 infection in the United States. Detailed information on recruitment and data collection has been described previously.^{35,36} Briefly, at semiannual visits, women undergo clinical examination and provide laboratory specimens and self-reported sociodemographic and behavioral information via surveys administered by trained research staff. Written informed consent was obtained from all participants in accordance with Department of Health and Human Services guidelines, after approval by the Institutional Review Board at each participating site.

There were 296 women actively enrolled at the Chicago WIHS site at the time of the study; 217 (73%) were HIV infected and 79 (27%) were HIV uninfected. Samples were

selected from women with available information on HIV-1 viral load at two consecutive WIHS visits for whom sufficient quantity of samples were available from the specimen repository. Women were categorized into three mutually exclusive groups based on HIV status and HIV RNA levels over the 2 years prior to the CMV study. Eligible patients included the 79 women who were HIV negative at the most recent WIHS visit, 108 HIV-infected women with undetectable HIV RNA (<50 copies/ml) at consecutive WIHS visits for at least 1 year, and 67 women with at least one visit with HIV RNA levels of $\geq 1,000$ copies/ml. Samples were then randomly selected from within these three groups to ensure approximately equal numbers in each group.

The most recent visit was used for women who had more than one visit at which these criteria were met, and women could be included in only one group. The visit at which CMV was measured corresponded to the visit at which women were viremic, or for aviremic women, the visit at which they had been aviremic for at least 1 year. A total of 175 women who met the criteria were screened for CMV seropositivity (60 HIV negative, 60 HIV aviremic, and 55 HIV viremic with HIV RNA $\geq 1,000$ copies/ml). There were 164 CMV-seropositive subjects (93.7%) divided approximately evenly among the three groups: 54 HIV uninfected, 58 HIV-1 aviremic, and 52 HIV-1 viremic. CMV-seronegative women were excluded, because there were only 11 women identified in this category. CMV and immune activation markers were measured from specimens collected at the time of the corresponding study visit for all women.

Serum, plasma, and CVL specimens from all the CMV-seropositive women were tested. Sociodemographics included age in years, race/ethnicity, annual household income (categorized as <\$18,000 vs. $\geq$ \$18,000), educational attainment (less than high school vs. high school or equivalent or higher education), and current employment. Clinical variables included HIV-1 RNA and CD4 count, hepatitis C virus (HCV) coinfection (based on antibody positivity measured at WIHS enrollment), clinically significant depressive symptoms using a cutoff of 16 measured by the Centers for Epidemiologic Studies-Depression (CESD),³⁷ current smoking status, diabetes (by self-report or any use of antidiabetic medication, fasting glucose ≥ 126 , or hemoglobin A_{1c} $\geq 6.5\%$), hypertension (based on self-report, systolic blood pressure ≥ 140 , diastolic blood pressure ≥ 90 , and/or use of antihypertensive medications), estimated glomerular filtration rate (eGFR) based on the Modification of Diet in Renal Disease (MDRD) Study, $eGFR = [186 \times (\text{serum creatinine} - 1.154) \times (\text{age})^{-0.203} \times (0.742 \text{ if female}) \times (1.210 \text{ if African American})]$,³⁸ and current body mass index (BMI; calculated as weight in kilograms/height in meters²).

Quantitation of CMV IgG

CMV IgG antibody levels were measured in serum using the Cytomegalovirus IgG Enzyme Immunoassay kit (ELISA, GenWay Biotech, San Diego, CA). Samples were diluted 1:80 for the initial assay and the OD was read at 450 nm, using a DuPont Kinetic Microplate Reader (Molecular Devices, Sunnyvale, CA). All samples below the assay cutoff were repeated at a 1:40 dilution. Quantitative results in international units per milliliter were calculated using kit calibrators.

Quantitation of CMV DNA

A validated in-house TaqMan real-time PCR assay was used to measure CMV DNA in plasma and whole CVL. DNA was extracted from specimens using the DNA Mini Kit (QIAGEN, Valencia, CA). The target sequence is the CMV DNA polymerase gene. The primer and probe sequences are forward CTCGTGCGTGTGCTACGAGA; reverse GCCGATCGTRAAGAGATGAAGAC; probe FAM-AGTGCAGCC CC GRCCATCGTTC-TAMRA. The lower limit of detection of the assay is 100 copies/ml.

Quantitation of HIV-1 RNA

HIV-1 plasma RNA was measured using the COBAS AmpliPrep/COBAS TaqMan HIV-1 Test version 2.0 (Roche). The assay had a lower limit of detection of 20 copies/ml. CVL specimens were centrifuged, and the supernatant fluid was analyzed for HIV-1 RNA using the Abbott RealTime HIV-1 Assay with a lower limit of detection of 40 copies/ml.

Quantitation of markers of inflammation and immune activation

Plasma specimens were analyzed for several biomarkers of inflammation and activation by commercially available ELISA kits: IL-6 (R&D, Minneapolis, MN), IP10 (R&D), sCD14 (R&D), and sCD163 (Trilium Diagnostics, Brewer, ME). IL-6 levels were also measured in CVL specimens.

Statistical methods

Demographic and clinical characteristics, inflammatory markers, and CMV IgG levels were compared among the three groups of women using chi-square tests for categorical variables and Kruskal–Wallis tests for nonnormally distributed continuous variables. Post hoc tests were conducted for variables for which overall group differences were observed to test the statistical significance of differences among subgroups, using the Holm method of adjustment for multiple testing.³⁹ Associations of CMV with sociodemographic and clinical variables and inflammatory markers were assessed using Spearman rank correlation coefficients for comparison of two nonnormally distributed continuous variables and Wilcoxon tests or Kruskal–Wallis tests for nonparametric comparison of CMV IgG distribution across two or more categories. Nonparametric tests were used due to significant skewness in the distribution of inflammatory markers and CMV IgG, which was not improved by transformation. Spearman partial correlations were used to assess associations among variables adjusted for potential confounders (age, CD4 count, viral load, and HCV).

Confounders were selected for analysis based on previous literature and a priori hypotheses about associations with CMV and/or inflammatory markers and associations observed in exploratory analysis. We do not present multivariable regression results because of the small sample size, exploratory nature of the study, and failure of several of the inflammatory markers to meet distributional assumptions necessary for linear regression. To account for multiple tests of significance in assessing associations of CMV IgG with inflammatory markers within each of the three subgroups, false discovery rate adjusted *p*-values were calculated using the Benjamini and

Hochberg method.⁴⁰ Analyses were conducted using SAS software version 9.3 (SAS Institute, Cary, NC).

Results

Study demographics

The median age (51.0 years) of the HIV-1 aviremic group was higher ($p < 0.001$) than the median age of either the HIV-1 viremic group (43.5) or the HIV-1-uninfected group (44.5) (Table 1). The racial distribution among the groups was similar with the majority of the women being African American. A significantly higher percentage of the HIV-1-uninfected women was currently employed, while the HIV-1 viremic group had the lowest percentage of employment ($p = 0.010$). The HIV-1 viremic group had a lower household income ($p = 0.035$) and less education ($p = 0.06$) than the other two groups. There were no significant differences among the groups for the time of WIHS enrollment (1994/1995, 2001/2002, 2011/2012).

Markers of CMV infection

For this study only the women who were CMV seropositive were included, because only 11 CMV-seronegative women were identified in the eligible cohort. Quantitative levels of CMV IgG were significantly higher in the HIV-1 viremic group compared to the other two groups ($p = 0.009$) (Table 1). Only five women (3.0%) had detectable CMV DNA in plasma, which was not associated with HIV-1 RNA or CMV IgG levels, although the power to detect differences was limited by the small number of women. These data are consistent with previous reports that CMV DNA is infrequently detected in plasma of patients with CD4 counts >200 cells/mm³.^{32,41} Thirty-one women (18.9%) had measurable CMV DNA in CVL with a significantly higher proportion among HIV-1 viremic women (Table 1). There was no association between plasma and CVL CMV DNA (Spearman $r = 0.005$; $p = 0.950$). Among those with detectable CMV DNA in CVL ($n = 31$) there was no association between CMV DNA in CVL and CMV IgG (Spearman $r = 0.02$; $p = 0.902$). CMV DNA in CVL was also not associated with HIV RNA in plasma (Spearman $r = 0.13$; $p = 0.575$) or HIV RNA in CVL (Spearman $r = 0.2$; $p = 0.800$).

Although CMV DNA was detected more frequently in CVL than in plasma (Table 1), we found that CVL had a much lower level of detection and copy number of CMV DNA compared to results reported for semen.^{29,30,32} This could be an effect of dilution from the lavage technique in which a 10 ml volume of saline is injected to collect the secreted virus.

Clinical characteristics of the study cohort

There were significant differences in T cell counts among the three groups (Table 1). Not surprisingly, CD4⁺ T cell counts were significantly higher in the HIV-1-uninfected group compared to each of the HIV-1-infected groups, and for HIV-1 avireemics the counts were significantly higher than for the HIV-1 viremic women (all *p* values < 0.001). Conversely, the CD8⁺ T cell counts were significantly higher for the viremic women compared to the HIV-negative women ($p < 0.001$). As predicted, the median CD4⁺/CD8⁺ ratio for the HIV-1-uninfected group was the highest (2.13) followed

TABLE 1. PATIENT CHARACTERISTICS BY HIV-1 STATUS, *N* = 164

	<i>HIV uninfected</i> (n = 54)	<i>HIV infected aviremic</i> (n = 58)	<i>HIV infected viremic</i> (n = 52)	
<i>Characteristic</i>	<i>Median (IQR) or n (%)</i>	<i>Median (IQR) or n (%)</i>	<i>Median (IQR) or n (%)</i>	p-value ^a
Sociodemographic variables				
Age, years ^{*,†}	44.5 (39–52)	51 (45–55)	43.5 (37–49)	<0.001
Race/ethnicity				
African American/black	45 (83.3)	44 (75.9)	46 (88.5)	0.229
Hispanic	7 (13.0)	6 (10.3)	3 (5.8)	
White/other	2 (3.7)	8 (13.8)	3 (5.8)	
Currently employed [†]	25 (47.2)	20 (34.5)	10 (19.2)	0.010
Annual household income <\$18,000	34/54 (63.0)	35/56 (62.5)	37/44 (84.1)	0.035
Education less than high school	16 (29.6)	15 (25.9)	24 (46.2)	0.060
Clinical variables				
CMV IgG, IU/ml ^{*,†}	32.9 (21.2–39.5)	31.7 (21.4–39.9)	38.6 (32.0–43.1)	0.009
CMV DNA in CVL				
Present, <i>n</i> (%) [*]	11 (20.4)	5 (8.62)	15 (28.9)	0.024
Median (IQR) ^b	658.0 (393.0–1365.0)	569.0 (355.0–584.0)	1378.0 (595.0–2587.0)	0.196
CMV DNA in plasma (%)	1 (1.9)	3 (5.2)	1 (1.9)	0.622
CESD ^c ≥16	13 (24.1)	17 (29.3)	22 (44.0)	0.080
Current smoker	29 (53.7)	24 (41.4)	29 (56.9)	0.226
Diabetes ever ^d	8 (14.8)	17 (29.3)	11 (21.2)	0.178
Hypertension (current) ^e	23 (42.6)	31 (53.5)	24 (46.2)	0.501
BMI ^f	31.0 (26.6–36.8)	29.6 (25.1–34.0)	25.6 (22.8–33.0)	0.037
eGFR ^g	92.0 (78.5–103.1)	86.1 (73.9–98.5)	93.9 (80.1–114.3)	0.105
HCV positive by Ab or RNA	6 (11.3)	13 (22.4)	12 (23.5)	0.209
CD4 ⁺ T cell count (cells/mm ³) ^{*,†,‡}	1046 (843–1224)	763.5 (581–958)	321.5 (178–515)	<0.001
CD8 ⁺ T cell count (cells/mm ³) ^{†,‡}	468 (357–606)	737.5 (529–1003)	781.5 (463–1135)	<0.001
CD4/CD8 ratio ^{*,†,‡}	2.13 (1.59–2.77)	1.16 (0.77–1.50)	0.36 (0.26–0.66)	<0.001
Raltegravir use (current)	NA	12 (20.7)	5 (9.6)	0.109
Any ART use (current) [*]	NA	55 (94.8)	28 (53.9)	<0.001
Adherence ≥95% ^{b,*}	NA	51/55 (92.7)	13/28 (46.4)	<0.001
log ₁₀ HIV-1 RNA in plasma, median (IQR)	NA	NA	4.4 (3.7–4.8)	NA
log ₁₀ HIV-1 RNA in CVL, median (IQR) ⁱ	NA	NA	2.6 (2.3–3.1)	NA
>40 copies/ml, <i>n</i> (%)	NA	1/58 (1.72)	21/51 (41.2)	<0.001
<40 copies/ml, <i>n</i> (%)	NA	0/58 (0.00)	6/51 (11.8)	
Negative, <i>n</i> (%)	NA	57/58 (98.3)	24/51 (47.1)	
Inflammatory markers				
IL-6 Plasma [*]	1.96 (1.29–4.28)	1.22 (0.79–3.61)	2.34 (1.45–6.13)	0.030
IL-6 CVL	0.91 (0.16–4.01)	0.48 (0.16–2.22)	1.51 (0.25–7.05)	0.063
IP10 ^{*,†,‡}	156.51 (114.05–205.58)	230.70 (190.20–372.00)	527.89 (348.80–709.82)	<0.001
sCD14 ^{*,†,‡}	5,868.85 (5,163.93–6,803.28)	7,339.29 (5,428.57–9,280.37)	8,805.19 (6,785.71–12,194.81)	<0.001
sCD163 ^{*,†,‡}	49.19 (36.00–62.74)	63.69 (46.25–81.83)	86.05 (69.38–111.42)	<0.001

^a*p*-values were calculated by Kruskal–Wallis test for continuous variables and chi-square tests for categorical variables (Fisher's exact test for cell sizes <5), and represent the overall test for differences between the three groups.

^bAmong those with CMV DNA present in CVL (*n* = 31).

^cCenters for Epidemiologic Studies–Depression.

^dEver self-reported antidiabetic medication or any of fasting glucose ≥126 or hemoglobin A_{1c} ≥6.5% or self-reported diabetes is confirmed.

^eAny indication of hypertension (SBP ≥140, DBP ≥90, self-report, or use of antihypertensive medications) at visit.

^fBody mass index.

^gEstimated glomerular filtration rate, ml/min/1.73 m²: $GFR = 186 \times (Pcr)^{-1.154} \times (age)^{-0.203} \times (0.742 \text{ if female}) \times (1.210 \text{ if black})$.

^hAmong those on ART.

ⁱAmong those with HIV-1 RNA present in CVL (*n* = 22).

^{*}*p* < 0.05 for comparison of HIV-infected viremic vs. aviremic.

[†]*p* < 0.05 for comparison of HIV-infected viremic vs. HIV uninfected.

[‡]*p* < 0.05 for comparison of HIV-infected aviremic vs. HIV uninfected.

CMV, cytomegalovirus; CVL, cervicovaginal lavage; eGFR, estimated glomerular filtration rate; HCV, hepatitis C virus; ART, antiretroviral therapy; IL-6, interleukin 6; IP10, interferon gamma-induced protein.

by the HIV-1 aviremic group (1.16) and the viremic group (0.36). The differences were all significant ($p < 0.001$).

Nearly all HIV-1 aviremic women reported currently using cART, compared to just over half of the HIV-1 viremic women. Therefore, HIV-1 aviremic women were significantly more likely to be on cART than viremic women ($p < 0.001$) (Table 1). We noted that there were three women in the aviremic group who were not on cART. These apparent elite controllers were included in the analyses shown in Tables 2 and 3, but we later ran the analysis excluding them and the results were similar.

The HIV-1 viremic group had a median plasma HIV-1 RNA copy number of 25,906/ml. Among women on cART at

the time of the study, there was no difference in duration of therapy by group; the median time since cART initiation was 12.4 years (range 3.0–17.1 years) among HIV-1 viremic women and 11.3 years (range 1.9–17.2 years) among HIV-1 aviremic women ($p = 0.487$).

Raltegravir has been reported to have antiviral activity against CMV.⁴² Compared to HIV-1 viremic women, more women in the HIV-1 aviremic group were currently using raltegravir, although the difference was not statistically significant. There was no correlation between CMV IgG and raltegravir use among HIV-1-positive women currently on cART (data not shown).

TABLE 2. ASSOCIATION OF CYTOMEGALOVIRUS IMMUNOGLOBULIN G WITH SOCIODEMOGRAPHIC AND CLINICAL VARIABLES BY HIV-1 STATUS

	<i>HIV uninfected</i> (n = 54)		<i>HIV-1 infected</i> <i>aviremic</i> (n = 58)		<i>HIV-1 infected</i> <i>viremic</i> (n = 52)	
	ρ^a	p-value	ρ^a	p-value	ρ^a	p-value
Continuous variables						
Age, years	0.41	0.002	0.20	0.138	0.34	0.014
CESD	-0.22	0.107	0.11	0.430	-0.08	0.601
BMI	0.18	0.196	0.03	0.810	-0.18	0.234
eGFR	-0.29	0.033	0.002	0.989	-0.48	<0.001
CD4 ⁺ T cell count (cells/mm ³)	0.18	0.204	-0.22	0.101	-0.24	0.088
CD8 ⁺ T cell count (cells/mm ³)	0.12	0.396	0.12	0.382	-0.15	0.300
CD4/CD8 ratio	0.02	0.898	-0.30	0.022	-0.15	0.295
log ₁₀ HIV-1 RNA in plasma	NA	NA	NA	NA	0.13	0.360
log ₁₀ HIV-1 RNA in CVL	NA	NA	NA	NA	0.09	0.516
	<i>HIV uninfected</i> (n = 54)		<i>HIV-1 infected</i> <i>aviremic</i> (n = 58)		<i>HIV-1 infected</i> <i>viremic</i> (n = 52)	
	Median (IQR)	p-value ^b	Median (IQR)	p-value ^b	Median (IQR)	p-value ^b
Categorical variables^c						
Race/ethnicity						
African American/black	34.3 (22.7–39.8)	0.030	34.9 (22.2–40.2)	0.508	38.2 (31.9–45.0)	0.656
Hispanic	16.7 (9.4–23.1)		28.8 (22.7–33.5)		35.1 (24.3–39.6)	
White/other	66.8 (23.5–110.2)		25.0 (16.6–65.2)		39.2 (38.4–42.5)	
Current smoker						
Yes	32.3 (22.6–35.7)	0.311	37.3 (26.5–40.2)	0.209	40.0 (33.0–45.0)	0.029
No	35.4 (16.7–42.3)		30.1 (21.0–39.3)		35.5 (26.2–39.2)	
Diabetes ever ^d						
Yes	34.0 (18.3–41.0)	0.884	34.2 (20.4–41.8)	0.852	38.4 (28.4–40.3)	0.609
No	32.9 (21.2–39.5)		31.1 (22.7–39.9)		38.7 (32.1–45.0)	
Hypertension (current) ^e						
Yes	35.7 (32.3–41.5)	0.015	37.5 (21.7–42.9)	0.081	42.6 (32.2–90.7)	0.046
No	25.6 (13.4–35.9)		30.9 (20.3–37.1)		36.3 (30.1–39.6)	
HCV positive						
Yes	32.84 (29.00–35.69)	0.900	38.86 (30.85–41.27)	0.151	41.99 (39.39–45.67)	0.033
No	34.04 (16.71–40.03)		30.53 (21.42–38.44)		35.96 (26.17–42.99)	
HIV-1 VL in CVL						
>40 copies/ml	NA	NA	NA	NA	38.8 (34.8–43.3)	0.655
<40 copies/ml	NA		NA		38.2 (33.0–40.0)	
Negative	NA		NA		37.6 (26.0–43.6)	

^aSpearman rank correlation coefficient.

^bp-values by Wilcoxon rank-sum test.

^cCurrent employment, annual income, and education were not significant in any of the subgroups and are not shown in the table.

^dEvery self-reported antidiabetic medication or any fasting glucose ≥ 126 or hemoglobin A_{1c} $\geq 6.5\%$ or self-reported diabetes is confirmed.

^eAny indication of hypertension (SBP ≥ 140 , DBP ≥ 90 , self-report, or use of antihypertensive medications) at visit.

TABLE 3. UNADJUSTED AND ADJUSTED ASSOCIATIONS BETWEEN CYTOMEGALOVIRUS AND INFLAMMATORY MARKERS BY HIV-1 STATUS

	<i>HIV uninfected, N=54</i>									
	<i>IL-6 plasma</i>		<i>IL-6 CVL</i>		<i>sCD1</i>		<i>sCD163</i>		<i>IP10</i>	
	ρ^a	p-value	ρ^a	p-value	ρ^a	p-value	ρ^a	p-value	ρ^a	p-value
Unadjusted association	0.24	0.081	-0.13	0.344	0.10	0.471	0.10	0.470	0.21	0.133
False discovery rate		0.238		0.430		0.471		0.471		0.249
adjusted p-value										
Adjustment variables										
Age	0.20	0.148	0.05	0.717	-0.02	0.861	-0.005	0.974	0.002	0.990
CD4	0.17	0.247	-0.19	0.191	0.12	0.409	0.08	0.572	0.21	0.145
HCV	0.24	0.092	-0.17	0.235	0.12	0.390	0.08	0.594	0.19	0.168
BMI	0.18	0.190	-0.17	0.223	0.11	0.423	0.04	0.774	0.19	0.175
eGFR	0.17	0.224	-0.04	0.778	0.03	0.807	0.08	0.586	0.20	0.162
Smoking	0.21	0.136	-0.18	0.202	0.11	0.442	0.09	0.534	0.20	0.141
Hypertension	0.15	0.286	-0.12	0.378	0.04	0.801	0.003	0.985	0.12	0.405
Black race	0.20	0.159	-0.10	0.498	0.07	0.634	0.07	0.613	0.21	0.139
<i>HIV aviremic, N=58</i>										
	<i>IL-6 Plasma</i>		<i>IL-6 CVL</i>		<i>sCD14</i>		<i>sCD163</i>		<i>IP10</i>	
	ρ^a	p-value	ρ^a	p-value	ρ^a	p-value	ρ^a	p-value	ρ^a	p-value
Unadjusted association	0.21	0.114	-0.10	0.450	0.27	0.039	0.17	0.193	0.15	0.261
False discovery rate		0.244		0.471		0.146		0.290		0.356
adjusted p-value										
Adjustment variables										
Age	0.20	0.133	-0.05	0.702	0.23	0.082	0.12	0.370	0.11	0.424
CD4	0.24	0.069	-0.09	0.504	0.29	0.026	0.15	0.281	0.13	0.323
HCV	0.17	0.213	-0.10	0.479	0.27	0.041	0.15	0.251	0.09	0.524
BMI	0.21	0.119	-0.12	0.378	0.30	0.025	0.17	0.203	0.15	0.257
eGFR	0.21	0.116	-0.11	0.436	0.28	0.038	0.18	0.190	0.15	0.259
Smoking	0.21	0.126	-0.09	0.488	0.26	0.049	0.20	0.127	0.16	0.231
Hypertension	0.15	0.274	-0.13	0.332	0.26	0.047	0.14	0.304	0.12	0.356
Black race	0.21	0.116	-0.09	0.499	0.30	0.022	0.19	0.168	0.18	0.187
<i>HIV viremic, N=52</i>										
	<i>IL-6 plasma</i>		<i>IL-6 CVL</i>		<i>sCD14</i>		<i>sCD163</i>		<i>IP10</i>	
	ρ^a	p-value	ρ^a	p-value	ρ^a	p-value	ρ^a	p-value	ρ^a	p-value
Unadjusted association	0.31	0.023	-0.32	0.021	0.43	0.002	0.23	0.095	0.20	0.150
False discovery rate		0.115		0.115		0.030		0.238		0.250
adjusted p-value										
Adjustment variables										
Age	0.26	0.064	-0.26	0.069	0.33	0.018	0.16	0.252	0.15	0.286
CD4	0.30	0.036	-0.30	0.036	0.40	0.005	0.23	0.114	0.16	0.277
Viral load	0.29	0.036	-0.33	0.021	0.42	0.002	0.20	0.160	0.16	0.264
cART since last visit	0.28	0.047	-0.23	0.105	0.35	0.011	0.29	0.040	0.20	0.150
HCV	0.28	0.045	-0.25	0.082	0.39	0.005	0.16	0.272	0.12	0.424
BMI	0.31	0.033	-0.33	0.024	0.39	0.007	0.23	0.127	0.19	0.193
eGFR	0.20	0.151	-0.26	0.064	0.24	0.087	0.08	0.590	0.16	0.262
Smoking	0.24	0.091	-0.30	0.034	0.36	0.011	0.25	0.084	0.17	0.234
Hypertension	0.24	0.083	-0.35	0.012	0.39	0.005	0.23	0.111	0.18	0.216
Black race	0.31	0.025	-0.33	0.021	0.43	0.002	0.24	0.092	0.20	0.152

^aSpearman rank correlation coefficient.

HCV has been reported to have an impact on T cell phenotypes in HIV-1-infected persons.⁴³ Although there was no significant difference in HCV serostatus between the groups, there was a significant association of HCV seropositivity with CMV IgG levels among HIV-1 viremic women.

HIV-1 RNA was analyzed in CVL supernatant fluids. HIV-1 viremic women were much more likely to have detectable HIV-1 RNA in CVL than HIV-1 aviremic women. Only one woman with undetectable HIV-1 RNA in plasma had detectable HIV-1 RNA in CVL (Table 1).

Biomarkers of inflammation and immune activation associated with HIV-1 status

Plasma biomarkers IL-6, sCD14, sCD163, and IP10 and CVL IL-6 were measured and compared among the three groups of women. Levels of these markers measured in the HIV-1 viremic group were significantly higher than those in either the HIV-1 aviremic or the uninfected groups (Table 1). The sCD14, sCD163, and IP10 values followed a significant trend with the highest median value found in the HIV-1 viremic group followed by the aviremic group, and the lowest median value in the uninfected group (all p values <0.001) (Table 1). Plasma IL-6 levels in the HIV-1 viremic group were higher than in the other groups, however HIV-1 aviremic women had lower levels than uninfected women. A similar pattern was found for CVL IL-6.

Association of CMV IgG with sociodemographic and clinical characteristics

After establishing differences among the three groups related to sociodemographic and clinical characteristics, we examined associations with CMV IgG levels (Table 2). Significant negative associations between CMV IgG levels and eGFR were found for HIV-1-uninfected and HIV-1 viremic women. The CD4⁺/CD8⁺ cell ratio was significantly associated with CMV IgG levels in aviremic women ($p=0.022$).

Age was significantly associated with CMV IgG for the HIV-1 viremic women and HIV-1-uninfected women. African American race among HIV-1-uninfected women ($p=0.03$), current smoking among HIV-1 viremic women ($p=0.029$), and hypertension among both HIV-1-uninfected ($p=0.046$) and HIV-1 viremic ($p=0.015$) women were significantly associated with CMV IgG levels (Table 2).

Biomarkers of inflammation and immune activation associated with CMV IgG

We investigated the association of CMV IgG with the selected biomarkers of activation and inflammation and also with HIV-1 status/viremia. For HIV-1-uninfected women, there were no significant unadjusted associations between CMV IgG levels and plasma levels of IL-6, sCD14, sCD163, and IP10 or CVL levels of IL-6 (Table 3).

Among HIV-1 aviremic women, the unadjusted correlation of sCD14 with CMV IgG levels was 0.27 (raw $p=0.039$; FDR adjusted $p=0.15$); the magnitude of the correlation was similar when single variable adjustments were made for CD4 count, HCV antibody positivity, BMI, smoking, hypertension, eGFR, and black race (p -values <0.05). Among HIV-1 viremic women, there was a significant unadjusted positive association of plasma IL-6 ($p=0.023$) and negative association of CVL IL-6 ($p=0.021$) with CMV IgG. The FDR adjusted was $p=0.115$ for both. The magnitude of the correlations for plasma IL-6 was similar when single adjustments were made for CD4, viral load, cART, HCV, and BMI. For CVL IL-6 the correlations remained significant with single adjustments for CD4, viral load, BMI, and smoking.

Among HIV-1 viremic women, sCD14 had the strongest (Spearman $r=0.3$ – 0.4) and most consistently significant positive association with CMV IgG ($r=0.43$; raw $p=0.002$; FDR adjusted $p=0.030$), which remained significant with adjustment for age, CD4 count, HIV-1 viral load, cART use,

HCV infection, smoking, hypertension, BMI, and black race ($p\leq0.018$ for all; Table 3). Significance or a trend toward significance for sCD14 also remained with adjustments for multiple variables: age plus CD4 ($p=0.051$), age plus HIV-1 viral load ($p=0.025$), or age plus HCV ($p=0.022$) (data not shown). However, after adjustment for eGFR the association of CMV IgG with sCD14 was attenuated ($r=0.24$; $p=0.087$).

Discussion

In this study of HIV and CMV coinfection, we found significant differences in levels of markers of inflammation and immune activation among three groups of women enrolled in the WIHS: HIV uninfected, HIV infected aviremic, and HIV infected viremic. Viremic women had the highest levels of all markers among the three groups. The unadjusted association of these markers with CMV IgG among aviremic women was statistically significant as was adjustment for single variables CD4 counts, HCV, BMI, eGFR, smoking, hypertension, and race. Unadjusted and adjusted associations for these same single variables among viremic women were all significant with p values ≤0.025 , except for eGFR. In contrast, there were no significant associations between CMV IgG and any of the biomarkers among HIV-1-uninfected CMV-seropositive women.

We noted that among viremic women the correlation of sCD14 and CMV IgG was attenuated with the adjustment for eGFR. A significant negative association between CMV IgG levels and eGFR was reported in a study of elderly HIV-1-uninfected adults.⁴⁴ A significant negative association between sCD14 and eGFR was reported in a study of patients with chronic kidney disease.⁴⁵ These associations as well as our data call for future investigation to determine the relationship among CMV IgG, sCD14, and eGFR.

Our results differ from others in that the group that showed the strongest association between sCD14 and CMV IgG levels was HIV-1 viremic.^{15,16,21} However, a limitation of our study is the lack of a CMV-/HIV-1⁺ group of women, which is difficult to acquire with the high seroprevalence of CMV among HIV-1-infected persons. A second limitation is the cross-sectional design of the study. Classifications of HIV viremic and aviremic might change at different time points as a result of the degree of adherence to therapy or the effectiveness of drug regimens. In addition, the level of HIV viral load among the viremic women ranged from approximately 5,000 to 63,000 HIV RNA copies per ml, which may have led to residual confounding of the association between CMV IgG and inflammatory markers by HIV viremia, although the association of sCD14 and CMG IgG levels among viremic women remained significant after controlling for HIV RNA.

Finally, we cannot rule out the role of other viral co-pathogens in increased levels of IgG and biomarkers of inflammation and immune activation. There have been reports that HIV infection affects B cell function leading to non-specific hypergammaglobulinemia.^{18,46} Brunt *et al.*¹⁸ reported elevated levels of CMV and Epstein–Barr virus (EBV) antibodies over a period of 10 years after initiation of cART in a small group of nine CMV-seropositive patients. sCD14 levels were not associated with CMV antibody over this time period. Burdo *et al.*²² found a positive correlation of sCD163 in HIV-1-infected patients with CMV IgG and sCD14 but no correlation with EBV IgG. Although we did not test for IgG

specific for other herpesviruses, we have participated in a study of CMV pathogenesis in another WIHS cohort,⁹ in which quantitation of EBV-specific antibody showed no association of EBV IgG with CMV IgG-associated pathogenesis. The exploratory nature of the present study limited our ability to investigate the interaction of other viruses with HIV in HIV-infected patients; however, our data have led us to focus on the association between CMV and sCD14.

sCD14 is a receptor for lipopolysaccharide⁴⁷ and a marker of microbial translocation and monocyte activation, which has been reported to be an independent predictor of non-AIDS-defining disease in HIV-1-infected patients.^{15,17,33} Among these studies, sCD14 was reported to be associated with coronary calcification, an indicator of atherosclerosis in HIV-1-infected patients on cART.¹⁵ It was also reported that in HIV-1-infected patients, sCD14 together with gut epithelial barrier dysfunction was predictive of increased mortality.³³

The monocyte appears to play a central role in HIV-1 pathogenesis. Monocyte activation may persist even with successful cART, and monocytes may be the vector for transmission of viruses to the central nervous system.^{4,11,23} Nonclassical monocyte phenotypes (CD14⁺⁺/CD16⁺ or CD14⁺/CD16⁺⁺) were found to be activated by exposure to HIV-1 and have also been reported to be infected by HIV-1.⁴

At the same time, monocytes are known to be sites of CMV latency,⁴⁸ although they are not permissive for virus replication. In the absence of HIV-1, induction of replicating CMV has been shown to occur with the terminal differentiation of monocytes to myeloid dendritic cells,⁴⁹ and *in vitro* studies have demonstrated that monocyte to macrophage differentiation leads to production of infectious virus.^{50–52} If HIV-1 infection activates monocytes and macrophages,⁵³ it is likely that CMV could be reactivated and replicate leading to an inflammatory response. In our study the association of CMV IgG with sCD14 within the HIV-1 viremic group, which was not present in the HIV-uninfected group, suggests that coinfection and long-term interaction of HIV-1 and CMV may lead to the development of serious non-AIDS events.

Non-AIDS-defining morbidity and mortality have also been associated with increased CD8⁺ T cells and a decreased ratio of CD4⁺/CD8⁺, which remains low in patients who have been successfully treated with cART.^{10,34} Our data support these findings, suggesting that the ratio did not return to normal despite control of the HIV-1 viral load. Of note is the significant negative association of the CD4⁺/CD8⁺ ratio with CMV IgG among the group of aviremic women (Table 2). This could be the result of enhancement of CD8⁺ T cell responses to CMV.^{25,54}

A low CD4⁺/CD8⁺ ratio has also been shown to correlate inversely with sCD14,¹⁰ which provides a potential link between innate immune activation and elevated CD8⁺ T cell counts through inflammation. Finally, CMV infection alone has been associated with driving an inverted CD4⁺/CD8⁺ ratio in older persons.^{55,56} The eventual outcome of these clinical correlates may be an increase in non-AIDS-associated morbidity and mortality.¹⁷

From the results of this study, we propose a model of HIV-1 and CMV coinfection, based on the significant association of CMV IgG with sCD14 levels in women who are HIV-1 viremic and CMV seropositive. CMV primary infection results in asymptomatic latency in multiple cell types including monocytes.⁴⁸ HIV-1 infection activates monocytes, which

may lead to reactivation of CMV from latency in these cells. In addition, differentiation of monocytes to macrophages produces a cell that is permissive for CMV replication.⁵⁰ Reactivation of CMV may result in production of biomarkers of inflammation and immune activation such as sCD14, IL-6, sCD163, and IP10.^{22,47,51,57} An increase in CMV IgG and CMV-specific T cells may result in temporary control of infection, but the potential for additional cycles of reactivation and inflammation would be predicted to result in cellular and tissue damage. The extent of the damage would depend on the body compartment(s) involved and the magnitude and frequency of the immunological response to the infections. The results that we report suggest that future studies should include *in vitro* analysis of HIV/CMV coinfection in monocytes with activation to macrophages, as well as analysis of coinfection in monocytes from HIV/CMV-seropositive patients.

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Author Disclosure Statement

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