Cytomegalovirus Immunoglobulin G Antibody Is Associated With Subclinical Carotid Artery Disease Among HIV-Infected Women

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(See the editorial commentary by Aiello and Simanek, on pages 1772–4.)

Background. Cytomegalovirus (CMV) infection has been implicated in immune activation and accelerated progression of immunodeficiency virus (HIV) coinfection. We hypothesized that CMV is associated with vascular disease in HIV-infected adults.

Methods. In the Women’s Interagency HIV Study, we studied 601 HIV-infected and 90 HIV-uninfected participants. We assessed the association of CMV immunoglobulin G (IgG) level with carotid artery intima-media thickness, carotid artery distensibility, Young’s elastic modulus, and blood pressures. Multivariable models adjusted for age, race/ethnicity, smoking, diabetes, and body mass index.

Results. Mean CMV IgG levels were higher in HIV-infected women compared with HIV-uninfected women (P < .01). Among HIV-infected women, higher CMV IgG level was associated with decreased carotid artery distensibility (P < .01) and increased Young’s modulus (P = .02). Higher CMV IgG antibody level was associated with increased prevalence of carotid artery lesions among HIV-infected women who achieved HIV suppression on antiretroviral therapy, but not among viremic or untreated HIV-infected women. Adjustment for Epstein–Barr virus antibody levels and C-reactive protein levels had no effect on the associations between CMV IgG levels and vascular parameters.

Conclusions. Cytomegalovirus antibody titers are increased in HIV-infected women and associated with subclinical cardiovascular disease. Host responses to CMV may be abnormal in HIV infection and associated with clinical disease.

Cytomegalovirus (CMV) is a β human herpesvirus that after primary infection remains latent or persistent within the host over the life course [1]. In the general population, CMV infection has been linked with the development of cardiovascular diseases [2–8] and all-cause and cardiovascular disease mortality [9].

Human immunodeficiency virus (HIV)–infected individuals have an increased risk of cardiovascular events and may have more advanced subclinical cardiovascular disease when compared with HIV-uninfected controls. Cytomegalovirus viremia predicts increased mortality in treated HIV-infected patients [10], and recent evidence suggests that CMV coinfection may contribute to cardiovascular complications of HIV infection. In a previous study among HIV-infected men, we showed that those who had a higher percentage of CD8+ T cells producing interferon gamma in response to CMV had increased carotid artery wall thickness, which is a measure of subclinical vascular disease [8]. Several potential mechanisms may explain the link between CMV infection and vascular disease, including the role of chronic CMV infection or CMV reactivation in promoting immune...
activation [11–14], inflammation [2, 15], release of angiogenic growth factors and extracellular matrix-degrading enzymes [15], and hypercoagulability [15–17]. These mechanisms may affect not only atherosclerosis but also other subclinical manifestations of vascular disease that are increased in HIV-infected adults, including arterial stiffness and blood pressure alterations [18–22]. In the current study, we hypothesized that circulating CMV immunoglobulin G (IgG) antibody titers would be associated with subclinical measures of vascular disease in HIV-infected women. Furthermore, because the response to CMV appears to be amplified with effective antiretroviral treatment [23, 24], we studied whether treatment status modified the association between CMV and subclinical atherosclerosis.

METHODS

Study Population
The Women’s Interagency HIV Study (WIHS) cohort consists of HIV-infected women and HIV-uninfected controls enrolled at 6 US field centers [25]. All WIHS participants are invited to complete study visits every 6 months for collection of biological specimens, questionnaire data, and clinical measurements. A carotid artery ultrasound substudy, which was initiated in April 2004, was completed by 75% of both HIV-infected and HIV-uninfected groups. The present study of CMV included the first 644 HIV-infected women who were enrolled in the longitudinal phase of the carotid artery substudy, as well as 100 HIV-uninfected women who were frequency-matched to the HIV-infected women on age and race/ethnicity. We then limited analyses to the 601 HIV-infected and 90 HIV-uninfected women who were CMV IgG seropositive. Institutional review board approval and informed consent were obtained on all participants.

Carotid Artery Ultrasound
Standardized high-resolution B-mode carotid artery ultrasound methodology was used to image the far wall of the right common carotid artery, internal carotid artery, and bifurcation (patents 2005, 2006, 2011) [26–28]. Standardized carotid artery ultrasound images were centrally measured by automated computerized edge detection software (patents 2005, 2006, 2011) [26–28]. The following carotid artery parameters were measured: intima-media thickness (cIMT) of the far wall of the right common carotid artery; carotid artery lesions, defined as the presence of focal cIMT >1.5 mm in any of the imaged carotid artery segments; carotid artery distensibility, calculated using carotid artery diameters at systole (Ds) and diastole (Dd) and brachial artery pulse pressure, all measured on the same occasion, as Distensibility = (2(Ds − Dd)/Ds) × 106/133.3, in units of (10−6 × Newtons−1 × meters2) [19, 26, 29]; and Young’s elastic modulus: PP/DD × 0.5 × Dd/cIMTd, where PP = pulse pressure, DD = percent arterial dilation over the cardiac cycle and cIMTd = cIMT at diastole [26]. For calculating distensibility and Young’s elastic modulus, we used blood pressure levels measured simultaneously with carotid artery ultrasound. At a separate visit, conducted within approximately 6 months of the carotid artery ultrasound, seated brachial artery blood pressures were measured again; these were used for the analyses that defined systolic blood pressure, diastolic blood pressure, and pulse pressure per se as the outcome. By repeating carotid ultrasound and blood pressure measurements on a subset of participants (n = 115) at each field center, we estimated the coefficient of variation as 1.8% for cIMT (intraclass correlation coefficient [ICC], 0.98), 2.2% for carotid diameters (ICC, 0.96), and 8.8% for blood pressures (ICC, 0.65–0.73).

Assays
HIV infection was determined via serologic testing using enzyme-linked immunosorbent assay (ELISA) and confirmed using Western blot assays. Plasma HIV RNA levels were quantified using nucleic acid sequence-based amplification commercial assays with a lower limit of quantification of 80 copies/mL (bioMérieux), and total peripheral CD4+ T-cell counts were measured with standard flow cytometric methods. High-sensitivity C-reactive protein (CRP) levels were measured using nephelometry (Dade Behring).

Cytomegalovirus IgG antibody levels were determined using the CMV ELISA Quantitation Kit (GenWay Biotech, Inc.). A 2.5-µL volume of each serum sample was diluted 1:80 to obtain samples within the readable range of the assay. Binding of CMV-specific IgG antibodies to the antigen-coated wells was detected by horseradish peroxidase-conjugated goat antihuman IgG followed by addition of enzyme substrate 3,3′,5′,5′-tetramethylbenzidine. The optical density of the colored product was read at 450 nm using a DuPont Kinetic Microplate Reader (Molecular Devices). Kits from the same lot number were used for all of the samples. The intra-assay coefficient of variation (CV) for CMV IgG levels ranged 2.4%–8.0% and the interassay CV ranged 5.2%–9.9%. We measured Epstein–Barr virus (EBV) IgG antibody levels using the EBV VCA IgG ELISA (GenWay). A 5-µL volume of each serum sample was diluted 1:100. The procedure for detection of EBV-specific IgG antibodies was the same as that described above for CMV IgG. The intra-assay CV was 9.4%, and the interassay CV ranged 1.6%–14.0%. All samples were tested with kits from the same lot number. CD4+ and CD8+ T-cell activation (frequency of cells expressing CD38 and HLA-DR) were measured in thawed peripheral blood mononuclear cells as previously described [20]. CRP, CMV IgG, EBV IgG, and T-cell activation markers were measured at the time of the carotid artery ultrasound examination.

Variable Definitions
Study variables included age at visit (in years); current smoking status (smoker, nonsmoker); race/ethnicity (African
American/black, Hispanic, white/other); diabetes (defined as fasting glucose levels ≥126 mg/dL, self-reported physician’s diagnosis of diabetes, or use of diabetes medications); body mass index (BMI, kg/m²); current CD4⁺ T cell count (cells/µL); HIV RNA level (copies/mL); nadir CD4⁺ T cell count (cells/µL) since study baseline; study site; and self-reported protease inhibitor, nonnucleoside reverse transcriptase inhibitor, and nucleoside reverse transcriptase inhibitor use. HIV-infected subjects were categorized into 3 mutually-exclusive subgroups: (1) current users of antiretroviral medications who had HIV RNA below the lower limit of detection of 80 copies/mL (treated/aviremic); (2) current users of antiretroviral medications who had HIV RNA above the lower limit of detection of 80 copies/mL (treated/viremic); and (3) nonusers of antiretroviral medications (untreated).

Statistical Analyses
We examined characteristics in the study population by HIV infection, HIV treatment, and viremia status. To determine statistically significant differences in characteristics across groups, we used χ² tests for categorical variables, and Student’s t tests or analysis of variance for continuous variables. We used the Mann–Whitney–Wilcoxon or Kruskal–Wallis tests for non-normally distributed variables. In preliminary analyses, we examined unadjusted associations of CMV IgG with current and nadir CD4⁺ T-cell count, HIV RNA, CRP, BMI, age, smoking, race/ethnicity, and diabetes. We used Pearson correlations, with log transformation for skewed variables including HIV RNA and CRP, and Student’s t tests for assessing the association of CMV IgG with categorical variables. We then used Pearson partial correlations to examine age-adjusted associations between CMV IgG and measures of subclinical cardiovascular disease. The main analyses examined the covariate-adjusted association between CMV IgG and cardiovascular outcome variables. For continuous outcome variables, including cIMT, carotid artery distensibility, Young’s elastic modulus, diastolic blood pressure, systolic blood pressure, and pulse pressure, we used multivariable linear regression to estimate the difference in outcome for every 10-IU/mL increase in CMV IgG titer. For presence of carotid artery lesions, we used a log-binomial model to estimate the prevalence ratio for a 10-IU/mL increase in CMV IgG. All multivariable models were adjusted for potential confounders including age, race/ethnicity, smoking status, diabetes, BMI, and study site. Models with and without inclusion of CRP as an additional covariate were also presented to evaluate the role of a generalized inflammatory response in the observed associations. Additional adjustment for nadir CD4⁺ T-cell count did not appreciably affect the results, so it was not included in the more parsimonious models presented here. As a control antigen, we adjusted the analyses of CMV IgG antibody levels and vascular parameters for levels of EBV IgG antibody. We conducted analyses separately in women with and without HIV infection.

In subsequent analyses, we also examined whether the association between CMV IgG and cardiovascular outcomes varied by HIV treatment and viremic status. We used first-order interaction terms, with a P<.05 criteria for statistical significance, to test whether the association between CMV IgG and carotid artery parameters differed across HIV subgroups defined as treated/aviremic, treated/viremic, and untreated.

Analyses were conducted using SAS version 9.2 (SAS Institute).

RESULTS
Subjects
Subjects included 601 HIV-infected women (226 treated and aviremic; 148 treated and viremic; and 227 untreated) and 90 HIV-uninfected women (Table 1). Subjects had a mean age >40 years, were mostly African American/black (64%), and had a prevalence of smoking near 50%. Approximately 20% of women were diabetic. The mean BMI in both the HIV-infected and HIV-uninfected groups was in the overweight/obese range (Table 1).

Association Between HIV Status and Cardiovascular Parameters
Systolic blood pressure was marginally significantly lower in the HIV-infected group compared with the HIV-uninfected group (P = .04) (Table 1). In contrast, diastolic blood pressure, cIMT, presence of carotid artery lesions, carotid artery distensibility, Young’s elastic modulus, and pulse pressure were not significantly different between HIV serostatus groups. Among HIV-infected women, these cardiovascular measures did not differ significantly across treated/aviremic, treated/viremic, and untreated subgroups (Table 1).

Characteristics Associated With CMV IgG
We excluded a similar number of CMV-negative women from the HIV-infected (7%) and HIV-uninfected (10%) study groups. Compared with HIV-uninfected controls, HIV-infected subjects had significantly higher serum CMV IgG levels (mean, 25.4; standard deviation [SD], 9.9 IU/mL among HIV-infected women; and mean, 19.4; SD, 9.2 IU/mL among HIV-uninfected women; P < .01) (Table 1). No significant correlation was observed between CMV IgG levels and CD4⁺CD38⁺HLA-DR⁺ T-cell frequency (r = 0.13; P = .18) or CD8⁺CD38⁺HLA-DR⁺ T-cell frequency (r = 0.06; P = .53).

Among HIV-infected women, CMV IgG levels were statistically significantly different across subgroups defined by antiretroviral treatment and detectable viremia (Table 1). No correlation was observed between CMV IgG and either current HIV RNA level or current CD4⁺ T-cell count, and
there was a modest but statistically significant inverse correlation between CMV IgG and nadir CD4+ T cell count ($r = -0.22; P < .01$) (Table 2).

In HIV-infected women, CMV IgG was statistically significantly higher among women who were older, who were diabetic, and who were current smokers (Table 2). A significant correlation between CMV IgG and age was observed in HIV-uninfected women (Table 2).

### CMV IgG and Cardiovascular Variables: HIV-Infected Women

Among HIV-infected women, CMV IgG had a significant age-adjusted correlation with carotid artery stiffness (decreased distensibility and increased Young’s modulus) (Table 3). After adjustment for age, race/ethnicity, smoking, diabetes, BMI, and study site, higher CMV IgG remained independently associated with lower carotid artery distensibility and higher Young’s modulus among HIV-infected women.
Table 2. Associations of Cytomegalovirus (CMV) Immunoglobulin G (IgG) with Clinical and Sociodemographic Variables by HIV Serostatus

<table>
<thead>
<tr>
<th>Variable</th>
<th>HIV-Infected (n = 601)</th>
<th>HIV-Uninfected (n = 90)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>P Value</td>
</tr>
<tr>
<td>CD4+ T-cell count</td>
<td>−0.08</td>
<td>.06</td>
</tr>
<tr>
<td>Nadir CD4+ T-cell count</td>
<td>−0.22</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>Log10 HIV RNA</td>
<td>0.04</td>
<td>.35</td>
</tr>
<tr>
<td>Ln C-reactive protein</td>
<td>−0.06</td>
<td>.15</td>
</tr>
<tr>
<td>BMI</td>
<td>−0.06</td>
<td>.17</td>
</tr>
<tr>
<td>Age</td>
<td>0.24</td>
<td>&lt;.01</td>
</tr>
</tbody>
</table>

Mean (SD) CMV IgG P Value

Current smoking status

Current smoking status

* r values represent Pearson correlations. Results are presented separately for human immunodeficiency virus (HIV)-infected and HIV-uninfected women; however, there was no evidence for statistical interaction by HIV serostatus. Abbreviations: BMI, body mass index; SD, standard deviation.

Table 3. Age-Adjusted Pearson Correlations of Cytomegalovirus (CMV) Immunoglobulin G (IgG) With Cardiovascular Parameters by HIV Serostatus

<table>
<thead>
<tr>
<th>Cardiovascular Variable</th>
<th>HIV-Infected (n = 601)</th>
<th>HIV-Uninfected (n = 90)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>P Value</td>
</tr>
<tr>
<td>Carotid artery distensibility</td>
<td>−0.13</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>Young’s elastic modulus</td>
<td>0.12</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>Diastolic BP</td>
<td>0.01</td>
<td>.87</td>
</tr>
<tr>
<td>Systolic BP</td>
<td>0.04</td>
<td>.39</td>
</tr>
<tr>
<td>Pulse pressure</td>
<td>0.04</td>
<td>.29</td>
</tr>
<tr>
<td>cIMT</td>
<td>0.01</td>
<td>.86</td>
</tr>
</tbody>
</table>

Effect of Adjustment for EBV Antibody Levels

Compared with HIV-uninfected women, HIV-infected women had higher levels of EBV IgG (192 U/mL vs 140 U/mL; P < .01) (Table 1). Among HIV-infected women, treated/viremic women had the highest EBV IgG titers and untreated women had the lowest titers (P = .01) (Table 1). In HIV-infected women, after adjustment for age, higher levels of EBV IgG were associated with lower cIMT (r = −0.09; P = .04) and lower blood pressure (for systolic BP, r = −0.12; P = .01; and for diastolic BP, r = −0.10; P = .02). The EBV IgG level was not associated with carotid artery distensibility, Young’s elastic modulus, or pulse pressure (P > .05). Further adjustment for EBV IgG levels had no effect on the associations described.
Among HIV-uninfected women, no significant associations were observed between CMV IgG and cIMT, presence of carotid lesions, carotid artery distensibility, Young’s modulus, systolic blood pressure, diastolic blood pressure, or pulse pressure in age-adjusted analyses (Table 3) or multivariable-adjusted analyses (data not shown). The EBV IgG levels were not significantly correlated with vascular parameters in HIV-uninfected women.

**DISCUSSION**

Among HIV-infected women, higher CMV IgG levels were associated with carotid artery stiffness, as measured by decreased carotid artery distensibility and increased Young’s elastic modulus. This finding was independent of other factors associated with both vascular disease and CMV IgG antibody titers, including age, race/ethnicity, and smoking. We also found evidence for an association between CMV IgG antibody titers and increased prevalence of subclinical carotid artery lesions among treated/aviremic HIV-infected women, but not among HIV-infected women who were untreated, or who were treated but had residual viremia and reduced CD4+ T-cell count. Finally, no associations between CMV IgG levels and subclinical cardiovascular disease parameters were observed in an HIV-uninfected control group that was studied using similar methods, albeit the HIV-uninfected control group was relatively small in size and we may have lacked statistical power to detect associations in this group. Importantly, we demonstrated that the association between CMV IgG antibody levels and vascular parameters was unaffected by adjustment for EBV antibody titers, suggesting that the association is virus-specific and not explained by non-specific hypergammaglobulinemia.

Limitations of this study include the cross-sectional design and absence of data on men. Nearly all women in the HIV-infected and HIV-uninfected groups had detectable CMV antibody titers, so we lacked sufficient numbers of individuals to draw comparisons between CMV-exposed and CMV-unexposed groups. It is uncertain whether high CMV antibody titers may have reflected recurrent CMV infection, reactivation of latent CMV, or the patient’s underlying immune status.

In persons infected with CMV, CMV-specific CD8+ T-cell responses increase once HIV infection has been established, even early in HIV infection before the loss of peripheral CD4+ T cells [23, 24, 30, 31]. Cytomegalovirus-specific T-cell responses may expand among HIV-infected patients once they are placed on effective antiretroviral therapy, as compared with patients in the early or untreated phases of HIV infection or HIV-uninfected controls. In a prior study, we found that secretion of interferon gamma by CD8+ T cells in response to CMV pp65 and IE was associated with carotid artery lesions in a population of HIV-infected men, most of whom were on antiretroviral treatment [8]. In this study, which included HIV-infected women both on and off antiretroviral therapy, CMV IgG levels were associated with carotid artery lesions in the subgroup of effectively treated HIV-infected women.
The association of CMV IgG with reduced carotid artery distensibility and increased Young’s modulus, a marker of vascular stiffness, was consistently observed in treated/aviremic, treated/viremic, and untreated HIV-infected groups. Patients with HIV infection have reduced carotid distensibility and other indices of arterial stiffness [18, 19, 21, 25, 32], as do patients with other inflammatory conditions [33]. We are aware of only a single study, from a non-HIV-infected population, which showed an association between CMV IgG titers and increased carotid artery stiffness [34]. Recent studies suggest that chronic inflammation associated with chronic HIV infection may impair vasoreactivity and cause structural changes in blood vessels and surrounding connective tissues, and it is possible that CMV infection contributes either directly or indirectly to these mechanisms [35, 36].

Cytomegalovirus infects various cell types of the vasculature and surrounding connective tissue (endothelial cells, smooth muscle cells, pericytes, fibroblasts) as well as cells of the immune system that are known to be involved in vascular disease (eg, macrophages) [15, 37, 38]. It has been proposed that the vasculature represents an important reservoir for CMV [39, 40]. In nonimmunocompromised individuals, immediate-early CMV antigen (IE 70 viral protein) is found in vascular smooth muscle cells and in endothelial cells from diseased blood vessels and is also found to a lesser extent in normal vessels. This is possible evidence of active CMV replication and reactivation in human arterial tissues [6]. Cytomegalovirus infection of vascular cells may stimulate localized inflammation [2, 15] and lead to the release of angiogenic growth factors and extracellular remodeling factors, including matrix metalloproteinases and tissue inhibitors of metalloproteinases [15, 41]. Indeed, treatment of asymptomatic CMV replication in antiretroviral therapy–treated HIV-infected individuals has recently been shown to decrease systemic immune activation levels in a randomized controlled trial [42]. T-cell immunosenescence, which is a risk factor for subclinical cardiovascular disease in HIV-infected patients [20, 43], may result from chronic exposure to CMV [13, 14], although we lacked sufficient data on T-cell phenotypes to address this hypothesis in the current study.

In summary, our findings suggest that CMV IgG is associated with increased carotid artery stiffness and carotid artery lesions in HIV-infected women. Those HIV-infected patients on effective antiretroviral therapy may have the greatest susceptibility to develop vascular lesions in association with CMV coinfection, or alternatively the association between CMV and arterial lesions may have been overshadowed by other vascular disease mechanisms in untreated or viremic HIV-infected women. Future research will be needed to clarify the reasons for the association between CMV antibody titers and subclinical cardiovascular disease and to test the hypothesis that therapies directed against CMV infection may
reduce HIV disease progression and associated vascular complications [1].

Notes

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