Virologic Correlates of Anti-CMV IgG Levels in HIV-1–Infected Men

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In human immunodeficiency virus (HIV)–infected individuals, higher levels of anti-cytomegalovirus (CMV) immunoglobulin G (IgG) antibody have been associated with increased immune activation, increased HIV transmission, cardiovascular complications, and neurocognitive impairment. However, the mechanism of these observations is unknown. This analysis of 228 HIV-infected men found that higher CMV IgG levels were positively associated with older age and antiretroviral treatment. Higher frequency of detectable CMV in peripheral blood mononuclear cells and recurrent seminal CMV reactivations were associated with lower plasma CMV IgG levels, suggesting that immune response to CMV rather than direct effect of viral replication is likely responsible for adverse clinical outcome observed in other studies.

Keywords. CMV replication; immune response; HIV infection; CMV IgG.

Semen represents an important reservoir for cytomegalovirus (CMV). Actively replicating CMV can be found in the seminal plasma of >50% of human immunodeficiency virus (HIV)–infected men who have sex with men [1]. Detection of CMV in blood or semen of HIV-infected men has been associated with higher T-cell immune activation [2, 3], greater levels of blood HIV DNA [4], HIV disease progression [5], increased HIV transmission [1, 6, 7], and premature mortality [3]. Moreover, immune activation in response to CMV replication may contribute to accelerated immunosenescence [3, 8]. Recent

evidence suggests that CMV coinfection may also contribute to cardiovascular complications. In one study, HIV-infected men with higher levels of CMV-specific CD8⁺ T cells had increased carotid artery wall thickness [9]. Another study showed that circulating anti-CMV immunoglobulin G (IgG) antibody levels were associated with subclinical cardiovascular disease in HIV-infected women [10]. Other studies of non-HIV-infected populations showed an association between CMV IgG levels and increased carotid artery stiffness [11] and ischemic heart disease [12]. Moreover, higher levels of CMV IgG were recently associated with neurocognitive impairment [13] and increased risk of HIV-transmission in HIV-infected subjects [6]. Age also seems to be an issue because in individuals aged >50 years, CMV antibody production was associated with increased circulating C-reactive protein and interleukin 6, suggesting a relationship between CMV immune response and proinflammatory immune activation [14].

Although several studies point to CMV IgG levels as a prognostic marker, it remains unclear whether high CMV antibody levels are reflective of recurrent reactivation of latent CMV or more likely reflect the patient's underlying immune status. In this study we aim to address this question by investigating the associations between dynamics of anti-CMV IgG antibody and CMV replication in semen and peripheral blood mononuclear cells (PBMCs) of treated and untreated HIV infected men.

METHODS

Participants, Samples, and Clinical Laboratory Tests

A total of 353 paired semen and blood samples were included in this study from 114 recently HIV-infected antiretroviral therapy (ART)–naïve participants from the San Diego Primary Infection Cohort (SD-PIC) [1, 2], and 114 ART-treated chronically HIV-infected subjects from the California Collaborative Treatment Group (CCTG) 592 Study [7]. Semen was collected by masturbation, as described previously [1, 2]. From blood, CD4⁺ T-lymphocyte subsets were measured by flow cytometry, and HIV RNA was quantified. The studies were conducted with appropriate written subject consent and were approved by the Human Research Protections Program at the University of California San Diego, Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center, and the University of Southern California.

Quantification of Herpesviruses in Semen and CMV and HIV DNA in PBMCs

DNA was extracted from seminal plasma, and the DNA levels of seven different herpesviruses (CMV, Epstein–Barr virus,

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herpes simplex viruses types 1 and 2, human herpesvirus 6, 7, and 8) were measured by real-time polymerase chain reaction, as previously described [1]. In all but 4 ART-naive subjects ($n = 110$), genomic and viral DNA were extracted from PBMCs, and levels of HIV DNA and CMV DNA were determined [4].

Anti-CMV IgG Antibody Levels

Anti-CMV IgG antibody levels were measured in blood plasma. Units per milliliter (UI/mL) were determined by interpolation from a standard curve of a known anti-CMV IgG solution [10]. If IgG was undetectable at a 1:80 dilution, the assay was repeated at a 1:40 dilution, per manufacturer's instruction.

Multiparameter Flow Cytometry Analysis

For a subset of 36 ART-naive subjects [2], blood lymphocyte populations were analyzed by flow cytometry on a dual-laser, 6-color Becton Dickinson FACS Canto using Diva (6.1) or FlowJo (9.0) software [2]. Cells were stained using CD45-PerCP/Cy5.5 (clone 2D1), CD45RA-PE (clone HI100), CD4-FITC (Leu 3a/3b multiclone), CD38-PE/Cy7 (clone HB7), CD3-APC (clone SK7), and CD8-APC/Cy7 (clone SK1).

Statistics

Statistical analyses were performed using SAS version 9.2. All viral DNA copy numbers were calculated as the mean of replicate polymerase chain reaction measurements. HIV and CMV DNA levels in PBMCs were normalized to cellular input, expressed in copies per 10^6 cell equivalents as obtained with beta-actin polymerase chain reaction [4]. All viral load and DNA values were $\log_{10}$ transformed. CMV IgG levels were normally distributed, as determined by Shapiro–Wilks test. In our main analysis, we evaluated associations between CMV IgG levels in blood plasma and CMV DNA levels in semen and blood. Additionally, we investigated associations between CMV IgG levels and the following variables: HIV RNA and HIV DNA levels in blood, CD4⁺ cell counts, age, immune activation status of CD8 and CD4 lymphocytes (determined as the percentage of CD45RA-CD38⁺ T-cells), and shedding of other herpesviruses in semen.

To compare factors cross-sectionally between the recently infected ART-naive and the ART-experienced groups, if longitudinal time points were available, the last measured time point of the ART-naive group was used. Univariable analyses were performed by group using Student *t* test for dichotomous predictors (significance threshold at $P \leq .05$) and Pearson correlation coefficients to compare continuous variables. A multivariable linear regression for the association of treatment status with CMV IgG was performed, adjusting for age, CD4 count, and CMV shedding in semen.

For a subset of 58 individuals in the ART-naive group with multiple available time points, we investigated associations between CMV IgG levels and frequency of longitudinal seminal

CMV shedding and divided subjects into 2 groups, those with detectable seminal CMV shedding $\geq 50\%$ of the time and those with detectable seminal CMV shedding $< 50\%$ of all included time points.

RESULTS

Study Participants

Among SD-PIC participants, single or longitudinal blood and semen samples ($n = 239$) were collected from 114 ART-naive, recently HIV-infected subjects. For 58 subjects with repeated sampling (≥ 2), there was a median follow-up of 67 days (interquartile range, 29–175), with 2 time points in 30 patients, 3 time points in 14 patients, and ≥ 4 timepoints in 14 patients. Among CCTG participants, semen and blood samples were collected at a single time point for 114 asymptomatic chronically HIV-infected men who have sex with men. All CCTG subjects were receiving effective ART with blood plasma HIV RNA < 500 copies/mL within 3 months before the seminal sample collection.

Comparison Between ART-Naive (SD-PIC) and ART-Treated (CCTG) Subjects

A comparison of the last visit of SD-PIC and CCTG groups is summarized in [Supplementary Table 1](#). Overall, the treated and ART-naive groups differed by mean age (44.4 years vs 33.8 years; $P < .01$) and race (38.6% white vs 59.7% white; $P < .01$). CMV IgG levels were also significantly different by ART status, with subjects receiving ART having a mean $\pm$ standard deviation titer of 65.9 ± 28.1 UI/mL compared with 37.4 ± 24.6 UI/mL in those not receiving ART ($P < .01$). Shedding in semen of CMV and other herpesviruses was not significantly different by ART status.

CMV IgG (odds ratio, 1.03; 95% confidence interval, 1.02–1.05) remained independently associated with ART use after adjusting for age, CD4 count, and CMV shedding in semen.

Factors Associated With CMV IgG Levels by ART Status

The univariable cross-sectional analysis included all subjects with 1 time-point sample and the last available time-point sample for those with multiple sampling (Table 1). Older age was associated with higher levels of CMV IgG among ART-treated subjects ($P = .02$) and among ART-naive participants ($P = .08$). In both ART-naive and ART-treated men, levels of CMV in semen were not associated with CMV IgG in cross-sectional analysis. However, levels of CMV DNA in PBMCs, available for ART-naive subjects only, was negatively associated with CMV IgG levels in the cross-sectional analysis ($P < .01$). Similarly, subjects with detectable CMV DNA in PBMCs (but not detectable CMV DNA in semen) had significantly lower levels of CMV IgG in blood plasma compared with those without detectable CMV ($P = .01$). These associations remained

Table 1. Factors Associated With Cytomegalovirus Immunoglobulin G in Antiretroviral Therapy–Naïve and Antiretroviral Therapy–Treated HIV-Infected Men Who Have Sex With Men

Factors Associated With CMV IgG (UI/mL)	ART Naïve		ART Experienced	
	<i>r</i>	<i>P</i> Value	<i>r</i>	<i>P</i> Value
Age	0.16	.08	0.22	.02
CD4 T-cell count	0.11	.25	−0.08	.41
HIV DNA (n = 110) ^a	0.11	.25	NA	
CMV in PBMCs (n = 110) ^a	−0.26	<.01	NA	
CMV in semen	−0.05	.57	0.05	.53
HIV RNA blood plasma levels	−0.13	.18	NA	
Activated CD4 T cells, % (n = 36) ^b	−0.11	.52	NA	
Activated CD8 T cells, % (n = 36) ^b	−0.05	.79	NA	
Group Comparisons by CMV Shedding	IgG (UI/mL)	<i>P</i> Value	IgG (UI/mL)	<i>P</i> Value
CMV shedding in semen				
CMV: any shedding (n = 49, n = 55)	34.6 (28.2–40.9)	.29	66.9 (60.7–73.0)	.71
CMV: no shedding (n = 65, n = 59)	39.5 (33.0–46.0)		64.9 (56.4–73.4)	
CMV shedding in PBMCs				
CMV detected (n = 9)	18.4 (3.2–33.7)	.01	NA	
CMV not detected (n = 101)	38.3 (33.5–43.1)			
Longitudinal CMV shedding				
≥2 visits (N = 58)				
CMV ≥50% (n = 24)	32.8 (22.5–43.2)	.09	NA	
<50% (n = 34)	43.8 (35.5–52.1)			
≥3 visits (N = 28)				
CMV ≥50% (n = 14)	27.5 (16.3–38.7)	<.01	NA	
<50% (n = 14)	48.9 (38.7–59.2)			
≥4 visits (N = 14)				
CMV ≥50% (n = 9)	19.3 (4.5–34.1)	<.01	NA	
<50% (n = 5)	53.8 (34.5–73.0)			

Significant values are bolded.

Abbreviations: ART, antiretroviral therapy; CMV, cytomegalovirus; HIV, human immunodeficiency virus; IgG, immunoglobulin G; NA, not available; PBMC, peripheral blood mononuclear cell.

^a Cytomegalovirus in peripheral blood mononuclear cells and proviral human immunodeficiency virus DNA available for 110 antiretroviral therapy–naïve subjects.

^b Activation status of CD4 and CD8 T cells (defined as CD38⁺ CD45RA⁺ T-cells) available for 36 antiretroviral therapy–naïve subjects.

significant among the untreated subjects also after adjusting for age ($P = .01$). Unfortunately, CMV DNA data were not available in PBMCs for the treated cohort. Percentage of immune activated CD4 and CD8 T cells (available for 36 ART-naïve subjects), levels of HIV RNA and DNA, and CD4⁺ counts were not associated with CMV IgG levels. Additionally, we observed associations between CMV IgG levels and seminal shedding of other herpesviruses. Specifically, CMV IgG levels in ART-treated subjects were negatively associated with shedding of human herpesvirus 7 ($P = .01$) and positively associated with shedding of Epstein–Barr virus ($P < .01$) and human herpesvirus 8 ($P = .03$) (data not shown).

For 58 patients with at least 2 longitudinal time points, we observed a trend toward higher CMV IgG levels in those subjects with less-frequent CMV seminal shedding (<50% compared with ≥50%; $P = .09$). When only examining those individuals with >2 time points available (≥3, $n = 28$; ≥4,

$n = 14$), this negative association between CMV shedding and lower CMV IgG levels became highly statistically significant (both $P < .01$; Figure 1).

DISCUSSION

This study investigated the associations between CMV IgG levels and levels and frequency of CMV shedding in semen and PBMCs in a large cohort of recently HIV-infected ART-naïve subjects and treated chronically HIV-infected subjects. Unlike CMV DNA levels in semen [2], CMV IgG levels were not associated with activation status of T lymphocytes in blood, with CD4⁺ T-cell counts, or with HIV RNA in blood plasma. Similar to previous studies, we observed differences in CMV IgG levels with age and ART status [10]. Counter to the hypothesis that CMV IgG levels are directly driven by CMV replication, we did not find any significant association between

Figure 1. Anti-cytomegalovirus (CMV) immunoglobulin G (IgG) levels in subjects with frequent and less-frequent CMV seminal shedding. CMV IgG levels were determined in blood plasma for 58 patients for whom longitudinal samples were available. Patients were divided into the following 2 groups: those with detectable seminal CMV DNA in <50% of visits (gray bars) and those with seminal shedding in ≥50% of visits (black bars). X-axis indicates number of longitudinal visits, and y-axis indicates CMV IgG levels (UI/mL). Mean and 95% confidence interval are plotted. At both ≥3 and ≥4 visits, there was significantly more CMV IgG in the less-frequent seminal shedding group, as determined by Student *t* test ($P < .01$). *shows a trend; **shows significant *P* values.

CMV IgG levels and levels of CMV in semen in either the treated or untreated groups in our cross-sectional analysis, and a significant negative correlation was observed between levels of CMV DNA in PBMCs and CMV IgG. Similarly, for a subset of recently HIV-infected ART-naive subjects with available longitudinal semen and blood samples, we found that more-frequent presence of CMV shedding in semen was associated with significantly lower CMV IgG antibody levels. These findings strongly suggest that higher levels of CMV IgG do not primarily reflect frequent CMV reactivations, but more likely, they are the consequence of a stronger immune response to CMV leading to less reactivation. Therefore, caution should be used when considering CMV IgG as a surrogate marker of CMV activity and burden. In a recent clinical trial of HIV-infected individuals, valganciclovir-mediated declines in T-cell activation did not appear to be modified by the presence of detectable CMV DNA in semen or plasma at baseline [15]. This might add further support to the notion that the immune response elicited by CMV may be independent of the degree of CMV replication (or that a greater immune response to CMV may result in less CMV replication).

Our study has a number of important limitations. First, we did not include individuals with very low CD4⁺ T-cell counts (<300 cells/ μ L), and these measurements should be carried out in a population with compromised immunity. Moreover, the

associations between CMV IgG and CMV were mainly observed in PBMCs and in the longitudinal analysis of semen of a recently HIV-infected ART-naive cohort. Unfortunately, those data were not available for the treated cohort and should be evaluated in a future study. Estimated duration of infection was also not available for the treated cohort. However, treated patients were older and had a longer duration of HIV infection compared with the early-infected ART-naive group. With this study design, it was not possible to distinguish the real impact of ART initiation on CMV IgG levels from the duration of HIV infection. Nevertheless, CMV IgG levels remained independently associated with ART use after accounting for age, which could be a correlate of duration of infection.

To account for possible confounders, we also investigated for presence of associations between CMV IgG and shedding of other herpesviruses. Interestingly, the presence of some other herpesviruses in semen was also associated with CMV IgG levels, possibly indicating an interaction between these viruses and the host immune response, which merits further investigation.

In conclusion, although high CMV IgG levels are associated with increased mortality and morbidity [10–12], the underlying mechanisms remain unclear. Several previous studies described associations between detection of CMV DNA, chronic immune activation, and disease outcomes [2, 3, 5]. This study suggested that a higher immune response to CMV, reflected by higher CMV antibodies, might predict worse disease outcomes, such as cardiovascular and neurologic disease. Future studies should investigate whether CMV antiviral therapy can mitigate this likely immune-mediated damage.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online (<http://jid.oxfordjournals.org/>). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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S. G. participated in the study design, performed the laboratory experiments, participated in the data analyses for this study and wrote the primary version of the manuscript; S. R. M. participated in study design, performed statistical analysis and wrote the primary version of the manuscript; D. M. S. participated in the study design, participated in the data analyses and wrote and revised the manuscript; M. V. V. performed the laboratory experiments; D. D. R., R. H. H., E. S. D., M. P. D., E. T., S. J. L. participated in study design, and revised the manuscript, S. R. M., S. J. L., E. S. D., M. P. D. and R. H. H. enrolled participants.

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All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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