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Cytomegalovirus Immunoglobulin G (IgG) Titer and Coronary Artery Disease in People With Human Immunodeficiency Virus (HIV)

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Background. Cytomegalovirus (CMV) infection is thought to result in increased immune activation in people with human immunodeficiency virus (HIV, PWH). Although some data have linked asymptomatic CMV infection to cardiovascular disease among PWH, it remains unknown whether CMV is associated with increased or high-risk coronary plaque.

Methods. The Randomized Trial to Prevent Vascular Events in HIV (REPRIEVE) enrolled PWH aged 40–75 years on stable antiretroviral therapy (ART) with low-to-moderate atherosclerotic cardiovascular disease (ASCVD) risk. Among a subset of US REPRIEVE participants, coronary plaque was assessed by coronary computed tomography angiography. Here, we assessed the relationship between CMV immunoglobulin G (IgG) titer and (1) levels of immune activation, (2) inflammatory biomarkers, and (3) coronary plaque phenotypes at study entry.

Results. Of 672 participants, mean age was 51 years, 83% were men, median ASCVD risk score was 4.5%, and 66% had current CD4+ T-cell count ≥500 cells/mm^3. Higher CMV IgG quartile group was associated with older age and lower current and nadir CD4+ T-cell counts. CMV IgG titer was associated with specific inflammatory biomarkers (sCD163, MCP-1, interleukin [IL]-6, hsCRP) in univariate analysis, but not after controlling for HIV-specific factors. In contrast, CMV IgG titer was not associated with coronary artery disease indexes, including presence of plaque, coronary artery calcium (CAC) score >0, vulnerable plaque presence, or Leaman score >5.

Conclusions. No meaningful association was seen between CMV IgG titer and coronary artery disease indexes among ART-treated PWH at study enrollment. Longitudinal assessments in REPRIEVE will determine the relationship of CMV IgG titer to plaque progression and cardiovascular events.

Clinical Trials Registration. NCT02344290.

Keywords. human immunodeficiency virus (HIV); cytomegalovirus (CMV); cardiovascular disease (CVD); coronary artery disease (CAD); inflammation.

People with human immunodeficiency virus (HIV, PWH) have an almost 2-fold increased risk of myocardial infarction (MI) compared to the general population [1]. Health-related behaviors such as smoking and substance use, enriched among PWH, incompletely account for this increased risk [2–4]. Persistent immune activation, driven by HIV reservoirs, microbial translocation, and viral coinfections like cytomegalovirus (CMV), declines but fails to normalize during antiretroviral therapy (ART)-mediated suppression and has emerged as a potential contributor to increased cardiovascular disease (CVD) risk [3, 5, 6]. Indeed, asymptomatic CMV replication is induced by and causes systemic inflammation [7–11]. The causal relationship between CMV and inflammation was demonstrated in a placebo-controlled trial of CMV-seropositive PWH, in which valganciclovir led to significant reductions in T-cell activation, monocyte/macrophage activation, and microbial translocation [12, 13].

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Although asymptomatic CMV infection appears to play a negligible role in CVD in the general population, multiple studies have linked CMV to CVD in PWH, though the mechanisms remain unclear [14–18]. In one cohort, CMV-seropositive PWH had more than a 2-fold increased hazard of incident cardiovascular and cerebrovascular events [16]. Mechanistically, CMV replicates within endothelial and smooth muscle cell-resident macrophages of the vasculature and attracts monocytes and activated inflammatory CX3CR1+ T cells [11, 19]. Activated CD8+ T cells induce monocytes to express tissue factor, which may also promote plaque instability and rupture [20]. Several studies have linked CMV immunoglobulin G (IgG) or CMV-specific T-cell responses to increased carotid intima media thickness (cIMT), but none have examined whether asymptomatic CMV infection is associated with coronary atherosclerotic plaque phenotypes [19, 21, 22]. A recent study associated CMV IgG titer with an increased hazard of type 1 but not type 2 MI, suggesting a possible CMV-specific effect in the pathogenesis of vulnerable plaque or plaque rupture [23]. Given that PWH have an increased risk of noncalcified and high-risk plaque formation, assessing the relationship between asymptomatic CMV infection and coronary artery disease (CAD) is critical to informing our understanding of the pathogenesis and potential effect of CMV on CVD in PWH [24–27].

To address these issues, we leveraged the mechanistic substudy embedded in the global Randomized Trial to Prevent Vascular Events in HIV (REPRIEVE) to assess the association between CMV IgG titer and coronary atherosclerotic plaque phenotypes in ART-treated PWH with low-to-moderate traditional CVD risk [28, 29]. We assessed the relationship of CMV IgG titer to key immune and inflammatory biomarkers, which we previously demonstrated to be related to plaque [27]. We hypothesized that higher CMV IgG titer would be associated with a vulnerable plaque phenotype, and that this might be mediated through increases in immune activation and inflammation.

METHODS

Study Design

REPRIEVE enrolled PWH aged 40–75 years on ART for at least 6 months with current CD4+ T-cell count >100 cells/mm^3 at low-to-moderate traditional CVD risk. Exclusion criteria were notable for atherosclerotic cardiovascular disease (ASCVD) history, statin or immunosuppressant use, active cancer, or active systemic infection [28, 29]. A subset of REPRIEVE participants from 31 US sites co-enrolled in the mechanistic substudy, which entailed CTA and immune phenotyping [27].

Coronary CT Angiography

Details regarding CTA protocols, quality control, and interobserver variability have been reported [27, 29]. Coronary artery calcium (CAC) score was measured on a non-contrast CT. All other plaque indexes were measured on contrast-enhanced CTA. Vulnerable plaque features were defined by presence of positive remodeling, low-attenuation plaque, and the napkin-ring sign [27]. Degree of stenosis was categorized as none, minimal to mild (1–49%), moderate (50–69%), and severe (≥50% of left main or ≥70% of other segments). The Leaman score was calculated factoring in the degree of stenosis, coronary dominance, plaque location and composition [27, 30]. Primary outcomes included presence of any plaque, CAC >0, presence of vulnerable plaque, and Leaman score >5.

Biomarker Measurements

CMV IgG titer (IU/mL) was measured using the CMV IgG enzyme immunoassay (EIA) by Genway Biotech (GWB-892399) (Supplementary Methods). The coefficient of variation for CMV IgG titer was <20% (any >20% was repeated). A cutoff for CMV seropositivity was not provided by the kit manufacturer. As none of the participants demonstrated a CMV IgG titer below the assay's lower limit of detection, we assessed all associations in relation to increasing CMV IgG titer within the range of observed values. Data on biomarkers of immune activation and inflammation were previously quantified in REPRIEVE [27].

Statistical Analysis

Continuous variables are reported as means with standard deviation (SD) or medians with first-to-third quartiles (Q1–Q3). Categorical variables are reported as numbers with proportions. Trends across CMV quartiles were tested using an extension of the Wilcoxon rank-sum test [31]. Adjusted linear regression models were used to assess the relationship between CMV IgG and individual biomarkers, adjusted a priori for age, sex, race, ethnicity, smoking history, substance use history, nadir CD4, and HIV viral load. CMV IgG and biomarkers were log-transformed using the natural log. Coefficients were transformed using $(1.25^{Beta}-1)\times100$, allowing for an interpretation of a 25% increase in CMV IgG titer leading to the beta coefficient as a percent increase in the dependent variable. All statistical tests were 2-tailed with an alpha level of 0.05 to guide statistical inference. No adjustments were made for multiple comparisons. All statistical analyses were performed using Stata version 16.1 (StataCorp).

Ethical Considerations

The REPRIEVE trial was approved by the Mass General Brigham Human Research Committee and local institutional review boards at each participating site. All participants provided written informed consent.

RESULTS

Participant Characteristics

Of 805 participants enrolled in the REPRIEVE mechanistic substudy, 755 had a diagnostic CTA. Of those, biomarker

aliquots were not available for 83 participants, resulting in 672 participants with available CMV IgG titer and a diagnostic CTA (Table 1). Mean age was 51 years, 83% were men, and 53% were White. Fifty-five percent reported current or former cigarette smoking, and mean body mass index (BMI) was 27.4 kg/m^2. Median ASCVD risk score was 4.5% (Q1–Q3 2.5–7.0%) Most participants had been on ART >5 years, and almost half were on integrase strand transfer inhibitors. Fifty-one percent had nadir CD4 T-cell counts <200 cells/mm^3; 66% had current CD4 >500 cells/mm^3. Median CMV IgG titer was 2686 IU/mL (Q1–Q3 790–6671 units); no participant had a measurement below the limit of detection. The population with available data for analysis (N = 672) was similar to the overall substudy population (N = 755) (Supplementary Table 1).

Current and Nadir CD4 Were Associated with CMV IgG Titer

To demonstrate trends in clinical characteristics associated with CMV IgG titer, participants were divided into quartiles based on CMV IgG. Older age was associated with a higher CMV IgG titer ($P = .03$) (Table 1). Neither natal sex ($P = .82$) nor race ($P = .98$) was associated with CMV IgG. There was no association with BMI ($P = .69$), but a trend of lower LDL across higher CMV IgG quartiles was observed ($P = .03$).

Current and nadir CD4 were significantly associated with CMV IgG quartiles, such that those with lower current and nadir CD4 were overrepresented in the higher CMV IgG quartile groups ($P < .001$ for both) (Table 1). Only 5% of those in the lowest quartile group but 24% in the highest quartile group had a current CD4 <350, with a stepwise decline in proportions across groups. Similarly, 11% of those in the lowest quartile group but 39% in the highest quartile group had a nadir CD4 <50 cells/mm^3. No differences were seen between CMV IgG and ART class.

No Association of CMV IgG Titer With Plaque Presence or Phenotype

We assessed the presence of plaque, vulnerable plaque features, Leaman score, and any CAC (CAC >0) across CMV IgG quartile groups, and we found no significant trends (Table 2). There were also no significant associations identified for any outcome when CMV IgG was modeled as a continuous variable or when CAC or Leaman score were modeled ordinally or continuously. In a sensitivity analysis, there was also no significant association of CMV IgG titer to plaque when stratified by age, sex, ASCVD risk score, LDL, or current or nadir CD4 (data not shown). Although the CMV IgG assay did not include a seropositivity cutoff, based on some estimations of 95% seroprevalence in the United States, we designated the highest 95% of CMV IgG titer levels as hypothetically seropositive to compare plaque phenotypes [17, 23]. In this sensitivity analysis, potential CMV seropositivity was associated with some vulnerable plaque features but not associated with increased plaque (Supplementary Table 2).

Association Between CMV IgG Titer and Biomarkers of Immune Activation and Inflammation

CMV IgG titer was associated with interleukin (IL)-6, hsCRP, MCP-1, and sCD163 in unadjusted analyses but not sCD14, LpPLA2, or oxLDL (Figure 1). We performed multivariate regression modeling with each individual biomarker as the outcome and CMV IgG titer as the independent variable, controlling for the potential confounders of age, sex, race, ethnicity, current or former cigarette smoking, substance use, HIV viral load, and nadir CD4 (Supplementary Table 3). CMV IgG titer was not significantly associated with biomarkers after adjustment. In a sensitivity analysis, we excluded nadir CD4 from modeling as it related to CMV and may be on the causal pathway of increased immune activation in the context of CMV. In this sensitivity analysis, marginal statistical significance was seen relating CMV IgG titer to sCD163 and LpPLA2, but effect sizes were modest and comparable to the primary model (eg, <1% for a 25% higher CMV IgG titer).

Because there were no significant associations between CMV IgG titer and plaque, we did not assess for mediation of the relationship between CMV IgG titer and plaque by inflammatory biomarkers or whether CMV IgG titer mediated any effect of specific immune pathways on plaque.

DISCUSSION

Persistent immune activation despite durable ART-mediated suppression in PWH is thought to mediate the increased risk of non-AIDS-related comorbidities, particularly CVD. Although asymptomatic CMV infection may increase persistent immune activation in PWH, it is largely unknown whether CMV itself is linked to increased coronary plaque or if CMV mediates the known relationship of specific immune and inflammatory pathways with plaque in this population. We leveraged the mechanistic substudy embedded in the global REPRIEVE trial and assessed CMV IgG titer in relation to baseline coronary artery plaque phenotypes by CTA among a population with long-standing, well-controlled HIV with low-to-moderate traditional CVD risk. We addressed this gap in the literature and made several important observations. First, CMV IgG titer is associated with key host-specific factors, most strikingly nadir CD4. Second, the associations between CMV IgG titer and pathways of immune activation and inflammation were attenuated and modest after adjustment. Most surprisingly, CMV IgG titer was not associated with plaque presence or phenotype in this cross-sectional analysis.

Consistent with prior work, we found that CMV IgG titer was associated with key host-specific factors. Older age, detectable HIV viral load (although most participants were suppressed), and lower nadir CD4 were associated with higher CMV IgG titer. Older age has been previously associated with CMV IgG and CMV T-cell specific responses, in both PWH and the general population [32, 33]. As expected, the

Table 1. Participant Demographic and Cardiovascular Characteristics by CMV Quartiles

Variable	All Participants N = 672	CMV <790 IU/mL N = 168	CMV 790–2674 IU/mL N = 168	CMV 2675–6670 IU/mL N = 168	CMV >6670 IU/mL N = 168	*P* Value
Demographics						
Age, years	50.8 ± 5.9	49.7 ± 5.8	51.4 ± 5.7	51.1 ± 6.2	51.2 ± 5.7	.03
Natal sex						.82
Women	117 (17.4)	30 (17.9)	26 (15.5)	31 (18.5)	30 (17.9)	
Men	555 (82.6)	138 (82.1)	142 (84.5)	137 (81.6)	138 (82.1)	
Race						.98
White	353 (52.5)	90 (53.6)	90 (53.6)	86 (51.2)	87 (51.8)	
Black	242 (36.0)	57 (33.9)	56 (33.3)	62 (36.9)	67 (39.9)	
Other ethnicity	77 (11.5)	21 (12.5)	22 (13.1)	20 (11.9)	14 (8.3)	.05
Hispanic or Latinx	170/662 (25.7)	49/166 (29.5)	46/165 (27.9)	41/165 (24.9)	34/166 (20.5)	
Not Hispanic or Latinx	492/662 (74.3)	117/166 (70.5)	119/165 (72.1)	124/165 (75.2)	132/166 (79.5)	
Cardiovascular risk factors						
Smoking status						.05
Current/Former	367/670 (54.8)	85/167 (50.9)	86/168 (51.2)	95/168 (56.6)	101/167 (60.5)	
Never	303/670 (45.2)	82/167 (49.1)	82/168 (48.8)	73/168 (43.5)	66/167 (39.5)	
Alcohol use						.06
Usually/Often/Sometimes	182 (27.1)	51 (30.4)	51 (30.4)	42 (25.0)	38 (22.6)	
Rarely/Never	490 (72.9)	117 (69.6)	117 (69.6)	126 (75.0)	130 (77.4)	
Substance use						.05
Current/Former	333/669 (49.8)	73/167 (43.7)	85/168 (50.6)	83/168 (49.4)	92/166 (55.4)	
Never	336/669 (50.2)	94/167 (56.3)	83/168 (49.4)	85/168 (50.6)	74/166 (44.6)	
Hypertension	221 (32.9)	56 (33.3)	50 (29.8)	57 (33.9)	58 (34.5)	.63
Diabetes	2 (0.3)	1 (0.6)	0 (0.0)	0 (0.0)	1 (0.6)	1.00
BMI, kg/m^2	27.4 ± 4.4	27.1 ± 4.3	27.6 ± 4.3	27.5 ± 4.2	27.3 ± 4.8	.69
Fasting glucose, mmol/L	5.18 ± 0.70	5.24 ± 0.68	5.16 ± 0.73	5.20 ± 0.75	5.12 ± 0.67	.10
eGFR, mL/min/1.73m^2	88.2 ± 16.5	90.4 ± 17.2	87.8 ± 15.8	87.2 ± 17.2	87.6 ± 15.7	.10
Entry fasting lipids						
LDL-C, mmol/L	2.79 ± 0.78	2.86 ± 0.78	2.86 ± 0.76	2.78 ± 0.79	2.68 ± 0.78	.03
HDL-C, mmol/L	1.31 ± 0.49	1.32 ± 0.47	1.36 ± 0.55	1.31 ± 0.50	1.24 ± 0.43	.12
Cardiovascular medications						
Prior statin use	55 (8.2)	13 (7.7)	16 (9.5)	13 (7.7)	13 (7.7)	.85
Antihypertensive medication	139 (20.7)	35 (20.8)	27 (16.1)	34 (20.2)	43 (25.6)	.19
Median ASCVD risk score	4.5 [2.5–7.0]	3.9 [2.0–6.3]	5.0 [2.5–7.3]	4.5 [2.9–6.5]	5.0 [3.0–7.7]	.01
HIV parameters						
Total ART duration, years						.05
<5	103 (15.3)	32 (19.1)	26 (15.5)	27 (16.1)	18 (10.7)	
5–10	175 (26.0)	43 (25.6)	46 (27.4)	45 (26.8)	41 (24.4)	
>10	394 (58.6)	93 (55.4)	96 (57.1)	96 (57.1)	109 (64.9)	
Entry regimen						
ART regimen by class						.20
NRTI + INSTI	310 (46.1)	77 (45.8)	74 (44.1)	81 (48.2)	78 (46.4)	
NRTI + NNRTI	165 (24.6)	51 (30.4)	42 (25.0)	46 (27.4)	26 (15.5)	
NRTI + PI	109 (16.2)	26 (15.5)	37 (22.0)	22 (13.1)	24 (14.3)	
NRTI-sparing	20 (3.0)	1 (0.6)	4 (2.4)	3 (1.8)	12 (7.1)	
Other NRTI-containing	68 (10.1)	13 (7.7)	11 (6.6)	16 (9.5)	28 (16.7)	
CD4 category, cells/mm^3						<.001
CD4 <350	99 (14.7)	8 (4.8)	23 (13.7)	28 (16.7)	40 (23.8)	
CD4 350–499	133 (19.8)	27 (16.1)	35 (20.8)	38 (22.6)	33 (19.6)	
CD4 ≥500	440 (65.5)	133 (79.2)	110 (65.5)	102 (60.7)	95 (56.6)	
Nadir CD4 category						<.001
Nadir CD4 <50	148 (22.0)	19 (11.3)	26 (15.5)	37 (22.0)	66 (39.3)	
Nadir CD4 50–199	194 (28.9)	39 (23.2)	51 (30.4)	59 (35.1)	45 (26.8)	
Nadir CD4 200–349	175 (26.0)	53 (31.6)	49 (29.2)	39 (23.2)	34 (20.2)	
Nadir CD4 ≥350	133 (19.8)	52 (31.0)	34 (20.2)	28 (16.7)	19 (11.3)	
Unknown	22 (3.3)	5 (3.0)	8 (4.8)	5 (3.0)	4 (2.4)	

Table 1. Continued

Variable	All Participants N = 672	CMV <790 IU/mL N = 168	CMV 790–2674 IU/mL N = 168	CMV 2675–6670 IU/mL N = 168	CMV >6670 IU/mL N = 168	*P* Value
HIV viral load, copies/mL						<.001
<LLQ	587 (88.5)	152 (92.1)	154 (92.2)	146 (87.4)	135 (82.3)	
LLQ - <400	63 (9.5)	13 (7.9)	11 (6.6)	17 (10.2)	22 (13.4)	
≥400	13 (2.0)	0 (0.0)	2 (1.2)	4 (2.4)	7 (4.3)	

Reported as n/N (%), mean ± SD, or median [Q1–Q3].

Abbreviations: ART, antiretroviral therapy; ASCVD, atherosclerotic cardiovascular disease; BMI, body mass index; CMV, cytomegalovirus; eGFR, estimated glomerular filtration rate; IU, international units; LLQ, lower limit of quantification Q1, first quartile; Q3, third quartile; SD, standard deviation.

Table 2. Comparison of Coronary Artery Disease Indexes by CMV Quartiles

Variable	All Participants N = 672	CMV <790 IU/mL N = 168	CMV 790–2674 IU/mL N = 168	CMV 2675–6670 IU/mL N = 168	CMV >6670 IU/mL N = 168	*P* Value
Participants with any plaque	329 (49.0)	82 (48.8)	75 (44.6)	86 (51.2)	86 (51.2)	.43
Plaque score categories						.25
0 segments with plaque	343 (51.0)	86 (51.2)	93 (55.4)	82 (48.8)	82 (48.8)	
1–2 segments with plaque	240 (35.7)	62 (36.9)	60 (35.7)	58 (34.5)	60 (35.7)	
≥3 segments with plaque	89 (13.2)	20 (11.9)	15 (8.9)	28 (16.7)	26 (15.5)	
Non-calcified plaque categories						.29
0 segments with non-calcified plaque	406 (60.4)	105 (62.5)	107 (63.7)	95 (56.6)	99 (58.9)	
1–2 segments with non-calcified plaque	217 (32.3)	51 (30.4)	53 (31.6)	55 (32.7)	58 (34.5)	
≥3 segments with non-calcified plaque	49 (7.3)	12 (7.1)	8 (4.8)	18 (10.7)	11 (6.6)	
Vulnerable plaque features						
Participants with vulnerable plaque	152 (22.6)	37 (22.0)	34 (20.2)	36 (21.4)	45 (26.8)	.28
Presence of remodeling	147/152 (96.7)	34/37 (91.9)	33/34 (97.1)	35/36 (97.2)	45/45 (100.0)	.05
Low-attenuation plaque	36/152 (23.7)	7/37 (18.9)	8/34 (23.5)	12/36 (33.3)	9/45 (20.0)	.75
Napkin ring sign	19/152 (12.5)	5/37 (13.5)	3/34 (8.8)	6/36 (16.7)	5/45 (11.1)	.98
Positive remodeling AND low-attenuation plaque	31/152 (21.1)	5/37 (13.5)	7/34 (20.6)	11/36 (30.6)	9/45 (25.0)	.36
Leaman score, continuous	2.0 ± 2.7 0.0 [0.0–3.2]	1.9 ± 2.6 0.0 [0.0–3.2]	1.9 ± 2.7 0.0 [0.0–3.2]	2.3 ± 2.9 0.6 [0.0–4.2]	2.2 ± 2.8 0.3 [0.0–3.5]	.20
Leaman score, ordinal						.21
Leaman score = 0	343/660 (52.0)	86/162 (53.1)	93/167 (55.7)	82/167 (49.1)	82/164 (50.0)	
Leaman score ≥0–5	215/660 (32.6)	55/162 (34.0)	52/167 (31.1)	56/167 (33.5)	52/164 (31.7)	
Leaman score ≥5	102/660 (15.5)	21/162 (13.0)	22/167 (13.2)	29/167 (17.4)	30/164 (18.3)	
Stenosis						
Participants with CAD, stenosis >0%	317/660 (48.0)	76/162 (46.9)	74/167 (44.3)	85/167 (50.9)	82/164 (50.0)	.36
CAD categories (those with CAD)						.14
Mild CAD (stenosis 1–49%)	297/317 (93.7)	72/76 (94.7)	73/74 (98.7)	77/85 (90.6)	75/82 (91.5)	
Moderate CAD (stenosis 50–69%)	14/317 (4.4)	3/76 (4.0)	1/74 (1.4)	6/85 (7.1)	4/82 (4.9)	
Severe CAD (stenosis ≥70% or ≥50% stenosis of left main coronary artery)	6/317 (1.9)	1/76 (1.3)	0/74 (0.0)	2/85 (2.4)	3/82 (3.7)	
CAD stenosis ≥50%	20/660 (3.0)	4/162 (2.5)	1/167 (0.7)	8/167 (4.8)	7/164 (4.3)	.11
CAC score						
Participants with CAC >0	223/639 (34.9)	67/162 (41.4)	47/158 (29.7)	47/161 (29.2)	62/158 (39.2)	.66
CAC categories (those with CAC)						.07
CAC = 1–100	155/223 (69.5)	48/67 (71.6)	37/47 (78.7)	34/47 (72.3)	36/62 (58.1)	
CAC = 101–400	57/223 (25.6)	17/67 (25.4)	8/47 (17.0)	10/47 (21.3)	22/62 (35.5)	
CAC >400	11/223 (4.9)	2/67 (3.0)	2/47 (4.3)	3/47 (6.4)	4/62 (6.5)	

Reported as n/N (%), mean±SD, or median [Q1–Q3].

Abbreviations: CAC, coronary artery calcium; CAD, coronary artery disease; CMV, cytomegalovirus; IU, international units; SD, standard deviation.

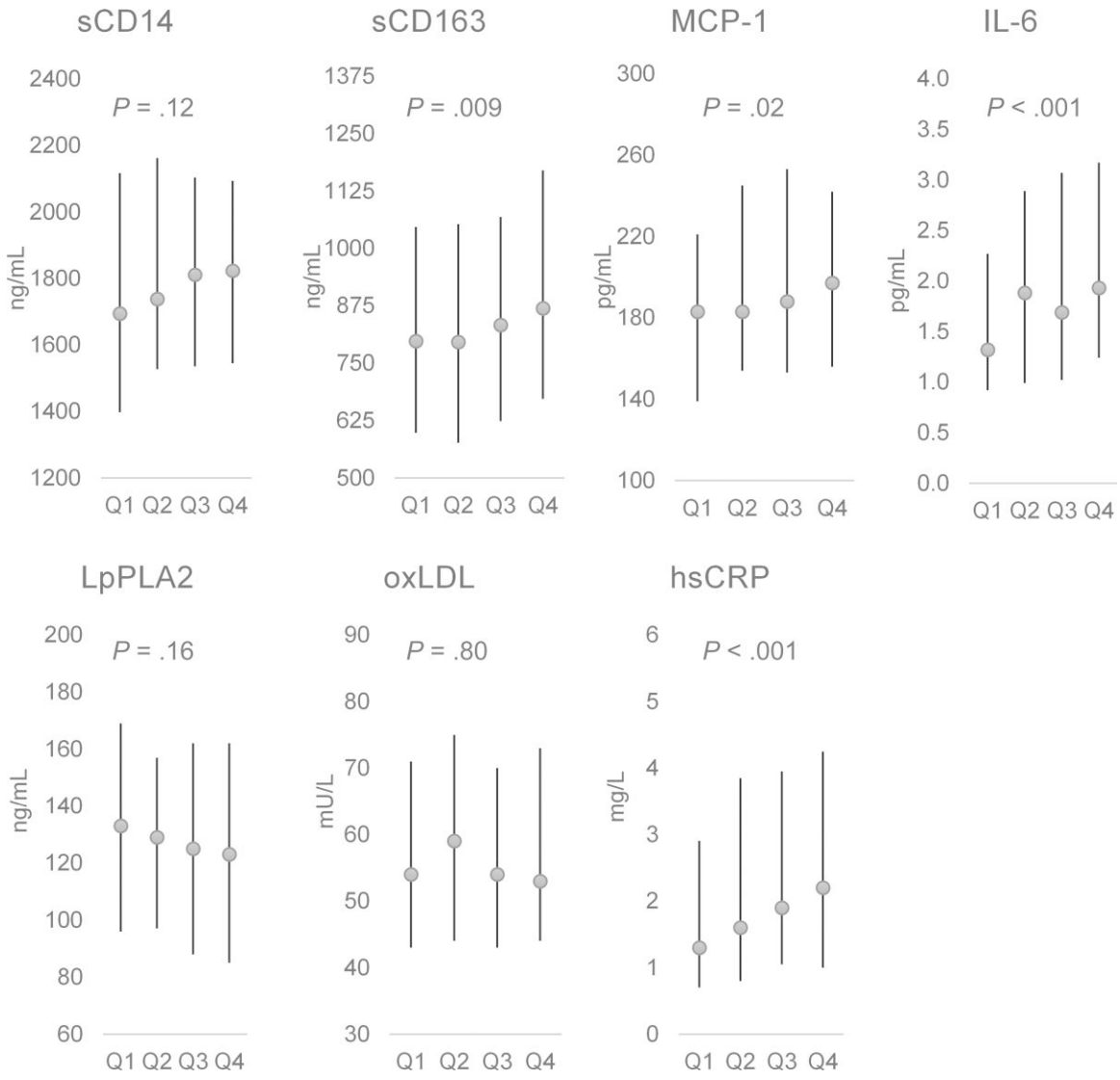

Figure 1. Biomarker distributions across CMV IgG quartile groups. Figure representing the unadjusted biomarker medians denoted by dots and range of first to third quartiles denoted by connecting lines, shown across increasing CMV IgG quartile groups. Units for sCD14 (ng/mL), sCD163 (ng/mL), MCP-1 (pg/mL), IL-6 (pg/mL), LpPLA2 (ng/mL), oxLDL (mU/L), and hsCRP (mg/L) listed here. Abbreviations: CMV, cytomegalovirus; hsCRP, high-sensitivity C-reactive protein; IgG, immunoglobulin G; IL-6, interleukin 6; LpPLA2, lipoprotein-associated phospholipase A2; MCP-1, monocyte chemoattractant protein-1; oxLDL, oxidized LDL; Q1, first quartile; Q2, second quartile; Q3, third quartile; Q4, fourth quartile; sCD14, soluble CD 14; sCD163, soluble CD163.

association with nadir CD4 was most remarkable. Multiple prior studies have associated CMV IgG with lower current CD4, nadir CD4, and poor CD4 recovery [9, 34, 35]. The one randomized trial of valganciclovir in PWH with CD4 ≤350 cells/mm^3 did not find any significant improvement in the CD4/CD8 ratio compared to placebo, while a later study found CMV seropositivity to be associated with lower odds of CD4/CD8 normalization and immune recovery when initiating ART [9, 13]. Asymptomatic CMV infection could be both a cause and consequence of immune recovery. Indeed, it is unclear to what degree CMV IgG titer reflects CMV replication burden or antiviral immune response. Data in the cancer and transplant populations link more frequent CMV reactivations or viremia to a higher CMV IgG titer; our results associating lower current and nadir CD4 with higher CMV IgG titer appear congruent [36, 37]. However, one study in men with treated HIV associated greater CMV seminal shedding with lower CMV IgG titer [38]. The relationship between CMV tissue burden and IgG titer has not been fully elucidated, as there is likely a complex interplay of unique host factors.

In our study, higher CMV IgG titer was associated with higher sCD163, MCP-1, IL-6, and hsCRP in unadjusted analyses, but these relationships were attenuated after adjustment for HIV-specific factors. Multiple cross-sectional and cohort studies

have shown variable associations of CMV IgG titer or CMV serostatus with CRP, IL-6, and sCD14 [11, 34, 39]. The valganciclovir trial in PWH showed a possible reduction in CRP but more significant reductions in other markers of generalized inflammation (sTNFR1, sTNFR2), some markers of endothelial dysfunction (sICAM-1), monocyte/macrophage activation (sCD14, sCD163), and microbial translocation (sCD14) [12, 13]. It is unclear if valganciclovir's immunologic effects are mediated primarily by suppressing CMV versus other herpesviruses; an impending study on the CMV-specific drug letermovir will help to clarify this (NCT04840199). Moreover, we do not know if the proposed causal relationships of CMV with these inflammatory pathways would extend to those with better immune recovery, as CMV and other putative drivers of immune activation may not impact all PWH equally [3]. As most participants in REPRIEVE had CD4 ≥ 350 (85%) and ≥500 (66%), there may be a relatively smaller impact of asymptomatic CMV infection on immune activation and inflammation.

Most surprisingly, we found that CMV IgG titer was not associated with the presence of plaque or plaque phenotypes in this baseline analysis. Across multiple surrogate measures of CAD, including CAC, stenosis, presence, or amount of calcified or non-calcified plaque, Leaman Score, and vulnerable plaque features, no association was identified with CMV IgG titer across increasing CMV IgG quartile groups. Given the possibility of differential effects of CMV on CAD by age, sex, ASCVD risk score, LDL, or current or nadir CD4, we also stratified by these and found no evidence of interaction. While this is the first comprehensive study to assess the possible relationship between CMV IgG titer and coronary atherosclerotic plaque in PWH, our findings were unexpected in the context of previously published literature. Several studies have demonstrated the association between CMV seropositivity or IgG titer with cardiovascular or cerebrovascular disease in PWH, though some with small sample sizes [16, 17, 23]. Other studies in PWH have found associations between CMV IgG titer or CMV-specific T-cell responses and cIMT [19, 21, 22, 40]. CMV has been mechanistically linked to atherosclerosis and vascular damage through replication in macrophages and endothelial and smooth muscle cells in the vasculature, and monocyte tissue factor expression [11, 19, 20, 41–43]. In contrast, we did not identify an association of CMV IgG titer with plaque in ART-treated PWH.

It is important to acknowledge several factors when evaluating our finding of the absence of association between CMV IgG titer and coronary atherosclerotic plaque among PWH in the context of prior studies. First, it is possible that the identified associations between CMV IgG and cardiovascular events or cIMT seen in other studies may not have been fully adjusted for confounders. Second, the longer-term ramifications of asymptomatic CMV infection on plaque may be seen over time and not cross-sectionally. However, the lack of any relationship at a single point in time among a large group with longstanding treated HIV is notable. Further, the REPRIEVE substudy population may not represent those with HIV who are most at risk for a CMV-specific effect on plaque. Existing literature suggest a negligible-to-modest effect of prior CMV infection in the general population but a significant role in cardiac allograft rejection or vasculopathy in the transplant population [14, 18, 44–47]. This suggests an increasing role for CMV in CAD based on T-cell defects; although stratification based on current or nadir CD4 did not impact our findings, it is plausible that a CMV-specific relationship with plaque may be present for those PWH with more immunosuppression. Additionally, given that we do not know what CMV IgG titer represents in vivo and to what extent it reflects viral burden and/or antiviral inflammatory response, it is also possible that CMV IgG titer may not be an ideal surrogate for CMV tissue burden in the vasculature, and as such, bias our findings toward the null. Finally, asymptomatic CMV infection may have a mechanism leading to CVD or CV events outside of coronary atherosclerotic plaque. One prior study has associated CMV IgG titer with cardiac microvascular dysfunction on PET-CT, with some corroborating mechanistic findings in a murine model via *P*-selectin [48, 49]. Other studies have linked CMV to thrombogenic activity, and with a recent study's link of CMV IgG to type 1 MI but not type 2 MI, it is also possible that CMV has a more direct relationship to acute plaque rupture [41–43].

This study had strengths in terms of its size and careful phenotyping, with CTA and inflammatory biomarkers assessed in a highly relevant population of well-controlled, longstanding PWH and excess CAD despite a low CVD risk. Nonetheless, the study does have several limitations. First, the cross-sectional nature of the study does not allow for assessment of incident plaque development, though this is one of the main forthcoming outcomes. Second, CMV IgG titer was used as a surrogate for CMV disease burden, and it ultimately may not be an appropriate proxy in the relationship between asymptomatic CMV infection and plaque as a surrogate for CAD. Moreover, although we attempted to assess plaque phenotypes by estimated seroprevalence based on prior studies, there were no consistent patterns with seropositivity, and the actual seroprevalence in this setting is unknown. Additionally, the study participants may not be representative of all PWH in terms of geography, as our participants in the substudy were limited to the US, and differential relationships may be seen in other settings. Finally, we did not measure all inflammatory pathways that are associated with persistent immune activation, CMV, and CAD.

In summary, we observed associations between CMV IgG titer as a surrogate for asymptomatic CMV infection and several host-specific factors, particularly nadir CD4. Nonetheless, we did not find any meaningful association between CMV IgG

titer and coronary artery plaque phenotypes at enrollment into REPRIEVE. Further longitudinal assessments in REPRIEVE will determine the relationship of CMV IgG titer to plaque progression and CVD events.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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