Role of T-Cell Dysfunction, Inflammation, and Coagulation in Microvascular Disease in HIV

Arjun Sinha, MD, MS; Yifei Ma, MS; Rebecca Scherzer, PhD; Sophia Hur, MPH; Danny Li, BS; Peter Ganz, MD; Steven G. Deeks, MD; Priscilla Y. Hsue, MD

**Background**—Compared to uninfected adults, HIV-infected adults on antiretroviral therapy are at increased risk of cardiovascular disease. Given the increase in T-cell dysfunction, inflammation, and coagulation in HIV infection, microvascular dysfunction is thought to contribute to this excess cardiovascular risk. However, the relationships between these variables remain undefined.

**Methods and Results**—This was a cross-sectional study of 358 HIV-infected adults from the SCOPE cohort. Macrovascular endothelial function was assessed using flow-mediated dilation of the brachial artery and microvascular function by reactive hyperemia. T-cell phenotype was determined by flow cytometry. Plasma markers of inflammation (tumor necrosis factor-α, interleukin-6, high-sensitivity C-reactive protein, sCD14) and coagulation (fibrinogen, D-dimer) were also measured. In all HIV+ subjects, markers of inflammation (tumor necrosis factor-α, high-sensitivity C-reactive protein), coagulation (D-dimer) and T-cell activation (CD8+PD1+, CD4+ interferon-γ/cytomegalovirus-specific) were associated with worse reactive hyperemia after adjusting for traditional cardiovascular risk factors and co-infections. In treated and suppressed subjects, tumor necrosis factor-α and CD8+PD1+ cells remained associated with worse reactive hyperemia after adjustment. Compared to the untreated subjects, CD8+PD1+ cells were increased in the virally suppressed group. Reactive hyperemia was predictive of flow-mediated dilation.

**Conclusions**—CD8+PD1+ cells and tumor necrosis factor-α were associated with microvascular dysfunction in all HIV+ subjects and the treated and suppressed group. Additionally, D-dimer, high-sensitivity C-reactive protein, sCD-14, and interleukin-6 were associated with microvascular dysfunction in all HIV+ subjects. Although T-cell dysfunction, inflammation, and microvascular dysfunction are thought to play a role in cardiovascular disease in HIV, this study is the first to look at which T-cell and inflammatory markers are associated with microvascular dysfunction in HIV-infected individuals. (J Am Heart Assoc. 2016;5: e004243 doi: 10.1161/JAHA.116.004243)

**Key Words:** coagulation • HIV • immune system • inflammation • microcirculation

HIV-infected individuals have high rates of cardiovascular disease including acute myocardial infarction, heart failure, and arrhythmias. The mechanism underlying this excess risk remains largely unknown but likely includes an interplay between traditional risk factors, antiretroviral therapy (ART), along with other HIV-related features.7–9

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A hallmark of HIV infection is increased inflammation that is present even in the setting of treated and suppressed HIV disease.10 This chronic inflammation is thought to underlie non-AIDS events including cardiovascular disease.11 In the SMART study, inflammatory and coagulation markers were strongly predictive of mortality and cardiovascular disease independent of viral load or CD4+ count.12–14

Several different pathways contribute to this chronic inflammatory state in HIV.15,16 Ongoing activation of the immune system in virally suppressed individuals occurs through persistent low-level viral replication in protected cells and tissue, translocation of gut microbial products due to breakdown of gut mucosal defenses, and reactivation of chronic infections such as cytomegalovirus.17–20

Both innate and adaptive immunity play an important role in the pathogenesis of atherosclerosis in HIV-uninfected individuals.21 Antigens such as oxidized low-density lipoprotein are recognized as pathogens by dendritic cells, a part of the innate immune system. The dendritic cells present these antigens to naïve T cells, leading to T-cell activation.22
Activated T cells are defined by markers including CD38, HLA-DR, CCR5, and PD-1. These activated T cells are significantly increased in HIV-infected individuals and correlate with viral load and disease stage. Some studies have shown that T cell and monocyte activation are independently associated with subclinical atherosclerosis in HIV-infected individuals on ART as measured by carotid intimal medial thickness or carotid artery stiffness. However, other studies have not found a relationship between T-cell activation and carotid intimal medial thickness or subsequent cardiovascular events in HIV-infected individuals.

The vast majority of cardiovascular disease studies in HIV-infected adults have focused on clinical end points or measures of large-vessel (macrovascular) function. These include studies of flow-mediated dilation (FMD), coronary calcification, and carotid intimal medial thickness progression. FMD, a measure of endothelial dysfunction, assesses key steps in the initiation, perpetuation, and clinical manifestations of atherogenesis. However, the consistent findings that systemic markers of inflammation and hypercoagulability are elevated in HIV disease and strongly associated with cardiovascular disease suggest that microvascular disease may in fact be more prevalent in this patient population. Reactive hyperemia (RH) in the brachial artery is an assessment of microvascular function and serves as the stimulus for FMD. It is measured by Doppler and can be determined along with FMD during brachial artery reactivity studies. Prior studies have shown that both RH and FMD are lower in HIV-infected individuals compared to healthy controls.

The purpose of this study was to elucidate the role of inflammation, hypercoagulability, and T-cell immunity as potential determinants of endothelial function, particularly microvascular function, in HIV-infected individuals. In assessing endothelial function, we measured the response of the microvasculature and the macrovasculature using RH and FMD, respectively. Endothelial dysfunction occurs early on in atherosclerosis and precedes the development of morphological changes in the vasculature. Thus, determining whether inflammation, hypercoagulability, and T-cell dysfunction are associated with endothelial dysfunction in HIV-infected individuals is an important step in understanding the mechanism underlying HIV-associated atherosclerosis and heightened cardiovascular risk in the setting of HIV.

Methods

Patient Selection

This was a cross-sectional study of HIV-infected adults from the SCOPE cohort. SCOPE is an observational prospective cohort based on the HIV/AIDS clinics at the San Francisco General Hospital and the San Francisco Veterans Affairs Medical Center. Each participant undergoes an in-depth questionnaire interview detailing their HIV disease history and overall medical history including cardiac risk factors. Participants in the SCOPE cohort are seen every 4 months for blood tests and are given questionnaires regarding medications, health-related behaviors, and symptoms. The University of California, San Francisco Committee on Human Research approved the study and all participants provided written informed consent.

With respect to cardiac risk factors, hypertension and diabetes mellitus (DM) were defined using standard classifications. Total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, and triglycerides were measured after fasting for 12 hours. For HIV-specific factors, we measured current as well as nadir CD4 counts. Given that CD4/CD8 ratio is emerging as a prognostic marker and is independently associated with non-AIDS events or death, current CD8 levels were also measured. There were a total of 358 participants in this study, with 250 on ART with suppressed HIV RNA levels (treated suppressed). There were 61 participants off ART with detectable HIV RNA (untreated noncontrollers), 25 participants off ART with a suppressed viral load (elite controllers), and 22 participants on ART with an elevated viral load. Total duration of ART including the duration for nucleoside reverse transcriptase inhibitors, nonnucleoside reverse transcriptase inhibitors, and protease inhibitors was measured for each individual.

Measurement of Plasma Biomarkers

Soluble markers of inflammation (interleukin-6, tumor necrosis factor (TNF)-α, high-sensitivity C-reactive protein, sCD14), coagulation (D-dimer, fibrinogen), macrophage activation (sCD163), and prior cytomegalovirus exposure (cytomegalovirus immunoglobulin G [IgG]) were measured in cryopreserved plasma samples using a multiplex electrochemiluminescence assay (Meso Scale Discovery, MD).

Measurement of T-Cell Phenotype

T-cell phenotypes were measured by immunophenotyping performed at the University of California, San Francisco, Core Immunology Laboratory, using methods that have been optimized and validated for frozen peripheral blood mononuclear cells. Briefly, cryopreserved peripheral blood mononuclear cells were rapidly thawed in warm media, counted on Accuri C6 (Biosciences) with the Viacount assay (Millipore), washed, and stained with Aqua Amine Reactive Dye to discriminate dead cells. Cells were then stained with the following fluorescently conjugated monoclonal antibodies: CD3-Pacific Blue, CD4-PE Texas Red, CD8-Qtet 605, CD38-PE, HLA-DR-FITC, CCR5-PECY5, and PD-1-Alexa647. Stained cells
were washed, fixed in 0.5% formaldehyde, and held at 4°C until analysis where they were run on a customized BD LSR II (BD Bioscience). A total of 100,000 lymphocytes were collected for each sample. Data were compensated and analyzed using FlowJo (Tree Star) to determine the proportion of CD4+ and CD8+ T cells expressing each of the T-cell markers (CD38, HLA-DR, CCR5, and/or PD-1). Combinations of markers were calculated in FlowJo, using the Boolean gate function. Cytomegalovirus-specific T-cell responses were determined by measuring the proportion of CD4+ and CD8+ T cells that expressed interferon (IFN)-γ after exposure to cytomegalovirus pp65 peptides using flow cytometry.

**Measurement of Endothelial Function**

High-resolution 10-MHz linear array ultrasound probe and the GE Vivid 7 Imaging Console were used to measure endothelial function in the right brachial artery immediately proximal to the antecubital fossa. Participants were asked not to consume any food, alcohol, caffeine, or nicotine 12 hours prior to the study. Measurements were done in a dark and quiet room while the participant was lying supine. FMD was quantified by inflating a forearm cuff to a suprasystolic pressure for 5 minutes in order to create an ischemic stimulus. Subsequently, the change in brachial artery diameter was measured every 15 s using B-mode ultrasound 30 to 120 s following cuff deflation. FMD was calculated as the percent change between the maximum post cuff release brachial artery diameter and the baseline diameter. RH was quantified using spectral Doppler images for the first 15 s following cuff release. Maximal RH was measured as the Doppler mean velocity-time integral of the first 3 complete beats after cuff release. Analysis of digitized images was performed using dedicated software (Medical Imaging Applications, LLC, Coralville, IA). The level of reproducibility for both FMD and RH is excellent in our laboratory as described in previous studies. On repeat assessments of FMD, the coefficient of variation has been 2.7% and the intraclass correlation coefficient has been 0.98, indicating high reproducibility. Similarly, on repeat measurements of RH, the coefficient of variation has been 0.65% and the intraclass correlation coefficient has been 0.99.

**Statistical Analyses**

For all HIV groups, continuous variables were expressed as the median (and interquartile range). Categorical variables were expressed as percent. We used both unadjusted and multivariable adjusted generalized linear regression (using a log link function) in order to examine relationships of T-cell phenotypes and inflammatory markers with endothelial function, in separate models for FMD and RH. Multivariable models were adjusted for demographics (age, sex, race/ethnicity), traditional cardiac risk factors (DM, hypertension, hyperlipidemia, and smoking), and co-infections (hepatitis C virus, opportunistic infections). Each T-cell marker and plasma marker was included individually and not simultaneously in the models. Hepatitis B virus and concurrent neoplasms were also identified but given their low prevalence in the cohort, they were not incorporated into the multivariable models. We constructed models using all HIV+ subjects, and also using the treated suppressed group only. Similar models were constructed to determine the association between HIV-related factors and endothelial function. Each HIV-related factor was included individually and not simultaneously in the models. Measures that were right-skewed such as T-cell phenotypes and inflammatory markers were log transformed in order to normalize their distributions. We calculated the standardized regression coefficients for T-cell phenotypes, inflammatory markers, and HIV-related continuous variables to reflect the effects with 1 SD increment. Since FMD and RH also showed right-skewed distributions, each outcome was log-transformed for analysis; results were back-transformed to produce estimated percentage differences.

We also sought to determine whether inflammatory markers were important mediators of the relationship between T-cell phenotypes and endothelial dysfunction in the treated and suppressed group. An inflammatory marker was considered a mediator if it was significantly associated with the T-cell marker and endothelial dysfunction (at the 0.05 level in multivariable analysis). Mediation models were constructed for that T-cell marker if it showed a significant association with endothelial dysfunction (also at the 0.05 level in multivariable analysis). The coefficients estimating the associations between the T-cell phenotype and endothelial dysfunction were compared in models before and after adjustment for the inflammatory marker. Diminishing coefficient would indicate complete or partial mediation effect by inflammatory markers. Additionally, we tested for interactions between T-cell phenotypes and inflammatory markers in order to assess the presence of moderated mediation. All analyses were performed using the SAS system, version 9.4 (SAS Institute, Inc, Cary, NC).

**Results**

**Clinical Characteristics**

We studied 358 HIV-infected individuals. A large majority, 250 subjects, were in the treated and suppressed group. About 24% of the subjects were untreated and only 6% were on antiretroviral therapy but still had a detectable viral load. As shown in Table 1, the median age was 50 years and 84% were male. Sixty-one percent were white, 22% black, and 11% Hispanic. The median duration of HIV infection was 15 years.
and the median CD4 T-cell count was 542 cells/mm³. While many of the individuals had cardiovascular risk factors such as hypertension (42%) and smoking (31%), relatively few had DM (8%), and the median total cholesterol was within the normal range. The traditional risk factor profile was similar among the treated and suppressed group as compared to the other HIV-infected individuals. Nearly all of the HIV-infected participants were cytomegalovirus seropositive (98%).

### Table 1. Summary of Demographics and Clinical Characteristics

<table>
<thead>
<tr>
<th></th>
<th>All Groups N=358</th>
<th>Treated and Suppressed N=250</th>
<th>Untreated and Unsuppressed N=61</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Demographics</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, y*</td>
<td>50 (43, 56)</td>
<td>51 (44, 58)</td>
<td>44 (39, 54)</td>
</tr>
<tr>
<td>White</td>
<td>61%</td>
<td>65%</td>
<td>52%</td>
</tr>
<tr>
<td>Black</td>
<td>22%</td>
<td>18%</td>
<td>31%</td>
</tr>
<tr>
<td>Hispanic</td>
<td>11%</td>
<td>12%</td>
<td>10%</td>
</tr>
<tr>
<td>Other</td>
<td>6%</td>
<td>5%</td>
<td>7%</td>
</tr>
<tr>
<td>Male</td>
<td>84%</td>
<td>88%</td>
<td>77%</td>
</tr>
<tr>
<td><strong>Cardiovascular risk factors</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>42%</td>
<td>40%</td>
<td>41%</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>8%</td>
<td>8%</td>
<td>7%</td>
</tr>
<tr>
<td>Current smoking</td>
<td>31%</td>
<td>25%</td>
<td>46%</td>
</tr>
<tr>
<td>Smoking pack years*</td>
<td>2 (0, 15)</td>
<td>1 (0, 15)</td>
<td>2 (0, 13)</td>
</tr>
<tr>
<td>LDL, mg/dL*</td>
<td>102 (83, 123)</td>
<td>103 (86, 123)</td>
<td>102 (80, 127)</td>
</tr>
<tr>
<td>HDL, mg/dL*</td>
<td>45 (37, 53)</td>
<td>45 (37, 55)</td>
<td>44 (36, 50)</td>
</tr>
<tr>
<td>Total cholesterol, mg/dL*</td>
<td>177 (154, 201)</td>
<td>179 (156, 204)</td>
<td>171 (148, 198)</td>
</tr>
<tr>
<td>Triglycerides, mg/dL*</td>
<td>116 (84, 192)</td>
<td>122 (89, 202)</td>
<td>100 (69, 127)</td>
</tr>
<tr>
<td><strong>HIV-related factors</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current CD4, cells/mm³*</td>
<td>542 (339, 720)</td>
<td>535 (319, 709)</td>
<td>536 (370, 701)</td>
</tr>
<tr>
<td>Nadir CD4, cells/mm³*</td>
<td>188 (45, 315)</td>
<td>130 (29, 234)</td>
<td>350 (272, 458)</td>
</tr>
<tr>
<td>CD4/CD8 ratio*</td>
<td>0.58 (0.38, 0.92)</td>
<td>0.62 (0.40, 0.98)</td>
<td>0.50 (0.32, 0.76)</td>
</tr>
<tr>
<td>CD8, cells/mm³*</td>
<td>892 (666, 1224)</td>
<td>852 (632, 1105)</td>
<td>1069 (752, 1393)</td>
</tr>
<tr>
<td>HIV duration, y*</td>
<td>15 (7, 20)</td>
<td>16 (8, 21)</td>
<td>9 (4, 20)</td>
</tr>
<tr>
<td>NNRTI use</td>
<td>51%</td>
<td>62%</td>
<td>10%</td>
</tr>
<tr>
<td>NRTI use</td>
<td>80%</td>
<td>100%</td>
<td>16%</td>
</tr>
<tr>
<td>Protease inhibitor use</td>
<td>62%</td>
<td>78%</td>
<td>8%</td>
</tr>
<tr>
<td>Hepatitis C infection</td>
<td>18%</td>
<td>15%</td>
<td>16%</td>
</tr>
<tr>
<td>Hepatitis B infection</td>
<td>5%</td>
<td>4%</td>
<td>5%</td>
</tr>
<tr>
<td>Opportunistic infection</td>
<td>13%</td>
<td>12%</td>
<td>8%</td>
</tr>
<tr>
<td>Neoplasms (non-skin cancer)</td>
<td>4%</td>
<td>5%</td>
<td>2%</td>
</tr>
<tr>
<td><strong>Disease categories</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Off ART, VL &lt;75 copies/mL</td>
<td>7%</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Off ART, VL &gt;75 copies/mL</td>
<td>17%</td>
<td>—</td>
<td>100%</td>
</tr>
<tr>
<td>On ART, VL &lt;75 copies/mL</td>
<td>70%</td>
<td>—</td>
<td>100%</td>
</tr>
<tr>
<td>On ART, VL &gt;75 copies/mL</td>
<td>8%</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td><strong>Endothelial function</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reactive hyperemia (%)*</td>
<td>61.5 (46.3, 83.3)</td>
<td>59.2 (44.9, 82.2)</td>
<td>66.9 (51.9, 85.6)</td>
</tr>
<tr>
<td>Flow-mediated dilation (%)*</td>
<td>4.0 (2.6, 5.5)</td>
<td>3.9 (2.6, 5.4)</td>
<td>4.2 (2.6, 5.6)</td>
</tr>
</tbody>
</table>

ART indicates antiretroviral therapy; HDL, high-density lipoprotein; LDL, low-density lipoprotein; NNRTI, non-nucleoside reverse transcriptase inhibitors; NRTI, nucleoside reverse transcriptase inhibitors; VL, viral load.

*Continuous variables were summarized as median (interquartile range).
Microvascular and Macrovascular Function Are Strongly Correlated

We first examined the relationship between RH and FMD. In unadjusted analysis for all HIV+ subjects, RH was positively and strongly associated with FMD (25% increase in FMD per doubling of RH, 95% CI [15%, 37%], \( P<0.01 \)). This relationship remained significant after multivariable adjustment for demographics and traditional cardiovascular disease risk factors (26% increase in FMD per doubling of RH, 95% CI [16%, 37%], \( P<0.01 \)). Similarly, in the treated suppressed group, there was a 23% increase in FMD per doubling of RH after adjustment (95% CI [12%, 35%], \( P<0.01 \)).

Expression of T-Cell Markers Is Increased in the Treated Suppressed Group

Compared to subjects who were untreated with an elevated viral load, those in the treated and suppressed group had significantly increased expression of multiple different T-cell markers (Table 2). This was true for both CD4+ and CD8+ T cells expressing PD1. However, there was no difference between the 2 groups in the CD4+ IFN+ cytomegalovirus specific T-cell subset.

CD8+PD1+ T Cells Are Associated With Microvascular Dysfunction

We examined the association between T-cell phenotypes and microvascular dysfunction as measured by RH (Table 3). Among all HIV subjects, after adjustment for demographics, cardiovascular risk factors, and co-infections, CD8+PD1+ and CD4+IFN+cytomegalovirus-specific subsets were significantly associated with worse RH. Findings were similar and directionally consistent when we restricted the analysis to those who were treated and suppressed, although only the CD8+PD1+ subset remained statistically significant.

T-Cell Dysfunction Is Not Associated With Macrovascular Dysfunction

We then investigated which T-cell phenotypes were associated with macrovascular dysfunction as measured by FMD. Among all HIV+ subjects, after adjustment for demographics, traditional cardiovascular risk factors, and co-infections, the T-cell markers showed little association with FMD. These findings were similar when the analysis was restricted to the treated and suppressed individuals only (Table 3). Thus, CD8+PD1+ T cells were associated with microvascular dysfunction in all HIV+ subjects as well as the treated and suppressed subset, but they had no association with macrovascular dysfunction.

TNF-α Is Associated With Microvascular Dysfunction

Among all HIV+ subjects, D-dimer, TNF-α, high-sensitivity C-reactive protein, sCD-14, and interleukin-6 were associated with significantly worse RH after adjustment for demographics, cardiovascular risk factors, and co-infections. Results were similar when the analysis was restricted to the treated suppressed group, although only TNF-α remained associated with significantly worse RH in adjusted analysis (Table 4).

Cytomegalovirus IgG Is Associated With Macrovascular Dysfunction

In all HIV+ subjects, cytomegalovirus IgG and fibrinogen were significantly associated with worse FMD after adjustment for demographics, cardiovascular risk factors, and co-infections. Results were similar when we restricted the analysis to the treated suppressed group, although only cytomegalovirus IgG remained significantly associated with worse FMD (Table 4). Thus, while TNF-α was associated with microvascular dysfunction, prior infection with cytomegalovirus was associated with macrovascular dysfunction.

Effect of TNF-α on the Association Between CD8+PD1+ T-Cell Marker and Microvascular Dysfunction

Given that both TNF-α and CD8+PD1+ T-cell marker were significantly associated with microvascular dysfunction in the treated and suppressed group, we evaluated whether TNF-α
was a mediator of the association between CD8+PD1+ T cell and RH. In unadjusted analysis, we found that each 1 SD increase in TNF-α was associated with a 12.1% decrease in CD8+PD1+ T cells (95% CI: -22.5 to -0.3, \( p=0.04 \)). After adding TNF-α to the multivariable adjustment model used for Table 3, the association of CD8+PD1+ T cells with RH weakened from -11.2\% (\( p=0.02 \)) to -8.1\% (\( p=0.09 \)), while TNF-α remained strongly associated with lower RH (-12.5\%, \( p=0.001 \)). We found that the test for interaction between CD8+PD1+ T cells and TNF-α was positive and marginally significant (\( +8.3\% \), \( p=0.07 \)), suggesting that the negative effect of CD8+PD1+ T cells on RH was reversed as TNF-α increased.

**Total CD8+ T-Cell Counts Are Associated With Endothelial Dysfunction**

Finally, we assessed the association of traditional markers of HIV disease progression with vascular function. Among all HIV+ subjects, greater CD8+ T-cell count was associated with worse FMD after adjusting for demographics and traditional cardiovascular risk factors (Table 5). In the treated and suppressed cohort, greater CD8+ T-cell count was associated with worse RH after adjusting for demographics and traditional cardiovascular risk factors. Higher CD4/CD8 ratio was not associated with improved FMD or RH after adjustment in either group.

**Discussion**

In this relatively large cross-sectional study of treated and untreated HIV-infected adults, we investigated associations of markers of inflammation, coagulation, and T-cell phenotype with vascular function. In addition to measuring large-vessel function (FMD), we measured for the first time small-vessel function (as assessed by RH). We identified several markers of inflammation and immune phenotype that were associated with microvascular dysfunction, even after controlling for demographic, traditional cardiovascular risk factors, and co-infections. Among HIV-infected adults on effective ART (suppressed viremia), the total CD8+ T-cell count, the frequency of CD8+ T cells that express PD-1, and the inflammatory cytokine TNF-α were each associated with worsened microvascular disease. Among a larger cohort of treated and untreated HIV-infected adults, a broader panel of markers was linked to microvascular dysfunction, including the frequency of CD8+PD1+ and CD4+IFN-γ/cytomegalovirus-specific responses, as well TNF-α, high-sensitivity C-reactive protein, D-dimer, interleukin-6, and sCD14. Importantly, we found that macrovascular function (FMD) significantly correlated with microvascular function (RH), and that the major factors associated with FMD were cytomegalovirus IgG and fibrinogen.

We focused specifically on the treated and suppressed subgroup of the cohort for a few reasons. First, studies have demonstrated that endothelial dysfunction persists in the
Table 4. Association of Plasma Markers With Reactive Hyperemia and FMD

<table>
<thead>
<tr>
<th>Parameter (Per Increase in SD)</th>
<th>HIV+ All Groups</th>
<th>HIV+ Treated Suppressed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FMD %Estimate* (95% CI)</td>
<td>Reactive Hyperemia %Estimate (95% CI)</td>
</tr>
<tr>
<td>IL-6</td>
<td>−3.7 (−11.4, 4.6)</td>
<td>−6.6 (−12.1, 0.0)</td>
</tr>
<tr>
<td>TNF-α</td>
<td>0.3 (−5.9, 6.9)</td>
<td>−6.6 (−11.9, −1.0)</td>
</tr>
<tr>
<td>hsCRP</td>
<td>−4.7 (−10.1, 1.0)</td>
<td>−5.8 (−10.3, −1.0)</td>
</tr>
<tr>
<td>D-Dimer</td>
<td>−6.0 (−13.3, 2.0)</td>
<td>−5.8 (−11.0, −0.3)</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>−8.1 (−14.4, −1.3)</td>
<td>−6.0 (−12.1, 0.6)</td>
</tr>
<tr>
<td>CMV IgG</td>
<td>−9.9 (−15.2, −4.3)</td>
<td>−1.7 (−8.0, 5.0)</td>
</tr>
<tr>
<td>sCD-14</td>
<td>2.5 (−3.0, 8.4)</td>
<td>−4.0 (−7.6, −0.3)</td>
</tr>
<tr>
<td>sCD-163</td>
<td>2.0 (−5.2, 9.8)</td>
<td>−1.3 (−7.4, 5.2)</td>
</tr>
</tbody>
</table>

CMV indicates cytomegalovirus; FMD, flow-mediated dilation; hsCRP, high-sensitivity C-reactive protein; IgG, immunoglobulin G; IL-6, interleukin-6; TNF-α, tumor necrosis factor-α. *Percent estimate represents estimated percentage difference in reactive hyperemia or FMD associated with a doubling of each plasma marker. Each factor of interest was adjusted for age, sex, race, hypertension, diabetes mellitus, hyperlipidemia, smoking, hepatitis C virus, and opportunistic infections. Markers are included individually, not simultaneously.

setting of treated HIV infection and these individuals have an increased risk of cardiovascular disease. Accordingly, the impact of immune activation and inflammation on endothelial function is likely distinct in the treated and suppressed subgroup. Thus, focusing on this subgroup allows us to better characterize the relationship between immune activation and vascular function in the setting of controlled HIV infection. Second, this subgroup has become the most clinically relevant as initiation of antiretroviral therapy upon the diagnosis of HIV has become the standard of care. Due to the small number of subjects in each of the remaining 3 subgroups, we did not do separate analyses for the treated and unsuppressed, untreated and suppressed, and untreated and unsuppressed subgroups.

While in the general population, RH is predictive of FMD, macrovascular and microvascular functions are relatively independent in patient with rheumatoid arthritis, which suggests differential regulation of endothelial function in these two vascular beds. Given that HIV and rheumatoid arthritis are chronic inflammatory diseases, we wanted to evaluate whether RH and FMD are uncoupled in the setting of HIV infection as well. However, we found in our entire cohort as well as the treated suppressed group that RH was strongly and independently associated with FMD after adjusting for cardiovascular risk factors. Impaired RH has recently been identified as more predictive than FMD of cardiovascular events in the general population. We showed that specific T-cell phenotypic markers were associated with endothelial dysfunction in the general HIV population as well as in the treated suppressed group. Interestingly, these T-cell markers were predictive of worsened RH but showed little or no association with FMD. One explanation for this finding could be that FMD represents the combination of conduit artery endothelial function as well as the hyperemic stimulus. Thus, in order to truly differentiate macrovascular from microvascular function, FMD must be adjusted for RH-induced shear stress. Therefore, our results suggest that T-cell activation plays a role in endothelial dysfunction at a microvascular level in HIV-infected individuals and that poor macrovascular function in HIV infection might be secondary to diseased microvasculature.

The total CD8+ T-cell count and the frequency of CD8+ PD1+ T cells were associated with worse RH in the treated suppressed group. This is the first study that has reported this association in HIV-infected or uninfected individuals. The role of the PD-1 pathway in HIV infection is complex and remains poorly understood. In acute viral infections, CD8+ T cells are activated and undergo massive expansion, resulting in upregulation of PD-1. In chronic infections, the frequency of PD-1 expressing cells remains elevated and likely contributes to persistent adaptive immune dysfunction. This in turn likely contributes to excess burden of other microbes (eg, cytomegalovirus, microbial translocation) and perhaps excess risk of malignancy. Persistent elevations in CD8+ T-cell
CD4 count was associated with worse RH in all HIV adults.32,55,56 In the Heart Outcomes Prevention Evaluation also contribute to cardiovascular disease in HIV-infected adults.40,49 ART indicates antiretroviral therapy; FMD, flow-mediated dilatation; NA, not applicable; NNRTI, non-nucleoside reverse transcriptase inhibitors; NRTI, nucleoside reverse transcriptase inhibitors; PI, protease inhibitor.

*Percent estimate represents estimated percentage difference in reactive hyperemia or FMD associated with a doubling of each HIV-related factor. Each factor of interest was adjusted for age, sex, race, hypertension, diabetes mellitus, hyperlipidemia, smoking. Markers are included individually, not simultaneously.

count during ART (often measured as a low CD4/CD8 ratio) are associated with chronic inflammation and have been consistent predictors of cardiovascular complications, non-AIDS morbidity, and overall mortality.10,49–52 Expansion of CD8+ T cells are associated with T-cell activation, senescence, and altered CD4/CD8 ratio in the gut mucosa.40 Given these results, PD-1 inhibitors could be potential therapeutic targets for treatment of cardiovascular disease in chronic HIV infection.

Cytomegalovirus co-infection likely contributes to persistent inflammation and expansion in CD8+ T-cell counts during ART.18,49,53,54 Cytomegalovirus-associated inflammation may also contribute to cardiovascular disease in HIV-infected adults.52,55,56 In the Heart Outcomes Prevention Evaluation study, patients with positive cytomegalovirus serology had an increased risk of myocardial infarction, stroke, or cardiovascular death.57 Cytomegalovirus has also been associated with subclinical atherosclerosis in the Multi-Ethnic Study of Atherosclerosis.58 In our current study, we found that the CD4+ IFN+cytomegalovirus-specific T-cell activation marker was associated with worse RH in all HIV+ subjects. Comparable nonsignificant trends were observed in treated and suppressed individuals. These data collectively suggest that cytomegalovirus infection contributes to CD8+ T-cell expansion, persistent activation of potentially harmful inflammatory pathways, microvascular dysfunction, and cardiovascular disease.

In all HIV+ subjects, fibrinogen had a significant negative association with FMD while D-dimer had a significant negative association with RH. Both associations weakened among the treated and suppressed individuals. Since both fibrinogen and D-dimer are proinflammatory markers in the coagulation cascade, these results indicate that a prothrombotic state contributes to microvascular and macrovascular dysfunction.

As discussed, both TNF-α and CD8+PD1+ T cells had a negative association with RH in the treated and suppressed group after adjusting for potential confounding factors. However, TNF-α also had a significant negative association with CD8+PD1+ T cells. But when TNF-α was added to the multivariable adjustment model, the association between CD8+PD1+ T cells and RH weakened. This finding may reflect the effect of an unmeasured confounding variable as opposed to a true mediation effect of TNF-α, given the negative association between TNF-α and CD8+PD1+ T cells.
Alternatively, this may represent moderated mediation, where the effect of CD8+PD1+ T cells on RH differs depending on the level of inflammation. Our analysis found that CD8+PD1+ T cells were associated with worsening RH in the absence of TNF-α, but this relationship appeared to reverse with increasing levels of TNF-α. This reversal in relationship (ie, CD8+PD1+ T cells become protective) is supported by the fact that activation of the PD1 pathway in T cells can have an inhibitory effect and lead to decreased production of cytokines such as TNF-α. Thus, the negative effect of CD8+PD1+ T cells on RH we have shown here indicates a separate mechanism (Figure).

The main limitation of our study is its cross-sectional design. Endothelial dysfunction might reflect a cumulative effect of various factors, whereas the T cell and the inflammatory marker levels were measured only at a single time point. Therefore, we performed multivariable regression analysis to control for traditional cardiovascular factors in order to determine whether T-cell activation and the plasma markers remained predictive of endothelial dysfunction. However, there could be other confounding factors in this population that we did not take into account.

In conclusion, we evaluated the interplay between T-cell phenotypes, markers of inflammation/coagulation, cytomegalovirus, and endothelial dysfunction as measured by RH and FMD in HIV-infected individuals. Our findings show that T-cell dysfunction in HIV is preferentially associated with the microvasculature as assessed by RH. Since RH was strongly coupled to FMD, our study suggests that worse microvascular function in HIV could be due to the influence of inflammation/immune activation on the microvasculature. We identified markers of inflammation, specifically TNF-α, coagulation, and measures of CD8+ T-cell dysfunction (CD8+PD1+) and cytomegalovirus-associated CD4+ T-cell dysfunction that, could serve as potential targets for future interventions. Future studies are under way to investigate the impact of lowering inflammation on endothelial function and cardiovascular risk.

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