Altered Monocyte and Endothelial Cell Adhesion Molecule Expression Is Linked to Vascular Inflammation in Human Immunodeficiency Virus Infection

Manjusha Kulkarni,1 Emily Bowman,1 Janelle Gabriel,1 Taylor Amburgy,1 Elizabeth Mayne,2 David A. Zidar,3 Courtney Maierhofer,3 Abigail Norris Turner,4 Jose A. Bazan,3 Susan L. Koletar,4 Michael M. Lederman,2 Scott F. Sigel,5 and Nicholas T. Funderburg1

1School of Health and Rehabilitation Sciences, Division of Medical Laboratory Science, Ohio State University, Columbus; 2National Health Laboratory Service and Faculty of Health Sciences, University of Witwatersrand, Johannesburg; 3Harrington Heart & Vascular Institute, University Hospitals Case Medical Center, Cleveland, Ohio; 4Department of Internal Medicine, Division of Infectious Diseases, Ohio State University, Columbus; 5Department of Internal Medicine, Division of Infectious Diseases, Case Western Reserve University/University Hospitals of Cleveland, Ohio

Background. Human immunodeficiency virus (HIV)-infected individuals have increased risk for vascular thrombosis, potentially driven by interactions between activated leukocytes and the endothelium.

Methods. Monocyte subsets (CD14+CD16−, CD14+CD16+, CD14dimCD16+) from HIV negative (HIV−) and antiretroviral therapy-treated HIV positive (HIV+) participants (N = 19 and 49) were analyzed by flow cytometry for adhesion molecule expression (lymphocyte function-associated antigen 1 [LFA-1], macrophage-1 antigen [Mac-1], CD11c/CD18, very late antigen [VLA]-4) and the fractalkine receptor (CX3CR1); these receptors recognize ligands (intercellular adhesion molecules [ICAMs], vascular cell adhesion molecule [VCAM]-1, fractalkine) on activated endothelial cells (ECs) and promote vascular migration. Plasma markers of monocyte (soluble [s]CD14, sCD163) and EC (VCAM-1, ICAM-1,2, fractalkine) activation and systemic (tumor necrosis factor receptor [TNFR-II], TNFR-II) and vascular (lipoprotein-associated phospholipase A2, Lp-PLA2) inflammation were measured by enzyme-linked immunosorbent assay.

Results. Proportions of CD16+ monocyte subsets were increased in HIV+ participants. Among all monocyte subsets, levels of LFA-1 were increased and CX3CR1 levels were decreased in HIV+ participants (P < .01). Levels of sCD163, sCD14, fractalkine, ICAM-1, VCAM-1, TNFR-II, and Lp-PLA2 were also increased in HIV+ participants (P < .05), and levels of sCD14, TNFR-I, and TNFR-II were directly related to ICAM-1 and VCAM-1 in HIV+ participants. Expression of CX3CR1 on monocyte subsets was inversely related to plasma Lp-PLA2 (P < .05 for all).

Conclusions. Increased proportions of CD16+ monocytes, cells with altered adhesion molecule expression, combined with elevated levels of their ligands, may promote vascular inflammation in HIV infection.

Keywords. Monocytes; adhesion molecules; fractalkine; HIV; lipoprotein-associated phospholipase A2; monocytes.
levels of the monocyte activation marker soluble (s)CD14 [25] have been independently associated with mortality in HIV disease [9] and are associated with coronary artery calcification in HIV+ individuals. Arterial inflammation [26] and noncalcified coronary plaques [27] are associated with the monocyte/macrophage marker sCD163 in HIV infection.

Homing of leukocyte subsets to the endothelium is a tightly regulated process that involves endothelial cell (EC) expression of chemokines, including CCL2 and fractalkine (CX3CL1), and vascular and intracellular adhesion molecules (VCAM-1 and ICAMs) [16]. Recognition of these ligands by their receptors, including CCR2, CX3CR1, and various integrins, eg, very late antigen-4 ([VLA-4], αβ, CD49d/CD29), lymphocyte function-associated antigen-1 ([LFA-1], αβ, CD11a/CD18), macrophage-1 antigen ([Mac-1], αβ, CD11b/CD18), and CD11c/CD18 (αβ), promote leukocyte adhesion to the endothelium and transmigration into the vascular intima [16, 21, 28–31]. Adhesion to, and transmigration across, the endothelium by leukocytes contributes to vascular inflammation and blood vessel occlusion [16]. Infection with HIV is associated with EC dysfunction and activation, including altered vascular tone and increased expression of adhesion molecules [32–34], which may promote vascular homing of leukocytes that express the appropriate receptors. Thus, the activated endothelium may serve as a substrate for leukocyte binding and migration in HIV infection. We recently reported an increased accumulation of monocytes and T cells along the endothelium in simian immunodeficiency virus-infected rhesus macaques [35].

Lipoprotein-associated phospholipase A2 (Lp-PLA2) is an enzyme produced by macrophages within blood vessel walls [36] that cleaves oxidized, low density lipoprotein (LDL) into lysophosphatidylcholine and nonesterified fatty acids, proinflammatory lipid molecules that can contribute vascular inflammation [37]. Plasma levels of Lp-PLA2 have been associated with atherosclerosis and CVD events in HIV-uninfected participants [38, 39]. Monocyte migration into the vascular intima, where these cells differentiate into macrophages and produce Lp-PLA2, may be a critical step in the initiation of atherosclerosis. The differential expression of adhesion molecules among monocyte subsets and the relationship between monocyte expression of vascular homing molecules and Lp-PLA2 have been incompletely explored in persons living with HIV. In this study, we hypothesized that HIV-infected participants would have increased blood levels of monocyte and endothelial adhesion molecules and that these markers would be associated with Lp-PLA2 levels, providing a potential link to the vascular homing capabilities of monocytes and development of atherosclerosis.

MATERIALS AND METHODS

Study Participants
All study participants provided written informed consent, in accordance with the Ohio State University (OSU) Institutional Review Board. Human immunodeficiency virus-infected men were recruited at the Infectious Disease Clinic, and HIV-1 uninfected men were recruited from the general population of OSU. Age, CD4 counts, and viral loads (VLs) were measured at enrollment; total cholesterol levels, LDL, smoking status, use of antihypertensive medicine, diagnosis of diabetes, and use of aspirin were extracted for each HIV+ individual from medical charts from the most recent time point available (typically within 3 months of study enrollment). Lipid levels for HIV negative (HIV−) participants were measured by Alere Cholestech LDX Analyzer (Alere San Diego, Inc., San Diego, CA).

Sample Collection
Blood was drawn into EDTA-containing tubes (BD vacutainer; Bectin Dickinson [BD] Biosciences, Franklin Lakes, NJ). Plasma was isolated by centrifugation for 15 minutes at 800 × g and frozen at −80°C until thawed once and analyzed.

Flow Cytometry
Expression of adhesion molecules on monocyte subsets was measured directly ex vivo by flow cytometry (MACs Quant 10; Miltenyi Biotec, Bergisch Gladbach, Germany). Fresh blood samples were lysed with FACS lyse buffer (BD Biosciences) for 15 minutes and washed with buffer (phosphate-buffered saline with 1% bovine serum albumin and 0.1% sodium azide). Cells were stained for 30 minutes in the dark on ice and then washed in buffer, fixed in 1% paraformaldehyde, and analyzed. Monocyte subsets were identified by size, granularity, and surface expression levels of CD14 and CD16 using fluorochrome labeled antibodies (anti-CD14 Pacific Blue and anti-CD16 phycoerythrin; BD Pharmingen, San Diego, CA). Fluorescence minus 1 and isotype gating strategies were used to identify expression of surface markers as previously reported [22].

Adhesion molecule expression was monitored using fluorochrome-labeled antibodies against: CD11a (Pe-Cy7), CD11b (allophycocyanin-Cy7 [APC-Cy7]), CD11c (APC), CD18 (fluorescein isothiocyanate [FITC]), CD29 (APC), and CD49d (Pe-Cy7) (BD Pharmingen). Surface expression of CX3CR1 was measured using anti-CX3CR1 (Pe-Cy7; eBioscience, San Diego, CA), MACS Quant software (version 2.21031.1; Miltenyi Biotec) and Prism 5.0 GraphPad software (GraphPad, La Jolla, CA) were used to analyze the data.

Fractalkine Receptor Modulation
Agonist-induced downregulation of CX3CR1 was measured by flow cytometry (adapted from [40]). Peripheral blood mononuclear cells (PBMCs) were isolated from HIV-uninfected participants (N = 4) and incubated in medium alone (RPMI medium + 10% autologous serum) or in medium supplemented with fractalkine (25–250 ng/mL; R&D Systems, Minneapolis, MN) overnight at 37°C. Peripheral blood mononuclear cells were washed and expression of CX3CR1 was quantified.
Soluble Markers
Plasma levels of the immune activation markers sCD14, sCD163, TNF receptor (TNFR)-I, and TNFR-II were measured using Quantikine enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems). Levels of Lp-PLA2 were measured by a quantitative ELISA (Plac Test; DiaDexus, South San Francisco, CA). Endothelial activation markers VCAM-1, ICAM-1, fractalkine (CX3CL1) (R&D Systems), and ICAM-2 (Abcam, Cambridge, MA) were measured by ELISA. Oxidized LDL was measured by ELISA (Mercodia, Uppsala, Sweden).

Statistical Analysis
Categorical variables were compared using χ² analysis. The Mann-Whitney U test was used to compare continuous variables. The correlations between pairs of continuous variables were evaluated using Spearman rank correlation. All comparisons are 2 sided without formal correction, and P values < .05 were considered statistically significant.

RESULTS
Patient Characteristics
Demographic information is provided in Table 1. All participants enrolled in this study were men; the median age for both groups was 43 years (age range, 24–66 years for HIV+ and 21–67 years for HIV− participants). All HIV+ participants were on ART, and all but 1 had controlled viremia (VL < 40 copies/mL). The median CD4 count for the HIV+ group was 455 cells/μL (range, 47–1338 cells/μL). Twenty percent of the HIV+ population reported daily aspirin use and 20% were taking a statin. Forty percent of HIV+ participants were current smokers and 9 HIV+ participants (18%) were using antihypertensive medications. Total cholesterol values were significantly higher for HIV-uninfected participants than for HIV-infected participants (median, 203 vs 175 mg/dL; P = .048). The levels of LDL, high-density lipids (HDL), and triglycerides were similar between groups.

Expression of Adhesion Molecules Differs by Monocyte Subset and Between Human Immunodeficiency Virus (HIV)+ and HIV− Participants
First, we measured the proportional representation of monocyte subsets (traditional, CD14+/CD16−; inflammatory, CD14+/CD16+; and patrolling, CD14 Dim/CD16+). The proportions of inflammatory monocytes were increased (25% vs 13%; P < .0001) and the proportions of patrolling monocytes (median 7% vs 5%; P = .1) tended to be increased in HIV+ participants; we report a concomitant decrease in the proportion of traditional monocytes in HIV+ (68%) versus HIV− participants (82%, P < .0001) (Figure 1A). We also found a significant decrease in absolute numbers of traditional monocytes (192 vs 274 monocytes/μL, P = .003) in HIV+ compared with HIV− participants.

Next, we measured expression of selected integrins (LFA-1/αLβ2, Mac-1/αMβ2, CD11c-CD18/αXβ2), proteins that enhance leukocyte migration and arrest along the endothelium, among monocyte subsets [29], extending previous findings [20, 21, 23]. The integrins LFA-1, Mac-1, and CD11c-CD18 are each

Table 1. Demographic and Clinical Information for HIV-1-Infected Patients and HIV-1-Uninfected Controls

| Variables                                | HIV-1-Infected Participants (N = 49) | HIV-1-Uninfected Participants (N = 19) |
|------------------------------------------|--------------------------------------|----------------------------------------|
| Age (years)                              | Median = 43                          | Median = 43                            |
|                                          | Range = 24–66                         | Range = 21–67                           |
| CD4+ T-cell count (date of enrollment)   | Median = 569                          | NA                                     |
|                                          | Range = 47–1338                       |                                        |
| HIV viral load (date of enrollment)      | Median = 40                           | NA                                     |
|                                          | Range = 40–284 638                    |                                        |
| On ART (%)                               | 100 %                                 | NA                                     |
| Hepatitis B coinfection (%)              | 4%                                    | NR                                     |
| Diabetes (%)                             | 10%                                   | NR                                     |
| Diagnosed hypertension (%)              | 18%                                   | NR                                     |
| Current smoker (%)                       | 41%                                   | NR                                     |
| Aspirin use (%)                          | 20%                                   | NR                                     |
| Statin use (%)                           | 20%                                   | NR                                     |
| Antihypertensive medication use (%)      | 18%                                   | NR                                     |
| Total cholesterol (mg/dL)                | Median = 175                          | Median = 203                           |
|                                          | Range = 107–222                       | Range = 140–254                        |
| LDL                                      | Median = 95                           | Median = 128                           |
|                                          | Range = 41–149                        | Range = 50–162                         |
| HDL                                      | Median = 40                           | Median = 47                            |
|                                          | Range = 24–79                         | Range = 28–60                          |
| Triglycerides                            | Median = 147                          | Median = 198                           |
|                                          | Range = 35–720                        | Range = 45–348                         |

Abbreviations: ART, antiretroviral therapy; HDL, high-density lipoprotein; HIV, human immunodeficiency virus; LDL, low-density lipoprotein; NA, not applicable; NR, not reported.

Information reported is extracted from patient medical records.
composed of a unique α chain (CD11a, CD11b, or CD11c) and a conserved β2 chain (CD18). Representative dot plots for monocyte subset expression of LFA-1, Mac-1, and CD11c/CD18 are displayed (Figure 1B). The proportional representation of cells that expressed these receptors was similar between samples from HIV-infected and uninfected participants. Within both donor groups, there were significant differences in expression levels (mean fluorescence intensity [MFI]) of these receptors among monocyte subsets (Figure 1C). Among samples from both HIV+ and HIV− participants, the surface intensity of CD18/β2 was highest on inflammatory monocytes compared with both traditional and patrolling monocytes (P < .03 for all; data not shown). The surface intensity of CD11a (LFA-1/αLβ2) was increased on patrolling monocytes compared with traditional and inflammatory monocytes in both donor groups (P < .0001 for all). Expression of CD11a was increased on each monocyte subset among HIV+ participants compared with expression among monocytes from HIV− participants (P < .0001 for all) (Figure 1C). Expression of CD11b/αx tended to be lowest on patrolling monocytes among all participants. No significant differences were measured between HIV+ and HIV− participants for expression levels of CD11b or CD11c (Figure 1C).

We next measured expression levels of the integrin VLA-4 and the chemokine receptor CX3CR1, receptors also known to be involved in vascular migration. Very late antigen-4 supports tethering and rolling of leukocytes along the endothelium under blood flow conditions [28] and is composed of the α4 and β2 chains (CD49d and CD29). Cells that express CX3CR1 migrate in response to a concentration gradient of fractalkine, a chemokine expressed by activated ECs. Representative dot plots for VLA-4 expression and histograms for CX3CR1 expression among monocyte subsets are displayed (Figure 2A). The proportions of monocyte subsets expressing VLA-4 (CD49d/CD29+) were comparable for HIV+ and HIV− participants (Figure 2B). The density of CD49d/α4 tended to be increased on patrolling monocytes from both HIV+ and HIV− participants compared

Figure 1. Adhesion molecule expression differs among monocyte subsets between human immunodeficiency virus (HIV)-1 infected and HIV-uninfected participants. Whole blood samples from 49 HIV-1 infected and 19 HIV-uninfected participants were stained for monocyte subset surface markers (CD14 and CD16) and adhesion molecules (lymphocyte function-associated antigen 1 [LFA-1], macrophage-1 antigen [Mac-1], CD11c/CD18). The relative proportions of monocyte subsets and surface expression of adhesion molecules were analyzed by flow cytometry. (A) Summary data of monocyte subset representation among HIV-negative (HIV−) and HIV-positive (HIV+) participants are shown. (B) Representative dot plots for the integrins LFA-1, Mac-1, and CD11c/CD18, on each monocyte subset are shown. (C) Comparative summary data of monocyte subsets for adhesion molecules (CD11a, CD11b, and CD11c) for HIV− and HIV+ participants are displayed. MFI, mean fluorescence intensity.
with traditional and inflammatory monocytes (P < .0001 for all; Figure 2B). Expression of CD29/β1 tended to be reduced among all monocyte subsets in HIV+ donors (traditional, P = .003; inflammatory, P = .003; and patrolling monocytes, P = .07) (Figure 2B). Furthermore, we confirmed [21, 22] that patrolling monocytes more often express CX3CR1, and at increased levels, compared with traditional and inflammatory monocytes among both donor groups. The proportions of CX3CR1+ monocytes did not differ significantly among subsets between donor groups; however, the intensity (MFI) of CX3CR1 was reduced for all monocyte subsets from HIV+ participants (traditional, P = .0007; inflammatory, P < .0001; and patrolling monocytes P = .0001) (Figure 2C).

Soluble Biomarkers and Endothelial Activation Molecules

Plasma markers of immune activation were elevated in persons infected with HIV compared with levels in uninfected participants (Figure 3). These included the following: sCD14 (median values, 3019 vs 2044 ng/mL; P < .0001), sCD163 (566 vs 457 ng/mL; P = .04), and sTNFR-II (3860 vs 2273 pg/mL; P < .0001) (Figure 3A). Endothelial activation markers including VCAM-1 (median values, 1117 vs 622 ng/mL; P < .0001), ICAM-1 (299 vs 209 ng/mL; P = .003), ICAM-2 (617 vs 429 U/mL; P < .0001), and fractalkine (209 vs 26 pg/mL; P = .0005) (Figure 3B) were increased in samples from HIV+ versus HIV− participants. We also measured increased levels of the vascular inflammation marker Lp-PLA2 in samples from our HIV+ participants compared with levels in HIV− participants (231 vs 177 U/mL; P = .002) (Figure 3C).

Among the HIV− participants, we report several significant correlations among plasma markers of inflammation and EC and monocyte activation (Supplemental Table 1). Levels of sTNFR-II were associated with levels of multiple markers: sTNFR-I (r = 0.80, P < .0001), VCAM-1 (r = 0.67, P < .0001), ICAM-1 (r = 0.55, P < .0001), sCD163 (r = 0.43, P = .003), and sCD14 (r = 0.42, P = .003). Soluble CD14 was associated with VCAM-1 (r = 0.46, P = .001) and ICAM-1 (r = 0.42, P = .004). Representative graphs of associations between select markers

Figure 2. Expression of CX3CR1 is reduced on monocyte subsets from human immunodeficiency virus-positive (HIV+) participants. Whole blood samples from HIV-infected and HIV-uninfected participants were stained for monocyte subset surface markers (CD14, CD16), the adhesion molecule very late antigen-4 (VLA-4) CD29 and C49d, and the chemokine receptor CX3CR1. Three monocyte subsets were analyzed for VLA-4 and CX3CR1 expression. (A) Representative dot plots for VLA-4 and histograms for CX3CR1 are displayed. Comparative summary data of monocyte subset expression of (B) VLA-4 and (C) CX3CR1 among HIV+ and HIV-negative (HIV−) participants are shown.

Downloaded from https://academic.oup.com/ofid/article-abstract/3/4/ofw224/2755776 by guest on 15 March 2020
Figure 3. Plasma levels of inflammatory markers and endothelial adhesion molecules are elevated in human immunodeficiency virus (HIV)-infected participants. Plasma samples from all participants were thawed, and levels of soluble markers were measured using commercial enzyme-linked immunosorbent assays. Comparative data between HIV-positive (HIV+) and HIV-negative (HIV−) participants for inflammatory markers are shown as medians (with interquartile ranges) for levels of the following: (A) soluble (s)CD14, sCD163, tumor necrosis factor receptor (TNFR)-I, and TNFR-II; (B) vascular cell adhesion molecule (VCAM)-1, intercellular adhesion molecule (ICAM)-1, ICAM-2, and fractalkine; and (C) lipoprotein-associated phospholipase A2 (Lp-PLA2). (D) The representative correlation graphs between inflammatory markers and endothelial adhesion molecules among samples from HIV+ participants are shown. Levels of VCAM-1 (left) were associated with levels of sCD14, TNFR-I, TNFR-II, and ICAM-1. Levels of ICAM-1 (right) were associated with levels of sCD14, TNFR-I, TNFR-II, and fractalkine.
are provided (Figure 3D). The relationships among plasma markers in HIV-uninfected participants were far more limited; significant relationships existed between levels of TNFR-I and TNFR-II (r = 0.56, P = .01), and VCAM-1 levels were related to levels of Lp-PLA₂ (r = 0.7, P = .001) and CX3CL1 (r = 0.47, P = .05).

We next assessed relationships among expression levels of vascular homing molecules on monocyte subsets and Lp-PLA₂, because Lp-PLA₂ is largely produced by activated monocytes/macrophages that have migrated from the blood into the vessel wall [36]. Among HIV-infected participants, we report an inverse relationship between plasma levels of Lp-PLA₂ and both the proportion of monocytes that express CX3CR1 (data not shown) and the intensity of CX3CR1 on monocyte subsets (Figure 4A). We did not see a relationship between Lp-PLA₂ levels and expression of any monocyte adhesion molecules in HIV-uninfected participants. We hypothesized that monocyte exposure to increased plasma levels of fractalkine in the blood of HIV+ participants might be responsible for the decreased expression of CX3CR1 on monocyte subsets. Therefore, we exposed PBMCs from HIV-uninfected participants to fractalkine/CX3CL1 overnight; we measured a dose-dependent decrease in monocyte expression of CX3CR1 (Figure 4B).

**DISCUSSION**

Activation of both the endothelium and localized migration of immune cells, including monocytes, are important contributors to the development and progression of atherosclerosis [16]. Cardiovascular disease risk is increased with HIV infection [3, 4], and this increased risk has been associated with chronic immune activation and inflammation, even during suppressive ART [5, 8]. The mechanisms for increased atherosclerosis in this population may be related to altered monocyte homing [21, 23, 41] and activation [25, 27, 42, 43]. Previous studies, including our own [15, 22, 44], have described differential expression of chemokine receptors and adhesion molecules on monocyte subsets [17, 19, 20], and fractalkine is known to contribute to the migration of immune cells along the endothelium [21]. In this study, we report increases in plasma markers of EC activation (VCAM-1, ICAM-1, fractalkine) and altered expression of corresponding receptors (LFA-1 and CX3CR1) on monocyte subsets in ART-treated HIV+ participants (summarized in Supplemental Table 2). Proportions of inflammatory and patrolling monocytes are enriched in HIV+ participants [22]; these CD16+ monocyte subsets often express the highest levels of vascular adhesion molecules [20, 21], and their enrichment in the blood may correlate with an increase in their eventual accumulation in blood vessel walls.

We report here, for the first time, that expression of CX3CR1 on monocyte subsets is inversely related to plasma levels of Lp-PLA₂, potentially linking vascular homing of monocytes to

![Figure 4](https://academic.oup.com/ofid/article-abstract/3/4/ofw224/2755776/158038)
CVD risk in HIV-infected participants. Macrophages can produce Lp-PLA$_2$ [36], and increased Lp-PLA$_2$ levels (>200 ng/mL) are associated with increased risk of an acute coronary event [45, 46]. Here, the proportion of participants with Lp-PLA$_2$ levels ≥200 ng/mL is increased dramatically among HIV-infected (61%) compared with HIV-uninfected participants (21%, $P = .006$), in spite of similar levels of triglycerides, LDL, and oxidized LDL (54.2 and 51.2 μg/mL, $P = .63$) between the groups.

These data are compatible with a model wherein monocyte subsets that express altered levels of vascular homing molecules, including CX3CR1, LFA-2, Mac-1, and VLA-4, work in conjunction with enhanced expression of EC adhesion molecules (ICAM-1, VCAM-1, fractalkine) to support monocyte/macrophage accumulation in the vessel wall [16]. Then, these cells may produce proinflammatory mediators including Lp-PLA$_2$, leading to vascular inflammation. Why CX3CR1 levels are low on monocytes from HIV$^+$ participants is not clear, but it may be due to exposure to fractalkine in vivo, a possibility supported by our in vitro studies (Figure 4B). Another explanation could be that the monocytes that had expressed the highest levels of CX3CR1 have already migrated into the vessel wall, leaving a population of monocytes that express low levels of CX3CR1 in the circulation.

Previous work in mice has demonstrated that CX3CR1, CCL2, and CCR5 are all involved in monocyte accumulation in the vessel wall [47]. In a cross-sectional study, we have reported that expression levels of CCR5 and CCR2 do not differ between monocyte subsets from HIV$^+$ and HIV$^-$ participants [44]. Our current data extend the findings of Westhorpe et al [23], who demonstrated a direct relationship between the proportion of monocytes that express CX3CR1 to carotid intima media thickness among HIV-infected, but not uninfected, participants. Combined, these findings suggest that in chronic HIV infection, CX3CR1 may be a potential target for modulating monocyte vascular homing, inflammation, and progression of atherosclerosis. Further studies on the roles of CX3CR1 and LFA-1 in the development of CVD risk in HIV infection are warranted.

We also report (1) increased levels of TNFR-II in HIV$^+$ compared with HIV$^-$ participants and (2) relationships among TNFR-I and/or TNFR-II and several markers of monocyte (sCD14, sCD163, CX3CR1 expression) and vascular (VCAM-1, ICAM-1, Lp-PLA$_2$) activation/inflammation. Levels of TNF-α are related to levels of TNFR-I and TNFR-II after administration of lipopolysaccharide (LPS) in a human sepsis model [48]; based on the relationships among TNFR-II in our present study and markers of both monocyte and EC activation, one could speculate that TNF-α could be a common intermediate involved in activation of these cells. These data could provide a physiological context for the studies that have reported that TNFR-I and TNFR-II are predictive of morbidity in HIV infection [6, 7, 10]. Furthermore, Maisa et al [41] have demonstrated that TNF-α may be on the causal pathway for the increased propensity for macrophages from HIV-infected participants to transition into foam cells upon exposure to pooled human serum. The effects of increased TNFR-II expression, how levels of TNFR-I and TNFR-II relate to systemic and vascular levels of TNF-α, and what is contributing to the production of TNF-α itself (ie, LPS, proinflammatory lipids, among others) are worthy of investigation.

Our study has limitations. First, we were unable to characterize completely the CVD risk factors of participants. Furthermore, we use plasma markers for vascular adhesion molecules as opposed to measuring them directly on ECs; complementary studies in cell lines or in animal models could provide insights into the phenotype of ECs after exposure to HIV-1, cytokines, microbial products, or proinflammatory lipids. Our analysis of lipid levels is limited; perturbations in other proinflammatory lipids may be playing a role, and traditional lipid panels may not adequately measure CVD risk in persons infected with HIV [49]. The donor population is also composed entirely of men; however, in our previous studies on monocyte subsets, phenotype did not differ by sex [15, 22], and in preliminary studies, men and women had similar levels of monocyte adhesion molecule expression. One HIV$^+$ donor was on ART but had detectable viremia; censoring this donor’s data did not change the results of this study. We also have limited data on body mass index (BMI), smoking status, and current medication use among our HIV-uninfected participants. Because smoking and obesity may play a role in inflammation, we separated our HIV$^+$ participants based on smoking status and obesity (BMI >30). Among the plasma markers of immune activation that were different between HIV$^+$ and HIV$^-$ participants, only soluble CD14 (2704 vs 3307 pg/mL, $P = .04$) and ICAM-1 (272 vs 353 ng/mL, $P = .04$) were statistically significantly different among HIV$^+$ nonsmokers versus HIV$^-$ smokers. The levels of sCD14 and ICAM-1 were still elevated among nonsmoking HIV$^+$ donors compared with levels in the HIV-uninfected donors (2044 pg/mL, $P < .001$ and 208 ng/mL, $P = .0009$). Monocyte subset expression levels of CX3CR1 and CD11a were not different between HIV$^+$ smokers and HIV$^+$ nonsmokers, and the significant differences we report for CX3CR1 and CD11a on individual monocyte subsets were maintained among HIV$^+$ smokers, HIV$^+$ nonsmokers, and HIV$^-$ participants ($P < .01$ for all).

None of the plasma markers of immune activation were different between obese (BMI >30, N = 12) and nonobese (N = 34) HIV$^+$ donors. After separation of the groups, levels of sCD163 were no longer significantly different between the obese HIV$^+$ donors and HIV$^-$ donors (487 vs 456 ng/mL, $P = .29$), but levels of sCD163 were significantly higher in nonobese HIV$^+$ participants (619 ng/mL, $P = .03$) than HIV-uninfected participants. We also did not find statistically significant differences in monocyte subset expression of CD11a or CX3CR1 in obese versus nonobese HIV-infected participants; significant differences were maintained between these groups and HIV$^+$ participants.
CONCLUSIONS

Because CVD risk is increased in HIV-infected individuals, studies that explore the potential contributors of increased monocyte accumulation within the arterial wall and subsequent blood vessel inflammation and occlusion are needed. We believe this study extends previous works in persons living with HIV and in HIV-uninfected populations that have identified CX3CR1, LFA-1, Lp-PLA2, and TNF-α as potential targets for intervention [16, 17, 20, 21, 23, 31, 36, 41]. The complex interplay between leukocytes and the endothelium, the mechanisms related to their activation, and their role in contributing to CVD risk in ART-treated HIV infection warrant further investigation.

Supplementary Data
Supplementary material is available at Open Forum Infectious Diseases online.

Acknowledgments

We thank the participants in this research and the study coordinators of the AIDS Clinical Trial Unit at Ohio State University for enrolling participants in this study. We also thank the Bad Boys of Cleveland/the Cleveland Immunopathogenesis Consortium for helpful discussions related to this project.

Author contributions. M. M. K., E. B., J. G., and T. A. performed experiments. N. T. F. and M. M. K. organized figures and performed statistical analyses. M. M. K. extracted data from patient records. A. N. T., S. L. K., and J. A. B. provided patient samples and contributed to the Institutional Review Board regulatory processes. All authors contributed to experimental design, data analysis, and writing of the manuscript.

Disclaimer. The contents of this work are solely the responsibility of the authors and do not necessarily represent the official views of the National Institutes of Health (NIH). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Financial support. This work was supported by the following: grants from the National Institutes of Health (AI-07164, AI-67039, AI-68636, R01HL108743, R56HL126563, R01HL134544); an AIDS Clinical Trials Group’s Minority Investigator Award (UM1AI068636; to J. A. B. [Bethesda, MD]); the Fasenmyer Foundation (Cleveland, OH); the Center for AIDS Research at Case Western Reserve University (AI-36219); and the Clinical and Translational Science Collaborative of Cleveland, (KL2TR000440) from the National Center for Advancing Translational Sciences component of the National Institutes of Health and NIH roadmap for Medical Research.

Potential conflicts of interest. N. T. F. has served as consultant for Gilead. S. L. K. has been a co-investigator on several Gilead-sponsored research contracts.

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.

References

1. Mooser V. Atherosclerosis and HIV in the highly active antiretroviral therapy era: towards an epidemic of cardiovascular disease? AIDS 2003; 17(Suppl 1):S65–9.
2. Periard D, Cavassini M, Taffé P, et al. High prevalence of peripheral arterial disease in HIV-infected persons. Clin Infect Dis 2008; 46:761–7.
3. Talib A, Leroux C, Mormex JF, Loire R. Accelerated coronary atherosclerosis and arteriosclerosis in young human immunodeficiency-virus-positive patients. Coron Artery Dis 2000; 11:41–6.
4. Triant VA, Lee H, Hadigan C, Grinspoon SK. Increased acute myocardial infarction rates and cardiovascular risk factors among patients with human immunodeficiency virus disease. J Clin Endocrinol Metab 2007; 92:2506–12.
5. Emery S, Neuhaus JA, Phillips AN, et al. Major clinical outcomes in antiretroviral therapy (ART)-naive participants and in those not receiving ART at baseline in the SMART study. J Infect Dis 2008; 197:1133–44.
6. Hunt PW, Sinclair E, Rodriguez B, et al. Gut epithelial barrier dysfunction and innate immune activation predict mortality in treated HIV infection. J Infect Dis 2014; 210:1228–38.
7. Kalayjian RC, Machekano RN, Rizk N, et al. Pretreatment levels of soluble cellular receptors and interleukin-6 are associated with HIV disease progression in subjects treated with highly active antiretroviral therapy. J Infect Dis 2010; 201:1796–805.
8. Käller LH, Tracy R, Belloso C, et al. Inflammatory and coagulation biomarkers and mortality in patients with HIV infection. PLoS Med 2008; 5:e203.
9. Sandler NG, Wamb E, Roque A, et al. Plasma levels of soluble CD14 independently predict mortality in HIV infection. J Infect Dis 2011; 203:780–90.
10. Tenorio AR, Zheng Y, Bosch RJ, et al. Soluble markers of inflammation and coagulation but not T-cell activation predict non-AIDS-defining morbidity events during suppressive antiretroviral treatment. J Infect Dis 2014; 210:1248–59.
11. Hatano H, Delwart EL, Norris PJ, et al. Evidence of persistent low-level viremia in long-term HAART-suppressed, HIV-infected individuals. AIDS 2010; 24:2535–9.
12. Hunt PW, Martin JN, Sinclair E, et al. Valganciclovir reduces T cell activation in HIV-infected individuals with incomplete CD4+ T cell recovery on antiretroviral therapy. J Infect Dis 2011; 203:1474–83.
13. Klaas NR, Funderburg NT, Brenchley JM. Microbial translocation, immune activation, and HIV disease. Trends Microbiol 2013; 21:16–13.
14. Piconi S, Farciotti S, Rizzardi G, et al. Atherosclerosis is associated with multiple pathogenic mechanisms in HIV-infected antiretroviral-naive or treated individuals. AIDS 2013; 27:381–9.
15. Zidar DA, Juchnowski S, Ferrari B, et al. Oxidized LDL levels are increased in HIV infection and may drive monocyte activation. J Acquir Immune Defic Syndr 2015; 69:154–60.
16. Libby P, Ridker PM, Maseri A. Inflammation and atherosclerosis. Circulation 2002; 105:1135–43.
17. Ancuta P, Wang J, Gabuzda D. CD16+ monocytes produce IL-6, CCL2, and matrix metalloproteinase-9 upon interaction with CX3CL1-expressing endothelial cells. J Leukoc Biol 2006; 80:1156–64.
18. Belge KU, Dayani E, Horel A, et al. The proinflammatory CD14+CD16+DR++ monocytes are a major source of TNF J Infect Med 2002; 168:3536–42.
19. Ziegler-Heitbrock L. The CD14+CD16+ blood monocytes: their role in infection and inflammation. Leukoc Biol 2007; 81:584–92.
20. Cross J, Cagnard N, Woollard K, et al. Human CD14dim monocytes patrol and sense nucleic acids and viruses via TLR7 and TLR8 receptors. Immunity 2010; 33:375–86.
21. Ancuta P, Rao R, Moses A, et al. Fractalkine preferentially mediates arrest and migration of CD16+ monocytes. J Exp Med 2003; 197:1701–7.
22. Funderburg NT, Zidar DA, Shive C, et al. Shared monocyte subset phenotypes in HIV-1 infection and in uninfected subjects with acute coronary syndrome. Blood 2012; 120:4599–608.
23. Westhorpe CL, Maisa A, Spelman T, et al. Associations between surface markers on blood monocytes and carotid atherosclerosis in HIV-positive individuals. Immunol Cell Biol 2014; 92:133–8.
24. Baker IV, Hultske KH, Singh A, et al. Immunologic predictors of coronary artery calcium progression in a contemporary HIV cohort. AIDS 2014; 28:831–40.
25. Longenecker CT, Jiang Y, Orringer CE, et al. Soluble CD14 is independently associated with coronary calcification and extent of subclinical vascular disease in treated HIV infection. AIDS 2014; 28:969–77.
26. Subramanian T, Tawkol A, Burdo TH, et al. Arterial inflammation in patients with HIV JAMA 2012; 308:379–86.
27. Burdo TH, Lo J, Abbara S, et al. Soluble CD163, a novel marker of activated macrophages, is elevated and associated with noncalcified coronary plaque in HIV-infected patients. J Infect Dis 2011; 204:1227–36.
28. Alon R, Kassner PD, Carr MW, et al. The integra VLA-4 supports tethering and rolling in flow on VCAM-1. J Cell Biol 1995; 128:1243–53.
29. Mazzone A, Ricevuti G. Leukocyte CD11/CD18 integrins: biological and clinical relevance. Haematologica 1995; 80:161–75.
30. Sumagin R, Prizant H, Lomakina E, et al. LFA-1 and Mac-1 define characteristically different intralumenal crawling and emigration patterns for monocytes and neutrophils in situ. J Immune 2010; 185:7057–66.
31. Yusuf-Makagiansar H, Anderson ME, Yakovleva TV, et al. Inhibition of LFA-1/ICAM-1 and VLA-4/VCAM-1 as a therapeutic approach to inflammation and autoimmune diseases. Med Res Rev 2002; 22:146–67.
32. Solages A, Vita JA, Thornton DJ, et al. Endothelial function in HIV-infected persons. Clin Infect Dis 2006; 42:1325–32.
33. Huang AL, Vita JA, Thornton DJ, et al. Endothelial function in HIV-infected persons. Clin Infect Dis 2006; 42:1325–32.
34. López M, San Román J, Estrada V, et al. Endothelial dysfunction in HIV infection—the role of circulating endothelial cells, microparticles, endothelial progenitor cells and macrophages. AIDS Rev 2012; 14:223–30.
35. Panigrahi S, Freeman ML, Funderburg NT, et al. SIV/SHIV infection triggers vascular inflammation, diminished expression of Krüppel-like factor 2 and endothelial dysfunction, J Infect Dis 2016; 213:1419–27.
36. Häkkinen T, Luoma JS, Hiltunen MO, et al. Lipoprotein-associated phospholipase A(2), platelet-activating factor acetylhydrolase, is expressed by macrophages in human and rabbit atherosclerotic lesions. Arterioscler Thromb Vasc Biol 1999; 19:2909–17.
37. Zalewski A, Macphee C. Role of lipoprotein-associated phospholipase A2 in atherosclerosis: biology, epidemiology, and possible therapeutic target. Arterioscler Thromb Vasc Biol 2005; 25:923–31.
38. Brilakis ES, Khera A, Saeed B, et al. Association of lipoprotein-associated phospholipase A2 mass and activity with coronary and aortic atherosclerosis: findings from the Dallas Heart Study. Clin Chem 2008; 54:1975–81.
39. White HD, Simes J, Stewart RA, et al. Changes in lipoprotein-associated phospholipase A2 activity predict coronary events and partly account for the treatment effect of pravastatin: results from the Long-Term Intervention with Pravastatin in Ischemic Disease study. J Am Heart Assoc 2013; 2:e000360.
40. Kohout TA, Nicholas SL, Perry SJ, et al. Differential desensitization, receptor phosphorylation, beta-arrestin recruitment, and ERK1/2 activation by the two endogenous ligands for the CC chemokine receptor 7. J Biol Chem 2004; 279:23214–22.
41. Maisa A, Hearps AC, Anglovich TA, et al. Monocytes from HIV-infected individuals show impaired cholesterol efflux and increased foam cell formation after transendothelial migration. AIDS 2015; 29:1445–57.
42. Hileman CO, Turner R, Funderburg NT, et al. Changes in oxidized lipids drive the improvement in monocyte activation and vascular disease after statin therapy in HIV. AIDS 2016; 30:65–73.
43. Nou E, Lu MT, Looby SE, et al. Serum oxidized low-density lipoprotein decreases in response to statin therapy and relates independently to reductions in coronary plaque in patients with HIV. AIDS 2016; 30:583–90.
44. Petrov V, Funderburg N, Weinberg A, Sieg S. Human β defensin-3 induces chemokines from monocytes and macrophages: diminished activity in cells from HIV-infected persons. Immunology 2013; 140:413–20.
45. Koenig W, Twardella D, Brenner H, Rothenbacher D. Lipoprotein-associated phospholipase A2 predicts future cardiovascular events in patients with coronary heart disease independently of traditional risk factors, markers of inflammation, renal function, and hemodynamic stress. Arterioscler Thromb Vasc Biol 2006; 26:1586–93.
46. Packard CJ, O’Reilly DS, Caslake MJ, et al. Lipoprotein-associated phospholipase A2 as an independent predictor of coronary heart disease. West of Scotland Coronary Prevention Study Group. N Engl J Med 2000; 343:1148–55.
47. Combadière C, Potteaux S, Rodero M, et al. Combined inhibition of CCL2, CX3CR1, and CCR5 abrogates Ly6C(hi) and Ly6C(lo) monocytosis and almost abolishes atherosclerosis in hypercholesterolemic mice. Circulation 2008; 117:1649–57.
48. Spinas GA, Keller U, Brockhaus M. Release of soluble receptors for tumor necrosis factor (TNF) in relation to circulating TNF during experimental endotoxinemia. J Clin Invest 1992; 90:533–6.
49. Munger AM, Chow DC, Playford MP, et al. Characterization of lipid composition and high-density lipoprotein function in HIV-infected individuals on stable antiretroviral regimens. AIDS Res Hum Retroviruses 2015; 31:221–8.