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MAJOR ARTICLE

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

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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 ($CD14^+CD16^-$, $CD14^+CD16^+$, $CD14^{Dim}CD16^+$) 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-I], TNFR-II) and vascular (lipoprotein-associated phospholipase A_2 [Lp-PLA_2]) 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-PLA_2 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 levels in HIV^+ participants. Expression of CX3CR1 on monocyte subsets was inversely related to plasma Lp-PLA_2 ($P < .05$ 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. adhesion molecules; fractalkine; HIV; lipoprotein-associated phospholipase A_2; monocytes.

The expected lifespan of individuals living with human immunodeficiency virus (HIV) has been improved by combination antiretroviral therapy (ART); however, cardiovascular disease (CVD) risk is increased in this population [1–4]. Immune activation persists in HIV positive (HIV^+) individuals, even during ART treatment, and markers of immune activation, including increased levels of interleukin-6, D-dimer, and the soluble receptors for tumor necrosis factor (TNF)-α, are predictive of morbidity and mortality [5–10]. The mechanisms responsible for persistent immune activation in ART-treated HIV infection are not fully identified, but they may include the following: low-level HIV-1 replication [11], copathogens [12], microbial translocation [13], and proinflammatory lipids [14, 15], each of which can activate innate defenses, inducing inflammatory cytokines, and altering endothelial and immune cell function. The consequences of increased levels of these proinflammatory mediators, their downstream effects on various cell types, and their contribution to CVD risk in ART-treated HIV infection have been inadequately explored. Inflammation is often associated with the initiation and potentiation of atherosclerosis in HIV-uninfected populations [16].

Monocyte subsets likely contribute to the progression of atherosclerosis; these cells express varying levels of vascular homing molecules [17–21] and can differentiate into lipid-laden foam cells that produce proinflammatory mediators within the vessel wall. "Inflammatory" ($CD14^+CD16^+$) and "patrolling" ($CD14^{Dim}CD16^+$) monocytes are enriched in HIV-infected persons and in uninfected persons who have recently experienced an acute coronary event [22]. These $CD16^+$ monocyte populations are enriched for cells that express the fractalkine receptor (CX3CR1) [20–22] that has been independently associated with carotid artery intima-media thickness in HIV^+ persons [23]. Increased proportions of $CD16^+$ monocytes [24] and elevated

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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], $\alpha_4\beta_1$, $CD49d^+CD29$), lymphocyte function-associated antigen-1 ([LFA-1], $\alpha_L\beta_2$, $CD11a^+CD18$), macrophage-1 antigen ([Mac-1], $\alpha_M\beta_2$, $CD18^+CD11b$), and $CD11c^+CD18$ ($\alpha_X\beta_2$), 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 A_2 (Lp-PLA_2) 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-PLA_2 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-PLA_2, 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-PLA_2 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-PLA_2 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-PLA_2 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 χ^2 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/$\alpha_L\beta_2$, Mac-1/$\alpha_M\beta_2$, CD11c-CD18/$\alpha_X\beta_2$), 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[a]

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.

[a]Information reported is extracted from patient medical records.

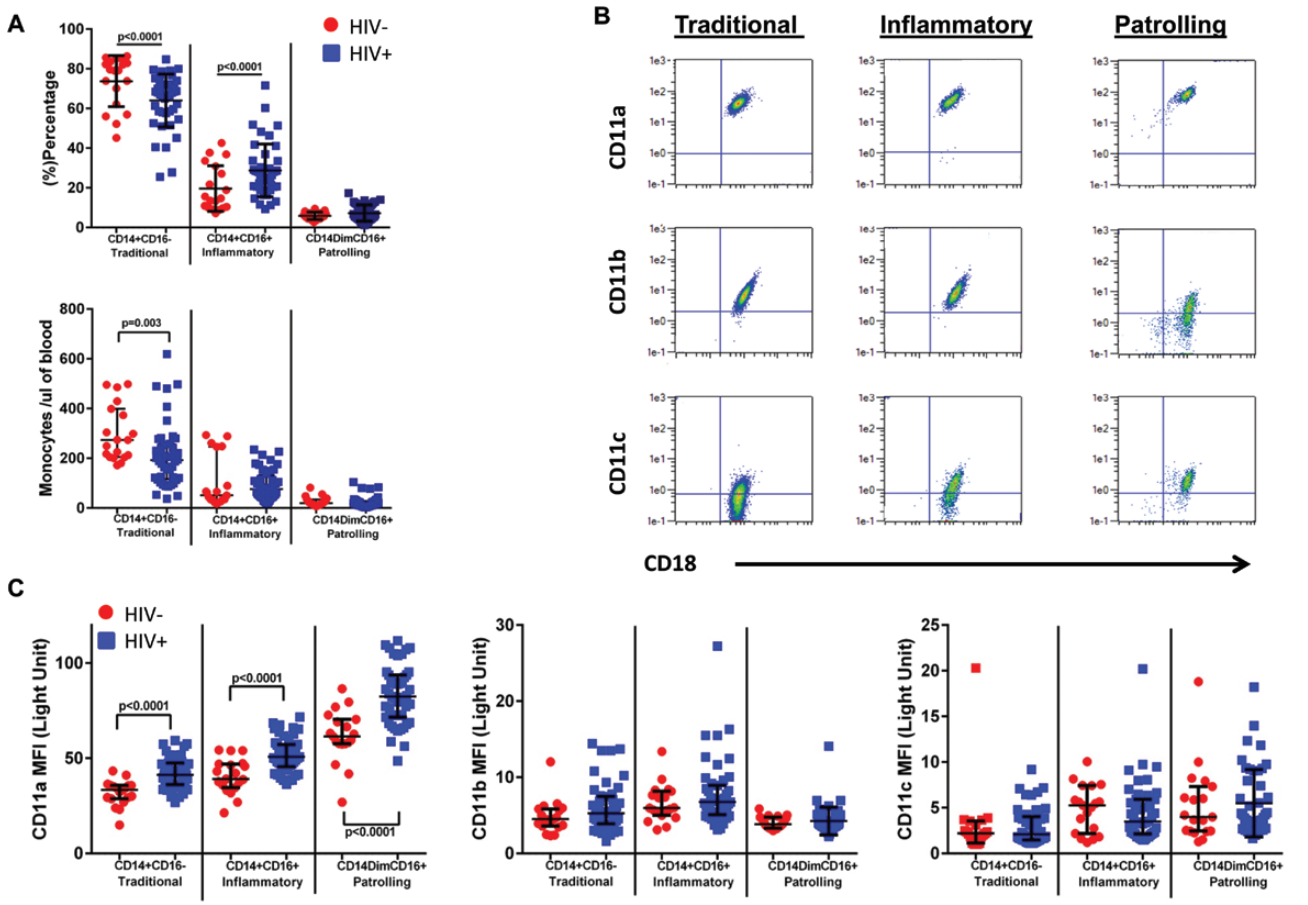

Figure 1. Adhesion molecule expression differs among monocyte subsets between human immunodeficiency virus (HIV)-1 infected and HIV-uninfected participants. Whole blood samples from 49 HIV-1 infected and 19 HIV-uninfected participants were stained for monocyte subset surface markers (CD14 and CD16) and 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.

composed of a unique α chain (CD11a, CD11b, or CD11c) and a conserved β_2 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/β_2 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/$\alpha_L\beta_2$) 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/α_X 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 α_4 and β_1 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/α_4 tended to be increased on patrolling monocytes from both HIV^+ and HIV^- participants compared

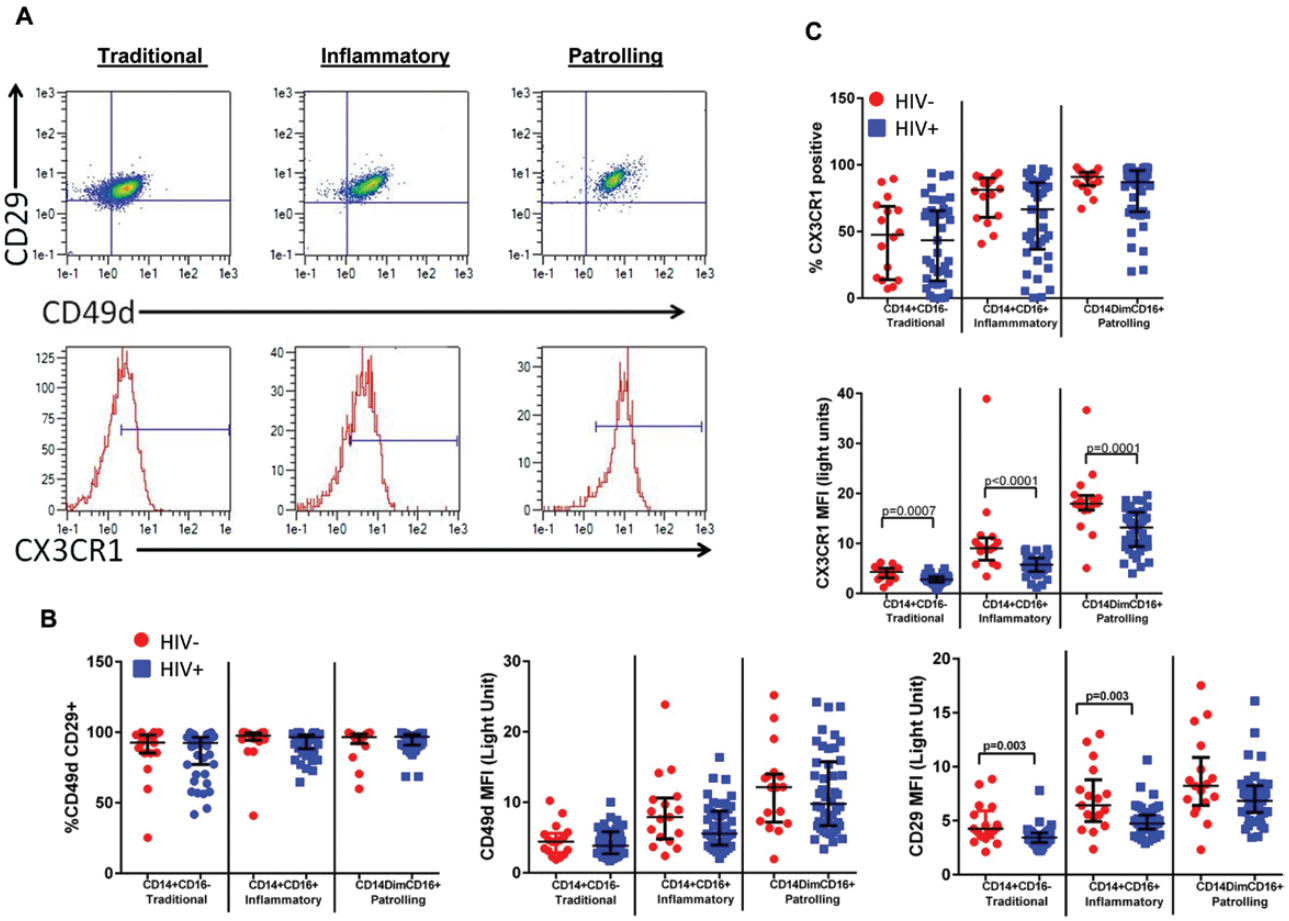

Figure 2. Expression of CX3CR1 is reduced on monocyte subsets from human immunodeficiency virus-positive (HIV$^+$) participants. Whole blood samples from HIV-infected and HIV-uninfected participants were stained for monocyte subset surface markers (CD14, CD16), the adhesion molecule very late antigen-4 ([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.

with traditional and inflammatory monocytes ($P < .0001$ for all; Figure 2B). Expression of CD29/β_1 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-PLA$_2$ 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

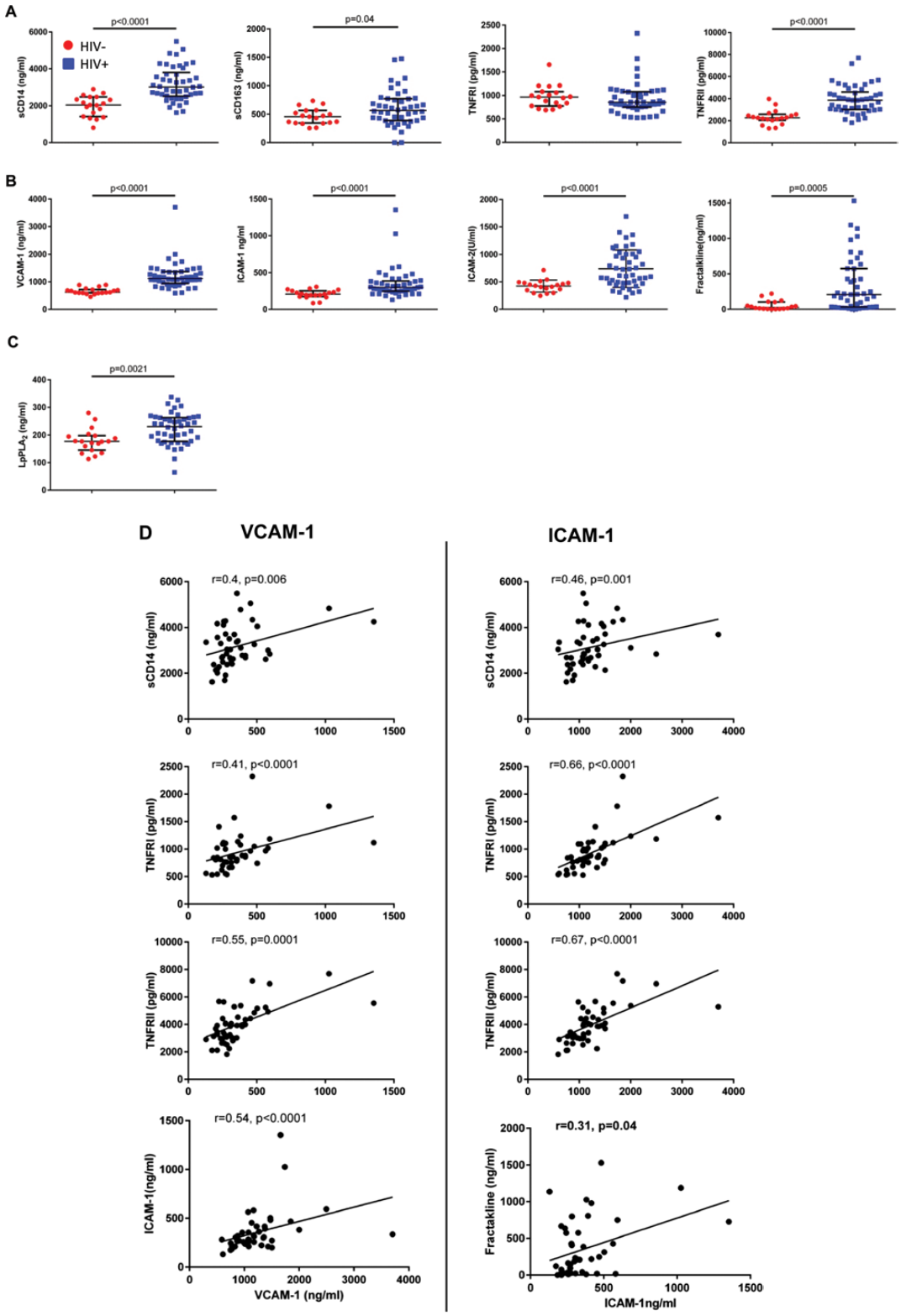

Figure 3. Plasma levels of inflammatory markers and endothelial adhesion molecules are elevated in human immunodeficiency virus (HIV)-infected participants. Plasma samples from all participants were thawed, and levels of soluble markers were measured using commercial enzyme-linked immunosorbent assays. 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 A_2 (Lp-PLA_2). (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-PLA$_2$ (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-PLA$_2$, because Lp-PLA$_2$ 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-PLA$_2$ 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-PLA$_2$ 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-PLA$_2$, potentially linking vascular homing of monocytes to

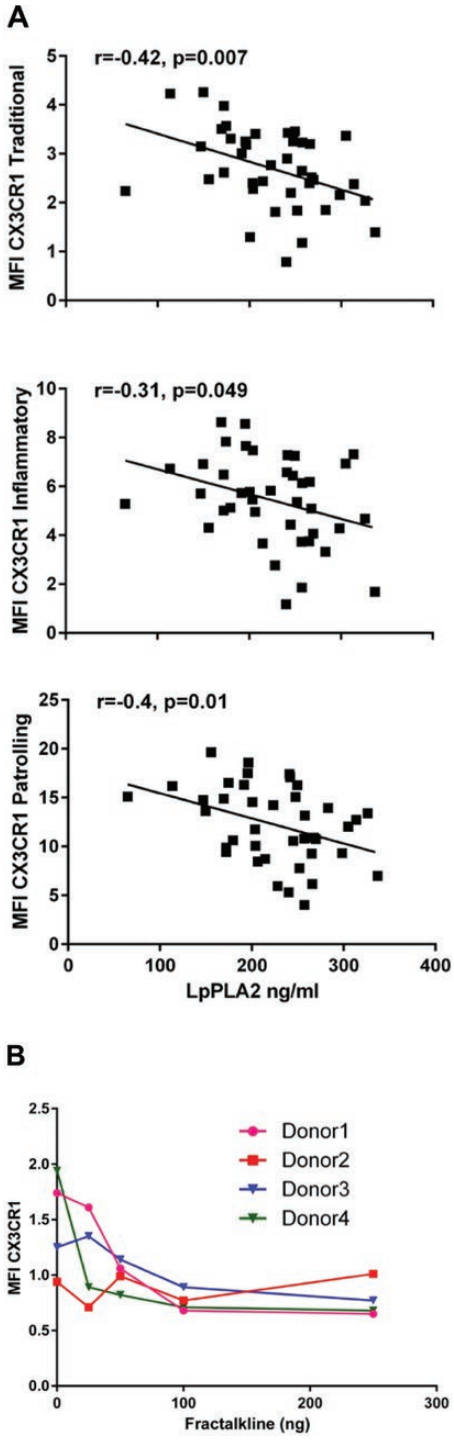

Figure 4. Decreased expression of CX3CR1 on monocyte subsets is related to vascular inflammation in human immunodeficiency virus-positive (HIV$^+$) participants. Whole blood samples from HIV-infected and HIV-uninfected participants were stained for monocyte subset surface markers (CD14, CD16) and the chemokine receptor CX3CR1. Plasma samples from all participants were thawed, and levels of lipoprotein-associated phospholipase A$_2$ (Lp-PLA$_2$) were measured by enzyme-linked immunosorbent assay. (A) Expression of CX3CR1 on monocyte subsets is inversely related to plasma levels of Lp-PLA$_2$. (B) Peripheral blood mononuclear cells from HIV-negative participants were exposed to fractalkine (0–250 ng/mL) overnight. Cells were stained for monocyte surface markers (CD14, CD16), and expression of CX3CR1 was measured by flow cytometry. MFI, mean fluorescence intensity.

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 dramtically 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 μ/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 explaination 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

($P < .03$ for all). Therefore, we suspect that other proinflammatory mediators associated with HIV infection are more likely to contribute to monocyte and EC activation than do smoking and obesity.

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-PLA$_2$, 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.

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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. C. M. 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.

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