JAMA Cardiology | Original Investigation

Association of Arterial and Lymph Node Inflammation With Distinct Inflammatory Pathways in Human Immunodeficiency Virus Infection

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IMPORTANCE Human immunodeficiency virus (HIV) infection is associated with a high risk of cardiovascular disease and increased arterial inflammation. In HIV, inflammation is also increased within lymph nodes (LNs), tissues known to harbor the virus even among treated and suppressed individuals.

OBJECTIVE To test the hypothesis that arterial inflammation is linked to HIV disease activity and to inflammation within HIV-infected tissues (LNs).

DESIGN, SETTING, AND PARTICIPANTS For this case-control study, participants were recruited from the SCOPE (Observational Study of the Consequences of the Protease Inhibitor Era) cohort, a clinic-based cohort of individuals receiving care at San Francisco General Hospital and the San Francisco Veteran's Affairs Medical Center. Arterial and LN inflammation were measured using ^{18}F-fluorodeoxyglucose positron emission tomography. Detailed immunophenotyping was performed, along with measurement of viral activity/persistence and of circulating inflammatory biomarkers.

MAIN OUTCOMES AND MEASURES Arterial and LN inflammation.

RESULTS A total of 74 men were studied (45 HIV-infected men with a median age of 53 years [interquartile range, 49-59 years] and 29 uninfected male controls with a median age of 52 years [interquartile range, 46-56 years]). Lymph node inflammation was higher in HIV-infected individuals and correlated with markers of viral disease activity (viral load, CD8$^+$ T cells, and CD4/CD8 ratio) and CD4$^+$ T-cell activation. Uninfected controls had the lowest LN activity (mean [SD] maximum axillary LN standardized uptake value, 1.53 [0.56]), the elite controller and ART-suppressed groups had intermediate levels of LN (mean [SD] maximum axillary LN standardized uptake value, 2.12 [0.87] and 2.32 [1.79], respectively), and the noncontrollers had the highest activity (mean [SD] maximum axillary LN standardized uptake value, 8.82 [3.08]). Arterial inflammation was modestly increased in HIV-infected individuals and was positively correlated with circulating inflammatory biomarkers (high-sensitivity C-reactive protein and IL-6) and activated monocytes (CD14dimCD16$^+$; nonclassical) but not with markers of HIV. While LN and arterial inflammation were increased in HIV, inflammatory activity in these tissues was not related (r = 0.09, P = .56).

CONCLUSIONS AND RELEVANCE While LNs and, to a lesser degree, the arterial wall are inflamed in HIV, inflammation in these tissues is not closely linked. Namely, measures of HIV disease activity are strongly associated with LN inflammation but not with arterial inflammation. These data suggest that LN and arterial inflammation do not share underlying pathways of immune activation and also that therapeutic interventions that reduce viral disease activity may not predictably reduce arterial inflammation in HIV or its downstream consequence (ie, cardiovascular disease).

JAMA Cardiol. 2017;2(2):163-171. doi:10.1001/jamacardio.2016.4728
Published online December 7, 2016.

Supplemental content

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Current treatment regimens allow individuals infected with human immunodeficiency virus (HIV) to have a life expectancy that approaches that of the general population.[1] However, there has been a concomitant increase in non-HIV-related morbidity and mortality, notably due to atherosclerotic diseases, among HIV-infected individuals.[2] After correcting for traditional cardiovascular disease (CVD) risk factors, we found that the incidences of myocardial infarction, sudden cardiac death, and stroke are higher among HIV-infected individuals, even in the setting of treated and suppressed HIV infection.[3-5]

Several mechanisms likely contribute to the increased prevalence of CVD in HIV infection. Individuals infected with HIV may have a higher burden of traditional CVD risk factors,[6,7] and antiretroviral therapies (ARTs) may lead to atherogenic changes in lipid metabolism.[8,9] However, those factors alone do not explain the excess CVD risk seen in HIV-infected individuals. Untreated and, to a lesser extent, treated HIV infections are associated with the chronic upregulation of a number of inflammatory pathways implicated in the development of atherosclerosis.[3] Multiple immunologic changes, such as T-cell activation and senescence and macrophage activation, have been linked to CVD in HIV-infected individuals.[10] The levels of IL-6, D-dimer, and, to a lesser degree, C-reactive protein are elevated in HIV-infected individuals, and these elevated levels are strongly associated with the risk of developing CVD independently of traditional CVD risk factors for HIV.[11] Moreover, arterial inflammation, a key pathobiological hallmark of atherosclerosis,[12] which is independently associated with the development of CVD events in noninfected individuals,[13,14] is increased in HIV-infected individuals, after controlling for traditional CVD risk factors.[15,16] Accordingly, there is substantial interest in deciphering which inflammatory pathways may be responsible for the excess arterial inflammation seen in HIV-infected individuals.

Human immunodeficiency virus infection is associated with multiple chronic immunologic changes.[10] Human immunodeficiency virus preferentially infects memory $CD4^+$ T cells, most of which reside in the secondary lymphoid tissues such as lymph nodes (LNs). In the absence of ART, HIV replication in these tissues causes local inflammation and $CD4^+$ T-cell loss.[17,18] Although effective ART prevents the spread of HIV, it does not cure the disease; namely, previously infected cells continue to produce virions, especially within HIV "reservoirs" such as the LN. As a consequence of HIV persistence in these secondary lymphoid tissues, as well as other factors,[19] HIV-associated inflammation persists within lymphoid tissues, even in the presence of effective ART. Accordingly, there is substantial interest in studying LN inflammation in individuals with treated and suppressed HIV infection. Furthermore, it is hypothesized that the inflammatory pathways associated with LN inflammation may be related to those that potentiate arterial inflammation in HIV-infected individuals.

^{18}F-fluorodeoxyglucose positron emission tomography/computed tomography (FDG-PET/CT) is used to assess inflamed and/or infected tissues,[20] including atherosclerotic inflammation,[21,22] leveraging the fact that FDG accumulates in immune cells owing to their unusually high metabolic rates.[23] ^{18}F-fluorodeoxyglucose positron emission tomography/computed tomography is clinically used to localize and measure inflammatory and infectious diseases,[20,24-27] including within LNs[28] and the artery wall[15,16] of HIV-infected individuals. However, despite the importance of atherosclerotic inflammation, it remains unknown as to whether the abundant arterial inflammation in HIV-infected individuals relates to inflammatory mechanisms associated with the virus itself (such as inflammation within viral reservoirs) or to other causes of chronic inflammation.[10] Accordingly, FDG-PET/CT imaging provides a unique tool to study the potentially linked activity between these tissues.

In the present study, we tested whether arterial inflammation in HIV-infected individuals is linked to (1) HIV disease activity (eg, treatment status, viral load, $CD4/CD8^+$ T-cell counts, or T-cell activation) and (2) tissue inflammation within foci of persistent HIV infection (namely, lymphoid tissues). To address these questions, we performed FDG-PET imaging for HIV-infected individuals; assessed markers of inflammation, immune activation, and HIV persistence; and assessed their relationships to inflammation within the arterial wall and lymphoid tissues.

Key Points

Question Are lymph node inflammation and arterial inflammation increased in the setting of human immunodeficiency virus (HIV) infection, and does the inflammation in these 2 tissue beds share common pathways of immune activation?

Findings While we observe increased arterial and lymph node inflammation in HIV, in this case-control study, we demonstrate distinct patterns of immune activation. Namely, lymph node inflammation was significantly elevated in HIV and was strongly associated with HIV disease activity and persistence; in contrast, arterial inflammation was associated with systemic inflammatory markers and not HIV disease activity.

Meaning Arterial inflammation is not closely linked to viral disease activity or to chronic inflammation within viral reservoirs such as lymphoid tissues, which suggests that therapeutic interventions that reduce viral disease activity may not predictably reduce arterial inflammation in HIV or its downstream consequence (ie, cardiovascular disease).

Methods

Study Population

Participants were recruited from the SCOPE (Observational Study of the Consequences of the Protease Inhibitor Era) cohort, a clinic-based cohort of individuals receiving care at San Francisco General Hospital and the San Francisco Veteran's Affairs Medical Center. Prior to study entry, HIV infection status was confirmed using HIV antibody testing. Participants were not preselected based on CVD risk factors and were consecutive volunteers from the SCOPE study who agreed to participate. Our study population included the following groups based on their treatment status and virologic

control at the time of enrollment into our substudy: (1) antiretroviral-treated participants with undetectable viral loads using conventional assays (typically <40 copies/mL) (ART suppressed), (2) antiretroviral-untreated or treated participants with detectable viremia ("noncontrollers"), and (3) antiretroviral-untreated participants with undetectable viremia ("elite controllers"). Uninfected controls were recruited from the San Francisco community. All controls were confirmed to be HIV antibody negative. Controls were matched to HIV-infected study participants by age, sex, and Framingham Risk Score (FRS). The University of California, San Francisco, Committee on Human Research approved this study, and all individuals provided written informed consent before enrollment.

Laboratory Assays

Details of the laboratory analyses are provided in the eAppendix in the Supplement. In brief, blood samples were obtained in the fasting state and used to measure lipids and soluble markers of inflammation. In addition, cryopreserved peripheral blood mononuclear cells from the time point closest to the PET/CT scan, median 4 days, were thawed in batches. Cells were stained with viability dye, washed, and then stained with fluorescent conjugated antibodies to cell surface markers to measure $CD4^+$ and $CD8^+$ T-cell activation, to identify monocytes (lineage negative, $HLA\text{-}DR^+$ cells) and to evaluate subpopulations of monocytes. Cellular markers were detected by flow cytometry using an LSRII flow cytometer (BD Biosciences). We evaluated monocytes for expression of CD14 vs CD16 (defining classical monocytes as $CD14^+CD16^-$, intermediate as $CD14^+CD16^+$, and patrolling or nonclassical as $CD14dimCD16^+$), using methods previously described.[29]

Integrated HIV DNA

The size of the HIV reservoir was estimated by measuring the frequency of $CD4^+$ T cells harboring integrated HIV DNA, as previously described.[30] In brief, $CD4^+$ T cells were isolated from cryopreserved peripheral blood mononuclear cells by negative selection (StemCell) and subjected to an Alu-nested polymerase chain reaction to quantify the number of integrated HIV genomes.

Imaging

FDG PET/CT Image Acquisition

Imaging of the carotid arteries and ascending aorta by FDG-PET/CT was performed using a validated approach.[22,31] In brief, FDG was administered intravenously (10 mCi) after an overnight fast, and imaging was performed 120 minutes later using FDG-PET/CT (Siemens Biograph 64). Positron emission tomographic imaging of the neck and chest was performed (7 minutes per bed position). Attenuation-correction scanning was performed using a voltage of 140 kilovolts (peak) [kV(p)] and a current of 35 mA. The reconstruction of attenuation-corrected images was performed using the ordered subset expectation maximization algorithm. All participants had a blood glucose concentration of less than 200 mg/dL (to convert to millimoles per liter, multiply by 0.0555) at the time of imaging.

Image Analysis

Image analysis was performed by a radiologist blinded to all clinical data, using a workstation that enables multimodal standard image fusion (Leonardo–TrueD; Siemens Solutions).

Arterial Inflammation | The FDG uptake in the arterial wall was evaluated by placing circular regions of interest in the axial plane, every 3 mm, starting 1 cm above the aortic valve and continuing to the bottom of the aortic arch. The FDG uptake in the superior vena cava was evaluated for background correction. The aortic FDG uptake was expressed as the target to background ratio, by dividing the maximum standardized uptake values (SUVs) in the aortic wall by the mean SUVs in the superior vena cava. In addition, in the subset of individuals, the arterial FDG uptake was measured in the carotid artery as previously described.[32]

Lymphatic Tissue Activity | The FDG uptake in the lymphatic tissue was evaluated by placing circular regions of interest in the axial plane over visualized LNs in the axillary LNs (and, in a subset of individuals, in the cervical LNs as well). For each of the axillary and cervical LNs, at least 2 regions of interest were drawn on each side. The maximum SUVs were recorded for each region of interest, and the activity was expressed as the target to background ratio, defined as the ratio of the average maximum SUV calculated in the LN tissue to background blood activity derived from the superior vena cava. In addition, activity in the spleen was measured as described previously.[33]

Statistical Analysis

Continuous parametric variables are presented as mean values with standard deviation, continuous nonparametric variables as median values with interquartile range, and nominal variables as frequency (percentage). Group comparisons for normally distributed continuous variables (such as the arterial FDG uptake) were assessed using an independent-samples *t* test, and nonnormally distributed continuous variables (such as the LN FDG uptake) were compared using the Mann-Whitney *U* test. Nominal data (such as sex and diabetes) were assessed using the Pearson χ^2 test. Correlations were tested using either the Pearson correlation coefficient (r) for continuous, normally distributed variables or the Spearman rank correlation coefficient (ρ) for continuous, nonnormally distributed variables. For further comparison of arterial inflammation between groups, we matched uninfected controls to HIV-positive individuals who were treated and virally suppressed based on age, sex, race, and FRS, excluding statin users. All statistical analyses were performed with IBM SPSS Statistics version 23.

Results

Clinical Characteristics

The study cohort included 45 HIV-infected individuals and 29 uninfected controls (**Table 1**). All study participants were men. Of the 45 individuals with HIV, 33 were treated and sup-

Table 1. Clinical Characteristics of Study Participants

Parameter	Participants Without HIV (n = 29)	Participants With HIV (n = 45)	*P* Value
Demographic factors			
Age, median (IQR), y	53 (49-59)	52 (46-56)	.14
Race, No. (%)			
White	12 (41)	30 (67)	.12
African American	14 (48)	10 (22)	
Latino	2 (7)	4 (9)	
Other	1 (4)	1 (2)	
Male, No. (%)	29 (100)	45 (100)	
BMI, median (IQR)	27.0 (23.1-29.2)	26.2 (24.0-28.7)	.55
Comorbidities, No. (%)			
Family history	3 (10)	16 (36)	.02
Hypertension	6 (21)	11 (24)	.71
Diabetes	0	2 (4)	.25
Statin use	1 (5)	5 (11)	.24
Ever smoked	18 (62)	29 (64)	.84
Aspirin use	3 (10)	11 (24)	.13
History of MI or stroke	1 (4)	1 (2)	.75
Hepatitis C	2 (7)	10 (22)	.08
Laboratory values, median (IQR), mg/dL			
Total cholesterol	173 (154-199)	178 (152-206)	.99
LDL-C	108 (84-120)	110 (90-129)	.47
HDL-C	49 (44-63)	46 (37-53)	.07
Triglycerides	85 (63-117)	91 (70-132)	.50
Creatinine	0.91 (0.81-1.04)	0.96 (0.86-1.07)	.19
Inflammatory markers, median (IQR)			
D-dimer, µg/mL	0.39 (0.28-0.53)	0.34 (0.25-0.49)	.41
hsCRP, mg/L	1.19 (0.41-5.08)	1.29 (0.53-2.69)	.83
Soluble CD14, µg/mL	1.52 (1.34-1.75)	1.75 (1.40-1.95)	.08
Soluble CD163, ng/mL	307.46 (224.23-431.61)	455.73 (344.37-650.55)	.002
IL-6, pg/mL	0.87 (0.61-1.38)	0.86 (0.56-1.76)	.85
MCP-1, pg/mL	213.76 (158.53-259.17)	210.09 (159.55-360.06)	.44
Tissue factor, pg/mL	71.66 (52.95-81.96)	68.83 (55.30-83.34)	.10
FRS, %	5 (3-9)	6 (3-7)	.99

Abbreviations: BMI, body mass index (calculated as weight in kilograms divided by height in meters squared); FRS, Framingham Risk Score; HDL-C, high-density lipoprotein cholesterol; HIV, human immunodeficiency virus; hsCRP, high-sensitivity C-reactive protein; IQR, interquartile range; LDL-C, low-density lipoprotein cholesterol; MCP1, monocyte chemoattractant protein 1; MI, myocardial infarction.

SI conversion factors: To convert total cholesterol, LDL-C, and HDL-C to millimoles per liter, multiply by 0.0259; triglycerides to millimoles per liter, multiply by 0.0113; creatinine to micromoles per liter, multiply by 88.4; D-dimer to nanomoles per liter, multiply by 5.476; hsCRP to nanomoles per liter, multiply by 9.524.

pressed, 7 were elite controllers, and 5 were noncontrollers. Compared with the HIV-positive group, the HIV-negative group had a higher proportion of participants who were African American (48.3% vs 22.2%; *P* = .02). The HIV-infected individuals and controls were well matched by age, sex, traditional risk factors (including cigarette smoking), and FRS, with the exception of family history of CVD, which was more common in HIV-infected individuals (36% vs 10%; *P* = .02).

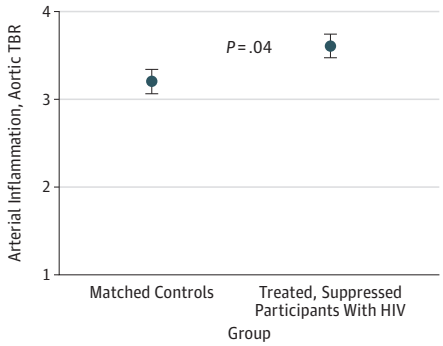
Arterial and LN Inflammation Increased in HIV

Arterial inflammation, when assessed across all study participants, did not differ between the HIV-infected and HIV-uninfected groups (eFigure 1 in the Supplement). However, the arterial FDG uptake is associated with FRS and differs among races, and can be attenuated by statin therapy.[15,32,34] Accordingly, we matched 15 statin-naive HIV-positive participants receiving ART with an undetectable viral load to 15 statin-naive HIV-uninfected participants based on age, race, and FRS. In these matched groups, arterial inflammation was higher in HIV-positive individuals (mean [SD] aortic target to background ratio, 3.63 [0.61]) than in HIV-negative controls (mean [SD] aortic target to background ratio, 3.26 [0.51]; *P* = .04) (**Figure 1**).

In addition, arterial inflammation was generally concordant across arterial beds. In the subset of individuals who also provided carotid arterial PET data (n = 71), carotid FDG uptake correlated with aortic FDG uptake (*r* = 0.30, *P* = .01).

Lymph node activity was substantially higher in HIV-infected individuals than in the uninfected controls (**Figure 2** and **Figure 3**). Uninfected controls had the lowest LN activity (mean [SD] maximum axillary LN SUV, 1.53 [0.56]), the elite controller and ART-suppressed groups had intermediate levels of LN (mean [SD] maximum axillary LN SUV, 2.12 [0.87] and 2.32 [1.79], respectively), and the noncontrollers had the highest activity (mean [SD] maximum axillary LN SUV, 8.82 [3.08]). Compared with noninfected controls, the level of lymphoid inflammation was higher in each of the HIV-infected groups (Figure 3).

Figure 1. Arterial Inflammation in Treated and Suppressed Participants Infected With Human Immunodeficiency Virus (HIV) and Matched Controls



When 15 statin-naive individuals with HIV who were treated and suppressed were matched for age, race, and Framingham Risk Score to 15 statin-naive controls, arterial inflammation was higher in the HIV-infected individuals (P = .04). Error bars indicate ±1 SEM. TBR indicates target to background ratio.

Furthermore, there was general concordance of activity across lymphoid tissues. In the subset of individuals who provided spleen data (n = 64) and cervical LN data (n = 71), axillary LN activity correlated with activity derived from the spleen (r = 0.29, P = .02), and cervical LNs (r = 0.47, P < .001).

Association of LN Inflammation With Measures of HIV Disease Activity

Among elite controllers, the size of the reservoir as estimated by frequency of $CD4^+$ T cells harboring integrated HIV DNA was associated with levels of LN inflammation (r = 0.85, P = .02; eTable in the Supplement). There was no consistent association between HIV DNA and LN inflammation among those receiving ART.

Moreover, across all HIV-infected participants, a higher level of LN inflammation was associated with a higher viral load (r = 0.302, P = .05; **Table 2**), a higher $CD8^+$ T-cell count (r = 0.513, P = .002; Table 2), and a lower CD4/CD8 ratio (r = −0.412, P = .02). We found no significant associations between LN activity and $CD4^+$ T-cell count or CD4 nadir.

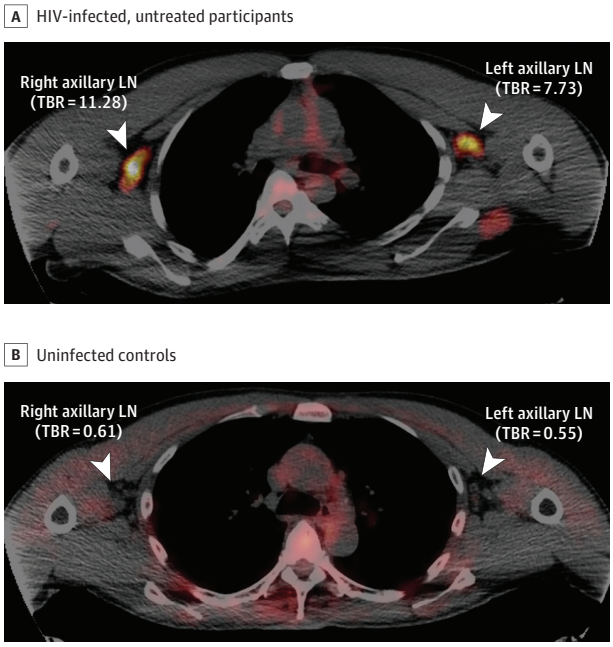
Arterial Inflammation Not Associated With Measures of HIV Disease Activity

In distinct contrast to LN activity, arterial inflammation did not relate to any measure of HIV disease activity (Table 2). Furthermore, arterial inflammation did not correlate with LN activity, either in all HIV-positive individuals or in treated and suppressed HIV-infected individuals (eFigure 2 in the Supplement).

Inflammatory Cell Subsets and Inflammatory Biomarkers Differentially Associated With Arterial and LN Inflammation

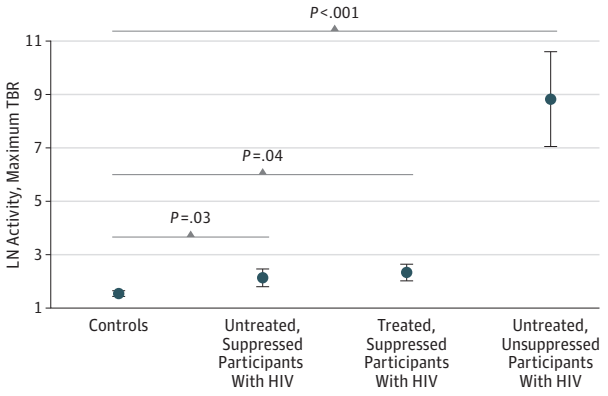
Lymph node activity correlated with a higher frequency of circulating classical monocytes ($CD14^+CD16^-$; **Table 3**) and activated $CD4^+$ T cells ($HLA^-DR^+CD38^+$). In contrast, arterial inflammation correlated with a higher frequency of nonclassical monocytes ($CD14^{dim}CD16^+$); none of the peripheral blood $CD4^+$ or $CD8^+$ T-cell markers were associated with arterial activity. Arterial inflammation also positively correlated with the proportion of $CX3CR1^+$ monocytes and negatively correlated with the proportion of $CCR2^+$ monocytes; neither was predictive of LN activity. Inflammatory markers were predictive of both arterial and LN inflammation; namely, IL-6 and high-sensitivity C-reactive protein were correlated with arterial in-

Figure 2. ^{18}F-Fluorodeoxyglucose Positron Emission Tomography/Computed Tomography (FDG-PET/CT) of Lymph Node (LN) Activity



Axillary LN activity (arrowheads) as assessed using FDG-PET/CT can be seen to be higher in a participant with untreated human immunodeficiency virus (HIV) infection (A) compared with an uninfected control (B).

Figure 3. Lymph Node (LN) Activity in Participants Infected With Human Immunodeficiency Virus (HIV) and Matched Controls



There was a graded increase among different groups of HIV-infected individuals and controls. Namely, controls (n = 29) had the lowest activity; elite controllers (n = 7) and treated, suppressed individuals (n = 33) had intermediate levels of LN activity; and unsuppressed individuals (n = 5) had the highest activity. The level of LN activity was higher in each of the HIV-infected groups vs controls (P < .05 for each group vs controls). Error bars indicate ±1 SEM. TBR indicates target to background ratio.

Table 2. Correlations Between Viral Load, CD4, and CD4 Nadir With Tissue FDG-Uptake in 45 HIV-Positive Participants

	Lymph Node Activity		Arterial Inflammation	
Disease Activity Measure	Correlation Coefficient	*P* Value	Correlation Coefficient	*P* Value
Viral load	0.302	.05	0.012	.94
CD4	−0.109	.48	0.112	.47
CD4 nadir	−0.12	.45	−0.128	.42
CD8	0.513	.002	0.090	.61
CD4/CD8 ratio	−0.412	.02	−0.138	.44

Abbreviations: FDG, ^{18}F-fluorodeoxyglucose; HIV, human immunodeficiency virus.

Table 3. Correlations With Biomarkers in 33 Treated, Suppressed Participants

Group, Marker	Arterial Inflammation	*P* Value	Lymph Node Activation	*P* Value
Markers of inflammation				
D-dimer	0.324	.08	0.371	.04
hsCRP	0.543	.002	0.299	.11
IL-6	0.383	.04	0.386	.04
Soluble CD163	−0.104	.58	0.136	.47
Soluble CD14	−0.047	.80	0.259	.17
$CD4^+$ T-cell markers, %				
$CD4^+$ cells	0.063	.74	−0.344	.06
$HLA^-DR^+CD38^+$	0.137	.47	0.474	.01
$CD28^-CD57^-$	0.148	.44	0.189	.32
$CD28^-CD57^+$	0.281	.13	0.030	.88
$CX3CR1^+$	0.314	.09	0.036	.85
$CD8^+$ T-cell markers, %				
$HLA^-DR^+CD38^+$	0.196	.30	0.319	.09
$CD28^-CD57^-$	−0.027	.89	−0.008	.97
$CD28^-CD57^+$	0.167	.15	−0.199	.29
$CX3CR1^+$	0.070	.71	−0.137	.47
Monocyte markers, %				
$CD14^+CD16^+$	−0.316	.09	−0.091	.63
$CD14^+CD16^-$	0.293	.12	0.384	.04
$CD14^{dim}CD16^+$	0.381	.04	0.033	.86
$CX3CR1^+$	0.372	.04	0.170	.37
$CCR2^+$	−0.412	.02	−0.191	.31
Tissue factor	0.180	.33	0.328	.08

Abbreviation: hsCRP, high-sensitivity C-reactive protein.

flammation, whereas IL-6 correlated with higher LN activity. In addition, the coagulation marker, D-dimer, was associated with LN activity.

Discussion

We performed a comprehensive assessment of arterial and lymphoid tissue inflammation in HIV-infected and uninfected adults. We found that inflammation in lymphoid tissues was consistently higher in HIV-infected adults. The effect of HIV infection on arterial inflammation was less striking, becoming significant only after controlling for confounders known to affect atherosclerotic inflammation. Moreover, using detailed, state-of-the-art measures of immune cellular subsets, inflammatory biomarkers, and measures of viral persistence, we observed distinctly separate patterns of immune activation for lymphoid tissues vs arterial inflammation. Specifically, we found that markers of HIV disease activity and persistence were associated with lymphoid tissue (but not arterial) inflammation. Importantly, despite the effect of HIV on lymphoid tissues, we observed no consistent effect of these markers on arterial inflammation. Instead, we observed that markers of innate system inflammation (high-sensitivity C-reactive protein, IL-6, and nonclassical monocytes) were associated with arterial (but not LN) inflammation. Many of these markers have been consistently associated with risk of CVD in both the general population and the HIV-infected population.[32-35] Accordingly, these data demonstrate that arterial inflammation is not closely linked to viral disease activity or to chronic inflammation within viral reservoirs such as lymphoid tissues. These findings have important therapeutic implications in emerging efforts to reduce the burden of CVD in HIV and to cure HIV infection.

The observation of distinct patterns of immune activation for arterial inflammation and infected lymphoid tissue in

HIV-infected individuals may have an important impact on treatment strategies. Current studies are testing the hypothesis that reducing viremia may result in lowered arterial inflammation in HIV (NCT01766726). However, our findings suggest that arterial inflammation does not closely follow viral activity. Indeed, in a small study[28] of previously untreated HIV-infected individuals, initiation of ART resulted in a marked reduction in LN activity (assessed using FDG PET), while the arterial inflammatory signal increased modestly. Hence, the findings from our study provide some insights into the observation of Zanni and colleagues[28] and provide further data to suggest that control of viremia alone may not result in a reduction in arterial inflammation and, thus, may be insufficient for reducing CVD events. Interventions that target other pathways—including monocyte/macrophages—may prove to be more effective in reducing the burden of CVD in HIV infection. These findings are broadly consistent with various cohort studies that have consistently demonstrated an association between monocyte activation, IL-6, D-dimer, soluble CD14, and soluble CD163 and either CVD progression or all-cause mortality.[11,30,36-43]

The association between arterial inflammation and nonclassical monocyte subsets may have physiologic relevance. Nonclassical monocytes are elevated in the setting of HIV[44] and have been linked to atherosclerosis progression in HIV.[45] The activated monocyte is the target of several therapeutic studies aiming to reduce atherosclerotic inflammation in HIV-infected individuals, including low-dose methotrexate and IL-1β inhibition (NCT01949116, NCT02312219, and NCT02272946).

In distinction to immune cell subsets and measures of HIV disease activity, soluble inflammatory biomarkers were associated with both arterial inflammation and LN activity and did not separate into distinct pathways. One likely explanation is that the soluble markers are downstream products of chronic HIV infection and are less directly reflective of HIV disease activity compared with the CD4/CD8 ratio and T-cell activity. Indeed, in HIV, inflammatory and coagulation markers (eg, IL-6, soluble tumor necrosis factor receptor type I and type II, the kynurenine to tryptophan ratio, and D-dimer) are known to be more strongly associated with non-AIDS events compared with measures of viral disease activity.[39]

Upregulation of inflammation in HIV infection was much more striking in the lymphoid system compared with the arterial system. Indeed, significant differences for arterial inflammation were only seen when the groups were carefully matched and individuals taking statins (which is known to reduce arterial inflammation in uninfected individuals[32,34]) were excluded. This observation may help to explain the inconsistent findings of heightened arterial inflammation in HIV[15,46,47] because, in prior studies, individuals using statins were not consistently excluded and groups were not matched.

We observed a surprisingly high degree of lymphoid inflammation in aviremic states ("elite" control and during ART). Given the association between viral load and inflammation across the entire cohort, and the association between HIV DNA and inflammation in the "elite" controllers, it is likely that HIV production and replication directly stimulate an inflammatory process. The persistent immune dysfunction likely also contributes to inflammation, given the association between the CD4/CD8 ratio and LN activity. These findings are generally in agreement with prior studies from our group and others, including evidence for persistent inflammation and immune dysfunction during ART in the gut (a major lymphoid organ).[48-52] We are now actively investigating the degree to which these pathways remain elevated in the LNs of long-term treated adults and the degree to which this phenomenon can be quantified by imaging.

Our findings of a persistent inflammatory environment in the lymphoid tissues of individuals receiving long-term, apparently effective ART has direct implications for emerging efforts to eradicate or control HIV in the absence of ART. Theoretically, inflammation within these tissues can cause counterregulatory immunosuppressive responses, reducing the capacity of the adaptive immune response to eliminate the reservoir. Alternatively, higher levels of T-cell activation (a correlate of lymphoid inflammation in this study) could lead to excess production and the spread of the virus, even during ART. Finally, excess inflammation may contribute to antigen-specific and cytokine-driven $CD4^+$ T-cell proliferation, a major factor contributing to HIV persistence during ART.[53,54] Prospective interventional studies in which these pathways are specifically inhibited in a controlled manner will be necessary to unravel the many complex interactions that likely contribute to HIV persistence and inflammation during ART.[55] Such studies are now ongoing.

Limitations

Our study has the limitation that it was a cross-sectional study, and the findings are associative in nature. In addition, we did not correct for multiple testing in this exploratory analysis; hence, the findings require replication. As with any cross-sectional study, unmeasured confounders may be present. In addition, only male participants were recruited.

Conclusions

In conclusion, using multimodal imaging and HIV viral disease and immune system measures, we observe divergent patterns of immune activation in association with arterial inflammation and lymphoid tissue inflammation. These findings may have important therapeutic implications in both cardiovascular risk modification and curative strategies in HIV.

ARTICLE INFORMATION

Accepted for Publication: October 17, 2016.

Published Online: December 7, 2016. doi:10.1001/jamacardio.2016.4728

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Author Contributions: Dr Hsue had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.
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Obtained funding: Tawakol, Deeks, Hsue.
Administrative, technical, or material support: Tawakol, Li, Hur, Rupert, Hsu, Sereti, Chomont, Ganz.
Supervision: Tawakol, Ishai, Pampaloni, Sereti, Ganz, Hsue.

Conflict of Interest Disclosures: All authors have completed and submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Dr Tawakol reports receiving grants from the National Institutes of Health (NIH)/National Heart, Lung, and Blood Institute, during the conduct of the study, grants from Actelion, Takeda, and Genentech, and personal fees from Actelion, Takeda, Amgen, and AstraZeneca, outside the submitted work. Dr Deeks reports receiving grants from Merck, outside the submitted work, and from the NIH and amfAR during the conduct of the study. Dr Hsue reports grants from NIH/National Heart, Lung, and Blood Institute, a grant from Pfizer, honoraria from Gilead, and other support from Merck and Bristol-Myers Squibb outside the submitted work. Ms Hur and Mr Li report receiving support (grant K24AI112393 to Dr Hsue) from the NIH during the conduct of the study. No other disclosures are reported.

Funding/Support: This study was funded in part by the National Institute of Allergy and Infectious Diseases (grant K24AI112393 to Dr Hsue), the National Heart, Lung, and Blood Institute (grant R01HL122177 to Dr Tawakol), the amfAR Institute for HIV Cure Research (amfAR 109301), the intramural research program of the National Institute of Allergy and Infectious Diseases/NIH, and the Delaney AIDS Research Enterprise (grant AI096109). The SCOPE cohort is also supported in part by National Institute of Allergy and Infectious Diseases (grant K24AI069994 to Dr Deeks), the University of California, San Francisco/Gladstone Institute of Virology and Immunology (grant P30AI027763), the University of California, San Francisco Clinical and Translational Research Institute Center (grant UL1RR024131), and the Center for AIDS Research Network of Integrated Systems (grant R24 AI067039).

Role of the Funder/Sponsor: The study sponsors had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

Meeting Presentation: This paper was presented at the Conference on Retroviruses and Opportunistic Infections of the International Antiviral Society–USA; February 23, 2016; Boston, Massachusetts.

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