Association of T cell and Macrophage Activation with Vascular Health in HIV

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INTRODUCTION

- HIV-infection is associated with increased risk of cardiovascular disease, including acute MI, stroke and peripheral artery disease.[1-4] While the etiology is multifactorial, increased vascular endothelial activation and impaired smooth muscle function is a feature of HIV that persists despite viral suppression.[5-6]
- Prior studies have found increased T cell activation associated with carotid plaque formation, intimal thickness and reduced carotid distensibility in HIV infected patients.[7-10]
- While initiation of ART can improve arterial responsiveness,[11] markers of endothelial cell activation often remain persistently elevated despite suppression of plasma viremia. [6, 12, 13]
- We assessed potential associations between T cell activation and systemic inflammation with impaired arterial response and endothelial activation in HIV-infected individuals on long-term, effective ART without known cardiac, vascular, or metabolic disease.
- We hypothesized greater activation of circulating T cells impairs vascular responsiveness, while greater circulating inflammatory and macrophage activation markers would be accompanied by higher levels of the soluble endothelial activation markers Intercellular Adhesion Molecule 1 (ICAM-1) and Vascular Cell Adhesion Molecule 1 (VCAM-1).

METHODS

- We enrolled 70 HIV-infected patients on ART from the Vanderbilt Comprehensive Care Clinic between April 2013 and September 2014. All subjects were on Atripla for at least 6 months prior and had persistent HIV-1 RNA measurements of <50 copies/ml for at least the previous 2 years.
- Additional inclusion criteria were CD4+ count >350 cells/μl, no antidiabetic medication or statin use, no heavy alcohol or cocaine/amphetamine use, no active infection aside from HIV, and no previously diagnosed diabetes, CVD, or rheumatologic disease.
- Brachial artery reactive hyperemia was measured using a Philips iE33 ultrasound. Flow mediated dilation (FMD) was calculated as the largest percent increase in vessel diameter after cuff deflation.
- Fasting blood samples were collected for high-sensitivity C-reactive protein (hsCRP), as well as plasma levels of soluble CD14 and CD163 measured using ELISA. Interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α) receptor 1, ICAM-1, and VCAM-1 were measured using multiple immunoassay.
- T cell subsets were determined using flow cytometry. The proportion of CD4+ and CD8+ cells expressing surface markers of activation (CD38+), senescence (CD57+PD1+), memory status (CD45RO+), and naïve status (CD45RO-CCR7+CD27+) were determined.
- We assessed whether T-cell subsets or circulating immune biomarkers were associated with impaired FMD or increased ICAM-1 and VCAM-1 using multivariate models adjusted for age, sex, BMI, smoking status, and duration of antiretroviral therapy. Sensitivity analyses further adjusted for hepatitis C co-infection and CD4+ count.

RESULTS

- The clinical and demographic characteristics of the HIV-infected participants, stratified by sex, are shown in **Table 1**.

Table 1: Demographics and clinical characteristics of Cohort stratified by sex

	All HIV+ N=70	HIV+ males N=40	HIV+ females N=30	p-value
Age, median years (IQR)	45.0 (38.8, 50.0)	43.5 (35.0, 49.0)	46.0 (43.5, 51.3)	0.02
Female, n (%)	30 (43%)	-	-	
Non-white, n (%)	38 (54%)	16 (40%)	22 (73%)	<0.01
BMI, median kg/m²	30.3 (23.9, 35.7)	29.4 (23.3, 35.4)	31.4 (26.0, 37.0)	0.32
CD4+ at enrollment, median cells/μl	701 (540, 954)	681 (519, 912)	730 (582, 1014)	0.28
CD4+ at ART initiation, median cells/μ	257 (140, 378)	239 (122, 400)	236 (163, 303)	0.40
Duration of ART treatment, median years	6.24 (4.33, 10.14)	5.12 (3.82, 7.97)	8.36 (5.81, 13.41)	0.001
Smoker, n (%)	25 (36%)	12 (30%)	13 (43%)	0.32
Hep C positive, n (%)	8 (11%)	3 (8%)	5 (17%)	0.28
Hemoglobin A1c, median	5.2 (4.9, 5.5)	5.2 (4.9, 5.5)	5.3 (5.0, 5.6)	0.14
Maximum FMD, median %	8.85% (5.76, 11.00)	6.65% (4.50, 10.37)	10.14% (8.41, 11.48)	<0.01

p-values represent Wilcoxon rank sum tests or Chi-square tests

- Relationships between CD4+ and CD8+ T cell subsets and markers of inflammation and macrophage activation with brachial artery FMD were assessed in individual multivariate models (**Table 2**).
- Relationships between T cell subsets, circulating markers of inflammation, and markers of macrophage activation with soluble VCAM-1 and ICAM-1 levels were assessed in individual multivariate models (**Table 3**).

Table 2: Multivariable linear regression for effect of immunologic biomarkers on maximal brachial artery flow-mediated dilation

Biomarker	Regression coefficient	p-value
CD8+ T cell markers		
CD8+CD38+ (activated)	-0.33	**<0.01**
CD8+CD57+PD1+ (senescent)	0.10	0.46
CD8+CD45RO+ (memory)	0.01	0.98
CD8+CD45RO-CCR7+CD27+ (naïve)	-0.09	0.52
CD4+ T cell markers		
CD4+CD38+ (activated)	-0.12	0.42
CD4+CD57+PD1+ (senescent)	0.14	0.26
CD4+CD45RO+ (memory)	0.17	0.40
CD4+CD45RO-CCR7+CD27+ (naïve)	-0.15	0.29
Inflammatory biomarkers		
High sensitivity C-reactive protein	-0.40	**<0.01**
Interleukin-6	0.14	0.30
TNF-α receptor 1	-0.21	0.13
TNF-α receptor 2	-0.22	0.06
Macrophage activation markers		
Soluble CD14	-0.20	0.12
Soluble CD163	0.07	0.56
Macrophage Inflammatory Protein 1α	-0.20	0.11

Model adjusted for age, sex, body mass index, smoking status, and duration of antiretroviral therapy. Maximal FMD was natural-log transformed. Abbreviations: FMD, flow-mediated dilation; TNR-α, tumor necrosis factor alpha. P-values <0.05 shown in bold.

RESULTS CONTINUED

Table 3: Multivariable linear regression for effect of immunologic biomarkers on plasma ICAM-1 and VCAM-1

Biomarker	Regression coefficient VCAM-1	p-value	Regression coefficient ICAM-1	p-value
CD8+ T cell markers				
CD8+CD38+ (activated)	0.11	0.36	0.04	0.75
CD8+CD57+PD1+ (senescent)	0.13	0.28	-0.06	0.66
CD8+CD45RO+ (memory)	0.21	0.07	0.02	0.89
CD8+CD45RO-CCR7+CD27+ (naïve)	0.11	0.39	0.13	0.34
CD4+ T cell markers				
CD4+CD38+ (activated)	-0.32	**0.01**	-0.27	**0.04**
CD4+CD57+PD1+ (senescent)	0.03	0.80	0.04	0.74
CD4+CD45RO+ (memory)	0.31	**0.01**	0.10	0.43
CD4+CD45RO-CCR7+CD27+ (naïve)	0.10	0.45	-0.06	0.67
Inflammatory biomarkers				
High sensitivity C-reactive protein	-0.07	0.61	0.11	0.40
Interleukin-6	0.15	0.25	0.17	0.19
TNF-a receptor 1	0.42	**<0.01**	0.48	**<0.001**
TNF-a receptor 2	0.31	**<0.01**	0.36	**<0.01**
Macrophage activation markers				
Soluble CD14	0.12	0.33	0.32	**<0.01**
Soluble CD163	0.41	**<0.001**	0.32	**<0.01**
Macrophage Inflammatory Protein 1α	0.27	**0.02**	0.37	**<0.01**

Model adjusted for age, sex, body mass index, smoking status, and duration of antiretroviral therapy. n=70 HIV+ subjects. Plasma ICAM-1 and VCAM-1 were natural-log transformed.
Abbreviations: ICAM-1, intercellular adhesion molecule 1; TNR-α, tumor necrosis factor alpha; VCAM-1, vascular cell adhesion molecule 1. P-values <0.05 shown in bold.

CONCLUSIONS

- In HIV-infected adults on long-term ART with sustained virologic suppression, CD8+ T cell activation (but not CD4+ or monocyte activation) was associated with impaired brachial artery smooth muscle relaxation independent of age, tobacco use, and BMI.
- It is possible that the long duration of viral suppression and ART treatment (median 6.8 years) in our cohort may have created a T cell subset phenotype in which CD8+ T cell activation, rather than CD4+ activation, was more representative of on-going antigenic stimulation despite absence of plasma viremia.
- In contrast to FMD results, endothelial activation was primarily associated with soluble markers of macrophage activation, likely reflecting the effects of vascular macrophage activity on endothelial cell function.
- We unexpectedly observed inverse associations between the proportion of activated CD4+ T cells and levels of plasma ICAM-1 and VCAM-1, which persisted after adjusting for total CD4+ T cell count and nadir CD4+ T cell count in sensitivity analyses (data not shown).
- Further studies are needed to understand the links between T cell activation and vascular function, and the source of the chronic monocyte/macrophage stimuli contributing to endothelial activation.

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