Effect of T-Cell Activation on Endothelial Dysfunction in HIV

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Background

- HIV-infected individuals are at an increased risk of cardiovascular disease and HIV is thought to play a direct role in the pathophysiology.
- One possible mechanism includes chronic inflammation secondary to T cell activation. These activated T cells are significantly increased in HIV-infected patients and correlate with viral load and disease stage.

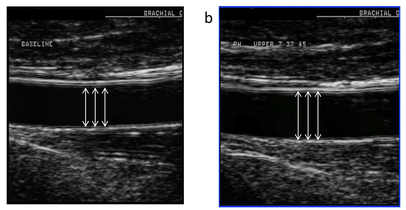
Hypothesis

- T cell activation and inflammatory markers are elevated in HIV-infected individuals and are independently associated with endothelial dysfunction, which is known to play a role in the progression of atherosclerosis.

Methods

- Sixty-eight healthy controls were compared to 359 HIV-infected subjects.
- A broad array of inflammatory and coagulation markers were measured including CRP, fibrinogen, D-dimer, IL-6, and TNF-α.
- T cell activation markers were measured by immunophenotyping in frozen peripheral blood mononuclear cells.
- Endothelial function was measured using Flow-mediated dilation (FMD) and Reactive hyperemia (RH) in the brachial artery.

Figure 1: Measurement of Endothelial Function



Macrovascular function measured by comparison of brachial artery vasodilation before (a) and after (b) restoration of blood flow. (FMD)
Microvascular function measured from the Doppler mean velocity-time integral of the first three beats after cuff release (b). (RH)

Results: Clinical Characteristics

	Controls	HIV +	p-value
N	68	359	
Age (years)	48 ± 10	49 ± 10	
Caucasian	50%	61%	<0.01
African American	35%	22%	
Hispanic	9%	11%	
Other	6%	2%	
Male	97%	84%	<0.01
HTN	22%	42%	<0.01
DM	2%	8%	<0.01
Current Smoking	46%	31%	<0.01
Smoking Pack Years	9.7 ± 17.5	10.2 ± 16.3	0.54
LDL (mg/dL)	112.0 ± 28.5	103.3 ± 30.8	0.02
HDL (mg/dL)	51.3 ± 13.1	46.8 ± 14.9	<0.01
TC (mg/dL)	184.6 ± 31.8	179.6 ± 39.1	0.13
TGs (mg/dL)	103.0 ± 58.1	154.7 ± 122.3	<0.01
CKD	3%	9%	<0.01
PASP (mm Hg)	19.6 ± 2.4	32.6 ± 15.1	<0.01
Family History of CAD	22%	32%	<0.01
Hepatitis C	3%	18%	<0.01
Hx of CAD/CVA	2%	5%	<0.01
Current CD4 count (cells/mm³)		563 ± 305	
Nadir CD4 count (cells/mm³)		212 ± 184	
Viral Load Suppressed		77%	
Current ARV Use		81%	
Reactive Hyperemia (%)	69.4 (50.6, 81.6)	61.5 (46.3, 83.3)	0.25
Flow-Mediated Dilation (%)	4.4 (2.6, 5.7)	4.0 (2.6, 5.5)	0.85

Results: T cell Activation Markers

	Controls N = 24	HIV + N = 168	p-value
Activation			
CD4+ CD38+ DR+	0.33 (0.26, 0.48)	0.66 (0.35, 1.33)	<0.01
CD4+ CCR5+	0.45 (0.27, 0.75)	0.46 (0.26, 0.94)	0.70
CD4+ CCR5+ CD38+ DR+ PD1+	0.81 (0.38, 1.20)	0.91 (0.50, 1.86)	0.19
CD8+ CD38+ DR+	1.66 (1.12, 2.58)	3.42 (1.84, 5.16)	0.19
CD8+ CCR5+	1.22 (0.92, 1.95)	1.14 (0.64, 1.93)	0.19
CD8+ CCR5+ CD38+ DR+ PD1+	2.44 (0.99, 4.08)	4.19 (2.00, 7.24)	<0.01
CD4+ IFN+ (CMV specific)	0.09 (0, 0.48)	0.43 (0.19, 1.34)	<0.01
CD8+ IFN+ (CMV specific)	0.17 (0.01, 1.42)	3.86 (1.95, 8.21)	<0.01
CD4+ IFN+ (HIV specific)	0.001 (0, 0.01)	0.11 (0.05, 0.23)	<0.01
CD8+ IFN+ (HIV specific)	0.002 (0, 0.02)	0.47 (0.13, 1.90)	<0.01
Immunosenescence			
CD4+ CD28- CD57+	0.56 (0.01, 1.77)	3.26 (0.79, 6.25)	<0.01
CD8+ CD28- CD57+	2.45 (2.04, 4.04)	2.60 (1.81, 3.86)	0.69
Immune Exhaustion			
CD4+ PD1+	0.39 (0.21, 0.82)	0.69 (0.30, 1.37)	0.04
CD8+ PD1+	1.34 (0.86, 2.23)	1.45 (0.84, 3.30)	0.26

Results: Association of T cell Activation Markers with Endothelial Dysfunction

	FMD		RH	
Per Doubling of T Cell Subset	Unadjusted % estimate (95% CI)	Adjusted % estimate (95% CI)	Unadjusted % estimate (95% CI)	Adjusted % estimate (95% CI)
CD4+ CD28- CD57+	3.5 (0.2, 6.9)	4.2 (0.5, 8.1)	1.2 (-0.7, 3.2)	1.9 (-0.8, 4.7)
CD8+ CD28 -CD57+	3.3 (-6.3, 13.9)	1.1 (-9.1, 12.4)	-6.1 (-12.7, 1)	-6 (-12.3, 0.6)
CD4+ CD38 +DR+	-0.5 (-6.6, 6)	1.1 (-5.3, 7.9)	-2 (-7.4, 3.8)	-0.8 (-6.2, 4.9)
CD4+ CCR5+	2.7 (-4.5, 10.5)	3.8 (-3.6, 11.9)	2.1 (-3.2, 7.7)	3.7 (-2.9, 10.7)
CD4+ PD1+	0.1 (-5.4, 5.9)	1.5 (-4.1, 7.4)	-2.3 (-6, 1.7)	-1.3 (-5.2, 2.8)
CD4+ CCR5+ CD38+ DR+ PD1+	-3.8 (-9.7, 2.5)	-3.4 (-9.5, 3.2)	-4.9 (-10, 0.5)	-3.8 (-9.5, 2.3)
CD8+ CD38+ DR+	0.6 (-6.9, 8.7)	1.1 (-6.6, 9.5)	-2.3 (-9.2, 5.1)	-1.1 (-7.4, 5.7)
CD8+ CCR5 +	1.8 (-4.4, 8.3)	2.7 (-3.4, 9.3)	-0.7 (-5, 3.8)	-0.8 (-5.8, 4.5)
CD8+ PD1+	0.2 (-6.4, 7.3)	1.3 (-5.7, 9)	**-7.9 (-13.6, -1.7) p = 0.013**	**-6.6 (-12.1, -0.8) p = 0.027**
CD8+ CCR5+ CD38+ DR+ PD1+	-2.1 (-8.1, 4.3)	-1.1 (-7.1, 5.3)	**-5.4 (-10.1, -0.4) p = 0.036**	-4.2 (-9, 0.9)
CD4+ IFN+ (CMV specific)	-4.9 (-9.7, 0.2)	-5.2 (-10.8, 0.7)	**-6 (-9.8, -2.1) p = 0.003**	**-6.9 (-11.2, -2.3) p = 0.004**
CD8+ IFN+ (CMV specific)	-0.8 (-3.8, 2.3)	0.5 (-2.8, 4)	-1 (-3.3, 1.3)	-1.8 (-4.2, 0.8)
CD4+ IFN+ (HIV specific)	-1.2 (-7.4, 5.4)	-2.4 (-9.3, 5.1)	-1 (-5.3, 3.5)	-1.8 (-6, 2.7)
CD8+ IFN+ (HIV specific)	-1.4 (-5.9, 3.2)	-1.7 (-6.4, 3.2)	0.4 (-3.3, 4.3)	-0.7 (-4.5, 3.4)

Discussion

- HIV+ cohort had lower FMD and RH levels than the control group, but the differences did not reach significance.
- Markers of T cell activation, immunosenescence, and apoptosis were significantly increased in the HIV+ cohort. This increase is thought to be from direct HIV infection as well as possible chronic co-infections.
- Certain T cell markers were independently associated with endothelial dysfunction as measured by RH but not FMD. RH has been shown to be more predictive of cardiovascular outcomes than FMD.
- D-dimer, sCD-14, and TNF-α were independently associated with RH as well.
- When these inflammatory markers were added to the adjustment model, sCD-14 weakened the association between CD8+PD1+ and RH. D-dimer weakened the association between CD8+PD1+ and CD4+IFN+ (CMV specific). TNF-α did not weaken any association.
- While the associations were weakened, D-dimer and sCD-14 did not account for all of the association between T cell markers and RH.
- Fibrinogen and CMV IgG were the inflammatory markers independently associated with FMD.

Results: Association of Inflammatory Markers with Endothelial Dysfunction

	FMD		RH	
Per Doubling of Inflammatory Marker	Unadjusted % estimate (95% CI)	Adjusted % estimate (95% CI)	Unadjusted % estimate (95% CI)	Adjusted % estimate (95% CI)
D-Dimer	-2.2 (-7.3, 3.3)	-4 (-9.5, 2)	-1.8 (-5.7, 2.2)	**-3.9 (-7.7, 0.1) p = 0.05**
IL-6	-3.2 (-9.6, 3.8)	-2.9 (-10, 4.7)	-2.9 (-7.8, 2.4)	-5.2 (-10.3, 0.2)
TNF-α	-1.1 (-8.3, 6.6)	0.5 (-7.2, 8.9)	-6.2 (-12.6, 0.6)	**-8.1 (-14.5, -1.2) p = 0.022**
Fibrinogen	**-21.2 (-33, -7.2) p = 0.004**	**-19.6 (-33.2, -3.1) p < 0.022**	-9.1 (-22.7, 6.8)	-15 (-28.3, 0.8)
RANKL	-3.7 (-11.5, 4.8))	-1.6 (-10.1, 7.7)	3.7 (-4.2, 12.2)	3.9 (-4.1, 12.6)
Osteopontin	-10.4 (-23.4, 4.8)	-13.7 (-27.4, 2.6)	2.7 (-10.2, 17.4)	0.3 (-12.9, 15.5)
Neopterin	-0.8 (-10.4, 9.8)	0.8 (-9.9, 12.6)	-4 (-12.1, 4.7)	-8.6 (-17, 0.7)
Cystatin C	-4.6 (-24.5, 20.6)	-0.3 (-24.2, 31.1)	6.1 (-11.6, 27.3)	-2.6 (-20.5, 19.3)
CMV IgG	**-6.4 (-9.5, -3.3) p < 0.001**	**-6.3 (-9.9, -2.6) p = 0.001**	-1.3 (-4.8, 2.4)	-0.7 (-4.6, 3.3)
sCD-14	5.5 (-8, 20.9)	6.4 (-7, 21.9)	-7.1 (-16.8, 3.8)	**-9.3 (-17.6, -0.3) p = 0.043**
sCD-163	2.6 (-8.5, 15)	3.6 (-7.6, 16.3)	1.3 (-7.2, 10.6)	-0.9 (-10.1, 9.2)
Leptin	-0.9 (-5.6, 4)	-1.7 (-6.9, 3.9)	3.3 (-0.9, 7.7)	3.1 (-2.2, 8.7)
Adiponectin	-1.5 (-7.2, 4.5)	-0.8 (-7.1, 5.9)	4.5 (-1.6, 11.1)	5.9 (-1.1, 13.3)
LPS	4.4 (-6.7, 16.8)	5.4 (-6.7, 19.1)	-1.3 (-9.1, 7.1)	-4.9 (-13.5, 4.6)
Pro-BNP	2.3 (-1.6, 6.4)	1.8 (-2.4, 6.1)	0 (-4.2, 4.5)	-0.9 (-5, 3.5)
PTX3	3.9 (-5, 13.7)	5 (-5.1, 16.1)	-1.4 (-8.3, 6)	-0.5 (-7.6, 7.1)

Results: Mediation Analysis for RH

	RH			
Per Doubling of T Cell Subset	Unadjusted % estimate (95% CI)	Adjusted % estimate (95% CI)	Adjusted + D-Dimer % estimate (95% CI)	Adjusted + sCD14 % estimate (95% CI)
CD8+CD28-CD57+	-6.1 (-12.7, 1) p = 0.089	-6 (-12.3, 0.6) p = 0.074	-6.8 (-14.2, 1.1) p = 0.09	-6.6 (-13, 0.3) p = 0.061
CD8+PD1+	-7.9 (-13.6, -1.7) p = 0.013	-6.6 (-12.1, -0.8) p = 0.027	-7 (-13.4, -0.2) p = 0.045	-6.6 (-12.9, 0.1) p = 0.054
CD4+IFN+ (CMV specific)	-6 (-9.8, -2.1) p = 0.003	-6.9 (-11.2, -2.3) p = 0.004	-6.9 (-12.5, -0.9) p = 0.024	-8.1 (-12.6, -3.5) p = 0.001

Conclusions/Limitations

- The results here suggest that T cell dysregulation is upregulated in HIV and leads to activation of the inflammatory and coagulation cascades, which results in microvascular endothelial dysfunction as measured by RH.
- The cross-sectional design of this study allows us to show only correlations. Larger longitudinal studies need to be done to prove causation.