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Increased Coronary Atherosclerosis and Immune Activation in HIV-1 Elite Controllers

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Summary

HIV-1 elite controllers (EC) spontaneously maintain suppressed levels of viremia, but exhibit significant immune activation. We investigated coronary atherosclerosis by coronary CT angiography (CTA) in: 1) EC, 2) non EC, chronically HIV-1 infected, ART-treated patients with undetectable viral load (“chronic HIV”), and 3) HIV-negative controls. Prevalence of atherosclerosis (78% vs. 42%, $P<0.05$) and markers of immune activation were increased in EC compared to HIV-negative controls. sCD163, a monocyte activation marker, was increased in EC compared to chronic HIV-1 ($P<0.05$) and compared to HIV-negative controls ($P<0.05$). These data suggest a significant degree of coronary atherosclerosis and monocyte activation among EC.

Introduction

Recent data suggest that immune activation – even among virologically suppressed patients – may contribute to increased atherosclerotic disease in HIV-infected patients[1, 2]. HIV-1 elite controllers (EC) represent an ideal population to explore the potential role played by longstanding immune activation in atherosclerotic disease, without the confounding effects of high level viral replication or ART. Despite tight control of viral replication, residual low-level viremia in EC is associated with persistent T-cell activation and inflammation[3-5]. In contrast, little is known about monocyte/ macrophage activation in EC and direct investigation of coronary atherosclerosis has not been performed in this population.

Methods

Ten HIV-1 EC were identified from the International HIV Controllers Study[6, 7], with approximately 400 controllers, of whom less than half had no history of viral blips and/or ART use. ART-naïve subjects, ages 40-60, with HIV-1 viral load below the detection limit of an ultrasensitive assay (<48 copies/mL), and no history of “viral blips”, CVD or renal disease were chosen. Subjects underwent assessment of CTA and coronary artery calcium (CAC)[8]. Viral reservoir was measured by cell associated HIV-1 DNA[9, 10]. Comparison

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was made to data previously obtained in non EC, chronic HIV-1 patients, receiving ART, with undetectable HIV viral load (“chronic HIV”) and HIV-negative controls[1, 8]. Subjects in the two comparison groups were similar to the EC with respect to age and lack of known CVD. All participants gave informed consent to participate. This study was approved by the Massachusetts General Hospital IRB.

Data were compared between the groups using ANOVA/Kruskal-Wallis test depending on normality. Pairwise comparisons were performed using Student's T-test or the Wilcoxon test for variables significant in overall ANOVA. Logistic regression was used to control for traditional CVD risk factors (SAS JMP).

Results

EC were similar in age, gender and traditional CVD risk factors, including smoking and Framingham score, to the two comparison groups (Table). Duration of HIV diagnosis was 15 or more years for both EC and chronic HIV-1 patients. Longitudinal plasma viral loads spanning a median of 8 years were obtained in EC. HIV reservoir, measured by copies per CD4 cell, of total HIV-1 DNA (median 1.4×10^{-4}), 2-LTR circles (median undetectable), and integrated HIV-1 DNA (median 4.0×10^{-6}) were low, consistent with previous data in EC[10]. Among chronic HIV-1, ART duration was 8.5 years.

Presence of plaque was significantly increased in EC compared to HIV- negative controls (78% vs. 42%, $P < 0.05$), and was relatively, but not statistically, increased compared to the prevalence of plaque seen in chronic HIV-1 (78% vs. 60%, $P = 0.28$) (Table). The overall presence of plaque remained significantly increased in EC compared to HIV-negative controls after adjusting for known cardiovascular risk factors, including Framingham point score alone ($P = 0.03$) or Framingham score and use of lipid lowering therapy ($P = 0.04$). The proportion of EC with $>50\%$ stenosis of any coronary vessel (25%) tended to be greater than among chronic HIV-1 (11%) or HIV-negative controls (6%), but did not meet statistical significance due to the small number of EC. The percentage of patients with CAC tended to be higher in EC (Table).

sCD163, sCD14 and CXCL10 were significantly different between groups ($P = 0.0002$, $P = 0.001$ and $P = 0.04$ respectively) and were higher in the EC than the HIV-negative controls (Table). Furthermore, sCD163 was significantly increased in EC compared to the chronic HIV-1. In contrast, hsCRP was not increased in EC. The %CD38+HLA-DR+ CD4+ was increased in both EC and chronic HIV-1 compared to HIV-negative controls ($P < 0.0001$). The %CD38+HLA-DR+ CD8+ tended to be higher in EC, although not statistically significant.

Discussion

This study is the first to investigate coronary atherosclerosis using CTA in a carefully chosen group of art-naïve EC without prior “viral blips”[11]. EC were compared to chronically HIV-1-infected, ART-treated, virologically suppressed patients, and HIV-negative controls, both of similar age.

We demonstrate an unexpectedly high degree of coronary atherosclerosis and elevated markers of immune activation in EC. Interestingly, the degree of atherosclerosis was similar, if not greater, compared to chronic HIV-1 receiving long-term ART with suppressed viremia, and was associated with a high degree of luminal stenosis, giving relevance to the data and emphasizing the added value of CTA beyond other measurements of CVD. This increase in plaque could not be explained by differences in traditional CVD risk factors or

ART exposure, as the EC were ART naïve and CVD risk factors were similar between the groups.

Previous studies have shown that cIMT is higher in EC than in HIV-negative controls and comparable to that observed in chronic ART-treated HIV-1 patients[12]. Furthermore, increased T-cell activation has been associated to cIMT[2] and monocyte activation to noncalcified coronary plaque[1] among well-controlled ART-treated HIV patients. In this study, sCD163, a marker of monocyte/macrophage activation not previously measured in EC, was markedly increased compared to chronically HIV-1 infected, ART-treated patients and HIV-negative controls, underscoring the potential contribution that monocytes/macrophages might play in the observed findings. Furthermore, sCD14 was also elevated, but surprisingly, CRP, a marker of generalized inflammation and CVD[13] was not increased in this group, despite the presence of significant coronary artery disease.

It is possible that sustained prolonged low-level viral replication in EC might directly contribute to endothelial damage[14] or lead to sustained T-cell and monocyte activation, which in turn contribute to increased arterial inflammation[15]. Although all EC in our study had undetectable viral loads over a prolonged period of time, there is residual low-level viremia and persistent cell integrated HIV-1DNA[3, 4, 10], replication competent virus can be isolated, and viral evolution has been demonstrated[16, 17]. Another possibility is that highly effective HIV-specific immune responses in EC[3, 18, 19], while critical to control viral replication, may result in chronic immune activation, accelerating atherosclerosis[20].

Our study included a small number of EC but this group represents < 1% of the total HIV population[21, 22], and we specifically selected a relatively younger group without any cardiovascular history, in whom there was no prior evidence of "viral blips" over a long period of longitudinal follow-up. Our study is preliminary and underpowered to correlate immune activation with the degree of coronary atherosclerosis. Anticipated differences in the %CD38+HLA-DR+ CD8+ between the EC and chronic HIV-1 groups were observed but did not reach statistical significance due to sample sizes.

Taken together, our data suggests that HIV-1 elite controllers, despite excellent virologic and immunologic control and no confounding ART, have significant coronary atherosclerosis. The precise interplay between immune activation, highly effective HIV specific T-cell responses, low level viral replication and CVD need to be fully elucidated in larger studies of EC, in which highly sensitive assays of persistent low level viremia, below standard clinical detection limits, might also be related to atherosclerotic indices.

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Table 1

	HIV-1 Elite Controllers (n=10)	Chronic HIV-1 (n=103)	HIV-negative (n=49)	P-value
Demographics				
Age (yrs)	52.2 ± 5.1 ^{a,b}	49.1 ± 5.0	48.2 ± 4.5	0.06
Gender (% male)	80%	69%	67%	0.71
Duration since HIV Diagnosis (yrs)	17.8 ± 9.1	14.8 ± 6.2	N/A	0.16
Currently on ART (%)	0%	100%	N/A	N/A
Duration of ART (yrs)	0 ± 0	8.5 ± 4.4	N/A	N/A
Percent on PI	0%	55%	N/A	N/A
Duration of PI (yrs)	0 ± 0	4.4 ± 4.5	N/A	N/A
Percent on NRTI	0%	95%	N/A	N/A
Duration of NRTI (yrs)	0 ± 0	8.2 ± 4.5	N/A	N/A
Percent on NNRTI	0%	39%	N/A	N/A
Duration of NNRTI (yrs)	0 ± 0	3.0 ± 4.0	N/A	N/A
CD4+ count (cells/μL)	934 ± 513	571 ± 281	N/A	0.0005
Nadir CD4+ count (cells/μL)	582 ± 372	186 ± 160	N/A	< 0.0001
HIV Viral load (copies/mL)	<48 [<48 , <48]	<50 [<50 , <50]	N/A	0.006[*]
Undetectable VL (%)	100%	100%	N/A	N/A
CMV IgG Ab Test (% positive)	89%	92% ^a	71%	0.004
Cardiovascular Risk Factors				
BMI (kg/m ²)	28.6 ± 5.1	27.1 ± 4.7	27.8 ± 4.8	0.44
Framingham risk score (total points)	10.7 ± 3.5	9.6 ± 3.0	9.0 ± 4.0	0.34
Total cholesterol (mg/dL)	182 ± 50	185 ± 41	182 ± 37	0.85
Triglycerides (mg/dL)	110 ± 66	143 ± 122	111 ± 68	0.18
HDL (mg/dL)	54 ± 15	54 ± 18	51 ± 13	0.55
LDL (mg/dL)	106 ± 44	103 ± 31	109 ± 31	0.55
Fasting Glucose (mg/dL)	92 ± 19	94 ± 29	88 ± 12	0.45
SBP (mm Hg)	119 ± 13	121 ± 14	117 ± 15	0.36
WHR	0.95 ± 0.05	0.95 ± 0.07	0.93 ± 0.07	0.66
HTN (% prevalence)	30%	26%	16%	0.37
Diabetes (% prevalence)	10%	12%	4%	0.26
Statin Use (% prevalence)	10%	21% ^a	6%	0.11
Current Smoker (% prevalence)	40%	42%	41%	0.98
CT Angiography and Coronary Artery Calcium[†]				
Presence of coronary plaque (% prevalence)	78% ^a	60% ^a	42%	0.049
Total Plaque Segments	2.5 [0.3, 5.8]	1 [0, 3]	0 [0, 3]	0.14 [*]

	HIV-1 Elite Controllers (n=10)	Chronic HIV-1 (n=103)	HIV-negative (n=49)	P-value
Calcium Score	16 [0, 96]	0 [0, 16]	0 [0, 30]	0.15 [*]
Calcium Score >0 (% prevalence)	70%	41%	34%	0.11
Non-Calcified Segments	0.5 [0, 2]	0 [0, 2] ^a	0 [0, 1]	0.07 [*]
Mixed Calcified and Non-Calcified Segments	1 [0, 2.8]	0 [0, 1]	0 [0, 2]	0.27 [*]
Calcified Segments	0 [0, 1]	0 [0, 0]	0 [0, 0]	0.27 [*]
Stenosis >50% (% prevalence)	25%	11%	6%	0.35
Markers of Inflammation and Immune Activation				
sCD163 (ng/mL)	2841 [1722, 3427] ^{a,b}	1247 [829, 1883] ^a	847 [624, 1230]	0.0002 [*]
sCD14 (ng/mL)	1530 [499, 1919] ^a	416 [218, 1614] ^a	241 [134, 395]	0.001 [*]
hsIL6 (pg/mL)	1.74 [0.99, 6.33] ^a	1.05 [0.73, 1.65]	0.93 [0.55, 1.57]	0.13 [*]
CXCL10 (pg/mL)	197 [173, 498] ^a	132 [100, 267]	109 [80, 175]	0.04 [*]
hsCRP (mg/L)	0.4 [0.3, 2.4]	1.4 [0.6, 3.9]	1.2 [0.5, 3.1]	0.25 [*]
MCP-1 (pg/mL)	319 [179, 452]	267 [200, 366]	232 [192, 286]	0.25 [*]
%CD14+CD16+ monocytes	31.7 [12.8, 36.1]	18.3 [12.6, 29.9]	16.0 [9.3, 24.4]	0.24 [*]
%CD38+HLA-DR+CD4 cells	0.9 [0.8, 1.7] ^a	1.2 [0.9, 1.9] ^a	0.6 [0.5, 0.7]	< 0.0001 [*]
%CD38+HLA-DR+CD8 cells	4.3 [1.8, 13.1]	1.9 [1.2, 4.2]	3.4 [2.1, 8.5]	0.10 [*]

Mean ± SD for normally distributed parameters

Median [IQR] for non-normally distributed parameters

CXCL10 data were available in 56 participants (9 HIV-1 Elite controllers, 33 Chronic HIV-1, 14 HIV-negative)

^{*} Nonparametric Wilcoxon/Kruskal-Wallis Test

^a $P < 0.05$ vs HIV-negative

^b $P < 0.05$ vs Chronic HIV-1 treated

[†] For one HIV-1 elite controller, only calcium score was measured. Presence of coronary plaque could not be excluded. Measurements of coronary segments with plaque in table were obtained from 9 total HIV-1 elite controllers.