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Increased coronary atherosclerosis and immune activation in HIV-1 elite controllers

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

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 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 antiretroviral therapy (ART). Despite tight control of viral replication, residual low-level viremia in elite controllers is associated with persistent T-cell activation and inflammation [3–5]. In contrast, little is known about monocyte/macrophage activation in elite controllers and direct investigation of coronary atherosclerosis has not been performed in this population.

Methods

Ten HIV-1 elite controllers were identified from the International HIV Controllers Study [6,7]. The International HIV Controllers Study cohort consists of 400 HIV-1 elite controllers, of whom less than half had no history of viral blips and/or ART use. From this cohort, we selected ART-naive patients, aged 40–60 years, 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. Patients underwent assessment of CTA and coronary artery calcium (CAC) [8]. Viral reservoir was measured by cell associated HIV-1 DNA [9,10]. Comparison was made to data previously obtained in nonelite controller, chronic HIV-1 patients, receiving ART, with undetectable HIV viral load ('chronic HIV') and HIV-negative controls [1,8]. Patients in the two comparison groups were similar to the elite controllers 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

Elite controllers were similar in age, sex and traditional CVD risk factors, including smoking and Framingham score, to the two comparison groups (Table 1). Duration of HIV diagnosis was 15 or more years for both elite controllers and chronic HIV-1 patients. Longitudinal plasma viral loads spanning a median of 8 years were obtained in elite controllers. 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 elite controllers [10]. Among chronic HIV-1 patients, ART duration was 8.5 years.

Presence of plaque was significantly increased in elite controllers compared with HIV-negative controls (78 vs. 42%, $P < 0.05$), and was relatively, but not statistically, increased compared with the prevalence of plaque seen in chronic HIV-1 (78 vs. 60%, $P = 0.28$) (Table 1). The overall presence of plaque remained significantly increased in elite controllers compared with HIV-negative controls after adjusting for known cardiovascular

Table 1. Demographic and clinical characteristics.

	HIV-1 elite controllers ($n=10$)	Chronic HIV-1 ($n=103$)	HIV-negative ($n=49$)	*P*-value
Demographics				
Age (years)	52.2 ± 5.1[a,b]	49.1 ± 5.0	48.2 ± 4.5	0.06
Sex (%male)	80%	69%	67%	0.71
Duration since HIV diagnosis (years)	17.8 ± 9.1	14.8 ± 6.2	N/A	0.16
Currently on ART (%)	0%	100%	N/A	N/A
Duration of ART (years)	0 ± 0	8.5 ± 4.4	N/A	N/A
Percentage on PI	0%	55%	N/A	N/A
Duration of PI (years)	0 ± 0	4.4 ± 4.5	N/A	N/A
Percentage on NRTI	0%	95%	N/A	N/A
Duration of NRTI (years)	0 ± 0	8.2 ± 4.5	N/A	N/A
Percentage on NNRTI	0%	39%	N/A	N/A
Duration of NNRTI (years)	0 ± 0	3.0 ± 4.0	N/A	N/A
$CD4^+$ cell count (cells/μl)	934 ± 513	571 ± 281	N/A	0.0005
Nadir $CD4^+$ cell 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 (mmHg)	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*
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*
$\%CD38^+HLA\text{-}DR^+CD4$ cells	0.9 (0.8, 1.7)[a]	1.2 (0.9, 1.9)[a]	0.6 (0.5, 0.7)	< 0.0001*
$\%CD38^+HLA\text{-}DR^+CD8$ 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 nonnormally distributed parameters. CXCL10 data were available in 56 participants (9 HIV-1 Elite controllers, 33 Chronic HIV-1, 14 HIV-negative). ART, antiretroviral therapy; CT, computed tomography; CMV, cytomegalovirus; HTN, hypertension; MCP, monocyte chemoattractant protein-1; NRTI, nucleoside reverse transcriptase inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor.

*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 nine total HIV-1 elite controllers.

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 elite controllers with more than 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 elite controllers. The percentage of patients with CAC tended to be higher in elite controllers (Table 1).

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 elite controllers than the HIV-negative controls (Table 1). Furthermore, sCD163 was significantly increased in elite controllers compared with the chronic HIV-1 group. In contrast, hsCRP was not increased in elite controllers. The %CD38$^+$HLA-DR$^+$ CD4$^+$ was increased in both elite controllers and chronic HIV-1 compared with HIV-negative controls ($P < 0.0001$). The %CD38$^+$HLA-DR$^+$ CD8$^+$ tended to be higher in elite controllers, although not statistically significant.

Discussion

This study is the first to investigate coronary atherosclerosis using CTA in a carefully chosen group of art-naive elite controllers without prior 'viral blips' [11]. Elite controllers were compared with 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 elite controllers. Interestingly, the degree of atherosclerosis was similar, if not greater, compared with 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 elite controllers were ART naive and CVD risk factors were similar between the groups.

Previous studies have shown that cIMT is higher in elite controllers 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 elite controllers, was markedly increased compared with 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 elite controllers 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 elite controllers in our study had undetectable viral loads over a prolonged period of time, there is residual low-level viremia and persistent cell integrated HIV-1 DNA [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 elite controllers [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 elite controllers but elite controllers represent less than 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 elite controllers 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 elite controllers, 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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Conflicts of interest

There are no conflicts of interest.

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Raltegravir central nervous system tolerability in clinical practice: results from a multicenter observational study

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Central nervous system (CNS) symptoms have been reported in clinical trials and case reports in patients receiving raltegravir. We investigated CNS symptoms in 453 HIV-infected patients. Of these 47 (10.4%) developed at least one drug-related CNS symptom. Predictors of CNS symptoms were concomitant therapy with tenofovir or with proton pump inhibitors that can increase raltegravir concentration. Thus, our data suggest a possible correlation between high raltegravir plasma concentrations and CNS symptoms, and therefore their monitoring in clinical practice.

Raltegravir is the first HIV integrase inhibitor available in clinical practice for the treatment of HIV infection in both naive and experienced patients [1–4]. Raltegravir inhibits the strand-transfer step of integration by blocking

the enzyme's active site, and thus the preintegration complex is unable to bind to host DNA [5,6]. The nonintegrated proviral HIV DNA is repaired via normal cellular DNA repair mechanisms and is rendered inactive [7]. In contrast to most other antiretroviral drugs, raltegravir is metabolized by glucuronidation via UGT1A1 [8,9]. Excretion in feces (51%) and in urine (31%) accounts for most of the elimination. No dose adjustment is required for sex, age, hepatic or renal function, or BMI [10]. Raltegravir has been shown to pass the blood–brain barrier in the majority of patients, even if central nervous system (CNS) concentrations exceed the drug concentration needed to inhibit 95% of viral replication for HIV-1 strains without resistance to integrase inhibitors in only 50% of cases [11].

Randomized clinical trials have shown a good safety profile. However, some CNS symptoms have been reported in clinical trials, and case reports of worsening depression and acutely onset insomnia after starting raltegravir have also been described [12,13].

The aim of our study was to further investigate CNS safety of raltegravir in a multicenter observational study. The Surveillance Cohort Long-Term Toxicity of Antiretrovirals (SCOLTA) Project is an online pharmacovigilance program involving 18 Italian infectious disease departments. The Project has an internet site (http://www.cisai.info) in which grade III and IVadverse events, according to Division of AIDS table, are recorded (http://rcc.tech-res-intl.com/tox_tables.htm). The SCOLTA Project currently includes two cohorts: raltegravir and darunavir. Patients undergo follow-up at 6-month intervals, and adverse events are notified when they are clinically observed. Complete data collection and follow-up procedures for the cohorts are described elsewhere [14]. Patients were asked about the onset of CNS symptoms including headache, dizziness, altered dreams, nightmares, insomnia, anxiety, and depression that were prospectively evaluated and recorded in a standardized form.

A total of 453 HIV-infected patients with a mean age of 45.8 ± 9.2 years were enrolled, of these, 302 (66.7%) were male. Mean CD4 cell count was 378 ± 263 cells/μl, and HIV RNA was 3.01 ± 1.57 $\log_{10}$ copies/ml. A total of 176 (38.8%) were in Centre for Disease Control stage C, and 176 (38.8%) had hepatitis C virus coinfection. In 181 (40.0%), a clinical diagnosis of lipodystrophy was also present. At the time of the analysis, the median follow-up was 23 months (interquartile range 13–30), and 371 (81.9%) were still receiving raltegravir. Complete demographic and therapeutic characteristics of the patients are summarized in Table 1.

Therapy interruptions were caused by patient's choice/low adherence in 15 (3.3%) patients, regimen simplification in four (0.9%), virological failure in 13 (2.9%), death in nine (2.0%), and other reasons in nine (2.1%). Adverse event-related interruptions were recorded in 15 (3.3%) patients, and 17 (3.8%) were lost to follow-up.

During follow-up, 47 (10.4%) patients developed at least one drug-related CNS symptom. Among these, 17 (3.8%) referred headache, 15 (3.3%) depression, eight (1.8%) anxiety, seven (1.5%) dizziness, six (1.3%) insomnia, and one (0.2%) altered dreams.

Among interruptions due to adverse events, four were caused by CNS symptoms. These included headache in two cases, psychomotor agitation and suicide attempt in one case.

At univariate analysis, patients with CNS symptoms were receiving tenofovir more frequently (14.2%) in respect of those without (7.8%, $P = 0.03$). Patients receiving proton pump inhibitors (PPIs) also had CNS symptoms more frequently when compared with those without PPI (25.9 versus 9.4%, $P = 0.006$).

At multivariable analysis, after adjustment for age, sex and tenofovir and PPI in turn, the only significant predictors of CNS symptoms were concomitant therapy with tenofovir [odds ratio (OR) 1.9; 95% confidence interval (CI) 1.0–3.5, $P = 0.04$] or with PPI (OR 3.4; 95% CI 1.3–8.8, $P = 0.01$).

To our knowledge, this study includes the largest observational cohort of raltegravir-treated patients. Our results confirm raltegravir safety as evidenced by the low proportion of drug discontinuation due to adverse events. Unexpectedly, CNS symptoms, found in more than 10% of patients, represented the second cause of drug discontinuation following muscle adverse events. Of note, concomitant administration of tenofovir has been associated in both univariate and multivariate analysis with CNS symptoms. In pharmacokinetic studies, tenofovir has been shown to increase raltegravir maximum concentration of drug (Cmax) by 64% and area under the concentration–time curve (AUC) by 49% [15] with an unexplained mechanism. Furthermore, CNS symptoms have also been associated with coadministration of PPI, which have been shown to increase raltegravir Cmax by 415% and AUC by 312%, in the case of omeprazole, in healthy volunteers and in HIV-infected patients, but to a lesser extent [16]. This interaction has been explained by increased intestinal absorption [16].

Efavirenz, a nonnucleoside reverse transcriptase inhibitor, has been associated with the onset of CNS side effect in 20–40% of patients. Efavirenz plasma levels seem to predict persistent CNS symptoms and a dose reduction guided by therapeutic drug monitoring (TDM) has been shown to reduce symptoms without loss of virologic response [17,18]. Studies on raltegravir pharmacokinetic and pharmacodynamic are still ongoing, and no conclusive results are available to date. A recent study

Table 1. Demographic and clinical baseline characteristics of 453 patients enrolled in the raltegravir cohort.

	On raltegravir treatment $n=453$	
	Mean or number or median	SD or % or IQR
Age (years)	45.8	9.2
Sex		
Male	302	66.7
Female	151	33.3
HIV transmission category		
IVDU	166	36.6
Heterosexual/homosexual	244	53.9
Other or unknown	43	9.5
CDC stage		
A	135	29.8
B	142	31.1
C	176	38.8
CD4 cell count (cells/μl)		
<200	126	28.1
200–350	112	25.0
>350	210	46.9
Undetectable HIV viral load	179	39.5
HCV positive	176	38.8
Lipodystrophy	181	40.0
Naive	28	6.2
ART duration before study entry (years)	11.7	6.3–13.9
Concurrent treatment		
NRTI	281	62.0
NNRTI	75	16.6
PI	117	25.8
FI	28	6.2
Anti-CCR5	43	9.5
Total cholesterol	187	59
HDL cholesterol	42	34
Triglycerides	152	106–232
Glucose	93	31
ALT	31	21–56

ALT, alanine aminotransferase; ART, antiretroviral therapy; CDC, Centre for Disease Control; FI, fusion inhibitors; HCV, hepatitis C virus; HDL, high-density lipoprotein; IQR, interquartile range; IVDU, intravenous drug users; NNRTI, nonnucleoside reverse transcriptase inhibitors; NRTI, nucleoside reverse transcriptase inhibitors; PI, protease inhibitors.

has evidenced a very high interpatient and intrapatient variability of raltegravir pharmacokinetics in HIV-infected patients on stable HAART, eventually exposing the patients to drug under exposure and increased risk of virological failure [19]. A population pharmacokinetic analysis has further confirmed a very high interpatient pharmacokinetic variability of raltegravir, suggesting a possible relevant role of TDM in some situations [20]. No genetic polymorphism was found to explain the large raltegravir pharmacokinetic variability, except possibly for UGT1A9*3, which needs further confirmation [20].

A correlation between high raltegravir concentrations and the onset of severe insomnia has been found in three patients [19,21]. Furthermore, recent data have shown that raltegravir is present in cerebrospinal fluid (CSF), and that a significant correlation between CSF and plasma concentration exists [22,23].

Thus, our data suggest a possible correlation between high raltegravir plasma concentrations and CNS symptoms. However, a possible limitation of our study is the lack of drug plasma level quantification, given its observational nature.

In conclusion, our results suggest a careful evaluation of patients with psychiatric diseases prior to starting raltegravir and a continuous monitoring of CNS symptoms in clinical practice in those starting the drug. Attention should also be paid to concomitant drugs that can increase raltegravir concentrations. Although TDM is not currently recommended in clinical practice, it could be useful in the management of patients receiving raltegravir with CNS symptoms, especially in those with limited drug options. Further prospective studies are needed to better clarify risk factors, the role of drug interactions, and the clinical significance of CNS symptoms in patients receiving raltegravir.

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Conflicts of interest

All authors declare no conflicts of interest.

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