Factors associated with a low HIV reservoir in patients with prolonged suppressive antiretroviral therapy

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INTRODUCTION

The persistence of HIV within infected CD4+ T cells during antiretroviral treatment is a major obstacle to eradication. Although most of these resting cells do not produce infectious HIV, a small fraction of these cells contain integrated HIV DNA that is capable of producing virus upon stimulation.

Previous studies demonstrated that reservoirs are established early during infection. Starting antiretroviral therapy during primary infection provides significant benefits to HIV-infected patients in terms of reduction of viral reservoirs. During the chronic phase of HIV infection, the dynamics of the latent reservoir is complex and evolving. The decay of reservoirs is very slow over time on antiretroviral treatment. In the context of chronically infected patients under suppressive antiretroviral therapy, limited data exist on clinical, virologic and immunological characteristics associated with obtaining a low reservoir.

The classic method to measure reservoirs is a quantitative co-culture assay which relies on in vitro reactivation of latently infected resting CD4+ T cells. However this assay has several limitations including intensive labor and high cost. Total HIV DNA may be a potential surrogate for quantifying the reservoir although this marker is likely to overestimate the size of the reservoir as the majority of HIV DNA is defective.

OBJECTIVES

The objective of this study is to determine factors that influence the establishment of a low reservoir in chronically infected long-term treated patients (excluding treatment since acute infection) to a level similar to HIV Elite controllers (<100 HIV Total DNA copies/10^6 cells).

SUBJECTS and METHODS

This cross sectional study involved a subset of patients in Pitié Salpêtrière hospital with sustained virologic suppression (HIV RNA levels <50 copies/ml) after at least 6 months of antiretroviral therapy in whom total DNA measurement was performed.

Total cell-associated HIV-DNA was quantified with a detection limit of 66 copies/10^6 cells. HIV-1 DNA reservoir was considered low when < 100 copies/10^6 cells.

Both univariate (unadjusted) and multivariate logistic regression models were used to search for predictive factors of the DNA outcome (<100 HIV Total DNA copies/10^6 cells). Variables associated with the outcome ($p<0.1$) were considered candidate for the final model. The final multivariate model was built using a stepwise procedure in selecting the variables associated to the outcome.

RESULTS

Overall, 243 subjects with undetectable HIV-1 plasma RNA (<50 copies/ml) were included in the study. The characteristics of patients are presented in table 1. Patient mean age was 47 years and 72% (n=173) were male. A total of 195 (81%) acquired HIV-1 infection by sexual contact (men having sex with men or heterosexual intercourse) and 12 (5%) via intravenous drug use.

Data on HIV-DNA were available in all patients while data on baseline ultrasensitive viral load (VL) were available in 212 of the 243 studied patients. In this population, 61% (130 of 212) of patients had a baseline ultrasensitive VL <1 copy/mL and the median HIV-DNA at D0 was 372 copies/10^6 cells. Fifty-eight patients had a low HIV DNA level < 100 copies/10^6 PBMCs.

Patients' characteristics	**N (%)**	**Median [IQR]**
Age (years)		47 [41-55]
Sex		
Male	173 (72%)	
Female	68 (28%)	
HIV DNA (Log_{10} copies/10^6 cells)		2.571[2.019-2.947]
Nadir CD4 cell count (cells/µL)		216 [107-339]
Plasma HIV-1 RNA zenith (log_{10} copies/mL)		5.028 [4.467- 5.380]
Time since HIV diagnosis in years		12 [6-18]
Duration of antiretroviral treatment in years		9 [4-13]
Duration of undetectable plasma HIV-1 RNA in years (<50 copies/ml)*		3.1 [0.9-5.4]
Study time point		
Ultrasensitive plasma viral load		
20-50 copies/mL	32 (15%)	
1-20 copies/mL	50 (24%)	
<1 copie/mL	130 (61%)	
not performed	31	
Current CD4 cell count (cells/µL)		583 [379-779]
% CD4 T cells		30% [24%-38%]
Current CD8 cell count (cells/µL)		715 [520-941]
% CD8 T cells		38% [31%-47]
Current CD4/CD8 ratio		0.778 [0.531-1.195]

To assess whether clinical, immunological or virologic parameters might be related to obtaining a low reservoir in this population of HAART treated patients, a logistic model was used. In univariate analysis, a low level of proviral DNA was associated with an ultrasensitive VL < 1copy/mL (p=0.0003), a lower HIV-1 RNA zenith (p< 0.0001), a higher CD4 T cells nadir (p=0.0433), a lower current CD8 T cells count (p=0.0256) and a higher current CD4/CD8 ratio (p=0.0024). In addition, such a low reservoir was also associated with a higher time spent with undetectable HIV-1 RNA (p=0.0281). Other factors such as length of time on HAART and duration of HIV-1 infection were not associated with levels of HIV-1 DNA. In a multivariate regression model, a low HIV-1 RNA zenith, absence of residual viremia (<1 copy/mL) and a high CD4/CD8 ratio remain significantly associated with a low HIV reservoir.

	Univariate analysis		**Multivariate analysis**	
Variable associated with a low HIV-1 DNA load (<100 copies/10^6 cells)	OR (95% CI)	P	OR (95% CI)	P
Age (years)	0.98 (0.96-1.02)	0.3864		
Sex (Female vs Male)	2.31 (1.23-4.32)	0.0086		
Nadir CD4 cell count, per 100 cells/µL increase	1.18	0.0433		
Plasma HIV-1 RNA zenith, per 1 log_{10} copies/mL increase	0.40 (0.27-0.57)	<0.0001	0.42 (0.26-0.64)	0.0001
Time since HIV diagnosis (years)	1.00 (0.96-1.04)	0.9858		
Duration of antiretroviral treatment (years)	1.00 (0.94-1.06)	0.9923		
Duration of undetectable HIV-1 RNA (<50 copies/ml) per 1 year increase	1.09 (1.00- 1.20)	0.0281		
Study time point				
Ultrasensitive plasma viral load <1 copy/ml	4.57 (2.19-11.05)	0.0003	2.97 (1.21-8.12)	0.0228
Current CD4 cell count, per 100 cells/µL increase	1.02 (0.92-1.13)	0.725		
% CD4 T cells	1.04 (1.01-1.08)	0.0086		
Current CD8 cell count, per 100 cells/µL increase	0.90 (0.82-0.98)	0.0256		
% CD8 T cells	0.96 (0.93-0.98)	0.0043		
Current CD4/CD8 ratio	2.33 (1.36-4.09)	0.0024	2.31 (1.12-4.80)	0.0227

DISCUSSION

Obtaining a low HIV DNA level, which reflects a limited pool of infected cells is associated with a low HIV RNA zenith and a high CD4/CD8 ratio, reinforcing the need to institute antiretroviral treatment early during infection that allows to get a better recovery of CD4/CD8 ratio. In addition, our results reinforce the idea that a low reservoir may be related to stronger control of residual viremia that in turn keeps the immune activation system to a low activation status. Strategies aiming at controlling residual viremia should be further investigated to contribute in reducing the size of HIV reservoir.

This study helps to define factors associated with low proviral DNA set points after long-term treatment and should be useful to identify future candidates in the context of HIV eradication.

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