

An Intermediate Subset of Effector-Memory CD4 T Cells is a Major Reservoir of HIV in Long Term Elite and Viremic Controllers.

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Background. The long term steady state established in Long-Term non Progressors (LTNP) between host and virus is characterized by low HIV RNA and HIV DNA levels and high specific immune responses. It represents a model to investigate distribution and stability of the HIV reservoir and the impact of CD4-T cell differentiation on the long-term control of those HIV reservoirs.

Methods. Twelve untreated LTNP HIV-seropositive for >8 years, CD4 T cells > 600/μl, selected for HIV RNA < 200 cp/ml in 7 cases and <3000 cp/ml in 5 cases defining Elite and Viremic controllers respectively, were included. Live resting CD4+ peripheral blood mononuclear cells (PBMC) were sorted according to anti-CD45RA, CD27 and CCR7 combinations in various Memory subsets from Central (TCM) to Effector-Memory (TEM), Effector (E) and Naïve (N) T cells. Ultrasensitive HIV-DNA quantification was performed on the different sorted subsets and their ability to produce virions was tested by 6 days *in vitro* activation with anti-CD3+CD28 and IL-2. Comparisons were performed with Mann-Whitney test.

Results. The median HIV DNA in the 12 LTNP was 1.74 log cp/million PBMC and was distributed among resting CD4-T cells according to a constant hierarchy: an intermediate TEM subset contained median values of 3.02 log cp/million intermediate TEM, followed by TCM (2.85 log), TEM (2.27 log), E (1.98 log) and Naïve CD4-T cells (1.71 log). This distribution was highly stable and maintained over 10 years. The intermediate TEM subset contributed significantly more to this reservoir than the other subsets (median: 47% and p<0,001 compared to all subsets). Elite differed from Viremic Controllers by a significantly lower infection level of this intermediate population (p=0.035), independently of the genetic HLA or CCR5 background. *In vitro* activation of these sorted subsets induced HIV replication from the 3 different memory CD4 T cell subsets, independently of their replicative capacity, but neither from Naïve nor from Effector T cells.

Conclusions. In LTNP with stable long-term control of HIV, TCM are not the predominant reservoir of HIV but instead an intermediate TEM subset. The concentration of the HIV reservoir can vary by 10 to 100 fold depending on the subset maturation. Altogether our results demonstrate that the memory peripheral blood CD4-T cells in these LTNP contain a stable inducible reservoir with low production rate and distinct mechanisms of control of HIV dictated by T cell maturation.

INTRODUCTION

The long term steady state established between host and virus in ELITE and VIREMIC controllers, altogether included in Long-Term non Progressors (LTNP), is characterized by low HIV RNA and DNA levels, normal CD4 counts and high specific immune responses. It has been reported in viremic individuals that a higher infection level is observed in Memory CD4 T cells (CD45RO+ 57+) (Brenchley et al).

In order to further understand mechanisms of the equilibrium and long term control of the virus, we have investigated whether the latent and inducible HIV reservoir in circulating CD4-T cells from those LTNP is influenced by their differentiation status and tested the *in vitro* inducibility of the HIV reservoir. So we proposed here a study of the relationship between HIV and CD4-T cells in reservoir distribution paying attention of their position along the differentiation pathway and homeostasis.

PATIENTS

The ANRS CO15 cohort enrolled 71 LTNP patients defined by an asymptomatic HIV infection for at least 8 years, no antiretroviral therapy and a CD4 cell count ≥ 600 cells/mm³.

We selected patients from this cohort defined in 2 groups of LTNP :

The **ELITE controllers** : 7 patients with HIV-RNA < 200 copies/mL

The **VIREMIC controllers** : 5 patients with 200<HIV-RNA<3000 copies/mL.

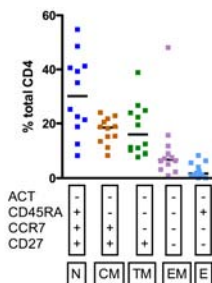
METHODS

Cell sorting: Live resting CD4+ (25-69-HLA-DR-) PBMC were sorted according to anti-CD45RA, CD27 and CCR7 combinations in various Memory subsets from Central (TCM) to Effector-Memory (TEM) through a Transitional subset of Effector Memory (TMT), Effector (E) and Naïve (N) T cells. Experiments were performed in Inter IFR-UPMC Flow Cytometry Platform,

Virology: Ultrasensitive HIV-DNA quantification was performed on the different sorted subsets and their ability to produce virions was tested by 6 days *in vitro* activation with anti-CD3+CD28 and IL-2. Comparisons were performed with Mann-Whitney test.

Peripheral blood CD4									
Group and patient no.	time	HLA-B	CCR5 Δ32	%	(cells/ mm ³)	HIV-1 DNA	HIV-1 RNA		
						log ₁₀ (cps/ 10 ⁶ PBMC)	log ₁₀ (cps/ml)		
Elite Controllers									
02009	2004	51	57	+	870	1,11	1,96		
	2008	28	19		505	NT	2,23		
04030	1999	5	12	-	35	1011	1,4	2,19	
04046	1999	12	14	-	47	690	1,83	2,05	
08005	1995	8	35	-	31	790	1,93	1,78	
08011	1998	27	27	-	28	655	1,79	1,72	
11020	2000	14	57	-	43	1505	1,08	<1,6	
11024	1998	14	40	-	41	828	0,77	<2,3	
Viremic Controllers									
02002	1998	21	57	-	43	1895	2	2,92	
	2008				30	1047	2,45	2,95	
04002	1996	7	22	-	42	818	1,68	2,32	
04036	1998	5	8	+	34	392	2,07	3,03	
04054	1998	14	27	-	36	718	1,52	1,48	
	2004				42	473	NT	2,31	
09015	2008	7	57	+	17	317	2,44	2,97	

CD4 T cell subpopulation distributions among resting CD4 T cells in ELITE and VIREMIC controllers

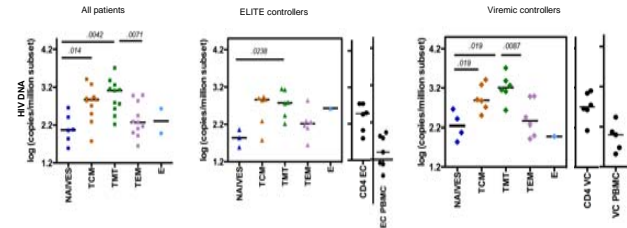


No significant difference in the CD4 cell subset repartition was observed between ELITE and VIREMIC controllers.

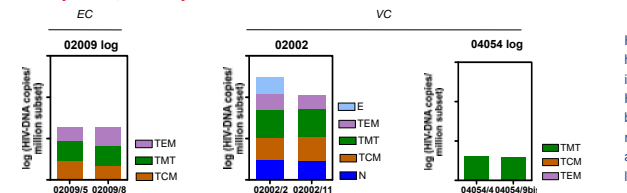
RESULTS

A. Impact of CD4-T cell differentiation on the distribution of the "latent" HIV reservoir

Infection levels of resting CD4-T cells subsets in ELITE and VIREMIC controllers

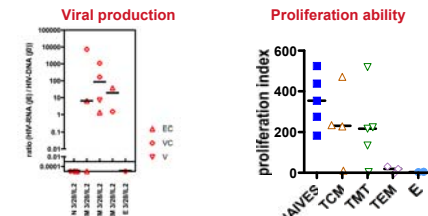


Stability over 4, 6 and 10 years of ELITE & VIREMIC controllers reservoirs



Because TMT are frequent and highly infected, they constitute a major HIV reservoir in Long Term ELITE and VIREMIC controllers.

B - Inducible HIV reservoir



After 6 days of *in vitro* stimulation with anti CD3/CD28 and IL2,

- Virus production is observed from :TCM, TMT and TEM which are able to produce the same amount of virus, even if they are infected at different levels (and have different proliferation capacity).
- No viral production was found in N and in E in those culture conditions while N are as infected as TEM.

TCM and TMT are the most infected CD4-T cells in ELITE and VIREMIC controllers compared to others resting subsets.

No significative difference of HIV reservoir distribution was observed according to the host genetic background (HLA B27, HLA B57, HLA no B27/B57).

HIV Reservoir in one ELITE controller patient has been evaluated at 4 years apart. Repartition is conserved over this time. HIV reservoir in two VIREMIC controllers has been evaluated at 6 and 10 years apart, respectively. Repartition is maintained over time although some significant variations of the viral load were observed.

CONCLUSIONS

In stable long-term control of HIV, Transitional TEM represent an important viral reservoir. The CD4 subsets maturation influence both the HIV reservoir concentration that can vary by 10 to 100 fold. Such cell distribution of the reservoir is stable over 10 years and independent of CCR5 expression level and of genetic background. HIV production can be induced from this latent reservoir only in memory T cells (TCM, TMT, TEM). Lack of HIV production after N and E stimulation appears to be independent of proliferation capacity and might reflect a really low level of infection (Naïve) or apoptosis in Effector CD4-T cells. Altogether our results demonstrate that the peripheral blood memory CD4-T cells in these LTNP contain a stable inducible reservoir with low production rate and distinct mechanisms of control of HIV dictated by T cell maturation. Understanding the mechanisms by which CD4 T cells maturation/homeostasis influence maintenance of the HIV reservoirs should provide clues to progress toward exhaustion of the HIV reservoirs.