CD8 RECOGNITION OF AUTOLOGOUS VIRUS IN HIV-ELITE CONTROLLERS DESPITE ESCAPE MUTATIONS

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I. Background

- Escape mutations in HIV proviral sequence coding for epitopes of CD8 T cells response have been proposed as one of the reasons for the failure of the shock and kill strategy to purge the HIV reservoir.
- We have previously shown the existence of mutations in CD8 epitopes in the viral reservoir even in patients with spontaneous control of HIV (elite controllers).
- Herein, we have analysed the ability of HIV-specific CD8 response to recognize autologous epitopes from the viral reservoir, in two groups of HIV patients with different HIV replication control (spontaneously or through cART) and in a group of patients with uncontrolled HIV replication.

II. Methods

- Thirty HIV+ patients were included: 10 elite controllers (EC), 10 cART-suppressed (TX) and 10 cART-naive with high levels of HIV plasma viremia (TP) (**Table 1**).
- For each patient, the sequences of HIV-Gag CD8 epitopes restricted by the patient HLA-I haplotype were obtained from the proviral HIV-DNA of CD4 resting memory cells.
- CD8 T cells ability to respond to autologous Gag epitopes was examined using a boosted flow cytometry assay including IFNɣ, MIP1β and TNFα production (**Figure 1)**
- Inter and intra-group comparisons were performed using non-parametric tests.

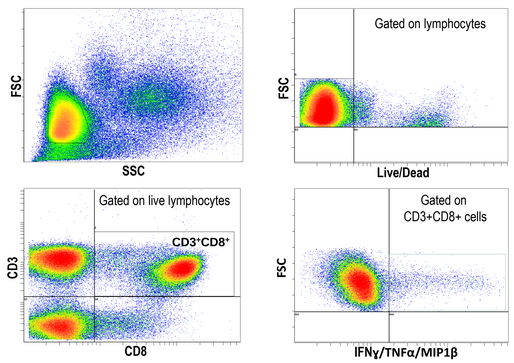

Figure 1. Representative example of flow cytometry data and gating strategy to measure HIV-specific CD8 response. An initial gating was applied using forward (FSC) and side (SSC) scatter, and staining with Live/dead was used to exclude dead cells from further analysis. From the population of live lymphocytes, a gate was placed to select CD3+CD8+ T cells and the simultaneous production of IFNɣ and/or TNFα and/or MIP1β cytokines was analyzed on these cells.

III. Results

Table 1. Characteristics of patients included in the study

Characteristic	Study Group			p-value[1]	p-value[2]	p-value[3]	p-value[4]
	EC	TX	TP				
n	10*	10	10	-	-	-	-
% Male	57.1%	90%	90%	0.156	0.116	0.116	1
Age (years)	42 [32-46]	47 [44-51]	37 [30-44]	0.031	0.07	0.591	0.012
CD4 counts (cells/µL)	744 [696-957]	988 [585-1469]	599 [518-832]	0.167	0.526	0.172	0.084
Time since HIV diagnosis (years)	5 [4-12]	13 [11-16]	4.5 [2-5]	0.00	0.01	0.20	0.00
Lenght of treatment (years)	NA	12 [9-16]	NA	NA	NA	NA	NA
Lenght of EC status (years)	5 [2-7]	NA	NA	NA	NA	NA	NA
Viral load (HIV-RNA copies/mL)	<50	<50	61607 [28717-81635]	NA	NA	NA	NA
Number of autologous epitopes	7 [4-7]	10 [6-14]	8 [7-10]	0.19	0.10	0.13	0.62
Prevalence (%) of mutated epitopes	86 [50-100]	57 [48-82]	64 [50-71]	0.52	0.30	0.32	1

Data are given as median [IQR], except sex, expressed as %; p-value1: comparison between three study groups (EC, TX and TP; Kruskal Wallis test); p-value2: comparison between EC and TX groups (Mann Whitney test); p-value3: comparison between EC and TP groups (Mann Whitney test); p-value4: comparison between TX and TP groups (Mann Whitney test). Sex comparisons evaluated with $\chi 2$ test. *Amplification of proviral HIV-DNA was unsuccessful in 3 EC patients. NA : not apply.

- The number of autologous HIV-Gag CD8 epitopes (either wild-type or mutated) that were used in the stimulation assays was similar between groups of patients (**Figure 2**). Also, the number of wild-type and the number of mutated epitopes was similar in each study group. The prevalence (%) of mutated HIV-Gag CD8 epitopes was high and similar between groups of patients (**Figure 3**). All EC patients presented HIV-specific T CD8 response against at least 1 autologous epitope from their reservoir, while the prevalence of this response in TX and TP groups was lower (**Figure 4**).

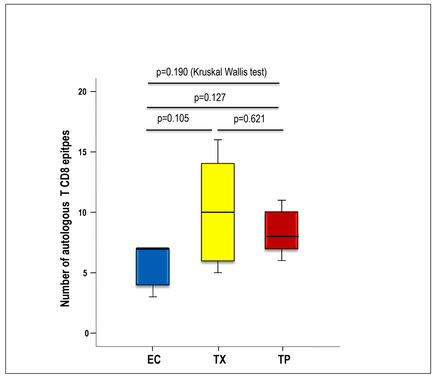

Figure 2. Box-plots graphs showing the number of total autologous HIV-Gag CD8 epitopes tested in each study group. p-values for the comparisons (Mann-Whitney U test and Kruskal Wallis test) are shown.

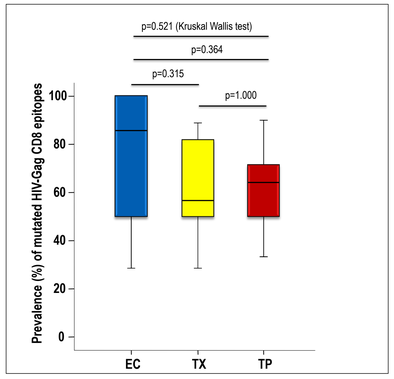

Figure 3. Box-plots graphs showing prevalence (%) of mutated HIV-Gag CD8 epitopes in autologous sequences obtained from the viral reservoir of each patient included in the study. p-values for the comparison (Mann-Whitney U test and Kruskal Wallis test) are shown.

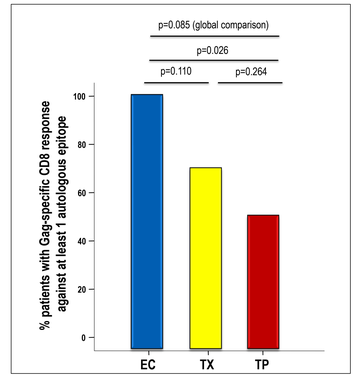

Figure 4. Bar graphs showing % of patients in each study group showing HIV-Gag CD8 response against at least one autologous epitope from the viral reservoir. p-values for the comparison (Chi-square test) are shown.

- The proportion of HIV-Gag autologous epitopes that elicited a CD8 response was higher in EC compared to TX and TP patients (**Figure 5A**).
- Moreover, in all the study groups there were no differences in the proportion of HIV-Gag autologous epitopes eliciting a CD8 response when comparing wild-type and mutated epitopes (**Figure 5B**).
- Level of HIV-Gag CD8 response was higher in EC patients compared to TX patients and similar to TP patients (**Figure 6A**). In each of the study groups the level of HIV-Gag CD8 response was similar when comparing wild-type and mutated epitopes (**Figure 6B**).

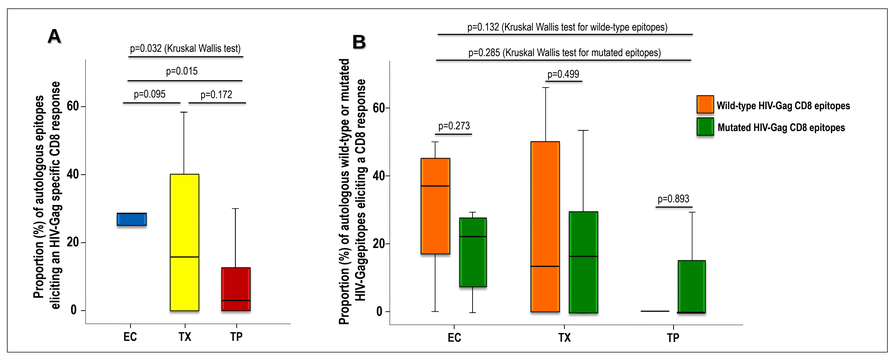

Figure 5. Box-plots graphs showing the proportion (%) of autologous HIV-Gag epitopes that elicited a CD8 response (**A**) and the proportion of mutated or wilde-type epitopes eliciting a CD8 response (**B**) in each study group. p-values for the comparisons (Mann-Whitney U test, Kruskal Wallis test and Wilcoxon signed-rank test) are shown.

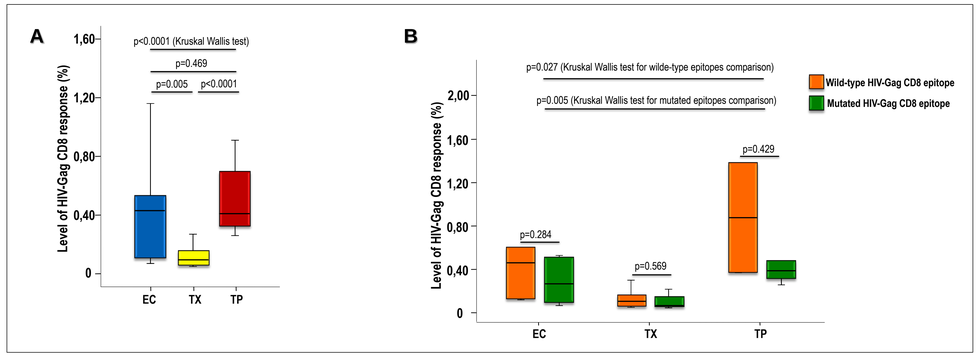

Figure 6. Box-plots showing the level of HIV-Gag CD8 response elicited by autologous epitopes (**A**) and the level of HIV-Gag CD8 response elicited by mutated or wilde-type epitopes (**B**). p-values for the comparisons (Mann-Whitney U test, Kruskal Wallis test and Wilcoxon signed-rank test) are shown.

IV. Funding / Acknowledgements

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V. Conclusions

- Our results show that EC patients have an increased ability to recognize the autologous HIV viruses present in the viral reservoir despite the high prevalence of mutation in these epitopes, suggesting that the CD8 response of these patients could potentially be able to kill latently infected cells after reactivation with latency reversing agents.
- Further studies analyzing functional parameters of this CD8 response to autologous provirus are needed to ascertain this hypothesis.