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CD8 RESPONSE TO HIV PROVIRUS IN ELITE CONTROLLERS IMPROVES EXPECTATIONS OF HIV CURE

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I. Background

- The existence of escape mutations in epitopes from the viral reservoir of HIV patients recognized by CD8 T cells poses a great challenge to viral eradication.
- A broad and efficient CD8 T cells response is associated with HIV control as demonstrated by studies in HIV elite controllers. However, mutations in epitopes recognized by CD8 T cells are found accumulated in the viral reservoir, even in HIV elite controllers patients.
- Herein, we have analysed the functionality profile of CD8 T cells response against autologous epitopes from the viral reservoir in two groups of HIV infected patients with different mechanism of viral control (spontaneously or through cART).

II. Methods

- Fourteen HIV⁺ patients were included: 7 elite controllers (EC) and 7 cART-suppressed (TX) (**Table 1**).
- For each patient, the sequences of HLA-I-restricted HIV-Gag CD8 T cells epitopes were obtained from the proviral HIV-DNA of CD4 resting memory cells.
- Functional profile, lytic ability, maturation stage, level of exhaustion, level of activation and homing potential of CD8 T cells recognizing autologous HIV-Gag epitopes from HIV reservoir were assessed by spectral flow cytometry (**Figure 1**).
- Inter-group differences were tested by non-parametric tests. A canonical discriminant analysis (CDA) was carried out to find those HIV-specific CD8 T cells features that better discriminate between studied groups.

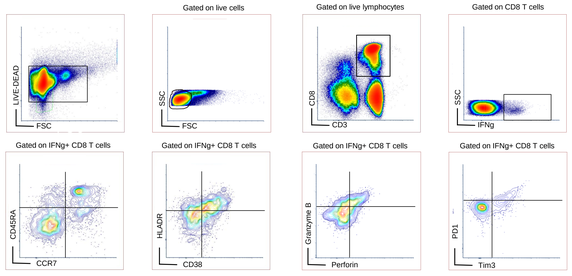

Figure 1. Representative example of flow cytometry data and gating strategy to measure profile of HIV-specific CD8 response. An initial gating was applied using forward (FSC) and Live/dead to exclude dead cells from further analysis. Then FSC and SSC were used to gate on lymphocytes. From the population of live lymphocytes, a gate was placed to select CD3⁺CD8⁺ T cells and the production of IFNɣ, TNFα, MIP1β and IL-2 cytokines was analyzed on these cells (an example showing production of IFNg is shown). Finally, the different phenotypic markers (CD45RA/CCR7=maturation; HLADR/CD38=activation; perforin and granzymeB=cytolisis-associated molecules, and Tim3 and pD1=exhaustion markers) were analysed on HIV-specific CD8 T-cells.

III. Results

Table 1. Characteristics of patients included in the study

Characteristic	Study Group		p-value
	EC	TX	
n	7	7	-
Sex (% male)	57%	86%	0.237
Age (years)	42 [32-46]	47 [45-51]	0.073
CD4 counts (cells/μL)	744 [696-957]	612 [546-1064]	0.609
Time since HIV diagnosis (years)	5 [4-12]	13 [11-17]	0.012
Lenght of treatment (years)	NA	12 [9-16]	NA
Lenght of EC status (years)	5[2-7]	NA	NA
Number of autologous epitopes tested	2 [1-2]	2 [1-3]	0.678
Prevalence (%) of mutated epitopes	100 [0-100]	50 [0-100]	0.637

Data are given as median [IQR], except sex, expressed as %; p-value: comparison between EC and TX groups (Mann Whitney test). Sex comparison evaluated with $\chi 2$ test. NA: not apply.

- Compared to TX group, CD8 T cells recognizing autologous HIV-Gag epitopes (either wild-type or mutated) from EC patients showed higher levels of effector memory cells (CD45RA⁻CCR7⁻ cells) (p=0.03) (**Figure 2A**) and lower levels of exhausted (PD1⁺Tim3⁺) cells (p=0.08) (**Figure 3A**). These differences between TX and EC patients were mainly observed for CD8 T cells responding to mutated epitopes (p=0.050, **Figure 2B** and p=0.050, **Figure 3B**).

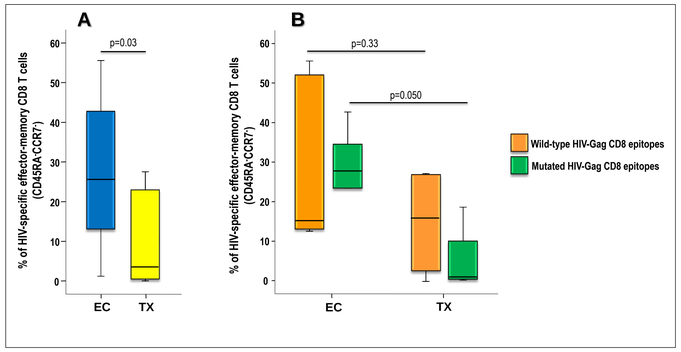

Figure 2. Box-plot graphs showing the level of effector memory phenotype among CD8 T cells responding to HIV-Gag autologous epitopes (**A**), and according to the type of HIV-Gag epitope (wild-type or mutated) (**B**). p-values for the comparisons (Mann-Whitney U test) are shown.

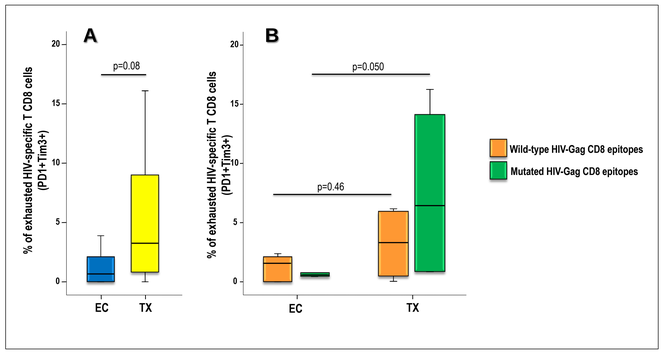

Figure 3. Box-plot graphs showing the level of exhausted cells among CD8 T cells responding to HIV-Gag autologous epitopes (**A**), and according to the type of HIV-Gag epitope (wild-type or mutated) (**B**). p-values for the comparisons (Mann-Whitney U test) are shown.

- Compared to TX group, the CD8 T cells recognizing wilde-type autologous HIV-Gag epitopes from EC patients showed higher levels of the cytolisis-associated molecule perforin (11.11 [9.43 - 20.09]% in EC and 5.19 [3.10 - 14.59]% in TX) (p=0.09) (**Figure 4**).
- The majority of HIV-Gag CD8 T cells response was mediated by monofunctional cells (cells producing only one cytokine) in both groups of patients. However, MIP1β was the cytokine with the highest contribution to the CD8 response in EC patients, and and IFNɣ was the cytokine with the highest contribution in TX patients (**Figure 5**).
- The canonical discriminant analysis (CDA) model was able to clearly discriminate between EC and TX groups, being exhaustion (PD1⁺Tim3⁺) of HIV-Gag specific CD8 T cells the feature with the highest relevance in the discriminant model (coefficient=1.882, p<0.001) (**Figure 6**).

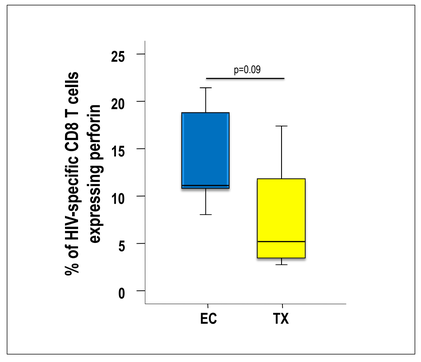

Figure 4. Box-plot graph showing the level of expression for perforin by CD8 T cells responding to wilde-type autologous HIV-Gag epitopes. p-value for the comparison (Mann-Whitney U test) is shown.

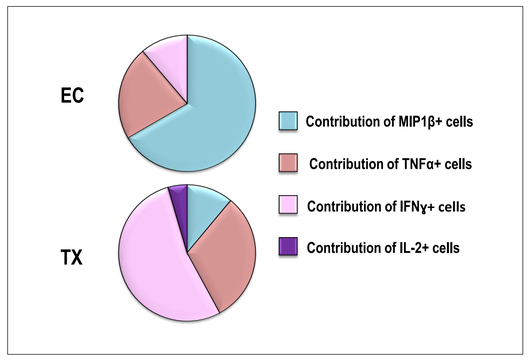

Figure 5. Sector graphs showing the functional profile of autologous HIV-Gag CD8 T cells response. Contribution to the CD8 response by cells producing each of the cytokines tested (MIP1β, TNFα, IFNɣ and IL2) .

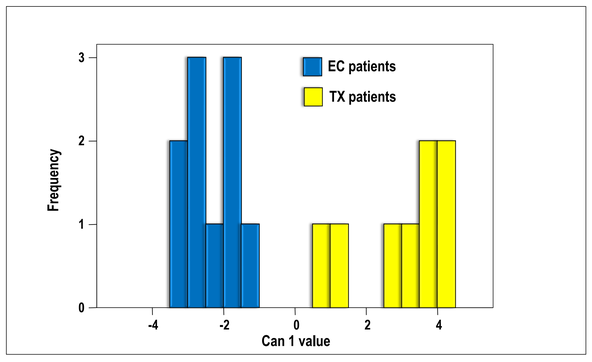

Figure 6. Canonical discriminant analysis comparing the profile of CD8 T cells responding to HIV-Gag epitopes, between elite controller -EC- (blue bars) and cART-suppressed -TX- (yellow bars) patients.

IV. Funding / Acknowledgements

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V. Conclusions

- Our results show that the functional profile of CD8 T cells against autologous HIV virus can distinguish between elite controllers and non-controllers patients.
- Importantly, low levels of exhaustion of virus-specific CD8 T cells and production of MIP1β (the natural ligand of HIV-coreceptor CXCR5 with anti-viral properties) were among the most important factors associated to the EC status.
- Taken together, these results suggest that HIV-specific CD8 response in EC patients could be able to kill latently infected cells after reactivation with latency reversing agents, which open a new opportunity to HIV eradication.