Transcriptional signatures of CD4 T cell Subsets and Control of HIV Reservoirs in Elite Controllers

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Introduction. Understanding the molecular mechanisms associated with the exceptional quasi-equilibrium between HIV reservoirs and host cells in the model of functional cure represented by Elite Long-Term Nonprogressors (E-LTNPs) should provide clues for developing strategies to purge the HIV reservoirs.

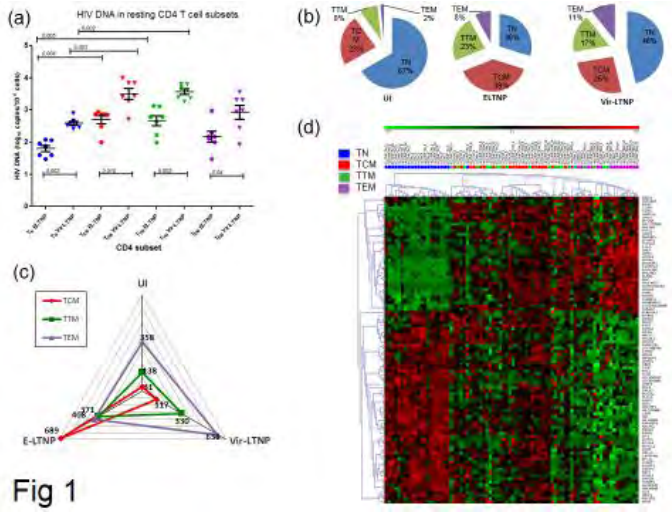


Fig 1. Characteristics of the main CD4 T-cell subsets in HIV infected LTNPs and uninfected individuals

(a) Distribution of the HIV reservoirs in the patient study groups. Total cell HIV DNA was quantified in sorted resting CD25-CD69-HLA-DR- CD4 T cell subsets (naive [T_N: CD45RA+CCR7+CD27+] and memory subsets: [T_{CM}: CD45RA–CCR7+CD27+], [T_{TM}: CD45RA–CCR7–CD27+], [T_{EM}: CD45RA–CCR7–CD27–]) by ultrasensitive real-time PCR. E-LTNPs, elite long-term non progressors; Vir-LTNPs, viremic long-term nonprogressors **(b) Relative frequency of each resting CD4 T-cell subset in percentages of the global resting CD4 T cell pool** in each group as determined by flow cytometry. **(c) Number of differentially expressed genes between T_{CM}, T_{TM}, and T_{EM} resting CD4 T cells** from UI, E-LTNPs and vir-LTNPs compared to T_N from UI (UI-T_N), with an FDR threshold <0.05. **(d) Heatmap and** Kendalls-Tau hierarchical **clustering analysis** of the expression intensities of 98 genes differentially expressed by T_N, T_{CM}, T_{TM}, T_{EM} resting CD4 T cells, regardless of the patient's status (UI, E-LTNP or vir-LTNP).

Methods. We compared the whole-genome transcriptional profiling of the 4 major resting (CD25-CD69-DR-) CD4 T cell subsets (naïve [TN], central-memory [TCM], transitional-memory [TM], effector-memory [TEM]) sorted from PBMCs of 14 HIV-1 infected individuals including 7 E-LTNPs. The HIV-1 cellular DNA and mRNA levels were quantified in parallel in each sorted subset.

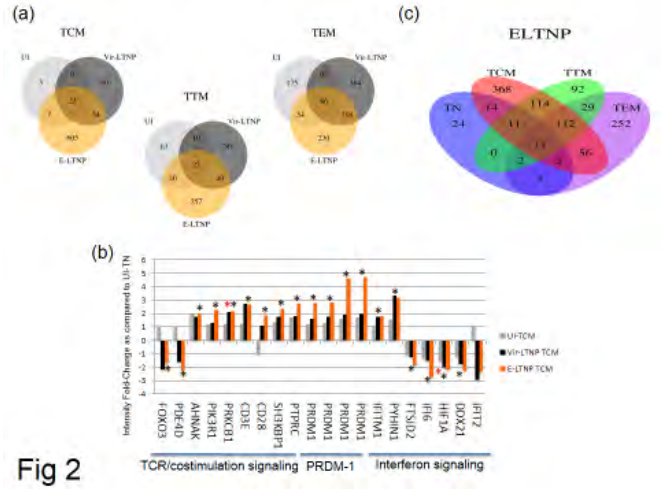


Fig.2. Transcriptional signatures of memory CD4 T cells in elite controllers

(a) Venn diagrams of common differentially expressed genes across all groups of subjects in each memory CD4 T cell subset as compared to UI-T_N. Numbers represent the number of differentially expressed genes in each CD4 T cell subset compared to UI-T_N, with a threshold of FDR<0.05. **(b) Transcriptional signatures of central memory CD4 T cells from elite controllers.** Histograms represent the fold changes related to the comparison of the mean expression values of the corresponding genes in central memory CD4 T cells from elite controllers (E-LTNP T_{CM}) versus naive CD4 T cells from uninfected individuals (UI-T_N).*FDR<0.05 **(c) Venn diagram of common differentially expressed genes in the following comparisons:** E-LTNP T_N versus UI-T_N, E-LTNP T_{CM} vs UI-T_N, E-LTNP T_{TM} vs UI-T_N, E-LTNP -T_{EM} vs UI-T_N

Results Host gene transcriptomes followed subset differentiation and viremia except in E-LTNPs where T_{CM}, the main CD4 compartment, showed the highest activity with 3 specific signatures involving:
(i) overexpression of TCR and co-stimulation signaling pathways,
(ii) overexpression of the PRDM-1/Blimp-1 transcriptional repressor and
(iii) down-modulation of type-I IFN-related genes.
Among subsets, the PRDM1/Blimp-1 upregulation was associated with lower levels of both cellular HIV-DNA and HIV mRNA levels.

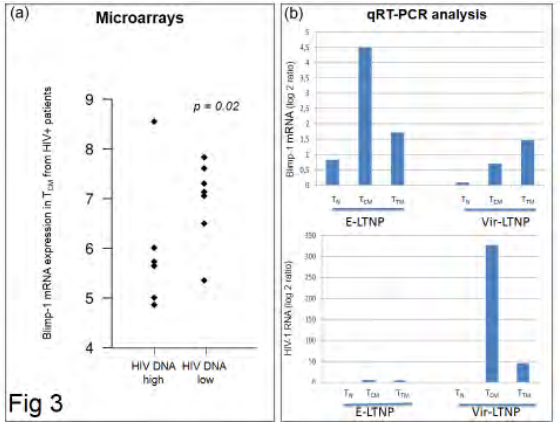


Figure 3. PRDM1 overexpression is associated with low cellular HIV DNA levels and HIV RNA transcription in memory CD4 T cells from elite controllers (E-LTNPs) and viremic long-term nonprogressors (vir-LTNPs).

(a) Inverse association between PRDM-1 expression intensity as determined by microarray and cellular HIV DNA level in T_{CM} of HIV-infected patients. HIV patients were separated into 2 groups according to the HIV DNA level in T_{CM} and PRDM1 expression, below and at or above the median HIV DNA level and the median PRDM1 expression in T_{CM}. A Fisher's exact test was applied (p=0.02). **(b) RT-PCR quantification of PRDM-1 mRNA transcript and HIV RNA transcripts** in pooled sorted CD4 T-cell subsets from E-LTNPs and vir-LTNPs.

Conclusions This unique Blimp-1 transcriptional repressor signature and the contrast between host and virus transcriptional activities in T_{CM} from ECs suggest Blimp-1 might be involved in controlling the HIV reservoirs in the key T_{CM} subset.