ART-Treated Subjects with Low Viral Reservoir Show Unusual HIV Latency Distribution

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BACKGROUND

Small-size viral reservoirs are predominantly found in HIV-1 controllers and individuals treated during acute/early HIV-1 infection. However, other HIV$^+$ subjects could naturally also harbor low viral reservoirs. We have previously established a cohort of 'Low Viral Reservoir Treated' subjects (LoViReT), which represent about 9% of individuals on ART. Here, we aim to study the mechanisms that cause these unusual low reservoir.

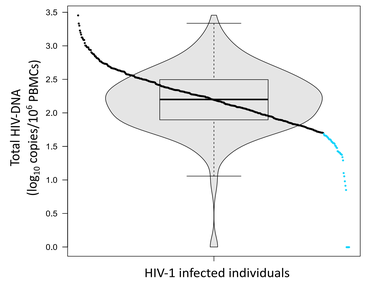

Figure 1. Total HIV-1 DNA distribution of 453 individuals of a previous phase of this study. In light blue are marked those subjects considered as LoViReT, which have <50 copies/10^6 PBMCs.

METHODS

- Subjects: 42 HIV$^+$ individuals under cART and <50 HIV-DNA copies/10^6 PBMCS constitutes the LoViReT cohort. 12 LoViReT subjects treated in the chronic phase of infection (>6 months after infection) with <50 HIV-DNA copies/10^6 PBMCs were compared with 13 chronic controls >50 HIV-DNA copies/10^6 PBMCs). Also, 14 LoViReTs were selected to comprehensively analize the viral persistence in blood and tissues. Individuals were selected from Hospital Universitari Germans Trias i Pujol and Hospital Clinic.

- Proviral reservoir: Total HIV-DNA was longitudinally measured in peripheral PBMCs, CD4$^+$ T cells, CD45$^+$ (rectal biopsies) and/or CD45RA$^-$ (lymph node biopsies) by droplet digital PCR amplifying Gag and/or LTR regions. The *RPP30* gene was quantified in parallel to normalize sample input.

- Replication-competent reservoir: Leukaphereses were obtained in 14 LoViReT to measure the number of infectious units per million (IUPM) in 38 million CD4$^+$ T cells with a detection limit of 0.0185 IUPM.

- Residual viremia: 9 ml of plasma were ultracentrifuged and HIV-RNA quantified using Abbott VL platform.

- Isolation of cellular subsets and flow cytometry: At least 150 million PBMCs were stained with monoclonal antibodies against CD4, CD3, CD45RA and CD27. CD45RA$^+$, CCR7$^+$, CD27$^+$ naive CD4$^+$ T cells; CD45RA$^-$, CCR7$^+$, CD27$^+$ central memory CD4$^+$ T cells; CD45RA$^-$, CCR7$^-$, CD27$^-$ effector memory CD4$^+$ T cell; CD45RA$^-$, CCR7$^-$, CD27$^+$ transitional memory CD4$^+$ T cells and CD45RA$^+$, CCR7$^-$, CD27$^-$ terminally differentiated CD4$^+$ T cells were live-sorted by FACS Aria.

RESULTS

1.- Longitudinal analysis of total HIV-DNA

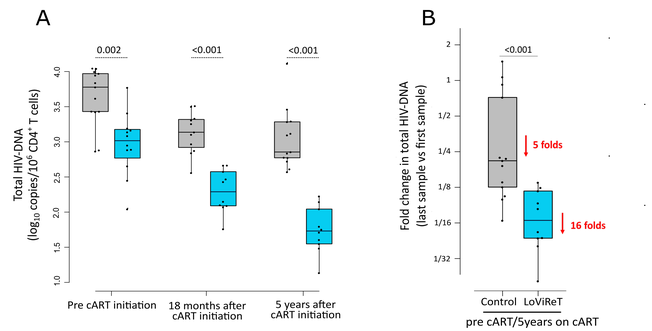

Figure 2. Longitudinal measure of total HIV-DNA in CD4$^+$ T cells by ddPCR. (**A**) Box plot of total HIV-DNA separate in pre-treatment intiation, 18 months and 5 years after treatment initation. (**B**) Fold change decay between the sample before treatment initiation and 5 years on cART.

2.-Viral persistence in LoViReT subjects

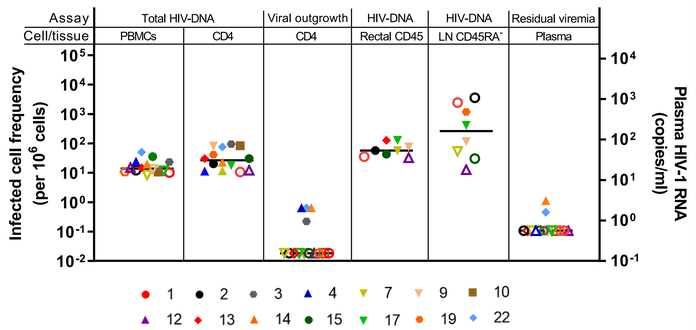

Figure 3. HIV-1 persistence performed on the indicated cell or tissue obtained from LoViReT subjects. HIV-DNA in rectal CD45$^+$ and lymph node (LN) CD45RA$^-$ were only assessed in LoViReTs with an IUPM <0.1. Median values are indicated by an horizontal black line. Open symbols represent undetectable values. In those cases, the limit of detection for the samples varied on the basis of cell/volume input, and that value is represented. LN= lymph node.

3.- Distribution of HIV-1 reservoirs in CD4 T-cell subsets

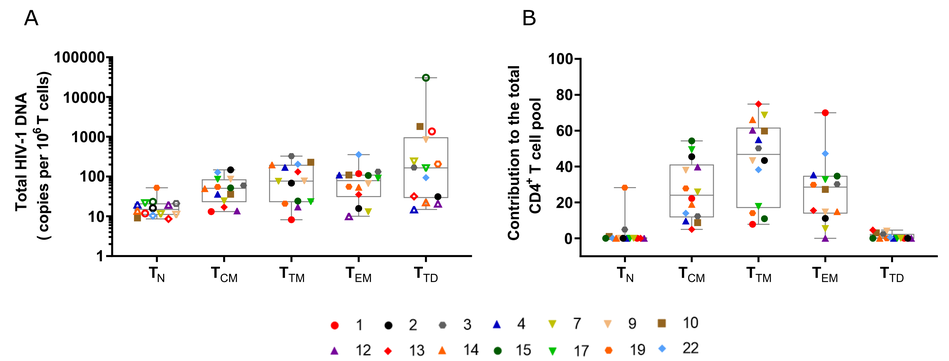

Figure 4. HIV latency distribution in T-cell subsets in LoViReT subjects. (**A**) Total HIV-DNA measured by ddPCR and (**B**) contribution of each subset to the HIV reservoir

CONCLUSIONS

- LoViReT individuals have abnormaly low HIV reservoirs even in the absence of cART.
- 71% of LoViReTs did not show replication-competent virus and harbored limited provirus in tissue sanctuaries under cART.
- A cause of this exceptional low reservoir could be the high contribution of the short-live T_{TM} and T_{EM} cells in the total HIV reservoir.
- This individuals are the perfect candidates to try new HIV remission strategies.

ACKNOWLEDGEMENTS

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