HIV-Infected Cells Expressing Envelope Display Distinct Phenotypes in ART-Treated and Viremic People with HIV

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BACKGROUND

- Using a novel assay (Env-Flow), we recently demonstrated that reactivated cells from ART-treated PWH express lower levels of Env compared to viremic PWH.
- However, whether Env$^+$ cells from ART-suppressed and viremic PWH display distinct phenotypes remains unclear.
- Characterizing Env-expressing cells is essential to understand reservoir biology and identify vulnerabilities for cure strategies.

METHODS

Env-Flow was used to determine the phenotype of HIV-infected cells from **17** people with HIV (PWH; **8 ART-suppressed**, **9 viremic**).

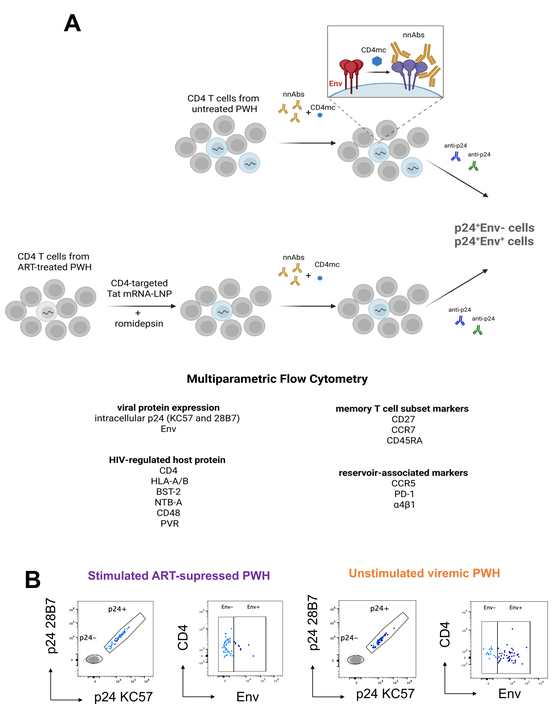


Figure 1. A) A multiparametric flow-cytometry assay (Env-Flow) was used to detect and characterize Env-expressing HIV-infected CD4 T cells. Purified CD4 cells from 17 people with HIV (8 ART-suppressed, 9 viremic) were evaluated. Cells from ART-treated PWH were pre-stimulated with CD4-targeted Tat mRNA-LNPs and romidepsin to reactivate HIV gene expression. Surface Env was detected using a potent CD4mimetic – non-neutralizing Ab cocktail, combined with intracellular p24 staining using clones KC57 and 28B7. Samples were acquired on a Cytek Aurora flow cytometer and analyzed using CellEngine software. B) Representative gating strategy to identify p24$^+$Env$^-$ (represented as light blue) and p24$^+$Env$^+$ cells (represented as dark blue).

RESULTS

Env$^+$ cells are enriched in the effector memory subset in ART-suppressed PWH

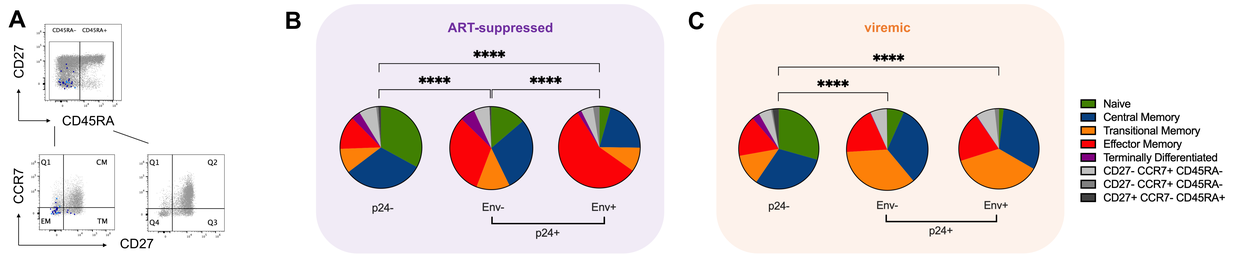


Figure 2. **A**) Representative dot plots from an ART-suppressed PWH showing the gating strategy used to identify memory CD4$^+$ T-cell subsets: naïve (N; CD45RA$^+$CCR7$^+$CD27$^+$), central memory (CM; CD45RA$^-$CCR7$^+$CD27$^+$), transitional memory (TM; CD45RA$^-$CCR7$^-$CD27$^+$), effector memory (EM; CD45RA$^-$CCR7$^-$CD27$^-$), and terminally differentiated (TD; CD45RA$^+$CCR7$^-$CD27$^-$). p24$^+$Env$^-$ cells (light blue) and p24$^+$Env$^+$ cells (dark blue) are overlaid onto p24$^-$ cells shown in grey. **B–C**) Pie charts showing the distribution of memory CD4$^+$ T-cell subsets among p24$^-$, p24$^+$Env$^-$, and p24$^+$Env$^+$ cells in ART-suppressed individuals (n = 8) (**B**) and viremic individuals (n = 9) (**C**). Percentages represent the mean proportion of each subset within each group. Pairwise chi-square tests were performed to compare p24$^-$ vs p24$^+$Env$^-$ and p24$^+$Env$^-$ vs p24$^+$Env$^+$.

Env$^-$ and Env$^+$ cells show elevated α4β1, CCR5, and PD-1 expression

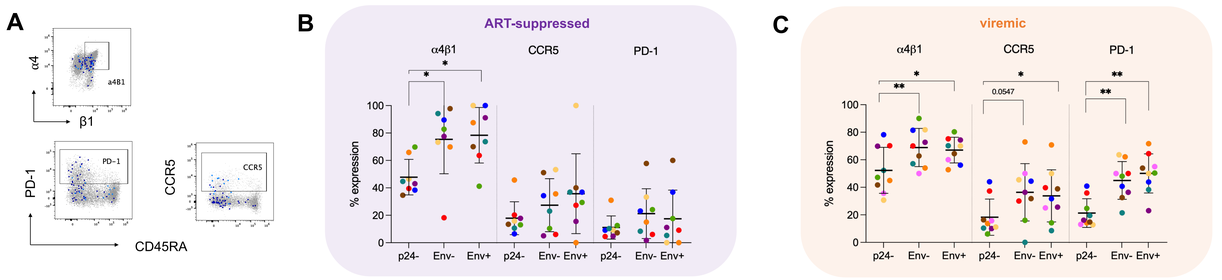


Figure 3. A) Representative dot plots showing the expression of α4β1, CCR5, and PD-1 on p24$^+$Env$^-$ cells (light blue) and p24$^+$Env$^+$ cells (dark blue), overlaid onto p24$^-$ cells (grey) from a viremic PWH. **B–C**) Expression levels (%) of α4β1, CCR5, and PD-1 in p24$^-$, p24$^+$Env$^-$, and p24$^+$Env$^+$ CD4$^+$ T cells from ART-suppressed individuals (**B**) and viremic individuals (**C**). Each point represents one participant (color-coded by individual). Statistical comparisons were performed using the Wilcoxon matched-pairs signed-rank test.

Stronger downregulation of HIV-regulated host proteins in Env+ cells from viremic PWH

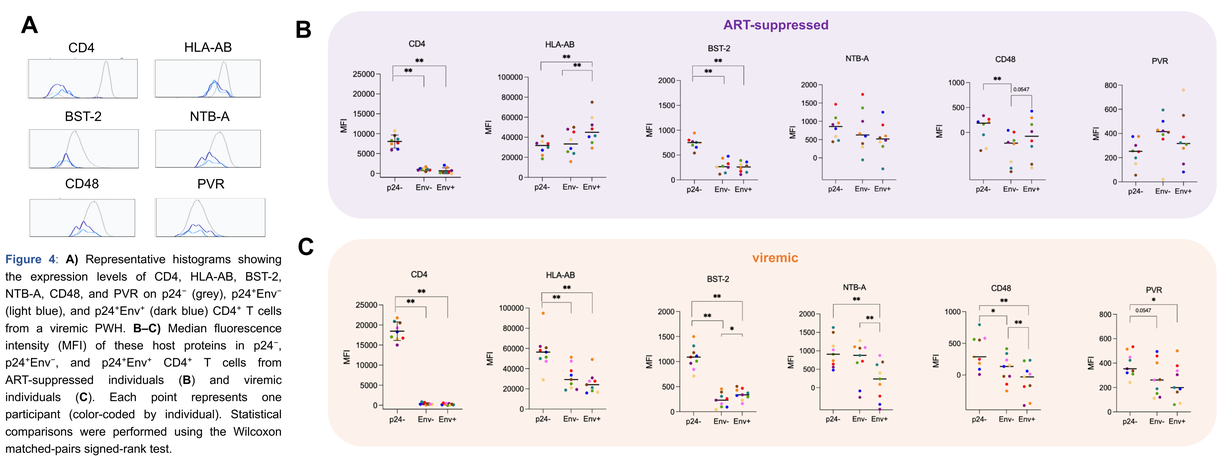


Figure 4: **A)** Representative histograms showing the expression levels of CD4, HLA-AB, BST-2, NTB-A, CD48, and PVR on p24$^-$ (grey), p24$^+$Env$^-$ (light blue), and p24$^+$Env$^+$ (dark blue) CD4$^+$ T cells from a viremic PWH. **B–C)** Median fluorescence intensity (MFI) of these host proteins in p24$^-$, p24$^+$Env$^-$, and p24$^+$Env$^+$ CD4$^+$ T cells from ART-suppressed individuals (**B**) and viremic individuals (**C**). Each point represents one participant (color-coded by individual). Statistical comparisons were performed using the Wilcoxon matched-pairs signed-rank test.

Env$^+$ cells display distinct differentiation and host-protein regulation profiles in ART-suppressed and viremic PWH.

CONCLUSIONS

- Env-Flow enables ex vivo characterization of reactivated HIV-infected cells in ART-suppressed and viremic PWH.
- In ART-suppressed individuals, reactivated Env$^+$ cells are enriched in effector-memory cells.
- Both Env$^-$ and Env$^+$ cells display elevated expression of persistence-associated markers.
- In viremic PWH, Env$^+$ cells show stronger downregulation of HIV-regulated host proteins (BST-2, NTB-A, CD48), consistent with higher accessory-protein activity.
- These differences were not observed in ART-suppressed PWH, suggesting limited accessory-protein function under ART.
- CD4-targeted Tat mRNA-LNP reactivation exposes Env$^+$ cells with reduced immune-evasion capacity, highlighting a potential window for immune-mediated clearance.

PARTICIPANTS!

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CanCURE
CIHR IRSC

PLAIN LANGUAGE SUMMARY: Using a novel assay, we observed that infected cells are different between ART-treated and viremic people with HIV, which will inform cure strategies targeting the reservoir.