VIRAL RESERVOIRS WITH SPONTANEOUS HIV EXPRESSION ARE ASSOCIATED WITH HIV-SPECIFIC CD4 AND CD8 T CELL RESPONSES

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BACKGROUND

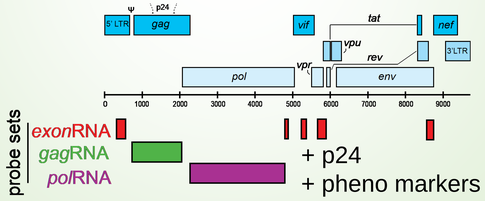
A major obstacle to HIV cure is the persistence of latent viral reservoirs. Even during ART, infected cells can adopt a state of spontaneous transcriptional "leakiness" characterized by a basal level of viral transcription. Cell-associated HIV RNA has been associated with a shorter time to viral rebound at ART interruption, demonstrating this phenomenon's biological relevance. However, leaky latency *in vivo* remains poorly understood.

Key questions:

- ❖ What is the frequency of HIV-infected cells with spontaneous transcription *in vivo*?
- ❖ Are they connected with anti-HIV immune responses?

METHODS

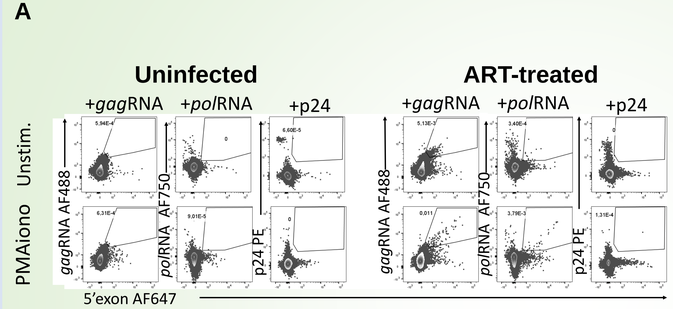
- ➢ 18 people with HIV (PWH) with optimal viral suppression (ART) for >2years. (-) control: 6 uninfected volunteers.
- ➢ Purified CD4 T cells were either PMA/ionomycin-stimulated (**inducible** reservoirs) or unstimulated (reservoirs with **spontaneous** transcription) for 16 h
- ➢ Viral RNA positive cells (**vRNA⁺**) are identified by single-cell flow cytometric fluorescent *in situ* RNA hybridization (RNAflow-FISH).

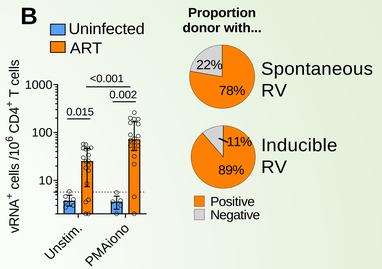


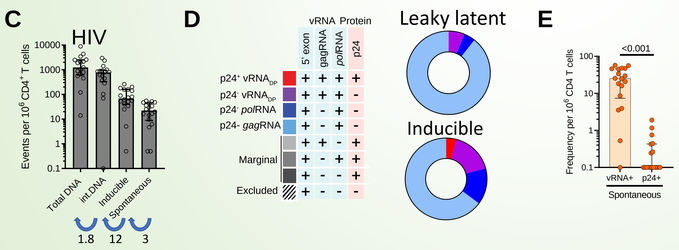
- ➢ Acquisition on a Symphony flow cytometer

RESULTS

Figure 1: Spontaneous "leaky" reservoirs are detectable in a majority of virally suppressed participants

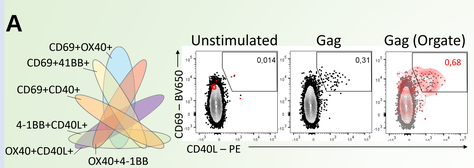


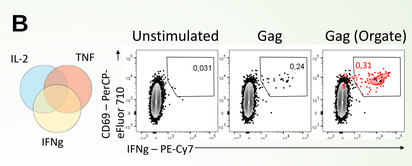


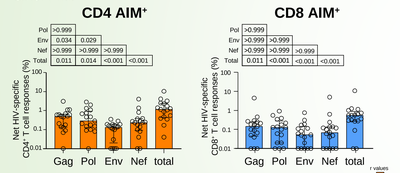


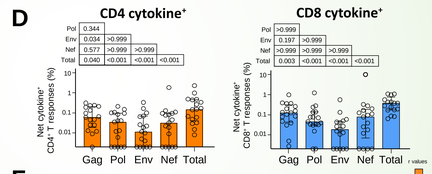
(A) Layered analysis of *exon*RNA⁺ cells, based on i) p24 and ii) *gag*RNA and *pol*RNA co-expression. **(B)** Quantification of reservoirs by multiplex RNAflow-FISH. The dotted line indicate the threshold of detection (mean+2SD). The proportion of participants with the indicated RV is shown on the right. **(C)** On the left: Theoretical populations. On the right: proportion of each viral subpopulations. **(D)** Frequency of p24⁺ spontaneously expressed during ART.

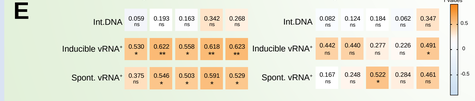
Figure 2: Identification of HIV-specific CD4 and CD8 T cells by AIM and ICS assays







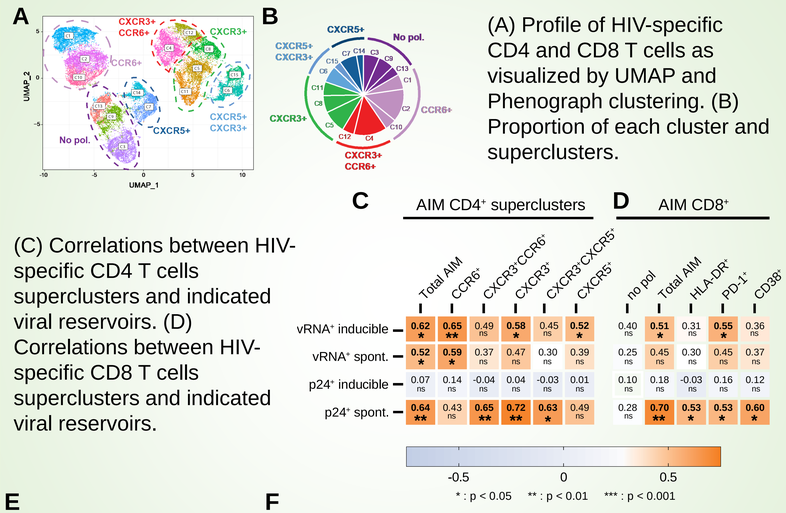




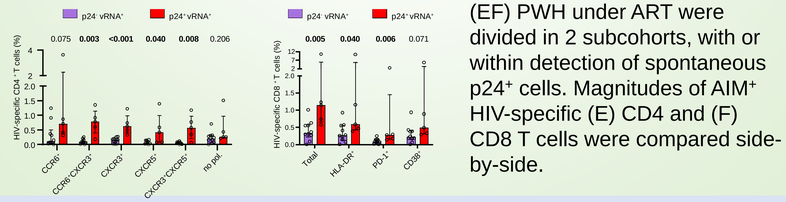


(AB) Identification of HIV-specific T cells by multiplex (A) AIM or (B) ICS assays. Cells identified by these strategies (in red) are more numerous than those identified by singleplex assays. (CD) Quantification of net (C) AIM⁺ (D) cytokine⁺ HIV-specific CD4 and CD8 T cells. (EF) Correlations between Gag, Pol, Env, and Nef-specific CD4 and CD8 T cells defined by (E) AIM and (F) ICS assays vs integrated DNA, inducible, and spontaneous viral reservoirs.

Figure 3: Associations between spontaneous vRNA and p24 expression and HIV-specific CD4 and CD8 T cell responses



(C) Correlations between HIV-specific CD4 T cells superclusters and indicated viral reservoirs. (D) Correlations between HIV-specific CD8 T cells superclusters and indicated viral reservoirs.



(EF) PWH under ART were divided in 2 subcohorts, with or within detection of spontaneous p24⁺ cells. Magnitudes of AIM⁺ HIV-specific (E) CD4 and (F) CD8 T cells were compared side-by-side.

CONCLUSIONS

- Spontaneous reservoirs are detectable in most virally supressed PWH
- Spontaneous vRNA and p24 expression correlates with HIV-specific CD4 and CD8 T cell responses
- Spontaneous reservoirs are biologically active and may shape anti-HIV immunity

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