CD127+ LYMPHOID MEMORY CD4+ T CELLS PREFERENTIALLY UNDERGO LATENT HIV INFECTION

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Abstract

Background

Lymphoid tissues are a primary site of HIV replication and persistence. We recently demonstrated that tonsillar memory CD4+ T cells expressing CD127, efficiently fused to HIV but did not allow the fused virus to complete a round of productive infection. Mechanisms that prevent transcription of the viral LTR can prevent completion of the viral life cycle, and also promote HIV latency which is one of the main barriers to an HIV cure. In this study, we set out to better characterize the molecular basis for the block in HIV replication in tissue CD127+ memory CD4+ T cells by considering two main possibilities: post-entry restriction by SAMHD1, or latent infection of these cells by HIV.

Methods

Tonsil cells from uninfected donors were mock-treated or exposed to a CCR5-tropic HIV reporter virus for 3 days. Multiple populations of memory CD4+ T cells were compared for SAMHD1 expression and infection levels by FACS. These subsets were sorted and quantified for levels of integrated HIV-1 provirus using 2-step Alu-gag PCR with ddPCR. Global expression profiling of the sorted subsets was conducted. A panel of RT-ddPCR assays was implemented to quantify various HIV transcripts to determine to what extent these cells have silenced HIV transcription.

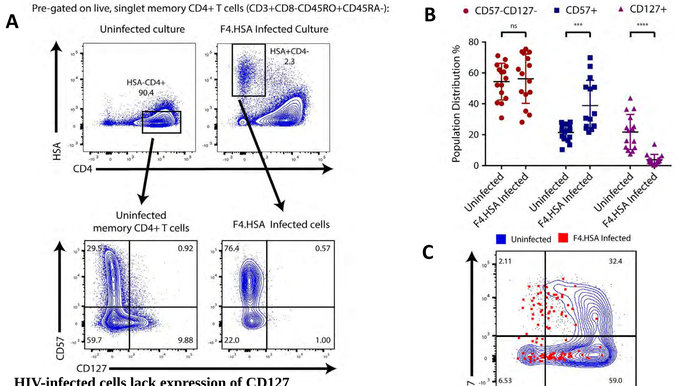
Results

Lymphoid CD127+ mem CD4+ T cells do not exhibit early post-entry restriction by SAMHD1, but rather preferentially undergo latent infection as they harbor high levels of integrated HIV DNA in the absence of reporter gene expression. Relative to other memory CD4+ T cell subsets highly permissive for productive infection, the CD127+ cells preferentially expressed host transcripts associated with cellular quiescence explaining how these cells can preferentially silence the HIV LTR. The transcriptional profiling of CD127+ mem CD4+ T cells revealed that some HIV transcription was occurring, but that splicing of these transcripts was blocked. Latently-infected CD127+ memory cells were reactivated upon stimulation.

Conclusions and future directions

We identify a population of tissue-specific memory CD4+ T cells expressing CD127 that upon exposure to HIV preferentially supports latent infection. Because these cells can undergo IL7-driven homeostatic proliferation and can be reactivated, they may serve as an important reservoir to target for HIV eradication efforts. They also serve as a useful *in vitro* tissue model of HIV latency that can be used to investigate multiple aspects of latency establishment and maintenance. Although CD127 has not been found to be preferentially expressed on latent cells *in vivo*, the data presented herein warrant investigation of this receptor as a potential biomarker of latently infected cells residing in tissues.

1 Tissue-derived memory CD4+ T cells expressing CD127 restrict productive infection by HIV-1



HIV-infected cells lack expression of CD127

A. HLACs were mock-treated or exposed for 3 days to the CCR5-tropic reporter virus F4.HSA, after which the selected populations were assessed for expression levels of CD57 and CD127 (*bottom plots*).

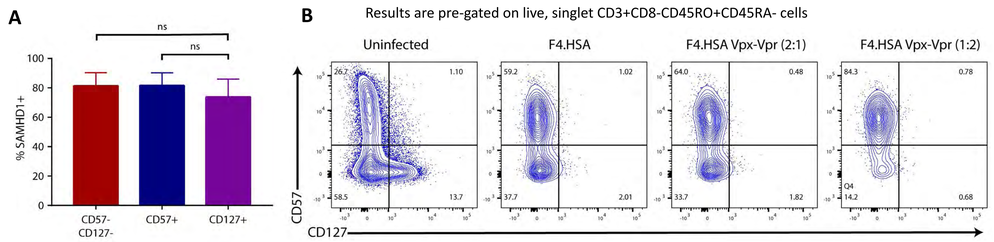
B. Bar graph comparing the proportion of the different populations amoungst uninfected or HIV infected Tm cells from 15 independant donors.

The restriction is tissue specific

C. Uninfected (blue) and productively-infected (red) CD4+ Tm cells from blood, demonstrating the existence of infected blood-derived CD127+ Tm.

Results

2 Post-entry restriction of HIV in CD127+ Tm is not mediated by SAMHD1

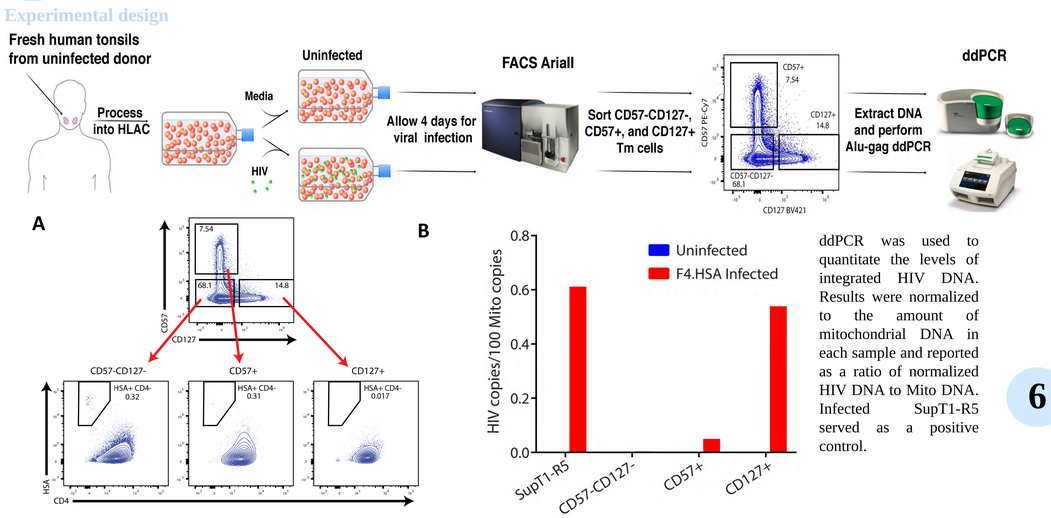


No differential expression of SAMHD1 expression in the tissues Tm subsets. Percentage of SAMHD1+ cells across the three indicated CD4+ Tm cell subsets from HLACs as determined by flow cytometry.

Relieving SAMDH1 restriction does not increase permissiveness of CD127+ Tm to productive infection by HIV-1.

Representative flow cytometric plots of HLACs mock-treated or exposed for 3 days to F4.HSA lacking or harboring Vpx as indicated. Expression levels of CD57 and CD127 were then assessed in uninfected memory CD4+ T cells, and HIV-infected (HSA+) memory CD4+ T cells.

3 CD127+ Tm preferentially support latent HIV-1 infection

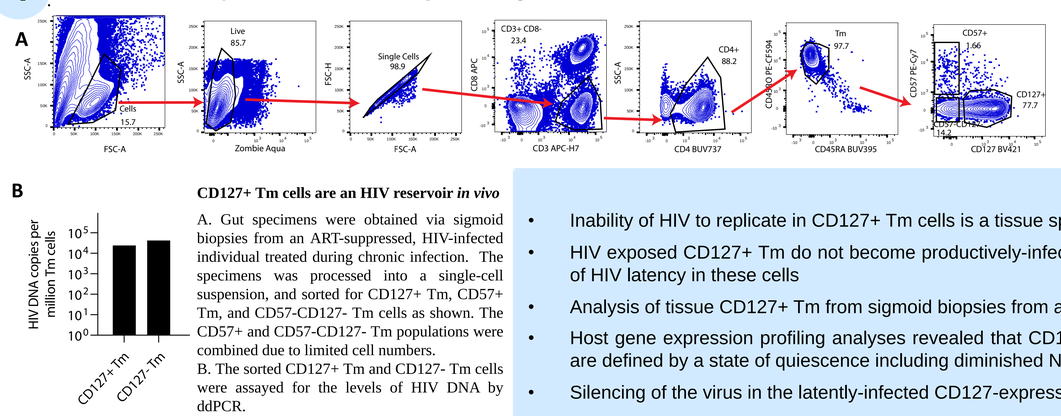


Sorting of the 3 populations of memory CD4+ T cells from an infected culture

ddPCR was used to quantitate the levels of integrated HIV DNA. Results were normalized to the amount of mitochondrial DNA in each sample and reported as a ratio of normalized HIV DNA to Mito DNA. Infected SupT1-R5 served as a positive control.

CD127+ Tm harbor high levels of integrated HIV DNA relative to productive infection rates.

4 Tissue memory CD4+ T cells expressing CD127 are a reservoir for HIV *in vivo*

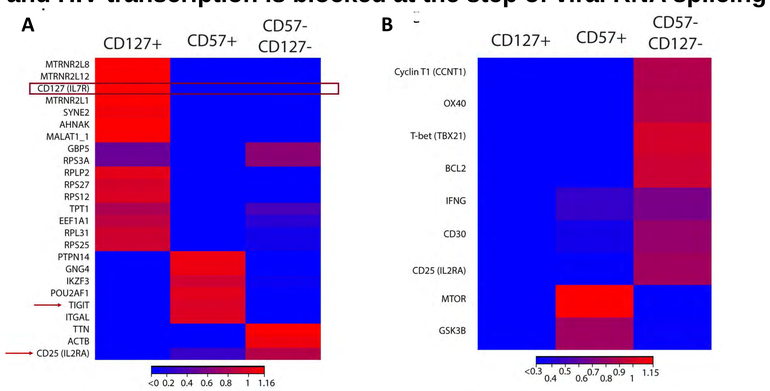


CD127+ Tm cells are an HIV reservoir *in vivo*

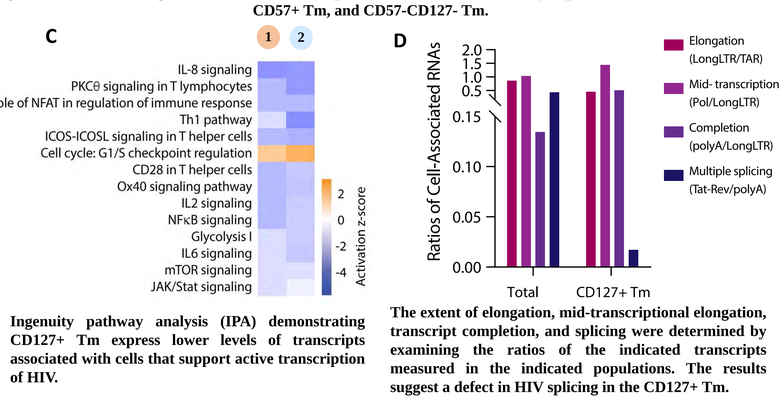
A. Gut specimens were obtained via sigmoid biopsies from an ART-suppressed, HIV-infected individual treated during chronic infection. The specimens was processed into a single-cell suspension, and sorted for CD127+ Tm, CD57+ Tm, and CD57-CD127- Tm cells as shown. The CD57+ and CD57-CD127- Tm populations were combined due to limited cell numbers.

B. The sorted CD127+ Tm and CD127- Tm cells were assayed for the levels of HIV DNA by ddPCR.

5 CD127+ Tm exhibit a transcriptional profile of cellular quiescence and HIV transcription is blocked at the step of viral RNA splicing



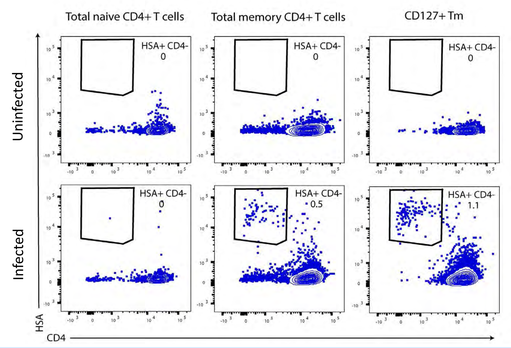
Top genes (A) and selected genes associated with HIV permissiveness (B) differentially expressed between CD127+ Tm, CD57+ Tm, and CD57-CD127- Tm.



Ingenuity pathway analysis (IPA) demonstrating CD127+ Tm express lower levels of transcripts associated with cells that support active transcription of HIV.

The extent of elongation, mid-transcriptional elongation, transcript completion, and splicing were determined by examining the ratios of the indicated transcripts measured in the indicated populations. The results suggest a defect in HIV splicing in the CD127+ Tm.

6 Latent CD127+ Tm are reactivable upon T cell stimulation



Latent HIV proviruses within CD127+ Tm are capable of reactivating upon stimulation.

HSA- naïve, memory, or CD127+ Tm cells were sorted from uninfected or infected tonsillar cultures, and then stimulated with anti-CD3/CD28 beads and then assessed levels of reactivation three days later. The proportion of infected (HSA+) cells that have downregulated cell-surface CD4 correspond to the frequency of reactivated cells.

Summary

- Inability of HIV to replicate in CD127+ Tm cells is a tissue specific phenomenon and it is not due to post-entry restriction by SAMHD1.
- HIV exposed CD127+ Tm do not become productively-infected, but harbor relatively high levels of integrated DNA, suggesting preferential establishment of HIV latency in these cells
- Analysis of tissue CD127+ Tm from sigmoid biopsies from an ART-suppressed, HIV-infected individual, show that those cells are an HIV reservoir *in vivo*
- Host gene expression profiling analyses revealed that CD127+ Tm cells are phenotypically distinct from other tissue memory CD4+ T cell subsets, and are defined by a state of quiescence including diminished NFkB, NFAT, and Ox40 signaling.
- Silencing of the virus in the latently-infected CD127-expressing cells is reversible upon T cell stimulation.

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