HIV DIRECTLY INFECTS RESTING MEMORY CD4 T CELLS

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ABSTRACT

BACKGROUND: The establishment of the latent HIV reservoir in resting memory CD4 T cells occurs early in infection. Resting CD4 T cells are more difficult to infect than activated cells. Therefore, the HIV reservoir is thought to form when HIV infects a few activated cells that are resting down. Furthermore, HIV encodes four proteins: Vif, Vpr, Vpu, and Nef, which play an important role for the persistence of HIV. For example, Nef is known to downregulate MHC class I molecules (pMHCs) which prevents the recognition by CD8 T cells. However, the precise timing of the expression these four HIV proteins and the downregulation of their targeted host proteins in resting memory CD4 T cells is unknown.

METHODS: We explored this question by direct infection and longitudinal analysis of primary resting CD4 T cells with a CCR5-tropic replication-competent reporter virus in which GFP reports the expression of Nef. We then measured pMHCs by flow cytometry and performed bulk and scRNAseq of sorted GFP+ cells to measure host and HIV mRNAs. We also performed scATACseq to identify the sites of HIV integration to determine their influence on the timing of Nef expression.

RESULTS: We detected resting memory GFP+ cells 3 to 4 days after infection. These GFP+ cells showed low surface levels of pMHCs. By scRNAseq HIV mRNAs were identified in GFP+ cells and they encoded for Nef, Vpr, Vif or Vpu-Env, but never for Gag-Pol, Tat or Rev. The analysis of scATACseq and scRNAseq revealed this differential expression of HIV mRNAs was due to HIV integration into genes that are stochastically transcribed across resting CD4 T cells. Importantly, RNAseq analysis identified the expression of a cell-cycle independent form of ribonucleotide reductase, which converts ribonucleotides to deoxynucleotides. Also, the pathway for thymidine synthesis was not active in resting T cells. Thus, using a real-time qPCR assay that distinguishes proviruses with deoxyuracils or thymidines, we found GFP+ cells had deoxyuracils instead of thymidines. Lastly, we revealed Vpr protected proviruses from an UNG-dependent inactivation mechanism.

CONCLUSIONS: We conclude that HIV can directly infect primary resting memory CD4 T cells to establish the reservoir. HIV-infected resting CD4 T cells incorporate deoxyuracils, which is deleterious in the absence of Vpr inhibiting UNG. Finally, we believe the integrated HIV genome persists through transcription and alternatively splicing for mRNAs encoding Nef, Vif, Vpr, or Vpu-Env. Thus, HIV prefers to hide than sleep.

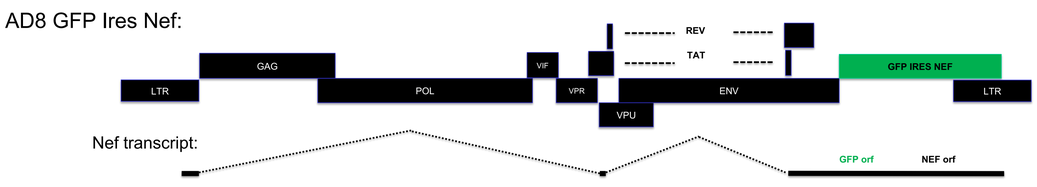


Figure 1. GFP reporter viruses used for study

The CCR5-tropic HIV molecular clone ADA-AD8 was used to generate the GFP reporter virus for the Nef transcript

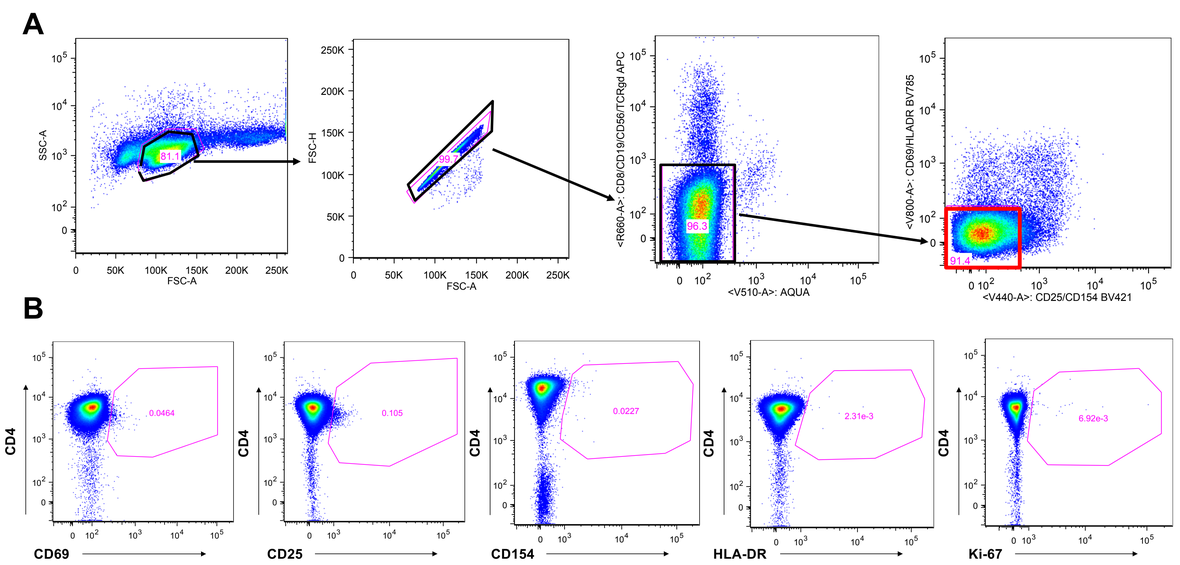


Figure 2. Sorting scheme for resting CD4 T cells and expression of activation markers post-sort and post-HIV infection

(A) Purified CD4 T cells are sorted for resting phenotype (red) by excluding live cells expressing CD19, CD8, CD56, TCRgd and activation markers CD25, CD154, CD69, HLA-DR. (B) Expression of CD69, CD25, CD154, HLA-DR, and Ki-67 on resting CD4 T cells. FACS plots show expression levels at the 96-hour time point post HIV infection.

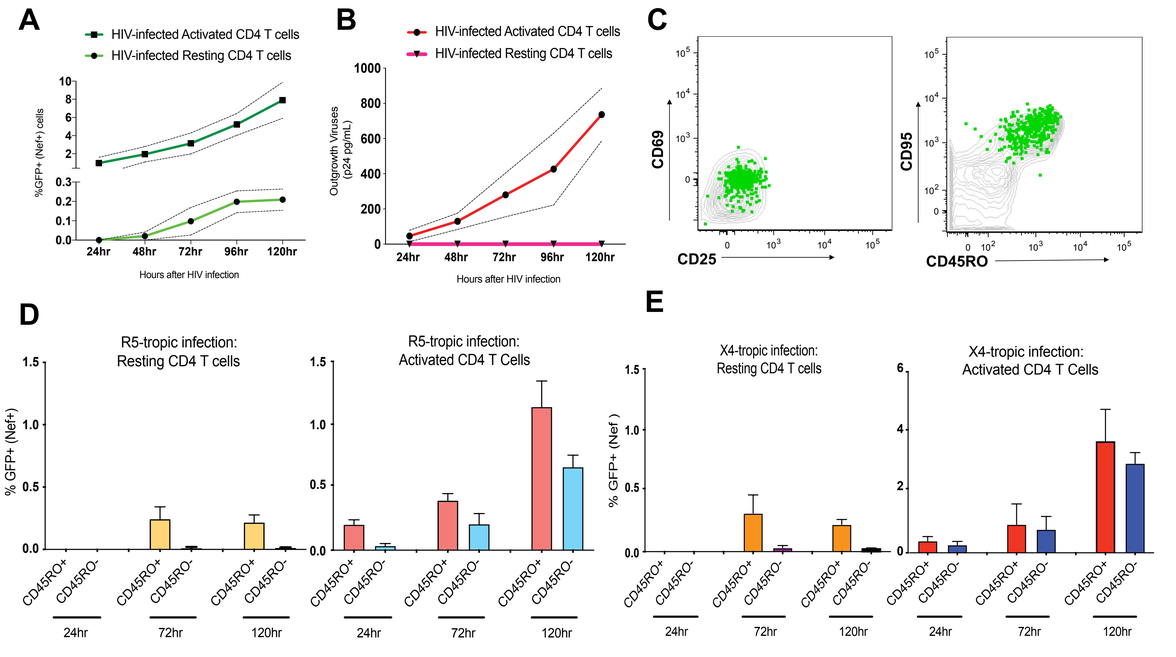


Figure 3. HIV infects resting and activated CD4 T cells, but only replicates in activated cells

(A) GFP expression in activated CD4 T cells (top) versus resting CD4 T cells (bottom). (B) HIV expression in activated (top) and resting (bottom) CD4 T cells. (C) HIV infection (gfp) of resting memory CD4 T cells. (D-E) HIV infection (gfp) of sorted resting or activated memory (CD45RO+) or non-memory (CD45RO-) cells. D: CCR5-tropic virus AD8, E: CXCR4-tropic virus NL4-3.

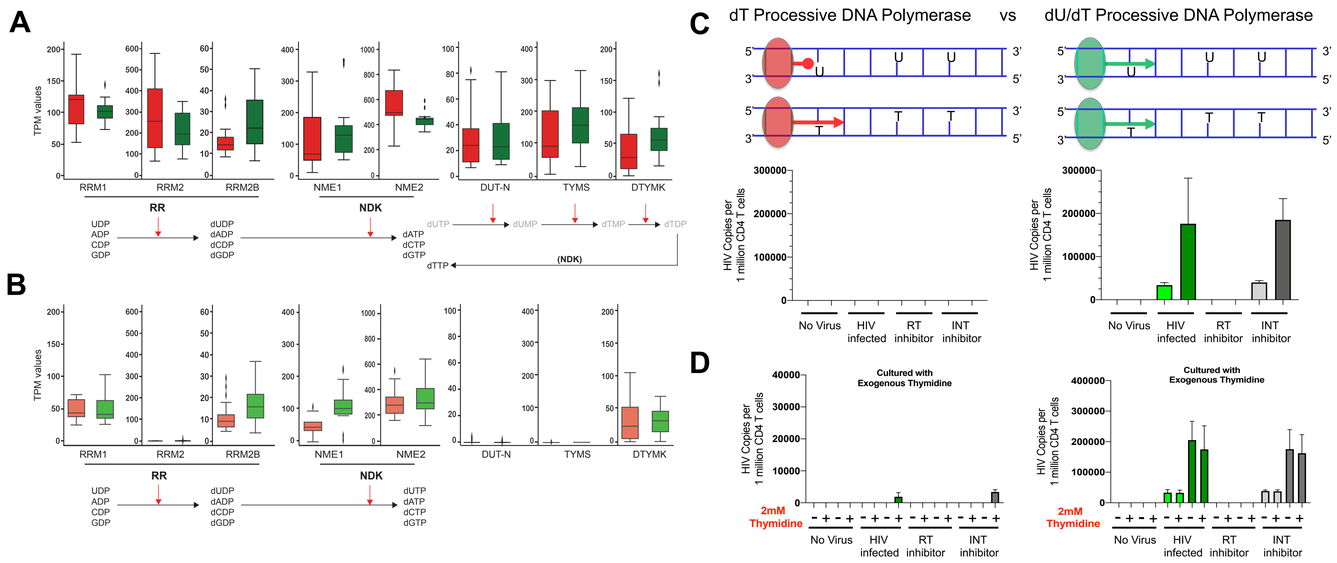


Figure 4. HIV-infected resting memory CD4 T cells synthesize dNTPs, but do not convert deoxyuracils to thymidines, which causes HIV to incorporate deoxuracils into the reverse transcribed provirus

(A) Transcriptome pathway of dNTP synthesis in activated memory CD4 T cells. (B) Transcriptome pathway of dNTP synthesis in resting memory CD4 T cell. (C) Real-time qPCR assay for dU or dT incorporation into provirus of HIV-infected CD4 T cells. (D) Real-time dU/dT assay for HIV-infected cells cultured with exogenous thymidine. Light green and grey are resting infected cells. Dark green and gray are activated infected cells.

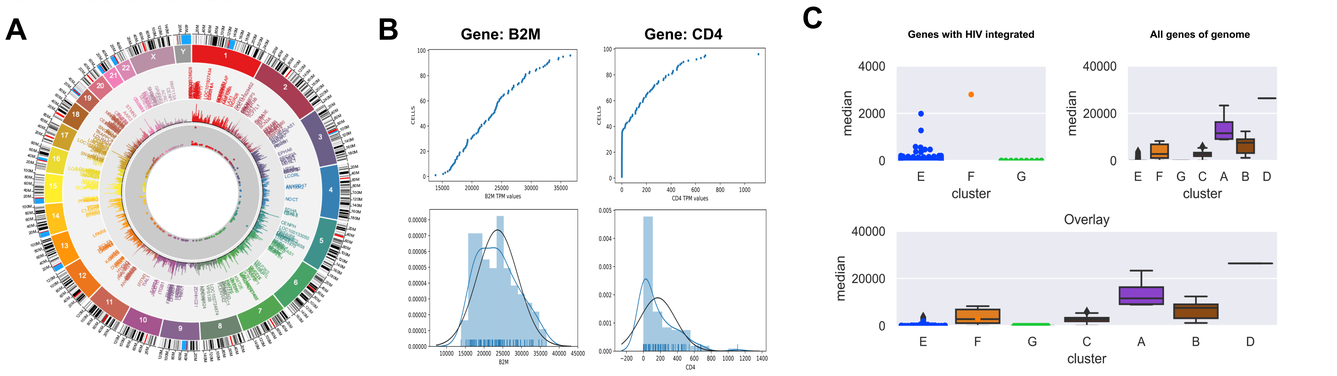


Figure 5. HIV integration sites in resting memory CD4 T cells and transcriptional activity of nearby genes by single-cell RNAseq of HIV-infected (GFP+) resting CD4 T cells

(A) Circos plot of HIV integration sites in resting memory CD4 T cells. Tracks show human genome, ATACseq DNA accessible regions,and expression of nearby-genes (B) Single-cell RNAseq expression analysis of two genes: B2M and CD4. (C) Clustering analysis of scRNAseq data for the transcriptome landscape of all genes in CD4 T cells and genes identified to have integrated HIV.

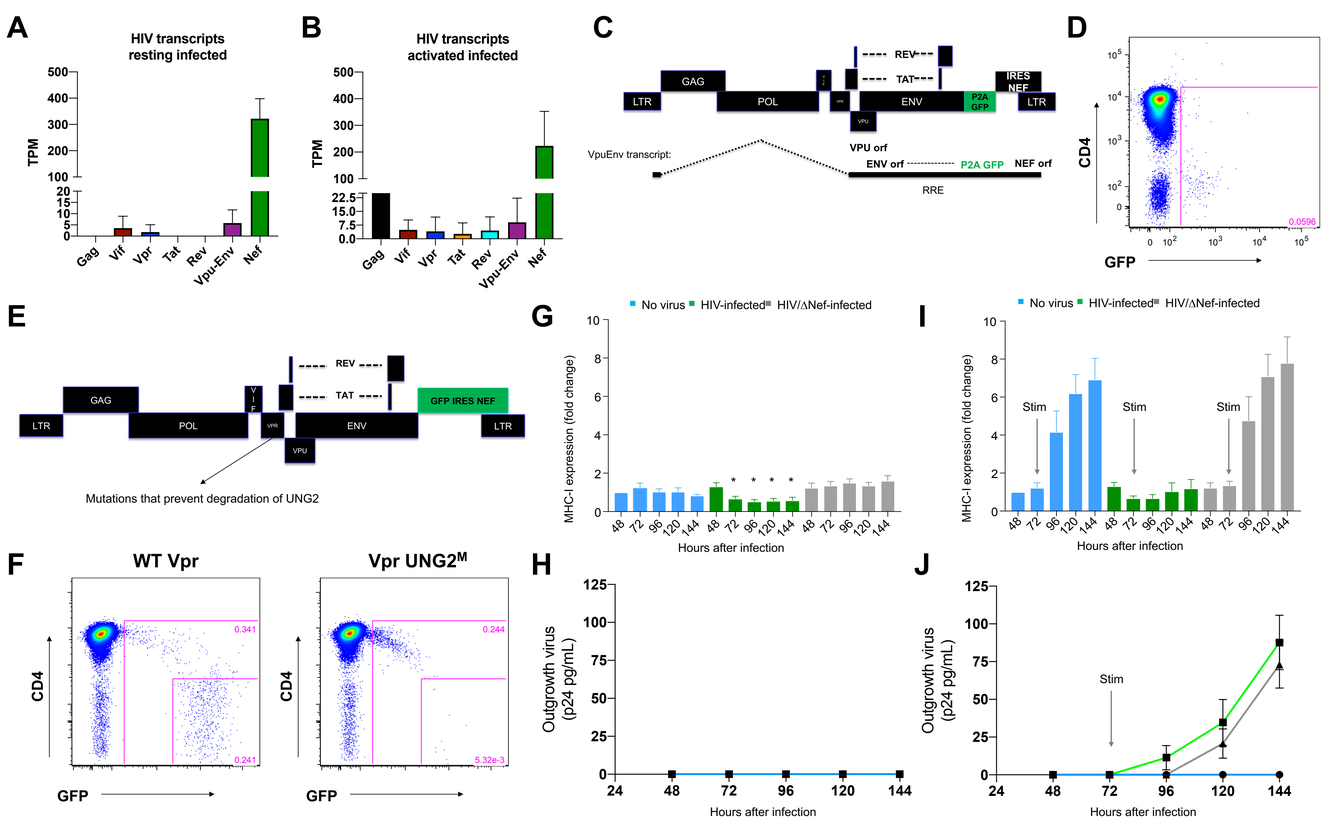


Figure 6. Differential HIV mRNA expression in resting and activated HIV-infected CD4 T cells reveals two gene expression programs for HIV persistence

(A-B) HIV mRNAs expression in HIV-infected resting (A) and activated (B) memory CD4 T cells. (C) GFP reporter virus for VpuEnv mRNA and (D) Infection of resting CD4 T cells. (E) GFP reporter virus (Nef) with mutation in Vpr orf that prevents the binding and degradation of UNG2. (F) Infection of resting CD4 T cells with GFP reporter virus (Nef) with WT Vpr or Vpr UNG2 mutant. (G) Time-course MHC class I levels in resting CD4 T cells with: No Virus, HIV-infected cells with Nef, and HIV-infected cells without Nef. (H) HIV expression of cells from (Panel G). (I) Time-course MHC class I levels in resting stimulated CD4 T cells with: No Virus, HIV-infected cells with Nef, and HIV-infected cells without Nef. (J) HIV expression of cells from (Panel I).

CONCLUSIONS

- HIV directly infects resting memory CD4 T cells without expressing virions
- HIV infects resting cells because of synthesis of dNTPs, which results in the incorporation of deoxyuracils into the provirus
- HIV integrates into sporadically transcribed genes
- HIV gene expression in resting memory CD4 T cells results in an immune evasion program because of the expression of Nef, Vif, Vpr and Vpu
- T cell activation results into HIV gene expression for Tat, Rev and full-length HIV GagPol mRNA for the virus replication program