VPR ENHANCES HIV REPLICATION BY INHIBITING T CELL PROLIFERATION

Rodrigo Matus-Nicodemos, David Ambrozak, Daniel Douek, and Richard Koup

Vaccine Research Center, NIAID, NIH

National Institute of Allergy and Infectious Diseases

Dale and Betty Bumpers Vaccine Research Center

#227

ABSTRACT

BACKGROUND: The resting HIV reservoir is a major obstacle towards curing HIV+ people. The reservoir is composed of clonally expanded T cells that predominantly harbor defective proviruses. A few infected cells containing replication-competent provirus are responsible for rebound viremia after treatment interruption. It is known the Vpr protein inhibits proliferation in cell lines. However, the timing of Vpr expression in resting infected cells is unknown and whether it will prevent proliferation of cycling primary CD4 T cells with replication-competent HIV.

METHODS: We explored this question by direct infection of primary resting CD4 T cells with a CCR5-tropic replication-competent GFP reporter virus. We measured GFP expression by flow cytometry over 5 days. We performed bulk and single cell RNAseq of sorted GFP+ cells to measure host and virus mRNAs. We measured proliferation by first labelling resting infected cells with violet CellTrace and then stimulating them for 10 days with anti-CD3/CD28 beads and ART. We infected cells with wild-type Vpr, without Vpr, and with Vpr mutants known to affect T cell proliferation. We measured cell cyle progression using the mitosis marker phospho-Ser31 in histone 3.3 in both resting and stimulated cells with ART. Finally, we assayed HIV replication through co-culture of sorted infected cells with autologous or heterologous target primary CD4 T cells.

RESULTS: We detected resting GFP+ cells 3 to 4 days after infection. Transcriptome analysis of resting GFP+ revealed infected cells expressed mRNAs coding for Vif, Vpr, VpuEnv and Nef. We confirmed Vpr modulates progression through the cell cycle and prevents T cell proliferation. Infected cells lacking Vpr contained transcriptionally-silent proviruses in the divided cells. In contrast, transcriptionally-active proviruses were present in the divided cells that expressed non-functional Vpr, because of mutations that prevented its binding to the host ubiquitin chaperone protein DCAF1. Lastly, we found replication-competent HIV originated from the undivided cells instead of the divided cells.

CONCLUSIONS: We conclude HIV can directly infect primary resting CD4 T cells. After T cell stimulation, Vpr prevents infected primary CD4 T cells from proliferating, which enhanced HIV replication. Surprisingly, two distinct effects on the provirus occurred after release of T cell proliferation based on whether Vpr was not expressed or non-functional.

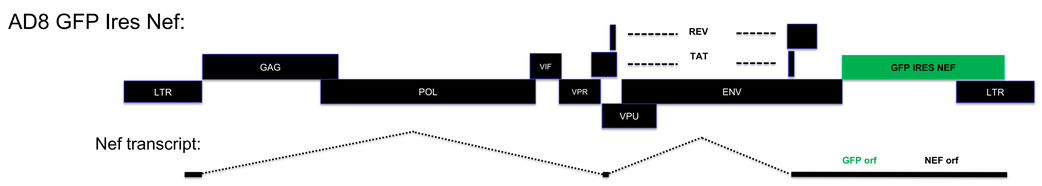


Figure 1. GFP reporter viruses used for study

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

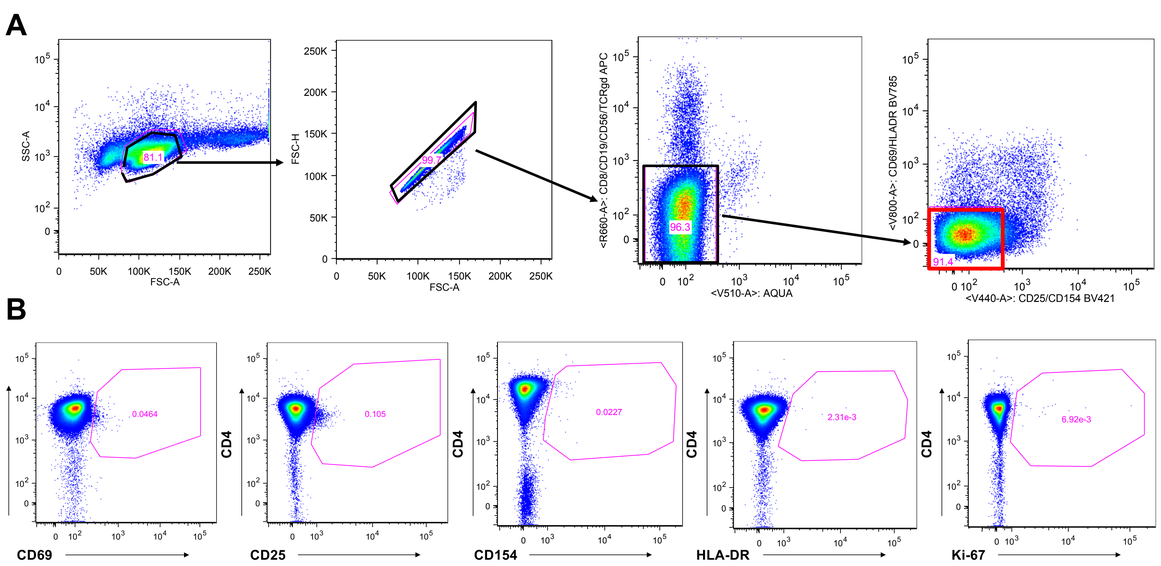


Figure 2. Sorting scheme for resting CD4 T cells and expression of activation markers post-sort and post-HIV infection

(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.

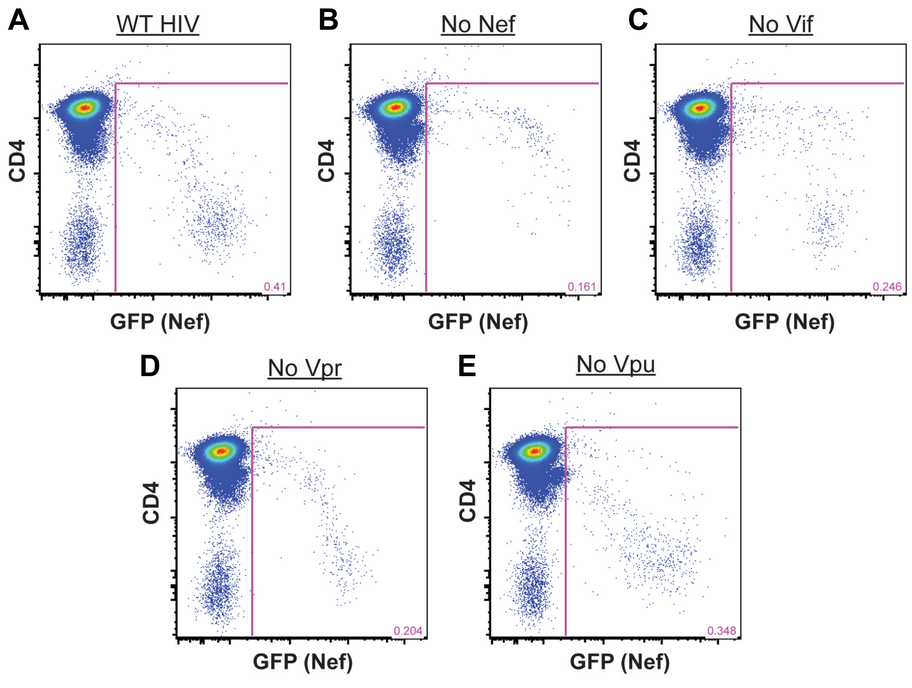


Figure 3. HIV infection of resting CD4 T cells with WT virus or lacking one of the four accessory proteins

(A-E) Resting CD4 T cell infection with HIVgfp. Flow plots showing CD4 versus GFP (Nef). (A) WT virus. (B) No Nef virus. (C) No Vif virus. (D) No Vpr virus. (E) No Vpu virus.

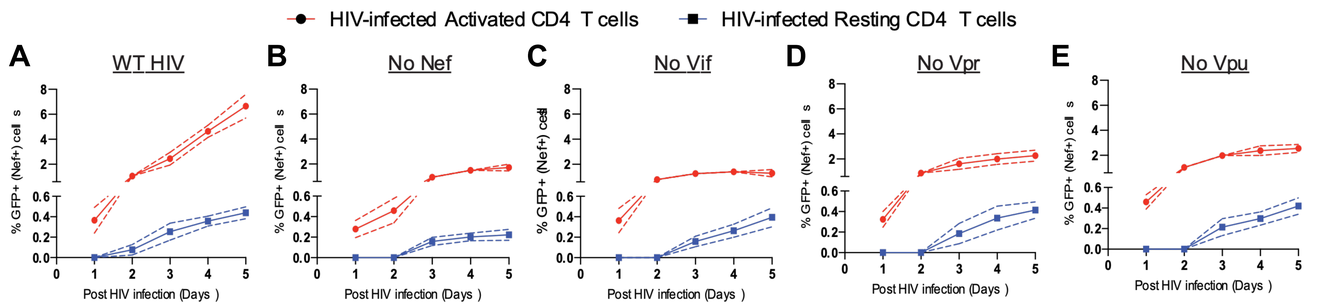


Figure 4. Time course of HIV infection in activated and resting CD4 T cells with WT virus or virus lacking Nef, Vif, Vpr, or Vpu

Expression of GFP+ (Nef+) cells was measured over five days after HIV infection with WT virus, or virus lacking one of the four accessory proteins in activated CD4 T cells (red) and resting CD4 T cells (blue).

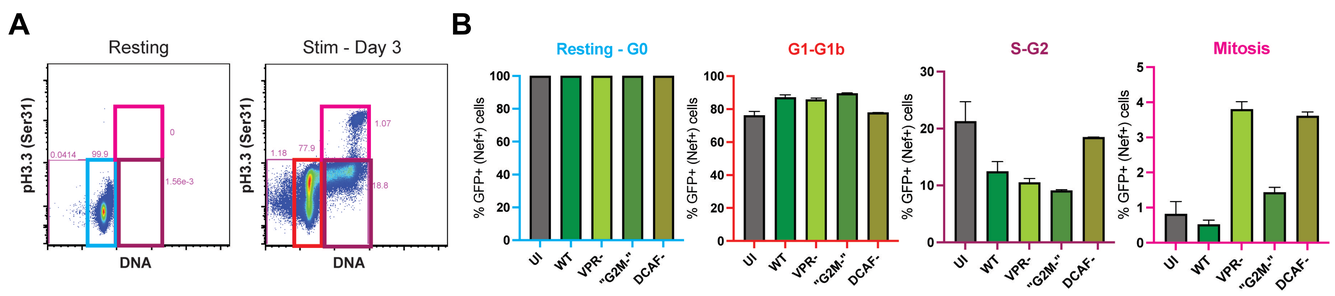


Figure 5. Primary CD4 T cell cycle arrest assay for HIV-infected cells

(A) Cell cycle flow analysis in resting CD4 T cells (left) and 3 days stimulated CD4 T cells on ART (right) showing pH3.3 versus DNA (DAPI). Stages of the cell cycle are colored: G0 (blue), G1-G1b (orange), S-G2 (purple), and Mitosis (pink). (B) Frequency of GFP+ (Nef+) cells in each cell cycle stage for WT virus and virus lacking Vpr, G2M mutant Vpr, and DCAF1 binding mutant Vpr.

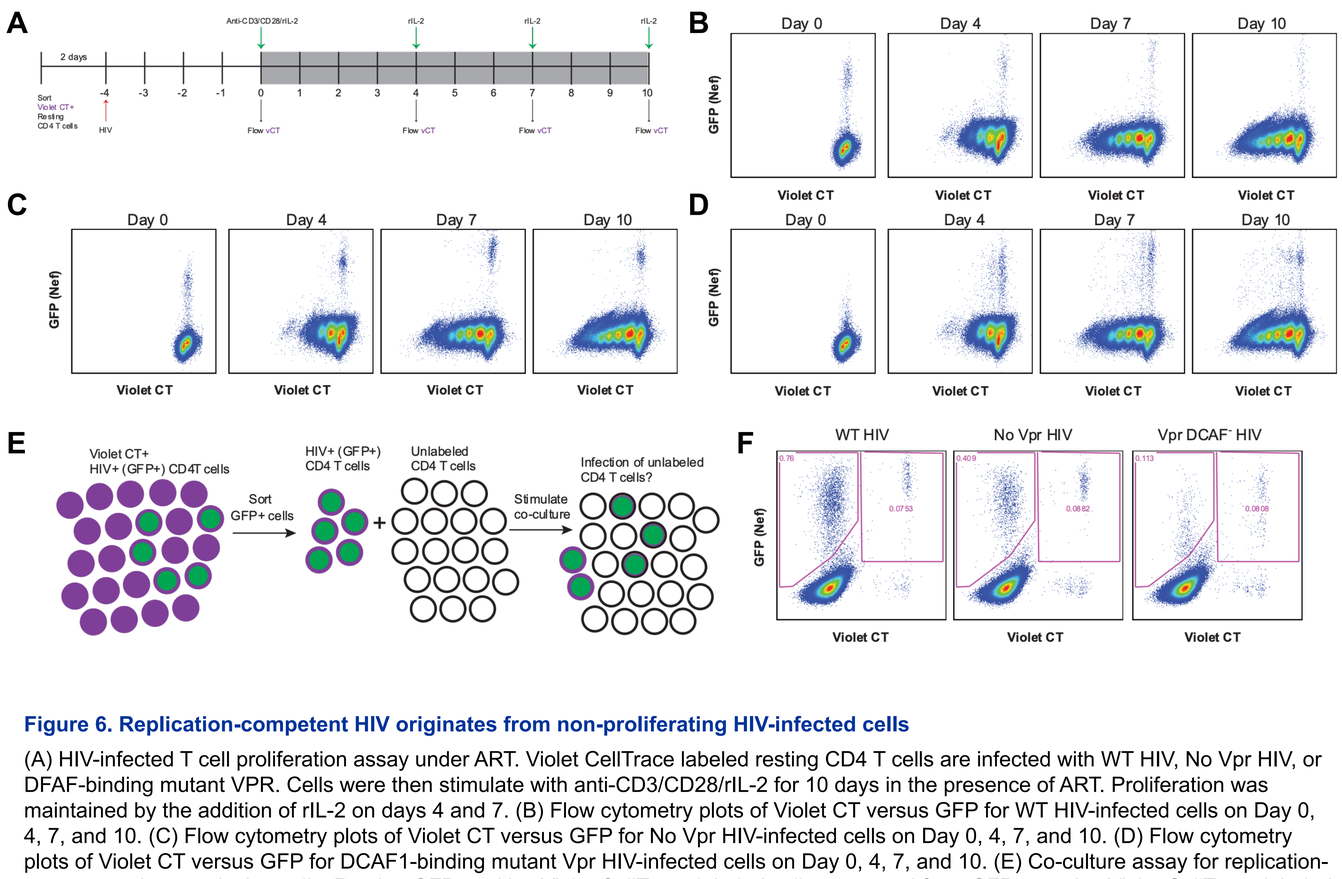


Figure 6. Replication-competent HIV originates from non-proliferating HIV-infected cells

(A) HIV-infected T cell proliferation assay under ART. Violet CellTrace labeled resting CD4 T cells are infected with WT HIV, No Vpr HIV, or DFAF-binding mutant VPR. Cells were then stimulate with anti-CD3/CD28/rIL-2 for 10 days in the presence of ART. Proliferation was maintained by the addition of rIL-2 on days 4 and 7. (B) Flow cytometry plots of Violet CT versus GFP for WT HIV-infected cells on Day 0, 4, 7, and 10. (C) Flow cytometry plots of Violet CT versus GFP for No Vpr HIV-infected cells on Day 0, 4, 7, and 10. (D) Flow cytometry plots of Violet CT versus GFP for DCAF1-binding mutant Vpr HIV-infected cells on Day 0, 4, 7, and 10. (E) Co-culture assay for replication-competent virus producing cells. Resting GFP-positive Violet CellTrace-labeled cells are sorted from GFP-negative Violet CellTrace-labeled cells and co-cultured with autologous unlabeled resting CD4 T cells. Cells are subsequently stimulated (as in A) and 4 days later GFP+ cells are measured in the unlabeled cells. (F) Flow cytometry plots of secondary GFP+ infections comparing WT HIV-infected cells, No Vpr HIV-infected cells, and DCAF1-binding Vpr mutant HIV-infected cells.

CONCLUSIONS

- HIV directly infects resting CD4 T cells and express Nef
- HIV accessory proteins: Nef, Vif, Vpu, and Vpr are required for efficient replication in activated CD4 T cells.
- Vpr inhibits progression of HIV-infected cells through the cell cycle
- Vpr “G2M” mutations did not inhibit cell cycle progression in HIV-infected cells
- Vpr DCAF1-binding is required for Vpr to inhibit cell cycle progression of HIV-infected cells
- WT HIV-infected cells do not proliferate
- HIV-infected cells lacking Vpr proliferate with transcriptionally-silent proviruses
- HIV-infected cells with DCAF1-binding mutant Vpr proliferate with transcriptionally-active proviruses
- Replication-competent HIV originate from HIV-infected cells that do not proliferate
- Vpr functions to enhance HIV replication by inhibiting HIV-infected T cell proliferation