The Effects of Accessory Genes on T/F HIV Infection of Primary CD4+ T Cells From Tissues

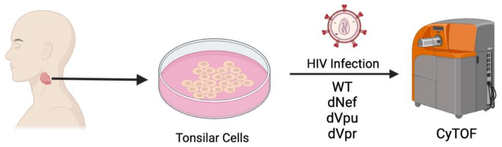
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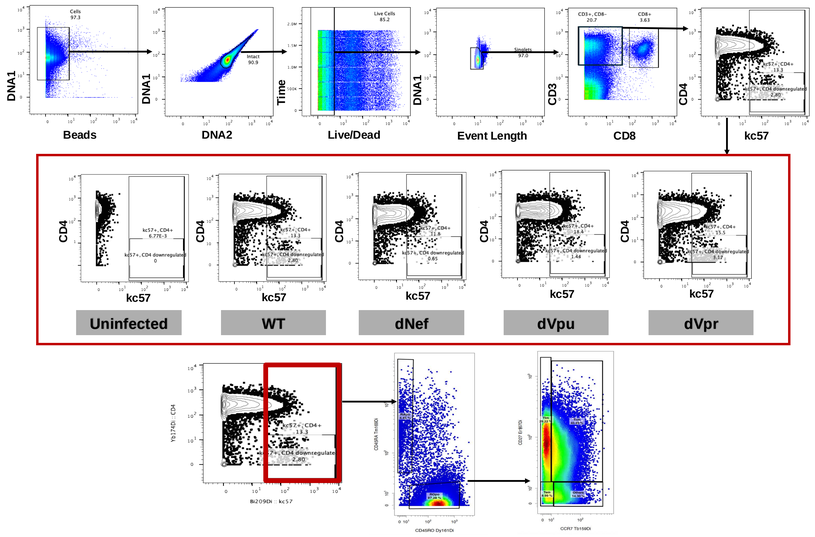
Introduction

HIV transmission often results from a single infecting viral variant, termed a transmitted/founder (T/F) virus. Accessory genes of T/F HIV often exhibit functions that are lost in cell culture-adapted HIV strains. The effects of accessory proteins from T/F HIV on infected cells is important for understanding HIV pathogenesis. Previous work has established that the accessory proteins Nef, Vpu, and Vpr are important for HIV fitness in the host. Both Nef and Vpu are known to downregulate a variety of host proteins from the cell surface, including CD4. However, prior accessory gene studies were largely performed in cell lines or PBMCs, whereas *in vivo* HIV primarily replicates in tissues. Here, we compared by CyTOF primary tissue CD4+ T cells infected with wildtype T/F HIV strain CH077, to those infected with CH077 deleted individually for *nef, vpu*, or *vpr*.



Results

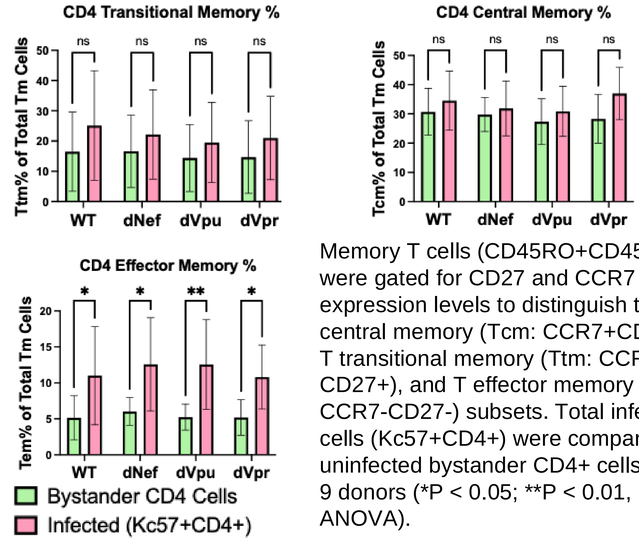
Figure 1: Gating strategy.



Gating strategy. Live, singlet CD3+CD8- cells were gated for the total population of p24-expressing infected cells (KC57+) or productive-infected p24-expressing cells that have downregulated cell-surface CD4 (KC57+CD4-). Memory CD4 T cells subsets (CD45RO+CD45RA-) were gated for CD27 and CCR7 to identify the T central memory (Tcm), T effector memory (Tem), and T transitional memory (Ttm) subsets of memory CD4+ T cells.

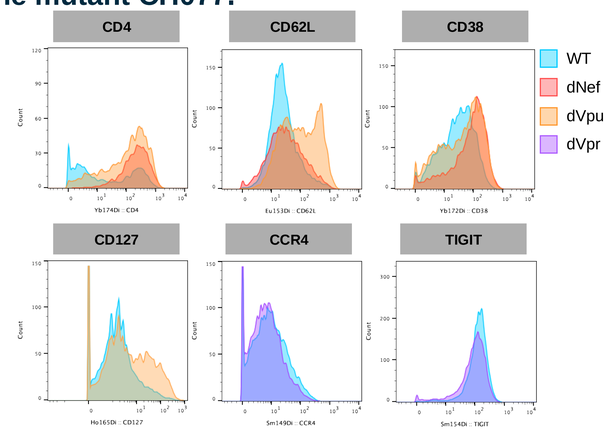
Results

Figure 2: WT and mutant CH077 all preferentially infect CD4+ T effector memory cells.



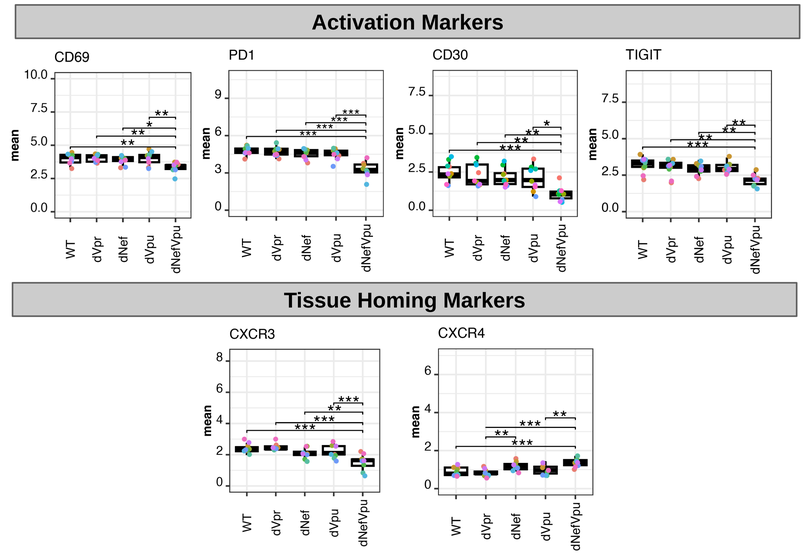
Memory T cells (CD45RO+CD45RA-) were gated for CD27 and CCR7 expression levels to distinguish the T central memory (Tcm: CCR7+CD27+), T transitional memory (Ttm: CCR7-CD27+), and T effector memory (Tem: CCR7-CD27-) subsets. Total infected cells (Kc57+CD4+) were compared to uninfected bystander CD4+ cells across 9 donors (*P < 0.05; **P < 0.01, 2-way ANOVA).

Figure 3: Activation, tissue homing, and cell survival markers differ in cells infected with WT vs accessory gene mutant CH077.



Marker expression of total p24-expressing infected cells (KC57+). dNef- and dVpu-infected cells exhibited less cell-surface CD4 downregulation than WT-infected cells, as expected due to the known ability of Nef and Vpu to downregulate cell-surface CD4. Cells infected with WT CH077 also downregulated lymph node homing receptor CD62L and activation marker CD38 relative to dNef- and dVpu-infected cells. Relative to dVpu-infected cells, WT CH077-infected cells exhibited downregulation of stemness-associated CD127. Finally, dVpr-infected cells exhibited diminished CCR4 and TIGIT relative to WT CH077-infected cells.

Figure 4: Activation and tissue homing receptor expression levels differ in productively-infected cells in the absence of both Nef and Vpu.



Marker analysis of productively-infected cells that have downregulated cell-surface CD4 (KC57+CD4-). Expression levels of activation markers CD69, PD1, TIGIT, and CD30, and homing receptor CXCR3, are decreased in the *dNefVpu* double mutant. By contrast, CXCR4 is increased in the *dNefVpu* double mutant.

Summary

- Tem are preferential subset for HIV infection in irrespective of expression of Nef, Vpu, and Vpr.
- CD4+ T cells infected with dNef, dVpu, and dVpr differentially express markers of of activation, tissue homing, and cell survival markers relative to their WT-infected counterparts.
- Cells productively-infected with CH077 deficient in both Nef and Vpu express lower levels of CD69, CD30, PD1, TIGIT, and CXCR3 but higher levels of CXCR4 relative to their WT infected counterparts.

Future Directions

- Determine mechanisms leading to differential remodeling of HIV-infected cells in the absence of Nef, Vpu, and Vpr and its functional consequences.
- Determine transcriptomic differences in cells infected with WT vs. accessory gene mutants using scRNAseq and to associate these changes with the observed proteomic changes observed by CyTOF

References

(1) Bendall, S. C., Nolan, G. P., Roederer, M., & Chattopadhyay, P. K. (2012). A deep profiler's guide to cytometry. *Trends in immunology*, *33*(7), 323–332. https://doi.org/10.1016/j.it.2012.02.010