HIV-1 Vpr Promotes Viral Replication and Death of Primary Gut CD4 T Cells *Ex Vivo*

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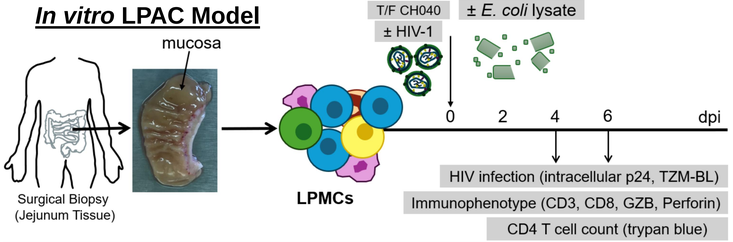
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Background

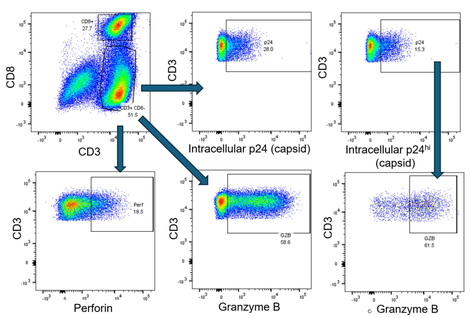
- HIV-1 protein Vpr has been linked previously to many functions including: cell cycle arrest[1], DNA damage[2], and apoptosis[3] *in vitro* in myeloid cells.
- No consistent phenotype in replication for Vpr deletion (HIV-1ΔVpr) has been found in primary CD4 T lymphocytes[4].
- This lack of phenotype is incongruous with Vpr being packaged in virions as it should exert influence early in infection, where CD4 T cells, particularly from the gut, are critical.
- The gastrointestinal tract is a major site of early and massive HIV-1 replication and CD4 T cell depletion following HIV-1 infection.
- However, most *in vitro* studies on HIV-1 Vpr have focused on CD4 T cell lines and mitogen-activated blood CD4 T cells using lab-adapted strains.
- In this current study, we utilized transmitter/founder HIV-1 in our *in vitro* Lamina Propria Aggregate Culture (LPAC) model[5] to determine if HIV-1ΔVpr will have a phenotype in the physiologically-relevant primary CD4 T cells of the gut.

Methods

In vitro LPAC Model



Flow Cytometry Gating Strategy:



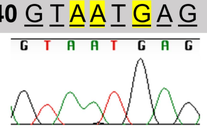
Standard flow cytometry staining protocols were used to stain for viability, cell surface and intracellular markers. Lamina propria CD4 T cells were identified within viable single cells as CD3+ CD8- T cells due to the down-regulation of CD4 by HIV-1[6]. Gates for Granzyme B (GZB) expression and perforin expression were established using isotype control. Intracellular p24 expression was established using mock-infected cultures. BD LSRII utilized for collection of cells.

HIV-1ΔVpr Mutant Virus:

Mutant CH040 HIV-1 plasmids were created utilizing specific primers, PCR, and kinase ligation with the Q5 site-directed mutagenesis kit (New England Biolabs). PCR was performed on CH040 WT plasmids (GenBank #JN944939.1; AIDS Reagent Program). Vpr mutagenesis altered the 22nd and 23rd codons (both for leucine) to stop codons.

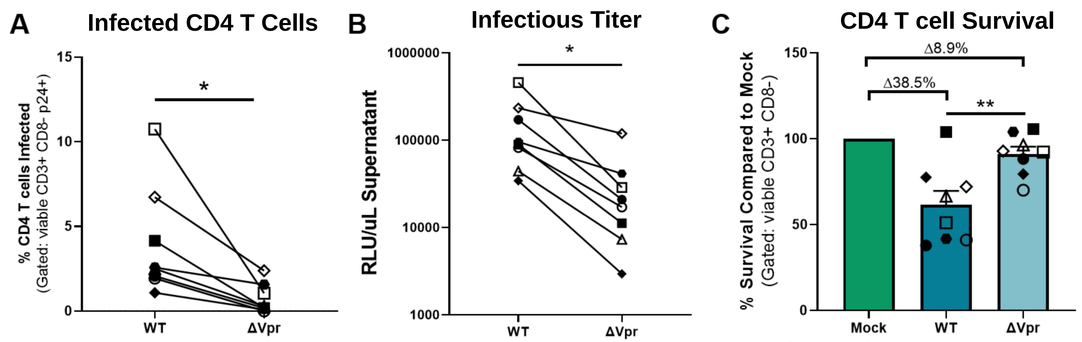
WT CH040 G C T T T T A G

ΔVpr CH040 G T AA T G A G



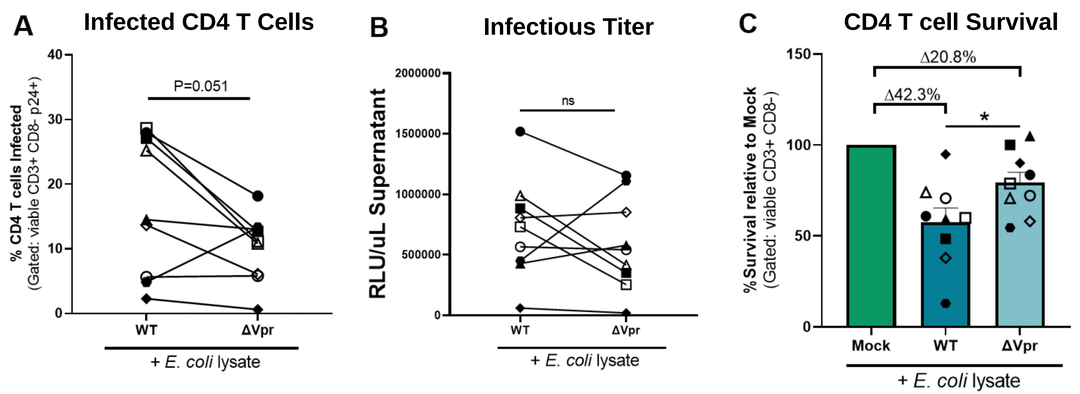
HIV-1 protein Vpr is critical for robust viral replication and depletion of CD4 T cells from the gut.

HIV-1ΔVpr Replication and Depletion Defect in Primary Gut CD4 T cells



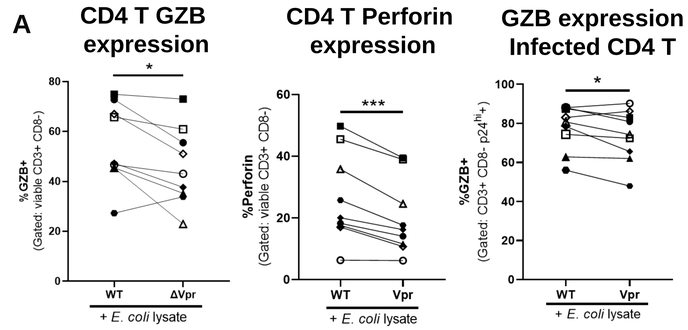
LPMCs were spinoculated with Transmitter/Founder (TF) HIV-1 (CH040; WT or ΔVpr 5.71x10^8 RLU/10^6 LPMC) or mock infected and cultured for 4 days. **A.** Percentages of productively infected (p24+) CD3+CD8- T cells (N=8) *P<0.05. **B.** Infectious titer (relative light units/uL supernatant) present in 4dpi cell culture supernatant from LPMC culture. (N=8) **C.** HIV-specific LP CD4 T cell depletion was enumerated based on the number of viable LPMC collected 4dpi (determined by trypan blue exclusion) multiplied by the percentage of CD3+CD8- T cells in viable LPMC determined using flow cytometry. The percent depletion relative to mock is then calculated. (N=8) **P<0.01. Bars indicate mean ± SEM.

Presence of Enteric *E. coli* Abrogates Replication Defect of ΔVpr in Primary Gut CD4 T cells but Cell Depletion Remains Decreased



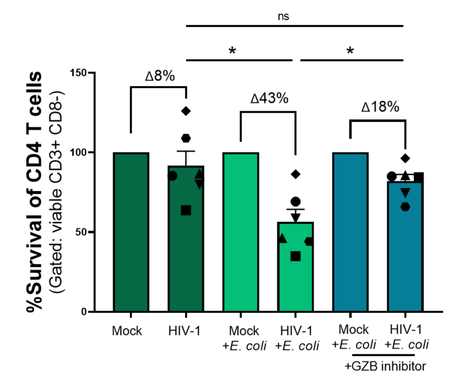
LPMCs were spinoculated with Transmitter/Founder (TF) HIV-1 (CH040; WT or ΔVpr 5.71x10^8 RLU/10^6 LPMC) or mock infected and cultured with commensal *Escherichia coli* lysate (10μg/ml) for 4 days. **A.** Percentages of productively infected (p24+) CD3+CD8- T cells (N=9). **B.** Infectious titer (relative light units/uL supernatant) present in 4dpi cell culture supernatant from LPMC culture. (N=9) **C.** HIV-specific LP CD4 T cell depletion was enumerated based on the number of viable LPMC collected 4dpi (determined by trypan blue exclusion) multiplied by the percentage of CD3+CD8- T cells in viable LPMC determined using flow cytometry. The percent depletion relative to mock is then calculated. (N=9) *P<0.05; Bars indicate mean ± SEM.

HIV-1ΔVpr Infection Results in Lower Granzyme B and Perforin Expression in Gut CD4 T cells



A. LPMCs were spinoculated with Transmitter/Founder (TF) HIV-1 (CH040; WT or ΔVpr 5.71x10^8 RLU/10^6 LPMC) or mock infected and cultured with commensal *Escherichia coli* lysate (10μg/ml) for 4 days. N=9; *P<0.05, ***P<0.001. Each dot is a separate LPMC donor, connected dots indicate same donor.

B GZB inhibition rescues HIV-1 mediated CD4 T cell killing



B. LPMCs were spinoculated with TF HIV-1 (CH040; 200ng p24/10^6 LPMC) or mock infected and cultured with or without commensal *E. coli* lysate (10μg/ml) in the presence/absence of the GZB inhibitor Z-AAD-CMK (250μM) for 4 days. In the presence of *E. coli lysate*, HIV-1 mediated CD4 T cell depletion was, on average, 43%; in the presence of GZB inhibitor, LP CD4 T cell depletion decreased to 18%[7]. N=6; *P<0.05, NS: not significant.

Conclusions

- Our findings demonstrate that Vpr is critical for robust HIV-1 replication and depletion of CD4 T cells in the gut
- In the context of microbial exposure, Vpr was dispensable for gut CD4 T cell replication, but Vpr still contributes to death, possibly through alteration of granzyme B and perforin expression
- These data underscore Vpr as an essential component of the HIV-1 life cycle in CD4 T cells and highlight novel functional attributes of HIV-1 Vpr in mucosal immunopathogenesis

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