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# SHIV-infected CD4 T cells in CSF during early acute infection of rhesus macaques

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## INTRODUCTION

The cellular origin of HIV-1 viremia in CSF and the CNS in general is unknown. Multiple cell types have been proposed to support viral replication within the CNS, including perivascular macrophages and microglia1. There is also evidence that virus replication occurs within the CNS during primary infection and that this virus is CD4-tropic2,3. We aim to determine whether cells within the CNS support viral replication during acute infection and if so estimate the frequency of infected cells relative to other compartments. Historically the limited number of cells in lumbar puncture specimens has made investigation of cell-associated virus technically challenging, but recent advances in applying molecular biology to small numbers of cells now enable investigation of such samples. Identification of infected cells in the CSF will add to our understanding of early mechanisms of HIV-1 entry, tropism, and replication in the CNS. It may also inform therapeutic strategies to target infected cells within the CNS to alleviate neuropathological symptoms in HIV-1-infected individuals.

## CONCLUSIONS

- SHIV-infected CD4 T cells were identified in the CNS during early acute SHIV infection.
  - CSF CD4 T cells harbor both spliced (env-vpu) and unspliced (gag) transcripts as early as 4 weeks post-infection.
  - Infected CD4 T cell frequency was similar across CSF, PBMC, and LNMC, suggesting limited compartmentalization during acute infection
- CD4 and CD8 T cell activation increased in both PBMC and CSF during acute SHIV infection.
- Blood monocytes were activated during acute infection, but CSF macrophage activation was not apparent. No SHIV-infected macrophages were observed in CSF, but sensitivity was limited by the small sample size of CSF macrophages.
- Our data supports a model of productive CD4 T cell infection within the CNS during acute SIV/HIV infection, with similar levels of T cell infection across blood, lymph nodes and CSF.

## REFERENCES

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2. Valcour, V et al. "Central nervous system viral invasion and inflammation during acute HIV infection." JID; 15;206 (2) (2012): 275-82.

3. Sturdevant, CB et al. "Compartmentalized replication of R5 T cell-tropic HIV-1 in the central nervous system early in the course of infection." Plos Pathogens; 11(3):) (2015):

## STUDY DESIGN

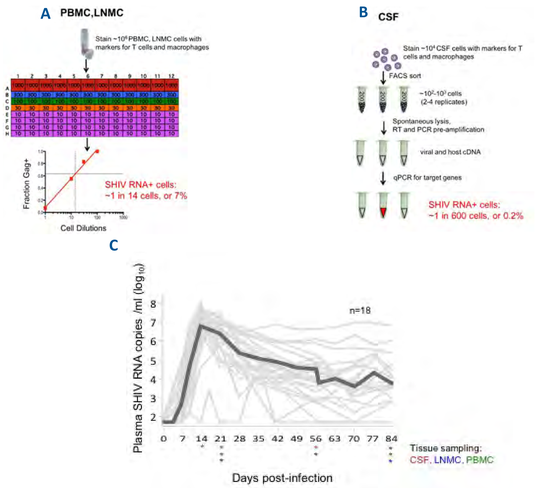



**Figure 1. Method used to estimate SHIV-infected cells from macaque specimens.** Cells from PBMC and LNMC (A) or CSF (B) samples were directly sorted, lysed and RT-PCR pre-amplified to generate viral and host specific cDNA. Infected cell frequency was estimated using Poisson distribution statistics for PBMC and LNMC samples or by assigning 1 Infected cell to every positive replicate for CSF samples. (C) Plasma viremia is shown for 18 rhesus macaques infected with SHIV-1157ipd3N4 intrarectally. PBMC, LNMC, and CSF specimens were collected at the indicated time points (*).

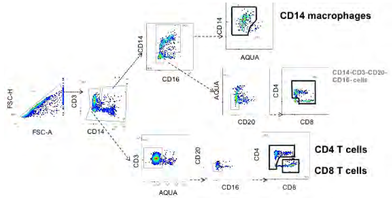



**Figure 2. FACS gating strategy to isolate viable hematopoietic cells from macaque CSF.** Macrophages, CD4 T and CD8 T cells from CSF samples were FACS sorted directly ex vivo. FACS gating strategy for all cell populations is depicted.

## RESULTS

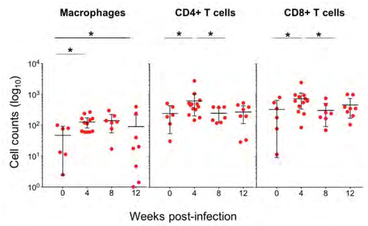



**Figure 3. Multiple hematopoietic cell populations increased in CSF as early as 4 weeks post-SHIV infection.** Macaque CSF cell pellets analyzed on a flow cytometer were assessed for the number of macrophages, CD4 T and CD8 T cells at the indicated time following SHIV-1157ipd3N4 infection. Cell counts were normalized to CSF supernatant volumes by dividing the number of cells by the starting CSF volume. Normalized cell counts were compared over time and significant differences are indicated (*, p<0.05 Mann-Whitney test).

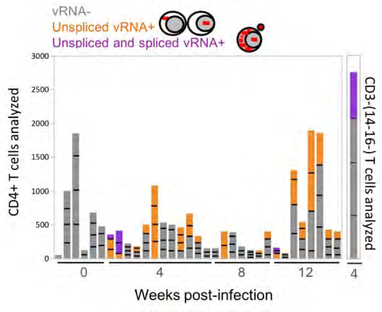



**Figure 4. CD4+ T cells are productively infected in CSF of SHIV-infected rhesus macaques by week 4 of infection.** Macaque CSF CD4+ T cells were FACS sorted into replicates of the indicated number of cells. Each bar depicts a CSF sample from an uninfected (week 0) or SHIV-infected animal. Each replicate is separated by a black bar and color coded by viral RNA status as assessed by qPCR. Three possible infection states are indicated: uninfected (env(-)gag(-) , gray), early/abortively/latently infected (env(-)gag(+) , orange), or productively infected (env(+)gag(+), purple).

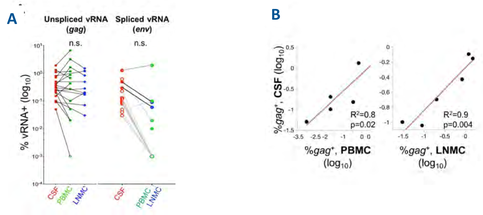



**Figure 5. SHIV-infected (vRNA+) CD4 T cell frequency is similar in CSF, PBMC, and lymph nodes.** Infected CD4+ T-cell frequencies were estimated in CSF, PBMC, and LNMC from SHIV-1157ipd3N4-infected rhesus macaques by viral gene qPCR for gag (unspliced, genomic vRNA) and env (spliced vRNA, indicating de novo viral gene expression or active infection) at weeks 4, 8, 12 post-infection. Estimates performed on the same animal at concurrent time points across multiple tissues are compared (A) and assessed for correlations across compartments (B). Open symbols represent limit of detection in samples where no vRNA was detected.

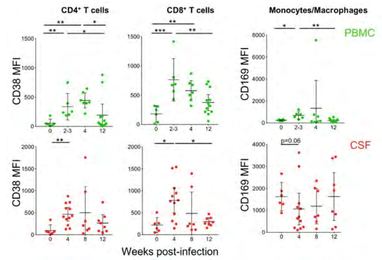



**Figure 6. Activation of CSF CD4+ and CD8+ T cells very early in SHIV infection, while monocyte/macrophages activation limited to blood.** SHIV-infected macaque CSF and PBMC T lymphocyte and myeloid cell populations were assessed for activation marker expression by flow cytometry during the first three months of infection. Significant differential surface protein expression is indicated (*, p<0.05 Mann-Whitney test).

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