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Ex Vivo Isolation and Characterization of Live, Envelope Expressing, SHIV-infected CD4 T cells.

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BACKGROUND

- Fully mature SHIV envelope is transported to the CD4 T cell surface. Therefore, expressed envelope represents a means of identifying live SHIV-infected CD4 T cells.

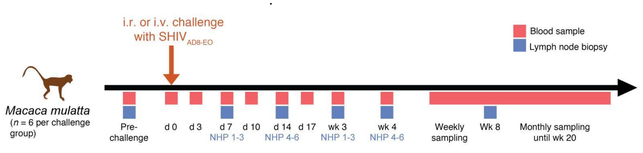
METHODS

- CD4 T cells from Fiebig-staged SHIV AD8EO-infected rhesus macaques were stained using 2 fluorescently labelled broadly neutralizing antibodies previously shown to identify *in vitro* SHIV AD8EO-infected rhesus macaque CD4 T cells. SHIV-infected cells from these 2 rhesus macaques were index sorted between Fiebig stages 1 and 6. For each animal lymph nodes (LNs) were sampled 3 times and blood was sampled 5 times. SHIV infection was confirmed by measurement of single cell SHIV gag-RNA by RT PCR. SmartSeq NGS libraries were prepared, then sequenced using an Illumina NovaSeq 6000. Bcl2fastq2, Picard and HTSeq were used to demultiplex, sequence and calculate read number for each gene. Benjamini Hochberg correction was used for multiple comparison correction.

RESULTS

- The frequency of SHIV-infected cells in both peripheral blood and lymph node (LN) CD4 T cells, intracellular SHIV RNA copy number and blood viral load all peaked between Fiebig stages 2 and 5 and then decreased in these macaques. During this time the frequency of SHIV infected cells increased in PD1 positive and CD28 dim CD4 T cells and then decreased. Intracellular SHIV RNA was inversely associated with CD3 and CD4 fluorescence intensity. Infection of germinal center CD4 T cells was discordant in these animals. One animal showed no germinal center CD4 SHIV infection. In the other, most infection occurred in the germinal center. Envelope staining did not strictly identify SHIV-infected CD4 T cells. In some populations the frequency of SHIV RNA in envelope stained CD4 T cells was close to 40%. This allowed the comparison of SHIV$^-$ infected and non-infected CD4 T cells in 57 Env$^+$SHIV RNA$^+$ and 63 Env$^-$SHIV RNA$^-$ LN CD4 T cells from a single lymph node. The frequency of SHIV RNA was as high as 5% of the sequenced transcriptome in some cells. The frequency of HIV RNA reads increased with CD3 and CD4 down regulation. GSEA analysis suggests G2M arrest and decreased antiviral responses in SHIV-infected CD4 T cells.

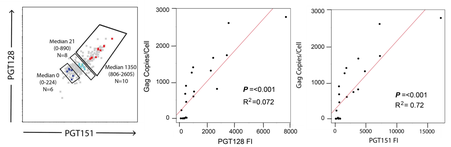
Six Rhesus Macaques were infected with 100x $TCID_{50}$ of SHIV AD8-EO and then followed over a course of 8 weeks as shown below. Throughout the study animals were Fiebig staged



from Dias, J *et al.* (2021) JCI131(17) doi 10.1172/JCI151632

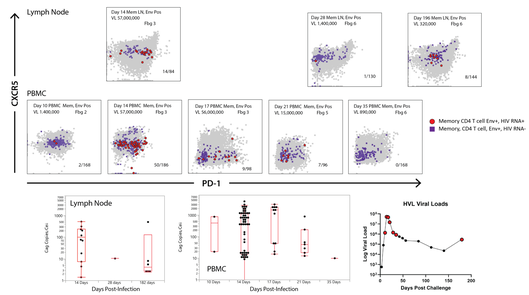
Ex vivo isolation of acutely infected SHIV was done using α-HIV envelope Abs. Subsets of CD4 T cells with greatly increased frequencies of infection were identified, allowing transcriptome analysis of infected CD4 T-cells.

In Vitro Infection- Rhesus Macaque cells were infected with AD8EO. stained with PGT 121 and PGT151, index sorted and SHIV Gag RNA extracted and measured. Envelope staining intensity predicted Gag RNA copy Number/cell.

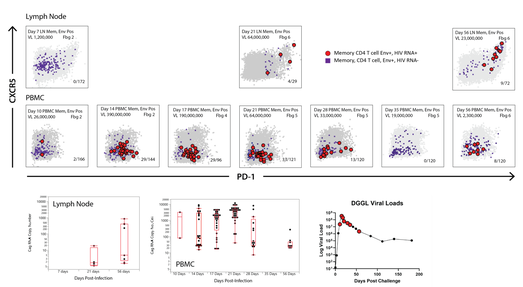


Ex Vivo infection- Six rhesus macaques were challenged with 100x TCID 50 of AD8EO by IV infusion and then followed for between 7 and 56-120 days. CD4 T cells were stained, index sorted based on envelope staining and HIV RNA measured. Data from 2 animals are presented here.

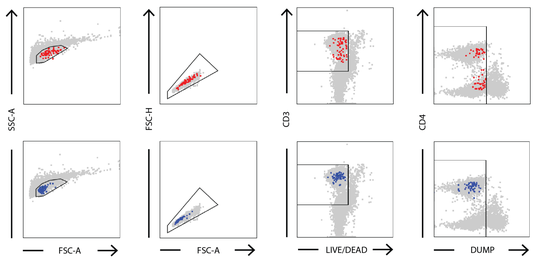
Macaque HVL

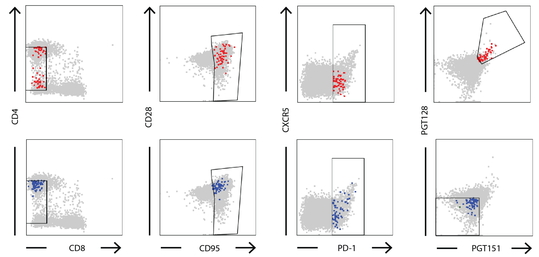


Macaque DGGL

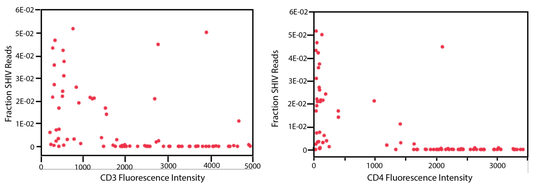


Based on earlier sorts, 14d post-infection LN cells from HVL were stained and index sorted for NGX sequencing base on presence and absence of measurable envelope staining. Only cells that are envelope positive with greater than 30 HIV reads/million are shown in red, cells that are envelope negative with no detectable HIV reads/million are shown in blue.

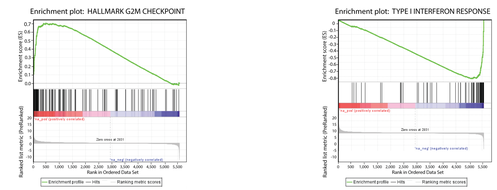




In CD4 T cells containing measurable amounts HIV read frequency was strongly associated with CD3 and CD4 fluorescence intensity. HIV read frequency was also linearly associated with PGT 128 and PGT151 fluorescence intensity (P<0.0001) but the association was weak (R^2 = 0.25 and 0.19).

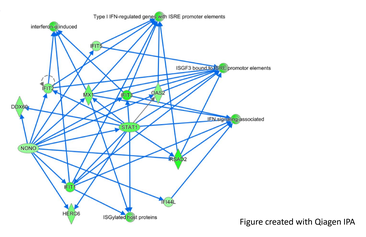


Transcriptome analysis using both GSEA and IPA showed strong evidence of decreased Type 1 interferon responsiveness (q<0.001) and a high percentage of SHIV-infected cells being in G2-G2/M stage (q<0.001)



Support for a higher frequency of envelope$^+$ HIV RNA positive$^+$ cells being in G2/M than in envelope$^-$ HIV RNA$^-$ CD4 T cells comes from increased levels of SMC2 (*P*<0.0009)), SMC4(*P*<0.00036), cyclin B (*P*<0.028), PRC1 (*P*<0.038) and FOX M1 (*P*<0.031) in envelope$^+$ HIV RNA positive$^+$ CD4 T cells. Decreased type I interferon responsiveness was suggested by decreased mRNA frequencies of many directly interconnected type I interferon responsive genes.

Gene	q	Log2FC
DDX60	5.64E-03	-1.64
EIF2AK2	1.01E0-6	-1.25
HERC6	8.33E-03	-1.69
IFI44L	2.53E-02	-1.21
IFIT1	2.38E-03	-2.44
IFIT2	2.50E-02	-1.70
IFIT3	7.76E-05	-2.85
IFIT5	2.67E-02	-1.38
MX1	2.37E-03	-1.91
NONO	1.48E-02	-1.30
OAS2	4.2E-03	-1.56
RSAD2	2..4E-02	-2.70
STAT1	2.05E-05	-2.07



CONCLUSIONS

- *Ex Vivo* SHIV-infected CD4 cells are identifiable at increase frequency by surface staining with fluorescently labelled anti-envelope antibodies.
- The highest frequency of SHIV infected lymph node CD4 T cells occurs during Fiebig 2-3 and then rapidly decreases.
- Acutely infected SHIV-infected CD4 T cells tend to be larger than uninfected CD4 T cells and decrease CD3, CD4 and CD28 surface expression. They also tend to be PD1 bright.
- Decreased CD3 and CD4 expression along with increased surface expression of envelope correlate with intracellular viral load.
- Decreased CXCR5 expression in early infection suggests initial SHIV infection does not occur in the B cell follicle.
- Transcriptome analysis is consistent with a greater proportion of infected cells being in G2/M than uninfected cells and a decreased type I interferon responsiveness .
- These data suggest that targeting infected CD4 T cells using anti-envelope antibodies may be a feasible means of targeting HIV-infected cells.