Ex Vivo Single Cell Transcriptome Sequencing of Human Lymph Node HIV-infected CD4 T Cells from Viremic Individuals

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ABSTRACT

Background- Identification of live HIV-infected CD4 cells allows the characterization of these cells both by single cell flow cytometry and by transcriptomic analysis.

Methods- Cervical lymph nodes were obtained from 3 viremic HIV infected-individuals, median CD4 count 671(range 247-1288) and median viral load 17437(14075-29923). Approximately 90 Individual CD45RO$^+$ PD-1^{hi} CD4^{dim} cells were index sorted into individual wells for each lymph node. Cells were stained with two fluorescently labelled bnAbs, PGT121 and PGT151. Whole transcriptome libraries were generated from the purified RNA of sorted single cells using established methods and deep sequenced on an Illumina HiSeq (Trombetta, JJ *et al.* (2014) *Curr Protoc Mol Biol* 4.222.1-422.17).

Results- Of the PD-1 bright CD4 cells sorted without bias with respect to HIV env staining, 4.4, 4.1 and 4.4% contained significant HIV RNA. In PD-1 bright CD4 cells surface stained by both PGT151 and PGT121 the frequency of HIV RNA positive CD4 cells was 7, 11 and 33%. Median (range) total HIV RNA reads as a percentage of total cellular RNA was 0.032(0.02-0.06)%, 0.16(0.06-0.3)% and 0.03(0.01-6.83)%. Although median frequency of HIV reads was significantly lower than was seen in 3 day HIV-infected primary CD4 T cells, the highest frequencies of HIV RNA measured *ex vivo* was comparable to that observed in *in vitro* infection.

Conclusions- These data show that it is possible to identify HIV-infected CD4 T cells at a level which makes whole cell transcriptome analysis of HIV RNA$^+$ and HIV RNA$^-$ cells possible.

Introduction- HIV-infected cells can be differentiated from HIV-uninfected cells by surface staining HIV env expression on the cell surface. Current next generation methods make sequencing of the whole cell transcriptome possible for individual cells. We have characterized HIV transcription in BAL infected primary cells, and performed preliminary analyses of HIV transcription in lymph node cells ex *vivo*.

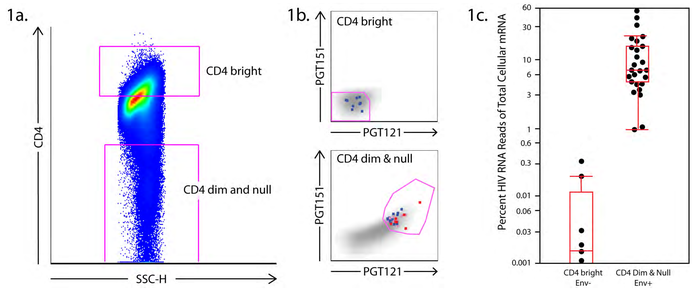


Figure 1. Index sorting of *in vitro* infected, purified primary CD4 T cells and percent HIV reads of total individual cellular mRNA 72h after BAL infection 1a.) Plots showing gating of CD4 bright and CD4 dim and null cells. CD4 bright cells were taken from brightest half of CD4 cells to minimize the possibility of selecting CD4 cells in which HIV-induced early down regulation of CD4 had occurred. 1b.) Density plots of CD4 bright and CD4 dim and null cells for staining with PGT121 and PGT151 with individual index sorted cell overlaid. Cells from the CD4 bright population which did not stain for Env and cells from the CD4 dim and null population which stained most brightly for Env were selected for analysis. In Env+ cells, cells within the highest quartile frequency of HIV reads are shown in red, the second quartile in lavender, and in the bottom 2 quartiles in blue. 1c.) A comparison of the frequency of HIV reads as a percentage of the total cellular mRNA for CD4 bright, Env$^-$ and CD4 dim and null, Env$^+$ cells. The frequency of HIV reads between these two groups was significantly different at a P of <0.001.

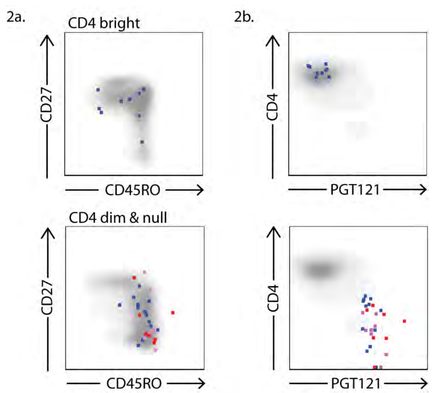


Figure 2. Overlay of sorted CD4 bright Env$^-$ and CD4 dim and null Env$^+$ cells onto density plots showing CD27 and CD45RO staining and CD4 and PGT121 staining. Cell within the highest frequency quartile of HIV reads are shown in red, the second quartile in lavender, and in the bottom 2 quartiles in blue. Higher frequency HIV RNA transcription is associated with CD27$^-$CD45RO$^+$ cells and higher PGT121 staining and increased CD4 down-regulation.

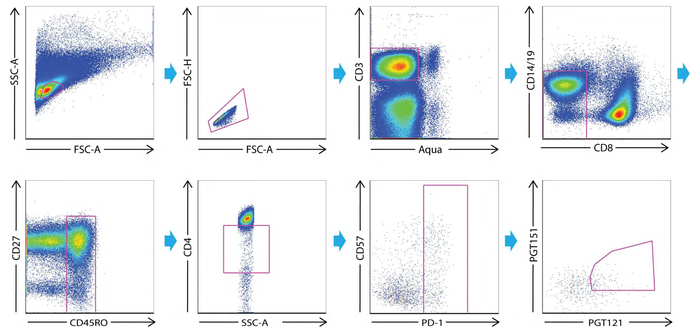


Figure 3. Gating of lymph node CD4 T cells. Preparations of frozen cells from 3 cervical lymph nodes were thawed and small lymphocytes were selected based on forward and side scatter parameters, then singlets were selected based on FSC-A and FSC-H, then live CD3$^+$ cells based on Aqua staining. Next, TCRγδ$^+$, CD56$^+$, CD14$^+$ CD19$^+$ and CD8$^+$ cells were excluded and then memory CD8$^-$ T cells were selected based on CD45RO staining. CD4 dim cells and then PD-1 bright were selected. Finally, PGT 151+ and PGT121+ cells were selected from PD-1 bright cells.

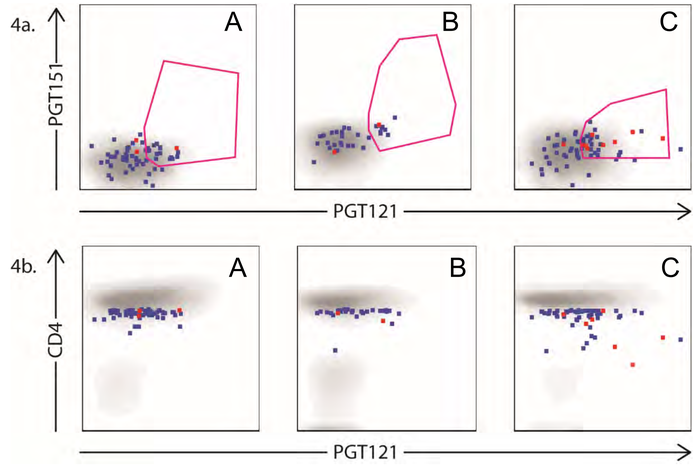


Figure 4. Cells containing viral RNA were associated with cells staining for PGT151 and PGT121. a) PD1 bright CD4 dim and null cells were sorted based on staining with PGT121 and PGT 151. Of the cells sorted without respect to env staining, 4.4, 4.1 and 4.4 % contained viral RNA; of those that were PGT121 and 151 positive, as indicated by populations outlined in red, 7.1, 11 and 33% of the cells sorted contained viral RNA. Individual index sorted HIV are overlaid onto density plots of PD1 bright, CD4 dim and null cells. Cells containing viral RNA are shown in red, those not containing measurable RNA are shown in blue. In all cells shown >80% of the sequences measured mapped to human or HIV genomes. Greater than 100 HIV reads were required for cell to be identified as HIV$^+$ b) Overlays of index sorted cells on memory cells showing CD4 and PGT121 staining . Cells containing viral RNA are again shown in red.

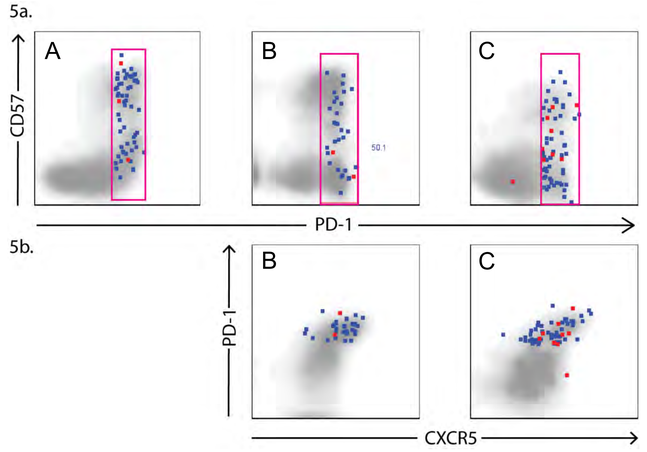


Figure 5. HIV-infected cells were not preferentially CD57$^+$ or CD57$^-$ but did associate with CXCR5. Individual index sorted cells are overlaid on density plots of memory CD4 cells in Fig 5a and PD-1$^+$ memory CD4 dim and null cells in Fig 5b. The distribution of HIV infected CD4 cells is similar to that seen using RT PCR of spliced HIV RNAs to establish HIV-infection. HIV-infected cells occur more frequently in GC Tfh, as identified by CXCR5 and PD-1 staining

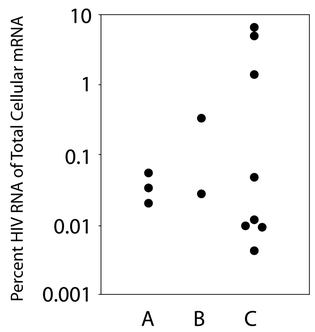


Figure 6. Percentage HIV RNA of Total cellular mRNA in individual cells sorted from 3 cervical lymph nodes from 3 viremic HIV infected individuals.

Conclusions

1. Dual staining of CD4 T cells with PGT151 and PGT121 can be used to identify HIV infected CD4 T cells both *in vitro* and *ex vivo.*
2. Identification of HIV infected CD4 T cells by next generation sequencing gives a pattern of cell surface markers similar to that obtained when spliced HIV RNA is used to identify HIV-infected cells.
3. Staining *in vitro* infected CD4 cells with PGT121 and PGT151 cleanly separates cells with high frequencies of intracellular HIV RNA from those with lower frequencies. Median HIV RNA in CD4 dim and null cells that were Env+ was surprisingly high, 7.1(0.96-54)%. In CD4 bright env- cells the HIV read frequency was 0.016(0.00014-0.3)%.
4. The frequency of HIV RNA reads in HIV-infected PD-1 bright CD4 T cells varied widely. Although the frequency of HIV RNA reads observed *ex vivo* was not as high as that observed in *in vivo* infection, the frequency of HIV RNA in some cells was comparable to that seen in *in vitro* infection. In 2 samples the frequency of HIV RNA was greater than 5% of the total mRNA reads. These data demonstrate the feasibility of coupling flow cytometry data, acquired by index sorting, to individual cell transcriptome data, obtained by next generation sequencing, to study the effects of HIV-infection on cellular RNA and protein expression.

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