#326 SINGLE-CELL TRANSCRIPTOMICS TO EVALUATE HIV LATENCY ESTABLISHMENT IN CD4 T CELLS

Microbiology & Immunology
University of Miami Miller School of Medicine

L de Armas[1], K Russell[1], S Williams[2], R Suter[2], S Rinaldi[1], L Pan[1], S Pallikkuth[1], R Pahwa[1], R T Younis[3], and S Pahwa[1]

[1] Microbiology and Immunology, [2] Sylvester Comprehensive Cancer Center, [3] Pediatric Otolaryngology, University of Miami, Florida

THE MIAMI CENTER FOR AIDS RESEARCH
CFAR
UNIVERSITY OF MIAMI MILLER SCHOOL OF MEDICINE

Abstract

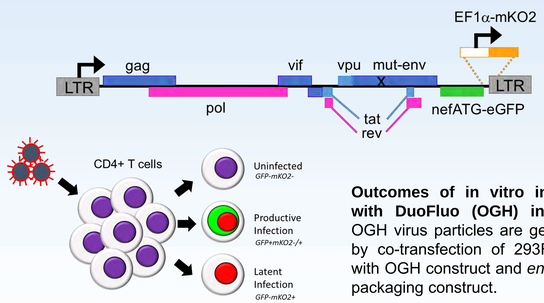
HIV eradication is hindered by the existence of latent HIV reservoirs in CD4 T cells. T follicular helper (Tfh) cells are a subset of CD4 T cells present in lymph nodes (LN) which harbor high frequencies of HIV-infected cells. Here, we used a primary cell model of HIV latency employing the dual reporter virus, HIV DuoFluo (E. Verdin, UCSF) to investigate latency establishment in CD4 and Tfh cells.

Tonsils from HIV-neg donors (n=8) were obtained as a source of LN CD4 T cells. Purified CD4 were activated with anti-CD3/CD28 beads for 3d prior to infection with HIV DuoFluo, containing GFP under HIV-LTR promoter and mKO2 under a constitutive EF1a promoter. Live, uninfected (U, GFP-mKO2-), latent (L, GFP-mKO2+), and productive (P, GFP+mKO2+/-) cells were analyzed 3d later for surface expression of CD4, CXCR5, and PD1 and were sorted in bulk for gene expression (GE) analysis of 96 genes involved in T cell function and metabolism. Single-cell sorting of U, L, and P cells (62 cells per group) was performed using cells from one tonsil donor for 3' whole transcriptome amplification. Limma and FDR correction were applied to determine significant differences in GE.

Tfh (PD1+CXCR5+) were more permissive to latent infection compared to Non-Tfh (PD1-CXCR5-) cells by comparing ratios of L: P (Mean: 0.4 vs. 1.0, respectively, p=0.02 student's t test). Bulk analysis of Tfh cells showed differential GE of 36 genes between P and U and 8 unique genes between P and L cells (ANOVA, p<0.05). 0 genes were exclusively differentially expressed (DEGs) between L and U, though *IL21* was downregulated in P and L Tfh compared to U which may affect overall function of Tfh. P had reduced expression of genes involved in transcriptional regulation and metabolism compared to U, and L had higher expression of TNF superfamily members and restriction factor, *APOBEC3G* compared to P. In single cell analysis, U and L overlapped with 0 DEGs while P showed 202 DEGs compared to U and 155 compared to L (121 overlapped between the 2 comparisons). *FCGR2A*, a proposed marker of latent cells in blood was not detectable in single cell analysis suggesting differences between blood and LN markers of HIV latency.

Background: Model of HIV Latency

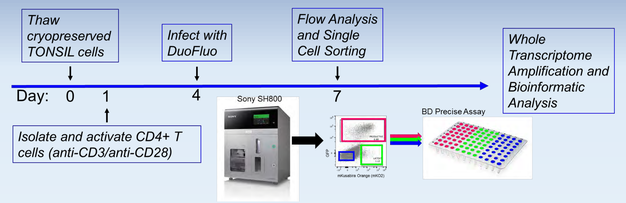
DuoFluo orange-green HIV (OGH) dual reporter virus genome. Construct provided by Emilie Battivelli and Eric Verdin. Schematic adapted from Vranckx *et al*, EBioMedicine 2016.



Outcomes of in vitro infection with DuoFluo (OGH) infection. OGH virus particles are generated by co-transfection of 293FT cells with OGH construct and *env* pSVIII packaging construct.

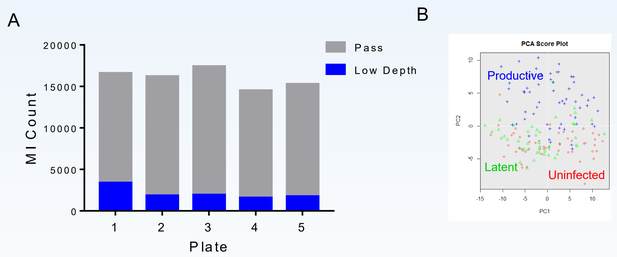
Part II Methods and Results: Single cell Isolation and RNA Sequencing

Experimental design for in vitro infection of Tonsil-derived CD4 T cells followed by single cell sorting and RNA Seq.



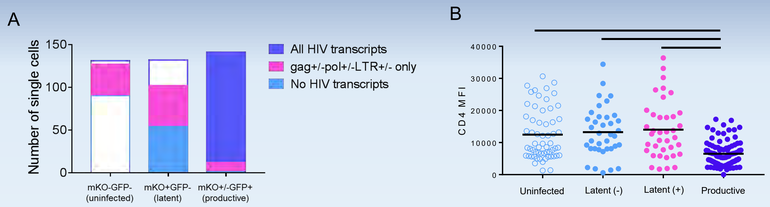
During the reverse transcription step of library preparation, the BD Precise assays apply a non-depleting pool of barcodes for stochastic and unique labeling of mRNA transcripts. Following trimming and QC filtering, data was aligned (STAR) to the annotated human reference genome (Hg19 with gencode.v19) and demultiplexed to retrieve unique molecular index (UMI) counts per transcript for each cell (BD™ Precise WTA Pipeline v2.0).

Initial Sequencing Data and Cluster Analysis of sorted Single cells

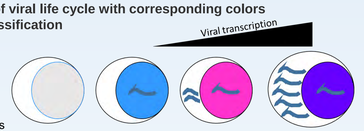


A) Total number of unique molecular index (UMI) counts per 96-well plate of sorted single cells over 5 plates and 3 independent experiments. B) Differential Gene Expression by sorted cell type in Principle Component Analysis (PCA) based on UMI counts (representative from 1 experiment).

Single cell analysis of UMI for HIV transcripts defines new cell populations for downstream transcriptional profiling



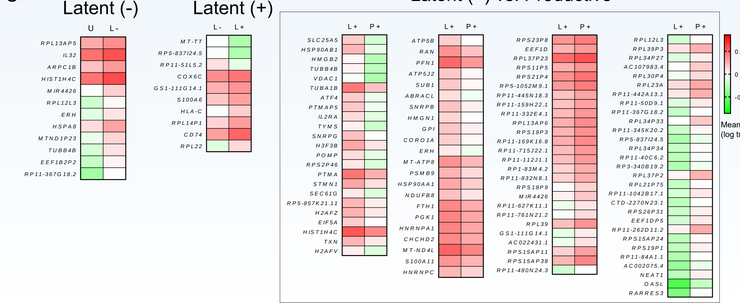
Schematic of viral life cycle with corresponding colors used for classification



HIV transcripts Detected:	Uninfected	Latent (-)	Latent (+)	Productive
Gag/Pol/LTR	-	-	+	+
Tat/Rev/Env/Vpr	-	-	-	+

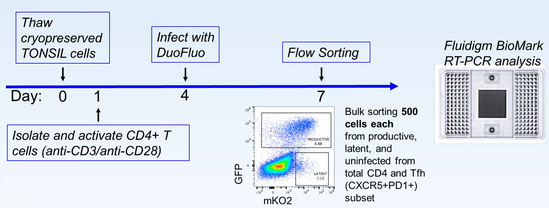
A) Sorted populations were reclassified based on HIV transcript expression. B) Index sorting allowed for measurement of CD4 Mean fluorescence intensity on individual cells as defined by HIV transcript expression. Black lines represent p<0.05 by t test. C) Differential gene expression was analyzed using SingulaR (Fluidigm) package in R between different cell subsets. All genes passed p value cutoff of <0.05 by ANOVA and fold change >1.2.

C



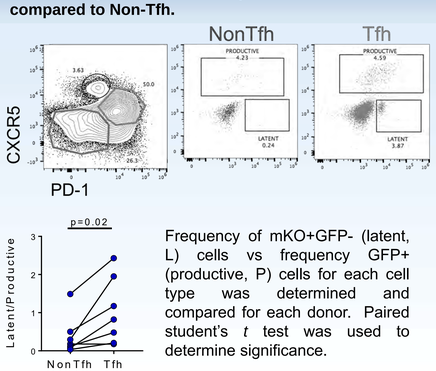
Part I Methods and Results: Latency Establishment in Tfh cells

In vitro infection of Tonsil-derived CD4 T cells followed by sorting and specific target amplification of 96 genes using Fluidigm BioMark platform.



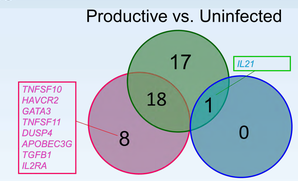
3 days post-infection cells were stained for CD4, CXCR5, PD-1 and live/dead and sorted using a Sony SH800. A 96 target Taqman gene expression panel was applied using Biomark multiplex RT-PCR. (n = 8 HIV-negative donors with tonsillectomy at University of Miami Hospital.

Tfh have higher ratio of latent:productive infection compared to Non-Tfh.



Frequency of mKO+GFP- (latent, L) cells vs frequency GFP+ (productive, P) cells for each cell type was determined and compared for each donor. Paired student's *t* test was used to determine significance.

Differentially Expressed Genes in Tfh cells by Infection Status.



Gene expression of 96 targets in Tfh were contrasted between cell populations and differentially expressed genes (DEGs) were determined by ANOVA using Fluidigm SingulaR. 36 genes were differentially expressed between productive-infected cells (GFP+mKO2-/+) and uninfected cells (GFP-mKO2-) at p <0.05 . Genes were involved in transcriptional regulation and cell metabolism.

Summary and Conclusions

- Latency establishment occurs reproducibly at higher ratios in lymphoid Tfh cells compared to Non-Tfh using in vitro primary cell model of latency. *IL21*, a Tfh signature cytokine, is downregulated in both productive and latent infected Tfh cells which may have implications on overall function in HIV-infected Tfh cells.
- Bulk cell analysis of populations defined by flow cytometry resulted in 0 differentially expressed genes between uninfected and latent cells, while single cell analysis allowed for more refined classification of cells using HIV gene expression and resulted in 12 genes differentially expressed between uninfected and latent cells.
- These studies in LN-derived cells will shed light on the biology of latency in a crucial anatomical site for HIV persistence and are a valuable tool for investigating cure strategies.

This project was made possible by support for the Miami Center for AIDS Research (CFAR) at the University of Miami Miller School of Medicine funded by a grant (P30AI073961) from the National Institutes of Health (NIH), which is supported by the following NIH Co-Funding and Participating Institutes and Centers: NIAID, NCI, NICHD, NHLBI, NIDA, NIMH, NIA, NIDDK, NIGMS, FIC AND OAR and a pilot grant from the Institute of AIDS and Emerging Infectious Diseases awarded to Sion Williams.