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Low Post-ART SIV Viral Load Set Point Associated With Enhanced Lymph Node CD8+ T Cell Proliferation

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Background

- The mechanisms by which CD8+ T cells contribute to post-ART control are unknown.
- Robust HIV-specific CD8+ T cell proliferation (after *in vitro* peptide stimulation) has been consistently associated with spontaneous ("elite") virus control.
- Here, we analyzed baseline (on ART) and early *in vivo* CD8+ T cell responses after analytic treatment interruption (ATI; off ART) in SIV-infected rhesus macaques. The cohort had a range of post-ATI plasma viral load (pVL) set point and a subset of animals exhibited low pVL set points after initial post-ATI viral rebound.

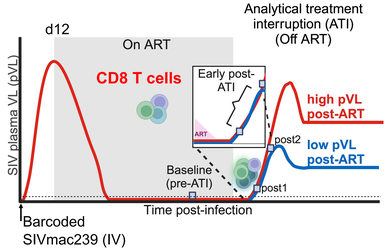
Methods

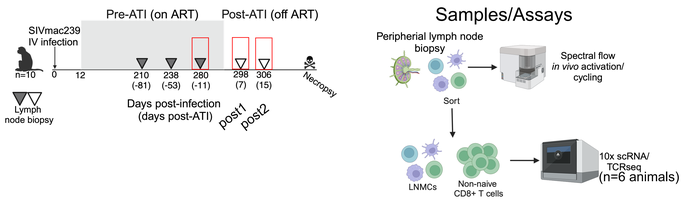
NHP ATI cohort

- IgG control group from Okoye et al. *JCI*. 2021
- 10 animals (n=5 *Mamu* A*01, n=5 *Mamu* B*08)

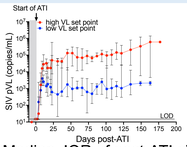
Timepoints:

- Pre-ATI (on ART, viral load undetectable)
- Post1 (day (d) 7 or d8 post-ATI)
- Post2 (d15 post-ATI)

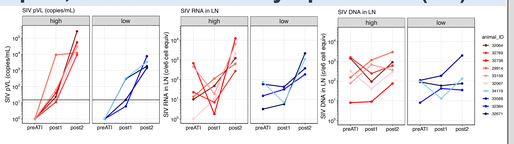




Viral measurements: pVL, SIV RNA/DNA in lymph nodes (LN)

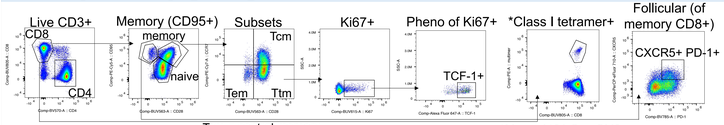


Median, IQR of post-ATI pVL for high (red) and low (blue) set point animals. Post-ATI set point calculated as the median from week (wk) 4 – wk 21 post-ATI



pVL, SIV RNA, and SIV DNA in LN for individual animals at pre-ATI, post1, and post2.

Flow cytometry gating



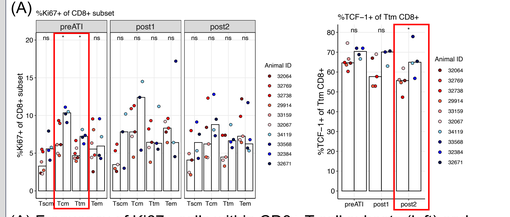
Tcm = central memory
Ttm = transitional memory
Tem = effector memory

Mamu-A*01 Gag CM9 or *Mamu*-B*08 Nef RL10

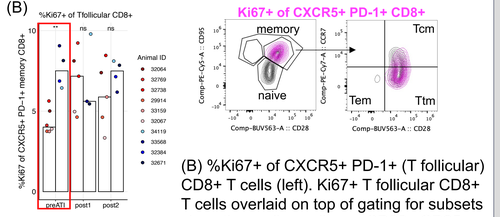
Our data suggest that animals that establish low pVL set points after ATI have a higher frequency of cycling CD8+ T cells present pre-ATI.

Results

Low set point animals: ↑ %Ki67+ transitional/follicular memory CD8+ T cells pre-ATI

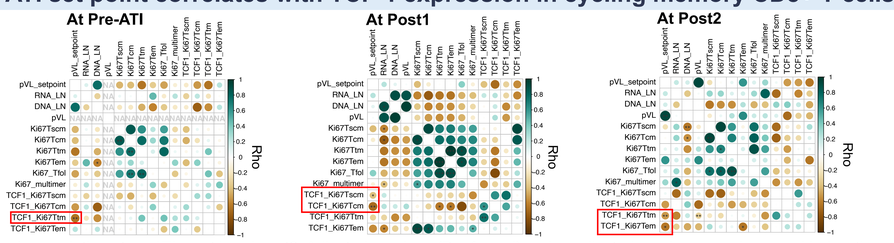


(A) Frequency of Ki67+ cells within CD8+ T cell subsets (left) and frequency of TCF-1+ within Ki67+ transitional memory (Ttm) (right). (Stats: Mann Whiney U test; *p<0.05)



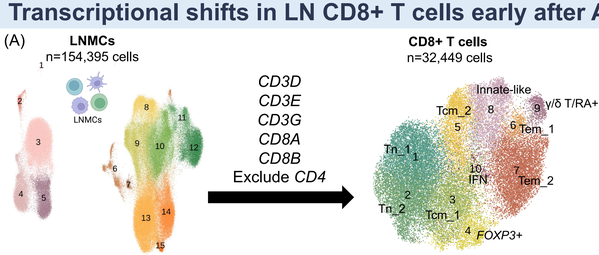
(B) %Ki67+ of CXCR5+ PD-1+ (T follicular) CD8+ T cells (left). Ki67+ T follicular CD8+ T cells overlaid on top of gating for subsets show the cycling cells are CD28+ CD95+ and fall within the Tcm and Ttm gate (right). (Stats: Mann Whiney U test; **p<0.01)

Post-ATI set point correlates with TCF-1 expression in cycling memory CD8+ T cells

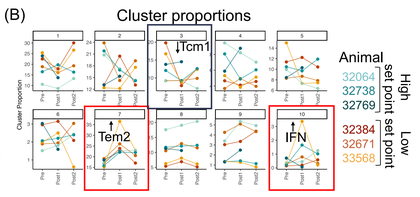


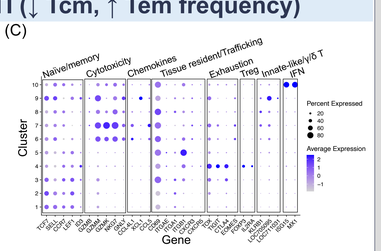
Spearman's correlation at preATI, post1 and post2. Adjusted p-values (Bonferroni) and unadjusted p-values are above and below the diagonal, respectively (p-values: *p<0.05, **p<0.01, ***p<0.001).

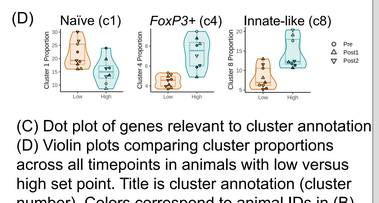
Transcriptional shifts in LN CD8+ T cells early after ATI (↓ Tcm, ↑ Tem frequency)



(A) Total CD8+ T cells were re-clustered after subsetting from the total LNMCs based on the genes listed above the arrow.
(B) Cluster proportions across pre-ATI, post1 and post2. Red boxes indicate clusters of interest for early responses to virus.

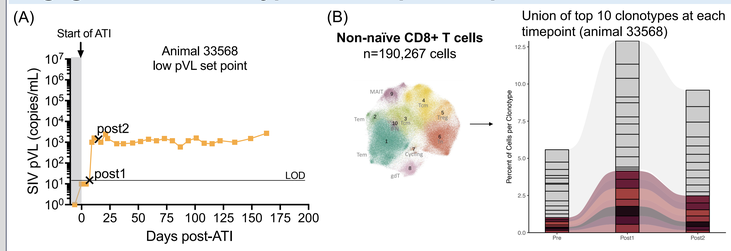






(C) Dot plot of genes relevant to cluster annotation
(D) Violin plots comparing cluster proportions across all timepoints in animals with low versus high set point. Title is cluster annotation (cluster number). Colors correspond to animal IDs in (B).

Early transcriptional changes post-ATI show evidence of TCR engagement in clonotypes that expand at post1



(A) Post-ATI pVL curve for animal 33568 (low pVL animal). "X" indicates pVL at post1 and post2.
(B) Union of top 10 clonotypes (of sorted non-naïve CD8+ T cells) at each timepoint in animal 33568. Colored clonotypes (n=7) were defined as **expanded** (log2 fold change (frequency post1/pre-ATI) > 1.5). Color indicates fold change as depicted in UMAP legend in (C).

(C) Expanded clonotypes from (B) are found within a cluster expressing effector genes annotated as Tem throughout timepoints. Color legend indicates log2 fold change. (D) Differential gene analysis (DEG) of post1 versus pre-ATI in the 7 clonotypes that expand at post1 in (B); method: EdgeR QLF.

Conclusions

- Prior to stopping ART, animals that go on to have lower set points post-ATI have ↑ cycling memory and follicular CD8+ T cells compared to higher set point animals. Animals with low post-ATI set points also maintained ↑ expression of TCF-1 in their cycling transitional memory post-ATI.
- Transcriptionally: no consistent changes pre-ATI -> post-ATI in animals with low versus high set points. There was a subtle ↑ in a more Tem transcriptional state post-ATI.
- Early transcriptional signs of type I IFN signaling and TCR activation in an animal that had expansion of a small number of clonotypes post-ATI. We are exploring antigen specificity of these clonotypes.
- Results from this cohort suggest that enhancing proliferative capacity of CD8+ T cells prior to stopping ART may improve control of pVL set point.

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NIH National Institute of Allergy and Infectious Diseases

DARE

OHSU Oregon National Primate Research Center