LOWER RESERVOIR CONTRIBUTION OF NAÏVE VS. MEMORY T CELLS IN PERINATAL HIV INFECTION

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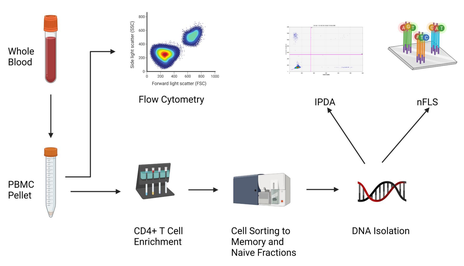
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BACKGROUND

- The memory CD4+ T cell population has canonically been shown to be the major site of the latent HIV reservoir.
- In the pediatric population, perinatal infection results in the establishment of a reservoir that is more resistant to reactivation as compared to the adult population[1], which could be due to higher abundance of proviral reservoirs in naïve CD4+ T cells
- Naïve CD4+ T cells are increasingly recognized to harbor HIV; the percentage of naïve CD4+ T cells is high in infancy and childhood when reservoirs are established and has been shown in the non-human primate (NHP) model to contribute substantially to the intact reservoir[2].
- In adult infections, replication competent HIV has been detected in the naïve population. However, the proviral load in naïve CD4+ T cells is in the minority compared to memory CD4+ T cells[3].
- We assessed the contribution and composition of naïve and memory CD4+ T cells to proviral reservoirs in longstanding, treated perinatal HIV infection.

METHODS

- Study Participants: 10 individuals with longstanding perinatal HIV infection enrolled in an IRB-approved study of HIV persistence were studied, 7 of whom had subtype B HIV-
- Individuals were at a median age of 15.3 years and a median duration of virologic suppression of 8.3 years at time of study.
- CD4+ T cells were sorted using a BD FACSAria into two fractions: naïve CD4+ T cells (CD3+/CD4+/CCR7+/CD45+/CD95-); the remaining CD3+/CD4+ cells were sorted as total memory CD4+ T cells.
- 1 million PBMCs were assessed for CD4+ T cell subpopulations by flow cytometry on a BD FACSymphony.
- The Intact Proviral DNA Assay (IPDA)[4] was used to quantify infected cell frequencies in both memory and naïve CD4+ T cell fractions.
- Near full-length genomes were amplified in limiting dilution and sequenced on the Illumina Miseq. Presence of intact and defective genomes was inferred using the published script HIVseqinR[5].



In individuals with longstanding perinatal HIV infection (median age 15.3 years) and durable ART (median 8.3 years), proviruses in naïve CD4+ T cells represent a small but detectable fraction of the reservoir.

RESULTS

- Naïve CD4+ T cells were present at a median of 63% of CD4+ T cells (IQR 45-71).
- HIV DNA was detected in memory CD4+ T cells in 10/10 participants. Median HIV DNA levels in memory CD4+ T cells were 840.9 copies/10^6 cells (IQR 185.7-1356.0). 7/10 participants had detectable HIV proviruses in naïve CD4+ T cells, with median HIV DNA level of 17.3 copies/10^6 cells (IQR 6.3-85.0).
- Intact HIV DNA in memory cells was detected in all 7/7 participants with HIV subtype B infection (median 39.1 copies/10^6 cells, IQR 12.7-81.8); only 3/7 participants with HIV subtype B infection had detectable HIV DNA in naïve CD4+ T cells (median 4.2 copies/10^6 cells, IQR 2.0-7.4).
- After adjusting for the abundance of naïve cells, contributions to the detectable proviral load and intact reservoir of HIV in naïve cells was 11.6% and 7.3%, respectively.
- Amongst participants with subtype B infection, the median % intact in naïve cells was 14.3% (IQR 7.4-22.1) vs 6.1% (IQR (5.2-14.9) in memory cells, although this difference was not significant (p=0.25).
- Near full length sequences from 4 participants was assessed from memory and naïve population All V3 regions were analyzed by WebPSSM[6], including in defective genomes, all proviral genomes are predicted to be R5 tropic.

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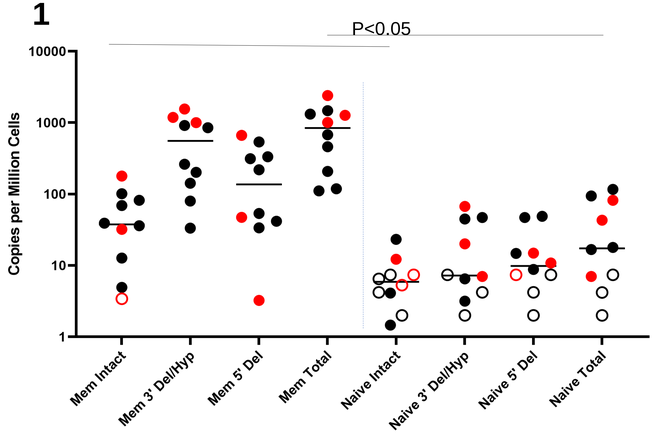


Figure 1: HIV DNA concentrations in memory (left) and naïve (right) CD4+ T cells as measured with the IPDA, separated into intact, 3' deleted/hypermutated, 5' deleted and total copies per million cells. Black circles represent subtype B participants, red represents non-subtype B participants; open circles represent undetectable. Significance was established at p<0.05 with a Wilcoxon test.

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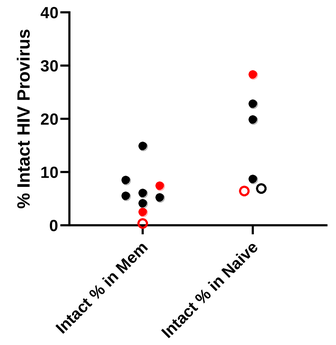


Figure 2: Proportion of intact HIV proviruses in memory (left) and naïve (right fractions) CD4+ T cells from adolescents with longstanding controlled HIV infection. Black circles represent subtype B participants, red represents non-subtype B participants; open circles represent undetectable intact provirus.

3

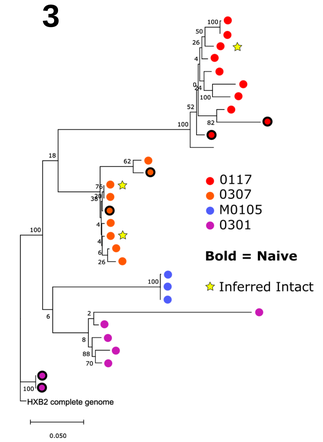


Figure 3: Phylogeny generated from near full-length HIV sequences derived from genomic DNA at limiting dilution of memory and naïve CD4+ T cells of 4 participants. Outlined circles represent proviral genomes from naïve CD4+ T cells, solid circles represent proviral genomes from memory CD4+ T cells. Stars represent inferred intact proviral genomes.

CONCLUSIONS

- In individuals with longstanding perinatal HIV infection controlled on long-term ART, the majority of proviruses reside in memory CD4+ T cells, including intact proviruses.
- Nevertheless, HIV proviruses were detected in naïve cells in a subset of participants, including intact proviruses, confirming the existence of a reservoir in naïve CD4+ T cells
- The proportion of intact proviruses was higher in naïve compared withs memory CD4+ T cells, although more studies are required to confirm this observation.

ACKNOWLEDGEMENTS AND REFERENCES

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- Flow cytometry and cell sorting was provided by the Johns Hopkins Bloomberg School of Public Health Flow Cytometry and Cell Sorting Core.
- The MGH CCIB DNA Core was use for sequencing, Dr. Guinevere Lee provided guidance on limiting dilution near full length PCR, sequencing and analysis.

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