Distinct Populations of Naïve and Memory CD4+ T Cell Clones in Children with HIV and on Long Term Antiretroviral Therapy (ART)

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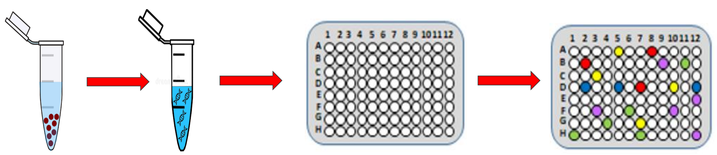
BACKGROUND

This work was conducted to genetically characterize HIV infection in naïve CD4+ T cells, which comprise 90% of the total CD4+ T cell population in neonates. PBMCs were collected from 8 children who were born with HIV, to whom ART was administered at a median age 8.75 months, and from whom samples were collected at a median age of 8.5 years **(Table 1)**. DNA was extracted from these samples and viral DNA quantified, evaluated for proviral intactness, and subjected to integration site analysis (ISA). Because ART arrests viral replication, the proviruses we characterized served as molecular records of infections that occurred just prior to ART initiation. The genic integration sites of these proviruses can even be informative of the transcriptional environments and differentiation states of the T cells at the time of infection. Highlights of our various analyses are provided here.

RESEARCH QUESTIONS

1. Do we detect naïve cells harboring HIV proviruses? Are any proviruses intact?
2. Do naïve cells with HIV proviruses have the capacity to proliferate into cell clones?
3. Do we see direct evidence that naïve CD4+ T cells harboring HIV proviruses differentiate into their memory phenotype during the years these children were on ART?
4. What can genic integration targets and their expression profiles tell us about the transcriptional and differentiation states of the infected T cells just prior to ART initiation?

METHODS:



1. Extracted gDNA from sorted naïve and memory CD4+ T cells

2. Partitioned gDNA at limiting dilution and amplified single-copy proviruses and flanking host DNA by multiple displacement amplification (MDA)

3. Screened and characterized amplified proviruses by:
- **Real Time PCR (HIV LTR)**
- **Real Time PCR (HIV ψ, RRE)**
- **Oxford Nanopore Sequencing**
- **Integration site analysis (ISA)**

MAIN FINDINGS

- HIV proviruses persist in both naïve and memory CD4+ T cell in all children despite long- term ART and have the propensity to proliferate and form distinct clonal progeny **(Figure 1)**
- The frequency of infection in the naïve compartment was 12-fold lower compared to the memory compartment (38 HIV naïve cells in one million naïve cells vs 464 HIV memory cells in one million memory cells)
- Having identified 8 potentially intact proviruses in CD4+ T naïve from 6 participants, we were able to assemble a contiguous sequence for one MDA-amplified provirus from PID 1001 which was verified to be intact using the online Pro-Seq-IT tool **(Table 2)**
- Integration site analysis data did not reveal naive-to-memory differentiation since there was no overlap between the sets of integration sites retrieved from the two subsets ($p<10^{-4}$), however this may be due to shallow sampling **(Figure 1)**
- In samples from PID 1001, most all naïve integration site genes are expressed to a greater extent in naïve cells, while the same consistency is not seen among memory cell integration site genes and their relative expression in memory cells. This suggests that some of the memory cells harboring proviruses in genes highly expressed in naïve cells were infected while naïve and then activated and differentiated into memory cells after the children were placed on ART **(Figure 2)**

RESULTS

Figure 1. Integration site analysis on naïve and memory CD4+ T cells (PID 1001)

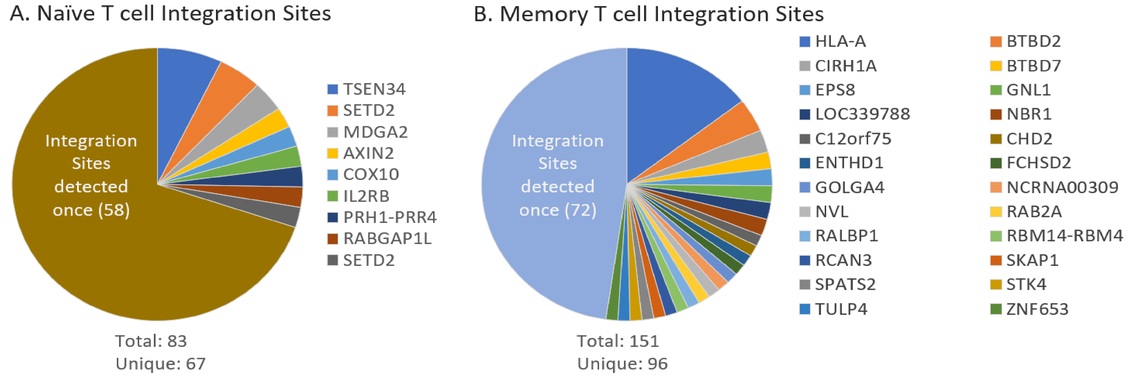


Figure 2. Genic Integration Sites in T naive (top bar graph) and in T memory (bottom bar graph)

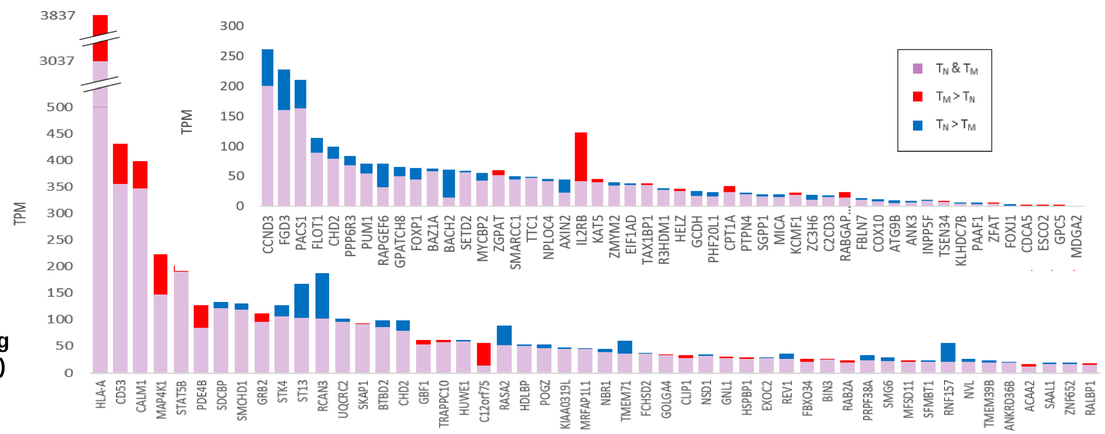


Table 1. Participant characteristic

PID[a]	Sex[b]	HIV subtype	Age at ART initiation (months)	Age at sampling (years)	CD4 cell count (cells/mm³)
0444	M	B	13(?)	10	917 [c]
0555	M	B	9	10	761
0600	F	B	1.5	5	1555
0888	M	C	17	11	819 [c]
1001	F	B	8.5	6	1506
4004	M	C	2	7	1177
5005	F	B	2	8	1196 [c]
9009	F	ND	12(?)	9	1018
Median			8.75	8.5	

[a] Participant ID.
[b] Sex assigned at birth: M (male), F (female).
[c] CD4 cell count was not performed at donation. Values obtained shortly prior to or after donation are reported.

Table 2.

PID[a]	Naïve CD4+ T cell purity (%)	Memory CD4+ T cell purity (%)	HIV+ per million naïve T cells (adj)	HIV+ per million memory T cells	Naïve T cell % of Total Infected[b]	# Candidate-intact PV[c] in Naïve T cells (of Total Screened)
0444	99.0	100	22	619	0%	6%
0555	96.7	97.4	56	600	13%	3%
0600	98.3	94.6	40	178	27%	6%
0888	97.0	93.0	31	384	5%	8%
1001	96.9	96.9	231	469	50%	6%
4004	98.5	96.3	53	347	23%	4%
5005	96.7	97.4	6	506	0%	0%
9009	NA	NA	34	459	--	0%
Median	**97**	**97**	**38**	**464**	**13%**	**4% (AVG)**

[a] Participant ID.
[b] Assuming age-appropriate T cell subtype distribution (70% Naïve, 30% Memory)
[c] Candidate-intact provirus, per IPDA (screened positive for HIV LTR, ψ, & RRE)

CONCLUSIONS

In this work, we characterize viral DNA populations in naïve T cells in children born with HIV. Intactness frequencies of proviruses in this subset resemble those previously reported for children and adults, providing additional evidence that naïve cells can contribute to the HIV reservoir in children. None of the expanded clones identified by integration site analysis were detected in both naïve and memory cell sorted collections, which, together with gene expression analysis, suggest that determinants of integration and persistence in the two T cell subsets are distinct. Collectively, our results serve as a foundation for future studies that, with greater sampling, may allow us to further distinguish subtype-specific and generalized characteristics of infection in different demographics of people living with HIV.

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