INFECTED NAÏVE CD4+ T CELLS IN CHILDREN WITH HIV CAN PROLIFERATE AND PERSIST ON ART

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Background:

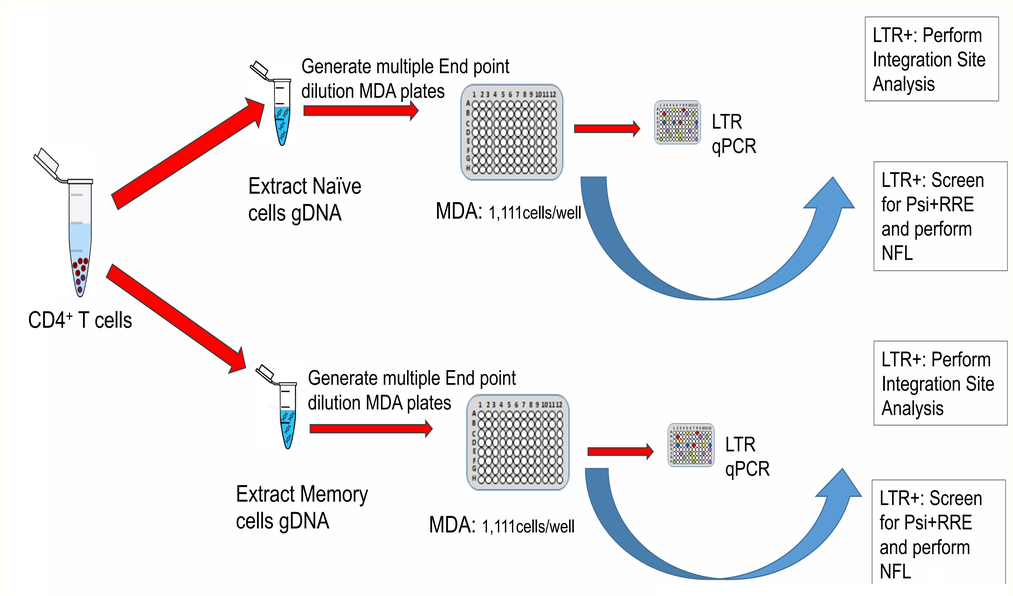
Intrinsic differences exist between neonatal and adult adaptive immune systems, which could translate into an altered program of T cell development. The majority of CD4⁺ T cells in infants are naïve and quiescent, CD4⁺CCR5⁺ expression on cell surfaces reaching adult levels after 5 years of age. In adults, naïve CD4⁺ T Cells ($T_{Naïve}$) have been shown to harbor a larger inducible reservoir of replication competent HIV than memory CD4⁺ T cells (T_{Mem}) (Zerbato,JVI,2016; Zerbato,CID,2020; Rullo, JCI, 2021; Pinzone, JCI, 2021). Although ART suppressed infant macaques have shown a high abundance of intact SHIV DNA in $T_{Naïve}$ (Obregon-Perko, JOV, 2020), little is known about the relative contribution of total, intact and defective HIV DNA in T_{Naive} and T_{Mem} of perinatally infected children and adolescents.

Key Questions:

- Is the $T_{Naïve}$ compartment an important HIV-1 reservoir in long-term treated, perinatally infected children?
- Can infected $T_{Naïve}$ undergo cellular proliferation to form infected T cell clones?
- Do $T_{Naïve}$ contain intact HIV proviruses?

Methods:

The cohort consisted of 8 children aged 5-11 years who initiated ART at a median of 4 weeks of age (range 0-39) with suppressed viremia for a median of 8.5 years. PBMC were FACs sorted into T_{Naive} (CD45RO-CD28+CD27+CD95-CCR7+CD45RA+) and T_{Mem} (CD45RO+ CD95+). Multiple displacement amplification (MDA) was performed on genomic DNA from 320,000 T_{Naive} and 106,667 T_{Mem} from each child and dispensed in 96-well plates at limiting dilution for HIV proviruses. HIV proviruses from MDA wells were detected by LTR qPCR, purified and sent for integration site analysis to determine the site of integration in the host chromosome.



Study Participants:

PID	Sex	HIV Subtype	Age at ART initiation	Duration on ART (years)	Age at visit (years)
0444	M	B	NA	NA	10
0555	M	B	9 months	10	10
0600	F	B	1.4 months	5	5
0888	M	C	1 month	10	11
1001	F	B	Birth	6	6
4004	M	C	1 month	7	7
5005	F	B	1 month	10	8
9009	F	NA (Ugandan)	12 months	8	9
Median			**1 month**	**8 years**	**8.5 years**

Results: Minimum infected T_{Naive} in Naïve Set

PID	% T_{Naive} cell purity	% T_{Mem} cell purity	$T_{Naïve}$ per 320,000 cells	Minimum $T_{Naïve}$ in Naïve set	95% CI T_{Mem} in Naïve set
0600	98.3	94.6	13	9	0 – 4
4004	98.5	96.3	17	14	0 – 3
0888	97.0	93.0	10	3	0 – 7
1001	96.9	96.9	74	65	0 – 9
0444	99.0	100.0	7	0	0 – 7
0555	96.7	97.4	18	12	0 – 6
5005	96.7	97.4	2	NA	NA
9009	NA	NA	11	NA	NA

Data from the purity of FACs cell sorting and frequency of infected cells in each subset, was used to determine the minimum number of infected cells that were statistically unlikely to be contaminating memory cells in the naïve set. The highlighted samples are outside the statistical chance that we were detecting purely contaminating memory cells in the naïve set and were prioritized for further analysis.

Results: T_{Naive} harbor potentially Intact Proviruses

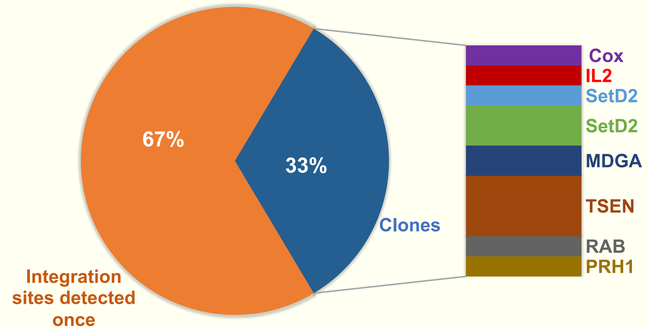
Total Cells assayed	Total LTR(+)	Total LTR & Psi (+)	Total LTR & RRE (+)	Total LTR, Psi & RRE (+)
4,479,000	**201**	**35**	**23**	**8**

IPDA primers and probes were used to screen LTR+ MDA wells for the presence of Psi and RRE. Using the total number of LTR+ cells as a denominator, 3.9% (8/201) of proviruses were potentially intact. Using the sum of Psi+, RRE+ and Psi&RRE+ cells as a denominator, 12.9% (8/62) of proviruses were potentially intact.

Results: Frequency of HIV Infected T_{Naive} and T_{Mem}

PID	$T_{Naïve}$ per 320,000 cells assayed	$T_{Naïve}$ per $1x10^6$	T_{Mem} per 106,667 cells assayed	T_{Mem} per $1x10^6$
0600	13	41	19	178
4004	17	53	37	347
0888	10	31	41	384
9009	11	34	49	459
1001	74	231	50	469
0444	7	22	66	619
0555	18	56	64	600
5005	2	6	54	506
Median	**12**	**37.5**	**49.5**	**464**

Results: Evidence of Proliferation in T_{Naive} of PID 1001



We obtained 70 integration sites from PID 1001 who had the highest number of infected naïve cells. Of these, 47 (67%) were detected once, while 23 (33%) were part of clonal populations. 8 distinct clonal populations were detected.

Discussion and Conclusions:

Infected $T_{Naïve}$ persist in children with perinatal HIV on ART for 5-11 years. At least some infected $T_{Naïve}$ can proliferate to form clones of infected cells. Using measurements adapted from the Intact Provirus Detection Assay, we found 6.5% of infected $T_{Naïve}$ to have proviruses predicted to be intact. $T_{Naïve}$ are an important HIV reservoir in perinatally infected children on ART because they could be a renewable source of infected central and effector memory T cells.

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