High-Dimensional Immune Profiling in Children with Perinatal HIV Initiating Early vs Late ART

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Background

Early childhood is a critical period for immune maturation, shifting from a naïve, hyporesponsive state towards an adaptive and regulatory homeostasis. In children living with perinatal HIV (CLH), chronic inflammation may disrupt this trajectory, with long-term consequences for immune function. Whether early ART initiation prevents inflammation and preserves physiologic immune development remains unclear.

Methods

- Participants. Kenyan children ages 1-5 years (enrolled 2011-2013)
  - **CHU:** Children unexposed to HIV, n=30
  - **E-ART:** CLH who initiated ART before age 6 months (*early ART),* n=9
  - **L-ART:** CLH who initiated ART after age 6 months (*late ART)* n=23
- Flow Cytometry. High-parameter flow cytometry panels defined 149 subsets across innate (monocytes, DC, NK, NKT, MAIT, γδT) and adaptive (B, CD4, CD8, Tfh, Th1/2/17, Treg) compartments, including markers of differentiation, activation (CD38, HLA-DR), exhaustion (PD-1, CD160, TIM-3, 2B4), proliferation (Ki67), cytotoxicity (granzyme B, perforin), and cytokines (Table 1).

Table 1: Markers for Flow Cytometry

Monocyte and Dendritic cell profile	Innate (like) cells: NK,NKT gdT, MAIT	B cell subsets	T cell differentiation, TFh	T helper subsets, Chemokine receptors	Tregs, Activation, Proliferation, Cytotoxicity	Immune Exhaustion	Cytokines^
CD14	CD8	CD19	CD45RO	CCR5	CD45RO	CD45RO	CD56
HLA-DR	CD16	CD27	CCR7	CD45RO	CD25	CD27	TNFa*
CD16	CD56	CD38	CD27	CXCR3	CD38	CD38	IFNg*
CD1c	HLA-DR	CD5	CD57	CCR4	HLA-DR	PD-1	IL-4*
CD141	CD161	CD21	CD31	CCR6	perforin*	Tim-3	IL-2*
CD303	γδ TCR	IgG	CXCR5	CD161	Ki67*	CD244 (2B4)	IL-17A*
CD33	Vα24Jα18 TCR	IgM		beta 7	Granzyme B*	CD160	Il-21*
CD11b	Vα7.2	IgD		CCR9	Helios*		IL-22*
Innate Immunity		Adaptive Immunity					

All panels include CD3, CD4 and CD8. *intracellular. ^stimulated with PMA and Ionomicin

- Plasma Biomarkers. Plasma biomarkers of monocyte/T cell activation (sCD14, sCD163, sCD40L), inflammation (CRP, IL-6), and gut permeability (IFABP) were measured by ELISA.
- Statistical Analyses. Immune markers were compared using Kruskal-Wallis test to identify overall differences among CHU, E-ART and L-ART, followed by Dunn's test for pairwise differences for 27 biomarkers with a Kruskal-Wallis test p-value < 0.1 in R. Graphs made using GraphPad Prism.

Table 2. Participant Characteristics

	CHU (N = 30)	E-ART (N = 9)	L-ART (N = 23)	P Value
Age (m)	33.1 (26.0-44.2)	29.0 (24.5-36.9)	38.7 (32.6-44.1)	0.14
Female #	20 (66.7%)	6 (66.7%)	9 (39.1%)	0.11
Age at ART initiation (m)		1.9 (0.0-2.7)	18.7 (13.6-22.2)	<0.0001
ART duration (m)		26.3 (22.1-36.3)	16.3 (11.6-24.5)	0.013
Viral suppression # (VL ≤ 220 copies/ml)		6 (66.7%)	14 (60.9%)	0.76
Absolute CD4 (/ul)	1409 (1124 -2012)	1416 (1295 -2238)	1561 (1072 -1794)	0.56
CD4 (%)	33 (28-38)	34 (24-35)	31 (26-35)	0.61

*All data presented as median values with IQR.
number and percentage. m = months.

Early ART attenuates inflammation but does not fully restore normal immune development: CD8 Tfh are depleted, regulatory T cells are expanded, PD-1 expression on CD4 T cells is high, and Th1 function is reduced. These disruptions highlight a need for immune-restorative approaches beyond ART.

Figure 1. Early ART preserves CD4:CD8 ratio and dampens inflammation

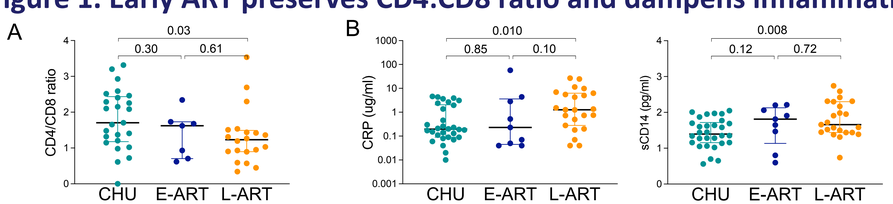


Figure 1. Comparisons of (A) CD4/CD8, (B) CRP and (C) soluble CD14 between CHU, E-ART and L-ART. Median values and interquartile ranges are shown as horizontal lines and bars.

Figure 2. Elevated Tregs and PD-1+ CD4 T cells despite early ART

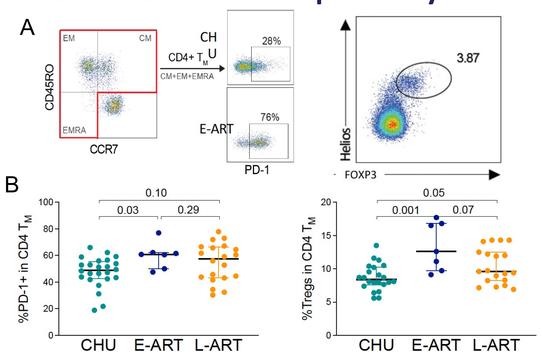


Figure 2. (A) Gating strategy shown for PD-1⁺ and regulatory T cells (Tregs) in memory CD4 T cells. (B) Comparisons of PD-1⁺ memory CD4 T cell and memory Treg frequencies between CHU, E-ART and L-ART.

Figure 3. Loss of CD8 Tfh

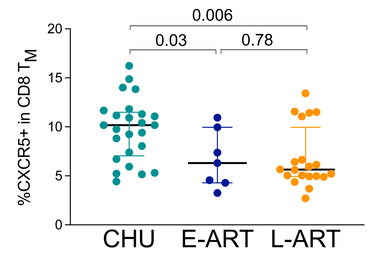


Figure 3. Comparisons of cytotoxic T follicular cells (defined as CXCR5⁺ in memory CD8 T cells) between CHU, E-ART and L-ART.

Figure 4. Late ART demonstrate depleted innate immune cell subsets

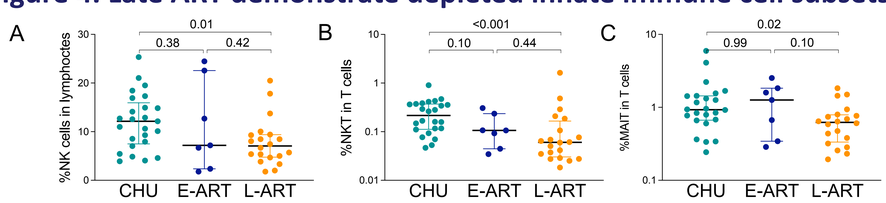


Figure 4. Comparisons of (A) NK cells (B) NKT and (C) Mucosal Associated Invariant T cells (MAIT) between CHU, E-ART and L-ART. NK cells were defined as the sum of CD16-CD56⁺ , CD16+CD56⁺ and CD16+CD56⁻ cells within lymphocytes, NKT as Vα24⁺Jα18⁺ in total T cells, and MAIT cells as Vα7.2⁺CD161⁺ in CD8 T cells.

Results

Figure 5. Dysregulated CD4 and CD8 T cell subsets

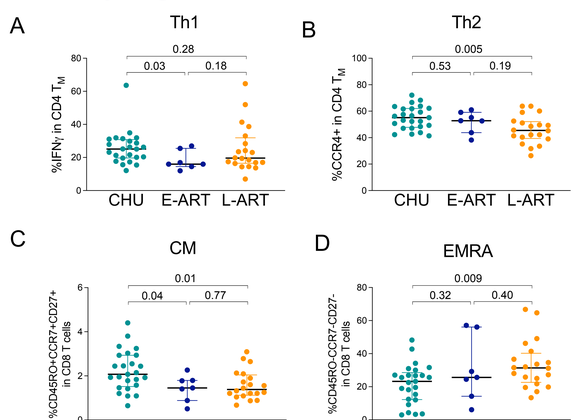


Figure 5. Comparison of (A) IFNγ⁺ memory CD4 T cell (T_M), (B) CCR4⁺ memory CD4 T cell, (C) CD45RO⁺CCR7⁺CD27⁺ (CM: central memory) CD8 T cell and (D) CD45RO⁻ CCR7⁻CD27⁻ (EMRA) CD8 T cell percentages between CHU, E-ART and L-ART.

Conclusions

- **Early ART** (E-ART) demonstrated CD4/CD8 ratios and inflammatory profiles comparable to CHU and preservation of MAIT cells. However, E-ART exhibited high memory Tregs, elevated PD-1 expression on memory CD4 T cells and lower Th1, IFNγ⁺ memory CD4 T cells.
- **Late ART** (L-ART) was characterized by lower CD4/CD8 ratios, heightened systemic inflammation, loss of NK, NKT and MAIT cells, and skewing toward terminally differentiated CD8 T cells.
- **Both groups** exhibited persistent immune perturbations, including low cytotoxic T follicular CD8 T cells and reduced central memory CD8 T cells.
- **Conclusion:** Deep immune profiling demonstrates that early ART attenuates inflammation but does not fully normalize immune development, supporting the need for adjunctive strategies to achieve durable immune health in children living with HIV (CLH).

Plain Language Summary

Children with HIV who start treatment early have immune profiles closer to healthy peers, but subtle changes in immune health persist. Later ART is linked to higher inflammation and more pronounced immune imbalance.

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