T Cell Activation and Exhaustion in Kenyan Pediatric Slow Progressors with Perinatal HIV

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Aabid Ahmed[1], Rupasri Pamulapati[3], Huiping Xu[3], Mussa Mwamzuka[1], Fatma Marshed[1], Alka Khaitan[3]

[1]Bomu Hospital, Mombasa, Kenya. [2]Department of Biostatistics and Health Data Science, Indiana University, Indianapolis, IN, USA. [3]Ryan White Center for Pediatric Infectious Diseases and Global Health, Indiana University School of Medicine, Indianapolis, IN, USA.

Background

Without ART, HIV progresses rapidly in infants, with >50% mortality by age two years. A rare subset of children living with perinatal HIV (CLH), termed **pediatric slow progressors** (SP), maintain high CD4 counts and low morbidity beyond age five years without ART. An SP cohort from South Africa showed low immune activation and minimal exhaustion, while other cohorts in Asia exhibit persistent activation. However, SP in East Africa remain poorly characterized, where viral subtypes and host genetics differ.

Methods

- Participants. Kenyan cohort, ages 5–19 (enrolled 2011–2012)
  - Children HIV-Unexposed (**CHU**): n=40
  - Children Living with HIV (CLH), ART-naïve:
    - Slow Progressors (**SP**): n=12 (CD4 >750/mm^3)
    - Pediatric Progressors (**PP**): n=30 (CD4 <750/mm^3)

- Flow Cytometry. Ten color flow cytometry was used to identify 149 innate and adaptive immune subsets including markers of differentiation, activation, exhaustion, proliferation cytotoxicity 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	Thelper 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. Measured by ELISA
  - sCD14, sCD163, sCD40L (monocyte / T cell activation)
  - CRP, IL-6 (inflammation)
  - IFABP (gut permeability)

- Statistical Analyses. All analyses performed in R. Graphs made using GraphPad Prism.
  - Heatmap generated using z scores by standardizing biomarker levels with mean and standard deviation of all participants to visualize differences across group
  - ATT (average treatment effect on the treated) weights used to balance participant groups regarding age and sex
  - Weighted Kruskal-Wallis test with false discover rate (FDR) adjustment for multiple comparisons
  - Pairwise comparisons using Wilcoxon rank sum test with Holm's adjustment to identify differences

Plain Language Summary

Some Kenyan children with untreated perinatal HIV maintain normal CD4 counts, but their immune systems show ongoing stress and imbalance. This shows that preserved CD4 numbers alone do not reflect full immune health.

Correspondence: Alka Khaitan, akhaita@iu.edu

Kenyan pediatric slow progressors (SP) exhibit elevated immune activation, T cell exhaustion and broad immune dysregulation despite preserved CD4 counts. While SP maintain robust HIV-specific and non-specific CD8 T cell functions, preserved CD4 counts do not equate to immunologic health in untreated perinatal HIV.

Table 2. Participant Characteristics

	CHU (N = 43)	SP (N = 12)	PP (N = 30)	P Value
Unweighted Analysis*				
Age in years, median (IQR)	11.3 (9.3 - 13.4)	8.8 (6.9 - 9.2)	11.6 (8.9 - 13.9)	0.019
Female Sex, n (%)	17 (39.5%)	6 (50%)	16 (53.3%)	0.48
Absolute CD4, median (IQR)	908 (737 – 1227)	992 (847 – 1246)	500 (285 – 589)	<0.001
Percent CD4, median (IQR)	38 (33 - 42)	28 (25 - 35)	18 (6.5 - 26.5)	<0.001
Log HIV copies, median (IQR)		4.8 (4.2 - 5.2)	4.8 (4.3 - 5.2)	0.99
Weighted Analysis^				
Age in years, median (IQR)	8.1 (6.6 - 10.8)	8.8 (6.9 - 9.2)	8.4 (6.2 - 10.5)	0.98
Female Sex, n (%)	50.9%	6 (50%)	41.6%	0.84
Absolute CD4, median (IQR)	977 (766 – 1298)	992 (847 – 1246)	523 (355 – 590)	<0.001
Percent CD4, median (IQR)	39.1 (34.1 - 43)	28 (25 - 35)	15.5 (12.4 - 24)	<0.001
Log HIV copies, median (IQR)		4.8 (4.2 - 5.2)	4.9 (4.2 - 5.2)	0.89

*P-values were based on Kruskal-Wallis test for continuous variables and Person's Chi-square test for categorical variables.
^ATT (average treatment effect on the treated) weights used to balance patient groups regarding age and sex

Figure 1. Immune Profile

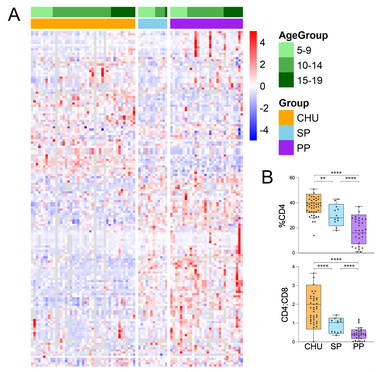


Figure 1. Heatmap of z scores using mean and standard deviation of all participants. (B) CD4% and CD4:CD8 ratios in CHU, SP and PP.

Figure 2. SP exhibit elevated pro- and anti-inflammatory immune subsets

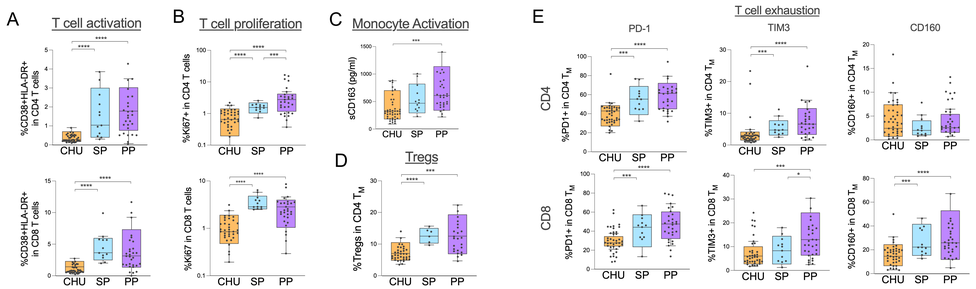


Figure 2. Comparisons of (A) CD38$^+$HLA-DR$^+$ and (B) Ki67$^+$ CD4 and CD8 T cells and (C) sCD163 between CHU, SP and PP. (D) Regulatory T cells and (E) immune checkpoints PD-1, TIM3 and CD160 in memory CD4 and CD8 T cells in CHU, SP and PP. *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001

Figure 3. SP preserve Naïve CD4 T cells and expand Memory CD8 subsets

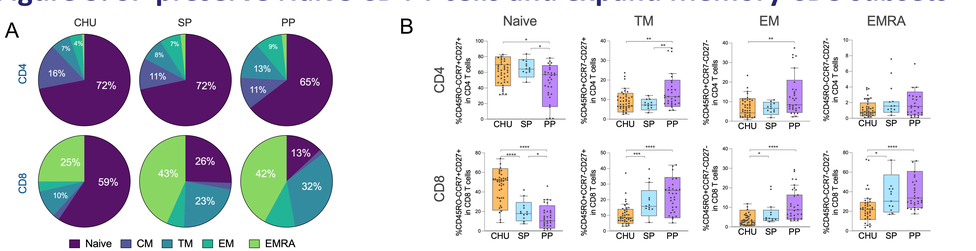


Figure 3. (A) Pie chart showing proportion of naive (CD45RO$^-$CD27$^+$CCR7$^+$), central memory (CM: CD45RO$^+$CCR7$^+$CD27$^+$), transitional memory (TM: CD45RO$^+$CCR7$^-$CD27$^+$), effector memory (EM: CD45RO$^+$CCR7$^-$CD27$^-$), and effector memory RA+ (EMRA: CD45RO$^-$CCR7$^-$CD27$^-$) in CD4 and CD8 T cells. (B) Comparisons of CD4 and CD8 naïve and memory subsets between CHU, SP and PP. *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001

Results

Figure 4. SP have altered Tfh, B cell and innate cells

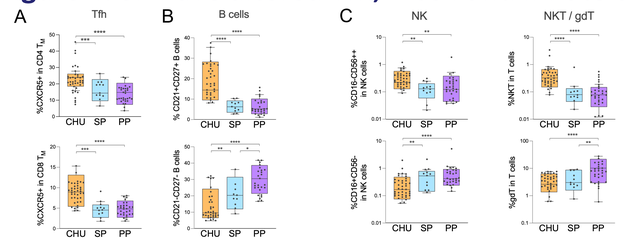


Figure 4. Comparisons of (A) CD4 and CD8 T follicular cells and (B) resting (CD21$^+$CD27$^+$) and transitional (CD21$^-$CD27$^-$) memory B cells between CHU, SP and PP. (C) Immature functional natural killer cells (CD16$^-$CD56^{++}) and dysfunctional NK (CD16$^+$CD56$^-$), NKT and gdT in CHU, SP and PP. *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001

Figure 5. SP preserve CD8 effector T cell functions

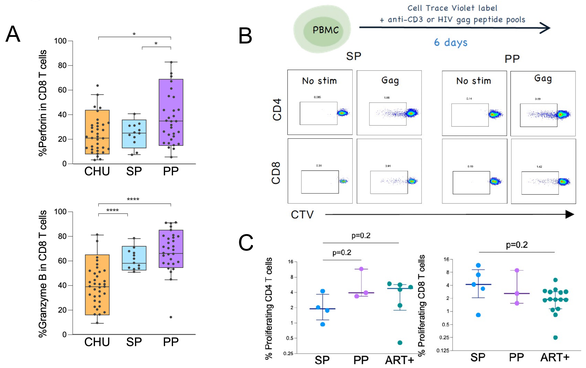


Figure 5. Comparisons of (A) Perforin and Granzyme B expressing CD8 T cells in CHU, SP and PP. *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001. (B) PBMCs were labeled with cell trace violet (CTV) then stimulated with pooled HIV gag or no stimulation for 6 days then read by flow cytometry. Representative flow plots shown. (C) Comparison of percent of proliferating CD4 or CD8 T cells after gag stimulation in SP, PP and CLH on antiretroviral treatment (ART).

Conclusions

- Despite preserved absolute CD4 counts, Kenyan slow progressors (SP) have lower CD4% and CD4:CD8 ratios than CHU
- SP demonstrate increased immune activation, exhaustion, and proliferation compared with CHU.
- SP preserve CD8 cytotoxic function and HIV-specific proliferative capacity
- Compared with pediatric progressors (PP), SP exhibit a more naïve CD4 phenotype, reduced CD4 proliferation, and lower TIM-3 and perforin expression on CD8 T cells.
- Preserved CD4 counts do not equate to immunologic health in untreated perinatal HIV. This distinct immune phenotype may reflect unique host or viral determinants.

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