Mycobacteria-specific CD4 T cells in children living with HIV before and after ART

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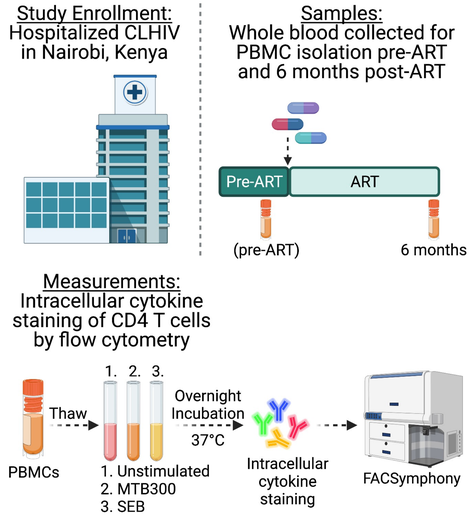
BACKGROUND

- Young children and people living with HIV are more likely to rapidly progress from *Mycobacterium tuberculosis* infection to tuberculosis (TB) disease.
- The capacity of ART to mediate restoration of functional mycobacteria-specific CD4 responses in children living with HIV (CLHIV) has not been well defined.
- We hypothesized that provision of ART in CLHIV would result in rapid increase in frequencies of mycobacteria-specific CD4 T cells.

METHODS

- Hospitalized CLHIV were enrolled in Nairobi, Kenya (n=47). The median age of participants was 1.5 years (IQR 0.7-5.4)
- Blood was collected for PBMC isolation prior to and 6 months after initiation of ART.
- PBMCs were thawed and incubated overnight with a peptide pool of 300 T cell epitopes from BCG and TB antigens (MTB300) or staphylococcal enterotoxin B (SEB).
- Multiparameter flow cytometry was used to measure CD4 T cell expression of IFN-γ, IL-2, TNF, IL-17, MIP-1β, and CD40L/CD69 co-expression.
- Polyfunctionality scores for antigen-specific CD4 T cells were evaluated using Combinatorial Polyfunctionality Analysis of Single Cells (COMPASS)[1].

Figure 1. Study schematic.



Residual deficits in mycobacteria-specific CD4 T cell immunity persist at 6 months following ART initiation among severely immunosuppressed children living with HIV.

RESULTS

Table 1.

Participant Characteristics	Pre-ART Median (IQR)	6 months post-ART Median (IQR)	p value
HIV RNA (log_{10} copies/ml)	5.8 (5.1-6.3)	2.3 (1.5-3.7)	<0.0001
CD4 count (cells/μl)	678 (337-1457)	1321 (761-2033)	<0.0001
CD4%	13 (9-18)	21 (14-24)	<0.0001
Severely Immunosuppressed (All Ages)	81%	46%	0.0005
<12 months, CD4% <25%	93%	78%	0.5331
12-35 months, CD4% <20%	76%	36%	0.0227
≥36 months, CD4% <15%	73%	40%	0.1394

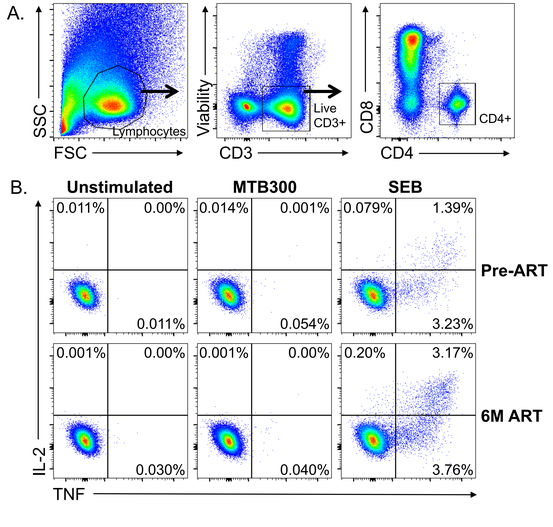


Figure 2. Measurement of Ag-specific CD4 T cells. PBMCs collected before and 6 months post-ART were thawed and stimulated overnight with MTB300 and SEB. (A) Gating strategy to define CD4 T cells. (B) Flow cytometry plots gated on CD4 T cells indicating intracellular expression of TNF and IL-2 before ART (top row) and the same child 6 months after initiation of ART (bottom row).

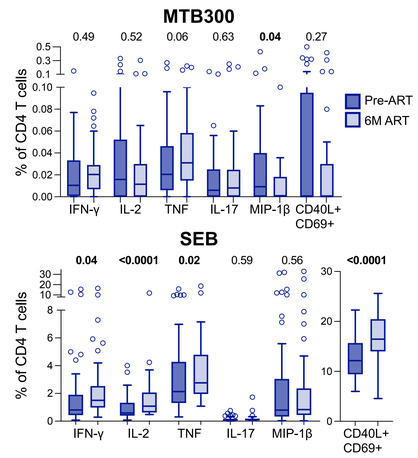


Figure 3. Frequencies of SEB-induced IFN-γ, IL-2, TNF, and CD40L$^+$CD69$^+$ CD4 T cells increase after 6 months of ART. PBMCs were stimulated as described in Figure 2. The frequency of CD4 T cells expressing each marker are indicated following stimulation with MTB300 (top panel) and SEB (bottom panel). Frequencies are shown after subtraction of background expression in the unstimulated condition. Differences in frequencies before and after ART were evaluated using a Wilcoxon matched-pairs test.

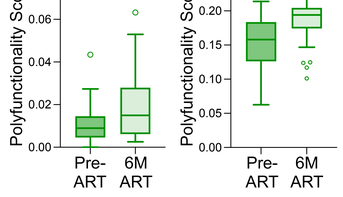


Figure 4. CD4 T cell polyfunctionality scores (PFS) increase after 6 months of ART. PFS were calculated in COMPASS for Ag-specific CD4 T cells expressing any combination of IFN-γ, IL-2, TNF, IL-17, MIP-1β, and CD40L. Differences in PFS before and after ART were evaluated using a Wilcoxon matched-pairs test.

CONCLUSIONS

- CD4 counts and percentages increased, coincident with decreased viral load after 6 months of ART in CLHIV.
- CD4 Th1 cytokine production capacity to SEB stimulation increased after 6 months of ART, although the frequency of mycobacteria-specific CD4 cells did not increase significantly during this time.
- Although mycobacteria-specific CD4 T cell polyfunctional scores are low, these scores increased after 6 months of ART.
- These data provide rationale for longer-term evaluation of polyfunctional and IFN-γ-independent mycobacteria-specific CD4 T cell responses in CLHIV.
- These findings may explain mechanisms for persistent risk of TB during the first year of ART, despite robust immune reconstitution.

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[1]Lin *et al*, COMPASS identifies T-cell subsets correlated with clinical outcomes. *Nat Biotechnol*. 2015. PMID: 26006008.