Coinhibitory Receptors and Cytokines Are Determinants of Reservoir Size in Children With HIV

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Pathology Advanced Translational Research Unit
Department of Pathology and Laboratory Medicine

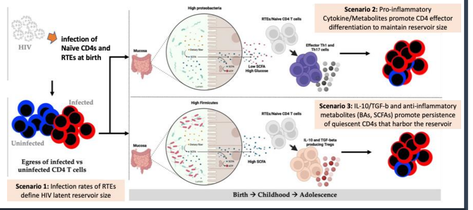
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BACKGROUND AND HYPOTHESIS

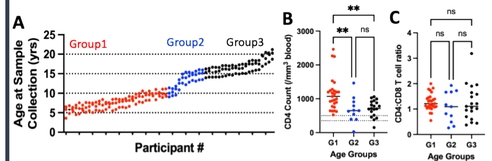
- Early antiretroviral therapy (ART) reduces HIV reservoir size in children living with HIV (CLWH), but latent reservoirs persist, causing viral rebound after ART interruption.
- Approximately 3,000 new perinatal HIV infections occur globally each week. In adults, cytokines like TGF-β and IL-10 drive T cell dysfunction and are linked to latent HIV-harboring CD4+ T cells.
- Early life features rapid gut microbiome maturation with shifts in metabolites (SCFAs, bile acids) that shape mucosal/systemic immunity.
- SCFAs and bile acids modulate T-cell differentiation and immune regulation (Th17/Treg, IL-10/TGF-β), potentially affecting pediatric reservoir establishment and persistence.

We hypothesize that changes in plasma cytokine and metabolite milieu after birth shape the differentiation and persistence of CD4 T cells that harbor the latent HIV reservoir



EPIC[4] Cohort

- Plasma and PBMC samples accrued from Early Pediatric Initiation Canada Child Cure Cohort (EPIC4) where the participants have been recruited from 9 clinical centers across Canada
- 3 longitudinal plasma samples from 63 CLWH have been collected over a ~2 year follow-up.



Methods: Plasma and PBMC's samples from this cohort were profiled using various multi-omics approaches.

Figure 1: Cohort Characteristics. A) Cohort with 3 longitudinal collections per participant with age range from 5-20 years, staggered into 3 age groups, Grp1 (5-10, red), Grp2 (10-15, blue), Grp3 (15-20, black). B) Increased CD4 counts at early age and C) no significant difference in CD4:CD8 T cell ratio in Grp 1-3.

RESULTS

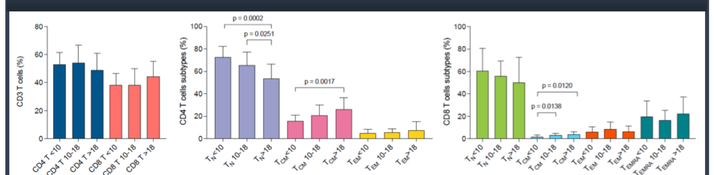


Figure 2: Frequency of naive T cells goes down with age.

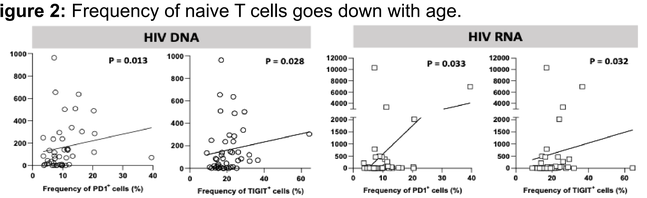


Figure 3: Inhibitory receptor expression in CD4⁺ cells correlates with reservoir size.

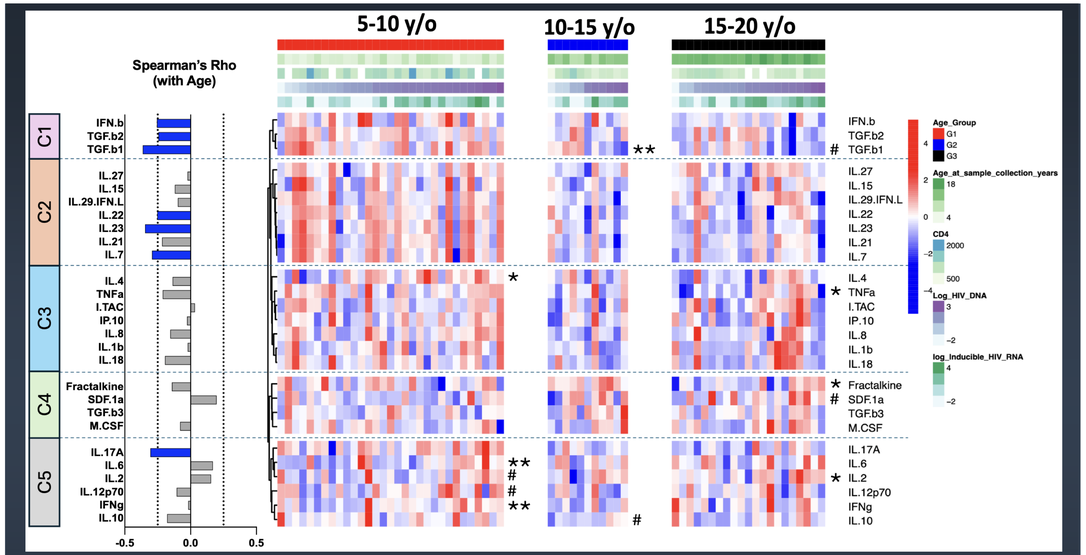


Figure 4: Age-driven plasma cytokine programs and reservoir-associated immune signatures in EPIC4. Unbiased PCA of plasma cytokines showed PC1 (27.4% variance) associated with age ($p<0.05$), while PC2 (15.04%) tracked with HIV reservoir size. The heatmap shows row-normalized cytokine/chemokine levels grouped into five clusters (C1–C5) across three pediatric age groups (G1–G3), with annotations for age, CD4 counts, log HIV DNA, and log inducible HIV RNA. TGF-β/Th2 cytokines (e.g., IL-4) were higher in children <10; in older participants, high TGF-β marked a subset with lower inducible HIV RNA, whereas low TGF-β associated with higher IL-17A, IFN-α, HIV DNA, and inducible HIV RNA. C1–C2 (TGF-β1/2, IL-7, IL-21) decline in the oldest group, and reservoir correlates shift with age: younger participants link HIV DNA to activation/differentiation cytokines (IL-2, IL-4, IFN-γ), while older participants link HIV DNA to chemokines. Left bars show Spearman's rho vs age. Significance: p<0.01 (**), p<0.05 (*), p<0.1 (#).

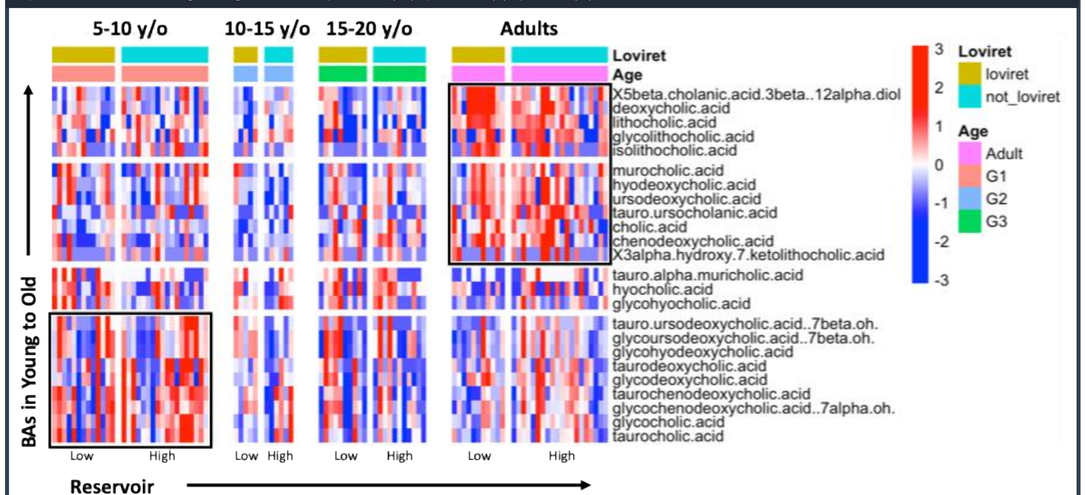


Figure 5: Specific bile acids associate with age and latent HIV reservoir status. Heatmap showing z-score–scaled plasma levels of targeted bile acids measured across EPIC4 participants stratified by age group (5–10, 10–15, 15–20 years, and adults) and reservoir category (Low vs High reservoir; ***Low = <50 copies/million PBMCs***). Columns represent individuals; rows represent bile acid species (including primary, secondary, and conjugated bile acids). Top annotation bars indicate ART regimen (Loviret vs not-Loviret) and age group. Color scale denotes relative abundance (red = higher, blue = lower). Black boxes highlight bile-acid modules that show coordinated enrichment patterns linked to age and/or reservoir status.

RESULTS

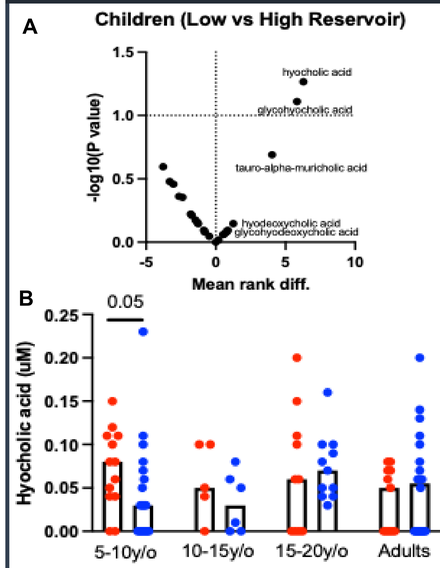


Figure 6: Hyocholic acid (HCA) associates with lower latent HIV reservoir in children and links to TGF-β signaling. (A) Targeted plasma bile-acid profiling comparing low vs high reservoir children identifies hyocholic acid (HCA) as enriched in the low-reservoir group (each dot = participant; group comparison p value shown on plot). **(B)** HCA concentrations across pediatric age strata (and adult comparator, if included) stratified by reservoir status (low vs high). Data support a model in which higher HCA is associated with lower reservoir burden and aligns with TGF-β–linked regulatory immune tone.

CONCLUSION AND FUTURE DIRECTION

- Age is the dominant driver of plasma immune variation in pediatric HIV (PC1), while a separable axis tracks with reservoir size/inducibility (PC2).
- Naïve T-cell frequency declines with age, consistent with immune maturation across childhood → adolescence.
- Co-inhibitory receptor expression correlates with reservoir size, with TIGIT enriched on memory CD4 T cells.
- Younger children (<10 y/o) show an enriched TGF-β milieu.
- Regardless of age, the effector cytokine cluster (C5) ↑ IL-17A and ↑ IFN-γ associates with higher HIV DNA and higher inducible HIV RNA.
- Targeted bile-acid profiling identifies an age- and reservoir-linked module: HCA/Glyco-HCA is enriched in <10 y/o with low reservoir, whereas bile acids such as DCA are more associated with adults and high reservoir.
- Together, these findings support Scenario 2 of our model: pro-inflammatory cytokines and metabolites promote CD4 effector differentiation, contributing to reservoir maintenance and inducibility.
- **FUTURE DIRECTIONS:** Integrate flow + cytokines + bile acids/SCFAs with single-cell transcriptomics/epigenomics and functional assays to define mechanisms (bile acids → TGF-β/IL-10 pathways) and develop predictive signatures of reservoir persistence/inducibility.

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NIH National Institute of Allergy and Infectious Diseases
NIH NICHD
CANFAR Canadian Foundation for AIDS Research / Fondation canadienne de recherche sur le sida
the CTN CIHR Canadian HIV Trials Network