Vγ9Vδ2 T cell responses in HIV-exposed Uninfected (HEU) Infants

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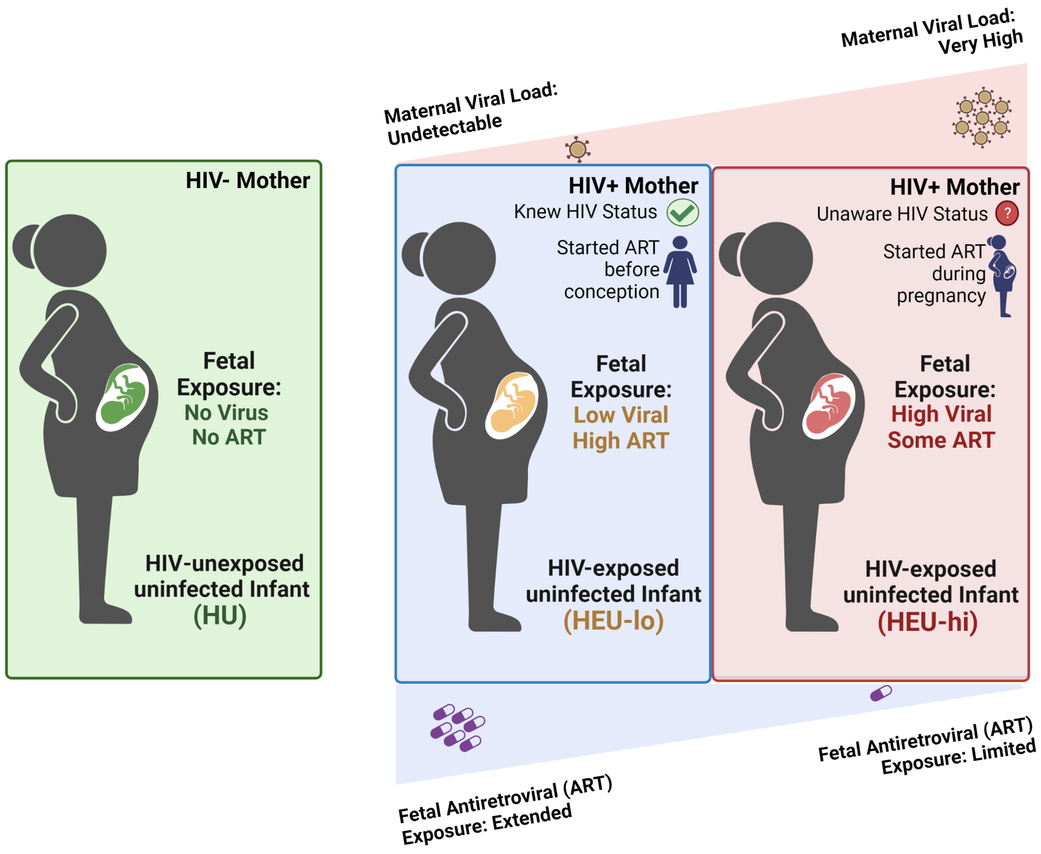
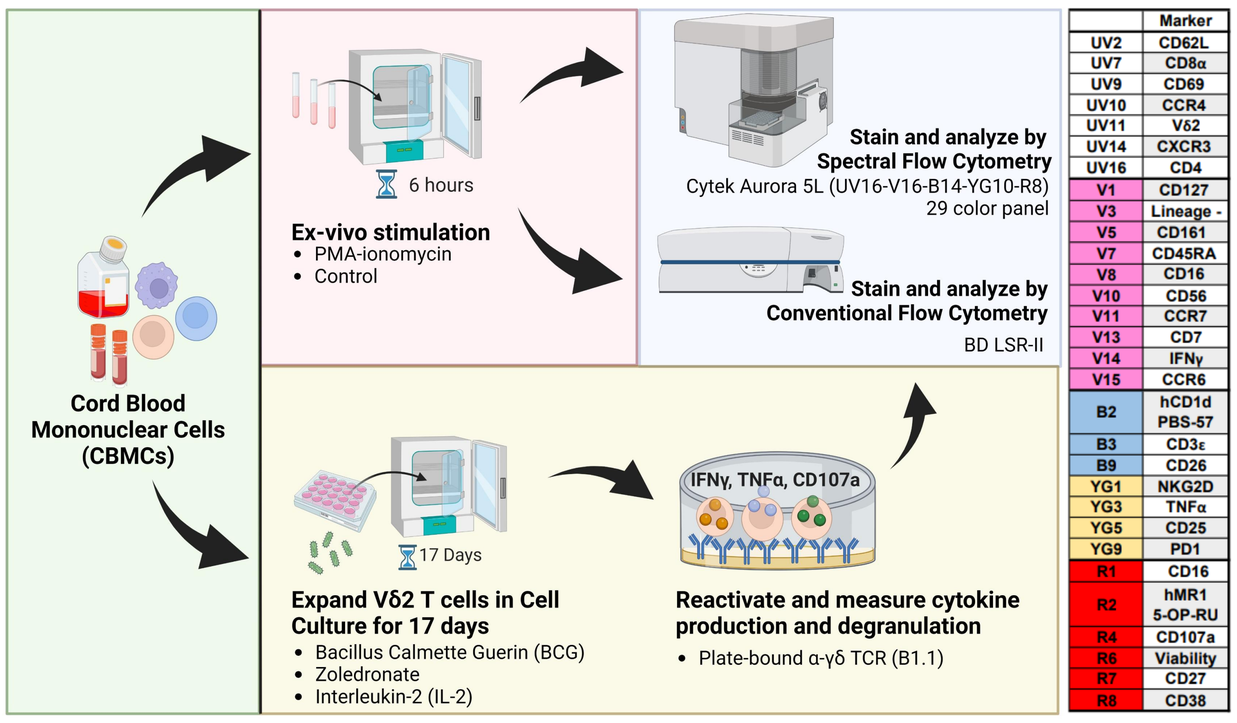
INTRODUCTION

- **Maternal antiretroviral therapy (ART) effectively prevents perinatal infection of infants born to mothers living with HIV.**
- **HIV-exposed Uninfected (HEU) Infants exhibit increased morbidity to lower respiratory tract and diarrheal infections during the first six months life compared to HIV-unexposed (HU) infants.**
- **Prenatal exposure to HIV and/or ART may perturb the fetal immune system, contributing to the observed increased infectious morbidity.**
- **Vγ9Vδ2 T (Vδ2) cells, due to their rapid secretion of Th1 cytokines in response to a broad array of pathogens, are likely to play a key role against infections in early life.**
- **Elevated inflammation at the fetal-maternal interface may result in dysfunction of Vδ2 cells; however, the effect of in-utero HIV exposure on this subset is unknown.**

STUDY POPULATION: MALAWIAN COHORT



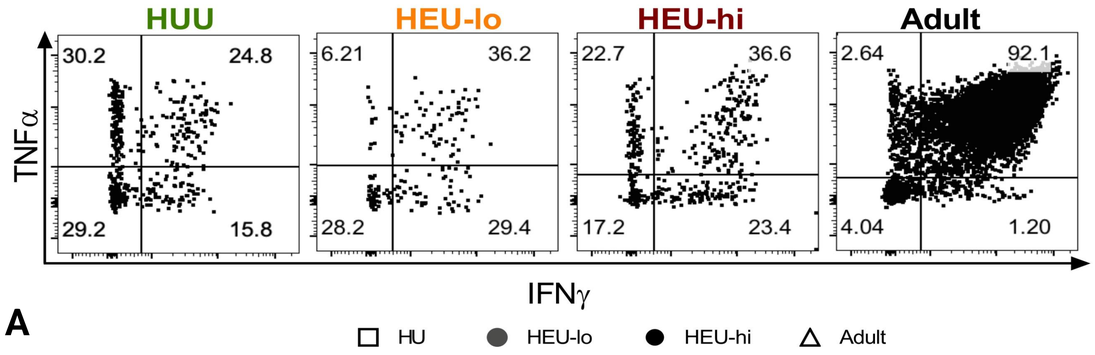
METHODS

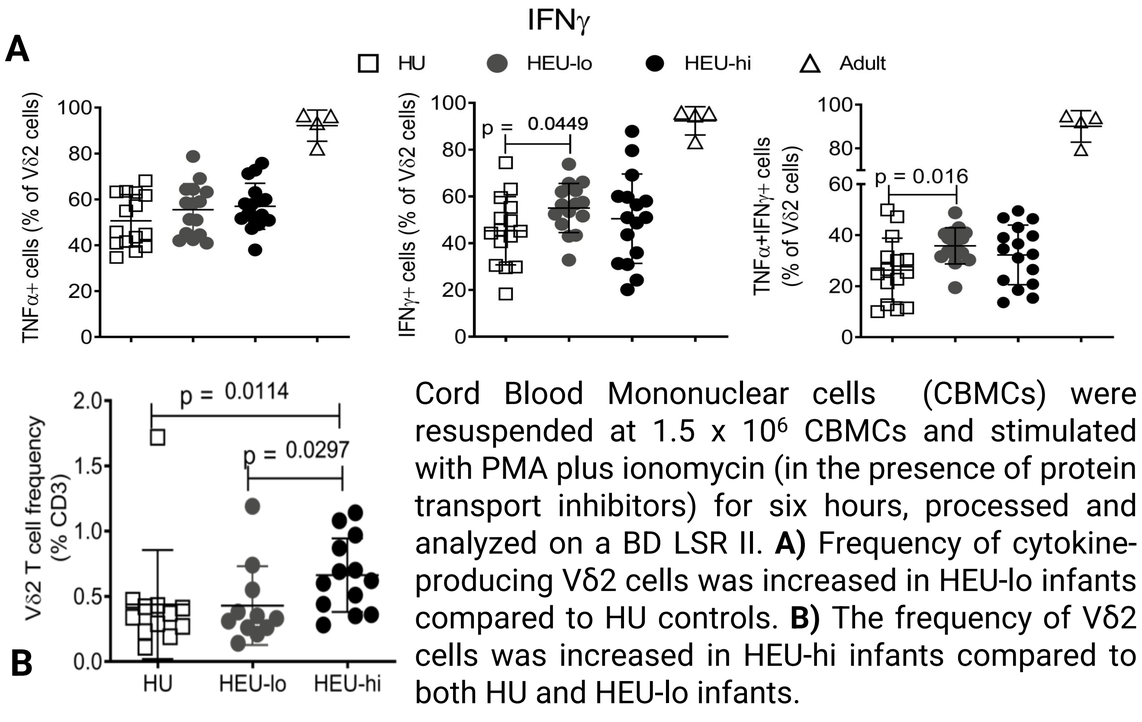


	Marker
UV2	CD62L
UV7	CD8α
UV9	CD69
UV10	CCR4
UV11	Vδ2
UV14	CXCR3
UV16	CD4
V1	CD127
V3	Lineage -
V5	CD161
V7	CD45RA
V8	CD16
V10	CD56
V11	CCR7
V13	CD7
V14	IFNγ
V15	CCR6
B2	hCD1d PBS-57
B3	CD3ε
B9	CD26
YG1	NKG2D
YG3	TNFα
YG5	CD25
YG9	PD1
R1	CD16
R2	hMR1 5-OP-RU
R4	CD107a
R6	Viability
R7	CD27
R8	CD38

RESULTS: FLOW CYTOMETRY – EX VIVO

Figure 1. The frequency and polyfunctionality of Vδ2 cells are altered in HEU infants compared to HU controls

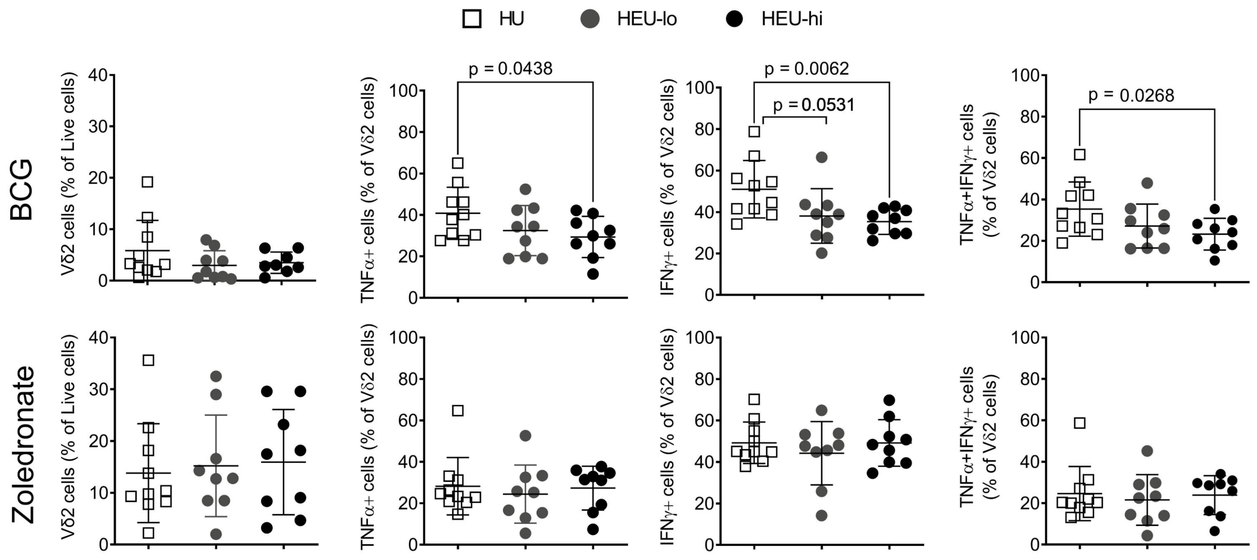




Cord Blood Mononuclear cells (CBMCs) were resuspended at 1.5 x 10^6 CBMCs and stimulated with PMA plus ionomycin (in the presence of protein transport inhibitors) for six hours, processed and analyzed on a BD LSR II. **A)** Frequency of cytokine-producing Vδ2 cells was increased in HEU-lo infants compared to HU controls. **B)** The frequency of Vδ2 cells was increased in HEU-hi infants compared to both HU and HEU-lo infants.

RESULTS: FLOW CYTOMETRY - CULTURE

Figure 2. In vitro proliferation of Vδ2 cells is similar across the cohorts

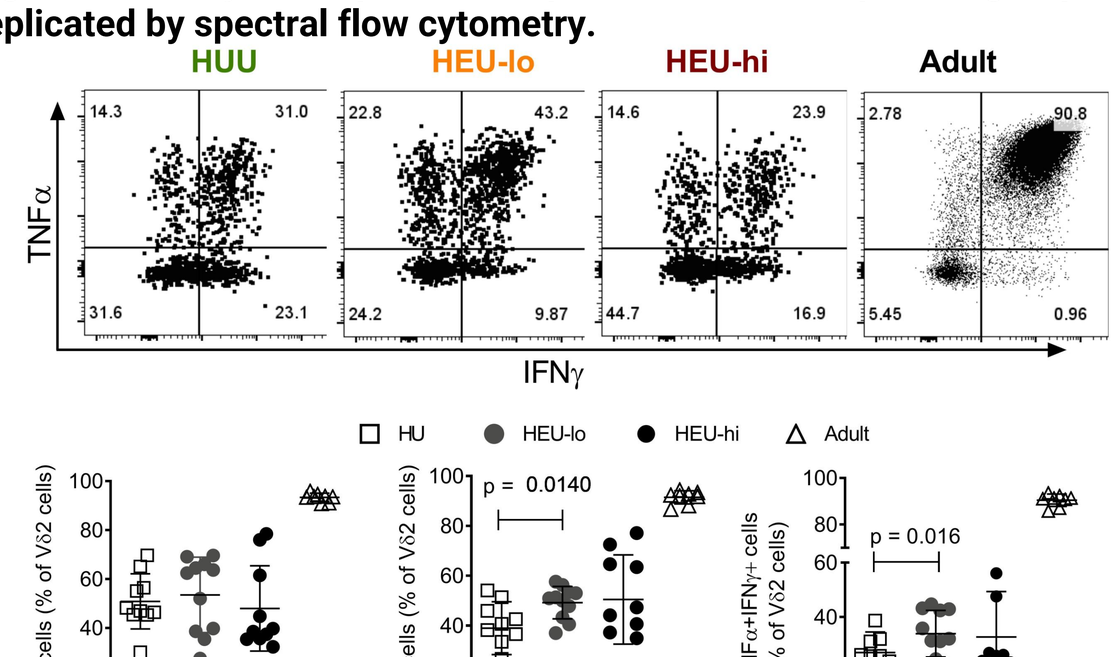


CBMCs were treated with A) Bacillus Calmette-Guérin [BCG] (MOI=1) or B) Zoledronate (0.5μM); plus IL-2 (100 U/mL) for two weeks to expand Vδ2 cells, before resting for 3 days. Intracellular Th1 cytokines were detected in Vδ2 cells after restimulation with plastic-immobilized anti-γδ TCR antibody (B1.1), followed by processing and analysis on a BD LSR II.

No statistically significant differences in frequency of Vδ2 cells following BCG or ZOL expansion between HEU and HU infants were noted. Following restimulation, HEU-hi infants had a lower frequency of cytokine-producing cells compared to HU controls in the BCG expansion cultures. This altered cytokine response, however, was not observed in Zoledronate expansion cultures.

RESULTS: SPECTRAL FLOW CYTOMETRY

Figure 3. The cytokine production patterns across exposure groups are replicated by spectral flow cytometry.



CBMCs were resuspended at 3.0 x 10^6 CBMCs and stimulated with PMA plus ionomycin (in the presence of protein transport inhibitors) for six hours, processed and analyzed on a Cytek Aurora 5L.

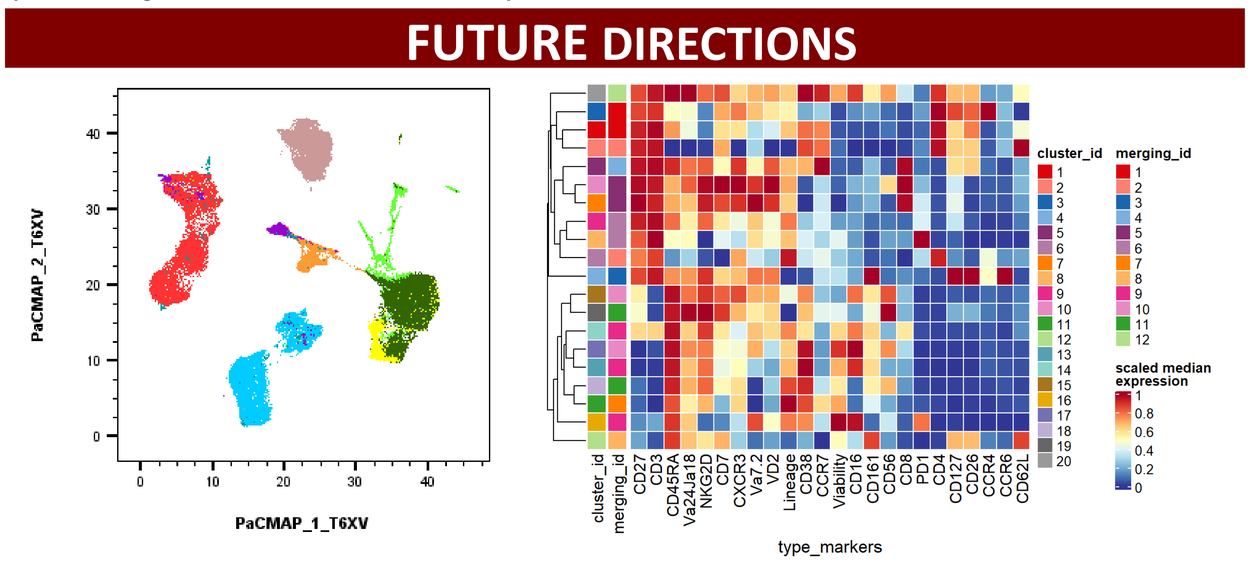
As in conventional Flow Cytometry data shown in Figure 1, the frequency of cytokine producing Vδ2 cells was increased in HEU-lo infants compared to HU controls when analyzed by spectral flow cytometry. Cytokine-production in HEU-hi infants appears heterogeneous.

CONCLUSIONS

- **In-utero HIV and/or ART exposure impacts cord blood Vδ2 cells in HEU infants**

- An increased frequency of cord blood Vδ2 cells in HEU infants; corroborating previous observations noted in a Nigerian cohort.
- An increased frequency of ex-vivo cytokine-producing Vδ2 cells was noted in response to PMA-ionomycin. Conversely, a decreased frequency of cytokine-producing cells was noted after expansion with BCG, but not with Zoledronate.

FUTURE DIRECTIONS



- Analyze the spectral flow cytometry data using both supervised and unsupervised approaches to understand the impact on other Innate-like T cells.
- Finalize acquisition and analysis of longitudinal adaptive T cell responses in our cohort to vaccine antigen (Bacillus Calmette Guerin (BCG)).

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