HIV EXPOSED UNINFECTED (HEU) INFANTS DISPLAY UNIQUE PRO-INFLAMMATORY BIOPROFILES

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Li Yin[1], Bernard M Fischer [2], Guglielmo M Venturi[2], Upasana Nepal[1], Shivangi Choudhary[2], Jerry Shen[1], Kai-Fen Chang[1], Isaac D Raplee[1], Samiksha A Borkar[1], Julie J Kim-Chang[2], Kristina De Paris[3], Maureen M Goodenow[1], John W Sleasman[2]

[1] National Institute of Allergy and Infectious Diseases, Bethesda, MD, USA, [2]Duke University School of Medicine, Durham, NC, USA, [3]University of North Carolina, Chapel Hill, NC, USA

BACKGROUND

Hypothesis: Inflammatory biomarkers associated with macrophage activation are elevated in pregnant women with HIV (**PWWH**) leading to increased levels in their HIV exposed uninfected (**HEU**) infants.

Introduction: HEU infants display higher levels of immune activation compared to HIV unexposed uninfected (**HUU**) infants. However, the impact of inflammatory biomarkers in PWWH on the corresponding bioprofiles in their HEU infants are largely unexplored. The current study evaluates differences in biomarkers associated with inflammatory immune pathways between HEU and HUU infants in relation to associations of biomarkers of immune activation between PWWH and their HEU infants, and between pregnant women without HIV (**PWWOH**) and HUU infants.

METHODS

Study cohorts: Archived plasma samples from mothers and their infants enrolled in PACTG protocol 316 are compared to PWWOH and their HUU infants. Study groups included: PWWOH and their HUU infants (n = 18 pairs); PWWH on ART who suppressed virus at delivery ($PWWH^{VLneg}$) and their HEU infants (HEU^{MVLneg}) (n = 23 pairs); PWWH on ART which failed to suppress HIV at delivery ($PWWH^{VLpos}$) and their HEU infants (HEU^{MVLpos}) (n = 23 pairs).

Biomarkers: 21 plasma immune biomarkers associated with B cell development, macrophage activation, T cell activation and differentiation, and inflammation (**Figure 1**) were measured using Mesoscale Diagnostics multiplex assays.

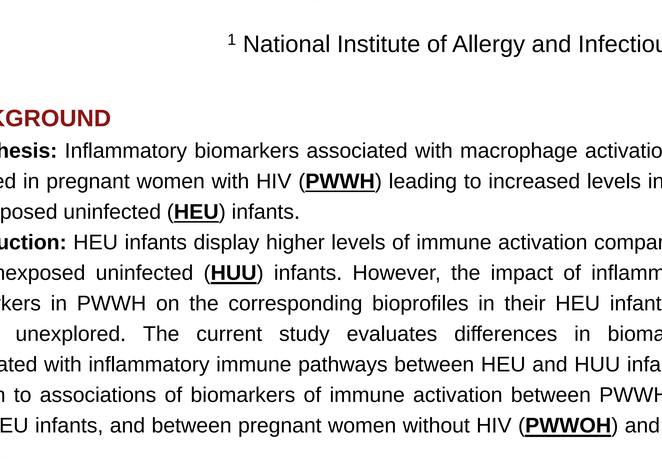


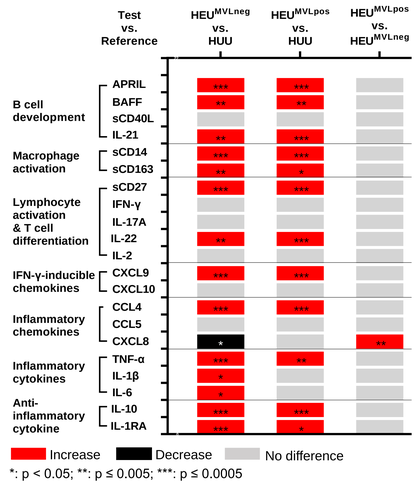
Figure 1. Cellular networking in lymphoid tissues. Known function and interactions among germinal center cells including macrophages, T cells, B cells and dendritic cells along with cytokines and cellular proteins that can be detected in the plasma. Elevated (red) or lower (green) biomarkers are known when comparing HEU to HUU infants.

Statistics, bioprofile relatedness and correlation networking: Mann-Whitney U test was used for pairwise comparisons between groups. One-Way ANOVA compared multiple groups. Spearman's rank test was used for correlation analysis. Uniform Manifold Approximation and Projection (UMAP) was applied to test for bioprofile relatedness between study groups. Cytoscape was used to plot correlation biomarker networks.

Biomarkers of innate and adaptive immunity are elevated in HEU infants and distinct from HUU infants, likely the result of HIV-associated maternal inflammation.

RESULTS

Figure 2. Modulation of immune biomarkers by in utero exposure to HIV/ART



*: p < 0.05; **: p ≤ 0.005; ***: p ≤ 0.0005

HEU compared to HUU infants:

- Increased levels of plasma biomarkers related to:

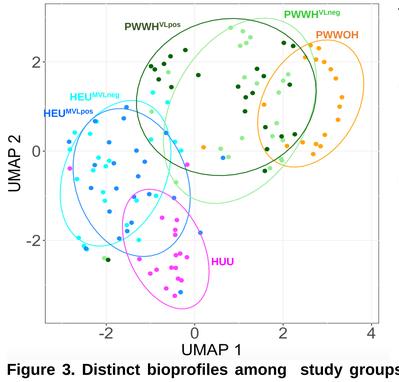
a) Higher biomarker levels of B cell development (APRIL, BAFF, IL-21).

b) Elevated immune activation and inflammation markers (sCD14, sCD163, sCD27, IL-22, CXCL9, CCL4, TNF-α), and immune regulation (IL-10, IL-1RA) in HEU infants, regardless of their mothers' viral status.

- HEU^{MVLneg} have increased levels of IL-1β and IL-6.

Biomarkers in HEU^{MVLneg} and HEU^{MVLpos} infants are similar (exception CXCL8).

Figure 3. Distinct bioprofiles among mothers and infants.



The bioprofiles of study groups developed using UMAP of all 21 biomarkers show:

Similar relationships between:

- HEU^{MVLneg} and HEU^{MVLpos}
- $PWWH^{VLneg}$ and $PWWH^{VLpos}$

Distinction relationships between:

- All Mothers and their infants
- HEU and HUU infants
- PWWH and PWWOH, indicating that bioproliles in PWWH are independent of VL and ART.

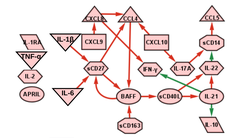
Figure 3. Distinct bioprofiles among study groups. UMAP was applied to test for bioprofile relatedness between study groups. Raw biomarker data were imported into R v4.1.3 and normalized by centering the data and dividing by their standard deviations. The umap v0.2.8.0 was used to group the study participants using the Euclidean distance and 10 nearest neighbors with results plotted by the ggplot2. Ellipses were generated using confidence intervals of 80%.

Figure 4. Correlations of biomarkers between mothers and babies.

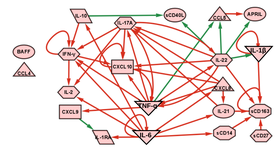
A.

Function	Biomarker	Mother/baby correlation B	C	D
B cell Development	APRIL			
	BAFF			
	sCD40L			
	IL-21			
Macrophage Activation	sCD14			
	sCD163			
T cell activation & differentiation	sCD27			
	INF-γ			
	IL-17A			
	IL-22			
	IL-2			
INF-γ-inducible chemokines	CXCL9			
	CXCL10			
Inflammatory chemokines	CCL4			
	CXCL5			
	CXCL8			
Inflammatory cytokines	TNF-α			
	IL-1β			
	IL-6			
Anti-inflammatory cytokines	IL-10			
	IL-1RA			

B.



C.



D.

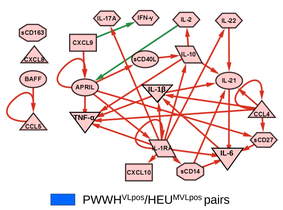


Figure 4. Correlations of biomarkers between mothers and babies. A. Pairwise correlations between mothers and infants on plasma biomarkers. All are positive correlations with Pink, PWWOH/HUU pairs; Cyan, $PWWH^{VLneg}/HEU^{MVLneg}$; Blue: $PWWH^{VLpos}/HEU^{MVLpos}$; white, no correlations. Cytoscape plotted multiple correlations between biomarkers in each group. **B.** unique to PWWOH/HUU, **C.** unique to $PWWH^{VLneg}/HEU^{MVLneg}$ **D.** unique to $PWWH^{VLpos}/HEU^{MVLpos}$. Arrow direction: pointing from mother factor to baby factor; → : positive correlation; → : negative correlation.

- PWWH and their HEU infants have multiple correlations between biomarkers when compared to HUU mother/infant pairs (B vs. C or D).
- Among HEU mother/infant pairs, correlations involve many biomarkers of innate immunity.
- Correlations in inflammatory pathways vary between PWWH and their infants based on maternal VL (e.g. IL-6 in $PWWH^{VLneg}$ and IL-1RA in $PWWH^{VLpos}$) (A-D).

CONCLUSIONS

- During pregnancy, maternal biomarkers involve germinal center formation and macrophage activation and are reflected in their infants.
- Bioprofiles in HEU infants are distinct from HUU infants, as well as their mothers.
- *In utero* HIV and ART exposure results in increased inflammatory biomarkers in the infants.
- Increased levels of inflammatory biomarkers in HEU infants are independent of maternal viral levels.
- HIV-associated inflammation in mothers with HIV may have long-term implications for early immune development in their infants.

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