Maternally Imprinted Immune Perturbations in HIV Exposed Uninfected (HEU) Infants Persist for 6 Months.

936

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. *Presenting author; #Co-senior authors

BACKGROUND

Introduction: HEU infants exhibit higher mortality/morbidity including adverse growth, developmental, infectious, and metabolic outcomes, and perturbed immune responses. How their immune profiles are altered and influenced by maternal immunity is unknown.

Hypothesis: Due to HIV-associated immune activation in mothers, HEU babies exhibit heightened immune activation and inflammation that may persist after birth.

METHODS

Table 1. Demographic and clinical characteristics of study cohorts.

		Mother/baby pairs						
		PWOH (n = 18)	HUU (n = 18)	PWH-VS (n = 23)	HEU-MVS (n = 23)	PWH-VNS (n = 23)	HEU-MVNS (n = 22)[a]	HUU (n = 32)
Time point		At delivery[b]	Birth	At delivery[b]	Birth & 6m	At delivery[b]	Birth & 6m	Birth & 6m
Women's age (year)[c]		28 (23 - 31)	NA	26 (23 - 33)	NA	26 (23 - 30)	NA	32 (29 - 35)
African Americans		17%		44%		52%		19%
Viral load (HIV-1 RNA copies/ml plasma)[c]		NA	NA	< 400[d]	NA	2,881 (1,505 - 22,948)	NA	NA
CD4 T cell count (cells/μL)[c]		ND	ND	640 (433 - 798)*	ND	440 (259 - 530)	ND	ND
ART during pregnancy	**ZDV alone**	NA	NA	1 (4%)	NA	10(44%)	NA	NA
	NRTI combo	NA	NA	9(43%)	NA	7(30%)	NA	NA
	NRTI combo + PI	NA	NA	13(52%)	NA	6(26%)	NA	NA
ART to mother during labor	**NVP single dose**	NA	NA	11 (48%)		13 (57%)		NA
Baby gender	**Male**	NA	50%	NA	39%	NA	55%	50%
	Female	NA	50%	NA	61%	NA	45%	50%
Baby birth weight (kg)[c]		NA	3.6 (3.0 - 4.0)	NA	3.0 (2.8 – 3.4)	NA	3.0 (2.8 - 3.5)	3.6 (3.1 – 3.8)
Mode of delivery	**Vaginal**	100%		65%		48%		100%
	Cesarean section	0%		35%		52%		0%
Feeding method[e]	**Breast fed**	NA	NA	NA	0%	NA	0%	59%
	Formula fed	NA	NA	NA	100%	NA	100%	16%
	Mixed fed	NA	NA	NA	0%	NA	0%	25%

[a]: The twins by one PWH-VNS were removed from the study; [b]: within 2 days before delivery; [c]: Median (25% - 75% quartile range); [d]: HIV-1 viral load undetectable level is <400 RNA copies/ml plasma; [e]: only apply to infants at 6 months of life. *p < 0.05 by Mann-Whitney test. **Abbreviations**: **ND**, no data; **NA**: not applicable; **PWOH**, pregnant woman without HIV; **PWH-VS**: pregnant woman with HIV virally suppressed; **PWH-VNS**: pregnant woman with HIV virally non-suppressed; **HUU**: HIV unexposed uninfected; **HEU-MVS**: HIV exposed uninfected born to virally suppressed mother; **HEU-MVNS**: HIV exposed uninfected born to virally non-suppressed mother; **ART**: antiretroviral therapy; **ZDV**: zidovudine; **NRTI**: nucleoside reverse transcriptase inhibitors; **PI**: protease inhibitors; **NVP**: nevirapine.

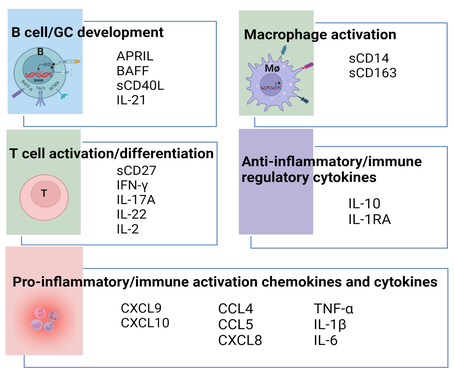

Figure 1. Biomarkers. Twenty-one biomarkers associated with B-cell/germinal center (GC) development, macrophage or lymphocyte activation, inflammation and immune regulation were measured in plasma using Mesoscale multiplex assays.

Analysis: Principal component analysis (PCA) was used to reveal the relatedness of study groups. Mann-Whitney test was applied to compare between two groups with p < 0.05 as significant. Linear regression was fit to identify differences among study groups, and biomarker correlations between mother/newborn pairs.

HEU infants displayed heightened immune activation and inflammation that correlated with maternal immune perturbations and persisted up to 6 months after birth.

RESULTS

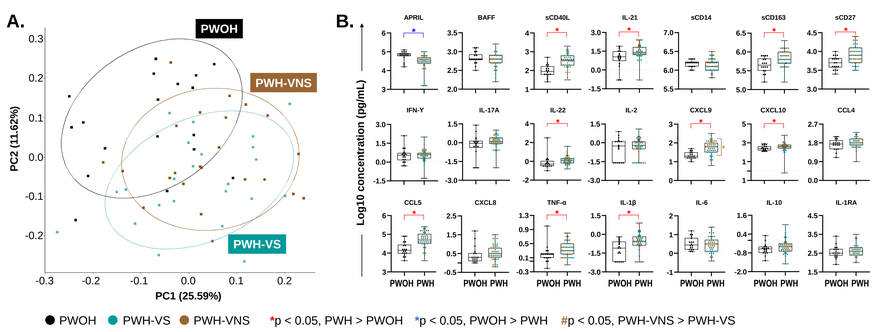

Figure 2. Immune activation and inflammation in PWH at the time of delivery. **A.** Immune profiles were distinct between PWOH and PWH but similar between VS and VNS PWH. **B.** PWH exhibited heightened immune activation and inflammation compared with PWOH at the time of delivery. VS or VNS PWH had a similar immune bioprofile.

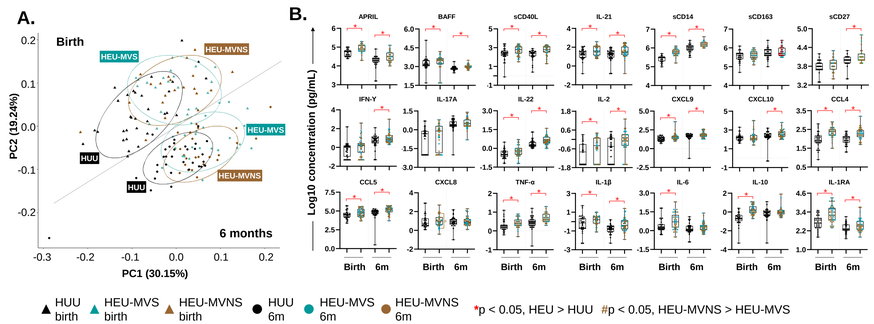

Figure 3. Immune activation and inflammation in HEU persisted at least 6 months. **A.** At birth and 6 months of life, immune bioprofiles were similar in HEU babies born to VS and VNS PWH, but distinct from HUU babies. **B.** Compared to HUU babies at birth and 6 months, HEU babies had significantly higher concentrations of biomarkers associated with B cell/GC development, immune activation, inflammation and anti-inflammation/immune regulation. By 6 months of age, elevated immune markers IL-6 and IL-10 in HEU infants had normalized, but other cytokines (sCD27, IFN-γ, CXCL10) increased compared to HUU infants. Consistent with the PCA results, the immune bioprofiles in HEU infants were similar at both birth and 6 months regardless of their mothers' viral status.

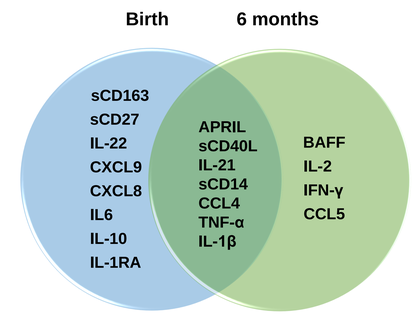

Figure 4. Age-related bioprofiles. Linear regression for biomarker concentrations across the three groups (HUU, HEU-MVS and HEU-MVNS) was fit and adjusted for covariates of mother's age and infant's sex. Several biomarkers, including APRIL, sCD40L, IL-21, sCD14, CCL4, TNF-α , and IL-1β remained significantly perturbed at 6 months of life in HEU infants.

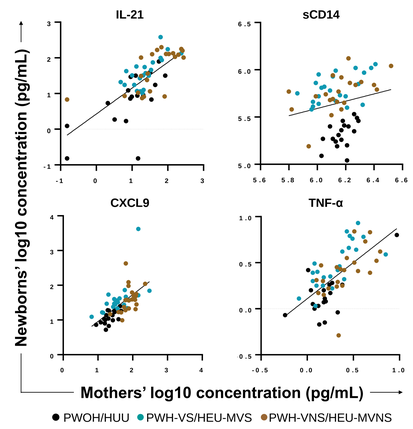

Figure 5. Biomarker correlations between mother/newborn pairs. Linear regression was fit with newborn's value as the outcome and mother's value as the variable of interest, and adjusted for mother's age, newborn's gender, and mother's HIV and viral status. There were positive correlations in plasma concentrations of IL-21, sCD14, CXCL9, INF-α as well as BAFF, sCD163, IFN-γ, IL-17A, CCL4, CCL5, and IL-6 (not shown) between all mothers (PWOH, PWH-VS and PWH-VNS) and their newborns.

CONCLUSIONS

HEU infants, even though uninfected, displayed bioprofiles of immune activation and inflammation that persisted up to 6 months of life when compared to HUU infants.

Altered immune bioprofiles in HEU infants correlated with immune perturbation in their mothers.

Maternally imprinted immune activation may alter immune ontogeny in HEU infants and contribute to increased morbidity later in life.

FUNDING

Pediatric AIDS Clinical Trials Group Protocol 316 *a Phase III Randomized, Double Blinded Study of Nevirapine for the prevention of maternal-fetal transmission in pregnant HIV-infected women*, UM1AI1068632, R01AI100147, R21HD102853, and NIH Office of AIDS Research.