INNATE IMMUNE ACTIVATION AMONG HIV-1 EXPOSED-UNINFECTED INFANTS FROM BOTSWANA

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Poster #881

BACKGROUND

- Maternal triple antiretrovirals (ARVs) used during pregnancy and during breastfeeding has dramatically reduced infant HIV acquisition globally. However, HIV-exposed uninfected (HEU) infants experience 2-4% higher mortality rates compared with HIV-unexposed uninfected (HUU) infants.
- Higher HEU infant morbidity/mortality is due to infectious causes, including respiratory illnesses and diarrheal disease.
- An immune profiling analysis was conducted to explore immune phenotypes of HEU and HUU infants enrolled in a longitudinal gut microbiome study in Botswana.

METHODS

Peripheral blood mononuclear cells (PBMCs) were collected at 3 and 6 months of life from a HUE (n=29) and HUU (n=25) infants. Multiparametric flow cytometry was used to quantify proportions and phenotypic characteristics of innate immune cells (monocytes, dendritic cells and NK cells) using mABs specific for CD3, CD19, CD16, CD56, CD14, CD16, HLADR, CD11c, CD123, NKG2D, NKp30 and CD161; and adaptive T and B cell-mediated immune responses using CD3, CD19, CD4, CD8, CD45RA, CCR7, CD27, CD127, CD25, HLADR, CD38, IgD immune markers.

All HIV-infected women received ≥6 weeks of ARVs prior to delivery and all infants were born full-term and all HEU infants tested HIV negative at 3- and 6-months of life.

Figure 1. Distribution of Dendritic Cells among HEU and HUU infants

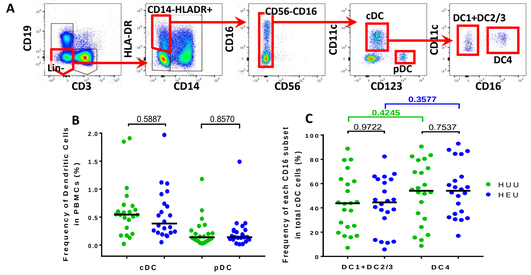

Figure 1: Identification of Dendritic Cells subsets using CD11c, CD123 and CD16 surface markers among HEU and HUU infants. A. Representative flow cytometry analysis used to phenotype the Dendritic Cells (DC) populations. The percentage of conventional DC (cDC) is shown as cells expressing CD11c+CD123- subdivided in the types 1-3 or 4 depending of CD16 expression, and plasmacytoid DC (pDC) as CD11c-CD123^{high} cells. **B.** Proportion of cDC and pDC in PBMCs from 22 HUU and 22 HUE infants at 3 months is shown. C. Distribution of cDC among subsets defined by the expression of CD16 at 3 months. Values expressed as median. P-values were calculated by Mann-Whitney U tests to unpaired samples and Wilcoxon test to paired samples: ns

Figure 2. Distribution of Monocytes among HEU and HUU infants

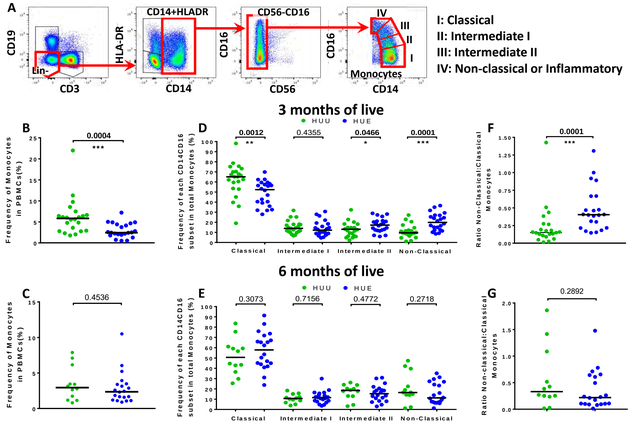

Figure 2: Enrichment of proinflammatory CD14+ CD16+++ monocytes in HEU infants. A. Representative flow cytometry analysis used to phenotype the monocytes populations. The percentage of classical monocytes is shown as cells expressing CD14+CD16-, intermediate I as CD14+CD16+ cells , Intermediate II as CD14+CD16++ cells and Non-classical or Inflammatory as CD14^{low}CD16+++ cells. **B-C.** Frequency of monocytes in PBMCs from 22 HUU and 22 HUE at 3 months (B) and 12 HUU and 20 HEU infants at 6 months (C) is shown. **D-E.** Distribution of monocytes among subsets defined by the expression of CD14 and CD16 at 3 (D) and 6 (E) months. **F-G.** Ratio between Non-classical and classical monocytes at 3 (F) and 6 (G) months. Values expressed as median. P-values were calculated by Mann-Whitney U tests: *p<0.05; **p<0.01; ***p<0.001.

Figure 3. Distribution of NK Cells among HEU and HUU infants

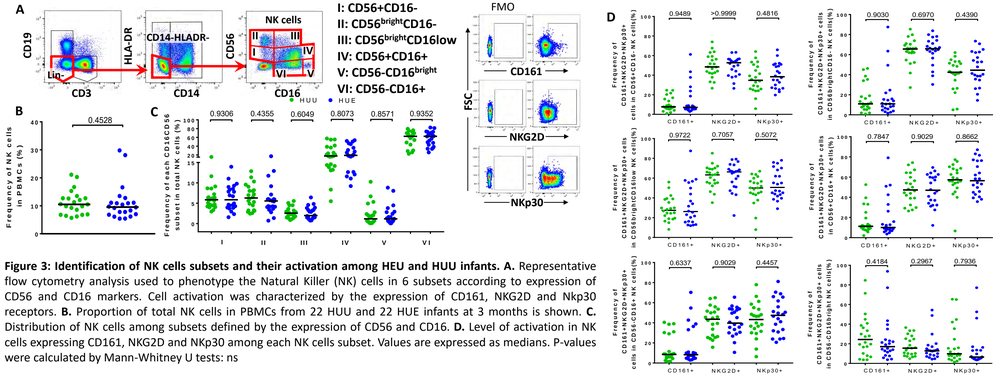

Figure 3: Identification of NK cells subsets and their activation among HEU and HUU infants. A. Representative flow cytometry analysis used to phenotype the Natural Killer (NK) cells in 6 subsets according to expression of CD56 and CD16 markers. Cell activation was characterized by the expression of CD161, NKG2D and Nkp30 receptors. **B.** Proportion of total NK cells in PBMCs from 22 HUU and 22 HUE infants at 3 months is shown. **C.** Distribution of NK cells among subsets defined by the expression of CD56 and CD16. **D.** Level of activation in NK cells expressing CD161, NKG2D and NKp30 among each NK cells subset. Values are expressed as medians. P-values were calculated by Mann-Whitney U tests: ns

RESULTS

Figure 4: Identification and characterization of CD4+ and CD8+ T cells subsets among HEU and HUU infants. A. Representative flow cytometry analysis used to phenotype the T cell populations. The percentage of Naïve subset is shown as cells expressing CD45RA+CCR7+CD27+; central memory (CM) as CD45RA-CCR7+CD27+ cells; effector memory (EM) Th0Th1 as CD45RA-CCR7-CD27- cells and Th1Th2 as CD45RA-CCR7-CD27+ cells; and terminally differentiated effector memory (TEMRA) as CD45RA+CCR7-CD27- cells. The proportion of regulatory CD4+ T cells is defined as CD127-CD25+, distributed among subsets defined by the expression of HLADR and CD45RA as resting (CD45RA+HLADR-), activated resting (CD45RA+HLADR+), activated effector (CD45RA-HLADR+) and activated memory (CD45RA-HLADR-). **B.** Ratio between CD4+ and CD8+ T cells from 25 HUU and 29 HUE infants at 3 months. **C.** Frequency of CD4+ and CD8+ T cells defined by the expression of CD45RA, CCR7 and CD27. **D.** Percentage of regulatory T cells in CD4+ T cells from 25 HUU and 29 HUE infants at 3 months. **E.** Distribution of Treg CD4+ T cell among subsets defined by the expression of CD45RA and HLA-DR. Values expressed as median. P-values were calculated by Mann-Whitney U tests: *p<0.05.

Figure 4. Distribution of T Cells among HEU and HUU infants

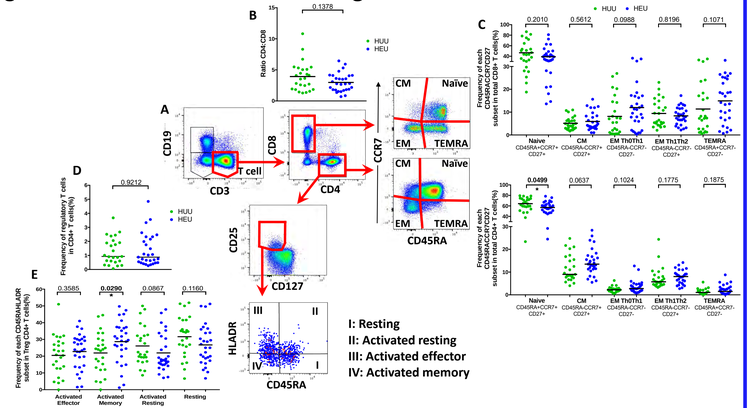

Figure 5. Distribution of B Cells among HEU and HUU infants

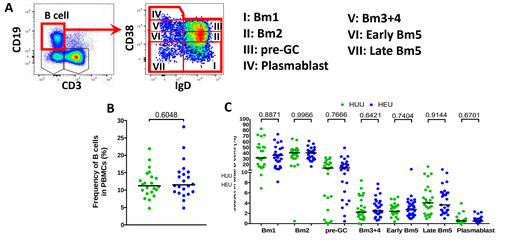

Figure 5: Identification of B cells subsets among HEU and HUU infants. A. Representative flow cytometry analysis used to phenotype the B cell populations. The percentage of the Bm1 subset is shown as cells expressing IgD+CD38-; Bm2 as IgD+CD38+ cells; pre-GC as IgD+CD38++ cells; Plasmablast as IgD-CD38+++ cells; Bm3+4 as IgD-CD8++ cells; Early Bm5 as IgD-CD38+ cells and Late Bm5 as IgD-CD38- cells. **B.** Frequency of B cells in PMBCs from 25 HUU and 29 HUE infants at 3 months is shown. **C.** Distribution of B cell among subsets defined by the expression of IgD and CD38. Values are expressed as medians. P-values were calculated by Mann-Whitney U tests: ns.

CONCLUSIONS

In this cohort, fetal *in utero* exposure to HIV-1 and ART was associated with a distinct immunological profile among HIV-exposed uninfected infants, characterized by increased immune activation. Additional research is needed to determine whether abnormal innate immune activation among HEU infants is associated with increased infant morbidity/mortality.

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