The Effect of HIV and Latent Tuberculosis Infection During Pregnancy on Infant Immune Development in IMPAACT P1078 study cohort

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Abstract

Background: In utero exposure to maternal HIV infection may impact the infant's ability to develop or retain immunological memory, even in the absence of vertical HIV acquisition. The additional effect of maternal latent tuberculosis status on immune development is not known.

Material and Methods: We acquired cryopreserved infant peripheral blood mononuclear cell PBMC) samples from the IMPAACT P1078 study and performed immunophenotyping on 48 HIV-exposed uninfected infants born to HIV+ mothers with (n=24) or without (n=24) latent TB infection (LTBI) as determined by Interferon-gamma release assay (IGRA) during the 2nd or 3rd trimester. Infant samples were obtained at 12 and 44 weeks of life. Immunophenotyping was performed using 28-color flow cytometry and the Cytek Aurora for identification of T cells, B cells, natural killer (NK) cells, and myeloid cells (monocytes, macrophages, and dendritic cells (DC)). Statistical analysis was performed to assess immune development from 12 to 44 weeks (paired students T-test) as well as to compare immune phenotypes between infants from IGRA+ vs. IGRA- mothers (Mann-Whitney U-test). Additionally, plasma samples at study entry from 76 mothers were assessed for inflammatory biomarkers using OLINK Inflammatory panel of 95 proteins and statistical comparisons were made between IGRA+ (n=38) and IGRA- (n=38) groups.

Results: Out of 830 parameters analyzed, statistically significant differences ($p<0.05$) were discovered in paired analysis between samples of infants at 12 and 44 weeks. Frequencies of gamma delta (GD) T cells, CD4 and CD8 T Effector and Effector memory, CXCR5+ CD4 T cells, and CD27+ B cells increased in all infants with age. Frequencies of T cells expressing CD38 and HLADR as well as IgM+, IgD+, and naive B cells decreased in all infants. CD2 expression on NK cells and DC subsets also increased with age in all infants. The myeloid population had the most differences between infants from IGRA+ and IGRA- mothers including decreased MFI of CD38, CD36, CD11b and CCR2 on myeloid dendritic cells as well as a decrease in expression in CCR2 and CD11b at 12 weeks in infants from IGRA+ mothers. Maternal plasma samples from IGRA+ women showed higher levels of inflammatory proteins including IL-18R1, TNSF14, CXCL9, EN-RAGE ($p<0.05$) while PD-L1 and IL-8 showed trends of higher levels ($p=0.052$ and 0.065, respectively).

Conclusion: Our results indicate that immune development from the perspective of immune cell distribution in blood is not drastically altered due to maternal LTBI. However, immune activation and DC subsets in infants from IGRA+ mothers were altered compared to infants from IGRA- mothers at 12 weeks but normalized by the 44wk timepoint. This may be due to higher levels of activation due to the maternal milieu of chronic stimulation from LTBI. Considering that the first few weeks of life are a high-risk time for infections, altered immune responses at 12 weeks may put infants at an increased or decreased risk of infection. Maternal plasma revealed that markers of inflammation were elevated in plasma from IGRA+ mothers possibly due to chronic stimulation from LTBI. Our results warrant further investigation of infant immune development following maternal LTBI exposure in utero.

Objective

Aim 1: To determine if infants born to women with HIV and LTBI have altered immune development.

Aim 2: To determine the impact of HIV and LTBI on maternal inflammatory responses.

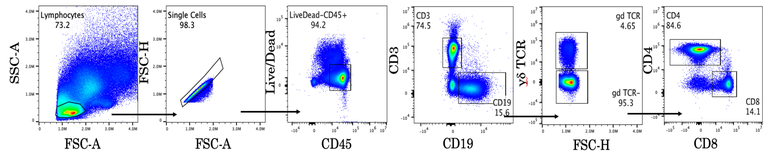
Study population

- Stored PBMC and Plasma samples from mother/infant pairs were acquired from IMPAACT study (P1078) which evaluated the safety of isoniazid preventive therapy (IPT) both antepartum and postpartum. All infants received BCG vaccination.
- Maternal samples were obtained at entry during pregnancy and infant samples were obtained at 12 and 44 weeks of life. IGRA status was determined by QuantiFERON gold test.
- Preliminary data presented here includes 48 IGRA- infants evaluated from IGRA- (n=24) and IGRA+ (n=24) mothers; and 76 maternal plasma samples from IGRA- (n=38) and IGRA+ (n=38) groups. Samples were chosen for this study based on sample availability.

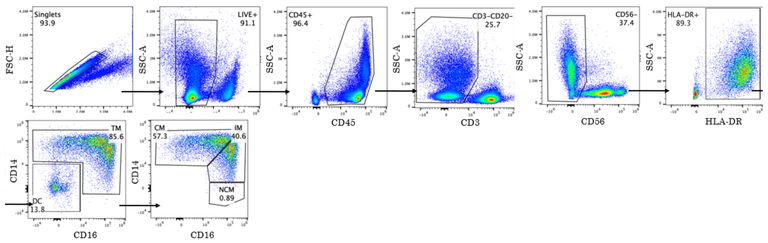
Methods

Flow Cytometry

T and B Cell Gating



Monocyte Gating



Two 28 color panels were developed for the Cytek Aurora to evaluate phenotype of T cells, B Cells, Monocytes, Dendritic Cells, and NK Cells. Data was analyzed using flowjo software. A basic gating strategy is shown above.

Methods (contd.)

Flow Cytometry Statistical Analysis

To assess changes in immune development from 12 to 44 weeks of age, paired t-test was performed. Mann-Whitney U-test was used to compare immune phenotypes between infants from IGRA+ (n=24) vs. and IGRA- (n=24) groups at 12 and 44wks independently.

Plasma Biomarker Analysis

Plasma samples at study entry from 76 mothers were assessed for inflammatory biomarkers using Olink services (Uppsala, Sweden) and the Inflammation panel of 95 proteins. Statistical comparisons were made between IGRA+ (n=38) and IGRA- (n=38) groups using unpaired t-test.

Results

Impact of Maternal LTBI on Immune Development in Infants During the First Year of Life

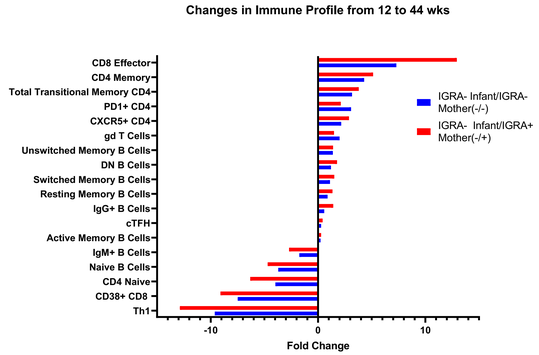


Figure 1. Similar change in distribution of many phenotypic subsets in blood with age in IGRA- infants from IGRA- and IGRA+ mothers from 12 to 44 weeks of age. Means for each immune marker were determined and the Delta was calculated (44wk – 12wk) for each study population (-/- and -/+). This analysis identified increases in memory populations of CD4 T cells and B Cells as well as frequencies of cells expressing specific markers like CXCR5+ CD4 and PD1+CD4. CD38+ CD8 T Cells, Th1, CD4 naïve T cells and IgM+ B cells decreased with age. Markers shown here were significantly different between 12 and 44wk but not different between groups. These changes reflect normal development of the infants' immune system between 12 and 44 weeks.

Impact of Maternal LTBI on T cells and Immune Activation in HIV Exposed Infants During the First Year of Life

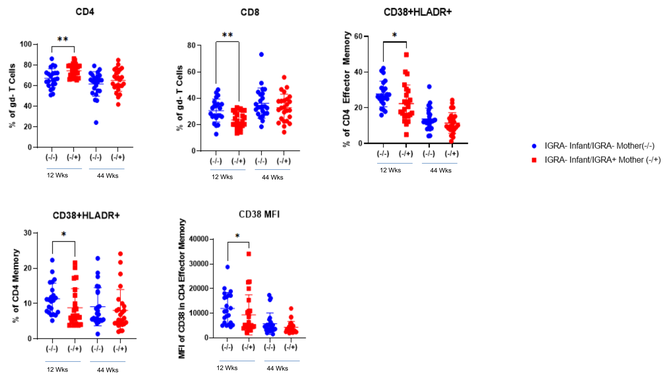


Figure 2. Reduced immune activation in CD4 T cell subsets in infants born to IGRA+ mothers at 12 weeks of age. Infants born to IGRA+ mothers showed reduced CD8 and CD4 T cell frequency and reduced CD38+HLADR+ expression in effector memory (CD45RO+CD27-) and memory (CD45RO+) CD4 T cells. Mean Fluorescence Intensity (MFI) of CD38 in CD4 effector memory was also reduced. This change was only observed in infants at the 12 wk timepoint.

Results (contd.)

Impact of Maternal LTBI on Myeloid Compartment in Infants During the First Year of Life

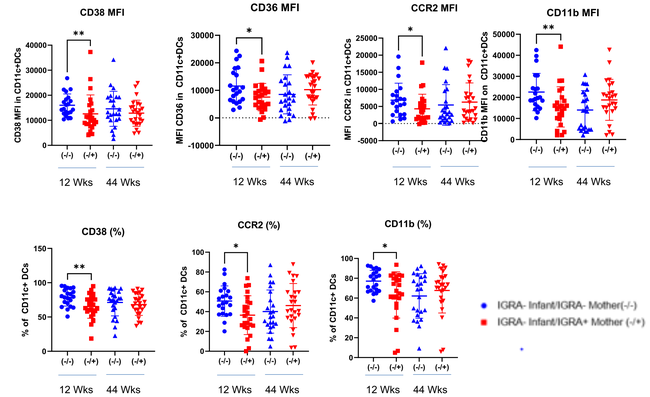


Figure 3. Immune markers associated with activation, phagocytosis and chemotaxis are reduced in myeloid Dendritic Cells (CD11c+ DC) in infants born to IGRA+ mothers at 12 weeks of age. Phenotypic analysis of dendritic cells and monocytic subsets revealed that the MFI of CD38, CD36, CCR2, and CD11b was reduced in infants born to IGRA+ mothers at 12 weeks only. Frequency of CCR2 and CD11b expression was also reduced in infants born to IGRA+ mothers.

Impact of LTBI on Soluble Biomarkers of Inflammation in Maternal Plasma

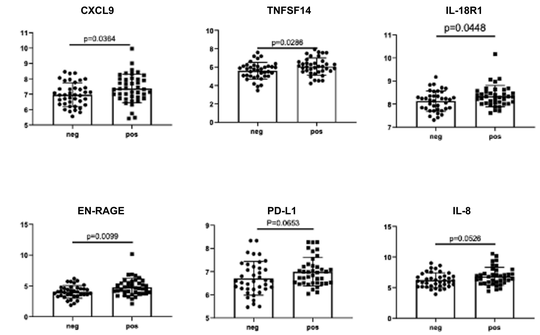


Figure 4. Markers of inflammation are increased in pregnant women with positive IGRA status. Maternal plasma samples from entry were assessed for inflammatory biomarkers using a panel to identify 95 possible proteins. Significant increases in CXCL9, TNFSF14, IL-18R1, and EN-RAGE were observed in IGRA+ mothers compared to IGRA- mothers while PD-L1 and IL-8 showed trends of higher levels.

Summary and Conclusions

1. Immune development from the perspective of immune cell distribution in blood is not drastically altered due to maternal LTBI
2. Immune activation and DC subsets in infants from IGRA+ mothers were altered compared to infants from IGRA- mothers at 12 weeks but normalized by the 44wk timepoint.
3. Markers of inflammation were elevated in plasma from IGRA+ mothers possibly due to chronic stimulation from LTBI.
4. Considering that the first few weeks of life are a high-risk time for infections, altered immune responses at 12 weeks may put infants at an increased or decreased risk of infection.
5. Our results warrant further investigation of infant immune development following maternal LTBI exposure in utero.

Acknowledgements

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