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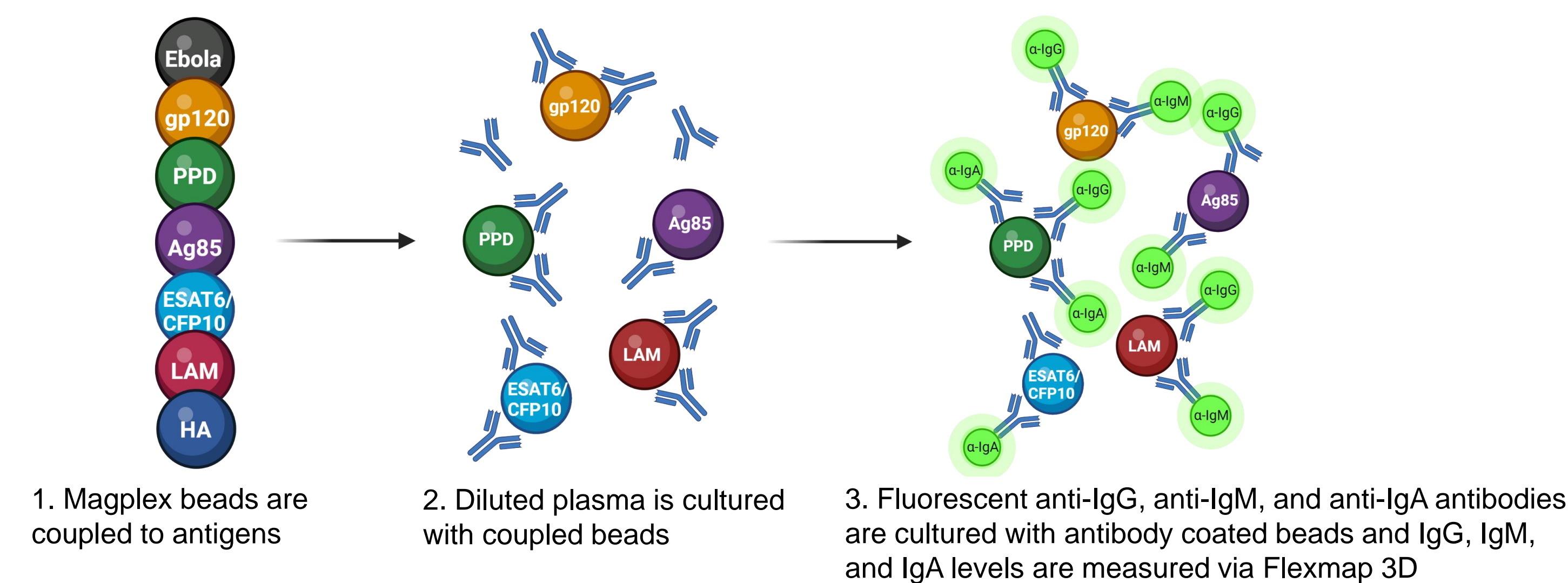
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## BACKGROUND

Latent tuberculosis (LTBI) is a common concurrent disease in people with HIV. How maternal HIV and LTBI influence the development of an infant's immune response is not well characterized. We hypothesized that maternal antibodies (Ab) may interact with the infants' immune responses to TB and BCG providing the basis for this study.

## METHODS

Frozen plasma from pregnant people with HIV (14-34 weeks gestation) (IGRA+: n=98, IGRA-: n=101) and their infants, aged 12 and 44 weeks, were obtained from the IMPAACT P1078 study. All women were on ART with median CD4 counts of 466 (IGRA-) and 499 (IGRA+). 6 mothers developed active TB disease (ATB), determined by AFB smear. Infants were HIV-exposed uninfected and received BCG at a median of 1 week after birth. Mother/infant (M/I) pairs were characterized by IGRA status as: -/-, +/-, -/+, +/+. At 44 weeks, 9 infants were IGRA+. M/I plasma was evaluated for the presence of TB- (PPD, ESAT6/CFP10, Ag85A, and LAM) and HIV-specific (GP120) IgG, IgA, and IgM using a bead-based Luminex assay with Flexmap 3D. Ebola and flu were used as negative and positive controls, respectively. Data was analyzed using prism software.

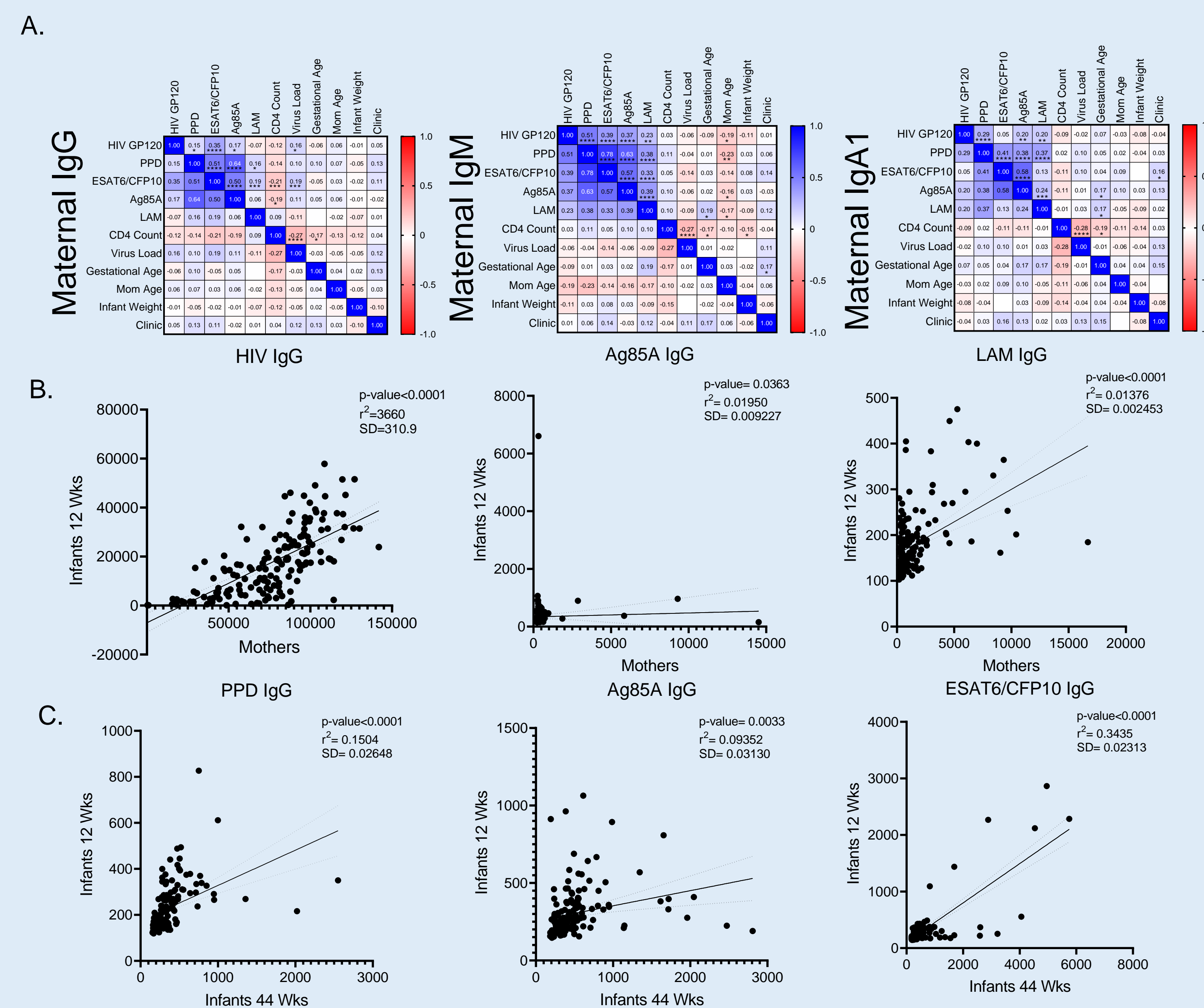


## Statistical Analysis

Mann Whitney test was used to identify significant differences (p<0.05) between mothers (IGRA- vs IGRA+ vs ATB) and infants (-/- vs +/- and IGRA- vs IGRA+). A spearman correlation was used to evaluate correlations (p<0.05) between maternal clinical parameters and mean fluorescence intensity (MFI) of maternal IgG, IgM, and IgA. Multiple linear regression analysis was used to identify significant differences (p<0.05) between maternal parameters and infant outcomes at 12 weeks.

|   | IGRA – (n=98)     | IGRA+ (n=101)    | Active TB (n=6)  |
|---|-------------------|------------------|------------------|
| Age, Years (median)                       | 18-44 (30)        | 18-44 (30)       | 21-40 (30)       |
| Gestational Age, Weeks (median)           | 14-34 (26)        | 14-34 (27)       | 16-31 (26)       |
| CD4 count, cells/mm <sup>3</sup> (median) | 143-1047 (467)    | 58-1411 (494)    | 79-678 (396.5)   |
| Plasma Virus Load (median)                | 39-464894 (39)    | 19-60475 (39)    | 39-199 (39)      |
| Infant Weight, Grams (median)             | 2000-42000 (2950) | 1550-4020 (3120) | 2780-3525 (3000) |
| Infants Analyzed                          | 87                | 83               | 4                |
| IGRA+ Infants                             | 3                 | 6                | 0                |
| BCG administration timing, Weeks (median) | 0-65 (1)          | 0-69 (1)         |                  |

## IGRA status does not impact maternal or infant antibody responses to TB, but maternal antibodies may influence infant HIV and TB-specific IgG at 12 Weeks



**Figure 1. Maternal HIV- and TB- specific IgG are highly correlated and maternal IgG influences infant IgG at 12 weeks.** Spearman correlation (A) between maternal IgG, IgM, and IgA1 and maternal clinical parameters. Significant values denoted as \* p≤0.05, \*\* p≤0.01, \*\*\* p≤0.001, \*\*\*\* p≤0.0001. Multiple linear regression analysis was used to determine relationships between mother and infant Ag-specific IgG responses. XY plots show significant associations between (B) maternal IgG and infant IgG at 12 weeks and (C) infant IgG at 12 weeks with infant IgG at 44 weeks is shown.

## RESULTS

- Maternal HIV Ab positively correlated with TB- specific antibody responses.
- Maternal CD4 count correlated negatively with maternal viral load.
- Multiple linear regression analysis showed significant correlations observed for HIV IgG, Ag85A IgG, and LAM IgG between mothers and infants at 12 weeks. PPD IgG, Ag85A IgG, and ESAT6/CFP10 IgG were correlated with between infants at 12 and 44 weeks.
- No significant differences in plasma Ab between IGRA- and IGRA+ mothers at entry
- ATB mothers: increased PPD, ESAT6/CFP10, and Ag85A IgG; ESAT6/CFP10 and LAM IgM, and PPD and ESAT6/CFP10 IgA at time of diagnosis.
- Infants (-/- vs +/-): no differences in TB-specific plasma Ab responses at 12 weeks.
- Infants (+/- vs -/-): trend for lower IgG against LAM at 44 weeks.
- Infants (+ vs -): trend for higher PPD & Ag85A-specific IgG at 44 wks.

|             | IGRA- Mothers   |                 |                 | IGRA+ Mothers   |                 |                 | Active TB       |                 |                 |
|-------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|             | IgG             | IgM             | IgA             | IgG             | IgM             | IgA             | IgG             | IgM             | IgA             |
| HIV GP120   | 76191 +/- 2868  | 1897 +/- 4833   | 531.4 +/- 2043  | 73353 +/- 31497 | 1855 +/- 7927   | 409.4 +/- 1019  | 68432 +/- 36424 | 1405 +/- 2185   | 242.6 +/- 101   |
| PPD         | 338.8 +/- 137.5 | 593.8 +/- 426.6 | 166.1 +/- 29.29 | 349.8 +/- 185.4 | 591.1 +/- 341   | 164.6 +/- 25.90 | 995.9 +/- 592.3 | 803.1 +/- 561.5 | 209.5 +/- 50.3  |
| ESAT6/CFP10 | 306.6 +/- 147   | 1046 +/- 991.3  | 182.6 +/- 40.88 | 308.4 +/- 183.5 | 919.9 +/- 581.5 | 184.1 +/- 41.84 | 480 +/- 215.4   | 2131 +/- 1493   | 270.8 +/- 76.74 |
| Ag85A       | 473.7 +/- 608.6 | 4291 +/- 4546   | 243.1 +/- 103.3 | 767.3 +/- 2371  | 3669 +/- 2519   | 279.2 +/- 277.5 | 3496 +/- 5192   | 4830 +/- 3196   | 313 +/- 146.8   |
| LAM         | 1525 +/- 2252   | 2038 +/- 2771   | 210.5 +/- 106.9 | 1809 +/- 2729   | 1413 +/- 1659   | 220.8 +/- 127.1 | 4038 +/- 8105   | 1204 +/- 1936   | 206.4 +/- 76.93 |

**Table 2. Maternal Antibody Responses.** Table showing the mean MFI with standard deviation in IGRA-, IGRA+, and ATB mothers. HIV- and TB- specific IgG, IgM, and IgA1 were included. IGRA- and IGRA+ mothers were compared to each other and ATB mothers. Significant values were found when comparing IGRA- and IGRA+ mothers to ATB. Significant values are represented by asterisks \* p≤0.05, \*\* p≤0.01, \*\*\* p≤0.001, \*\*\*\* p≤0.0001.

|             | IGRA-/IGRA- 12 Wks |                 | IGRA+/IGRA- 12 Wks |                 | IGRA-/IGRA- 44 Wks |                 | IGRA+/IGRA- 44 Wks |                 |
|-------------|--------------------|-----------------|--------------------|-----------------|--------------------|-----------------|--------------------|-----------------|
|             | IgG                | IgM             | IgG                | IgM             | IgG                | IgM             | IgG                | IgM             |
| HIV GP120   | 16892 +/- 13707    | 258.6 +/- 200.6 | 16990 +/- 12994    | 504.5 +/- 2169  | 313 +/- 992        | 1296 +/- 2214   | 1173 +/- 5304      | 1399 +/- 1693   |
| PPD         | 235.7 +/- 113.8    | 328.3 +/- 168.7 | 227.2 +/- 79.73    | 326.7 +/- 144.1 | 395.2 +/- 350.4    | 870.5 +/- 990.9 | 344.9 +/- 209.5    | 865.5 +/- 754.2 |
| ESAT6/CFP10 | 311.8 +/- 296.5    | 445.5 +/- 391.9 | 366.4 +/- 526      | 486 +/- 864.0   | 569.5 +/- 735.3    | 1624 +/- 2076   | 575.4 +/- 963      | 1645 +/- 1729   |
| Ag85A       | 376.3 +/- 693      | 1808 +/- 1784   | 315.1 +/- 192.1    | 1876 +/- 2255   | 509.8 +/- 383.6    | 3214 +/- 2752   | 519.1 +/- 451.8    | 4103 +/- 3614   |
| LAM         | 183.1 +/- 76.19    | 142.1 +/- 37.80 | 175.8 +/- 60.83    | 138.6 +/- 34.58 | 276.4 +/- 195.9    | 412.1 +/- 444.9 | 273.9 +/- 326.3    | 544.3 +/- 1131  |

**Table 3. Infant Antibody Responses.** Table showing the mean MFI with standard deviation in IGRA- infants from IGRA- and IGRA+ mothers at 12 and 44 weeks. HIV- and TB- specific IgG, IgM, and IgA1 were included. IGRA- infants were compared based on maternal IGRA status. No significant differences were found.

## SUMMARY and CONCLUSIONS

- Maternal IGRA status does not impact TB-specific Ab responses in infants at 12 or 44 weeks;
- Maternal antibodies impact infant Ag-specific antibody frequencies as shown in figure 1.
- Maternal antibodies may influence infant antibody responses to TB
- Further functional studies are needed in order to elucidate the role of maternal antibodies on the infant humoral response to TB and BCG.

**ONGOING STUDIES:** Aimed to elucidate function of antibodies and T cells in infants born to HIV+ women with or without LTBI.

- Antibody-dependent cellular phagocytosis and antibody-dependent NK degranulation for maternal and infant antibodies.
- T cell proliferation assays for infant T cell responses to TB antigens and BCG.

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