Poster # 0322

THE IMPACT OF HIV EXPOSURE ON PLACENTAL PATHOLOGY AND TREG CELLS

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DolPHIN2

BACKGROUND

The human placenta is an integral organ that links the mother to her unborn foetus. This transient organ is comprised of maternal and foetal tissue, which together form the foetal maternal (FM) interface. Numerous immune cell subsets have been described at the FM interface. These include regulatory T cells (Tregs). Defects in maternal Treg frequency and function could be a cause of pregnancy complications including preterm birth, possibly due to alterations in the maintenance of FM tolerance. HIV and/or antiretroviral (ARV) exposure may interfere with this tolerance but data are sparse.

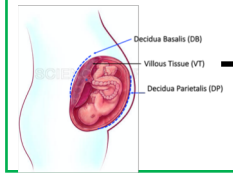
Our data is on placentas collected from late-presenting (at or after 28 weeks') HIV infected pregnant women, who were enrolled across multiple midwife obstetrics units in Cape Town, South Africa.

Aim: To characterize and identify Treg populations in decidua membranes and villous tissue from placentas collected at delivery from HIV-infected women compared to HIV uninfected mothers.

METHODS

HIV infected (n=16) and HIV uninfected (n=6) participants were enrolled from various midwife obstetrics units in Cape Town, South Africa. All placentas collected at birth and processed within 6 hours at the Division of Immunology, University of Cape Town.

The placenta was dissected to obtain the three tissues (DP, DB, VT).



Tissues was enzymatically digested. Lymphocytes were immunophenotyped using a 15 colour flow cytometry panel below:

Marker	Function/Expression Profile
CD45, CD14, VIVID	Leukocytes, monocytes, viability
CD3, CD4, CD8	T cells
CD25, FoxP3, CTLA4, CD127, CD39, TIGIT, PD1	Regulatory T cells
CD45RA, CD28	Memory delineation

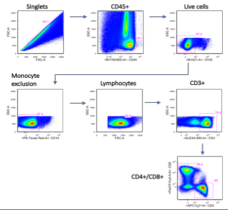
RESULTS

All HIV-infected women were enrolled from an ongoing study in which women commence antiretroviral therapy (ART) at or after 28 weeks' gestation (Table 1).

Table 1: HIV infected participant characteristics

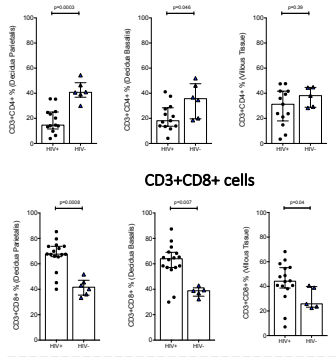
Description	Numbers
1. Participants (Total)	**n=22**
1a. HIV infected	n=13
2. Maternal characteristics	**Median (IQR)**
2a. Gestational age at ART initiation	28.5 (28 -31)
2b. Log viral load (copies/ml) at ART initiation	4.31 (3.74 - 4.58)
2c. Log viral load (copies/ml) at delivery	1.69 (1.69 - 2.50)

Fig. 1: Gating strategy to identify CD3$^+$CD4$^+$ and CD3$^+$CD8$^+$ T cells from each membrane



RESULTS 1: Gated Treg approach

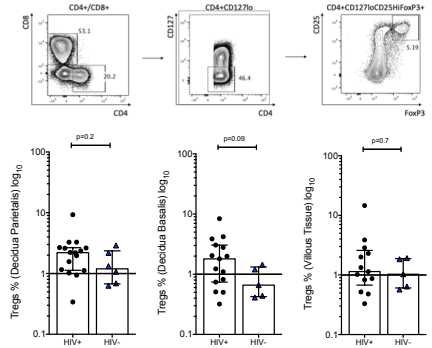
1. Frequency of CD3+ cell subsets



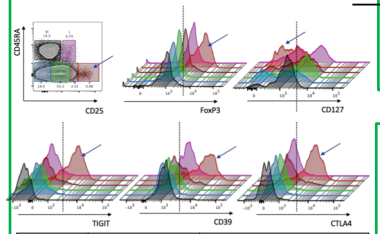
1. The significant depression of CD4+ T cells in the maternal decidua and elevation of CD8+ T cells in HIV infected cases mirrors the adult systemic footprint.

2. There is an increased trend of CD4+ Tregs in the maternal decidua parietalis, basalis and foetal villous tissue in placentas from the HIV infected mothers.

2. Minimally defined CD4+ Treg frequency in the placenta: CD4$^+$127^{lo}CD25^{Hi}FoxP3$^+$



3. Using CD25 and CD45RA to delineate resting and activated Tregs in the placenta

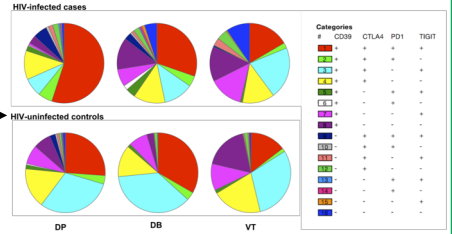


Fraction	Phenotype	Subset
Fraction I	CD4$^+$CD45RA$^+$ FoxP3^{lo}	Resting Treg cells
Fraction II	CD4$^+$CD45RA$^-$ FoxP3^{hi}	Activated Treg cells
Fraction III	CD4$^+$CD45RA$^-$ FoxP3^{lo}	Cytokine-secreting non-suppressive T cells

3. Activated Tregs in the placenta express high levels of TIGIT, CD39 and CTLA4, which are markers of increased suppressive capacity.

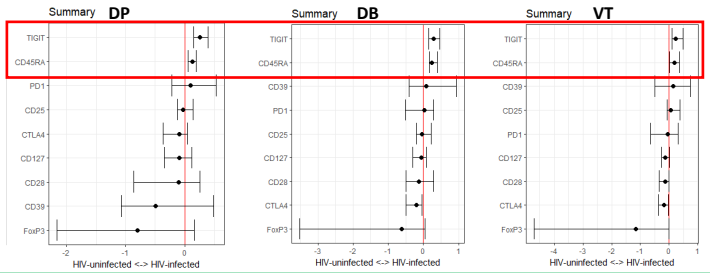
4. Boolean gating of the minimally defined Treg to show expression of CD39, CTLA4, PD1, TIGIT revealed unique subsets of Treg in the placenta, but no difference between the HIV cases and uninfected controls.

4. Proportions of CD4$^+$CD127^{lo}CD25^{Hi}FoxP3$^+$ Treg populations co-expressing CD39, CTLA4, PD1, TIGIT



RESULTS 2: Unbiased analysis of CD4+ T cells:

5. on CD3+CD4+ cells



5. A GLM model was used to identify in an unbiased manner the surface antigens on gated CD3+CD4+ to identify which markers account for differences between placentas collected from HIV uninfected and HIV infected mothers.

TIGIT and CD45RA in the DP (maternal), DB (maternal) and VT (fetal) appears to drive the phenotypic differences between placental CD4+ T cells from HIV infected versus HIV uninfected mothers.

RESULTS 3: Dimensionality reduction analyses

6. Unbiased T-Distributed Stochastic Neighbor Embedding (tSNE) on CD3+ subsets

HIV uninfected controls **HIV infected cases**

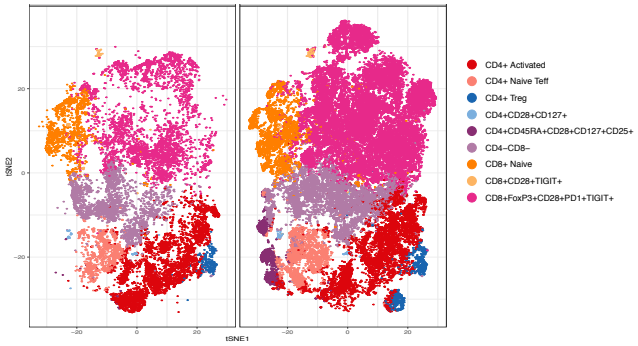


Fig.3: Further unbiased analysis of all T cells using tSNE revealed unique cell subsets that have hitherto not been described before including a large population of CD8+FoxP3+CD28+PD1+TIGIT+ in placentas from HIV infected mothers.

SUMMARY & CONCLUSIONS

- The maternal decidua membranes of the placenta mirror the adult systemic footprint in HIV infected mothers and characterized by a significant depression of CD3+CD4+ cells and elevated CD3+CD8+ cells in the tissue.
- Closer analysis of Treg cells within the CD3+CD4+ population revealed unique populations of Treg cells in the DP, DB and VT, but no difference between placentas from HIV-infected and HIV uninfected mothers.
- A Generalized Linear Mixed Model revealed that TIGIT and CD45RA were key drivers of differences in CD3+ T cells between placentas from HIV-infected versus uninfected mothers.
- Unbiased analyses revealed unique T cell subsets in the placenta expressing Treg markers. There was a large difference of CD8+FoxP3+CD28+PD1+TIGIT+ cells in the placentas from HIV infected mothers.
- The unique and multiple Treg signatures in the placenta which appear to be influenced by HIV exposure and ARV that warrant further investigation.

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