Background

Vitamin D deficiency is highly prevalent in pregnant women and is associated with adverse outcomes, including pre-eclampsia. Importantly, the maternal placenta (decidua) appears a key extra-embryonic extra-cellular site of biological action for Vitamin D, and is implicated in disorders of placental implantation and placental dysfunction. Vitamin D deficiency is highly prevalent in pregnant women and may serve an immunomodulatory role in the maternal decidua, we subsequently isolated using magnetic positive selection (Miltenyi Biotec) and positively express the vitamin D metabolic system which is more responsive and tightly regulated comparative to matched pNK cell subsets.

Objectives

1. Determine whether 1st trimester uNK cells and peripheral blood NK (pNK) cells express a functional vitamin D metabolic system.
2. Compare the functional responses of 1st trimester uNK and pNK cells to 1,25(OH)2D3.

Results

1. uNK cells have a unique phenotype compared to pNK cells

Results show that uNK cells are predominantly CD56bright and Nkp46bright, whereas pNK cells are predominantly CD56dim and Nkp46dim, as measured by flow cytometry.

Methods

Patients: Pregnant women undergoing elective 1st trimester ‘uncomplicated’ surgical termination of pregnancy were recruited from Birmingham Women’s Foundation Hospital Trust (REC Ref: 14/WM/1146).

Cell isolation: Whole decidua and matched peripheral blood samples were collected at the time of surgery. Mononuclear cells were obtained from blood and decidua by gradient centrifugation and CD56+ NK cells were subsequently isolated using magnetic positive selection (Miltenyi Biotec). Cell culture: uNK and pNK cells were cultured for 24 hours (h) in the presence or absence of 1,25(OH)2D3 (10nM) & cytokine (CK) stimulation (IL-2, IL-12, IL-15). Quantitative real-time polymerase chain reaction (qRT-PCR) and flow cytometry were performed to measure NK cell transcript and protein expression of the vitamin D metabolic system and IFN-γ.

Statistics: Significant differences were tested using 2-way ANOVA and t-tests, with post hoc analysis as appropriate. Stars indicate significance level (*p<0.05, **p<0.01, ***p<0.001).

Conclusions

1. 1st trimester uNK cells are the most prominent decidua immune cell subset and phenotypically are highly distinct from pNK cells.
2. Both uNK and pNK cells positively express the vitamin D metabolic system. This is more responsive and tightly regulated in uNK subsets.
3. VDR expression is higher in stimulated uNK cells comparative to pNK cells. 1,25(OH)2D3 may have a greater functional role in uNK cells.
4. uNK cells are less cytotoxic than pNK cells and 1,25(OH)2D3 promotes their immunoregulatory role, as characterised by suppression of IFN-γ. This effect appears unique to uNK subsets.

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