Vitamin D and Feto-maternal Immunity: Effects on Uterine Natural Killer Cells

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Background

Vitamin D deficiency is highly prevalent in pregnant women and is associated with adverse outcomes, including pre-eclampsia[1].

Importantly, the **maternal placenta (decidua) appears a key extra-renal target for active 1,25-dihydroxyvitamin D_3** ($1,25(OH)_2D_3$) [2], Our own studies also indicate that from the early 1st trimester there is positive expression of the vitamin D metabolic system, characterised by **1α-hydroxylase (CYP27B1), 24-hydroxylase (CYP24A1), and vitamin D receptor (VDR)**. However, the exact role of $1,25(OH)_2D_3$ in the decidua remains unclear[2].

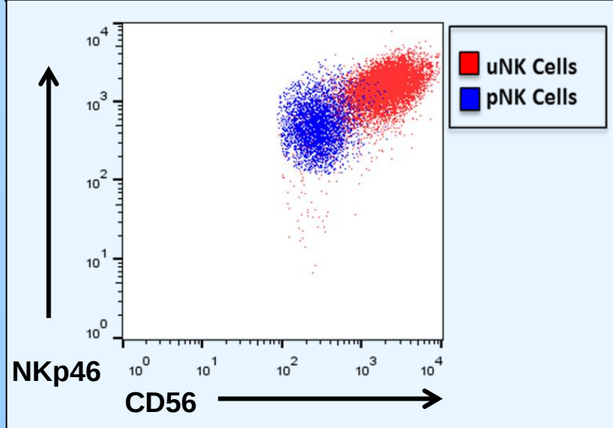
Given that a diverse immune cell population also resides within the maternal decidua, we anticipate **$1,25(OH)_2D_3$ may serve an immuno-regulatory role and promote fetal tolerance**[3]. Of particular interest are uterine natural killer (uNK) cells which are highly predominant (>60%) and critical for successful implantation & placentation[4]. Furthermore, aberrant uNK function is implicated in disorders of malplacentation (miscarriage, stillbirth, fetal growth restriction and pre-eclampsia)[5]. **We anticipate NK cells are a key target for $1,25(OH)_2D_3$**

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 $1,25(OH)_2D_3$.**

Results

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



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 $1,25(OH)_2D_3$ (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.

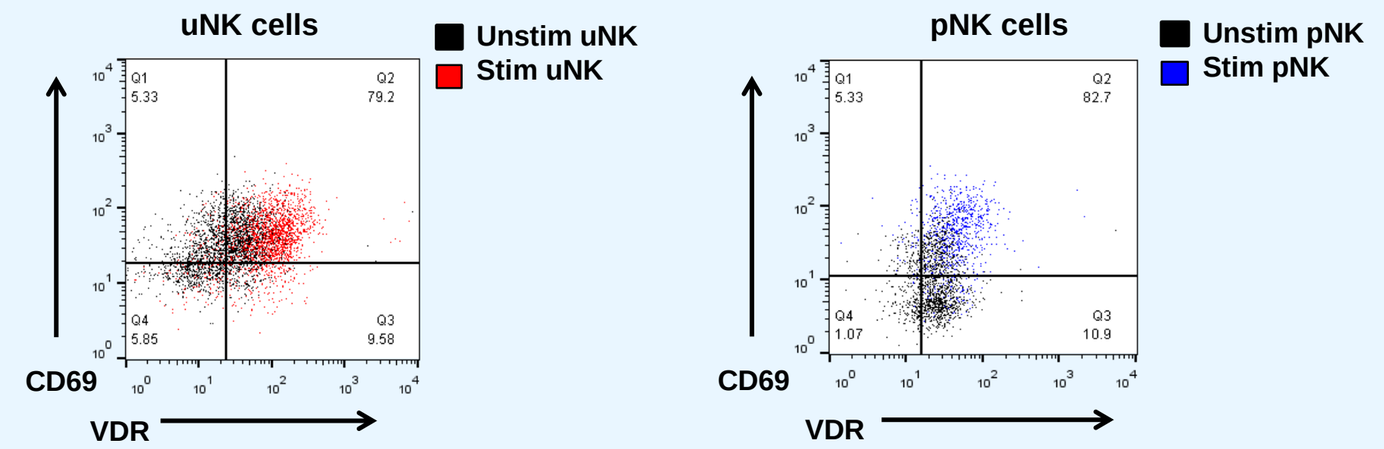
Statistics: Significant differences were tested using 2-way ANOVA and t-tests, with post hoc analysis as appropriate. Stars indicate significance level (*=p<0.05, **= p<0.01, ***= p<0.001).

Conclusions

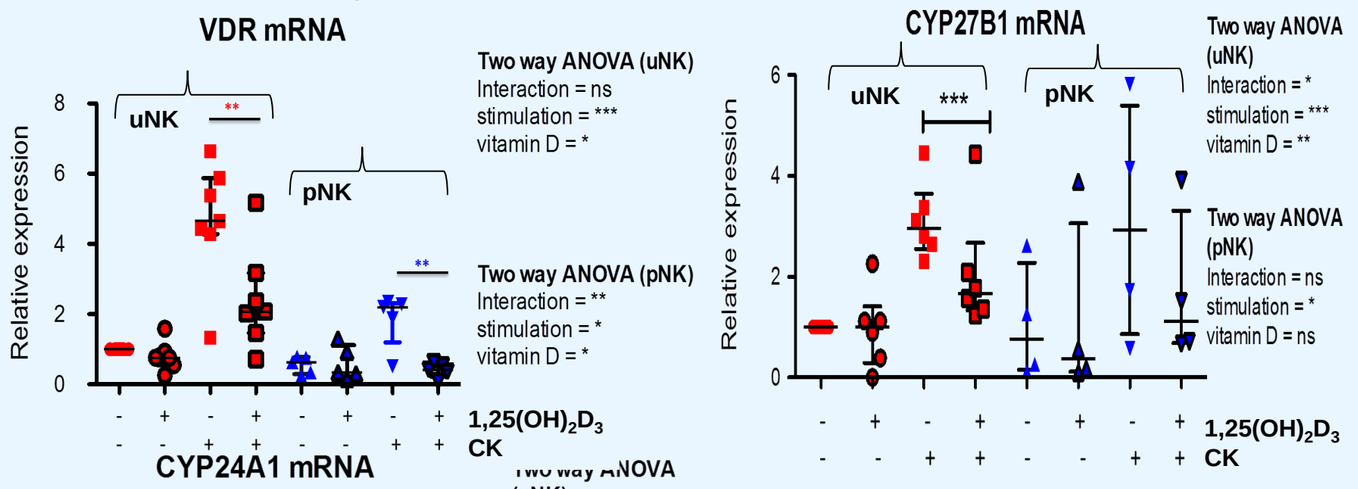
1. 1st trimester uNK cells are the **most prominent decidual immune cell** subset and phenotypically are **highly distinct from pNK cells**.
2. Both uNK and pNK cells **positively express the vitamin D metabolic system**. This is **more receptive and tightly regulated** in uNK subsets.
3. **VDR expression is higher in stimulated uNK cells** comparative to pNK cells. $1,25(OH)_2D_3$ may have a greater functional role in uNK cells.
4. **uNK cells are less cytotoxic than pNK cells** and **$1,25(OH)_2D_3$ promotes their immuno-regulatory role**, as characterised by suppression of IFN-γ. This effect appears **unique to uNK subsets**.

Results

2. uNK cells express a functional vitamin D metabolic system which is more responsive and tightly regulated comparative to matched pNK cell subsets

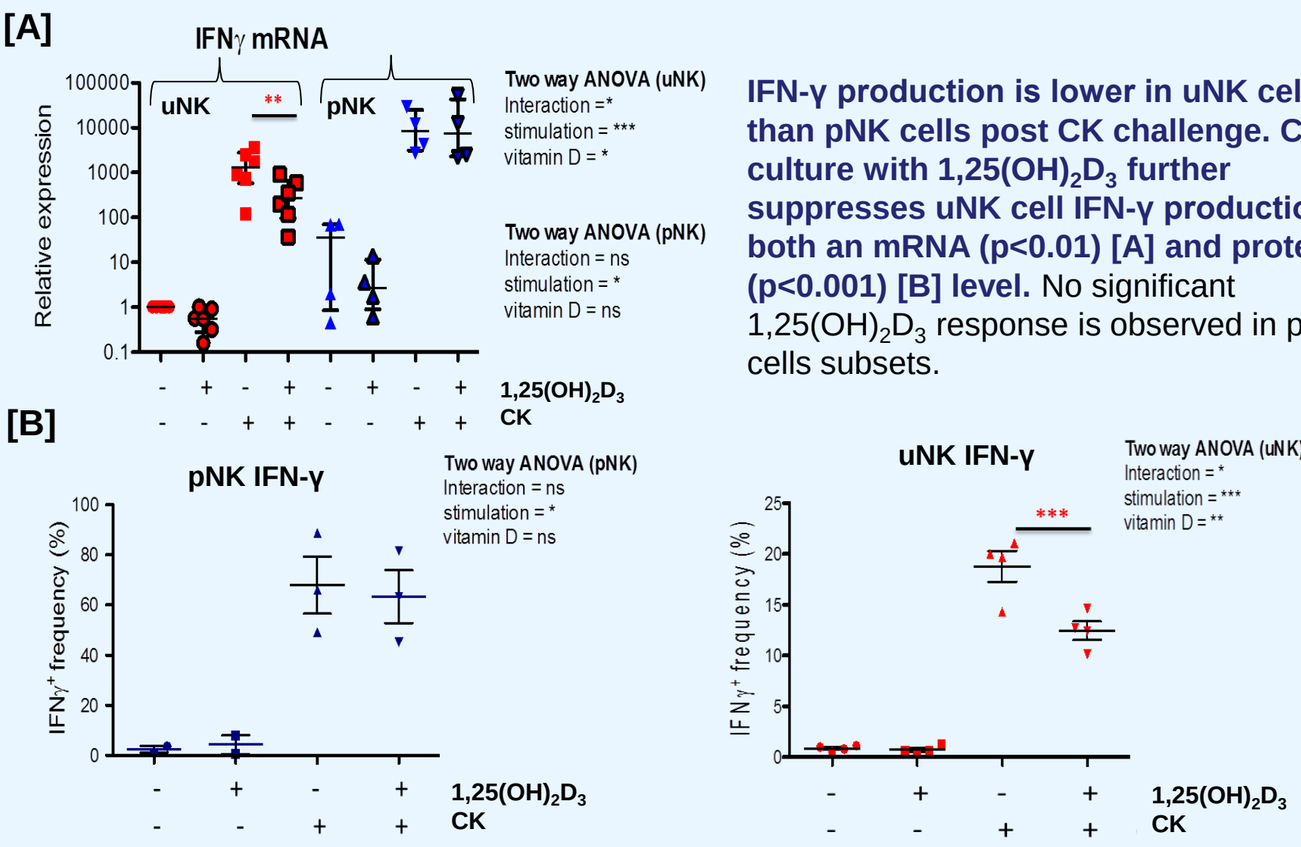


Intracellular VDR expression is up-regulated upon CK stimulation in both uNK and pNK cells, but is higher in uNK subsets. Baseline CD69 is also higher, suggesting in the decidua NK cells are constitutively more activated.



uNK and pNK subsets significantly up-regulate CYP27B1 (p<0.05) and VDR (p<0.05) expression in response to CK challenge. In uNK cells $1,25(OH)_2D_3$ suppresses this response (p<0.05), with a concomitant increase in catabolic CYP24A1. In pNK cells a similar system exists but appears less CK responsive and less tightly regulated by $1,25(OH)_2D_3$.

3. uNK cells appear less cytotoxic than pNK cells & $1,25(OH)_2D_3$ further suppresses their IFN-γ production



IFN-γ production is lower in uNK cells than pNK cells post CK challenge. Co-culture with $1,25(OH)_2D_3$ further suppresses uNK cell IFN-γ production at both an mRNA (p<0.01) [A] and protein (p<0.001) [B] level. No significant $1,25(OH)_2D_3$ response is observed in pNK cells subsets.

Acknowledgements & References

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