# NK Cells in Pregnancy

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NK cells are lymphocytes involved in the innate and adaptative immune response. These cells differ from T and B lymphocytes by the expression of preferentially CD16, CD56 markers. NK cells are located in peripheral blood and tissues with ample functions, from immune vigilant to tolerogenic reactions. The presence of tissue-resident NK cells has been observed in several species.

Keywords: NK cells ; tolerance ; pregnancy ; tissue-resident NK cells ; conventional NK cells ; decidual NK cells ; uterine NK cells

## 1. NK Cell Subpopulations

Natural killer cells (NK) have been considered an essential subtype of lymphocytes involved in innate and adaptive immune responses [1]. In peripheral blood, two main subpopulations have been described; one cytotoxic CD3-CD56dim CD16high, and one cooperative or tolerogenic CD3-CD56bright CD16dim [1]. NK cells can be transformed from one type to another depending on tissue milieu, cytokine or receptor stimulation, or pharmacologic therapy [1][2]. However, NK activity modulation does not come solely by expressing CD16 and CD56 receptors. A complex array of molecules are responsible for NK cell activity. There are activating or cytotoxic receptors, integrin, selectins, killing inhibitory receptors (KIR), PD-1 receptors, CD161, and cytokine receptors responsible for NK cell activity [1][2]. The expression of different receptors may be dependent on the stimulus or tissue milieu.

Reports of NK cells in innate immune response and memory events have led to a better understanding of NK cells' tissue-specific role in the immune response [2][3][4][5][6][7]. In a similar fashion as tissue macrophages, tissue-resident NK cells (trNK cells) differ in antigen expression and function. Differences have been described for NK cells from skin, lung, liver, adipose tissue, suggesting that trNK cells may be unique depending on the location [6][7]. Most probably, the variance between normal physiological responses, inflammation and remodelling involves trNK cells, macrophages, and the migration of other cell types [6][7][8][9][10][11][12][13]. Chemokines secreted by the tissue may recruit tissue independent NK cells from the bloodstream, and once they arrive at their destination, their cell functions may be modulated by tissue milieu [10][11][12]. There is still a debate concerning the responses of tissue-resident and conventional NK cells in humans' upon inflammatory stimulus.

In humans, tissue-specific uterine NK cells (uNK) is the most abundant lymphocyte, around 70 %, in decidua and mesometrial tissue (**Table 1**) [10][11][12][13]. These cells highly express CD56, CD103, CD9, NKp46, KIR inhibitory receptors, CD94 NKG2A, and a low amount of CD16 receptor and killing receptors. As expected, uNK cells are non-cytotoxic and heterogeneous [10][11][12][13]. Three different subpopulations have been described in the uterus based on CD56 expression. Two of them express CD117, CD49a, CD103, integrin β7, CD9, and CD69, and transcription factor Eomes, but one produces IFNγ and the other TGFβ/VEGF. The expression of CD103 and CD69 defines the difference in subpopulations (**Table 1**). A third cell subpopulation expresses CD49a, has an increased amount of Eomes transcription factor, and responds to IL4 [13]. Interestingly, ILC1 cells, associated with Th1 IFNγ transcription and function, are present in the endometrium before puberty. Thus, local ILC1 cells may be involved in the uterine milieu and crucial for the recruitment or maturation of uNK and cNK migration to the tissue in the menarche.

**Table 1.** General Characteristics of NK cells.

| Type of NK Cell | Markers | Cytokine Response |
|---|---|---|
| **Circulating tolerogenic** | **$CD16^{low}$ $CD56^{bright}$ $CD94^{bright}$, NKG2A(C or E)/CD94, CD161.** | **IL10, TGFβ** |

| Type of NK Cell | Markers | Cytokine Response |
|---|---|---|
| Circulating cytotoxic | CD16$^{bright}$ CD56$^{dim}$, CD57$^{+}$, CD94$^{dim}$, NKG2D/CD94, NKp30$^{+}$, NKp46$^{+}$, CD62L $^{med}$ | IFNγ |
| cNK | CD16$^{high}$ CD56$^{dim}$, CD49a$^{-}$, DX5$^{+}$, NKG2D/CD94, NKp30$^{+}$, NKp46$^{+}$, CD62L$^{med}$ | IFNγ |
| uNK | CD56$^{bright}$, CD16$^{low}$, NKG2A/CD94, CD49a$^{+}$, NKp46$^{+}$, integrin β7$^{+}$, CD117$^{+}$, DX5$^{-}$ | |
| uNK1 | CD49a$^{high}$, CD103$^{high}$, CD69$^{+}$ | IFNγ |
| uNK2 | CD49a$^{high}$, CD103$^{med}$, CD69$^{+}$, KIR$^{high}$ inhibitory | TGFβ/VEGF |
| uNK3 | CD56$^{high}$ CD49a$^{+}$, KIR$^{high}$ inhibitory, CD69$^{med}$ | IL-4 |
| dNK | CD56$^{high}$ CD16$^{low}$, CD94$^{high}$, NKp46$^{+}$ | |
| dNK 1 | CD103$^{low}$, CD9$^{+}$, CD39$^{+}$, CD69$^{low}$, CYP26A1, B4GALNT1$^{+}$, Jag1$^{+}$, TIM3$^{high}$, KIR2DL1$^{+}$, KIR2DL2$^{+}$, KIR2DL3$^{+}$, KIR2DS1$^{+}$, KIR2DS4$^{+}$, LIRB1$^{+}$, NKG2A$^{+}$, NKG2C$^{+}$, NKG2E$^{+}$ | IFNγ, TGFβ |
| dNK 2 | CD103$^{high}$, CD9$^{low}$, CD69$^{high}$, Jag1$^{+}$, CD83$^{+}$, KIR$^{high}$ inhibitory, ANXA1$^{+}$, ITGB2$^{+}$, TGIT$^{+}$, TIM3$^{low}$, NKG2A$^{+}$, NKG2C$^{+}$, NKG2E$^{+}$ | TGFβ/VEGF |
| dNK 3 | CD103$^{low}$, KIR$^{high}$ inhibitory, ITGβ7+, CD74$^{+}$, CD160$^{+}$, KLRB1$^{+}$, ITGB2$^{+}$, TGIT+ | TGFβ/IL-4 |

In mouse, a new classification was postulated of innate lymphocytes (ILC) as ILC1, ILC2, ILC3, NK tissue-resident (trNK), and conventional NK cells (cNK) [5][6][7]. Recent reports have shown that trNK cells express CD49a, but lack the expression of DX5, and the reverse is true for cNK [8]. Also, cNK cells are more cytotoxic than trNK and ILC counterparts [5][8][9][10][11][12]. Based on this definition of innate lymphocytes, Sojka and coworkers [12][13] named the NK cells in the uterine tissue as trNK, not uNK cells. Their remark is based on the fact that, in pregnancy, cNK migrate to the tissue and cooperate with trNK cells. The two NK cell subpopulations, tissue-resident and conventional, are distinguishable [10][12][13]. Nfil3 and Eomes are the required transcription factors for cNK cells and not for uterine trNK cells [12]. When pregnancy occurs, trNK and cNK cells increase in the decidua, providing the tolerogenic model for a normal pregnancy [12][13]. The trNK are closer to the trophoblasts, and the cNK cells are in the periphery. Once labor starts, the cNK presence decreases in the endometrium, while the number of uterine trNK cells diminishes in the puerperium [12]. In this particular physiological event, the cooperation among different cell populations and the tissue involved is crucial for fetal survival. Nonetheless, the tissue events in which NK cells are involved are not necessarily dependent on specific subpopulations circulating NK cells in the peripheral blood [6][8][10][11][12][13].

## 2. NK Cells in Pregnancy

Progesterone is essential in reproduction and pregnancy maintenance. The hormone induces the transcription and secretion of progesterone-induced blocking factor (PIBF) [14][15]. In turn, PIBF levels are increased in normal pregnancy and very low in recurrent pregnancy failure [16]. Moreover, the progesterone stimulates protein glycodelin A (GdA), or human placental protein-14 binds to NK cells CD16 low CD56bright NK cells purified from peripheral blood and transforms them into dNK-like cells [17][18]. Sialylated glycans expressed on glycodelin A are critical for binding to the receptor [17]. In addition, Glycodelin A stimulated cells to control endothelial cell angiogenesis by secreting vascular endothelial growth factor (VEGF) and trophoblast invasion by secreting insulin-like growth factor-binding protein 1 (IGFBP-1) [17].

Studies in pregnancy loss and preeclampsia have raised important questions concerning the role of NK cells in embryo implantation [9][10][11][12][13][14][15][16][17][18][19]. Fetal expression of MHC-I and its recognition by KIR receptors on NK cells, along with NKG2A/CD94, are critical for dNK cells (**Table 1**). On the contrary, the role of cNK cells is not tolerogenic; it protects the decidua against pathogens and abnormal cells. The cytotoxic response of cNK cells depends on NKG2D/CD94 and KIR activating receptors, NKp30, NKp46, and not by NKG2A/CD94 and KIR inhibitory receptors and their ligands. Activation of cNK cells may lead to a robust cytotoxic response against the embryo leading to pregnancy termination [11][19][20][21][22]. However, cNK cells are also critical for vascular remodeling, an important event to maintain blood flow to the fetus, often impaired in preeclampsia. The tolerogenic response of dNK cells is dependent on TGFβ levels, while IFN γ activates cNK cells. Thus, a balance between TGF β production inducing the tolerogenic response of NK cells or IFN γ inducing inflammatory responses is critical to determine pregnancy outcome [11][19][20][21][22].

Huhn and coworkers [23] were able to determine, in healthy human pregnancy, different cell populations in the decidua (**Table 1**). They identified three decidual NK cell subpopulations (d1, d2, and d3), ILC3, and a group of proliferating NK

cells using mass cytometry [23]. When dNK cells were stimulated with PMA and ionomycin, dNK2 and dNK3 secreted more chemokines than dNK1. The secretion included the chemokine C motif ligand (XCL1), a cytokine able to activate maternal dendritic cells and fetal extravillous trophoblasts. The d2 and d3 NK cells express a high amount of KIR inhibitory receptors [23]. Nevertheless, KIR antigen expression in dNK1 cells correlates with granzyme B granule content in the cells suggesting that these cells are more prone to be cytotoxic. These results indicate that KIR receptors control fetal development and could be involved in eliminating abnormal trophoblasts [24]. These issues have also been discussed in a recent review [19]. In summary, uNK cells are similar to dNK cells in the expression of several antigens; however, some others are only present in dNK cells (**Table 1**).

The involvement of different dNK cell subpopulations in embryo implantation and maintaining pregnancy still requires more research. Guo et al. [24], analyzing single-cell NK by qPCR, reported that a subset of dNK cells, previously defined as a protective NK cell for embryo growth, is diminished in patients with spontaneous abortion. Even though the decrease of this subpopulation may be due to different events, their assessment may be critical for determining therapeutic success.

A shift in the control of tissue subpopulations can be observed in different stages of normal pregnancy. In the first trimester, NK cells are prone to protect the fetus from pathogens; however, this protection decreased in term pregnancies [19][22][23]. In term pregnancies, dNK cells, usually tolerogenic, have a higher cytotoxic response against K562 than those of the first trimester [22][23]. This effect may be due to an impaired inhibitory response due to a downregulated expression of inhibitory receptors recognizing HLA-C antigens or HLA E and G similarly as cNK cells [22][23]. Also, the dNK cells from the first trimester differ from that dNK obtained in term pregnancies based on proteomic data. It is unclear if the cNK cells redistribute to other tissue or circulation after pregnancy termination and if the dNK cells transform into uNK cells to aid endometrial tissue repair after pregnancy.

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