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Decidual Natural Killer Cells Are Essential for a Successful Pregnancy (Review)

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Abstract

Pregnancy is a complex physiological process involving several interconnected systems. Many researchers were concerned that the formation of a fetus with different genetic components may contradict the normal state of immunity, which attempts to reject and fight foreign bodies. This piqued the interest of biologists and immunologists, who set out to discover the immune system's composition and mode of response in the uterus. According to several studies, natural killer (NK) cells are present in a significant percentage that differs from what is seen in peripheral blood. As a result, several scientific studies have been conducted on uterine NK cells, investigating their types, characteristics, receptors, secretions, and interactions with the surrounding environment. Research has also indicated the capacity of uterine NK cells to strike a balance between eradicating uterine infections and effectively contributing to different phases of pregnancy. Various studies have shown that NK cell activity is intimately related to the success or failure of pregnancy. In this review, we describe the uterine NK cell subtypes; decidual (dNK) cells and endometrial NK cells (eNK) cells and their important role during different phases of pregnancy.

Keywords

Uterine NK Cells, Decidual NK Cells, Pregnancy

1. Introduction

Natural killer cells, discovered in 1975, are granular lymphoid cells acting as cytotoxic effector cells against tumors [1]. These cells were categorized as part of the innate immune system and were named so because they did not require previous exposure to antigens to exert their cytotoxic function, which is required in T and B lymphocytes. Another study [2] revealed that instead of producing receptors via gene rearrangement, NK cells produced their inhibitory and activating receptors via germline encoding. NK cells account for approximately 5% - 15% of the total circulating lymphocytes and approximately 70% of the total leukocyte population of the uterine mucosa, whereas other lymphocytes account for only 10% [3].

The main cellular marker distinguishing NK cells is CD56, unlike the CD3 marker in T lymphocytes [4]. Based on CD56 protein expression, NK cells are further classified into two main groups: $CD56^{bright}$ (high concentration) and $CD56^{dim}$ (low concentration), each of which has its own specific functions and activities [3]. Another group of NK cells was negative for CD56 suggesting that there was a rise early in the development of $CD56^{dim}$; these cells were observed in very small numbers in peripheral circulation, but surprisingly represented about 40% of the total NK cells in patients with human immunodeficiency virus (HIV) and chronic hepatitis viral infections [5].

2. Main Types of NK Cells

2.1. $CD56^{bright}$ NK Cells

$CD56^{bright}$ NK cells represent only 10% of the resting NK cells in circulation but are considered the largest leukocyte population in the uterus. They express very few CD16 receptors and are characterized by cytokine production and minimal cytotoxic effect. The cytokines produced by these cells include IFN-γ, TNF-β, IL-10, IL-13, and GM-CSF [6]. The most highly expressed chemokine receptors on $CD56^{bright}$ are the lectin-like inhibitory receptor CD94/NKG2A [7], L-selectin, CCR7, and CXCR3 [8]. Additionally, the IL-2 receptor complex is also expressed at a high concentration, which induces the proliferation of $CD56^{bright}$ to a greater extent than that induced by CD16 NK cells [9]. By contrast, killer immunoglobulin-like receptors (KIRs) are expressed at low levels in $CD56^{bright}$ [10].

2.2. Maintaining the Integrity of the Specifications

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2.3. CD56^{dim} NK Cells

Ninety percent of the total circulating NK cells are CD56^{dim} cells. CD16 receptors are highly expressed in many types of cancer, which mediates the antibody-dependent cytotoxicity of NK cells. Unlike CD56^{bright}, CD56^{dim} show high expression of Killer Immunoglobulin like receptors (KIRs) and leukocyte function associated antigen-1 (LFA-1) but a very low expression of L-selectin [11]. Cooper *et al.* found that although IL-2 receptors are present on CD56^{dim}, high concentration of IL-2 is required to induce proliferation. In addition, the chemokine receptors CXCR1 and CX3CR1 are also expressed on CD56^{dim} and facilitate the movement of cells toward inflamed tissue. They also concluded that the two NK cell subsets need to be investigated separately and in greater depth [12].

3. Uterine NK (uNK) Cells

There are two types of uNK cells: endometrial NK (eNK) cells and decidual NK (dNK) cells. eNK cells make up 30% of the total endometrial lymphocytes and remain stable throughout the menstrual cycle. They also exhibit the same biological markers (CD56, CD57, CD94, and CD16) and receptors as CD56^{bright} NK cells from peripheral blood. Functionally, eNK cells show very little cytotoxic effect and fail to produce cytokines, such as interferon-inducible protein-10 (IP-10), vascular endothelial growth factor (VEGF), and placental growth factor (PLGF). IL-15 and IL-12 are closely associated with the activation of these cells [13]. dNK cells account for approximately 40% of all immune cells [14]. NK cells are known to be the dominant cells populating the decidua in the early stages of pregnancy, representing 70% of the total lymphocytes in the uterine mucosa. Interestingly, their distribution varies during the menstrual cycle. The pre-ovulatory phases indicate low-levels of dNK cells that gradually increase in the post-ovulatory phases, reaching a peak in the secretory phase. In the case of pregnancy, NK cells show dense infiltration in the decidua, particularly in early pregnancy, while they disappear or sharply decrease after 20 weeks of gestation and become completely absent during the last week of gestation. It is worth noting that these cells are absent in the uterus pre-menarche and post-menopause [15]. This information clearly links with uNK cells.

3.1. dNK Cells

The phenotypic characteristics dNK cells are different from those of circulating cells. Their CD56 concentration is very high, and hence, they are also called super bright CD56 cells. They are similar to CD56^{bright} NK cells of peripheral blood, which express CD94/NKG2 [14], and share the CD 56dim CD16 of eNK cells in the expression of Killer Immunoglobulin like receptors (KIRs) [16]. Moreover, with respect to the differential gene expression of dNK, granzyme A and the C-type lectin-like receptors, NKG2C and NKG2E are evidently overexpressed in dNK cells (A. Trundley, 2004). In addition, dNK cells are known to express several activating receptors, including NKp46, NKp30, NKp44, 2B4, and NKG2D,

whereas NKp30 and NKp44 are not expressed by eNK cells [17] [18]. Furthermore, dNK cells are similar to eNK and peripheral $CD56^{bright}$ cells in that they have poor cytotoxic effects, are granulated, and produce cytokines [19]. Unlike dNK cells, $CD56^{dim}$ NK cells are the most common type found in the peripheral blood. Additionally, dNK cells have been further classified into three subtypes according to their KIR expression, chemokine ligands, and cytoplasmic granules: dNK 1, dNK 2, and dNK 3 [20].

The KIRs and natural killer group 2 (NKG2)A/C/E receptors are commonly recognized as trophoblastic HLA class I human leukocyte antigens, HLA-C and HLA-E, respectively, which are expressed at higher levels in dNK cells than in their peripheral blood NK cells (pNK cells) counterpart [18]. The origin of dNK cells is presumably from a combination of the local eNK cells, peripheral NK cells, and local stem cells [13]. Despite being in direct contact with the fetal trophoblastic cells, dNK cells do not attack these cells [21]. Instead, the presence of dNK cells might be very crucial in the outcome of the pregnancy [14]. Although, dNK cells express several activating receptors (as mentioned above), as well as high levels of perforin and granzyme A and B [17] [22] [23], many studies suggest that the cytotoxicity of dNK cells is very low compared to that of pNK cells [17] [24].

Several studies have attempted to explain the default cytotoxic activity of dNK cells against trophoblastic cells. One study proposed that the inhibitory actions carried out by the binding activity between HLA-G and HLA-E and the inhibitory receptors expressed by dNK cells, such as LIRB, KIR2DL4, and CD94/NKG2A could be a factor [25] [26]. However, LIRB1, the most dominant HLA-G binding NK inhibitory receptor, is only expressed on 20% of dNK cells. Whether KIR2DL4 interacts with HLA-G and inhibits NK cell activity remains controversial [27]. Another explanation is related to the inhibition of the cytotoxic activity of dNK cells via the 2B4 receptor, which delivers inhibitory signals to the dNK cells [17].

In a mouse study, dNK cells proved to have a constructive function in spiral artery remodeling; they secrete cytokines and immune mediators that are involved in this process. IFN-γ, which is mostly secreted by dNK cells, positively regulates the diameter of the lumen of the spiral arteries during decidualization [26] [28]. In humans, mRNA analyses have confirmed that dNK cells secrete several cytokines such as GM-CSF, CSF-1, TNF-alpha, leukemia inhibitory factor (LIF), and IFN-γ [29]. IL-8 and IP-10, that are secreted by dNK cells, interact with the chemokine receptors on invasive trophoblastic cells playing an important role in trophoblastic cell migration. Additionally, dNK cells have been suggested to be a source of angiogenic factors, such as the Vascular endothelium growth factor (VEGF) family proteins, platelet growth factor (PLGF), angiopoietin-2 (Ang-2), and NKG5, which are essential in the regulation and remodeling of endometrial vessels during the first trimester [30]. High amounts of IL-8, IP-10, VEGF, and PLGF are produced by dNK cells when expressing the activating of KIR2DS4, which was true in case of expressing of inhibitory receptors,

such as KIR2DL1. This evidence supports the fact that sufficient amounts of cytokines produced from activated dNK cells may decrease the risk of pre-eclampsia [30].

The activation status of dNK cells during different stages of pregnancy has been studied extensively. Zhang *et al.* found that in the second trimester, the expression of activating receptors (NKp80 and NKG2D) was increased, whereas the degranulation capacity decreased. Cytokine expression (IFN-γ, VEGF, and IL-8) showed no significant differences between the first and second trimesters [31]. Moreover, in another study, the dNK cell subset showed a significant decline at term pregnancy relative to the first trimester; however, in the same study, they found variation in the expression of different KIR receptors between the first trimester and term pregnancy. Many important NK cell-related molecules and cytokines, including IFN-γ, GZMH, interferon gamma receptor 1 (IFNGR1), CD69, integrin subunit beta 2 (ITGB2), and NKp80, have been observed to be upregulated at full term [32]. Additionally, it was reported that dNK cells progressively decrease in number starting from mid-gestation and disappear completely at full-term [33] [34]. However, different researchers have offered contradictory explanations regarding the presence of dNK cells during pregnancy and menstrual cycle owing to differences in the methods of detection from conventional to advanced ones [35], along with the possibilities of appearance of subtypes having different functions from those primary dNK cells. In subsequent pregnancies, dNK cells were documented to have acquired a pregnancy-trained dNK cell memory by displaying unique phenotypic properties, and were characterized by increased expression of NKG2C and ILT2 and enhanced production of IFN-γ and VEGFα, which contribute in vascularization and placentation [36]. Extensive research review was done by Zhang, Wei and Cornelius, in which they displayed the relationship of NK cells with the stages of pregnancy was listed in an organized manner that I used in writing this literature review [37].

3.1.1. Effect of Ovarian Hormone on dNK Cells

The effect of ovarian hormones on uNK cells remains unclear. Here, we mainly discuss progesterone and estrogen, hormones that are known to regulate the menstrual cycle as well as pregnancy [38]. The accumulation of dNK cells around spiral arterioles in the endometrium during the mid-secretory phase and early pregnancy may be associated with an increase in hormone levels [39]. This relationship is supported by the fact that progesterone and estrogen induce the endometrium to highly express specific chemokine receptors, such as C-X-C motif chemokine ligand 10 (CXCL10) and CXCL11, which in turn induce the migration of NK cells to particular tissues [40]. Additionally, it has been documented that estrogen plays a role in the regulation of uNK cell migration in the uterus and induces uNK cells to produce CCL2, which mediates angiogenesis [41]. Moreover, because there is no specific receptor for progesterone on NK cells, it has been revealed that this hormone exerts its effect indirectly through the induction of IL-15 secretion from endometrial stromal cells, thereby stimulating

NK cell proliferation and differentiation [42] [43]. Guo *et al.* also reported that progesterone contributes to the inhibition of IFN-γ production by uNK cells via glucocorticoid receptors [44].

3.1.2. Contribution of dNK Cells in Implantation and Decidualization Stages

The presence of dNK cells in recognizable numbers mid-menstrual cycle (around 30% of WBC) and in early pregnancy (70% of lymphocytes of decidua) is considered as a strong evidence for the importance of these cells in conception [45]. The implantation process is believed to be inflammatory in origin with the participation of different cytokines such as IL-8, IL-15, IL-6, CXCL10, and CXCL11, which are secreted in response to hormonal production and other factors. These cytokines are activated and recruit immune cells into the endometrium, which are involved in placentation and implantation [40] [46]. The most important cells in this study were the uNK cells. In some instances, they serve as a biosensor to determine the implantation status, which is achieved by quantifying the uNK cell CD44 marker, the canonical hyaluronan (HA) receptor. The high molecular weight HA (HMWHA) inhibits uNK cell-mediated killing of senescent decidual cells. In contrast, low-molecular-weight HA (LMWHA) did not affect uNK cell-killing activity in co-culture experiments. Secretion of low levels of hyaluronidase 2 (a member of hyaluronidases family that regulates hyaluronan (HA) size at tissue formation) from blastocytes is a feature of low-quality blastocytes that are associated with a high level of HMWHA, leading to the inhibition of dNK cells. Therefore, dNK cells are essential in determining the implantation status [47]. Moreover, trophoblastic cells carry soluble HLA-G (sHLA-G), which binds to the NK cell receptor KIR2DL4, triggering a proinflammatory/proangiogenic response that aids the formation of a receptive endometrium in the initial weeks of pregnancy (the period of embryo implantation in humans) [48]. Another study also addressed the role of dNK cells in the elimination of senescent decidual cells to regulate endometrial renewal and remodeling upon embryo implantation, and in maintaining homeostasis of the endometrium. This is suggested to be induced by the senescence-associated secretory phenotype that mediates the initial acute auto-inflammatory decidual response. This response may further be related to endometrial receptivity [49]. Moreover, during early pregnancy and the mid-secretory phase of the menstrual cycle, dNK cells secrete prokineticin 1, a molecule that is essential in large amounts for endometrial preparation and that also regulates the secretion and expression of many mediators such as leukemia inhibitory factor, IL-11 and prostaglandins involved in decidua formation and implantation [50] [51]. Although the presence of dNK cells is crucial for decidualization and implantation, increased levels of dNK cells have been reported to correlate with recurrent early pregnancy losses suggesting that the implantation period is delayed by pathological elongation of the window of endometrial receptivity [52]. In early pregnancy, decidualization transforms from an acute inflammatory to an anti-inflammatory state, where the immune cells

start to infiltrate the endometrium, including dNK cells, which are observed to increase sharply from pre-decidualization up to the late secretory phase [52]. dNK cells have been proven to promote decidualization through the production of IL-25 [53]. Fonseca *et al.* confirmed higher levels of endocannabinoid anandamide (AEA) in the decidua of patients experiencing recurrent miscarriages. They further discovered that the uNK cells isolated from the decidua of patients experiencing recurrent miscarriages exhibited higher levels of TNF-α, which is suggested to interfere with the decidualization of ESCs. This process occurs by exacerbation of the inflammation and may also trigger the AEA signaling pathway [54]. Higher levels of AEA have been reported to be associated with recurrent miscarriage [55], and their role in the inhibition of decidualization has been proven in vitro [56]. In an experimental study in mice, the depletion or absence of NK cells were seen to be associated with a decrease in the number of embryo implantation, an increase in embryo losses, and angiogenesis disorders, which can be considered as additional evidence in the role of NK cells in decidualization and implantation [57] [58].

3.1.3. Role of dNK Cells during Placentation

Placentation stage immediately follows the decidualization and implantation stages. In this section, we will discuss the role of dNK cells in the remodeling of placental arteries and trophoblastic invasion, which are essential processes. If not, pregnancy will be compromised and complicated by recurrent miscarriages, preeclampsia, and fetal growth restriction [59]. Several studies have addressed the role of dNK cells in this phase. At 8 - 10 weeks of gestation, dNK cells secrete factors that promote spiral artery remodeling [60], while at 12 - 14 weeks of gestation, they produce mediators such as IL-8, INF-γ, and inducible protein (IP) 10, which enhances Extra villus trophoblastic cells EVT invasion by increasing matrix metallopeptidase 9 (MMP-9) secretion and reducing EVT apoptosis [61]. Although EVT invasion is essential for placentation but over-invasion is hazardous to the placenta. dNK cells play a crucial role in controlling this process by producing several cytokines, including TNF-α, TGF-β, and IFN-γ, which are known to inhibit excessive EVT invasion in later stages [62] [63].

By the 20th week of gestation, trophoblastic cells complete their endometrial invasion [64], which is accompanied with an increase in the uterine spiral artery caliber to maintain an adequate amount of oxygenation and nutrition for the growing fetus [65]. The initial stages of spiral artery remodeling are performed early on by the lymphocytes. This process involves the loss of vascular smooth muscle cells (VSMCs) and breaks in the endothelial cell layer by the action of MMPs. Histopathological samples have confirmed the presence of dNK cells distributed close to the spiral arteries accompanied by an expression of MMP, which is considered as evidence for their major contribution in this process [34] [35]. Spiral artery remodeling is believed to involve the migration of Vascular smooth muscle cells (VSMCs) rather than apoptosis. dNK cells play a significant role in the transformation of vascular wall content and secrete chemokines, cy-

tokines, and vasoactive factors such as IL-8, TGF-β, angiopoietin-1/2 (Ang1/2), and VEGF-C [60] [66] [67]. Additionally, the senescent state of dNK cells participates in the remodeling of spiral arteries by inducing the production of many cytokines including TNF-α, IL-1β, IFN-γ, IL-6, and IL-8. sHLA-G from EVT induces th the senescent state directly by binding its receptors on dNK cells [68]. Poor remodeling of spiral arteries and decrease in trophoblastic invasion were observed with a significant decline in dNK cells in patients with pre-eclampsia and intrauterine growth restriction (IUGR) [69]. dNK cells not only play a role in placentation at this stage, but also participate in fetal growth and development by secreting growth-promoting factors, including pleiotrophin (PTN) and osteoglycin (OGN) [70]. Furthermore, the transcription factor PBX homeobox 1 has been reported to regulate the transcriptional expression of growth-promoting factors in dNK cells and to promote fetal growth [71].

Placental formation is completed at five weeks of gestation and is considered to be an interface between the mother and the growing fetus. Both participate in the genetic constituents of trophoblastic cells, which are the main cells residing at the maternal-fetal interface. This offers a suitable medium for the semi-allogeneic fetus to survive and thrive in the presence of the maternal immune system, which accepts the fetus and provides protection against invaders [72]. This finding helps researchers to understand the mechanisms of immune tolerance at the maternal-fetal interface.

It has been documented that fetal trophoblastic cells, maternal DSCs, and decidual immune cells are the central cells that form the maternal-fetal interface [34]. NK cells represent 70% of immune cells populating the decidua in the first trimester, followed by macrophages (20%) [35]. dNK cells showed lower cytotoxic effects, possibly due to the binding of their inhibitory receptors (such as KIR2DL1, KIR2DL2/L3, and ILT2) to the HLA ligands (such as HLA-G, HLA-C, and HLA-E) expressed on EVT cells [73]. Studies have reported that dNK cells can take up secreted HLA-G from EVT cells. This process has been suggested to be associated with the very low cytotoxicity of NK cells [18] [74]. The decreased cytotoxicity of NK cells is also attributed to the synapse inhibition of EVT-NK cells during HLAG endocytosis [18]. HLA-E also participates in the downregulation of NK cell cytotoxicity via direct binding to the NK cell inhibitory receptors CD94/NKG2A [27]. Nevertheless, classical HLA-C plays a crucial role in attenuating the cytotoxic effect of NK cells by interacting with specific KIRs receptors of NK cells [75].

In contrast, $CD56^{bright}CD27^{+}$ NK cells control the activity of excess T helper (Th17) cells by secreting IFN-γ, which contributes to immune tolerance during pregnancy. Overproduction of Th17 cells was examined *in vivo* and has been reported to induce fetal loss [76]. Another interesting enzyme, indoleamine 2,3-dioxygenase (IDO), which catalyzes the degradation of tryptophan, has been demonstrated to be produced at a significant level at the fetal interface [77] [78]. It is further suggested to contribute to immune tolerance by reducing pNK cell

cytotoxicity and inhibiting the expression of NKp46 and NKG2D [79]. Moreover, the expression of immunomodulatory Tim-3 on the surface of dNK cells suggests a decrease in perforin production by dNK cells [80]. Another molecule, microRNA-30e, which is expressed in decidual tissue, is upregulated during normal and successful pregnancies. This molecule significantly induces the expression of inhibitory KIR2DL1 and NKP44, which directly downregulates the cytotoxicity of dNK cells [81]. Additionally, a form of protective immunomodulation has been observed at the maternal-fetal interface, effected by the chemokine CXCL16, which is produced by trophoblastic cells. This chemokine is involved in shifting the immune response to the M2 phenotype by the polarization of macrophages, which lowers the production of IL-15, and it plays a crucial role in NK cell development and activation [82].

3.1.4. dNK in Parturition

Recent studies suggest that during the late stages of pregnancy, the reproductive organs and tissues, namely, the uterus, placenta, cervix, and the fetal membranes, can secrete many chemotactic substances such as CXCL8, CXCL10, CCL2, and CCL3 [83] [84] [85]. These chemotactic factors are important for the recruitment of maternal leukocytes in these tissues. Collectively, reproductive tissues and maternal leukocytes are responsible for the secretion of proinflammatory mediators, such as cytokines (IL-1, IL-6, IL-8, and TNF), MMPs, and prostaglandins, leading to cervical effacement, dilatation and eventually membrane rupture, which precipitates labor and delivery. This finding proves that parturition is an inflammatory process [86]. Early activation of this pathway may lead to preterm delivery, significantly contributing to neonatal morbidity and mortality [87].

The role of leukocytes, including neutrophils, T cells, B cells, and macrophages, during delivery has been reported in many studies involving mice and humans. Many studies have also investigated the role of dNK in labor. A recent study conducted in the USA demonstrated the presence of lymphoid and myeloid cells, including NK cells, in the placenta and chorioamniotic membranes. The study also demonstrated that women with spontaneous labor expressed more single-cell signatures of NK and T cells than controls who were at the same gestational age and not in labor [88]. Another study reported that women who underwent preterm labor had more activated NKT-like cells in the decidua basalis. They also found that the *in vivo* activation of NKT cells leads to preterm labor as a result of a systematic proinflammatory response [89]. However, the mechanisms by which NK cells are regulated during labor remain unknown.

3.1.5. Role of dNK Cells in Uterine Infection

Despite the evident tendency to weaken during early pregnancy, it was found that dNK cells still contained more cytotoxic granules and activated NKp46, NKp44, NKp30, and NKG2D than that in their peripheral blood counterpart, $CD56^{bright}$

NK cells. Jabrane-Ferrat reported that in the presence of high levels of dNK cells in the placenta, vertical transmission is low in the first trimester [90]. This is attributed to the homeostatic ability of dNK cells, which can positively contribute to the physiological process of the maternal-fetal interface by downregulating its cytotoxic activities, while simultaneously preserving its cytotoxic function to fight microbial infection. Alternatively, several studies have revealed that individuals with higher activated KIR have a much better outcome following viral infections, e.g., Human cytomegalo-virus (HCMV), human immunodeficiency virus (HIV), and human papillomavirus (HPV) [91] [92]. dNK cells have been recognized to be involve in the eradication of HCMV- and HIV-related intrauterine infections [26]. Recently, it was reported that the dNK cells transmitted granulysin to trophoblasts, killing bacteria within trophoblastic cells without injuring placental cells [93]. Nevertheless, viruses can express activating ligands on the surface of infected cells (e.g., major histocompatibility class I polypeptide-related sequence A (MICA) and MICB), which bind directly to activating NK receptors and boost NK cell cytotoxicity [94] (**Table 1**).

Table 1. dNK cells subtypes.

dNK cells subtypes	Expression of KIR	Cytoplasmic granule proteins	Activating killer cell lectin-like receptor (NKG2C, NKG2E) and inhibiting NKG2A)	Chemokine	Function in pregnancy
dNK1	Higher	↑	+	–	plays a dominant role in early pregnancy
dNK2	low	↓	+	XCL1	recruitment of EVTs and dendritic cells
dNK3	low	↓	–	chemokine ligand 5	regulation of EVT invasion

Using single-cell RNA-sequencing, three primary subtypes of dNK cells (dNK1, dNK2, and dNK3) were found to co-express the tissue-resident marker CD49a during the proliferation of NK cells from isolated first-trimester decidual cells. They are different in their KIR expression, granules, receptors and their chemokine ligands [20].

Table 2. Contribution of decidual NK cells in normal pregnancy process.

Pregnancy process	Factors expressed or produced by uNK cells	Function	References
Implantation	Prokinetincin1 (a marker of receptive endometrium) secreted by uNK cells	Facilitating the embryo to implant into the endometrium by regulating implantation factor like leukemia inhibitory factor, IL-11, and prostaglandins	[50] [51].
	High expression of KIR2DL4	Activating a pro-inflammatory/proangiogenic response by binding with its ligand HLAG on trophoblastic cells; this step is beneficial to the establishment of receptive endometrium	[48]
Decasualization	IL-25 secreted by dNK cells	Promotes the proliferation of DSCs and of the regulation of decasualization	[53]
	Killer immunoglobulin-like receptors	Control NK cell activity and cytotoxicity	[18]

Continued

Placentation	IL-8 and INF-γ inducible protein, IP10	Increasing MMP-9 secretion, which reduces the apoptosis of extra villus trophoblastic cells (EVT)	[62]
	TNF-α, TGF-β and IFN-γ	Down-regulate the harmful excessive EVT invasion	[62] [63]
Spiral arteries re-modeling	IL-8, TGF-β, angiopoietin-1/2 (Ang1/2), and VEGF-C	Contribute to increase the calibers diameter of spiral arteries to maintain satisfactory blood flow to the growing Festus	[60] [66] [67]
Fetal development	dNK cells secrete growth-promoting factors like pleiotrophin (PTN)and osteoglycin (OGN)	Promote the growth and development of the fetus	[70]

4. Conclusion

In this review, we have highlighted the importance of dNK cells during pregnancy which perform a multitude of functions during the various stages of pregnancy. They directly secrete and express specific molecules that have crucial effects on normal pregnancy (**Table 2**). dNK cells do not produce a cytotoxic response in semi-allogeneic embryos in the early stages of pregnancy. However, at the maternal-fetal interface, dNK cells interact with HLA ligands produced on EVTs to suppress dNK cell cytotoxicity and mediate immunological tolerance. Furthermore, they play an important role in vascular remodeling, trophoblast invasion, and embryonic development in the early stages of pregnancy because they release many cytokines. When viruses infect the uterus, NK cells switch to cytotoxic activity and engage in immunological defense. Furthermore, dNK cells are reactivated late during pregnancy to disrupt immunological tolerance and induce parturition. However, the molecular basis for the transition of dNK cells from a weak to robust cytotoxic status at various phases is yet to be discovered. Further research is required to establish how these dNK subtypes change during pregnancy and what factors influence their transition mechanisms.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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