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Review

Uterine natural killer (uNK) cells and their missions during pregnancy: A review

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ABSTRACT

Natural killer (NK) cells are lymphocytes of the innate immune system. The aim of this review is to describe the properties and roles of NK cells in the human uterus during pregnancy. Uterine natural killer cells (uNK) constitute a major lymphocyte population during early gestation in the uterus. The uterine natural killer cells are recognized owing to their $CD56^{bright}$, $CD16^{-}$, $CD3^{-}$ phenotype. Their number increases in the first trimester with a subsequent decline as pregnancy progresses. They have been shown to be closely associated with cells of the extravillous trophoblast (EVT) and spiral arteries. They play important roles in remodeling of the spiral arteries, control of trophoblast invasion and in the development of the placenta. Some studies have shown the number and repertoire of receptors of uNK differ between women with healthy pregnancies and those with pathologic pregnancies, such as pre-eclampsia or intrauterine growth retardation. During pregnancy, the cytotoxic characteristics of the uterine killer cells are not directed towards the fetus, and scientists continue to question and explore this phenomenon with increasing evidence that these cells may perform differing beneficial roles during pregnancy. Contrary to their previously suspected "hostile" characteristics, the uterine killer cells are considered to be "friendly" and appear to be essential and very important regulators of successful implantation and pregnancy.

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Abbreviations: ADCC, antibody-dependent cellular cytotoxicity; Ang-2, angiopoietin-2; CSF-1, colony stimulating factor 1; EVT, extravillous trophoblast; gd, gestation day; GM-CSF, granulocyte macrophage colony stimulating factor; HLA, human leukocyte antigen; Ig, immunoglobulin; IHC, immunohistochemistry; IL-10, interleukin 10; IL-22, interleukin 22; ILT, Ig-like transcripts; IL-1β, interleukin 1 beta; IFN-γ, interferon gamma; IP-10, interferon-inducible protein; ITAM, immunoreceptor tyrosine-based activating motif; ITIM, immunoreceptor tyrosine-based inhibition motif; IUGR, intrauterine growth retardation; KIR, killer Ig-like receptors; LIF, leukemia inhibitory factor; LIR, leukocyte Ig-like receptor; MHC, major histocompability complex; NK, natural killer cell; NCR, natural cytotoxicity receptors; NKR, natural killer cell receptor; PE, pre-eclampsia; PGF, placental growth factor; pNK, peripheral natural killer cell; RSA, recurrent spontaneous abortion; SCID, severe combined immunodeficiency; TGF-β1, transforming growth factor beta 1; TNF-α, tumor necrosis factor alpha; uNK, uterine natural killer cell; VEGF-C, vascular endothelial growth factor-C

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Introduction

Human natural killer (NK) cells constitute a highly specialized population of large granular lymphocytes and comprise approximately 15% of all circulating lymphocytes. They are important effectors in the immune response against specific viruses, parasites and intracellular bacteria (Trinchieri, 1989) and are able to lyse target cells without prior sensitization (Lanier et al., 1986a; Ritz et al., 1988). During microbial infection or tumor growth and invasion, they are rapidly recruited to the site of injured organs and contribute to the elimination of infected or transformed cells and also to the recruitment and activation of other immune cells (Biron, 1997; Fogler et al., 1996; Moretta, 2002). Thus, NK cells are not only important effectors acting in the early phase of innate immune responses, but also play a crucial role as immunoregulators of adaptive immunity (Moretta, 2002). These cells are characterized phenotypically by the absence of CD3 and the presence of the CD56 surface antigen (Robertson and Ritz, 1990), which is an isoform of the human neural-cell adhesion molecule (Lanier et al., 1989). NK cells are cytotoxic and function by releasing the contents of cytoplasmic granules containing proteins such as perforin and granzymes, which lyse target cells. The ultrastructure of these granules was identified and classified by electron microscopy during the implantation process (Demir et al., 2003). NK cells mediate antibody-dependent cellular cytotoxicity (ADCC) through CD16. CD16 is the low-affinity FcγRIII on the surface of NK cells, which binds to antibody-coated (opsonized) targets and signals through associated subunits containing an immunoreceptor tyrosine-based activation motif (ITAM) to direct ADCC (Trinchieri, 1989; Perussia et al., 1984; Perussia and Trinchieri, 1984).

Subsets of human NK cells

There are two distinct subsets of human NK cells in the peripheral blood; $CD56^{dim}$ cells, which comprise around 90% of NK cells, and $CD56^{bright}$ cells. While the functional significance of the CD56 molecule in relation to NK cells is unknown, its bright or dim expression correlates with the expression of a number of other surface markers that confer unique functional properties to the $CD56^{bright}$ and $CD56^{dim}$ subsets (Lanier et al., 1986b). The $CD56^{dim}$ cells have a low cell-surface density of CD56, are cytotoxic and express high levels of CD16. $CD56^{bright}$ pNK cells are $CD16^{-}$ and are non-cytotoxic or less cytotoxic than $CD56^{dim}CD16^{+}$ pNK cells, but have the ability to produce large amounts of cytokines upon activation (Baume et al., 1992; Cooper et al., 2001; Perussia et al., 2005). The question has been raised if the $CD56^{bright}$ and $CD56^{dim}$ NK cells represent two functionally distinct subsets emerging from a common precursor or do they perhaps each represent a different stage of maturation of NK cells.

In a study done by Koopman et al. (2003), uNK cells from first trimester decidua were compared with peripheral blood NK cells consisting of $CD56^{bright}$ and $CD56^{dim}$ populations as shown using microarray analysis. The results showed that uNK cells (decidual NK cells, according to the authors) have unique properties and should be considered as unique NK subsets.

Cella et al. (2009) reported the characteristics of a human and mouse NK cell subset located in mucosa-associated lymphoid tissues, such as the tonsils and in Peyer's patches of the small intestine. Since these unique NK cells are specialized for interleukin (IL)-22 production, they referred to them as NK-22 cells. NK-22 cells appear in the *lamina propria* of the small intestine during bacterial infection, suggesting that NK-22 cells provide an innate source of IL-22 that may help constrain inflammation and protect mucosal sites.

Uterine natural killer cells (uNK cells)

Granulated cells have been identified in the endometrium of many species. Some researchers refer to the natural killer cells which are localized to the endometrium before pregnancy as "endometrial NK cells (eNK)" and "decidual NK cells (dNK)" after decidualization (Manaster and Mandelboim, 2008). In this review, we use the term "uterine NK cells (uNK)" since we refer to both endometrial and decidual NK cells.

Although the small subset of $CD56^{bright}CD16^{-}$ cells circulating in the peripheral blood phenotypically shows similarities to uNK cells, there are differences between $CD56^{bright}$ cells in the blood and $CD56^{bright}$ cells in the uterine tissue. All $CD56^{bright}$ cells in blood are agranular and whilst there are some agranular $CD56^{bright}$ cells in the decidua, the majority show typical features of large granular lymphocytes (King et al. (1991)).

Uterine NK cells display characteristic membrane-bound granules in their cytoplasm as shown by transmission electron microscopy. These granules contain perforin and granzymes, indicating that uNK cells are potentially cytolytic. Kopcow et al. (2005) showed that, although uNK cells are granular and express the essential molecules required for lysis, they display significantly reduced lytic activity on classical major histocompability complex (MHC) I negative targets. While they formed conjugates and polarized CD2, LFA-1 and actin toward the contact site, these uNK cells failed to polarize their microtubule organizing centers and perforin-containing granules, which could account for their lack of cytotoxicity.

Uterine NK cells are present in both the non-pregnant endometrium and decidua in women and their numbers change during the menstrual cycle and pregnancy (Peel, 1989). In the endometrium of premenarchial and postmenopausal women, uNK cells are sparse (Hameed et al., 1995; Kammerer et al., 2003). CD56+ cell numbers increase in the proliferative phase and reach the maximal level in the late secretory phase, an increase that is sustained into early pregnancy (King et al. (1991); Whitelaw and Croy, 1996). Other lymphocyte populations may also increase in number during the same period (Manaster and Mandelboim, 2008). Additional populations in the decidua include CD14+ macrophages, dendritic cells, CD4+ T cells, a few CD8+ T cells, γδ T cells and NKT cells (Smarason et al., 1986; Sato et al., 1990; Mincheva-Nilsson et al. (1992); Abraham et al., 2000). Ozenci et al. (2001) showed the presence of uNK cells in human decidua as early as on post-coital days 22–30. Up to 70% of decidual leukocytes are CD56+ and they form aggregates around the spiral arteries and glands. After about week 20 of gestation, trophoblast invasion is complete and the number of uNK cells begins to decline with a much less prominent lymphocyte population than that found in the first trimester of gestation. Changes in uNK cell number have been correlated to hormone-induced decidualization and to fluctuations in chemokine expression, rather than embryo implantation (Red-Horse et al., 2001; Jones et al., 2004; Ordi et al., 2006).

Although uteri of mice are not populated with mature NK cells prior to fertilization and blastocyst implantation, similar to the case of human pregnancy, uNK cells are also very abundant in early mouse and rat decidua. In mice, these cells are detected in the decidua basalis on days 5–6 of pregnancy. Their numbers increase between days 8 and 12. By day 13, the few remaining NK cells in the decidua basalis are found in the mesometrial triangle (Gambel et al., 1985; Croy and Kassouf, 1989).

Where do the uNK cells come from?

Although NK cells are known to differentiate in the bone marrow from a lymphoid precursor (Rosmaraki et al., 2001), recent

studies not only indicate that final maturation of NK cell progenitors occurs in the periphery, but they also suggest a thymic pathway of NK cell differentiation (Mingari et al., 1997; Carlyle et al., 1998; Di Santo, 2006). NK cells exist in peripheral lymphoid and non-lymphoid organs such uterus (Fehniger et al., 2003). The lineage and origins of uNK cells are largely unknown. Researchers provide some theories concerning the increase in uNK cells, which is seen in the mid- and late secretory phase of the menstrual cycle continuing into early pregnancy. One study by Jones et al. (1998) showed that up to 40% of CD56+ uNK cells isolated from late secretory phase endometrium express Ki67. Therefore local proliferation of uNK cells could account for their dramatic increase in number. The clear association between uNK cell numbers and the menstrual cycle suggests that estrogen and progesterone may play a role in this relationship. Henderson et al. (2003) demonstrated that uNK cells express estrogen receptor-β (ER-β). Because more Ki67+ uNK cells are present in the secretory phase and early pregnancy than in the proliferative phase (Kammerer et al., 1999; King et al. (1991)), mitosis is thought to be elevated by ovulation. uNK cell numbers decrease when progesterone levels fall, so it is likely that their survival is progesterone dependent. On the other hand, the influence of progesterone appears to be indirect as uNK cells do not express progesterone receptor transcripts (King et al., 1996). Progesterone binds to stromal cell receptors and leads to the upregulation of IL-15 secretion (Okada et al., 2000). IL-15 was reported as a stromal cell product in both human and mouse endometrium (Zourbas et al., 2001; Kitaya et al., 2000, 2005). IL-15 was shown to stimulate the proliferation of isolated uNK cells *in vitro* without promoting cytolytic activity against trophoblasts (Kitaya et al., 2003a; Verma et al., 2000). The differentiation of murine NK cells requires IL-15 (Puzanov et al., 1996). Implantation sites of IL-15 knock-out female mice do not contain NK cells, nor do they exhibit spiral artery modification (Ashkar et al., 2003).

Primary differences between peripheral blood NK cells (pNK) and uterine NK cells (uNK) are shown in Table 1. Some theories suggest that uNK cells originate from the influx of pNK cells (King et al., 1991). If this theory is correct, cells must differentiate in the uterus. The very close relationship between uNK cells and decidualized stromal cells suggests a fundamental role for these cells in the differentiation of uNK cells within the uterus (Kitaya et al., 2000, 2003a; Gubbay et al., 2002). Carlino et al. (2008) showed that pNK cells from pregnant women in their first trimester migrated through primary cultures of decidual endothelial and stromal cells better than pNK cells from male donors or non-pregnant women. Factors that could recruit pNK cells have been found in the endometrium and decidua. Several studies suggest that chemokines control the recruitment and/or retention of NK cells in the decidua (Hornung et al., 1997; Kitaya et al., 2003b; Hannan et al., 2004; Jones et al., 2004). Chemokine expression inside the uterus undergoes cyclical variations and is maximal during the secretory phase of menstrual cycle and early pregnancy. Campbell et al. (2001) demonstrated that $CD56^{bright}$ and $CD56^{dim}$ NK cells behave distinctly to different chemokines. In a study performed by Hanna et al. (2003), expression of CXCL12 by endovascular trophoblast cells and CXCR4-chemokine receptor by uNK cells was reported. There are numerous other examples: CXCL9, CXCL10, CCL3 and CCL4 chemokines produced by trophoblast or endometrial cells induce pNK cell chemotactic responses (Drake et al., 2001; Hanna et al., 2003; Kitaya et al., 2003b).

Although many studies support the hypothesis that the $CD56^{bright}CD16^{-}$ uNK cells originate in the blood from a $CD56^{bright}CD16^{-}$ NK subset, a study by Keskin et al. (2007) suggested that uNK cells might originate from $CD56^{dim}CD16^{+}$ peripheral blood NK cells and subsequently differentiate into uNK cells under the influence of tissue-derived TGFβ and other factors. This shows the tissue specific terminal differentiation of NK cells.

In conflict with this theory, there is a study by Matsuura-Sawada et al. (2005). When they transplanted human endometrium into NOD/SCID/γc^{null} mice, which lack T and B lymphocytes and have extremely low levels of NK cells, there was an increase in NK cell levels by day 28 of the menstrual cycle.

It is also possible that uNK cells may proliferate locally from precursor cells in the uterus. These cells are characterized by their CD34 expression. They are suggested to localize in the basal layer of the endometrium, which is not shed during menstruation. CD34+ cells could also be recruited to the endometrium from the blood during each menstrual cycle (Trundley and Moffett, 2004; Bulmer and Lash, 2005).

There are some studies showing that the endometrium recruits circulating hematopoietic stem cells and provides them with a unique micro-environment for differentiation/proliferation into uNK cells. Taylor (2004) reported that in women who received HLA-mismatched bone marrow transplants from female donors, the cells with donor HLA phenotypes appeared in the endometrial glandular epithelium and stroma 24–157 months after transplantation. Keskin et al. (2007) showed the differentiation of CD34+ hematopoietic stem cells present in human first trimester decidualized endometrium into CD16−/CD56+/CD9+/CD94+ NK cells in the presence of stem cell factor and IL-15 *in vitro*. Thus, the endometrium may provide the appropriate local micro-environment to support NK-cell development. The possible origins of uNK cells are summarized in Fig. 1.

Surface markers of NK cells

Different studies show that uNK cells and pNK cells express some common and some different surface markers. Eriksson et al. (2004) compared surface markers of pNK cells with those of uterine NK cells and they reported that the $CD56^{bright}$ subset of pNK cells and uNK cells share a similar expression profile of the surface markers CD56, CD57, CD94 and CD16. $CD56^{bright}$ pNK and uNK cells had positive expression for CD56 and CD94; negative expression for CD57 and negative/low expression for CD16. A cell differentiation factor expressed by uNK cells, but not by pNK cells, is CD9, a protein having different cellular and physiological functions (Koopman et al., 2003). Keskin et al. (2007) reported that $CD56^{bright}$ uNK cells show differences from the $CD56^{bright}$ minor population of pNK cells based on their expression of CD9, CD103 (αE integrin), and killer immunoglobulin-like receptors (KIR).

Table 1
Some features of NK cells. LGL: large granular lymphocyte.

Phenotypic markers	Peripheral $CD56^{dim}$	Peripheral $CD56^{bright}$	Uterine $CD56^{bright}$
CD56	+	++	+++
CD16	++	+/−	−
NK activity	high	low	low
Morphology	LGL	Agranular small NK	LGL

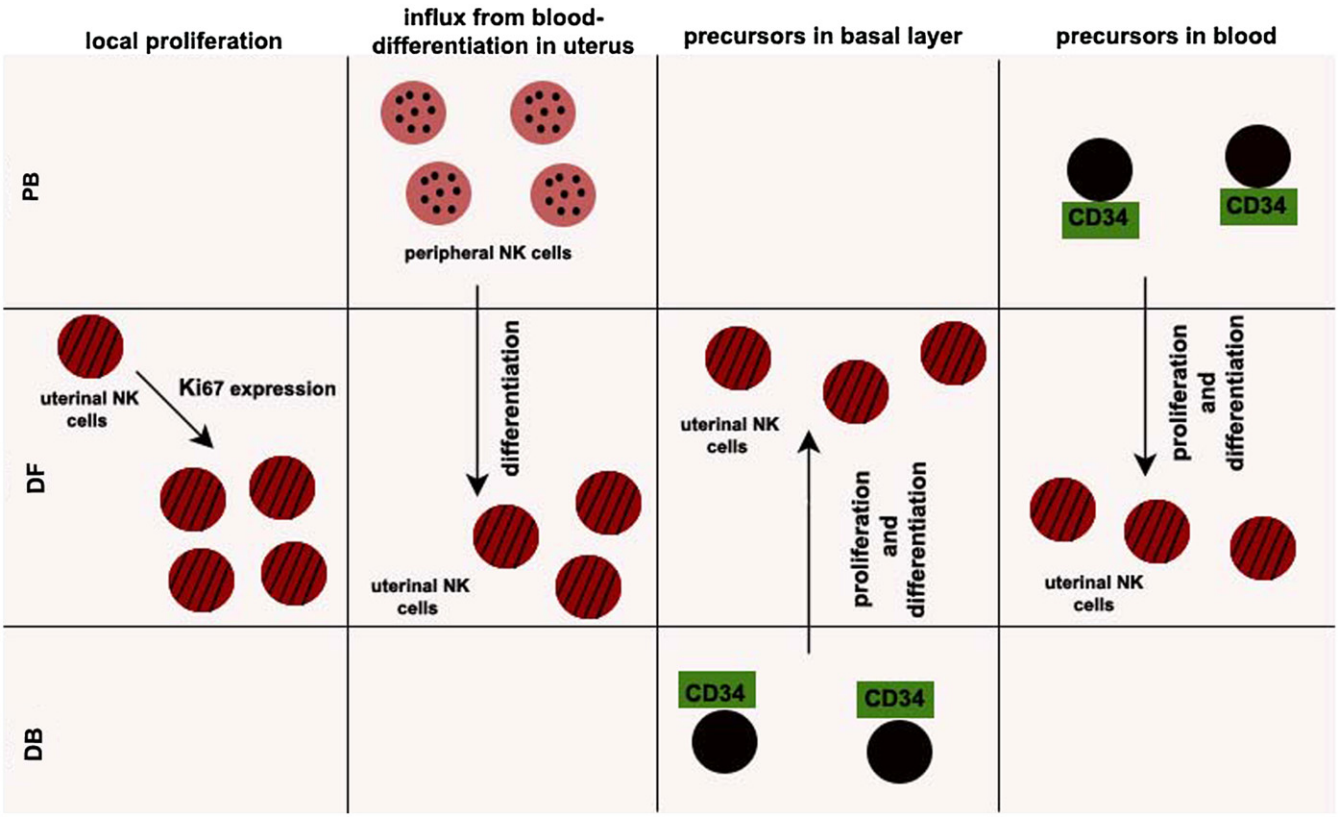


Fig. 1. This schematic figure shows the possible sources of the uterine natural killer cells (uNK). (1) uNK cells may proliferate in the of the decidua (as shown by Ki67 expression). (2) Peripheral NK cells may influx into uterus and differentiate here to uNK cells. (3) $CD34^+$ precursor cells localized in the basal layer may be a source of uNK cells. (4) $CD34^+$ precursor cells circulating in peripheral blood may influx into the uterus and differentiate to uNK cells. DB=basal layer of the decidua, DF=functional layer of the decidua, PB=peripheral blood.

NK cells bear some members of β1, β2 and β7 integrin families and these participate in NK cell activation and differentiation (Maenpaa et al., 1993; Allavena et al., 1994; Mainiero et al., 1994; Uksila et al., 1997; Frey et al., 1998). pNK cells express α5β1, α4β1 and α6β1 as fibronectin, VCAM-1 and laminin receptor, respectively (Gismondi et al., 1991). It has been shown that α4β1-VCAM-1 conjugation is important for NK cell rolling, adhesion and migration across activated endothelial cells (Allavena et al., 1991; Schneider et al., 2002). In the endometrium, endothelial cells do not express VCAM-1 in the proliferative phase, but during the mid- and late secretory phase they acquire this ligand. So, it is possible to infer that the α4β1-VCAM-1 adhesive pathway plays a role in NK cell adhesion to endometrial endothelial cells after ovulation.

The existence of natural killer cell receptors (NKR) was discovered by the observation of the elimination of MHC class I negative tumor cells by NK cells (Ljunggren and Karre, 1990). NKRs recognize MHC class I or class I-like molecules on target cells. Each NK cell expresses its own repertoire of activating and inhibitory receptors and NK cell activation is actually controlled by the integration of both activating and inhibitory signals, rather than a single signaling adapter (Cerwenka and Lanier, 2001; Diefenbach and Raulet, 2001; Farag et al., 2002; Kovats et al., 1990; Lanier, 2001; Ljunggren and Karre, 1990; Long, 1999; Lopez-Botet and Bellon, 1999).

NKRs for self-MHC class I molecules are important for distinguishing normal cells from foreign or transformed cells. NK cells can selectively kill autologous cells, which are known as "missing self" because they have lost or down-regulated expression of self-MHC class I molecules (Shimizu and DeMars, 1989; Storkus et al., 1989).

On human NK cells, three structurally different NKR families have been described: KIRs (killer-cell Ig-like receptor), CD94/NKG2 heterodimers and ILTs (Ig-like transcripts) (Colonna and Samaridis, 1995; D'Andrea et al., 1995; Wagtmann et al., 1995; Winter et al. (1998); Fan et al., 1996, 1997).

KIRs (killer-cell Ig-like receptors)

KIRs are type I transmembrane glycoproteins. KIR receptors encoded by a cluster of genes found on human chromosome 19q13.4 (Wagtmann et al., 1997). These receptors are highly polymorphic, both in the number of genes expressed in an individual and in the alleles present for a gene (Middleton et al., 2002). KIRs are not only expressed on NK cells but also on specific T-cell subsets. They are classified by the number of extracellular Ig-like domains-two (KIR2D) or three (KIR3D) and by the presence of long (KIR2DL and KIR3DL) or short (KIR2DS and KIR3DS) cytoplasmic tails (Biassoni et al. (1996); Colonna and Samaridis, 1995). KIRs with long cytoplasmic tails contain two immunoreceptor tyrosine-based inhibitory motifs (ITIM) which are responsible for the inhibitory function of these molecules. Engagement of the receptor with its class I ligand leads to phosphorylation of the tyrosine residues within the ITIM. The ITIM then recruits and activates the tyrosine phosphatase (SHP-1) which prevents the phosphorylation events associated with cellular activation, resulting in the inhibition of NK-cell mediated cytotoxicity and cytokine secretion (D'Ambrosio et al., 1995; Doody et al., 1995). KIRs with short cytoplasmic tails lack ITIM and associate with the immunoreceptor tyrosine-based activation motif (ITAM)-bearing protein DAP-12 through a positively

charged amino acid in their transmembrane domain. The phosphorylated ITAM provides a docking site for SH2 domains of ZAP70 and Syk tyrosine kinases and the action of these kinases leads to downstream transduction and NK cytolysis (Lanier et al., 1998). In general, KIR2D receptors recognize HLA-C alleles, whereas KIR3D receptors recognize HLA-A and HLA-B alleles (Boyington et al., 2000; Fan et al., 2001; Deng and Mariuzza, 2006).

A greater proportion of uNK cells than pNK cells express KIRs specific for HLA-C (Verma et al., 1997; Hiby et al., 1997). It has been shown that the interaction between maternal KIR and a trophoblast does not have an immune function, but instead plays a physiological role related to placental development. In mothers lacking most or all activating KIRs, fetuses possessed HLA-C belonging to the HLA-C2 group and were at a greatly increased risk of pre-eclampsia (PE). This was true even if the mother herself also had HLA-C2, indicating that neither non-self nor missing-self discrimination was operative (Hiby et al., 2004). NK receptor–ligand interactions favoring uNK activation are protective against PE by supplying sufficient amounts of NK-derived growth factors and chemokines, establishing adequate trophoblast invasion and vascular remodeling.

KIR2DL4 is an NK cell receptor for HLA-G (Rajagopalan and Long, 1999). Yan et al. (2007) compared mRNA and protein levels of KIR2DL4 in uNK cells isolated from normal women during early pregnancy and patients with early recurrent spontaneous abortion (RSA). They found no significant differences in KIR2DL4 mRNA expression however KIR2DL4 protein levels were much higher in normal controls than those in RSA patients. They concluded that KIR2DL4 might play a crucial implication for human pregnancy.Witt et al. (2004) however, reported that the KIR repertoire did not differ between RSA patients and controls in terms of the number of inhibitory receptors, the number of activating receptors, or the ratio of inhibitory to activating receptors. They concluded that their data provide little evidence that KIR polymorphism plays a role in predisposition to RSA.

CD94/NKG2

Both peripheral blood $CD56^{bright}CD16^{-}$ NK cells and uNK cells express high levels of CD94/NKG2 (Verma et al., 1997; Voss et al., 1998; Braud et al., 1998). CD94/NKG2 receptor is a member of the C type lectin superfamily. CD94 forms disulfide-linked heterodimers with members of the NKG2 glycoprotein family. While CD94 is a single gene with limited to no allelic polymorphism, the NKG2 family comprises five genes: NKG2A, NKG2C, NKG2D, NKG2E and NKG2F (Houchins et al., 1991; Yabe et al., 1993; Adamkiewicz et al., 1994; Plougastel and Trowsdale, 1997). These produce seven products (NKG2B and NKG2H being splice variants of NKG2A and NKG2E, respectively). CD94 lacks a cytoplasmic domain for intrinsic signal transduction. Depending on its cytoplasmic domain, the NKG2 segment determines whether the receptor is activating or inhibitory. CD94/NKG2 A/B/C has been shown to bind the HLA-E molecule. NKG2A/B provides an inhibitory signal with its ITIM and inhibits NK function. NKG2C, NKG2E, NKG2F and NKG2H associate with DAP12 and mediate activating signals. NKG2D, a prominent NK cell activating receptor, forms homodimers rather than heterodimers with CD94 (Adamkiewicz et al., 1994; Plougastel et al., 1996; Houchins et al. (1997); Braud et al., 1998; Sobanov et al., 1999).

ILT (Ig-like transcripts)

ILT genes are also known as LIRs (leukocyte Ig-like receptors). They are expressed on B, T, NK, and dendritic cells, as well as on monocytes/macrophages (Samaridis and Colonna, 1997; Cella et al. (1997); Cosman et al., 1997; Colonna et al., 1997, 1998; Fanger et al., 1998). They consist of at least eight members with different numbers of Ig domains. LIR-1, -2, -3, -4, -6a, -7 and - 8 have four domains, whereas LIR-5 and -6b have only two (Borges et al., 1997; Biassoni et al. (1996); Cosman et al., 1997). There are activating and inhibitory forms. So far, only two members of the ILT family (ILT2 and ILT4) have been found to bind to a broad range of HLA class I molecules including HLA-G (Colonna et al., 1997).

Natural cytotoxicity receptor (NCR)

Three recently identified receptors that are expressed on NK cells are NKp46, NKp30 and NKp44. While NKp46 and NKp30 are expressed on resting and activated NK cells, NKp44 are only expressed on activated cells. They are type I proteins belonging to the immunoglobulin (Ig) superfamily and have been called natural cytotoxicity receptors (NCR). The NKp46 gene locus is found on chromosome 19, whereas the gene loci for NKp44 and NKp30 are located on chromosome 6 (Pende et al., 1999; Vitale et al., 1998).

Functions of uNK cells

uNK cells are abundant in the uterus at the time of implantation and are in close contact with trophoblasts. While the specific functions of these cells remain unknown, they have been proposed to play a role in the implantation process and development of the placenta (Demir et al., 2002). The presence of NK cells at the maternal–fetal interface during implantation in many species suggests that trophoblasts are target cells for uNK cells. Their close proximity to the extravillous trophoblast suggests that they could recognize these cells as fetal and regulate their invasion (Loke and King, 1995). The differentiation of human endometrial epithelium is a dynamic event that occurs throughout the menstrual cycle and early pregnancy and uNK cells may play a role in this process (Demir et al., 2002).

The number of uNK cells increases during the secretory phase and, if pregnancy does not occur, they die pre-menstrually. According to some researchers, when uNK die pre-menstrually, there is a reduction in soluble factor products which maintain vascular integrity and this may trigger the menstrual breakdown. Therefore, the uNK cells may be important in renewal, differentation and breakdown of the endometrium during the menstrual cycle (Igarashi et al., 2001; Trundley and Moffett, 2004). They have been shown to express mRNA for vascular endothelial growth factor-C (VEGF-C), placental growth factor (PGF), and angiopoietin-2 (Ang-2) in the secretory phase, all of which could influence the arterial stability (Li et al., 2001).

Control of trophoblast invasion

The invasion of extravillous trophoblasts (EVTs) into decidua and spiral arteries is essential in order to establish the maternal blood supply to the fetus and is tightly controlled. Underinvasion or overinvasion of the decidua by trophoblasts can have disastrous consequences. While invasion is inadequate in pre-eclampsia (PE), some cases of intrauterine growth retardation (IUGR), preterm labour and late miscarriage, as well as excessive invasion occur in placenta accreta (Pijnenborg et al., 1983).

Owing to the high number of uNK cells during the first and second trimesters of pregnancy in decidua and their close association with invading trophoblasts, they have been proposed

to have an important role in the regulation of trophoblast invasion. Some of the mechanisms suggested for this regulation include cytotoxicity, cytokine production and apoptotic abilities of the uNK cells.

Although uNK cells are in close contact with trophoblasts at the fetal–maternal interface they do not exert cytolytic actions against trophoblastic cells (King et al., 1989). Although they have activating receptors such as NKp46, NKp44, NKp30, NKG2D and 2B4 (Kopcow et al., 2005; Vacca et al., 2006; Hanna et al., 2006) their cytotoxicity is reduced compared to peripheral blood NK cells. Koopman et al. (2003) showed that granzyme B and perforin (components of cytotoxic granules) were expressed at similar levels in uNK and $CD56^{dim}$ pNK cells, whereas granzyme A was 3- and 10-fold overexpressed in uNK cells versus the $CD56^{dim}$ and $CD56^{bright}$ pNK cells, respectively. Although uNK cells express high levels of perforin and granzyme A and B, different reports have indicated that their lytic granules were not released in normal pregnancy and that they display only limited cytolytic activity (Rukavina and Podack, 2000; Vacca et al., 2006). As described previously, Kopcow et al. (2005) showed that uNK cells failed to polarize their microtubules and perforin granules to the immunological synapse—the interface between a NK cell with its target.

uNK cells express receptors which recognize HLA-G, HLA-E and HLA-C expressed by invasive trophoblast cells (Kovats et al., 1990; Boucraut et al., 1993; Redman et al., 1984). Lack of cytotoxicity of uNK cells has been attributed to the interaction between these non-classical HLA-class I antigens and uNK receptors (Chumbley et al., 1994; Rouas-Freiss et al., 1997). According to Trundley and Moffett (2004), the function of uNK cells could possibly be modified after the ligation of uNK receptors with ligands expressed by EVT and this modification could influence trophoblast invasion.

The most likely mechanism for uNK cells to regulate trophoblast invasion is cytokine secretion. They secrete a rich source of growth factors and cytokines: VEGF-C, PlGF, Ang-2, TNF-α, IL-10, GM-CSF, IL-1β, TGF-β1, CSF- 1, LIF and IFN-γ (Jokhi et al., 1994, 1997; Li et al., 2001). van der Meer et al. (2004) reported increased production of IFN-γ and VEGF by uNK cells in response to HLA-G. *In vitro* invasion assays were used to detect the effects of cytokines and growth factors produced by uNK cells on extravillous trophoblast invasion. Ang-2 (Dunk et al., 2000), TNF-α (Bauer et al., 2004), IFN-γ and TGF-β1 (Graham et al., 1994) have all been shown to inhibit trophoblast invasion. IL-8 and interferon-inducible protein-10 (IP-10) secreted by uNK cells bind to their receptors on invasive trophoblasts and cause trophoblast migration (Hanna et al., 2006). However, it should be remembered that the uNK cell cytokine profile is altered on contact with extravillous trophoblast cells, particularly HLA-G. Therefore, the source (*Decidua basalis* versus *Decidua parietalis*) of uNK cells for isolation or *in vivo* studies may be important.

According to M30 immunostaining results during early gestation in the placental bed, approximately 30% of EVTs undergo apoptosis. M30 detects a neoepitope of cytokeratin 18 revealed after caspase mediated cleavage. Double immunohistochemical observations showed that many of these apoptosing trophoblast cells are surrounded by uNK cells. It is possible that uNK cells trigger apoptosis of trophoblast cells or that trophoblast cells undergoing apoptosis recruit uNK cells after apoptosis has been initiated (von Rango et al., 2003).

uNK cell control of vascular remodeling

uNK cells have been shown to have an important role in spiral artery transformation in transgenic mouse models. For example, more than 50% of female tgε26 (NK−T−B−) mice lose their fetuses by gestation days10–14. The surviving fetuses have very small placentas and remodeling of the spiral arteries is incomplete compared to controls. But when bone marrow from SCID donors was transplanted to tgε26 mice, NK and uNK cells reconstitute and reverse the reproductive deficiences (Guimond et al., 1998).

Which product of uNK cells is responsible for spiral artery remodeling? Ashkar et al. (2000) demonstrated that mice with uNK cell (RAG2−/−) or IFN-γ signaling (IFN-γ−/−) deficiencies have implantation site abnormalities and failure of decidual artery remodeling. These defects are not reversed when RAG2−/− mice are engrafted with bone marrow from IFN-γ−/− mice. These experiments strongly support the possibility that the functions of uNK cells in the mouse are mediated by IFN-γ (Ashkar et al., 2000).

Although some data have been gained from transgenic mouse studies, less is known about spiral artery remodeling in humans. Dilation and medial disorganization of spiral arteries occur before interaction with trophoblasts (Craven et al., 1998). During vascular change uNK cells are abundant (Bulmer et al., 1991) but their number decreases after 20 weeks of gestation when vascular changes are generally complete. uNK cells appear to be very closely associated with non-transformed, transformed and transforming spiral arteries in the placental bed. uNK cells can produce a variety of angiogenic factors: members of the vascular endothelial growth factor (VEGF) family, placental growth factor (PLGF), angiopoietin-2 (Ang-2) and NKG5 which mediate spiral artery transformation (Hanna et al., 2006).

In light of these data, we believe that uNK cells are vital for normal vascular remodeling during the early stages of pregnancy, a necessary process for the development of a normal pregnancy.

uNK cells in pathological pregnancies

Some studies have been performed to analyse the phenotype and function of uNK cells in abnormal pregnancies. Approximately 15% of pregnancies end in spontaneous abortion and genetic abnormalities account for about 50% of these abortions. However, no cause is found in most of the cases (King et al., 1998). Some groups compared uNK cells from women undergoing spontaneous miscarriage with those from normal pregnancies (Maruyama et al., 1992; Chao et al., 1995; Lea et al., 1995). Lachapelle et al. (1996) reported that the $CD56^{bright}$ $CD16^{-}$ population decreased while the $CD56^{dim}$ $CD16^{+}$ population increased in women with spontaneous abortion.

Trophoblast migration is shallow in some pregnancy disorders such as PE, IUGR and stillbirth and results from a poor maternal blood supply (Brosens et al., 1972). Different groups found contradictory results for uNK distribution in the *Decidua basalis* and *Decidua parietalis*.

Wilczynski et al. (2003) found an increased percentage of CD3−/CD56+CD16+ lymphocytes in third trimester decidua of PE women, however, on the contrary, Eide et al. (2006) found a decreased proportion of uNK cells in women with PE, PE complicated with IUGR and IUGR.

According to flow cytometry studies (Lachapelle et al., 1996; Fukui et al., 1999), women suffering from repeated *in vitro* fertilization-embryo transfer failures and unexplained spontaneous recurrent miscarriages had decreased proportions of uNK cells among whole endometrial lymphoid cell suspensions during the mid-secretory phase.

On the other hand, according to immunohistochemical studies by Clifford et al. (1999) and Quenby et al. (1999), the number of uNK cells increased in the mid-secretory phase endometrium in women suffering from unexplained recurrent miscarriages.

Whether there are differences in numbers of uNK cells in proliferative phase endometrium between fertile women and women with unexplained recurrent miscarriages has yet to be examined.

As we pointed out previously, there are different studies trying to reveal the collection of uNK cell receptors in healthy and pathological pregnancies (Yan et al., 2007; Witt et al., 2004). Varla-Leftherioti et al. (2003) examined the repertoire of NK cell receptors in women with recurrent spontaneous abortion (RSA). Less aborters than controls were found to have three inhibitory KIRs: KIR2DL1, 2, 3- (30.77% versus 69.23%, $p=0.01$), some of them had only one inhibitory KIR (19.23% versus 3.85%, $p=0.08$). Thus, they concluded that women with alloimmune abortions have a limited number of inhibiting KIRs and such miscarriages may occur because trophoblastic HLA class I molecules are recognized by uNK cells lacking the appropriate inhibitory KIRs. The results of Varla-Leftherioti et al. (2003) and Yan et al. (2007) are similar, but the data from Witt et al. (2004) is inconsistent with these. The differences may stem from dissimilar study groups or the primers used in these studies.

Conclusions

Pregnancy has drawn the attention of scientists for a long time. Although the fetus is a semi-allograft, it is not rejected by the mother. The fetus utilizes maternal sources without activating elements of the immune system against itself. Maternal leukocytes in the uterus are unique. There are other immune cells at the site of implantation: macrophages, dendritic cells, T and B lymphocytes. While they undoubtedly have important roles, they do not demand as much attention as the uterine NK cells.

NK cells constitute around 5–15% of circulating lymphocytes and they have been identified in many other organs like the spleen, lung, gastrointestinal tract and liver. Although scientists have been aware of the existence of granulated cells in the endometrium of several species for many years, these cells have only recently been recognized as a type of NK cell.

uNK cells regulate trophoblast invasion both *in vitro* and *in vivo* by production of interleukin-8 and interferon-inducible protein-10 chemokines. In addition, uNK cells are potent secretors of an array of angiogenic factors and induce vascular growth in the decidua.

There is increasingly strong evidence of a relationship between uNK cells and trophoblast invasion and spiral artery remodeling, but some facts to be considered. First of all, uNK cells are found in the endometrium, but trophoblast invasion and spiral artery remodeling reaches out into the inner myometrium, where there are no uNK cells. Moreover, in extrauterine ectopic pregnancies, invasion of trophoblasts occurs in the absence of uNK cells. uNK cells are also found in many species that do not have an invasive form of placentation. uNK cells are present in the non-pregnant endometrium and their numbers are affected by hormonal variations. Although some studies in the mouse have supported trafficking of precursors from the peripheral blood, it is still unclear whether the increase in uNK cell numbers during the late secretory phase of the menstrual cycle can be attributed to local proliferation of these cells or to their influx from the circulation.

Although an earlier concept defined uNK cells as dangerous cells, many studies now show their beneficial properties for implantation and pregnancy. Several recent published articles have proposed that their major function is to assist in fetal development. uNK cells can be considered as a 'peaceful' model for reproductive immunology, in which elements of innate immunity have been incorporated in a constructive manner to support reproductive tissue development.

Although much new information has been learned about NK biology in recent years, there still remain many areas that need further elucidation. Such areas include development of NK cells-uNK cells, structure and function of NKRs, the relationship between NKRs and HLA class I molecules, acquisition of self-tolerance and interaction of NK-uNK cells with other immune system cells.

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