Published in final edited form as:
Am J Reprod Immunol. 2008 May ; 59(5): 425–432. doi:10.1111/j.1600-0897.2008.00595.x.

Evolution of non-cytotoxic uterine natural killer (uNK) cells

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Abstract

The immune tolerance and de novo vascularization are two highly intriguing processes at the maternal-fetal interface that appear to be central for normal pregnancy outcome. Immune tolerance occurs despite the local presence of an active maternal immune system including macrophages, dendritic cells and specialized $CD56^{bright}CD16^{-}$ uterine NK cells (65–70%). Recent observations indicate that the phenotypic and functional repertoire of uNK cells is distinct from peripheral blood NK (pNK) and endometrial NK cells (eNK) cells, challenging the understanding of their temporal occurrence and function. Origin and specialized programming of uNK cells continue to be debated. uNK cells, replete with an armamentarium to kill the foreign, tolerate the conceptus and facilitate pregnancy. Why do these uNK cells remain non-cytotoxic? Are these NK cells "multitasking" in nature harboring beneficial and detrimental roles in pregnancy? Are there distinct subpopulations of NK cells that may populate the decidua? We propose that the endometrium/decidua functions as an "inducible tertiary lymphoid tissue" that supports the recruitment and expansion of $CD56^{bright}CD16^{-}$ NK cells, and induces transcriptional up-regulation of angiogenic machinery in response to exposure to local hormonal factors, cytokine milieu and perhaps hypoxia. The angiogenic features of uNK cells could further result in a "multitasking" phenotype that still remains to be characterized. This paper discusses the factors and pathways that bridge the angiogenic and non-cytotoxic response machineries at the maternal-fetal interface.

Keywords

Angiogenic factors; Decidua; Inducible tertiary lymphoid tissue; Natural killer cells; Non-cytotoxicity; Pregnancy

Introduction

Pregnancy is a dynamic process that triggers adaptation by the maternal immune, vascular, and hormonal systems. Immune cellular accumulation in the non-pregnant endometrium parallels its programming as a "tertiary lymphoid organ" which may provide an

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inflammatory impetus required for blastocyst implantation.[1,2] Establishment of pregnancy interrupts the menstrual cycle and creates a local hypoxic environment for placentation, early embryo and fetal development.

This evolutionary adaptation is addressed by remodeling of the maternal spiral arteries associated with loss of normal musculo-elastic structure, converting the spiral arteries into large, thin-walled, dilated vessels of minimum resistance.[3] These changes are associated with unique homing to and/or expansion of maternal NK cells in the decidua. Interestingly, uNK cells peak and constitute the largest leukocyte population during the early pregnancy period accounting for about 70% of total mononuclear population but dwindle as the pregnancy proceeds.[4]

Phenotypic features of uNK cells

Human NK cells that populate various peripheral blood, lymphoid and non-lymphoid organs originate from $CD34^{+}$IL-2Rβ-$chain^{+}$ hematopoietic stem cell progenitors.[5] Depending on their residential organ, subtle but distinct phenotypic and functional differences are observed. pNK cells constitute about 8–10% of the peripheral blood mononuclear cell (PBMC) population in the circulation. The majority of NK cells are characterized by the lack of CD3 and the presence of CD56 antigens. Based on the intensity of CD56 antigen, NK cells are further divided into $CD56^{bright}$ and $CD56^{dim}$ populations. The presence or absence of FcγRIII or CD16 further differentiates subpopulations of NK cells. Thus, the majority of pNK cells are of the $CD56^{dim}CD16^{+}$ phenotype while the remaining belongs to the $CD56^{bright}CD16^{-}$ phenotype. In lymph nodes, NK cells predominantly express $CD56^{bright}CD16^{-}$.[1,6] In the female reproductive tract, the majority of endometrial NK cells (eNK) and NK cells from the cervix and fallopian tube are of $CD56^{bright}CD16^{-}CD9^{+}CD94^{+}$ phenotype. On the other hand, in the ectocervix and vagina, NK cells express $CD56^{dim}CD16^{+}CD9^{+}CD94^{-}$.[6] As shown in Figure 1, the uNK cell number cyclically increases and decreases in tandem with the menstrual cycle - low in the proliferative phase, increases during early-, mid- and late-secretory phases, falling to basal level during menstruation. With successful implantation, the NK cell population further increases in the decidualized endometrium and reaches peak during first trimester and dwindle thereafter by the end of second trimester.[7] A similar kinetics of uNK cells characterized by typical NK cell phenotypic markers exists in rodents during pregnancy. However unlike humans, in non-pregnant mouse endometrium, granulated NK cells are almost absent. In mouse, NK cells are identified by NK1.1 and DX5 phenotypic markers. Comparative surface profiles of antigens, natural cytotoxicity receptors, chemokine and growth factor receptors on human pNK, eNk and uNK cells are provided in the Table 1. Further, $CD56^{bright}$ uNK cells are different from the $CD56^{bright}$ minor population of pNK cells based on the expression of CD9, CD103, and killer immunoglobulin-like receptors (KIR).[8,9] Replete with a cytotoxic arsenal of perforin, granzymes A and B, and the receptors NKp30, NKp44, NKp46, NKG2D, uNK cells remain tolerant cytokine producing cells at the maternal-fetal interface.[10]

Origin of uNK cells

The origin of uNK cells that peak during the secretory phase of luteal cycle and early pregnancy is not well established and evidence indicates multiple different possibilities.

Conscription of $CD56^{bright}CD16^{-}$ pNK cells

The notion of recruitment from peripheral blood $CD56^{bright}CD16^{-}$ NK cells to the endometrium and decidua is based on the fact that these cells express L-selectin and chemokine receptor CXCR4 that can home towards its ligand CXCL12 found on the

extravillous trophoblasts within the decidua.[11,12] However, the presence of conceptus is not required for the increase in $CD16^-$ NK cells during the secretory phase of luteal cycle.[7] Nevertheless, in the non-pregnant uterus, progesterone and estrogen may support the migration of CXCR3 positive $CD56^{bright}$ pNK cells by augmenting the expression of CXCL10 and CXCL11 in the endometrium.[13,14]

$CD56^{dim}CD16^+$ pNK cells as migratory precursors

Recruitment of $CD56^{dim}CD16^+$ pNK cells to estrogen or progesterone rich chemokine expressing endometrium is also possible. It has been recently reported that TGFβ produced by the decidual stromal cells can convert the $CD56^{dim}CD16^+$ NK cells to $CD56^{bright}CD16^-$ NK cells, indicating tissue specific terminal differentiation.[15] However, this may be an induced reversible process because $CD56^{bright}CD16^-$ NK cells may be precursor for $CD56^{dim}CD16^+$ NK cells. In addition, *in vitro* conversion of $CD56^{dim}CD16^+$ NK cells to $CD56^{bright}CD16^-$ NK cells appear to be a complex process and may not follow recruitment and appearance.

Progenitor cells as source of uNK cells

It is widely recognized that the bone-marrow microenvironment provides a rich source of cytokines and growth factors that can support NK-cell developmentand maturation in the presence of stromal cells.[5] Similar to the bone-marrow, the endometrium may provide appropriate a local micro-environment to support NK cell development. A minor population (0.1%) of $Lin^-CD34^+CD45^+$ cells is present in the decidua that can differentiate into $CD56^{bright}CD16^-$ NK cells in response to stromal cell-derived factors, interleukin-15 and stem-cell factor during a 30 day culture period.[15] However, a caveat with these observations is that many of the factors that allow this to happen have not been shown to be present in the endometrium. Nevertheless, animal studies reveal that infusion of bone-marrow cells to tgε26 mice which, are devoid of NK cells, repopulate the uNK cell pool.[16] uNK cells may then proliferate *in situ* by up-regulating the suitable gene cascade to reach the peak numbers.[17,18]

Seeding and Expansion of $CD56^{bright}CD16^-$ NK cells

The hormonal milieu-driven "seeding and expansion" of resident $CD56^{bright}CD16^-$ NK cells is thought to have a major influence on the evolution of these cells in the endometrium and the decidua. *In situ* expansion and proliferation is possible as $CD56^{bright}CD16^-$ NK cells express prolactin, estrogen β and glucocorticoid receptors despite the lack of progesterone receptors.[19,20] Progesterone, hCG, prolactin may act indirectly to prime stromal cells or to act directly on NK cells through glucocorticoid receptors and membrane potassium channels supporting proliferation of $CD56^{bright}CD16^-$ NK cells (Figure 2).[21,22] Additionally, progesterone may modulate $CD56^{bright}CD16^-$ NK cell cytotoxicity possibly through progesterone-induced blocking factor (PIBF) and support angiogenesis.[23, 24]

Endometrium/decidua as "inducible tertiary lymphoid" tissue

Unlike the canonical secondary lymphoid organs, "tertiary lymphoid tissues" are patterned as a result of accumulations of lymphoid cells through a process called "lymphoid neo-vascularization". Such extra-thymic temporal lymphoid tissues are known to occur in autoimmunity, microbial infections and chronic allograft rejection.[5,25,26] Like any mucosal tissue, many myeloid and lymphoid cell aggregates are found in non-pregnant uterus.[26,27] It is long thought that $CD56^{bright}CD16^-$ NK cells are precursors of $CD56^{dim}CD16^+$ NK cells, and unlike the ratios of these two phenotypic populations in blood, these cells are predominantly $CD56^{bright}CD16^-$ in lymph nodes. A plethora of evidence supports this notion: a) Shortly after bone-marrow transplantation, the $CD56^{bright}CD16^-$ cell population

increases and then tapers off when $CD56^{dim}CD16^{+}$ cells become prominent,[27] b) The telomere length is shorter in $CD56^{dim}CD16^{+}$ compared to $CD56^{bright}CD16^{-}$ cells from the same donor, supporting the precursor role of $CD56^{bright}CD16^{-}$ cells as telomeres progressively shorten with growth and differentiation,[28] and c) $CD56^{bright}$ cells can differentiate into $CD56^{dim}$ both *in vitro*, in the presence of synovial fibroblasts, and *in vivo*, upon transfer into NOD-SCID mice.[29] It is tempting to speculate that hormonal milieu during menstrual cycle or implantation, converts the endometrium or decidua into an inducible "tertiary lymphoid tissue" that recruits and amplifies circulating $CD45^{+}CD34^{+}$ hematopoietic progenitor cells and $CD56^{bright}CD16^{-}$ NK cells (Figure 3). The progenitor cells, in response to surrounding uterine stromal tissue and IL-15, differentiate into $CD56^{bright}CD16^{-}$ NK cells and express angiogenic factors, L-selectin and CCR7 that program them to adhere to the tissue. Hypoxia caused by the implanted and rapidly developing embryo may further stimulate angiogenic signals and chemokine expression in uNK cells to recruit invading trophoblasts. On the other hand, lack of pregnancy compatible intrauterine milieu (e.g. absence of IL-10, VEGF, Stromal Cell Protein (SCP), hCG, etc) or the presence of intrauterine infections may allow further maturation and differentiation into cytotoxic $CD56^{dim}CD16^{+}$ NK cells triggering pregnancy complications.

uNK cells as friends to pregnancy: Why do they remain non-cytotoxic ?

The predominant population of $CD56^{bright}CD16^{-}$ uNK cells in the decidua directly places them in close proximity with decidual stromal cells, maternal endothelial cells and extravillous trophoblasts. This should favor cell-cell cross-talk and interaction. Two hallmarks of activated pNK cells, the expression of natural cytotoxicity receptors and their ability to lyse targets that are deficient in major histocompatability complex (MHC) presentation, are also the intrinsic features of uNK cells. Interestingly, unlike pNK cells, uNK cells remain non-cytotoxic and produce regulatory cytokines and growth factors that support normal pregnancy. Thus, the question that should be asked here is whether the angiogenic machinery programmed in uNK cells is responsible for their non-cytotoxic activity during pregnancy.

Non-cytotoxic uNK cells as angiogenesis promoters

In situ hybridization studies with non-pregnant endometrium localized mRNA for VEGF-C, PlGF, Ang 2 to NK cells. VEGF-C was shown to peak during the early and mid secretory phase of the cycle reflecting the inherent nature of NK cell phenotypes found in endometrium.[30] Other studies have shown that unlike pNK cells, uNK cells are a major source of VEGF-C, Ang1, Ang2 and TGFβ1 within the placental bed that decrease with gestational age implicating uNK cells in promoting angiogenesis.[31] Studies have provided further evidence that uNK cells, but not pNK cells, regulate trophoblast invasion both *in vitro* and *in vivo* through the production of interleukin-8 and interferon-inducible protein-10 (IP-10) in addition to other of angiogenic factors.[32] Increased serum levels of IP-10 have been implicated for the poor angiogenesis seen in pre-eclampsia.[33] Our own unpublished observations indicate that eNK cells and uNK secrete variable amounts of VEGF-A, VEGF-C and IFNγ and express of natural cytotoxicity receptors (NCR), implying that empowerment of uNK cells with the angiogenic features keeps them non-cytotoxic.[34] Both hypoxia and a low pH associated with tumors have been shown to suppress NK cell cytotoxicity and increase the resistance of tumor cells.[35,36] Factors that trigger this angiogenesis-non-cytotoxicity axis of uNK cells are not yet clear, however, we propose transformation of endothelial cells and trophoblasts regulated by uNK cells.

Can uNK cells become foes to pregnancy?

Although the origin and temporal appearance of uNK cells is not yet clear, recent reports have implicated cytotoxic uNK cells in the pathogenesis of subgroup of spontaneous abortions and implantation failures. Spontaneous abortion is associated with an increase in $CD56^{dim}CD16^{+}$ cells and a decrease in $CD56^{bright}CD16^{-}$ NK cells in the pre-implantation endometrium during the luteal phase.[37,38] A fine balance between maternal activating and inhibitory killer IgG-like receptors (KIR) and their ligand human leukocyte antigen C (HLA-C) on fetal cells seems to be maintained in normal pregnancy. Insufficient inhibition of uNK cells can turn on the cytolytic machinery causing spontaneous abortions.[39] In the setting of IVF, the implantation failure has been associated with high uNK cell numbers but direct evidence for their role in abnormal implantation is not clear.[37] These observations raise an important question whether uNK cells can harm fetal tissue and if yes, under what conditions?

The anti-inflammatory cytokine IL-10 plays a critical role in pregnancy because of its regulatory relationship with other intrauterine modulators and its wide range of immunosuppressive activities.[40] Significantly, itslocal production by gestational tissues is well documented. We have demonstrated that IL-10 expression by the human placenta was gestational age-dependent, with significant expression through the second trimester followed by attenuation at term.[41,42] IL-10 expression was also found to be poor in decidual and placental tissues from unexplained spontaneous abortion cases,[42] andfrom deliveries associated with preterm labor [43] and pre-eclampsia (our unpublished observations). However, the mechanism(s) by which IL-10 protects the fetus remains poorly understood. $IL\text{-}10^{-/-}$ mice suffer no pregnancy defects when mated under pathogen-free conditions.[44] It is then plausible that in addition to IL-10 deficiency, an inflammatory insult resulting from genital tract infections, environmental factors, and/or hormonal dysregulation during gestation may lead to adverse pregnancy outcomes. Our recent studies provided direct evidence that uterine NK (uNK) cells became adversely activated and mediated fetal demise and preterm birth in response to low doses of LPS, a ligand for Toll-like receptor 4 (TLR). These pathologies were directly associated with uNK cell-derived TNF-α.[45] However, the impact of “danger signals” (TLR 4 expression) observed in the placental bed of patients with pre-eclampsia on human uNK cells remains to be explored.[46] Nevertheless, our findings strongly imply that uNK cells retain the ability to become foes to pregnancy under the axis of genetic stress and inflammatory trigger.

Conclusions

NK cells in non-pregnant humans have evolved as innate immune defense against pathogenic intrusions. Thus, it seems that the recruitment and copious presence of NK cells is not specific to pregnancy, but is an evolutionary coincidence that occurs in tandem with menstrual cycle. The cytokine rich microenvironment, hormonal milieu, relative hypoxia and interacting cells can recruit and expand circulating NK cells and/or differentiate the progenitor cells to eNK that go on to acquire the uNK cell phenotype observed in the decidualized endometrium mimicking “inducible tertiary lymphoid tissue”. Full maturation to cytotoxic NK cells is possibly prevented by the presence of unique growth factors. In pregnancy, armed with all tools to attack, the uNK cells behave as sentinels, adapt and tolerate the semi-allograft, turning on their roles as friends in a specific decidual microenvironment balancing between attenuated cytotoxicity and increased production of angiogenic growth factors. In response to stimuli that eventually would challenge the very existence of “maternal self”, these uNK cells would turn on the “primordially conserved” cytolysis mechanism, eliminate out the adverse stimulus eventually leading to pregnancy failure as seen in the IL-10 deficient scenario.

Acknowledgments

This work was supported in part by grants P20RR018728 from the NIH and Superfund Basic Research Program Award (P42ES013660) from the NIEHS.

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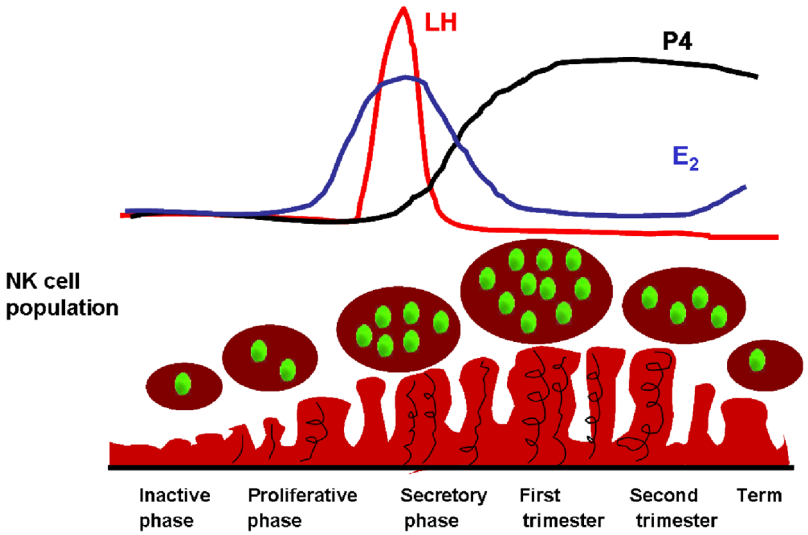

Figure 1. Biological rhythm of CD56^{bright}NK cells in the human endometrium and the decidua

uNK cell population cyclically patterns in tandem with the menstrual cycle. With successful implantation, uNK cells further increase in the decidua and dwindle thereafter by the end of second trimester. E2, estradiol; P4, progesterone; LH, leutinizing hormone.

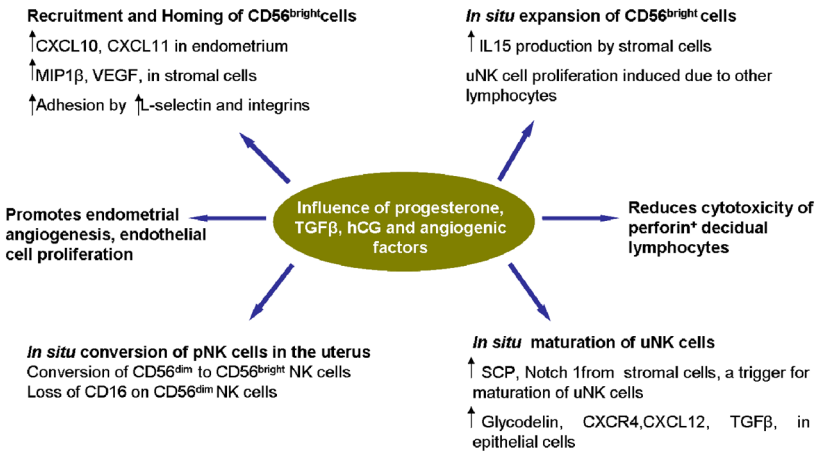

Figure 2. Hormone-cytokine influence on evolution and function of uNK cells

The hormone-cytokine milieu in the endometrium and the decidua can choreograph recruitment, expansion, differentiation and maturation of uNK cells in addition to their potential influence on angiogenesis and cytotoxicity. MIP, macrophage inflammatory protein; CXCR, CX chemokine receptor; CXCL, CX chemokine ligand; TGF, transforming growth factor; SCP, stromal cell protein.

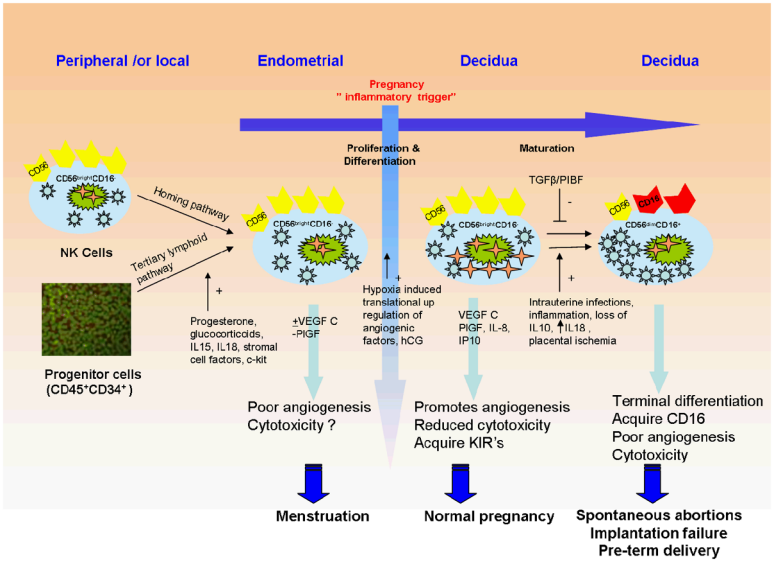

Figure 3. Origin of uNK cells-endometrium and decidua as "inducible tertiary lymphoid tissue"

In the homing pathway, local hormonal milieu can recruit and amplify circulating $CD56^{bright}CD16^{-}$NK cells. In the tertiary lymphoid pathway the lineage committed-progenitor cells ($CD45^{+}CD34^{+}$) and circulating NK cells differentiate into $CD56^{bright}CD16^{-}$ NK cells in the presence of stromal cells and IL-15. Pregnancy induced hypoxia can trigger translational up-regulation of angiogenic machineries and support pregnancy. Compromised pregnancy compatible intrauterine milieu may allow further maturation into cytotoxic $CD56^{dim}CD16^{+}$ NK cells causing pregnancy complications.
Symbols: ✦ angiogenic factors; ✿ cytolysis machinery
Abbreviations: VEGF C, vascular endothelial growth factor C; PlGF, placenta growth factor; IP 10, interferon gamma inducible protein

Table 1

Expression of surface markers and receptors on NK cells[†]

Antigen	pNK	eNK	uNK
CD56	dim (>90%)	bright	bright
CD16	+	−	−
CD45	+	+	+
CD7	+	+	+
CD69	−	+	+
L-Selectin	−	−	+/−
NK receptors			
KIR	+	+/−	+
NKp30	+	+	+
NKp44	_*	+	+
NKp46	+/−	+	+
NKG2D	+	+	+
CD94/NKG2A	+/−	+	+
Chemokine Receptors			
CXCR1	+	+/−	+
CXCR2	+	+/−	−
CXCR3	−	+	+
CXCR4	+	−	+
CX_3CR1	+	+	−
CCR7	−	−	+
Growth factor and steroid receptors			
VEGF R1	−	−	−
VEGF R2	−	−	−
VEGF R3	−	−	−
Progesterone receptors	−	−	−
ERα	_**	−	−
ERβ	+#	+	+
Prolactin receptor	+	+	+
Glucocorticoid receptor	+	+	+

−, +, −/+ indicates absence, presence or variable expression.

*Expression seen on activation with IL-2.

**Expression reported in mouse pNK cells.

#Observed at mRNA level

†Abbreviations: CXCR, CX-chemokine receptor; CX_3CR1, CX_3-C-chemokine receptor 1; CCR7, CC-chemokine receptor 7; KIR, Killer immunoglobulin receptor; VEGF R, vascular endothelial growth factor receptor; ER, estrogen receptor.

The details are tabulated from references [6, 20, 28], and [47] and our unpublished observations.