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Properties of Uterine Natural Killer Cells in Human Pregnancy, Major Receptors Involved, and Routes of Trophoblast Invasion

Tarek S Beytamouni and Esther Ghanem*

Department of Sciences, Faculty of Natural and Applied Sciences, Notre Dame University, 72 Lebanon

Abstract

Suppression of the maternal immune system to tolerate the fetal alloantigens during implantation translates into an immunological paradox. The initial process of implantation is marked by the migration of interstitial extravillous trophoblast (EVT) into the endometrial wall and is assisted by maternal uterine natural killer (uNK) cells. Any impairment in uNK assistance can ultimately lead to loss of pregnancy. In this review, we discuss current information regarding the role of uNK cells in healthy human pregnancy and their importance in sustaining a controlled invasion of the endometrium by EVT.

Keywords: Uterine natural killer cells; Extravillous trophoblast; Miscarriage; HLA; Implantation

Introduction

Of the widely diverse immune cells, uterine natural killer (uNK) cells have been given particular attention in miscarriage research [1-5] primarily due to their abundance in the uterine wall during the first three months of gestation [6]. Trophoblast migration into the endometrial wall is a key event in the implantation of the embryo. However, excess and deficient interstitial extravillous trophoblast (EVT) migrations have been both associated with pregnancy complications. The highly invasive potential of EVT cells can damage the maternal reproductive system if it goes uncontrolled. uNK cells seem to be essential to create the balance necessary to pregnancy [7]. In another rendering, uNK cells possess a potent role by regulating the invasion/ migration of trophoblasts into the maternal endometrium through a controlled expression of receptors and secretion of chemokines. In this review, we discuss current information regarding the role of uNK cells in healthy human pregnancy and their importance in sustaining a controlled invasion of the endometrium by EVT.

With 50% of recurrent miscarriages occurring inexplicably, it is necessary to widen the scope of research and focus on previously disregarded or overlooked factors.

Natural Killer Cells

Natural killer (NK) cells are large lymphocytes that are a key element of the innate immune system [6]. They are referred to as "natural" killers for their ability to recognize virally infected or malignant cells without being previously sensitized. They are distinguished from other lymphocytes by the expression of surface markers CD56 and CD16 [8-11], along with the absence of surface marker CD3, which is specific to T cells [6,10,12,13]. The two predominant subsets are $CD56^{bright}$ $CD16^{dim/-}$ NK cells, and $CD56^{dim}$ $CD16^{bright}$ NK [8]. $CD56^{bright}$ cells are prevailing in secondary lymphoid tissues and constitute a maximum of 10% of peripheral blood NK (pNK) cells; while $CD56^{dim}$ $CD16^{bright}$ NK cells form the remaining 90% [14].

Uterine Natural Killer Cells

The majority of uNK cells are similar to tissue-resident NK and a small subset of pNK cells, as they express CD56 but not CD16 [15]. They principally inhabit the uterine decidualized stroma, in the vicinity of endometrial glands and spiral blood vessels. To date, the generation of uNK cells is still fully understood. It is postulated that uNK cells are either derived from pNK cells or undergo tissue-specific differentiation.

The levels of uNK cells fluctuate during the menstrual cycle with low levels during the proliferative phase and peaks during the secretory phase reflecting their role in implantation [16,17]. In case of pregnancy, their levels remain high during the first trimester, and starts to decline during the second trimester, only to return to basal concentrations once pregnancy ends [6,18]. The main characteristic of NK cells is their cytotoxic role. They have the ability to lyse cells that are recognized as abnormal, i.e. cells that do not express HLA-I molecules at all or insufficient quantities [19]. Not all NK cell populations have the same lysing potential. The $CD56^{dim}$ $CD16^{bright}$ phenotype confers cytotoxic properties to pNK cells. In contrast, uNK cells are characterized by poor cytotoxicity in healthy pregnancy [14], and have been reported unable to secrete cytokines or mount immune responses without prior activation by interleukin 15 (IL-15) secreted by the uterine endometrium and decidua [20]. Despite their moderate but still existing cytotoxicity, trophoblast cells are entirely tolerated by uNK cells [20]. This unusual relationship translates into the immunological paradox of the embryo successfully evading the maternal immune system (IS). Through such immunotolerance, uNK cells modulate trophoblast invasion by exhibiting a synchronized co-engagement between its cell surface receptors and those at the trophoblast surface [21].

Major receptors involved in regulating trophoblast invasion

NK cells normally express a wide variety of receptors [22]. They include activating receptors, such as lectin-like and Toll-like receptors (TLRs) [23]; and inhibitory receptors, such as LT-2, CD94/NKG2A, CD161, and killer cell immunoglobulin-like receptors (KIRs) [20]. The

***Corresponding author:** Ghanem E, Department of Sciences, Faculty of Natural and Applied Sciences, Notre Dame University, 72 Lebanon, Tel: + 961 9218950-ext.2738; E-mail: eghanem@ndu.edu.lb

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relative amounts of expressed receptors can influence the functions of NK cells and their immunological impact on the body, and on the maternal-fetal interface in pregnant women. A decrease in the expression of inhibitory receptors or an increase in activating receptors can lead to an unbalanced activation of uNK cytotoxicity. KIRs are the predominant inhibitory receptors on NK cells. Such receptors block intracellular signaling and cytotoxicity of NK cells when human leukocyte antigen class I (HLA-I) molecules are normally expressed [24]. For instance, in the absence or low expression of HLA-I, NK cells are activated [25]. Interestingly, trophoblast cell populations do not express classical major histocompatibility complex (MHC) class I, except for low amounts of human leukocyte antigen C (HLA-C) molecules [26]. Nevertheless, trophoblasts are still tolerated by NK cells to mediate endometrial invasion to foster implantation. One plausible explanation could be related to the abundant presence of non-classical MHC-I molecules, namely HLA-E, HLA-G and HLA-F on the surface of invasive trophoblasts [27]. It has been postulated that HLA-E is capable of presenting a repertoire of peptides that belong to both classical and non-classical MHC molecules [28]. HLA-E in turn is recognized by the inhibitory receptors CD94/NKG2A of NK cells and thereby plays a very important role in modulating the activation/inhibition of NK cell cytotoxicity (Figure 1). Importantly, low levels of uNK cells expressing surface inhibitory receptor NKG2A has been linked to recurrent miscarriage. HLA-G is also particularly crucial to maintaining maternal tolerance of the embryo. Expression of abnormal or of decreased levels of HLA-G on trophoblast surface impairs the suppression of the maternal IS, with plausible outcomes being the destruction of the EVT and abortion [27,29,30]. Another inhibitory pathway is mediated via a C-type lectin-like receptor, NKR-P1A (CD161) that recognizes non-MHC ligands, namely lectin-like transcript-1 [31]. In case of excessive amount of CD161, NK cells are improperly regulated potentially leading to miscarriage (Figure 1) [32]. Thus, a collective balance between different receptors culminates in a regulated activity of NK cells.

On the other hand, activating receptors such as TLRs, trigger an immune response against infected cells through interaction with ligands that may or may not be MHC-I molecules. For instance, in human TLR activation ordinarily blocks T helper 2 (Th2)-specific cytokine release, and enhances the mounting of a T helper 1 (Th1)-dominated immune attack. Additionally, interactions of TLR–TLR ligand (TLRL) largely modulate many functions and activities of NK cells, usually by inducing cytokine production. Enhanced TLR-3 expression in uNK cells has been linked to a higher frequency of miscarriage [23].

The higher risk of miscarriage may be explained by a resulting stronger inhibition of trophopblast invasion of the uterine endometrium [26]. Since stronger NK cytotoxicity has been noted, it has been further suggested that high TLR-3 expression abolishes NK cell immune tolerance of trophoblast cells, and leads to inappropriate immune responses against the embryo which is then perceived as a harmful semi-allograft [23]. It has indeed been shown that upregulation of TLR mRNA in NK cells, as well as excessive levels of uNK cells expressing surface activating receptor CD161 as illustrated in figure 1, is related to higher levels of interferon gamma (IFN-γ). IFN-γ secretion contributes to macrophage activation and enhances cytokine production. This can induce disproportionate and haphazard lysis of trophoblasts and cause a spontaneous abortion [26]. It is important to note that moderate levels of IFN-γ mediate the regulation of uterine invasion by the EVT [33]. Another component that interacts with uNK cells and aids in maintaining pregnancy, is Treg cell. Treg cells belong to the adaptive immune system and usually mediate their role by suppressing NK cell in autoimmune diseases and other diseases such as prostate and colon cancer [34,35]. Despite its immunosuppressive role, Treg cells work cooperatively with uNK cells in pregnancy to tolerate fetal implantation [36].

Routes of EVT Migration

Interstitial EVT migration and invasion

Interstitial EVT cells are the part of the trophoblast, which detach from the distal parts of anchoring villi, and invade the decidual stroma extravascularly. This is made possible by the attachment of EVT to the stromal extracellular matrix (ECM), the destruction of the ECM and the translocation into cleared area.

Initial binding to the stroma is triggered by a change in adhesion molecule expression on EVT. Cytotrophoblasts that remain at the level of placental villi express α6β4 integrin, one of the main laminin-5 receptors. Trophoblasts of cell columns preferentially express α5β1, which binds fibronectin and helps stabilizing the columns. However, the migrating EVT down regulate the expression of these receptors in

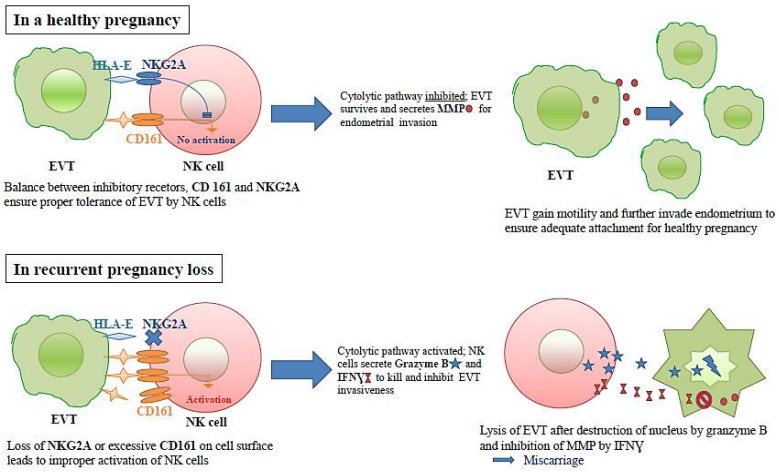

Figure 1: Immunotolerance of extravillous trophoblasts (EVT) alloantigens by natural killer (NK) cells in healthy pregnancy and recurrent pregnancy loss. The role of certain receptors (CD161, NKG2A) and mediators (matrix metalloproteinase (MMP) and granzyme B) is portrayed to reveal their interference in modulating the activation or inhibition of NK cells during human early pregnancy.

favor of α1β1 integrin which promotes invasiveness upon interaction with decidual collagens and laminins [37].

A distinction must be made between the two distinct processes of migration and invasion: migration involves the simple movement of cells from one place to another, while invasion implies the destruction of ECM to allow movement. Interstitial EVT releases proteases that enable the breakdown of the ECM, such as matrix metalloproteinase (MMPs) and urokinase plasminogen activator. In contrast, decidualized cells secrete tissue inhibitors of metalloproteinases (TIMPs) and plasminogen activator inhibitor (PAI) [37]. The gradient of inhibitors is highest at the border of invasion, and prevents excessive penetration of trophoblast.

Excess and deficient interstitial EVT have both been associated with pregnancy complications [7]. The highly invasive potential of EVT cells can damage the maternal reproductive system if it goes uncontrolled. uNK cells seem to be essential to create the balance necessary to pregnancy. The process of interstitial EVT migration is assisted by uNK cells through the secretion of promoting chemokines such as IL-8, IL-11 and IP-10, upon interaction with the trophoblast at the level of CXCR3 and CXCR1 receptors [14]. These same chemokines have been shown to limit invasion via an IFN-γ-dependent mechanism [7]. IFN-γ binds to its receptors on EVT and induces an adequate limitation of MMP-2 and MMP-9 levels. This slows down the destruction of the ECM and stromal invasion [33]. The number of NK cells in the uterine wall seems to be a delicate matter. Elevated levels of uNK cells early on in pregnancy are linked to both recurrent miscarriage and recurrent implantation failure [18].

Endovascular EVT migration and spiral artery remodeling

Arterial remodeling is necessary to create an intricate network which becomes vascularized, and allow the establishment of blood circulation by the third week of gestation. The infiltration of the uterine endometrium by endovascular EVT is preceded by trophoblast-independent remodeling (TIR) events, such as endothelial hyperplasia and smooth muscle vacuolization. Trophoblast-associated remodeling depends on the success of TIR. It is characterized by retrograde EVT migration within the lumen of the spiral arterioles [7]. First, EVT displaces maternal endothelial cells and cause apoptosis of vascular smooth muscles [37]. The endothelial EVT then replaces the maternal endothelium, and start expressing adhesion molecules specific to typical vasculature. These changes result in wide-bore channels which are free of vasomotor control. The enlargement of vessel diameter allows an increase in blood flow to the intervillous space.

This remodeling of the uterine vascular system is another key process of placental development that is crucial to a successful outcome of pregnancy [14].

Deficient remodeling is usually marked by a reduced number of invasive trophoblast or poor invasiveness. It is a major risk factor for a number of pregnancy complications, some of which are spontaneous abortion in the second trimester, pre-eclampsia, pre-term delivery and "small-for-date" birth (associated with fetal growth restriction) [38]. uNK cells enhance decidual vascularization through the release of vasculogenic and angiogenic factors, including vascular endothelial growth factor (VEGF), placental growth factor (PlGF), angiopoietin 1 and 2, and tumor growth factor β1 (TGF-β1) [14]. The synthesis and release of these factors is triggered by the binding of uNK activating receptors (such as NKp30 and NKp44) to their specific ligands on both stromal decidual cells and extravillous trophoblast cells [14], and the subsequent release of IFN-γ, which is particularly important in reinforcing uterine vascular remodeling [33]. uNK cells are involved in the destablilization of vascular smooth muscle cells and ECM, paving the way for EVT invasion.

Excessive angiogenesis- due to high uNK cell density or activity is also possible during implantation. Increased blood flow can lead to oxidative stress and terminate the pregnancy.

Tests

Tests to assess miscarriage risk vary, from a pelvic exam (to determine whether the cervix is dilating prematurely) to blood tests (to measure levels of human chorionic gonadotropin), and ultrasounds (to detect a fetal heartbeat).

In the context of NK cells, tests are still not totally agreed upon. It is usually accepted that a sample is needed to determine the concentration and level of activity of NK cells, but viewpoints start to diverge as to the nature of the withdrawn sample and levels of NK cells. For instance, evidence has shown a link between pNK activity and fertility [39,40]. However, it is still debated whether a blood sample prior to pregnancy is sufficient to estimate uNK cell activity, or if a uterine endometrial sample may give a more accurate account. In the latter case, both pNK and uNK cells will be observed due to blood contamination, and hence testing such specimens is misleading. Also, since the endometrium is shed every month during the normal menstrual cycle, along with all its resident NK cells, the number of these cells does not remain constant over time [41]. Due to the uncertain results of endometrial sampling, estimation of pNK cell levels is considered a better diagnostic tool before immunosuppressive therapies are initiated in women with recurrent spontaneous abortion or infertility [29].

None of the available tests is yet able to predict the right treatment for the patient [42].

Potential Treatment

Prednisolone

The use of corticosteroid prednisolone has proven to be effective in both reducing the number of uNK cells, and in suppressing their cytotoxicity [43]. Pre-conceptual prednisolone and aspirin administration was shown to improve pregnancy rates in women with an autoimmune component of their infertility. Prednisolone given post-conception at the time of blastocyst migration did not improve pregnancy rates. This suggests that prednisolone is only effective in altering preconceptual endometrial function [2]. In all cases, prednisolone is not effective in women who express antiphospholipid antibody [42].

Intravenous immunoglobulin therapy

Intravenous immunoglobulin (IVIG) therapy has been used as a reducer of NK cell cytotoxicity [44-46]. The immunosuppressive role of IVIG has been demonstrated in cases of secondary recurrent miscarriage, i.e. a miscarriage that follows a successful pregnancy. IVIG effectiveness could not be proven in primary recurrent miscarriage. Side-effects of IVIG therapy include a higher risk of undesirable immune responses and of transmitting infections such as cytomegalovirus (although the risk is extremely small) [42].

Conclusion

uNK cells affect pregnancy outcome in many different ways. Morphological characteristics, cell counts and interaction with trophoblasts are a few aspects of the many faceted problem. Of the

well documented anomalies, we can cite the deficiency in inhibitory receptors, and the excess of activating receptors and IFN-γ production. Complications related to or resulting from NK cell defects include vascular abnormalities and improper implantation [2]. Emerging evidence suggests that immune therapy ameliorates fertility and pregnancy outcome. However, immune therapy is still an inadvisable treatment and should only be offered in the context of clinical research [42].

Future Insights

Despite the cumulative evidence on the crucial role that uNK cells possess in healthy pregnancy, more research is needed to develop reliable tests to measure uNK levels in the uterus, their standard values, and different treatment modes. In addition, studies are necessary to validate whether hormonal dysregulation of the immune response, at any level, is responsible for one or more of the mechanisms responsible for miscarriage. Lastly, genetic analysis of non-classical HLA haplotypes in women with a history of unexplained pregnancy loss, compared with controls, could provide insight into mechanisms underlying recurrent miscarriage.

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