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Exploiting the significance of uterine natural killer cells in pregnancy

Tarique Hussain[a,*], Ghulam Murtaza[b], Dildar Hussain Kalhoro[c], Muhammad Saleem Kalhoro[d], Muhammad Ismail Chughtai[a], Anjaleena Yaseen[a]

[a] Animal Sciences Division, Nuclear Institute for Agriculture and Biology College, Pakistan Institute of Engineering and Applied Sciences (NIAB-C, PIEAS), Faisalabad 38000 Pakistan

[b] Department of Animal Reproduction, Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University, Tandojam, Sindh 70050 Pakistan

[c] Department of Veterinary Microbiology, Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University, Tandojam, Sindh 70050 Pakistan

[d] Food Engineering and Bioprocess Technology, Asian Institute of Technology, Bangkok 12120 Thailand

[*]Corresponding author, e-mail: drtariquerahoo@gmail.com

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ABSTRACT: Pregnancy is a complex physiological phenomenon involving maternal-fetal communication. Uterine natural killer (uNK) cells established a profound lymphocyte population in the early pregnant uterus. These cells are closely linked with extravillous trophoblast (EVT) and spiral arteries and play a vital role in remodeling spiral arteries, trophoblast invasion and placental development. The uNK cells are the potent releasers of cytokines and growth factors; with close association to EVTs in the maternal-fetal interface, it drives an essential function in the trophoblast. At present, limited studies are available explaining the significance of uNK cells in normal pregnancy and pregnancy-associated problems. This review article elucidates the descriptive role of uNK cells in pregnancy problems and their crosstalk with other immune cell components. Moreover, we will also discuss whether uNK cells become a threat to pregnancy with clinical evidence.

KEYWORDS: uterine natural killer cells, extravillous trophoblast, cytokines, leucocytes, pregnancy outcomes

UNK CELLS

Natural killer (NK) cells are part of the predominant innate lymphocyte with anti-tumor and anti-viral responses and therefore, utilized for therapeutic applications against health disorders [1]. NK cells are localized in various tissue distributions and appear in different phenotypic variations. These cells show multiple activating and inhibitory receptors, which identify the different responses of proteins on target cells and regulate their cytolytic activity [2]. Several articles have been published on the significance of NK cells in immunologic and other biological perspectives. In this review, the significance of uNK cells and their involvement in pregnancy is presented.

CD56 superbright uNK cells exist in the human endometrium before pregnancy initiates, then enlarge and become more granulated in progesterone dominant phase, followed by ovulation in the first trimester of pregnancy [3]. Murine uNK cells mature once the blastocyst implantation occurs, and they enable reactivity to *Dolichos biflorus* agglutinin (DBA) after gestation day (GD) 5 and enhanced granularity [4]. Moreover, murine uNK cells are less cytotoxic, even consisting of granules encasing perforin and granzymes [5]. There is rich lymphocyte aggregation around the implantation site and close to the decidua basalis consisting of mainly uNK cells, macrophages, and dendritic cells [6]. In humans, two distinct populations of uNK cells have been investigated in mice, they are different in reactivity with DBA. The expression of genes indicates that DBA+ uNK cells profoundly elicit transcripts for angiogenic factors while interferon gamma (IFN)-γ transcripts control in the DBA− subset [4]. The mesometrial lymphoid aggregate of pregnancy (MLAp) is unique to pregnancy in rodents which developed on GD 8. Mature uNK cells are prominent in decidua basalis and MLAp nearly half in entire pregnancy [6, 7]. uNK cells experience apoptosis starting from mid-pregnancy to onwards and are prominent on GD 12 [7, 8]. Expressing lectin-like Lys49 receptors, recognizing the major histocompatibility complex (MHC) class I, is more prominent in uNK than peripheral natural killer (pNK) cells. In contrast, few receptors are slightly titled in humans against the recognition of MHC ligands [9, 10] (Table 1). In mice, uNK cells are also expressed killer-cell lectin-like receptor G1 (KLRG1) more prominently in response to pNK cell counterparts, showing a high level of mature phenotype [9, 11].

UNK CELLS IN PREGNANCY

uNK cells consist of around 70% of maternal lymphocytes in pregnancy. These cells reside in decidua basalis and the MLAp surrounding the blood vessels that supply to the placenta. In early pregnancy, the cell number is high but reduces at parturition [12]. uNK cells are

Table 1 Classification of human NK cells in peripheral blood and decidua.

Characteristic	Peripheral blood NK cells		Uterine NK cells
	$CD56^{bright}$ $CD16^{-}$	$CD56^{dim}$ $CD16^{+}$	$CD56^{superbright}$ CD^{16}
% of total	5–30% circulating lymphocytes [61]		Over 70% leucocytes in early pregnancy [62]
% of total NK cells	10% [63]	90% [63]	80% [62]
CD94	$CD94^{bright}$ [64]	50% $CD94^{dim}$ [63]	$CD94^{bright}$ [63]
Natural killer group 2 (NKG2)A/C/E	+ [65]	+ [65]	++ [65]
NKG2D	+ [66]	+ [66]	++ [67]
Killer-cell	− [68]	+ [68]	++ [55]
Immunologobulin like receptors (KIR), NKP46	+ [69]	+ [69]	+ [69]
CD9	− [65]	− [65]	+ [65]
CD49a	− [70]	− [70]	+ [70]
CD57	− [55]	60% + [55]	− [55]

++, strong expression (bright); + weak expression (dim); ±, expression only on a subpopulation; −, no expression.

large, granular, rounded, or oval lymphocytes. These granular cells grow their size and number in the first two weeks of pregnancy and differ in different species. However, these behave as active cells in all species having obvious organelles comprising mitochondria, well-developed Golgi apparatus, free ribosomes, and rough endoplasmic reticulum [13]. uNK cells display a crucial role in human and mice pregnancy. Like other NK cells, uNK cells do not perform an essential part in the innate immune system; thus are not cytotoxic [14]. Across the pregnancy, uterus adaptation is required to allow fetal growth. Fetal immune system plays essential role to make pregnancy successful. Disruption in the mechanism may have deleterious effect on pregnancy and eventually causes pregnancy disorders [15]. Amino acids being with antioxidant properties regulate mammalian target of rapamycin (mTOR) signals which is responsible for protein synthesis in placenta, uterus and fetus and also maintain fetal growth and development [16]. Moreover, lipopolysaccharide (LPS) is used as a potential inducer of abortion during pregnancy. Due to flavonoids' strong antioxidant activities, it bears a potential to mitigate LPS related pregnancy problems [17].

The uNK cells once isolated from murine pregnant uterus, encompass large cytoplasmic droplets that do not have cytotoxic activity against their target cells [18]. This great function of NK cells in the gravid uterus left wonders because uNK cells since their discovery are closely associated with trophoblast cells lining the blood vessels. Previous findings assumed the hypothesis that uNK cells in murine pregnancy regulate placental vascular remodeling [12]. Transformation in spiral arterioles occurs during pregnancy, resulting in thin-walled vessels with large lumens [19]. It is believed that this happens due to the compensating nutritional requirements of the developing fetus. Research on mouse uNK cells helps to identify the significance of remodeling. In mice with conventional blood natural killer (cNK) cell deficiency, the anomalies in spiral artery remodeling during placentation were rectified when IFNγ was given systemically to animals with defective cNK cells [20]. The remodeling defects were fixed when bone marrow (BM) from NK-sufficient mice, but not from $IFN\gamma^{-/-}$ mice, was used in chimaera investigations. Furthermore, BM from IFNγ receptor-deficient mice was recovered, suggesting that NK cells can recover without the need for IFN. As a result, IFNγ produced by NK cells is involved in spiral arteriole remodeling through the use of endothelial cells and decidual stromal cells [21]. However, the specific signaling network responsible for cytokine production as well as uNK cell-dependent remodeling is largely unknown.

uNK cells are currently implicated in directly supporting fetal growth via growth-promoting substances for embryonic development prior to placental formation [22]. The tissue-resident natural killer (trNK) cells release growth factors such as pleiotrophin, osteoglycin, and osteopontin. Reduced trNK cells producing these growth factors in nuclear factor interleukin $3Nfil3^{-/-}$ and aged mice influenced offspring development and bone defects. Such fetal anomalies due to deficiency of growth factors were repaired when mothers offered reconstituted *in-vitro* expanded trNK cells that release a sufficient growth factors [21]. Thus, the research emphasized the novel function of trNK cells in early embryonic development.

CROSSTALK BETWEEN INVASIVE TROPHOBLAST AND UNK CELLS

It is reported that EVTs directly interact to uNK cells *in vivo* [23], suggesting the interactions strongly regulate activities of both molecules [13]. Moreover, EVTs have a specific function of classical human leukocyte antigen c (HLA-C) and non-classical human histocompatibility leukocyte antigen HLA-E, HLA-F, and HLA-G class-I ligands, which possess immune activity of acceptance of placenta/fetus [24, 25]. This particular composition of MHC supports EVT to regulate uNK cell processes via conserved NK receptors. Although, *in vivo* and *ex vivo* studies on NK receptor-EVT re-

vealed a challenge to collect human pregnancy samples or primary Cholera toxin subunit B cultures and then uNK cells extraction from pregnancy. Direct coculture of EVTs (using trophoblast-derived cell line HTR-8/SVneo cells) with uNK cells was reported to show a high level of survival of uNK cells and downregulation of stimulating natural killer group 2 member D (NKg2D) receptor [26]. These data must be taken into account that HTR-8/SVneo cells elicit a different response of HLA proteins which are not evident from EVTs [27].

The earlier mentioned class-I MHC molecules on extracellular trophoblasts communicates with several NK receptors. Then either it activates or suppresses signals showing a cytotoxic response of uNK cells or releasing cytokines [24]. uNK cell receptors are polymorphic killer Ig-like receptors (KIRs) with 2D or 3D immunoglobulin-like domains that include activation or inhibitory signals. Inhibiting KIR2DL1, KIR2DL2, and KIR2DL3 on uNKs, as well as receptors, suppresses signaling activity via a particular immunoreceptor tyrosine-based inhibition motif (ITIM) [28]. KIR2DS1 and KIR2DS4 [25] indicators are used to stimulate uNK KIRs, though their receptor KIR2DL4, which is primarily found in endosomes can also transmit active signals [29]. The KIRs attach with numerous cells within decidual tissue comprising EVTs [30]. Once particular haplotypic HLA-C expression occurs, the KIR genes differ between individuals, demonstrating the immunologic complexity of the uNKs-EVT response throughout pregnancies [31].

The HLA-E protein first occurs in EVTs in the 5th week of pregnancy and then disappears by the 7th week, showing its significant impact on implantation or trophoblastic development. HLA-E suppressing signals are controlled by the dimeric CD94/NKG2A receptor [32]. Published literature highlights that CD94/NKG2A shows prominent inhibitory signs that mostly overload activating inputs [28]. However, it is unclear that the role of EVT-derived HLA cells during pregnancy is to avoid uNK cell cytotoxicity. Trophoblast cells do not increase death in CD94 function-blocking studies [32], indicating that CD94/NKG2A may have additional activities at the maternal-fetal interface that have yet to be uncovered.

INTERACTION OF EVT VIA UNK CELLS

The uNK cells are potent releasers of cytokines and other factors; with close association to EVTs in the maternal-fetal interface and drive significant functions of the trophoblast. In this connection, uNK cells produced a media; that alters in particular biological mechanisms of primary trophoblast, enabling cell invasion [33]. More particular, soluble substances generated from uNK cells taken between 10 and 13 weeks of gestation age points are linked to pro-invasive effects of UNK cell-produced media. The soluble factors, on the other hand, are secreted by uNK cells during the first trimester of pregnancy. As a result, they had no pro-invasive properties in EVTs, implying that the uNK cell factors' composition affected the process related with gestational age and the effect of uNK cells on EVT biology is associated with developmental stage [34].

Several factors released by uNK cells have been identified, and interestingly, most of the receptor expressions are presented on primary events. For instance, uNK cells release significant amounts of interleukin (IL) 8, tumor necrosis factor (TNF), IFNγ, transforming growth factor-beta (TGFB1), C-X-C motif chemokine ligand (CXCL10), and angiogenic factors such as vascular endothelial growth factor A (VEGF-A), VEGF-C, and prostaglandin-F [34]. *Ex-vivo* investigations using primary EVTs and immunolocalization in implantation sites revealed that invasive trophoblasts near the maternal-fetal contact express ligand receptors. For instance, it exerts C-X-C motif chemokine receptors (CXCR1 and CXCL-10 (IL-8 and CXCR3) while the TNFR1, VEGFR-1, and VEGFR-3 bind VEGF-A and VEGF-C, respectively [34, 35]. Of note, the supplement approach with IL-8 and CXCL10 has increased migration of primary cytotrophoblasts [34]. Similarly, suppressing ligand binding to VEGFR-1 and VEGFR-3 reduced trophoblastic invasion [36]. Furthermore, uNK cell-conditioned medium with lower VEGF expression influenced overgrowth more than controls [23]. TNF and IFN-γ, on the other hand, hampered trophoblast migration and invasion by increasing plasminogen activator inhibitor production and altering matrix metalloproteinases induced proteolysis [37]. Altogether, the factors derived from uNK cells cannot regulate EVT-associated processes *in-vivo*. Elucidating the romance amid triggering or restricting invasive features in EVTs, uNK cells might be a key player in a cellular component in the decidua, which regulates the penetration of EVT invasion and expanding trophoblast triggered spiral artery remodeling.

MATERNAL-FETAL TOLERANCE AND UNK CELLS

Most of the research regarding uNK-trophoblast interactions has reported that the probability of involving uNK cells triggered via infection or inflammation could exert cytotoxic response against semi-allogeneic fetus and trophoblast. Therefore, undergoing disorder linked miscarriage and other pregnancy problems. Several research on primary syngeneic uNK cell – trophoblast co-cultures offered conclusive evidence that expressing trophoblasts (HLA-G- and non-HLA-G) are resistant to the direct lethal effects of uNK cells [23]. However, uNK cells avoid killing trophoblasts, even being used as stimulators for activation, though; the uNK cells preserve pro-cytotoxic products. Such effect behaves as an active cellular immune response to viral infection in maternal uterine stromal cells, showing that the trophoblast is immune fortunate

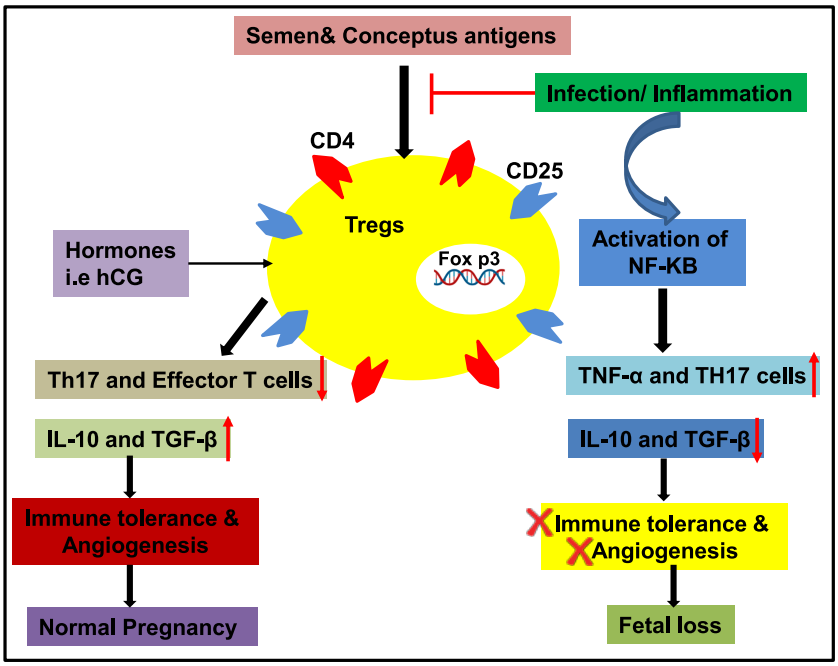

Fig. 1 Acquirement of inflammatory response via uterine regulatory T cells.

[23]. On the other hand, studies in mice indicate that uNK cells may acquire anti-trophoblast features. For instance, irregular stimulation of uNK cells against inflammation triggered by bacterial endotoxin [38] or allo-immunogenic responses [39], influences placenta function and eventually threat to fetal survival. Although it has been established that uNK cells are changed in pre-eclampsia and intra-uterine growth restriction (IUGR), there is evidence of contradictory results. Reduction in the level of uNK cells has been identified in IUGR using diverse methods [40].

Pre-eclampsia has been linked to higher levels of overall uNKs or the CD56+/CD16+ subset [41]. Presently, studies have shown that normal maternal health is linked with low-grade inflammation (obesity), which triggers activation of uNK cells and alters the interaction of uNK cells with fetal MHC ligand [42]. The uterine environment has been shown to alter uNKs processes, however aberrant regulation of uNKs in humans has been shown to affect trophoblasts, but more research is needed.

INTERACTIONS BETWEEN TREGS/DNK CELL AND MACROPHAGE/DNK CELL

The pregnancy bears immune tolerance due to fetal growth. Treg cells play a key role in endometrial receptivity and embryonic growth in humans and mice [43]. The reduced level of Treg cells has been reported in women with recurrent spontaneous abortion (RSA) and pre-eclampsia [44]. The findings have shown that mice with Treg deficiency have a problem in establishing spiral arteries [45].

The key regulators of the implantation process in mice are dendritic cells (DCs) [46]. DCs have been found to mediate Treg cells via indoleamine 2,3-dioxygenase (IDO), a dependent method for binding other immune responses [47]. However, additional research has revealed that IDO-deficient animals had normal gestations [48]. These data suggest that pregnancy is a mechanism of immunotolerance with no impressive evidences. However, the activation of these Treg cells is not investigated yet. In decidual tissues, dNK cells locate closely with particular myelomonocytic CD14(+), dCD14(+) cells. When dCD14(+) cells engage with uNK cells, they produce IDO, which can activate Tregs and is regulated by TGF-β. Surprisingly, co-culture of NK with CD14(+) cells isolated from peripheral blood is ineffective [49]. These findings revealed that new information on the mechanism of Treg cell growth. CXCL16 has been found in trophoblasts during early pregnancy. CXCL16 and CXCR6 major receptors are mostly found on selected leukocytes, which stimulate trophoblast proliferation and invasion in an autocrine way [50]. Fig. 1 shows the acquired inflammatory response via uterine regulatory T cells.

UNK CELLS IN PREGNANCY DISORDERS

Pregnancy is a sophisticated mechanism through which the mother and the fetus communication take place. In animals, over 20% of pregnancies resulted in embryonic loss [51]. Multiple factors such as genetics, epigenetics, hormonal balance, immunological tolerance, and angiogenesis all influence pregnancy. Fetal growth in pregnancy requires cellular expansion,

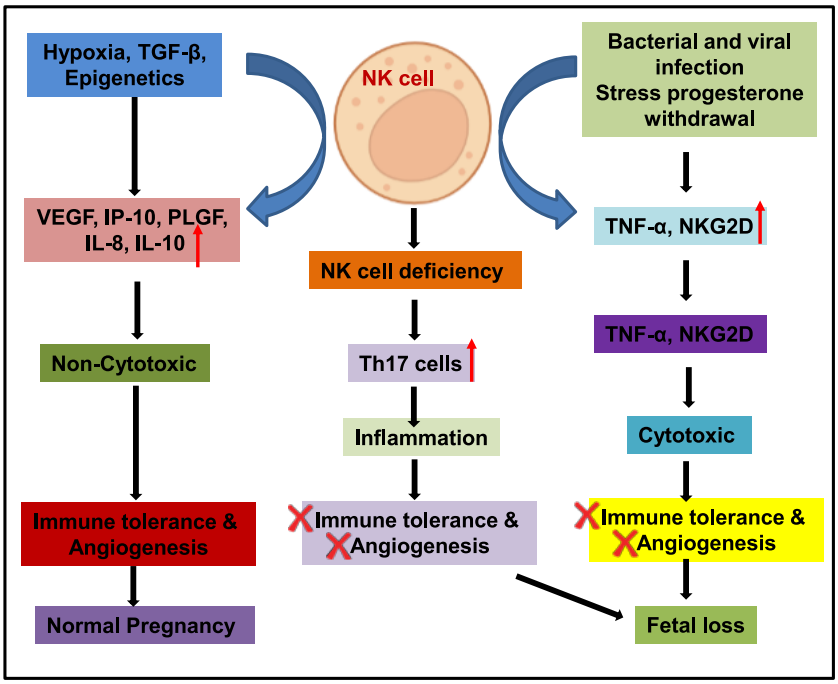

Fig. 2 The harmful effect of uNK cells during pregnancy.

differentiation, and the development of different organs. Unfortunately, the knowledge of fetal growth is minimal. For successful reproduction, fetal development must be pivotal. Irregularities in this mechanism may cause pregnancy problems such as infertility and spontaneous abortion. Embryo survival in uterus relies on several factors like cellular and molecular cascades in the uterine microenvironment [52], hormones, cytokines and growth factors in different cells that may influence pregnancy results. The proper cells and their particular mechanism directly involved in pregnancy outcomes are unknown. The lack of literature on cellular contributors that regulate pregnancy losses limits the experiments' effective design [53]. A deep understanding of the molecular and cellular mechanisms is required to devise therapeutic strategies against pregnancy-associated illness. At present, less information is available on the role of uNK cells in pregnancy. Fu et al [22] employed sophisticated technology to discover that NK cells stimulate embryonic growth by releasing growth-promoting substances. Surprisingly, transplanting uterine NK cells from healthy mice reversed the negative effects of NK cell deficiency on pregnancy in NK cell-deficient transgenic mice and elderly animals. This scenario depicts the uterine microenvironment's impact on NKs during pregnancy [53].

CLINICAL EVIDENCE

The studies have proved that NK cells and Tregs have been involved in RSA, repeated implantation failure and gestation problems [54]. Recurrent spontaneous abortion mainly influences 1–3% of all pregnancies. It has been enumerated that immune system may contribute in unexplained RSA. However, because uNK cells differ from peripheral blood natural killer cells (pBNK) in terms of phenotypic and functional characteristics, equivocal findings suggest that pBNKs may use a variety of uNK cells' patterns in women with infertility and RSA. Furthermore, the majority of studies have found increased cytotoxicity in NK cells in the peripheral circulation. The deleterious effect of uNK cells on pregnancy is depicted (Fig. 2).

Meanwhile, the percentage of NK cells in healthy individual blood varies; determining the proportion of these cells in RSA or infertility is difficult [55]. Furthermore, because uNK cells are less lethal than circulating cells, quantifying pBNKs, which may be unable to transmit information to patterns of uNK cells [55]. It has been shown that the decidua of women with history of RSA, increases numbers of granulysin-positive and $CD56^{bright}$ NK cells [56]. Intriguingly, uNK cells have been reported to enhance immunological tolerance and fruitful pregnancy via suppressing Th17 cells [57]. These explanations indicate that NK cells potentiate the immune system and are absent in RSA patients but gathered with an increased level of Th17 response and scattering local inflammation. As aforementioned, deficient decidua IL-10 in RSA patients has been associated with inflammatory response [58]. In normal pregnancy, when uNK cells and Tregs release a lot of IL-10, it inhibits Th17 activities [59]. It is

believed that disruption in uNK cells will result in local inflammation [60].

CONCLUDING REMARKS

For a successful pregnancy, a healthy and well-coordinated immune system is essential. Unfortunately, this mechanism may interfere with the immune system around maternal-fetal interface, resulting in gestation ailments. The uNK cells consist of more than seventy percentage maternal lymphocytes in pregnancy. They display function in human and mice pregnancy, observed higher in early pregnancy and declines around parturition. At present, uNK cells directly stimulate fetal growth before the placental establishment.

Moreover, the uNK cells are responsible for inducing cytokines, growth factors and their affiliation to EVTs around maternal-fetal interface; they perform several functions in the trophoblast. The uNK cells trigger numerous factors, expressed over the surface of primary EVTs. For example, uNK cells secrete a profound level of IL8, TNF, IFNFγ, TGFB1, CXCL10 and angiogenic factors. The interaction of uNK cells with trophoblast through infection or inflammation shows cytotoxic response; therefore, the condition is associated with miscarriage. A fruitful pregnancy requires maternal immune tolerance for the developing fetus. Treg cells play an essential role in embryo development and endometrial receptivity in humans and mice in this scenario. The declined level of Treg cells has been noticed in RSA and pre-eclampsia. Pregnancy is a choreographed process in which uNKs play a key role, interruption in uNk cells might be involved in pregnancy-related problems. The clinical studies suggest that uNK cells and Treg cells are involved in pregnancy disorders. A deep understanding of the molecular and cellular mechanisms of uNK cells and their interaction with other cellular components are required for alternative therapeutic options against adverse pregnancy outcomes.

REFERENCES

1. Abel AM, Yang C, Thakar MS, Malarkannan S (2018) Natural killer cells: development, maturation, and clinical utilization. *Front Immunol* **9**, 1869.
2. Paul S, Lal G (2017) The molecular mechanism of natural killer cells function and its importance in cancer immunotherapy. *Front Immunol* **8**, 1124.
3. King A, Wellings V, Gardner L, Loke Y (1989) Immunocytochemical characterization of the unusual large granular lymphocytes in human endometrium throughout the menstrual cycle. *Hum Immunol* **24**, 195–205.
4. Chen Z, Zhang J, Hatta K, Lima PD, Yadi H, Colucci F, Yamada AT, Croy BA (2012) DBA-lectin reactivity defines mouse uterine natural killer cell subsets with biased gene expression. *Biol Reprod* **87**, 81.
5. Parr E, Young L, Parr M, Young J (1990) Granulated metrial gland cells of pregnant mouse uterus are natural killer-like cells that contain perforin and serine esterases. *J Immunol* **145**, 2365–2372.
6. Croy BA, Esadeg S, Chantakru S, van den Heuvel M, Paffaro Jr VA, He H, Black GP, Ashkar AA, et al (2003) Update on pathways regulating the activation of uterine Natural Killer cells, their interactions with decidual spiral arteries and homing of their precursors to the uterus. *J Reprod Immunol* **59**, 175–191.
7. Paffaro Jr V, Bizinotto M, Joazeiro P, Yamada AT (2003) Subset classification of mouse uterine natural killer cells by DBA lectin reactivity. *Placenta* **24**, 479–488.
8. Kather A, Chantakru S, He H, Minhas K, Foster R, Markert UR, Pfeffer K, Croy AB (2003) Neither lymphotoxin α nor lymphotoxin β receptor expression is required for biogenesis of lymphoid aggregates or differentiation of natural killer cells in the pregnant mouse uterus. *Immunology* **108**, 338–345.
9. Kieckbusch J, Gaynor LM, Moffett A, Colucci F (2014) MHC-dependent inhibition of uterine NK cells impedes fetal growth and decidual vascular remodelling. *Nat Commun* **5**, 3359.
10. Madeja Z, Yadi H, Apps R, Boulenouar S, Roper SJ, Gardner L, Moffett A, Colucci F, et al (2011) Paternal MHC expression on mouse trophoblast affects uterine vascularization and fetal growth. *Proc Natl Acad Sci USA* **108**, 4012–4017.
11. Mallidi TV, Craig LE, Schloemann SR, Riley JK (2009) Murine endometrial and decidual NK1. 1+ natural killer cells display a B220+ CD11c+ cell surface phenotype. *Biol Reprod* **81**, 310–318.
12. Croy BA, He H, Esadeg S, Wei Q, McCartney D, Zhang J, Borzychowski A, Ashkar AA, et al (2003) Uterine natural killer cells: Insights to their cellular and molecular biology from mouse modelling. *Reprod (Camb, Eng)* **126**, 149.
13. Croy BA, Kiso Y (1993) Granulated metrial gland cells: a natural killer cell subset of the pregnant murine uterus. *Microsc Res Tech* **25**, 189–200.
14. Gaynor LM, Colucci F (2017) Uterine natural killer cells: functional distinctions and influence on pregnancy in humans and mice. *Front Immunol* **8**, 467.
15. Hussain T, Murtaza G, Kalhoro DH, Kalhoro MS, Yin Y, Chughtai MI, Tan B, Yaseen A, et al (2022) Understanding the immune system in fetal protection and maternal infections during pregnancy. *J Immunol Res* **2022**, 7567708.
16. Hussain T, Tan B, Murtaza G, Metwally E, Yang H, Kalhoro MS, Kalhoro DH, Chughtai MI, et al (2020) Role of dietary amino acids and nutrient sensing system in pregnancy associated disorders. *Front Pharmacol* **11**, 586979.
17. Hussain T, Tan B, Liu G, Murtaza G, Rahu N, Saleem M, Yin Y (2017) Modulatory mechanism of polyphenols and Nrf2 signaling pathway in LPS challenged pregnancy disorders. *Oxid Med Cell Longev* 2017, 8254289.
18. Croy BA, Kassouf SA (1989) Evaluation of the murine metrial gland for immunological function. *J Reprod Immunol* **15**, 51–69.
19. Cross JC, Hemberger M, Lu Y, Nozaki T, Whiteley K, Masutani M, Adamson SL (2002) Trophoblast functions, angiogenesis and remodeling of the maternal vasculature in the placenta. *Mol Cell Endocrinol* **187**, 207–212.
20. Boulenouar S, Doisne J-M, Sferruzzi-Perri A, Gaynor LM,

Kieckbusch J, Balmas E, Yung HW, Javadzadeh S, et al (2016) The residual innate lymphoid cells in NFIL3-deficient mice support suboptimal maternal adaptations to pregnancy. *Front Immunol* **7**, 43.
21. Sojka DK, Yang L, Yokoyama WM (2019) Uterine natural killer cells. *Front Immunol* **10**, 960.
22. Fu B, Zhou Y, Ni X, Tong X, Xu X, Dong Z, Sun R, Tian Z, et al (2017) Natural killer cells promote fetal development through the secretion of growth-promoting factors. *Immunity* **47**, 1100–1113.
23. Tilburgs T, Evans JH, Crespo ÂC, Strominger JL (2015) The HLA-G cycle provides for both NK tolerance and immunity at the maternal-fetal interface. *Proc Natl Acad Sci USA* **112**, 13312–13317.
24. Wallace AE, Fraser R, Cartwright JE (2012) Extravillous trophoblast and decidual natural killer cells: a remodelling partnership. *Hum Reprod Upd* **18**, 458–471.
25. Hackmon R, Pinnaduwage L, Zhang J, Lye SJ, Geraghty DE, Dunk CE (2017) Definitive class I human leukocyte antigen expression in gestational placentation: HLA-F, HLA-E, HLA-C, and HLA-G in extravillous trophoblast invasion on placentation, pregnancy, and parturition. *Am J Reprod Immunol* **77**, e12643.
26. Zhang J, Dunk CE, Kwan M, Jones RL, Harris LK, Keating S, Lye SJ (2017) Human dNK cell function is differentially regulated by extrinsic cellular engagement and intrinsic activating receptors in first and second trimester pregnancy. *Cell Mol Immunol* **14**, 203–213.
27. Apps R, Murphy SP, Fernando R, Gardner L, Ahad T, Moffett A (2009) Human leucocyte antigen (HLA) expression of primary trophoblast cells and placental cell lines, determined using single antigen beads to characterize allotype specificities of anti-HLA antibodies. *Immunology* **127**, 26–39.
28. El Costa H, Casemayou A, Aguerre-Girr M, Rabot M, Berrebi A, Parant O, Delannoy MC, Lombardelli L, et al (2008) Critical and differential roles of NKp46-and NKp30-activating receptors expressed by uterine NK cells in early pregnancy. *J Immunol* **181**, 3009–3017.
29. Tilburgs T, Crespo ÂC, Van Der Zwan A, Rybalov B, Raj T, Stranger B, Gardner L, Moffett A, et al (2015) Human HLA-G+ extravillous trophoblasts: Immune-activating cells that interact with decidual leukocytes. *Proc Natl Acad Sci USA* **112**, 7219–7224.
30. Sharkey AM, Gardner L, Hiby S, Farrell L, Apps R, Masters L, Goodridge J, Lathbury L, et al (2008) Killer Ig-like receptor expression in uterine NK cells is biased toward recognition of HLA-C and alters with gestational age. *J Immunol* **181**, 39–46.
31. Hiby SE, Apps R, Sharkey AM, Farrell LE, Gardner L, Mulder A, Claas FH, Walker JJ, et al (2010) Maternal activating KIRs protect against human reproductive failure mediated by fetal HLA-C2. *J Clin Invest* **120**, 4102–4110.
32. King A, Allan DS, Bowen M, Powis SJ, Joseph S, Verma S, Hiby SE, McMichael AJ, et al (2000) HLA-E is expressed on trophoblast and interacts with CD94/NKG2 receptors on decidual NK cells. *Eu J Immunol* **30**, 1623–1631.
33. Hanna J, Goldman-Wohl D, Hamani Y, Avraham I, Greenfield C, Natanson-Yaron S, Prus D, Cohen-Daniel L, et al (2006) Decidual NK cells regulate key developmental processes at the human fetal-maternal interface. *Nat Med* **12**, 1065–1074.
34. Pollheimer J, Vondra S, Baltayeva J, Beristain AG, Knöfler M (2018) Regulation of placental extravillous trophoblasts by the maternal uterine environment. *Front Immunol* **9**, 2597.
35. Lash GE, Schiessl B, Kirkley M, Innes BA, Cooper A, Searle RF, Robson SC, Bulmer JN (2006) Expression of angiogenic growth factors by uterine natural killer cells during early pregnancy. *J Leukoc Biol* **80**, 572–580.
36. Zhou Y, McMaster M, Woo K, Janatpour M, Perry J, Karpanen T, Alitalo K, Damsky C, et al (2002) Vascular endothelial growth factor ligands and receptors that regulate human cytotrophoblast survival are dysregulated in severe preeclampsia and hemolysis, elevated liver enzymes, and low platelets syndrome. *Am J Pathol* **160**, 1405–1423.
37. Bauer S, Pollheimer Jr, Hartmann J, Husslein P, Aplin JD, KnoÌĹfler M (2004) Tumor necrosis factor-α inhibits trophoblast migration through elevation of plasminogen activator inhibitor-1 in first-trimester villous explant cultures. *J Clin Endocrinol Metabol* **89**, 812–822.
38. Lee AJ, Kandiah N, Karimi K, Clark DA, Ashkar AA (2013) Interleukin-15 is required for maximal lipopolysaccharide-induced abortion. *J Leukock Biol* **93**, 905–912.
39. Yougbaré I, Tai W-S, Zdravic D, Oswald BE, Lang S, Zhu G, Leong-Poi H, Qu D, et al (2017) Activated NK cells cause placental dysfunction and miscarriages in fetal alloimmune thrombocytopenia. *Nat Comm* **8**, 224.
40. Eide IP, Rolfseng T, Isaksen CV, Mecsei R, Roald B, Lydersen S, Salvesen KÅ, Harsem NK, et al (2006) Serious foetal growth restriction is associated with reduced proportions of natural killer cells in decidua basalis. *Virchows Arch* **448**, 269–276.
41. Wilczyński JR, Tchórzewski H, Banasik M, Głowacka E, Wieczorek A, Lewkowicz P, Malinowski A, Szpakowski M, et al (2003) Lymphocyte subset distribution and cytokine secretion in third trimester decidua in normal pregnancy and preeclampsia. *Eu J Obst Gynecol Reprod Biol* **109**, 8–15.
42. Castellana B, Perdu S, Kim Y, Chan K, Atif J, Marziali M, Beristain AG (2018) Maternal obesity alters uterine NK activity through a functional KIR2DL1/S1 imbalance. *Immunol Cell Biol* **96**, 805–819.
43. Care AS, Bourque SL, Morton JS, Hjartarson EP, Robertson SA, Davidge ST (2018) Reduction in regulatory T cells in early pregnancy causes uterine artery dysfunction in mice. *Hypertension* **72**, 177–187.
44. Munoz-Suano A, Hamilton AB, Betz AG (2011) Gimme shelter: the immune system during pregnancy. *Immunol Rev* **241**, 20–38.
45. Samstein RM, Josefowicz SZ, Arvey A, Treuting PM, Rudensky AY (2012) Extrathymic generation of regulatory T cells in placental mammals mitigates maternal-fetal conflict. *Cell* **150**, 29–38.
46. Plaks V, Birnberg T, Berkutzki T, Sela S, BenYashar A, Kalchenko V, Mor G, Keshet E, et al (2008) Uterine DCs are crucial for decidua formation during embryo implantation in mice. *J Clinical Invest* **118**, 3954–3965.
47. Chung DJ, Rossi M, Romano E, Ghith J, Yuan J, Munn DH, Young JW (2009) Indoleamine 2, 3-dioxygenase-expressing mature human monocyte-derived dendritic cells expand potent autologous regulatory T cells. *Blood* **114**, 555–563.
48. Baban B, Chandler P, McCool D, Marshall B, Munn DH,

Mellor AL (2004) Indoleamine 2, 3-dioxygenase expression is restricted to fetal trophoblast giant cells during murine gestation and is maternal genome specific. *J Reprod Immunol* **61**, 67–77.

49. Vacca P, Cantoni C, Vitale M, Prato C, Canegallo F, Fenoglio D, Ragni N, Moretta L, et al (2010) Crosstalk between decidual NK and CD14+ myelomonocytic cells results in induction of Tregs and immunosuppression. *Proc Natl Acad Sci USA* **107**, 11918–11923.
50. Huang Y, Zhu X-Y, Du M-R, Wu X, Wang M-Y, Li D-J (2006) Chemokine CXCL16, a scavenger receptor, induces proliferation and invasion of first-trimester human trophoblast cells in an autocrine manner. *Hum Reprod* **21**, 1083–1091.
51. Bazer FW, Wu G, Spencer TE, Johnson GA, Burghardt RC, Bayless K (2009) Novel pathways for implantation and establishment and maintenance of pregnancy in mammals. *Mol Hum Reprod* **16**, 135–152.
52. Birbrair A (2017) Stem cell microenvironments and beyond. In: Birbrair A (ed) *Stem Cell Microenvironments and Beyond*, Advances in Experimental Medicine and Biology, vol 1041, Springer, Cham, pp 1–3.
53. Andreotti JP, Paiva AE, Prazeres PH, Guerra DA, Silva WN, Vaz RS, Mintz A, Birbrair A (2018) The role of natural killer cells in the uterine microenvironment during pregnancy. *Cell Mol Immunol* **15**, 941.
54. Quenby S, Nik H, Innes B, Lash G, Turner M, Drury J, Bulmer J (2009) Uterine natural killer cells and angiogenesis in recurrent reproductive failure. *Hum Reprod* **24**, 45–54.
55. Moffett A, Regan L, Braude P (2004) Natural killer cells, miscarriage, and infertility. *BMJ* **329**, 1283–1285.
56. Nakashima A, Shiozaki A, Myojo S, Ito M, Tatematsu M, Sakai M, Takamori Y, Ogawa K, et al (2008) Granulysin produced by uterine natural killer cells induces apoptosis of extravillous trophoblasts in spontaneous abortion. *Am J Pathol* **173**, 653–664.
57. Fu B, Li X, Sun R, Tong X, Ling B, Tian Z, Wei H, Ekerfelt C, et al (2013) Natural killer cells promote immune tolerance by regulating inflammatory TH17 cells at the human maternal-fetal interface. *Proc Natl Acad Sci USA* **110**, E231–E240.
58. Plevyak M, Hanna N, Mayer S, Murphy S, Pinar H, Fast L, Ekerfelt C, Ernerudh J, et al (2002) Deficiency of decidual IL-10 in first trimester missed abortion: a lack of correlation with the decidual immune cell profile. *Am J Pathol* **47**, 242–250.
59. Stewart CA, Metheny H, Iida N, Smith L, Hanson M, Steinhagen F, Leighty RM, Roers A, et al (2013) Interferon-dependent IL-10 production by Tregs limits tumor Th17 inflammation. *J Clin Invest* **123**, 4859–4874.
60. Faas MM, De Vos P (2018) Innate immune cells in the placental bed in healthy pregnancy and preeclampsia. *Placenta* **69**, 125–133.
61. Raulet DH, Vance RE, McMahon CW (2001) Regulation of the natural killer cell receptor repertoire. *Annu Rev Immunol* **19**, 291–330.
62. Chiossone L, Vacca P, Orecchia P, Croxatto D, Damonte P, Astigiano S, Barbieri O, Bottino C, et al (2014) *In vivo* generation of decidual natural killer cells from resident hematopoietic progenitors. *Haematologica* **99**, 448.
63. Kim S, Poursine-Laurent J, Truscott SM, Lybarger L, Song Y-J, Yang L (2005) Licensing of natural killer cells by host major histocompatibility complex class I molecules. *Nature* **436**, 709–713.
64. Yawata M, Yawata N, Draghi M, Partheniou F, Little A-M, Parham P (2008) MHC class I-specific inhibitory receptors and their ligands structure diverse human NK-cell repertoires toward a balance of missing self-response. *Blood* **112**, 2369–2380.
65. Sternberg-Simon M, Brodin P, Pickman Y, Önfelt B, Kärre K, Malmberg K-J, Höglund P, Mehr R (2013) Natural killer cell inhibitory receptor expression in humans and mice: a closer look. *Front Immunol* **4**, 65.
66. Johansson S, Johansson M, Rosmaraki E, Vahlne G, Mehr R, Salmon-Divon M, Lemonnier F, Karre K, et al (2005) Natural killer cell education in mice with single or multiple major histocompatibility complex class I molecules. *J Exp Med* **201**, 1145–1155.
67. Brodin P, Lakshmikanth T, Johansson S, Kärre K, Höglund P (2009) The strength of inhibitory input during education quantitatively tunes the functional responsiveness of individual natural killer cells. *Blood* **113**, 2434–2441.
68. Fernandez NC, Treiner E, Vance RE, Jamieson AM, Lemieux S, Raulet DH (2005) A subset of natural killer cells achieves self-tolerance without expressing inhibitory receptors specific for self-MHC molecules. *Blood* **105**, 4416–4423.
69. Fauriat C, Ivarsson MA, Ljunggren H-G, Malmberg K-J, Michaëlsson J (2010) Education of human natural killer cells by activating killer cell immunoglobulin-like receptors. *Blood* **115**, 1166–1174.
70. Ruggeri L, Capanni M, Urbani E, Perruccio K, Shlomchik WD, Tosti A, Posati S, Rogaia D, et al (2002) Effectiveness of donor natural killer cell alloreactivity in mismatched hematopoietic transplants. *Science* **295**, 2097–3000.