SCIENCE CHINA
Life Sciences

Special Topic: Organ/Tissue-specific immunity
• REVIEW •

December 2016 Vol.59 No.12:1224–1231
doi: 10.1007/s11427-016-0337-1

# Decidual natural killer cells and the immune microenvironment at the maternal-fetal interface

Binqing Fu[1,2*] & Haiming Wei[1,2]

[1]*Institute of Immunology and the CAS Key Laboratory of Innate Immunity and Chronic Disease, School of Life Science and Medical Center, University of Science and Technology of China, Hefei 230027, China;*
[2]*Hefei National Laboratory for Physical Sciences at Microscale, University of Science and Technology of China, Hefei 230027, China*

Received November 1, 2016; accepted November 10, 2016; published online November 29, 2016

During early pregnancy, an orchestrated evolutionary maternal adaption toward tolerance of the semiallogeneic fetus is required to ensure decidualization and early embryo development. Remodeling of the immune system involves natural killer cells (NKs), macrophages, T cells and dendritic cells (DCs) altering the microenvironment in the deciduas. In particular, a unique population of NK cells with a $CD56^{bright}CD16^{-}$ phenotype in the decidua has been proposed to play a key role in the maternal adaptation to pregnancy. However, there is a tendency for pregnancy immunology to reflect transplantation immunology regarding the assumption that the maternal immune system should be suppressed. This tendency is misleading. We discuss how the immune system is formed in early deciduas and the interactions between maternal NK cells and fetal growth. We propose that the maternal immune response must not be fully suppressed and is even necessary for the local response of uterine NK cells.

**NK cells, decidua, fetal growth, the immune microenvironment**

**Citation:** Fu, B., and Wei, H. (2016). Decidual natural killer cells and the immune microenvironment at the maternal-fetal interface. Sci China Life Sci 59, 1224–1231. doi: 10.1007/s11427-016-0337-1

## INTRODUCTION

During human pregnancy, extravillous trophoblast cells (EVT) from the fetus invade the deciduas and remodel the maternal spiral arteries to ensure adequate nutrition to the developing fetus. This remodeling of the maternal spiral arteries is associated with an evolutionary immunological scenario of maternal adaption toward the semiallogeneic fetus (Arck and Hecher, 2013; Erlebacher, 2013a; Fu et al., 2014). Over the past decades of pregnancy research, the examination of the interactions between the maternal immune system and fetal trophoblast cells has led to the realization that multiple mechanisms may be involved in promoting immune cell homeostasis during pregnancy.

It has been reported that the TH2-type cytokine profile (Piccinni et al., 1998; Saito et al., 1999; Mjosberg et al., 2010), transforming growth factor (TGF)-β1 (Ayatollahi et al., 2005), altered micro-RNA expression (Bidarimath et al., 2014), variation within extracellular vesicle populations (Tannetta et al., 2014) and the inhibition of complement activation (Xu et al., 2000) are all critical for a successful pregnancy. $CD25^{+}CD4^{+}$ regulatory T cells both in mice and humans have been shown to be very important in the generation of fetomaternal tolerance. Furthermore, studies have shown that galectin-1 (Blois et al., 2007) and indoleamine 2,3-dioxygenase (Munn et al., 1998) play pivotal roles in maternal-fetal tolerance. Wnt, PD-L1, signal transducers, activators of transcription (STAT) 3 and stimulatory (CD80

*Correspondence author (email: fbq@ustc.edu.cn)

and CD86) signals also participate in the regulation of pregnancy (Guleria et al., 2005; Poehlmann et al., 2005; Zhu et al., 2005; Zhang et al., 2009). It has become increasingly acknowledged that NK cells are pivotal in maternal adaption to pregnancy as they are the largest population of immune cells during the first trimester in human deciduas and show a close reaction to invasive fetal trophoblasts and stromal cells (Colucci and Kieckbusch, 2015; Tao et al., 2015). EVT cells from the fetus express the following unique array of MHC class I molecules: HLA-C, HLA-E, and HLA-G but not HLA-A or HLA-B (Apps et al., 2009). Decidual NK cells express receptors for these HLA class I molecules from the trophoblast and, thus, are able to mediate allorecognition of the fetus. With excessive inhibition of decidual NK cells, the risk of growth restriction and pre-eclampsia is increased (Sharkey et al., 2015). The present review summarizes the concepts and rationale for the remodeled immune system during pregnancy. We further review the formation and education of decidual NK cells and interactions between maternal NK cells and fetal growth.

## THE FORMATION PROCESS OF THE MATERNAL-FETAL IMMUNE INTERFACE AND BASIC CHARACTERISTICS

### Formation of the maternal-fetal immune interface

The invasion of trophoblastic cells is the starting factor of maternal-fetal interface formation. The trophoblastic cells derived from the embryo blastocyst layer position in the embryo's most outer layer and the layer of the uterus. During the process of human embryonic development, trophoblasts differentiate into two types, namely, villous trophoblasts (VTs) and extravillous trophoblast cells (EVTs) (Arck and Hecher, 2013; Parham and Moffett, 2013). VTs form chorionic villi, which are over the surface of the villi and have the ability to transport nutrients and oxygen to the fetus; EVTs stretch into decidual tissues, invade blood vessel linings, and reconstruct the spiral artery. Trophoblast cells have many features that are easily ignored by immune scholars. The invasion of trophoblastic cells in the embryo and maternal interface depends on the expression of maternal and paternal genes, including MHC and MHC-like molecules, allowing mutual recognition towards decidual NK cells, T lymphocytes and DCs.

Decidualization is one of the main features of the forming maternal-fetal interface. The characteristic of decidualization is mainly for the proliferation and differentiation of endometrial stromal cells (ESCs) into large round cells with abundant cytoplasm with nuclear and phenotypic changes of decidual stromal cells. Decidua originating in the spiral artery, with the growth of the embryo and the invasion of the trophoblastic cells, spread step-by-step to the whole endometrium (Maruyama and Yoshimura, 2008; Ramathal et al., 2010). When the embryo's implantation is completed, the endometrium is called the decidua. In humans and mice, the progress of decidualization is consistent with that of trophoblastic cell invasion. Moreover, trophoblastic cell invasion further plays a regulatory role in blastocyst implantation, placenta formation and the maintenance of normal pregnancy. Endometrial decidualization is one of the essential conditions for successful embryo growth. Insufficiency in decidualization may cause infertility, repetitive abortion (recurrent or repetitive spontaneous abortion, RSA), fetal developmental delay (intrauterine growth retardation, IUGR) and other diseases.

### The modified immune environment at the maternal-fetal immune interface

Decidual immune cells are the key factors in maintaining the maternal-fetal interface. During the period of endometrial decidualization, maternal peripheral immune cells accumulate locally, proliferate and further become decidual immune cells. In early pregnancy, decidual immune cells can reach 30%–40% of the total number of cells in the uterus, including NK cells, DCs, macrophages and T cells. B cells can hardly be measured. Specifically, decidual NK cells, as the main type of immune cells in deciduas, increase significantly and constitute approximately 70% of the local total number of immune cells.

The maternal-fetal interface is a special immune microenvironment that is mainly composed of fetal trophoblast cells, maternal decidual stromal cells, decidual glandular epithelial cells and immune cells. This maternal-fetal interface supplies enough nutrition for the embryo, and it also provides immune protection for the embryo, which is the key to protecting the embryo from maternal immune attack. In the process of pregnancy, there are two unique immune interfaces (Sargent et al., 2006); the first interface is composed of the immune cells in the decidua (dNK, T, Mo, and DCs), invasive trophoblastic cells from fetal villi, a large number of decidual stromal cells and the spiral artery. The second interface is composed of immune cells from the maternal circulation system (including DC, T, Mo, NK) and placental chorion surface syncytial trophoblast cells. The formation of the first interface and the second interface occurs in continuous phases. The first interface mainly appears in the early weeks of pregnancy and degenerates in the third trimester with the invasion of trophoblastic cells and the associated decidual lymphocytes. The second interface starts to appear at eight to nine weeks of gestation with the formation of uteroplacental circulation, and it gradually becomes the main maternal-fetal interface with the growth of the placenta until the end of pregnancy. The immune microenvironment formed by the maternal-fetal immune interface, and the mechanisms for avoiding maternal-fetal conflict and maintaining maternal-fetal immune tolerance, have special evolutionary wisdom by nature.

## IMMUNE CELL SUBSETS AND THEIR DYNAMIC CHANGES IN THE MATERNAL-FETAL INTERFACE

During early embryonic development, blastocyst adhesion and endometrial implantation is accompanied by endometrial cells forming decidual stromal cells (DSCs) as promoted by estrogen and progesterone (Matson and Caron, 2014). In the microenvironment created by DSCs, blastocysts differentiated into functionally unique villous trophoblast cells (EVTs). These EVTs in early pregnancy period not only express the unique HLA, but they also co-express chemokine receptors (CXCRs) and the corresponding chemokines (CXCLs) together with DSCs. This complex chemokine-chemokine receptor expression system, not only attracts EVT invasion of the deciduas, but it also attracts many immune cells into the deciduas. The number of immune cells can reach as high as 30%–40% of the total number of decidual cells at 9–12 weeks of pregnancy (Du et al., 2014).

### NK cells

NK cells are the most important decidual immune cells, accounting for 50%–70% of the total number of immune cells after 9–12 weeks of pregnancy. The decidual NK cell and peripheral blood NK cell (pbNK) show distinct phenotypes (Fu et al., 2011). Fully 90%–95% of NK cells in peripheral blood are $CD56^{dim}CD16^{+}$ NK cells (Li et al., 2015), whereas most decidual NK cells are $CD56^{bright}CD16^{-}$ NK cells. Further analysis found that $CD56^{bright}CD16^{-}$ NK cells express CXCR4, and they can be selectively recruited into the decidua by interaction with chemokine CXCL12 secreted by DSCs and EVTs. CXCL9 and CXCL10 secreted by DSCs can further interact with CXCR3 expressed on $CD56^{bright}CD16^{-}$ NK cells and allow decidual NK cells to stay local (Hanna et al., 2003; Wu et al., 2005). There are still debates about the source of decidual NK cells. On the one side, IL-15 and stem cell factor (SCF) secreted from DSCs prompt $Lin^{-}CD45^{+}CD34^{+}$ decidual hematopoietic stem cells (dHSCs) to develop into dNK cells (Vacca et al., 2011); on the other side, DSCs and ETVs may also produce TGFβ and IL-15 to transform $CD56^{dim}CD16^{+}$ NK cells into dNKs in the deciduas environment (Keskin et al., 2007), thereby maintaining the high content of decidual NK cells.

### Macrophages

Macrophages are the second largest category of immune cells in decidual tissue, accounting for approximately 20% of all the immune cells. During the period of embryo implantation, the human decidual stromal cells, decidual NK cells and glandular epithelial cells possess the ability to produce CSF1, which, on the one hand, promotes the proliferation and differentiation of macrophages; on the other hand, it induces macrophages to express the monocyte chemokine factors CCL2 (MCP-1), CCL7 (MCP-3) and CCL12 (MCP-5). These chemokines continue to attract circulating monocytes into the decidua, where they differentiate into macrophages and expand the amount of macrophages (Repnik et al., 2008; Houser et al., 2011).

Decidual macrophages in early pregnancy can be divided into two groups. One is called $CD209^{+}$ macrophages, referring to macrophages expressing molecular CD209 c-type lectin (DC-SIGN), which account for 70% of decidual macrophages. These cells have a high expression of CD163, CD206 (MRC-1, mannose receptor), CD304 (NRP-1, neuropilin 1) and ICAM-3 but a low expression of CD11c and may act as identifiers of pathogenic microorganisms and immune response effectors. $CD209^{+}$ macrophages are responsive towards infection in the decidua and chorionic inflammation. The CD209 molecule is also one of the typical signs of immature DCs derived from mononuclear cells. Whether CD209 molecules have other functions such as induction of immune tolerance is yet to be confirmed. $CD209^{-}$ macrophages have the phenotypes of $CD163^{-}CD206^{-}CD304^{-}ICAM3^{-}CD11c^{hi}$, expressing higher levels of IL-10 at the basic level or from stimulation with LPS than $CD209^{+}$ macrophages. This feature results in $CD209^{-}$ macrophages that tend to be the M2 type. Specifically, both $CD209^{+}$ macrophages and $CD209^{-}$ macrophages can be induced into M1 or M2 type *in vitro*. With the initiation of parturition or preterm delivery, a large number of decidual macrophages again gather locally in the deciduas, accompanied by decreased secretion of IL-10 but increased secretion of inflammatory cytokines, further suggesting that inflammation may play a role in starting the delivery (Hamilton et al., 2012; Gomez-Lopez et al., 2014). All of these phenomena indicate that decidual macrophage cells are a type of special group that performs a special function, being involved in organizational recasting, renovation of apoptotic cells, inducing a locally tolerant microenvironment during early pregnancy and starting the delivery in late pregnancy (Erlebacher, 2013a). Decidual macrophages in mice are divided into the $F4/80^{+}MHCII^{hi}$ and $F4/80^{+}MHCII^{lo}$ subgroups, expressing CD163 and Mrc1, respectively, and they are equivalent to the human decidual $CD209^{+}$ and $CD209^{-}$ macrophage subgroups (Tagliani et al., 2011).

### DCs

There are few DCs in the decidua. Approximately 1–5 $mm^{-2}$ mature $CD83^{+}$mDCs distribute in the decidua during early pregnancy. $CD205^{+}$DCs are even fewer, only 2 $mm^{-2}$, which is obviously fewer than the number of macrophages in the decidua in early pregnancy, approximately 50–100 $mm^{-2}$. $CD83^{+}$mDCs also exist in the endometrium, at approximately 3 $mm^{-2}$ in the physiological breeding period and reaching 9 $mm^{-2}$ in secretory phase, which suggests that the decidualization after embryo implantation limits the increase

in the number of DCs (Kemp et al., 2011). The same phenomenon also occurs in mice. Deciduas in mice are mainly Ly6C$^-$F4/80$^-$MHCII$^+$CD11c$^+$DCs (Collins et al., 2009).

A reduced number of DCs suggests that DC cells have difficulty capturing enough antigens in the specific decidual microenvironment and presenting these antigens to T cells in the lymph nodes through the lymph vessels. Moreover, decidual DCs also lack the capacity of migrating to lymph nodes. The density of lymphatic vessel in decidua is low, only scattered around the spiral arteries, suggesting that the small amount of DCs in decidua can contact the villous trophoblastic cells but do not have a homing ability to the lymph nodes in the womb. This limited contact may retain the immune response ability when uterine mucosa are infected by pathogens and initiate the Th2 state in the uterine microenvironment (Leno-Durán et al., 2014). Additionally, decidual CD209$^+$ macrophages can highly express CD83 and stimulate T cell proliferation after treatment with IL–1β, IL-6, TNFα and PEG2, potentially acting as DC precursor cells when necessary.

### T cells

CD3$^+$ T cells account for 10%–20% of decidual immune cells. Among them, 30%–45% of T cells are CD4$^+$ T cells, approximately 50% of which have the activation/memory CD25$^{dim}$ phenotype. Of T cells, 45%–75% are CD8$^+$, 40% of which show the effect/memory CD28$^-$ phenotype. Among these CD4$^+$ T cells, CD25$^{bright}$ Foxp3$^+$ regulatory T cells, TH2 cells and TH17 cells account for 5%, 5% and 2%, respectively. TH1 cells account for 5%–30% of CD4$^+$ T cells. γδT cells, CD4$^-$CD8$^-$TCRαβ T cells, and NKT cells are rare in deciduas (Tilburgs et al., 2010).

The physiological functions of T cells in decidual tissues are almost unknown. Mice with T cell defects are not affected in their reproductive capacity. There is no nourishing function similar to that of dNKs or macrophages. Excessive TH17 cell presence in spontaneous abortion cases is even considered to have a negative impact on pregnancy (Fu et al., 2014). However, the role of Treg in embryonic tolerance is still unclear (Erlebacher, 2013b). Decidual Treg cells were identified as CD4$^+$CD25$^+$FoxP3$^+$Cigs1$^+$Nrp1$^{low}$, distinguishing from Nrp1$^+$Treg cells in the thymus (Arck and Hecher, 2013). Currently, it is difficult to identify whether there are embryo/placenta antigen-specific T cells performing TCR-mediated effects or regulatory functions. The effect of T cells appearing in pregnancy related diseases is unlikely generated locally, but a product of the immune response of the whole body. This type of T cell effect lacks embryonic/placenta specificity, and it can also be the result of excessive activation of cytokines.

From the foregoing, decidual immune cells form a unique microenvironment, have a dynamic development process in pregnancy, peak in the recasting spiral artery phase, and even reach 30%–40% of all local cells in early pregnancy decidual tissues. Decidual immune cells are characterized by natural immune cells in the majority (NK cells and macrophages in absolute dominance) and maintain an immune tolerance as a result. For immune scientists, however, there are still many difficult questions as follows: how can a new immune microenvironment be formed in endometrial tissue and decidual tissue, in which the lymphatic vessels are lacking in both humans and mice and which cannot participate in lymphocyte recirculation? Why do very unique immune cells appear in decidual tissue, especially a large number of CD56$^{bright}$CD16$^-$ NK cells and CD209$^+$ macrophages? Why do T cells, B cells and DCs, which are important in the immune system of the whole body, decrease significantly in decidual tissue? How is the immune tolerance of the maternal-fetal interface is maintained?

## SPECIAL NK SUBSETS IN THE MATERNAL-FETAL INTERFACE

### The phenotype of decidual NK cells

pbNK cells have a CD56$^{dim}$CD16$^+$ phenotype and are endowed with ADCC function (Ni et al., 2013; Li et al., 2015; Wang et al., 2015). pbNK cells also express CD11a and CD11b, forming LFA-1 and MAC-1, respectively, with CD18, which all have ICAM-1 as their corresponding ligand. ICAM-1 combined with LFA-1 is the key molecule promoting pbNK cell adhesion with target cells and particle polarization.

In contrast with pbNK cells, decidual NK cells show the CD56$^{bright}$CD16$^-$ phenotype and express CD49a highly but not CD11b (pbNK cells are CD49a$^-$) (Montaldo et al., 2016). The CD49a ligands are collagen and laminin, which are expressed very highly in decidual tissue and may be one of the reasons for the decidual residence of NK cells. Our previous laboratory work found that CD49a$^+$NK cells accounted for half of all hepatic NK cells (Peng et al., 2013), potentially mediating immune tolerance in the liver. Interestingly, the liver is considered an immune tolerance organ. Transplantation of the liver does not require HLA matching. This liver tolerance condition is similar to that of the maternal-fetal interface, which suggests that decidual NK cells are a special immune cell type with the potential to mediate immune tolerance.

### The education of decidual NK cells

The formation of NK cell function requires them to accept the education of their MHC molecules. NK cells express several inhibitory and activating molecules. Before their education, receptors of NK cells intertwine, limiting the function of NK cells. After education from MHC molecules, the molecular activation is limited within a specific domain, whereas inhibitory receptors are limited by a grid structure mediated

by actin; therefore, the NK cells are able to identify the corresponding cells dependent on the presence of MHC ligands and accept the activation signal (Guia et al., 2011).

Decidual NK cells also need education. Maternal education from MHC molecules should follow the rules above. The core problem is how maternal NK cells are educated by semi-allogeneic embryonic cells in the formation of tolerance to the semi-allogeneic fetus. Whether NK cells can be tolerant with self-antigens and kill non-self cells is dependent on the combination ability of their inhibitory receptors with HLA class I molecules. The key subject in education is the MHC molecules. Extravillous trophoblast cells at the first interface do not express HLA-A or HLA-B antigens that will induce rejection responses from T cells, but they do express very unique HLA-C, HLA-E and HLA-G (Sharkey et al., 2015). Therefore, HLA molecules expressed on trophoblastic cells become the key molecules in the education of decidual NK cells. Among these HLA molecules, HLA-C is only expressed in trophoblastic cells. The corresponding receptors of HLA-C are killer cell immunoglobulin receptor molecules (KIRs), which are also important receptors on the surface of NK cells. Thus, the recognition and education of KIRs from HLA-C is particularly important. KIRs combine with different types of MHC class I molecules, such as KIR2DL1 combined with HLA-C2, KIR2DL3 combined with HLA-C1, and KIR3DL1 combined with HLA-Bw4. HLA-E can combine with another type of inhibitory receptor on NK cells, NKG2A, which limits the killing activity of NK cells. HLA-G has a function similar to that of HLA-E. Further study found that HLA-G can also induce tolerance of DCs and induce NK cells to secrete cytokines and angiogenesis factors, thus showing polyfunctionality (LeMaoult et al., 2004; van der Meer et al., 2004; Li et al., 2009; Rajagopalan, 2014). At the surface of the second interface, the syncytial trophoblast cells do not express MHC molecules; therefore, they cannot induce an antigen specific response from maternal T cells (Moffett-King, 2002). An education function of syncytial trophoblasts towards NK cells has not been confirmed.

NKG2A and KIRs mediate tolerance in two ways. One is by inhibiting the function of NK cells after combining with HLA molecules on the surface of normal cells; the other is by regulating NK cell function after accepting education from HLA molecules on the surface of normal cells. NK cells that do not express inhibitory receptors will lose the education effect of MHC I molecules, showing low reactivity towards MHC-I$^-$ target cells (Ivarsson et al., 2013).

### The function of decidual NK cells

Decidual NK cells are necessary for promoting embryonic development and maintaining the tolerance of decidual NK cells towards embryonic cells. Decidual NK cells constitute as much as 70% of lymphocytes in early pregnancy and are supposed to have special physiological functions. Anne Croy's group first found that in tgε26 mice (deficient in NK cells), many abnormalities appear in uterine glands, decidua and blood vessels, and embryo abortion will occur with up to 64% probability in the middle of pregnancy. In this model, adoptive transfer of bone marrow from SCID mice (T and B cell defects) can reverse this pregnancy disorder phenomenon, thereby proving that decidual NK cells play an important role in the promotion of deciduas development and blood vessel formation (Guimond et al., 1998). Afterward, researchers found that decidual NK cells regulate the invasion of trophoblastic cells and blood vessel recasting by the secretion of angiogenesis regulation molecules, cytokines and chemokines (Hanna et al., 2006; Lima et al., 2014). A series of soluble molecules, including GM-CSF, can be produced after coculturing KIR2DS1$^+$dNK cells with HLA-C2$^+$ target cells *in vitro*, which can significantly enhance the capacity of trophoblast cell invasion (Xiong et al., 2013). Decidual NK cells can also inhibit inflammatory T helper type 17 cells (Fu et al., 2013), promote regulatory T cells and the generation of indoleamine 2,3-dioxygenase (IDO)-producing monocytes, induce the apoptosis of effector T cells (Vacca et al., 2010) and participate in the formation of early mouse decidua basalis (Felker and Croy, 2016). These findings provide important evidence that decidual NK cells act as sentinel cells to control local inflammation and tolerance during pregnancy.

The frequency of KIR expression is significantly increased in decidual NK cells in early pregnancy compared with peripheral blood NK cells (pbNK), and these KIRs combine easily with HLA-C molecules (Male et al., 2011). Analysis of the different responses of NK cells after KIRs form different individual combinations with HLA-C is very important to understanding the role of NK cells in the maternal-fetal interface. Maternal dNK cells, for example KIR AA haplotype, when combined with HLA-C1 from maternal self and HLA-C2 from paternal genetic nourishing cells results in the inhibition of NK cells, increasing the risk of preeclampsia (Parham and Moffett, 2013) because the mother is carrying KIR2DL1 inhibitory receptors in such cases but lacks activated receptor KIR2DS1. When KIR2DL1 from the mother combines with HLA-C2 from the embryo, dNK cells are suppressed, cannot release cytokines or have direct contact with EVTs, and do not provide enough support for EVT invasion of vascular vessels. The combination between KIR A haplotype gene KIR2DL1 and HLA-C2 mediates strong inhibitory signals that can cause defects in placenta formation. Thus, the combination ability of KIR/HLA-C will influence the capability of dNK cells to secrete chemokines and cytokines and will further have an impact on the important ability to regulate the invasion of trophoblastic cells (Hanna et al., 2006; Hiby et al., 2010). KIR2DL2 and KIR2DS2, also belonging to KIR B haplotype, can reduce the expression frequency of KIR A haplotype KIR2DL1 and increase the activation ability of NK cells because KIR2DS2 itself is an activated receptor

and can be antagonized by KIR2DL1. Although KIR2DL2 and KIR2DL1 are both inhibitory receptors and can combine with HLA-C2, the binding force and inhibition effect of KIR2DL2 is weaker. KIR2DL2 expression has priority over KIR2DL1 during the growth phase of NK cells. Reducing the expression level of KIR2DL1 can also improve the ability of NK cell activation and recasting of blood vessels, reducing the risk of pre-eclampsia. However, mothers with KIR haploid type 2DS1, if they carry an HLA-C2 embryo at the same time, can experience overweight fetus and obstructed labor (Hiby et al., 2010; Hiby et al., 2014).

Previous evidence suggests that the normal physiological function of dNK cells guarantees a normal pregnancy, whereas decreased function of dNK cells leads to pregnancy failure. It is still unknown how dNK cells disorder naturally, but it is essential for maintaining the appropriate activation state of NK cells in each normal pregnancy. Therefore, inhibition of NK cells in clinical treatment function may lead to greater risk (Beaman et al., 2014). Some clinical testing found that the number and activity of peripheral blood NK cells have "relevance" to adverse pregnancy; therefore, patients have been given glucocorticoid, intravenous immunoglobulin and anti-TNF-α antibody for treatment (Moffett and Shreeve, 2015). This way of treatment apparently lacks enough support. The phenotype, activity, and even appearance are highly distinct between peripheral blood pbNKs and decidual NK cells. Using test results from pbNKs to characterize the dNK state will mislead our judgment. The present data show that dNK cells need moderate activation rather than suppression. The rush of using drugs targeting dNKs before clearly distinguishing the function of dNK cells is unsuitable and should be stopped (Moffett and Colucci, 2014).

## CONCLUSION

Decidual NK cells and their interaction with semi-allogeneic trophoblast cells may not only have an important effect on fetal growth by negotiating the suitable allocation of nutrients from the mother, but they also have an impact on the health of offspring later in adult life (Colucci and Kieckbusch, 2015). One example is that those individuals who are at a lower weight when they are born have fewer glomeruli (Hughson et al., 2003) and may be predisposed to renal disease during adulthood (Hoy et al., 1998; Hoy et al., 1999; Yudkin et al., 2001). Such damage and shortages in body development during the sensitive time point during pregnancy are irreversible. The discovery that NK cells mediate the regulation of fetal growth and immune tolerance suggests that we should handle clinical drug administration in a delicate matter if these drugs could affect or suppress the activation or function of NK cells.

**Compliance and ethics** *The author(s) declare that they have no conflict of interest.*

**Acknowledgements** *This work was supported by the Natural Science Foundation of China (91442202, 81330071, 81471527).*

Apps, R., Murphy, S.P., Fernando, R., Gardner, L., Ahad, T., and 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.

Arck, P.C., and Hecher, K. (2013). Fetomaternal immune cross-talk and its consequences for maternal and offspring's health. Nat Med 99, 548–556.

Ayatollahi, M., Geramizadeh, B., and Samsami, A. (2005). Transforming growth factor beta-1 influence on fetal allografts during pregnancy. Transplant Proc 37, 4603–4604.

Beaman, K.D., Jaiswal, M.K., Dambaeva, S., and Gilman-Sachs, A. (2014). Future directions of clinical laboratory evaluation of pregnancy. Cell Mol Immunol 11, 582–588.

Bidarimath, M., Khalaj, K., Wessels, J.M., and Tayade, C. (2014). MicroRNAs, immune cells and pregnancy. Cell Mol Immunol 11, 538–547.

Blois, S.M., Ilarregui, J.M., Tometten, M., Garcia, M., Orsal, A.S., Cordo-Russo, R., Toscano, M.A., Bianco, G.A., Kobelt, P., Handjiski, B., Tirado, I., Markert, U.R., Klapp, B.F., Poirier, F., Szekeres-Bartho, J., Rabinovich, G.A., and Arck, P.C. (2007). A pivotal role for galectin-1 in fetomaternal tolerance. Nat Med 13, 1450–1457.

Collins, M.K., Tay, C.S., and Erlebacher, A. (2009). Dendritic cell entrapment within the pregnant uterus inhibits immune surveillance of the maternal/fetal interface in mice. J Clin Invest 119, 2062–2073.

Colucci, F., and Kieckbusch, J. (2015). Maternal uterine natural killer cells nurture fetal growth: in medio stat virtus. Trends Mol Med 21, 60–67.

Du, M.R., Wang, S.C., and Li, D.J. (2014). The integrative roles of chemokines at the maternal-fetal interface in early pregnancy. Cell Mol Immunol 11, 438–448.

Erlebacher, A. (2013a). Immunology of the maternal-fetal interface. Annu Rev Immunol 31, 387–411.

Erlebacher, A. (2013b). Mechanisms of T cell tolerance towards the allogeneic fetus. Nat Rev Immunol 13, 23–33.

Felker, A.M., and Croy, B.A. (2016). Uterine natural killer cell partnerships in early mouse decidua basalis. J Leukocyte Biol 100, 645–655.

Fu, B., Wang, F., Sun, R., Ling, B., Tian, Z., and Wei, H. (2011). CD11b and CD27 reflect distinct population and functional specialization in human natural killer cells. Immunology 133, 350–359.

Fu, B., Li, X., Sun, R., Tong, X., Ling, B., Tian, Z., and Wei, H. (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.

Fu, B., Tian, Z., and Wei, H. (2014). Subsets of human natural killer cells and their regulatory effects. Immunology 141, 483–489.

Fu, B., Tian, Z., and Wei, H. (2014). TH17 cells in human recurrent pregnancy loss and pre-eclampsia. Cell Mol Immunol 11, 564–570.

Gomez-Lopez, N., StLouis, D., Lehr, M.A., Sanchez-Rodriguez, E.N., and Arenas-Hernandez, M. (2014). Immune cells in term and preterm labor. Cell Mol Immunol 11, 571–581.

Guia, S., Jaeger, B.N., Piatek, S., Mailfert, S., Trombik, T., Fenis, A., Chevrier, N., Walzer, T., Kerdiles, Y.M., Marguet, D., Vivier, E., and Ugolini, S. (2011). Confinement of activating receptors at the plasma membrane controls natural killer cell tolerance. Sci Signal 4, ra21–ra21.

Guimond, M.J., Wang, B., and Croy, B.A. (1998). Engraftment of bone marrow from severe combined immunodeficient (SCID) mice reverses the reproductive deficits in natural killer cell-deficient tg epsilon 26 mice. J Exp Med 187, 217–223.

Guleria, I., Khosroshahi, A., Ansari, M.J., Habicht, A., Azuma, M., Yagita,

H., Noelle, R.J., Coyle, A., Mellor, A.L., Khoury, S.J., and Sayegh, M.H. (2005). A critical role for the programmed death ligand 1 in fetomaternal tolerance. J Exp Med 202, 231–237.
Hamilton, S., Oomomian, Y., Stephen, G., Shynlova, O., Tower, C.L., Garrod, A., Lye, S.J., and Jones, R.L. (2012). Macrophages infiltrate the human and rat decidua during term and preterm labor: evidence that decidual inflammation precedes labor. Biol Reprod 86, 39–39.
Hanna, J., Goldman-Wohl, D., Hamani, Y., Avraham, I., Greenfield, C., Natanson-Yaron, S., Prus, D., Cohen-Daniel, L., Arnon, T.I., Manaster, I., Gazit, R., Yutkin, V., Benharroch, D., Porgador, A., Keshet, E., Yagel, S., and Mandelboim, O. (2006). Decidual NK cells regulate key developmental processes at the human fetal-maternal interface. Nat Med 12, 1065–1074.
Hanna, J., Wald, O., Goldman-Wohl, D., Prus, D., Markel, G., Gazit, R., Katz, G., Haimov-Kochman, R., Fujii, N., Yagel, S., Peled, A., and Mandelboim, O. (2003). CXCL12 expression by invasive trophoblasts induces the specific migration of CD16$^-$ human natural killer cells. Blood 102, 1569–1577.
Hiby, S.E., Apps, R., Chazara, O., Farrell, L.E., Magnus, P., Trogstad, L., Gjessing, H.K., Carrington, M., and Moffett, A. (2014). Maternal KIR in combination with paternal HLA-C2 regulate human birth weight. J Immunol 192, 5069–5073.
Hiby, S.E., Apps, R., Sharkey, A.M., Farrell, L.E., Gardner, L., Mulder, A., Claas, F.H., Walker, J.J., Redman, C.W., Redman, C.C., Morgan, L., Tower, C., Regan, L., Moore, G.E., Carrington, M., and Moffett, A. (2010). Maternal activating KIRs protect against human reproductive failure mediated by fetal HLA-C2. J Clin Invest 120, 4102–4110.
Houser, B.L., Tilburgs, T., Hill, J., Nicotra, M.L., and Strominger, J.L. (2011). Two unique human decidual macrophage populations. J Immunol 186, 2633–2642.
Hoy, W., Rees, M., Kile, E., Mathews, J., McCredie, D., Pugsley, D., and Wang, Z. (1998). Low birthweight and renal disease in Australian aborigines. Lancet 352, 1826–1827.
Hoy, W.E., Rees, M., Kile, E., Mathews, J.D., and Wang, Z. (1999). A new dimension to the Barker hypothesis: low birthweight and susceptibility to renal disease. Kidney Int 56, 1072–1077.
Hughson, M., Farris Iii, A.B., Douglas-Denton, R., Hoy, W.E., and Bertram, J.F. (2003). Glomerular number and size in autopsy kidneys: the relationship to birth weight. Kidney Int 63, 2113–2122.
Ivarsson, M.A., Loh, L., Marquardt, N., Kekäläinen, E., Berglin, L., Björkström, N.K., Westgren, M., Nixon, D.F., and Michaëlsson, J. (2013). Differentiation and functional regulation of human fetal NK cells. J Clin Invest 123, 3889–3901.
Kemp, B., Schmitz, S., Krusche, C.A., Rath, W., and von Rango, U. (2011). Dendritic cells are equally distributed in intrauterine and tubal ectopic pregnancies. Fertil Steril 95, 28–32.
Keskin, D.B., Allan, D.S.J., Rybalov, B., Andzelm, M.M., Stern, J.N.H., Kopcow, H.D., Koopman, L.A., and Strominger, J.L. (2007). TGFbeta promotes conversion of CD16$^+$ peripheral blood NK cells into CD16$^-$ NK cells with similarities to decidual NK cells. Proc Natl Acad Sci USA 104, 3378–3383.
LeMaoult, J., Krawice-Radanne, I., Dausset, J., and Carosella, E.D. (2004). HLA-G1-expressing antigen-presenting cells induce immunosuppressive CD4$^+$ T cells. Proc Natl Acad Sci USA 101, 7064–7069.
Leno-Durán, E., Muñoz-Fernández, R., García Olivares, E., and Tirado-González, I. (2014). Liaison between natural killer cells and dendritic cells in human gestation. Cell Mol Immunol 11, 449–455.
Li, C., Houser, B.L., Nicotra, M.L., and Strominger, J.L. (2009). HLA-G homodimer-induced cytokine secretion through HLA-G receptors on human decidual macrophages and natural killer cells. Proc Natl Acad Sci USA 106, 5767–5772.
Li, Y., Yin, J., Li, T., Huang, S., Yan, H., Leavenworth, J.M., and Wang, X. (2015). NK cell-based cancer immunotherapy: from basic biology to clinical application. Sci China Life Sci 58, 1233–1245.
Lima, P.D., Zhang, J., Dunk, C., Lye, S.J., and Anne Croy, B. (2014). Leukocyte driven-decidual angiogenesis in early pregnancy. Cell Mol Immunol 11, 522–537.
Male, V., Sharkey, A., Masters, L., Kennedy, P.R., Farrell, L.E., and Moffett, A. (2011). The effect of pregnancy on the uterine NK cell KIR repertoire. Eur J Immunol 41, 3017–3027.
Maruyama, T., and Yoshimura, Y. (2008). Molecular and cellular mechanisms for differentiation and regeneration of the uterine endometrium. Endocr J 55, 795–810.
Matson, B.C., and Caron, K.M. (2014). Adrenomedullin and endocrine control of immune cells during pregnancy. Cell Mol Immunol 11, 456–459.
Mjosberg, J., Berg, G., Jenmalm, M.C., and Ernerudh, J. (2010). FOXP3$^+$ regulatory T cells and T helper 1, T helper 2, and T helper 17 cells in human early pregnancy decidua. Biol Reprod 82, 698–705.
Moffett-King, A. (2002). Natural killer cells and pregnancy. Nat Rev Immunol 2, 656–663.
Moffett, A., and Colucci, F. (2014). Uterine NK cells: active regulators at the maternal-fetal interface. J Clin Invest 124, 1872–1879.
Moffett, A., and Shreeve, N. (2015). First do no harm: uterine natural killer (NK) cells in assisted reproduction. Human Reprod 30, 1519–1525.
Montaldo, E., Vacca, P., Chiossone, L., Croxatto, D., Loiacono, F., Martini, S., Ferrero, S., Walzer, T., Moretta, L., and Mingari, M.C. (2016). Unique eomes$^+$ NK cell subsets are present in uterus and decidua during early pregnancy. Front Immunol 6, 646.
Munn, D.H., Zhou, M., Attwood, J.T., Bondarev, I., Conway, S.J., Marshall, B., Brown, C., and Mellor, A.L. (1998). Prevention of allogeneic fetal rejection by tryptophan catabolism. Science 281, 1191–1193.
Ni, F., Sun, R., Fu, B., Wang, F., Guo, C., Tian, Z., and Wei, H. (2013). IGF-1 promotes the development and cytotoxic activity of human NK cells. Nat Commun 4, 1479.
Parham, P., and Moffett, A. (2013). Variable NK cell receptors and their MHC class I ligands in immunity, reproduction and human evolution. Nat Rev Immunol 13, 133–144.
Peng, H., Jiang, X., Chen, Y., Sojka, D.K., Wei, H., Gao, X., Sun, R., Yokoyama, W.M., and Tian, Z. (2013). Liver-resident NK cells confer adaptive immunity in skin-contact inflammation. J Clin Invest 123, 1444–1456.
Piccinni, M.P., Beloni, L., Livi, C., Maggi, E., Scarselli, G., and Romagnani, S. (1998). Defective production of both leukemia inhibitory factor and type 2 T-helper cytokines by decidual T cells in unexplained recurrent abortions. Nat Med 4, 1020–1024.
Poehlmann, T.G., Busch, S., Mussil, B., Winzer, H., Weinert, J., Mebes, I., Schaumann, A., Fitzgerald, J.S., and Markert, U.R. (2005). The possible role of the Jak/STAT pathway in lymphocytes at the fetomaternal interface. Chem Immunol Allergy 89, 26–35.
Rajagopalan, S. (2014). HLA-G-mediated NK cell senescence promotes vascular remodeling: implications for reproduction. Cell Mol Immunol 11, 460–466.
Ramathal, C.Y., Bagchi, I.C., Taylor, R.N., and Bagchi, M.K. (2010). Endometrial decidualization: of mice and men. Semin Reprod Med 28, 017–026.
Repnik, U., Tilburgs, T., Roelen, D.L., van der Mast, B.J., Kanhai, H.H.H., Scherjon, S., and Claas, F.H.J. (2008). Comparison of macrophage phenotype between decidua basalis and decidua parietalis by flow cytometry. Placenta 29, 405–412.
Saito, , Sakai, , Sasaki, , Tanebe, , Tsuda, , and Michimata, (1999). Quantitative analysis of peripheral blood Th0, Th1, Th2 and the Th1:Th2 cell ratio during normal human pregnancy and preeclampsia. Clin Exp Immunol 117, 550–555.
Sargent, I.L., Borzychowski, A.M., and Redman, C.W.G. (2006). NK cells and human pregnancy—an inflammatory view. Trends Immunol 27, 399–404.
Sharkey, A.M., Xiong, S., Kennedy, P.R., Gardner, L., Farrell, L.E., Chazara, O., Ivarsson, M.A., Hiby, S.E., Colucci, F., and Moffett, A. (2015). Tissue-specific education of decidual NK cells. J Immunol 195, 3026–3032.
Tagliani, E., Shi, C., Nancy, P., Tay, C.S., Pamer, E.G., and Erlebacher, A. (2011). Coordinate regulation of tissue macrophage and dendritic cell population dynamics by CSF-1. J Exp Med 208, 1901–1916.

Tannetta, D., Dragovic, R., Alyahyaei, Z., and Southcombe, J. (2014). Extracellular vesicles and reproduction-promotion of successful pregnancy. Cell Mol Immunol 11, 548–563.

Tao, Y., Li, Y.H., Piao, H.L., Zhou, W.J., Zhang, D., Fu, Q., Wang, S.C., Li, D.J., and Du, M.R. (2015). CD56brightCD25$^+$ NK cells are preferentially recruited to the maternal/fetal interface in early human pregnancy. Cell Mol Immunol 12, 77–86.

Tilburgs, T., Claas, F.H.J., and Scherjon, S.A. (2010). Elsevier trophoblast research award lecture: unique properties of decidual T cells and their role in immune regulation during human pregnancy. Placenta 31, S82–S86.

Vacca, P., Cantoni, C., Vitale, M., Prato, C., Canegallo, F., Fenoglio, D., Ragni, N., Moretta, L., and Mingari, M.C. (2010). Crosstalk between decidual NK and CD14$^+$ myelomonocytic cells results in induction of Tregs and immunosuppression. Proc Natl Acad Sci USA 107, 11918–11923.

Vacca, P., Vitale, C., Montaldo, E., Conte, R., Cantoni, C., Fulcheri, E., Darretta, V., Moretta, L., and Mingari, M.C. (2011). CD34$^+$ hematopoietic precursors are present in human decidua and differentiate into natural killer cells upon interaction with stromal cells. Proc Natl Acad Sci USA 108, 2402–2407.

van der Meer, A., Lukassen, H.G.M., van Lierop, M.J.C., Wijnands, F., Mosselman, S., Braat, D.D.M., and Joosten, I. (2004). Membrane-bound HLA-G activates proliferation and interferon-gamma production by uterine natural killer cells. Mol Human Reprod 10, 189–195.

Wang, Y.J., Chen, C., Dong, F., Ma, S.H., Xu, J., Gong, Y.M., Cheng, H., Zhou, Y., Cheng, T., and Hao, S. (2015). NK cells play a significant role in immunosurveillance at the early stage of MLL-AF9 acute myeloid leukemia via CD226/CD155 interactions. Sci China Life Sci 58, 1288–1298.

Wu, X., Jin, L.P., Yuan, M.M., Zhu, Y., Wang, M.Y., and Li, D.J. (2005). Human first-trimester trophoblast cells recruit CD56brightCD16$^-$ NK cells into decidua by way of expressing and secreting of CXCL12/stromal cell-derived factor 1. J Immunol 175, 61–68.

Xiong, S., Sharkey, A.M., Kennedy, P.R., Gardner, L., Farrell, L.E., Chazara, O., Bauer, J., Hiby, S.E., Colucci, F., and Moffett, A. (2013). Maternal uterine NK cell-activating receptor KIR2DS1 enhances placentation. J Clin Invest 123, 4264–4272.

Xu, C., Mao, D., Holers, V.M., Palanca, B., Cheng, A.M., and Molina, H. (2000). A critical role for murine complement regulator crry in fetomaternal tolerance. Science 287, 498–501.

Yudkin, J.S., Martyn, C.N., Phillips, D.I.W., and Gale, C.R. (2001). Associations of micro-albuminuria with intra-uterine growth retardation. Nephron 89, 309–314.

Zhang, J., Xiao, X., Liu, W., Demirci, G., and Li, X.C. (2009). Inhibitory receptors of the immune system: functions and therapeutic implications. Cell Mol Immunol 6, 407–414.

Zhu, X., Wang, M., Jin, L., Yuan, M.M., and Li, D.J. (2005). Blockade of CD86 signaling facilitates a Th2 bias at the maternal-fetal interface and expands peripheral CD4$^+$CD25$^+$ regulatory T cells to rescue abortion-prone fetuses. Biol Reprod 72, 338–345.