ANNUAL REVIEWS

Annual Review of Immunology

Natural Killer Cells in the Human Uterine Mucosa

Victoria Male[1] and Ashley Moffett[2]

[1]Department of Metabolism, Digestion, and Reproduction, Imperial College London, London, United Kingdom; email: v.male@imperial.ac.uk

[2]Department of Pathology, University of Cambridge, Cambridge, United Kingdom; email: am485@cam.ac.uk

Annu. Rev. Immunol. 2023. 41:127–51

First published as a Review in Advance on January 11, 2023

The *Annual Review of Immunology* is online at immunol.annualreviews.org

https://doi.org/10.1146/annurev-immunol-102119-075119

Keywords

reproduction, trophoblast, uterine natural killer cells, decidua

Abstract

The presence of granulated lymphocytes in the human uterine mucosa, known as decidua during pregnancy, or endometrium otherwise, was first noted in the nineteenth century, but it was not until 1990 that these cells were identified as a type of natural killer (NK) cell. From the outset, uterine NK (uNK) cells were found to be less cytotoxic than their circulating counterparts, peripheral NK (pNK) cells. Recently, unbiased approaches have defined three subpopulations of uNK cells, all of which cluster separately from pNK cells. Here, we review the history of research into uNK cells, including their ability to interact with placental extravillous trophoblast cells and their potential role in regulating placental implantation. We go on to review more recent advances that focus on uNK cell development and heterogeneity and their potential to defend against infection and to mediate memory effects. Finally, we consider how a better understanding of these cells could be leveraged in the future to improve outcomes of pregnancy for mothers and babies.

THE UTERINE MUCOSA DURING THE MENSTRUAL CYCLE AND PREGNANCY

The mucosal lining of the uterus, known as endometrium in the nonpregnant state, is transformed into decidua if pregnancy occurs. Like other mucosal surfaces, the endometrium must possess immune defense mechanisms against pathogens. Immune responses can occur in the endometrium, for example T cell–mediated formation of granulomas in systemic tuberculosis (1) and endometritis, which is the only situation in which plasma cells are found at this mucosal site (2).

There are, however, many unique aspects of the physiology of this mucosal lining. It is composed of a stratum basalis, or basal layer, where lymphoid aggregates composed of T cells and a few B cells are located, and a stratum functionalis, or functional layer, that is constantly changing throughout the menstrual cycle, during pregnancy, and after menopause. At menstruation, the stratum functionalis is shed, and during the first 14 days of the menstrual cycle, stromal cells, glands, and vascular cells proliferate to regenerate the functionalis: This is therefore called the proliferative phase of the cycle. At the end of the proliferative phase, high levels of estrogen trigger the release of luteinizing hormone from the pituitary gland, and this stimulates ovulation. After ovulation, the ovarian follicle from which the egg was released forms the corpus luteum, which secretes progesterone. Under the influence of progesterone, proliferation of the glands and stromal cells ceases and they differentiate. The stromal cells enlarge and the glands produce glycogen-rich secretions: This is therefore called the secretory phase of the cycle (3). If pregnancy does not occur, the corpus luteum involutes, and the loss of progesterone causes the stratum functionalis to be shed (**Figure 1**). Outside reproductive life, before menarche and after menopause, when hormones secreted from the ovarian follicle and subsequently the corpus luteum are lacking, the mucosa is inert (4).

If fertilization occurs, human chorionic gonadotropin produced by the embryo maintains the corpus luteum, and the progesterone it produces allows the stromal and glandular epithelial cells to be maintained, so that the endometrium is transformed into decidua. The embryo moves down the fallopian tube to the uterine cavity, and the blastocyst implants into the decidua approximately 7 days after ovulation. In the following week, the blastocyst, which is surrounded by trophectoderm, is quickly embedded in the mucosa. The attachment to the epithelium leads to syncytial formation of the trophectoderm, so the conceptus is then surrounded by this primary syncytium with an inner layer of cytotrophoblast (5). In the following weeks the inner cytotrophoblast pushes in columns through the syncytial layer to form a shell around the conceptus. These columns (the primary villi) are subsequently filled with extraembryonic mesenchymal cells and then vessels, to form the villous tree of the placenta. The villi are covered in an inner layer of mononuclear villous cytotrophoblast cells that fuse to form an outer layer of syncytiotrophoblast. From the villi in contact with the decidua (anchoring villi), invasive extravillous trophoblast (EVT) cells move into the decidual stroma and migrate toward the spiral arteries. This interstitial EVT encircles the spiral arteries and destroys the media, resulting in an inability to vasoconstrict. When the cytotrophoblast shell overlies the opening of the arteries, the trophoblast moves down to form a plug. These endovascular plugs prevent blood from reaching the intervillous space early in pregnancy, until the arteries have been modified to reduce the flow at 8–10 weeks (5) (**Figure 2**). Before the onset of the full hemochorial connection, the conceptus receives nourishment from the secretions of the endometrial glands—histotrophic nutrition (6, 7).

The degree of trophoblast invasion correlates with the extent of decidualization in different species, pointing to the importance of interactions between maternal decidual cells and fetal trophoblast in regulating placentation (8). When implantation occurs in the absence of decidua, either in an ectopic location such as the fallopian tube or in the uterus over a previous caesarean

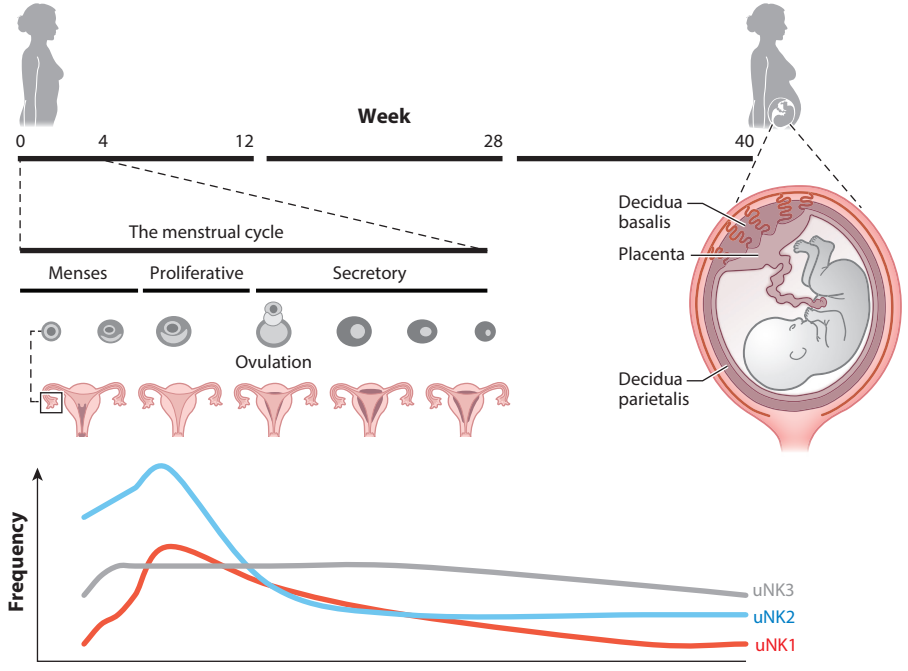

Figure 1

Kinetics of uterine NK (uNK) cells over the menstrual cycle and pregnancy. The menstrual cycle begins on the first day of menstruation. Subsequently, glands and stromal cells in the endometrium proliferate under the influence of progesterone, during the proliferative phase. Following ovulation, stromal cells expand and endometrial glands become secretory. This is called the secretory phase, and it involves decidualization of the mucosa in advance of pregnancy. If fertilization does not occur, the corpus luteum involutes and progesterone declines, triggering menstruation. If fertilization occurs, human chorionic gonadotropin from the conceptus maintains the corpus luteum, and the endometrium, now called the decidua, continues into the first trimester of pregnancy. uNK cells expand rapidly as the endometrium decidualizes in the secretory phase of the menstrual cycle and are most prominent in the first trimester of pregnancy, declining in the second and third trimesters. uNK cells are now known to consist of three subsets, uNK1, uNK2, and uNK3, with uNK1 and uNK2 cells peaking at around the time of implantation and uNK3 cells remaining relatively stable throughout the menstrual cycle and pregnancy.

section scar, the trophoblast penetrates deeply and can lead to rupture of the tube or uterus (9). All this evidence points to a crucial role of decidual-trophoblast interactions in defining the correct boundary between the placenta and the uterus.

DISCOVERY OF UTERINE NK CELLS

A unique feature of the endometrium, only during reproductive life, is the presence of granulated lymphocytes. Granulated cells with an eccentric nucleus and acidophilic cytoplasmic granules were noted in first-trimester decidua in the nineteenth century (10) and were thought to be a type of lymphoid cell (11). They were subsequently identified in nonpregnant endometrium (12) and have been variously called endometrial granulocytes, Kornchenzellen or K cells (13), globular leukocytes (14), and specific endometrial granular cells (15). Several of these reports noted the apparent hormonal dependence of endometrial granulocytes, as they were particularly prominent in the week before menstruation and were abundant in decidua until early in the second trimester, but cells with the typical granules were absent by term. In ovariectomized women, endometrial

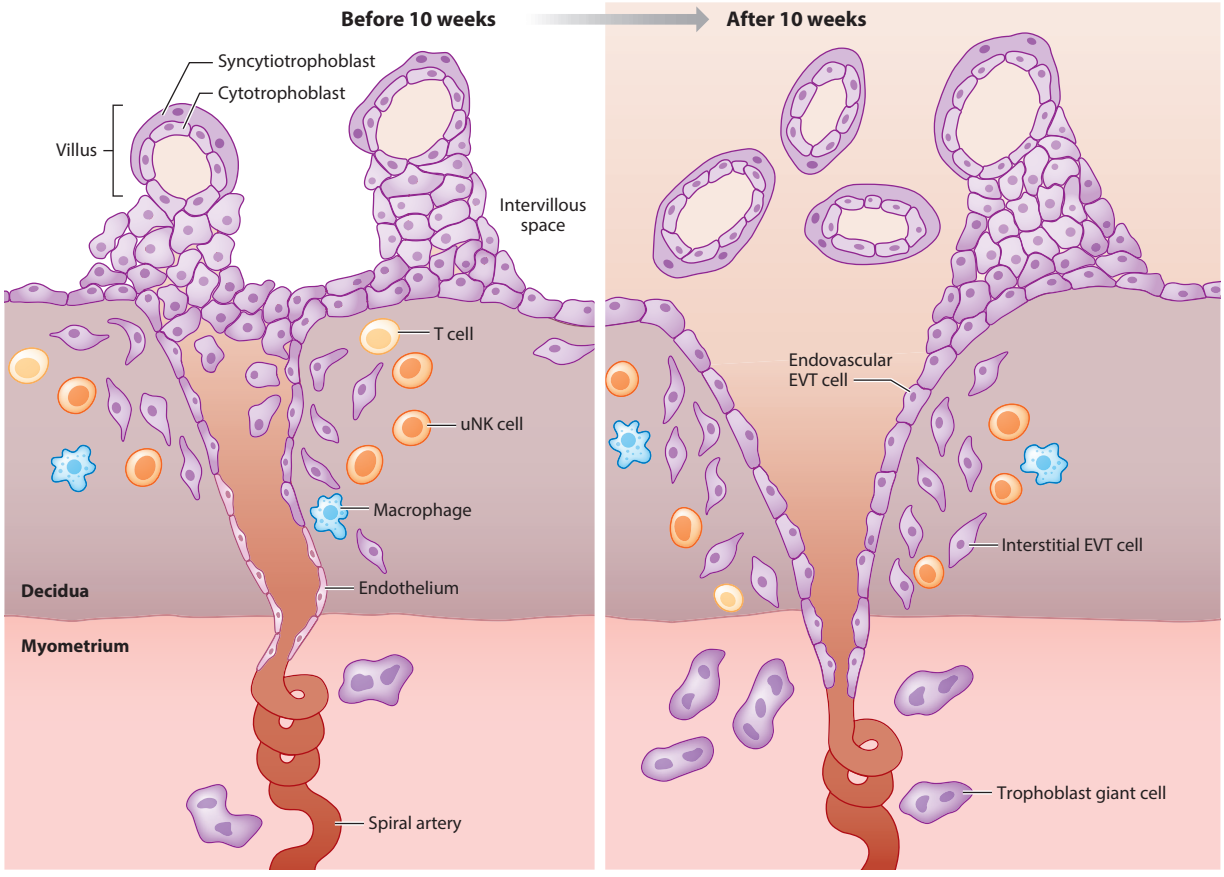

Figure 2

Anatomy of the maternofetal interface in the first trimester. The outer layer of the placental villi are covered by the syncytiotrophoblast, with an underlying layer of mononuclear cytotrophoblast cells. The inner layer of the cytotrophoblast grows out of the villi to anchor the placenta to the maternal decidua. The cytotrophoblast differentiates to the extravillous trophoblast (EVT), which moves into the decidua and migrates toward the spiral arteries, destroying the media and thus preventing the arteries from constricting, a process known as spiral artery transformation. Where the EVT overlies the opening of the arteries, it moves down, forming a plug that limits maternal blood from entering the intervillous space before the spiral arteries have been fully transformed, which occurs at between eight and ten weeks of pregnancy. Before this time, the conceptus is mainly nourished by secretions from the endometrial glands. After approximately ten weeks of gestation, the trophoblast plugs are effaced, allowing maternal blood to bathe the placental villi at high conductance and low pressure. The EVT expresses HLA-C, HLA-E, and HLA-G, which are major ligands for NK cells and macrophages: The immune cells present in the decidua while this process of implantation occurs are uterine NK (uNK) cells (approximately 70% of all immune cells), macrophages (20%), and T cells (10%).

granulocytes were only seen in those treated with estrogen and progesterone. Estrogen alone was insufficient for these cells to appear (13). Clearly their presence and function are under hormonal control.

Following on from studies in rodents (16–18), these cells were identified as $CD45^+$ leukocytes that expressed some lineage markers suggestive of T cells (CD2 and CD38) but not CD3, CD4, CD8, or CD5 (19–22). Around 1990 they were finally identified by immunohistochemistry and flow cytometry as a type of natural killer (NK) cell with a distinctive phenotype, $CD56^{bright}$, but lacking the other NK cell markers used at the time, CD16 and CD57 (22–25).

UTERINE NK CELLS ARE NOT CYTOTOXIC BUT HAVE UNUSUAL GRANULES

It was clear from the outset that the cytolytic ability of human uterine NK (uNK) cells against classical targets such as K562 is reduced compared to peripheral NK (pNK) cells (24, 26, 27). uNK cell granules do contain cytotoxic mediators, such as perforin and granzyme (28, 29) so this may be due to a failure to polarize the granules to form the synapse (30). Cytotoxicity to classical NK cell targets is increased after exposure in vitro to IL-2 or IL-15, but uNK cells do not kill the EVT cells that invade the decidua after implantation unless they are activated with IL-2 (but not IL-15) (29, 31, 32). The other commonly used readout for circulating NK cells, IFN-γ, is uninformative, as only low levels are secreted by uNK cells without preactivation (33–35).

The cytoplasmic granules of uNK cells also have features that distinguish them from those of pNK cells. One of the characteristics leading to their identification was the acidophilic staining of the cytoplasmic granules with phloxine tartrazine (14). In mice, the features of the granules are also unusual, as they stain strongly with the lectin DBA (36, 37). The granules of uNK cells are larger than those of pNK cells (38). NK cells with larger granules are better cytokine producers (39), so this might explain why uNK cells are weakly cytolytic. There are also points of similarity between uNK cells and pNK cells from patients with Chediak-Higashi syndrome that have large granules but fail to polarize toward NK cell target cells (40, 41) and are still efficient cytokine producers (42). Mutations in the lysosomal trafficking protein LYST are responsible for Chediak-Higashi syndrome and the equivalent beige syndrome in mice (that reproduce normally), and LYST mRNA levels are low in uNK cells compared to pNK cells (43). These clear points of difference from pNK granules raise the question of whether uNK granules contain additional mediators that are important for uNK cell–specific functions. A first step to answering this will be to fully define granule contents of uNK cells.

UTERINE NK CELLS EXPRESS RECEPTORS ALLOWING THEM TO RECOGNIZE THE EXTRAVILLOUS TROPHOBLAST

The presence of NK cells in the uterus at the time of placental implantation naturally led to investigations of whether they could recognize the EVT cells that invade the decidua early in pregnancy and are of fetal origin. Because of the role of polymorphic HLA molecules in immune recognition of allogeneic cells, an obvious question was which HLA molecules trophoblast cells express.

The major ligands for T cell receptors (TCRs), HLA-A and HLA-B molecules, have never been shown to be expressed by any human trophoblast subsets (44, 45). The presence of a non-polymorphic HLA class I molecule with a lower-molecular-weight (39 kDa) heavy chain than classical HLA class I molecules (45 kDa) expressed by choriocarcinoma lines and term placentae was the first hint that trophoblast cells expressed an unusual array of HLA class I molecules (46–48). This molecule, named HLA-G, was later found in the EVT in first-trimester pregnancies as well as the noninvasive trophoblast layer of the chorion laeve (49, 50).

The presence of another HLA class I molecule with a conventional heavy chain of 45 kDa was first reported on the choriocarcinoma line BeWo (44, 48). This was subsequently identified in nonmalignant first-trimester EVT cells and shown to be HLA-C (49, 51–53). HLA-E reaches the cell surface if there is a peptide derived from the leader sequence of another class I molecule, such as HLA-C, so it was unsurprising that this was also expressed (52, 54, 55). The peptide derived from the HLA-G leader sequence is preferentially presented by HLA-E (56).

Uterine NK Cell Receptors for HLA-C

A pivotal role for HLA-C expressed by the EVT emerged when the NK cell receptors that recognize human MHC-I molecules were defined in the mid-1990s (57, 58). Killer cell

immunoglobulin-like receptors (KIRs) are a closely related family of immunoglobulin superfamily molecules situated in the leukocyte receptor complex on chromosome 19, a region of the human genome that also has many genes devoted to reproduction (59, 60). Uterine NK cells express a higher proportion of the KIRs that bind to HLA-C allotypes than pNK cells from the same individuals (61, 62). HLA-C and KIR tetramers bind to primary uNK and EVT cells, respectively, demonstrating that these interactions occur at the time of placentation (61).

KIRs bind to four epitopes on HLA class I molecules: HLA-Bw4, HLA-A2/11, and all HLA-C allotypes distinguished by a dimorphism at position 80 of the α1 domain into two groups, $C1^+$HLA-C and $C2^+$HLA-C (60). Because the EVT does not express HLA-A or HLA-B, the C1 and C2 epitopes of HLA-C are the main polymorphic features detected by uNK cells. KIRs are either inhibitory or activating, depending on the length of the cytoplasmic tail. The KIRs that bind to $C1^+$HLA-C are inhibitory KIR2DL2/3. For $C2^+$HLA-C allotypes there are both inhibitory, KIR2DL1, and activating, KIR2DS1. There are two main KIR haplotypes, A and B, that differ both in the number of KIR genes and in allelic variation at each locus. The KIR A haplotype has seven genes that are inhibitory, apart from KIR2DS4, which is frequently nonfunctional. The KIR B haplotype has a variable number of additional, mostly activating, KIR and can have up to 14 genes (63).

Uterine NK Cell Receptors for HLA-E

CD94/NKG2 is an NK cell lectin-like receptor that binds to HLA-E (64–67). This heterodimer can be inhibitory (CD94/NKG2A) or activating (CD94/NKG2C). More than 95% of uNK cells express CD94/NKG2A, with a smaller subset expressing CD94/NKG2C (52, 68–70). When HLA-E is bound to the leader peptide of HLA-G, the affinity for the activating CD94/NKG2C is much higher than for any other HLA-derived peptide or even for peptides derived from pathogens like HCMV (56, 71, 72). This means that in the decidua basalis, the site where EVT cells invade the uterine lining, there will be a different signal to uNK cells from the EVT than from surrounding maternal stromal cells and macrophages that do not express HLA-G: The functional effects of this are still unknown.

Uterine NK Cell Receptors for HLA-G

Unlike classical class I HLA molecules, which are found in the same region of the genome, there is limited polymorphism of HLA-G, with one allele, HLA-G*0101, predominating and highly conserved at the protein level (73). Uniquely, HLA-G has a truncated cytoplasmic tail that influences trafficking and the repertoire of peptides presented, resulting in an extended surface half-life (74–76). Controversy has surrounded many aspects of the biology and possible functions of HLA-G (77). However, there is no dispute that there is intense expression by both endovascular and interstitial EVT cells, which increases as they move deeper into the decidua to form placental bed giant cells (47, 78, 79).

HLA-G associates with TAP and is expressed at the cell surface in association with β_2 microglobulin (β_2m) and peptide (78, 80, 81). It can also exist as a dimer of two HLA-G molecules, each of which consists of a heavy chain, β_2m, and peptide (82, 83) linked by a disulfide bond between extracellular cysteines at position 42 in the heavy-chain α1 domain: This moiety is unique to HLA-G and allows conservation of the conventional HLA class I heterotrimeric complex in the dimers (84). These HLA-G dimers can be immunoprecipitated from the surface of primary first-trimester EVT cells and provide a high-affinity ligand for members of the LILR family that, like KIRs, are located on chromosome 19q13.4 and have both inhibitory and activating forms depending on the cytoplasmic tail (85, 86).

LILRB1 has a long tail with ITIMs (immunoreceptor tyrosine-based motifs) characteristic of inhibitory receptors and binds to HLA-G dimers, as confirmed by crystal structure and by using a LILRB1-Fc fusion protein LILRB1 to immunoprecipitate HLA-G dimers from the surface of primary trophoblast cells (86–89). LILRB1 is expressed both by a subset of uNK cells and by decidual myeloid cells, macrophages, and DCs (90–92). The binding of EVT cells to the myeloid cells results in tolerogenic rather than immunogenic adaptive responses, clearly an important function at the maternofetal interface (86, 93, 94). The function of LILRB1 on uNK cells is less clear. In one study, coculture of uNK cells with transfectants expressing either the monomeric or the dimeric forms of HLA-G did not result in degranulation or IFN-γ production, but other groups have suggested that uNK cells may be either activated or inhibited by recognition of EVT cells via LILRB1, and no consensus has yet emerged (68, 86, 90, 91, 95).

KIR2DL4 is a framework KIR gene present in all individuals, with one transcript lacking the transmembrane domain present in most populations (96, 97). The cytoplasmic tail contains an ITIM as well as an arginine residue, initially leading to confusion over whether KIR2DL4 is inhibitory or activating (98). KIR2DL4 has been reported to bind to HLA-G, although binding of KIR2DL4-Fc proteins to HLA-G-expressing cells has revealed inconsistent results, perhaps because appropriate controls have not always been included (83, 99–101). Structural analysis has also suggested that KIR2DL4 does not bind to HLA-G monomers or dimers (102, 103). There is also conflicting evidence about KIR2DL4 expression in pNK cells and uNK cells, and surface expression is difficult to detect (100, 104, 105). One explanation that could account for these controversies is that HLA-G is endocytosed and KIR2DL4 is expressed in the endosomes where binding occurs (105): This might account for the difficulty both in detecting KIR2DL4 at the surface and in defining its ability to bind to HLA-G, since endosome-specific conditions may be required. In pNK cells, this endosomal binding is reported to result in proinflammatory and proangiogenic responses (106). If uNK cells do bind to HLA-G expressed by EVT cells, this is unlikely to be the functional outcome, as inflammation is not a feature of the decidua basalis during placentation and the increased blood flow is mediated not by angiogenesis but by destruction of the media (107). Therefore, the question of whether KIR2DL4 binds to HLA-G and the functional outcome of any such binding remains to be resolved.

UTERINE NK CELLS ARE A HETEROGENEOUS POPULATION

It has long been appreciated that uNK cells do not form a uniform population: As in pNK cells, there is cell-to-cell variation in the precise combination of NK cell receptors that are expressed (38, 61, 62, 108–112). Recently, however, new single-cell RNA sequencing (scRNA-seq) techniques have enabled an unbiased approach to these cells, and three major subpopulations were identified in first-trimester decidua, originally called dNK1, dNK2, and dNK3 cells (43). A smaller subset of proliferating uNK cells, designated dNKp cells, was identified by its expression of cell cycle genes. Another study that also examined first-trimester decidua using scRNA-seq identified two uNK cell clusters, one of which aligns with dNKp cells and one of which contains dNK1, dNK2, and dNK3 cells (113). More recently, equivalent subsets have been identified in nonpregnant endometrium (114, 115): In recognition of the finding that these subsets are found in the nonpregnant endometrium as well as the decidua, here we designate them uNK1, uNK2, and uNK3. Although one study has identified uNK1–3 cells in second- and third-trimester decidua using clustering and gating strategies developed with first-trimester tissue (115), where unbiased approaches to defining uNK subsets in the third trimester have been used, the three uNK subsets have not been identified (115–117). This suggests there may be transcriptional features of uNK cells late in pregnancy that mask the expression signatures of the subsets that can be identified earlier.

Table 1 Phenotypes and functions of uNK1, uNK2, and uNK3 cells[a]

Stage of pregnancy	uNK1	uNK2	uNK3
First trimester (43)	■ $CD49a^+CD9^+$ ■ $CD39^+$ $CD103^-ITGB1^-$ ■ Large, granular ■ Most *GZMA*, *GZMB*, *GNLY*, *PRF1* ■ High *KIR*, *LILRB1*, *NKG2A/C* ■ High expression of glycolysis genes	■ $CD49a^+CD9^+$ ■ $CD39^-CD103^-ITGB1^+$ ■ Medium size and granularity ■ Medium *GZMA*, *GZMB*, *GNLY*, *PRF1* ■ Low *KIR*, *LILRB1* ■ High *NKG2A/C* ■ Low expression of glycolysis genes	■ $CD49a^+CD9^+$ ■ $CD39^-CD103^+ITGB1^+$ ■ Small, agranular ■ Least *GZMA*, *GZMB*, *GNLY*, *PRF1* ■ Low *KIR*, *LILRB1*, *NKG2A/C* ■ Low expression of glycolysis genes
First trimester (38)	■ $Tbet^{lo}Eomes^{hi}Ahr^{int}$ ■ Little CD107a, GM-CSF, IFN-γ, MIP1a, MIP1b, XCL1 ■ Higher Ki67	■ $Tbet^{int}Eomes^{hi}Ahr^{int}$ ■ Intermediate CD107a, GM-CSF, IFN-γ, MIP1a, MIP1b, XCL1 ■ Lower Ki67	■ $Tbet^{hi}Eomes^{int}Ahr^{int}$ ■ High CD107a, GM-CSF, IFN-γ, MIP1a, MIP1b, XCL1 ■ Lower Ki67
First trimester (119)	■ Highest expression of PBX1	■ Intermediate expression of PBX1	■ Lowest expression of PBX1
First trimester (118)	■ Down in RPL ■ Enriched for angiogenesis-related molecules ■ Make little IFN-γ ■ Express more LILRB1	■ Up in RPL ■ Enriched for cytokine-mediated signaling ■ Make more IFN-γ ■ Express less LILRB1	■ Up in RPL ■ Enriched for cytokine-mediated signaling ■ Make more IFN-γ ■ Express less LILRB1
First trimester (120)	■ Highest *KIR* ■ Highest *GNLY*, *GZMA*, *GZMB*, *GZMK*, *PRF1* ■ Lower *GZMH*, *GZMM* ■ Reduced in RPL	■ Intermediate *KIR* ■ Lower *GNLY*, *GZMA*, *GZMB*, *GZMK*, *PRF1* ■ Higher *GZMH*, *GZMM* ■ Increased in RPL	■ Lowest *KIR* ■ Lower *GNLY*, *GZMA*, *GZMB*, *GZMK*, *PRF1* ■ Higher *GZMH*, *GZMM* ■ About the same in RPL
Endometrium and first trimester (122)	■ Most metabolically active ■ GO for angiogenesis highest ■ Proliferation lowest ■ *IFNG*, *CCL4*, *CCL3* lowest ■ Granzyme A, perforin, and degranulation highest ■ Galectin 9 highest	■ Less metabolically active ■ GO for angiogenesis intermediate ■ Proliferation intermediate ■ *IFNG*, *CCL4*, *CCL3* intermediate ■ Granzyme A, perforin, and degranulation intermediate ■ Galectin 9 intermediate	■ Less metabolically active ■ GO for angiogenesis lowest ■ Proliferation highest ■ *IFNG*, *CCL4*, *CCL3* highest ■ Granzyme A, perforin, and degranulation lowest ■ Galectin 9 lowest
Endometrium (114)	■ $CD69^+CD16^-$ ■ Higher expression of KIRs	■ $CD69^+CD16^-$ ■ Lower expression of KIRs	■ $CD69^+CD16^-$ ■ Lower expression of KIRs
Endometrium and first and third trimesters (115)	■ KIRs highest ■ LILRB1 higher ■ CD94 higher	■ KIRs intermediate ■ LILRB1 lower ■ CD94 lower	■ KIRs lowest ■ LILRB1 lower ■ CD94 lower

Abbreviations: GO, gene ontogeny analysis; KIR, killer cell immunoglobulin-like receptor; RPL, recurrent pregnancy loss; uNK1, type 1 uterine natural killer.

[a]Two further studies (116, 117) that were not able to distinguish between uNK1, uNK2, and uNK3 cells at term are not included in this table but are discussed in the text.

In contrast to pNK cells, all three of the major uNK subpopulations express the tissue-residence marker CD49a and can be distinguished from each other by their expression of CD39, integrin β1, and CD103 (43). This method of classifying uNK cells has now been used in several recent reports (38, 114, 115, 118–121). Another strategy that can be included, or used instead, is to subdivide uNK cells on the basis of the spectrum of KIR expression. Because uNK1 cells express the most and uNK3 cells the least KIRs, where this approach has been taken it is still broadly possible to map the findings onto the uNK1–3 classification (38, 43, 118, 120, 122). The phenotypic features of the uNK subsets are described in **Table 1**.

Of the three subsets, uNK1 cells express KIRs and LILRB1 at the highest frequency (38, 43, 115, 118, 120), meaning that they are particularly adapted to recognize EVT cells and therefore

regulate placentation (43). In support of this hypothesis, uNK1 cells are only present before menopause (122), their number and activity peak at the time of implantation (115, 122), and they are found in proximity to EVT cells (123). A subset identified as being uNK1-like by its expression of the transcription factor PBX1 is required for successful implantation in a mouse model (119). Reports that uNK1 cells are reduced in the failing decidua following miscarriage, compared to deciduae collected from ongoing pregnancies, have given rise to a similar claim (118–121). However, a major limitation of these studies is that they compare healthy pregnancies to those where the fetus has already died and associated inflammatory changes have already occurred. An alternative explanation for this observation is that uNK1 cells are more sensitive to the inevitable tissue destruction seen before the decidua is finally shed.

In contrast, uNK3 cells resemble intraepithelial group 1 innate lymphoid cells (ILC1s): They produce IFN-γ as well as expressing CD103, CD69, and CD161 (38). The uNK3 subset remains relatively stable throughout the menstrual cycle and pregnancy (115), and uNK3 are localized farther from EVT cells (123), suggesting their major role is not specifically in implantation. They seem likely to be more involved in conventional immune responses, including immune defense. Finally, uNK2 cells occupy a phenotypic and functional space between those of uNK1 and uNK3 cells, which could suggest that they are a developmental intermediate between the two (38, 43).

The relatively recent discovery of the three uNK subsets means that there are still a number of questions to be answered about the behavior of each subset. The kinetics and receptor expression of the subsets suggest that uNK1, and perhaps also uNK2, cells are most likely to be involved in implantation, with a homeostatic or immune role more likely for uNK3 cells, but this is still to be definitively determined (38, 43, 115).

ROLE OF UTERINE NK CELLS IN IMPLANTATION

The temporal and spatial association of uNK cells with invading EVT cells, together with their ability to recognize these cells and their relative inability to mediate conventional NK cell functions, led to the hypothesis that the major role for uNK cells is in mediating placental implantation. In support of this, granulated lymphoid cells are only seen in the uterus in species in which trophoblast cells breach the surface epithelium and infiltrate deep into the mucosa, allowing villi to be bathed in maternal blood (mice, rats, guinea pigs, simian primates): hemochorial placentation (124). As a result, a number of approaches have been taken to investigate the extent to which uNK cells influence placental implantation.

Immunogenetics

Because of the extreme polymorphism of both the maternal NK KIR genes and their fetal ligand, HLA-C, and convincing evidence that KIRs are expressed and bind trophoblast HLA-C molecules, immunogenetic studies were performed to detect if certain KIR/HLA-C combinations are associated with disorders of placentation, known as the Great Obstetrical Syndromes (125). The most studied of these is pre-eclampsia because there is a well-recognized international definition (126) and because of the heterogeneity in the causes of other disorders that can also result from defective placentation (fetal growth restriction, preterm labor, and stillbirth).

Results from cohorts in Europe and sub-Saharan Africa (Uganda and Ethiopia) show that women with two KIR A haplotypes are more at risk of pre-eclampsia when the fetus has a $C2^{+}$HLA-C group allele (127–129). The KIR A haplotype contains seven genes that are all inhibitory, apart from the activating *KIR2DS4*, which is frequently nonfunctional. Of these, *KIR2DL1*, located on the centromeric arm of the KIR A haplotype, encodes a protein that binds $C2^{+}$HLA-C, and this receptor-ligand interaction is therefore most likely to be responsible for the

association. Of the four main *KIR2DL1* alleles in Europeans, *KIR2DL1*003* is particularly associated with pre-eclampsia (130). It seems likely that specific $C2^+$HLA-C alleles are also particularly risky, but this has not yet been formally addressed.

The activating receptor encoded by the KIR B haplotype that recognizes $C2^+$HLA-C is KIR2DS1, and this not only provides protection against pre-eclampsia but also is increased in frequency in women who have large babies (131–134). In Ugandans, the frequency of *KIR2DS1* is low and it does not seem to confer protection. Instead, another activating KIR, *KIR2DS5*, which is located on the centromeric end of the KIR B haplotype in individuals with African ancestry, provides protection and can bind to $C2^+$HLA-C allotypes (128, 135). In contrast, *KIR2DS5* in Europeans is nonfunctional and located on the telomeric end of the KIR B haplotype (135).

Taken together, these studies find that combinations of maternal KIRs and fetal HLA-C variants that result in inhibition of uNK cells by the EVT are associated with pre-eclampsia: The important implication is that the interaction between uNK cells and the EVT regulates placental implantation, and that activating signals seem to protect against defective placentation but are associated with larger birth weights.

Although it is more difficult to collect clinically well-characterized cohorts of subjects with other Great Obstetrical Syndromes, there are hints that the same KIR/HLA-C combinations are found in fetal growth restriction and recurrent pregnancy loss. There have been many studies looking at maternal KIR genotypes in women with more than two to three early pregnancy losses, or those undergoing fertility treatment, but without consistent results (136–147). Other studies have also not reproduced the immunogenetic findings in pre-eclampsia (148, 149). One report merely used the population frequency of KIR and $C2^+$HLA-C alleles in Japanese people without any genotyping where there was <50% power to detect any significant difference in small cohorts (150, 151). There are several potential reasons for the disparities in all these studies: the difficulty of determining fetal HLA-C following pregnancy loss; the cohorts are too small, lack statistical power, and are ethnically diverse; there is no validation of the KIR typing using the UCLA exchange; there is no explanation of how KIR A and B haplotypes are assigned; and often results deviate from the Hardy-Weinberg equilibrium (152, 153).

Effect of Uterine NK Cells on Trophoblast Migration

Following the first immunogenetic evidence that activation of uNK cells was associated with lower risk of pre-eclampsia, a number of groups examined the ability of uNK cells to mediate trophoblast invasion experimentally. First-trimester uNK cells promoted the invasion of primary EVT cells into a Matrigel plug in mice, or across a transwell insert (95), while uNK supernatants promoted the invasion of trophoblast explants in vitro (154, 155). EVT cells express the receptors needed to respond to IL-8, and invasion could be partially reversed by anti-IL-8, suggesting this might be a key cytokine mediating EVT invasion (95, 154). However, when examined by intracellular flow cytometry, unstimulated uNK cells make only small amounts of IL-8, with decidual macrophages the major IL-8-producing population (115, 156).

Recently, other uNK cell–derived factors have been suggested as potentially mediating trophoblast invasion. Many cytokines and chemokines are produced by uNK cells, especially GM-CSF, CSF1, CCL2, CCL3, CCL4, and XCL1, and which uNK subsets produce these depends on the in vitro stimulation used, whether they are freshly isolated, and how pure the uNK cell isolates are (38, 43, 115, 118, 122, 131, 157). GM-CSF attracts trophoblast cells in a microfluidic invasion model (158); the receptor for XCL1, XCR1, is found on EVT cells (43, 157, 159); and XCL1 can promote migration of the EVT-derived cell line HTR-8/SVneo in vitro (159). Other chemokines expressed by uNK cells include CCL5, which is preferentially produced by uNK3 cells and whose receptor, CCR1, is expressed by EVT cells (43), and CCL1 and CCL3, which

may impact tissue remodeling at the site of implantation by signaling to macrophages (157). Their exact roles in EVT behavior and impact on other decidual cells remain unclear.

Role in Spiral Artery Remodeling

Transformation of the spiral arteries in early pregnancy is mediated by EVT (160), and therefore if uNK cells play a role in this process, it is likely to be via an effect on EVT. Some changes to the spiral arteries do occur in advance of pregnancy or in the decidua of ectopic pregnancies, by vessel elongation and loosening of the media (161–163). These are likely to be mediated by decidual factors that could include uNK cells, which have been observed to infiltrate remodeling spiral arteries in vivo (although it is not clear in some studies how arteries were distinguished from veins) (164) and in EVT/decidual cocultures (165). In coculture models, uNK cells increase vascular smooth muscle disorganization by their production of matrix metalloproteinases (165, 166) and they can destabilize tubules made by endothelial cell lines (167).

Some studies have found that uNK cells can promote sprouting angiogenesis in xenograft and human umbilical vein endothelial cell (HUVEC) tubule formation models (95) and that they produce angiogenic factors such as VEGF, PlGF, and Ang2 (95, 166, 168), although scRNA-seq findings have cast doubt on the cellular source of these factors (43). However, given that both pre-pregnancy and early pregnancy changes to the spiral arteries do not occur by sprouting angiogenesis, the extent to which the expression of these factors is involved in spiral artery transformation is unclear. Another important unanswered question is exactly how the phenotype and functions of EVT cells change as they move through the decidua toward the myometrium and how this is altered in disorders of placentation, causing incomplete arterial transformation. The new in vitro models of trophoblasts will be important in exploring the impact of uNK cells on the EVT (169–171).

ROLE OF UTERINE NK CELLS IN CONTROLING INFECTION

Because of their reduced cytotoxicity against the conventional NK cell target line K562, it was originally thought that uNK cells were unlikely to have a role in controlling infection. However, their expression of perforin and granzymes always left open the possibility that they could become cytotoxic under certain circumstances. More recently, evidence suggests that they can recognize infected cells and control pathogen spread. Because of the proximity of the decidua to the placenta, interest in this area has focused on those pathogens that can be vertically transmitted, but this requires infection of villous trophoblast cells and/or breach of the syncytiotrophoblast barrier, whereas uNK cells are in contact with EVT cells and decidual stromal cells. Nonetheless, the decidua will be affected in overwhelming systemic infections, so decidual control of pathogens might reduce the chance of vertical transmission (172).

Human Cytomegalovirus

In healthy adults, human cytomegalovirus (HCMV) is kept in check by the immune response and is of little consequence. However, active infection during pregnancy can result in vertical transmission and severe congenital disease. The longstanding observation that NK cells are important for the control of HCMV, with individuals who have defective NK cells more susceptible to HCMV infection (173–175), makes uNK cells attractive candidates to control uterine infection. In addition, the time at which uNK cells are most numerous, the first trimester, is also when vertical transmission of HCMV is least likely to occur (176).

First-trimester uNK cells can kill HCMV-infected decidual stromal cells in vitro (177). This process involves degranulation and is reduced following antibody blockade of the activating

receptors NKp44 and NKp46, or of HLA-E (177, 178). HLA-E binds peptides derived from HCMV, and pNK cells recognize HCMV-infected targets via NKG2C and HLA-E (179); NKG2C is preferentially expressed by KIR^+ uNK1 cells (68, 112, 180). KIR2DS1 expressed by uNK cells interacting with $C2^+$HLA-C HCMV-infected decidual stromal cells can also lead to degranulation, but EVT cells were not affected even if infected with HCMV (178). By term, this response is reduced, possibly related to the smaller proportion of NKG2C-expressing uNK1 cells later in pregnancy (115, 181).

Zika Virus

Zika virus can infect both the EVT and cytotrophoblast in placental explants; the addition of uNK cells to the culture reduces the number of infected EVT but not cytotrophoblast cells (182). NK cells can kill Zika virus–infected JEG-3 choriocarcinoma cells in a manner that is dependent on recognition via NKp46 and degranulation.

Listeria

First-trimester uNK cells can control *Listeria monocytogenes* infection in EVT cells by secreting granulysin. Crucially, this does not kill the trophoblast cells themselves (183). Furthermore, *Listeria*-induced abortion could be prevented in a mouse model when granulysin-expressing NK cells were present (183).

SARS-CoV-2

During the SARS-CoV-2 pandemic, a key concern was the effect of SARS-CoV-2 infection on pregnancy. Although for most, the virus is limited to the respiratory tract, in rare cases disseminated infection can be associated with an inflammatory syndrome of the placenta, called SARS-CoV-2 placentitis, which is associated with an increased risk of stillbirth (184). uNK cells might be activated in serious SARS-CoV-2 infection. One small scRNA-seq study compared two placentae delivered during active SARS-CoV-2 infection to two controls and found increased expression of granzymes, granulysin, CD69, and XCL1 in uNK cells from infected donors (185), although a larger study of nine placentae from SARS-CoV-2-infected donors compared to 10 controls found little change in gene expression by uNK cells (186). Taken together, these findings may suggest that uNK cells are more activated in SARS-CoV-2 infection, although whether they play any role in controlling SARS-CoV-2 in those cases where it infects uterine cells during pregnancy remains to be determined.

ORIGIN AND DEVELOPMENT OF UTERINE NK CELLS

The kinetics of uNK cells during the menstrual cycle raises the question of their origin: How do they achieve this remarkable cyclical regeneration? Previous evidence has suggested that they could arise from a resident hematopoietic stem cell (187–189) or recruited from the periphery, either as a progenitor (190) or mature (187) NK cell.

Origin

$CD45^+$ cells expressing donor HLA class I allotypes are present in the uterine mucosa of bone marrow transplant recipients up to 13 years after surgery (191). Recently, definitive evidence that uNK cells arise from a circulating progenitor has come from uterine transplant recipients, where uNK cells express the recipient, rather than the donor, MHC-I allele (122). A possible mechanism by which this recruitment could occur is that both stromal and endothelial cells might produce NK cell–attractive chemokines in response to ovarian hormones (192–194), and expression of adhesion

molecules by endometrial endothelium increases in the early secretory phase of the menstrual cycle (195).

Development

Two key questions remain to be answered: What is the identity of the circulating progenitor, and how does it develop within the uterus into a uNK cell? To address the second question, attempts have been made to define how uNK1, uNK2, and uNK3 cells may be developmentally related to each other, but with conflicting results. Both a supervised pseudotime analysis on flow cytometry data and an unsupervised analysis on scRNA-seq data (43) support the idea that uNK cells that express neither KIRs nor CD39 (largely uNK3 cells) upregulate first KIRs (becoming a uNK2-like subset) and then CD39 (uNK1-like) (122). This scheme is supported by an experiment where sorted human uNK cells were allowed to differentiate in immunodeficient mice engineered to express human IL-15, although the cells were recovered from the spleen and liver and were not found in the uterus. In contrast, a supervised pseudotime analysis of scRNA-seq data suggested that there are multiple routes of development, with uNK progenitors able to give rise to uNK1 or uNK2 cells, and uNK2 cells able to continue developing to uNK3 cells, or to cross-differentiate to uNK1 cells (118). Finally, using another supervised pseudotime method, uNK3-like cells were shown to differentiate first to a cluster that contains a mixture of uNK1 and uNK2, and finally to uNK2 cells (120). This wide variety of proposed lineage relationships is probably due to the fact that these analyses were supervised, with the root cell type chosen manually, and differently, between the studies. This underlines the importance both of unsupervised pseudotime analyses to generate hypotheses and of differentiation assays to test them.

Receptor Acquisition and Education

Education of NK cells depends on interactions between inhibitory NK cell receptors and HLA class I molecules that determine the strength and specificity with which mature NK cells later respond to missing HLA class I molecules (196). In pNK cells, receptors recognizing HLA class I molecules are expressed in a combinatorial manner to produce a diverse repertoire of receptor expression within the NK cell population. Not all NK cells will express an inhibitory receptor capable of recognizing a self HLA class I molecule, and while these cells are potentially autoreactive, they are not educated. The idea that NK cell education could affect the responses of mature NK cells is particularly pertinent for interactions between uNK and EVT cells, as maternal uNK cells may encounter non-self class MHC-I (paternal HLA-C) or missing-self class MHC-I (since only one maternal HLA-C allele will be inherited by the fetus). In the first trimester, uNK cells express a repertoire of KIRs that is biased toward the recognition of HLA-C, which is expressed by EVT cells, but not HLA-B, which is not (61, 62, 111, 131, 178). There is mixed evidence on whether this bias is present in advance of pregnancy (62, 70, 112, 115), but it seems to decline after the first trimester (115).

The finding that uNK cells have a distinct receptor repertoire suggests that receptor acquisition, and thus education, occurs in the uterus. Certainly, if progenitors are recruited from the circulation and differentiate locally (122), then education must occur in the uterus. This education may be different from that of pNK cells, as uNK cells do respond differently to missing MHC-I than NK cells in the periphery: uNK cells that express greater numbers of KIRs are less able to respond to the class I–negative target K562 by degranulating or producing XCL1, in contrast to pNK cells, which respond better the more KIRs they express (38, 111).

Another important question is whether uNK cells are educated on maternal MHC-I but then respond to paternal MHC-I expressed by EVT cells, or whether MHC-I expressed by EVT cells

can itself educate uNK cells. Fetal expression of $C2^+$HLA-C is associated with increased risk of pre-eclampsia in mothers with a KIR AA genotype with a higher risk if the $C2^+$HLA-C allele is paternally inherited, compared to maternally inherited, suggesting education by maternal HLA-C, and inappropriate over-inhibition when uNK cells encounter paternally derived $C2^+$HLA-C for the first time (127, 132). Murine uNK cells are also educated on maternal, but not paternal, alleles (197). Immunogenetic approaches have also indicated a role for NKG2A in the education of uNK cells: maternal allotypes of HLA-B that do not provide a strong leader peptide for HLA-E, and therefore do not favor education via NKG2A, were associated with an increased risk of pre-eclampsia in a large European cohort (198).

THE POTENTIAL FOR UTERINE NK CELL MEMORY OF PREGNANCY

As innate lymphocytes, NK cells have traditionally been considered not to have immune memory, but over the last decade it has become clear that NK cells do have some ability to learn from experience (199). This might mean that uNK cells remember encounters with previous placentae, perhaps explaining the longstanding observation that second and subsequent pregnancies are at lower risk of pre-eclampsia, unless there is a change of partner, in which case the risk returns to that seen in first pregnancies (200).

In pNK cells, NKG2C is a marker of adaptive NK cells that expand in response to HCMV infection (199), and $NKG2C^+$ uNK cells are expanded in first-trimester decidua from multigravidae (68). Like other receptors for MHC-I molecules, NKG2C is characteristic of the uNK1 subset. A confounding effect from HCMV infection is possible, as the findings were not replicated in endometrial NK cells from multigravidae (180). Expansions of $NKG2C^+KIR^+$ cells in both nulliparous and parous donors were also reported when menstrual blood was used as a source of uNK cells (112). An important question is the mechanism by which memory of a previous pregnancy could persist in the uterus if uNK cells are replaced from the circulation (122).

WHAT CAN WE LEARN FROM MICE?

The role of uNK cells in mice has recently been reviewed elsewhere and is not our focus (201, 202). However, it is worthwhile to consider what experiments carried out in mice can or cannot tell us about uNK cells and reproductive success in humans. There are some key similarities between the species: Both have hemochorial placentation, with decidualization, modification to the spiral arteries, and invasion of trophoblast cells into the decidua. This invasion is considerably more extensive in humans than in mice, with the trophoblast invading both down the blood vessels and through the decidua, crossing the junctional zone and continuing as far as the inner third of the myometrium. In mice, trophoblast invasion is limited to the junctional zone, and spiral artery modification is instead associated with NK cells, which infiltrate the arterial wall.

There are important differences in the timing of decidualization between the two species, and thus the point in the reproductive cycle at which uNK cells begin to accumulate. In humans, decidualization begins in the secretory phase of the menstrual cycle, with NK cells becoming prominent at this time; in mice NK cells do not begin to increase until decidualization is triggered by the implantation of the embryo. Like humans, mice have three subsets of NK cells in the uterus—conventional NK, tissue-resident NK, and ILC1—which change in relative frequency over the course of the reproductive cycle (203). The subsets in the two species do not align precisely, although some comparisons can be made. uNK3 cells are ILC1-like (38) and might therefore be considered equivalent to mouse ILC1s. Mouse conventional NK cells are circulating NK cells equivalent to those found in humans, although these are only present in the human decidua at very low frequency compared to the mouse (43, 203, 204). Mouse tissue-resident NK cells express

high levels of receptors that recognize MHC-I (203) and may therefore align with human uNK1 and uNK2 cells.

The location of uNK cells also differs between mice and humans. In mice, they are present in the decidua basalis soon after implantation but by mid-gestation are spatially limited to the mesometrial lymphoid aggregate of pregnancy (MLAp) in the myometrium. The function of the MLAp is unknown and no equivalent structure exists in humans, but as it encircles the main arterial branch to the placenta it is likely to affect blood flow. Finally, the major disorders of human pregnancy, such as pre-eclampsia, have no counterpart in rodents.

With such a list of caveats, it could be argued that mice are of limited use to illuminate processes in human reproduction. However, the experimental tractability of murine models is immense and should not be lightly dismissed. Mice have been engineered to express specific human HLA and KIR genes (205). By designing breeding experiments in which the pups express the relevant HLA-C genes and the dams the relevant KIR genes, researchers can use these mice to cleanly test hypotheses generated using immunogenetic approaches, about HLA-KIR interactions that might be beneficial or risky for human pregnancy. Another example is the recent finding that NKG2A knockout dams have incomplete spiral artery remodeling in pregnancy, whereas dams treated with NKG2A-blocking antibody during pregnancy do not experience this (198). This observation suggests a role for NKG2A in the education of uNK cells, confirmed in humans using immunogenetics. These examples illustrate the power of mouse models, both as a tool to test hypotheses generated in humans and as a means to generate hypotheses that can be tested immunogenetically.

Mice can also act as a powerful model to investigate how human uNK cells develop and function in vivo. For example, sorted uNK cells or progenitors can be adoptively transferred to immunodeficient mice, allowing analysis of which cells they give rise to, and which tissue sites these cells traffic to (122). Using these adoptive transfer approaches in mice that have been engineered to express human HLA (205) has the potential to narrowly define which of the three human uNK subsets is most important for implantation in a well-controlled, in vivo model.

FUTURE DIRECTIONS

In the three decades since they were first identified, significant progress has been made in understanding uNK cells. In the last few years, unbiased approaches have allowed us to better understand heterogeneity within uNK cells. These approaches, together with developments in uterine transplantation, have also allowed us to revisit questions about their origin and development. But there is still much to discover.

The exact roles of uNK cells in normal or abnormal pregnancies are still uncertain, but their close temporal and spatial association with events in the first few weeks of pregnancy points to their major role in controlling placentation. Further work is needed to understand exactly how this is mediated and how the EVT is altered by uNK cells, especially in particular maternal KIR-fetal HLA-C genetic combinations that are beneficial or detrimental to pregnancy outcomes. Our new understanding of subset heterogeneity may be of help here, since it is possible that examining uNK cells on a subset-by-subset basis will allow us to identify functions that were previously obscure. Recent developments in microfluidic and organoid approaches will also allow us to probe uNK cell function using primary trophoblast cells, in a way that was not previously possible (206). Little is known about the interactions of uNK cells with other decidual cells, especially stromal cells and macrophages. In the light of a renewed focus on the role of macrophages in implantation, the latter may prove to be extremely important (123).

A fuller understanding of uNK cell function will be an important first step in identifying cells and molecules that could potentially be targeted to promote placental implantation, improving outcomes of pregnancy for mothers and babies. Before we have gained this understanding, it

seems premature to offer clinical interventions that target uNK cells, or introduce maternal KIR and parental HLA-C typing in the clinic either for infertile couples or those with recurrent in vitro fertilization failure or pregnancy loss (207). However, the rapid pace of advance in our understanding of the development and function of uNK cells holds out the hope that, in the future, clinical applications based on this could become a reality.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

V.M. is supported by Borne. A.M. is supported by an Investigator Award from the Wellcome Trust. We are grateful to Holly Anderson for help with the preparation of this manuscript.

LITERATURE CITED

1. Nogales-Ortiz F, Tarancon I, Nogales FF. 1979. The pathology of female genital tuberculosis: a 31-year study of 1436 cases. *Obstet. Gynecol.* 53(4):422–28
2. McQueen DB, Maniar KP, Hutchinson A, Confino R, Bernardi L, Pavone ME. 2021. Redefining chronic endometritis: the importance of endometrial stromal changes. *Fertil. Steril.* 116(3):855–61
3. Mutter GL, Ferenczy A. 2002. Anatomy and histology of the uterine corpus. In *Blaustein's Pathology of the Female Genital Tract*, ed. RJ Kurma, pp. 383–419. New York: Springer
4. Archer DF, McIntyre-Seltman K, Wilborn WW, Dowling EA, Cone F, et al. 1991. Endometrial morphology in asymptomatic postmenopausal women. *Am. J. Obstet. Gynecol.* 165(2):317–22
5. Turco MY, Moffett A. 2019. Development of the human placenta. *Development* 146(22):dev163428
6. Kelleher AM, Demayo FJ, Spencer TE. 2019. Uterine glands: developmental biology and functional roles in pregnancy. *Endocr. Rev.* 40(5):1424–45
7. Burton GJ, Cindrova-Davies T, Turco MY. 2020. Review: Histotrophic nutrition and the placental-endometrial dialogue during human early pregnancy. *Placenta* 102:21–26
8. Carter AM. 2021. Unique aspects of human placentation. *Int. J. Mol. Sci.* 22(15):8099
9. Jauniaux E, Collins S, Burton GJ. 2018. Placenta accreta spectrum: pathophysiology and evidence-based anatomy for prenatal ultrasound imaging. *Am. J. Obstet. Gynecol.* 218(1):75–87
10. Marchand F. 1895. On the so-called decidual tumors following normal childbirth, hydatidiform mole and extrauterine pregnancy. *Obstet. Gynecol.* 1:419
11. Weill P. 1921. Etudes sur les leucocytes. I. Les cellules granuleuses des muqueuses intestinale et utérines. *Arch. Anat. Microsc.* 17:77–82
12. Hamperl H. 1955. The granular endometrial stromal cells: a new cell type. *J. Pathol. Bacteriol.* 69:358–59
13. Hamperl H, Hellweg G. 1958. Granular endometrial stromal cells. *Obstet. Gynecol.* 11(4):379–87
14. von Numers C. 1953. On the specific granular cells (globular leukocytes) of the human endometrium, with special reference to their occurrence in different pathological conditions and to their staining reactions. *Acta Pathol. Microbiol. Scand.* 33(3):250–56
15. Kazzaz BA. 1972. Specific endometrial granular cells: a semiquantitative study. *Eur. J. Obstet. Gynecol.* 2(3):77–84
16. Stewart IJ, Peel S. 1993. Natural killer cytotoxicity and antibody-dependent cytotoxicity of cells of rat metrial glands. *J. Reprod. Fertil.* 98(2):489–94
17. Gambel P, Croy BA, Moore WD, Hunziker RD, Wegmann TG, Rossant J. 1985. Characterization of immune effector cells present in early murine decidua. *Cell. Immunol.* 93(2):303–14
18. Croy BA, Gambel P, Rossant J, Wegmann TG. 1985. Characterization of murine decidual natural killer (NK) cells and their relevance to the success of pregnancy. *Cell. Immunol.* 93(2):315–26
19. Bulmer JN, Sunderland CA. 1983. Bone-marrow origin of endometrial granulocytes in the early human placental bed. *J. Reprod. Immunol.* 5(6):383–87

20. Bulmer JN, Sunderland CA. 1984. Immunohistological characterization of lymphoid cell populations in the early human placental bed. *Immunology* 52(2):349–57
21. Bulmer JN, Johnson PM. 1986. The T-lymphocyte population in first-trimester human decidua does not express the interleukin-2 receptor. *Immunology* 58(4):685–87
22. Bulmer JN, Hollings D, Ritson A. 1987. Immunocytochemical evidence that endometrial stromal granulocytes are granulated lymphocytes. *J. Pathol.* 153(3):281–88
23. Starkey PM, Sargent IL, Redman CW. 1988. Cell populations in human early pregnancy decidua: characterization and isolation of large granular lymphocytes by flow cytometry. *Immunology* 65(1):129–34
24. King A, Birkby C, Loke YW. 1989. Early human decidual cells exhibit NK activity against the K562 cell line but not against first trimester trophoblast. *Cell. Immunol.* 118(2):337–44
25. King A, Balendran N, Wooding P, Carter NP, Loke YW. 1991. $CD3^-$ leukocytes present in the human uterus during early placentation: phenotypic and morphologic characterization of the $CD56^{++}$ population. *Dev. Immunol.* 1(3):169–90
26. Ritson A, Bulmer JN. 1989. Isolation and functional studies of granulated lymphocytes in first trimester human decidua. *Clin. Exp. Immunol.* 77(2):263–68
27. Ferry BL, Starkey PM, Sargent IL, Watt GM, Jackson M, Redman CWG. 1990. Cell populations in the human early pregnancy decidua: natural killer activity and response to interleukin-2 of CD56-positive large granular lymphocytes. *Immunology* 70(4):446–52
28. Koopman LA, Kopcow HD, Rybalov B, Boyson JE, Orange JS, et al. 2003. Human decidual natural killer cells are a unique NK cell subset with immunomodulatory potential. *J. Exp. Med.* 198(8):1201–12
29. King A, Wooding P, Gardner L, Loke YW. 1993. Expression of perforin, granzyme A and TIA-1 by human uterine $CD56^+$ NK cells implies they are activated and capable of effector functions. *Hum. Reprod.* 8(12):2061–67
30. Kopcow HD, Allan DSJ, Chen X, Rybalov B, Andzelm MM, et al. 2005. Human decidual NK cells form immature activating synapses and are not cytotoxic. *PNAS* 102(43):15563–68
31. King A, Loke YW. 1990. Human trophoblast and JEG choriocarcinoma cells are sensitive to lysis by IL-2-stimulated decidual NK cells. *Cell. Immunol.* 129(2):435–48
32. Verma S, Hiby SE, Loke YW, King A. 2000. Human decidual natural killer cells express the receptor for and respond to the cytokine interleukin 15. *Biol. Reprod.* 62(4):959–68
33. Saito S, Morii T, Enomoto M, Sakakura S, Nishikawa K, et al. 1993. The effect of interleukin 2 and transforming growth factor-β2 (TGF-β2) on the proliferation and natural killer activity of decidual $CD16^-$ $CD56^{bright}$ natural killer cells. *Cell. Immunol.* 152(2):605–13
34. Jokhi PP, King A, Sharkey AM, Smith SK, Loke YW. 1994. Screening for cytokine messenger ribonucleic acids in purified human decidual lymphocyte populations by the reverse-transcriptase polymerase chain reaction. *J. Immunol.* 153(10):4427–35
35. Higuma-Myojo S, Sasaki Y, Miyazaki S, Saki M, Siozaki A, et al. 2005. Cytokine profile of natural killer cells in early human pregnancy. *Am. J. Reprod. Immunol.* 54(1):21–29
36. Bianco J, Stephenson K, Yamada AT, Croy BA. 2008. Time-course analyses addressing the acquisition of DBA lectin reactivity in mouse lymphoid organs and uterus during the first week of pregnancy. *Placenta* 29(12):1009–15
37. Paffaro VA, Bizinotto MC, Joazeiro PP, Yamada AT. 2003. Subset classification of mouse uterine natural killer cells by DBA lectin reactivity. *Placenta* 24(5):479–88
38. Huhn O, Ivarsson MA, Gardner L, Hollinshead M, Stinchcombe JC, et al. 2020. Distinctive phenotypes and functions of innate lymphoid cells in human decidua during early pregnancy. *Nat. Commun.* 11(1):381
39. Goodridge JP, Jacobs B, Saetersmoen ML, Clement D, Hammer Q, et al. 2019. Remodeling of secretory lysosomes during education tunes functional potential in NK cells. *Nat. Commun.* 10(1):514
40. Chiang SCC, Wood SM, Tesi B, Akar HH, Al-Herz W, et al. 2017. Differences in granule morphology yet equally impaired exocytosis among cytotoxic T cells and NK cells from Chediak-Higashi syndrome patients. *Front. Immunol.* 8:426
41. Haliotis T, Roder J, Klein M, Ortaldo J, Fauci AS, et al. 1980. Chediak-Higashi gene in humans. I. Impairment of natural-killer function. *J. Exp. Med.* 151(5):1039–48

42. Gil-Krzewska A, Wood SM, Murakami Y, Nguyen V, Chiang SCC, et al. 2016. Chediak-Higashi syndrome: Lysosomal trafficking regulator domains regulate exocytosis of lytic granules but not cytokine secretion by natural killer cells. *J. Allergy Clin. Immunol.* 137(4):1165–77
43. Vento-Tormo R, Efremova M, Botting RA, Turco MY, Vento-Tormo M, et al. 2018. Single-cell reconstruction of the early maternal-fetal interface in humans. *Nature* 563(7731):347–53
44. Redman CW, McMichael AJ, Stirrat GM, Sunderland CA, Ting A. 1984. Class I major histocompatibility complex antigens on human extra-villous trophoblast. *Immunology* 52(3):457–68
45. 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(1):26–39
46. Ellis SA, Sargent IL, Redman CWG, McMichael AJ. 1986. Evidence for a novel HLA antigen found on human extravillous trophoblast and a choriocarcinoma cell line. *Immunology* 59(4):595–601
47. Kovats S, Main EK, Librach C, Stubblebine M, Fisher SJ, Demars R. 1990. A class I antigen, HLA-G, expressed in human trophoblasts. *Science* 248(4952):220–23
48. Ellis SA, Palmer MS, McMichael AJ. 1990. Human trophoblast and the choriocarcinoma cell line BeWo express a truncated HLA Class I molecule. *J. Immunol.* 144(2):731–35
49. Hutter H, Hammer A, Blaschitz A, Hartmann M, Ebbesen P, et al. 1996. Expression of HLA class I molecules in human first trimester and term placenta trophoblast. *Cell Tissue Res.* 286(3):439–47
50. Hiby SE, King A, Sharkey A, Loke YW. 1999. Molecular studies of trophoblast HLA-G: polymorphism, isoforms, imprinting and expression in preimplantation embryo. *Tissue Antigens* 53(1):1–13
51. King A, Boocock C, Sharkey AM, Gardner L, Beretta A, et al. 1996. Evidence for the expression of HLA-C class I mRNA and protein by human first trimester trophoblast. *J. Immunol.* 156(6):2068–76
52. King A, Allan DSJ, Bowen M, Powis SJ, Joseph S, et al. 2000. HLA-E is expressed on trophoblast and interacts with CD94/NKG2 receptors on decidual NK cells. *Eur. J. Immunol.* 30(6):1623–31
53. Pröll J, Blaschitz A, Hutter H, Dohr G. 1999. First trimester human endovascular trophoblast cells express both HLA-C and HLA-G. *Am. J. Reprod. Immunol.* 42(1):30–36
54. Lee N, Goodlett DR, Ishitani A, Marquardt H, Geraghty DE. 1998. HLA-E surface expression depends on binding of TAP-dependent peptides derived from certain HLA class I signal sequences. *J. Immunol.* 160(10):4951–60
55. O'Callaghan CA, Tormo J, Willcox BE, Braud VM, Jakobsen BK, et al. 1998. Structural features impose tight peptide binding specificity in the nonclassical MHC molecule HLA-E. *Mol. Cell* 1(4):531–41
56. Llano M, Lee N, Navarro F, García P, Albar JP, et al. 1998. HLA-E-bound peptides influence recognition by inhibitory and triggering CD94/NKG2 receptors: preferential response to an HLA-G-derived nonamer. *Eur. J. Immunol.* 28(9):2854–63
57. Wagtmann N, Rajagopalan S, Winter CC, Peruzzi M, Long EO. 1995. Killer cell inhibitory receptors specific for HLA-C and HLA-B identified by direct binding and by functional transfer. *Immunity* 3(6):801–9
58. Moretta A, Sivori S, Vitale M, Pende D, Morelli L, et al. 1995. Existence of both inhibitory (p58) and activatory (p50) receptors for HLA-C molecules in human natural killer cells. *J. Exp. Med.* 182(3):875–84
59. Moffett A, Colucci F. 2015. Co-evolution of NK receptors and HLA ligands in humans is driven by reproduction. *Immunol. Rev.* 267(1):283–97
60. Parham P, Moffett A. 2013. Variable NK cell receptors and their MHC class I ligands in immunity, reproduction and human evolution. *Nat. Rev. Immunol.* 13(2):133–44
61. Sharkey AM, Gardner L, Hiby S, Farrell L, Apps R, 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(1):39–46
62. Male V, Sharkey A, Masters L, Kennedy PR, Farrell LE, Moffett A. 2011. The effect of pregnancy on the uterine NK cell KIR repertoire. *Eur. J. Immunol.* 41(10):3017–27
63. Parham P, Guethlein LA. 2018. Genetics of natural killer cells in human health, disease, and survival. *Annu. Rev. Immunol.* 36:519–48
64. Braud VM, Allan DSJ, O'Callaghan CA, Soderstrom K, D'Andrea A, et al. 1998. HLA-E binds to natural killer cell receptors CD94/NKG2A, B and C. *Nature* 391(6669):795–99

65. Brooks AG, Borrego F, Posch PE, Patamawenu A, Scorzelli CJ, et al. 1999. Specific recognition of HLA-E, but not classical, HLA class I molecules by soluble CD94/NKG2A and NK cells. *J. Immunol.* 162(1):305–13
66. Borrego F, Ulbrecht M, Weiss EH, Coligan JE, Brooks AG. 1998. Recognition of human histocompatibility leukocyte antigen (HLA)-E complexed with HLA class I signal sequence-derived peptides by CD94/NKG2 confers protection from natural killer cell-mediated lysis. *J. Exp. Med.* 187(5):813–18
67. Lee N, Llano M, Carretero M, Akiko-Ishitani, Navarro F, et al. 1998. HLA-E is a major ligand for the natural killer inhibitory receptor CD94/NKG2A. *PNAS* 95(9):5199–204
68. Gamliel M, Goldman-Wohl D, Isaacson B, Gur C, Stein N, et al. 2018. Trained memory of human uterine NK cells enhances their function in subsequent pregnancies. *Immunity* 48(5):951–62
69. Kusumi M, Yamashita T, Fujii T, Nagamatsu T, Kozuma S, Taketani Y. 2006. Expression patterns of lectin-like natural killer receptors, inhibitory CD94/NKG2A, and activating CD94/NKG2C on decidual $CD56^{bright}$ natural killer cells differ from those on peripheral $CD56^{dim}$ natural killer cells. *J. Reprod. Immunol.* 70(1–2):33–42
70. Feyaerts D, Kuret T, Van Cranenbroek B, Van Der Zeeuw-Hingrez S, Van Der Heijden OWH, et al. 2018. Endometrial natural killer (NK) cells reveal a tissue-specific receptor repertoire. *Hum. Reprod.* 33(3):441–51
71. Petrie EJ, Clements CS, Lin J, Sullivan LC, Johnson D, et al. 2008. CD94-NKG2A recognition of human leukocyte antigen (HLA)-E bound to an HLA class I leader sequence. *J. Exp. Med.* 205(3):725–35
72. Heatley SL, Pietra G, Lin J, Widjaja JML, Harpur CM, et al. 2013. Polymorphism in human cytomegalovirus UL40 impacts on recognition of human leukocyte antigen-E (HLA-E) by natural killer cells. *J. Biol. Chem.* 288(12):8679–90
73. Ober C, Aldrich CL. 1997. HLA-G polymorphisms: neutral evolution or novel function? *J. Reprod. Immunol.* 36(1–2):1–21
74. Davis DM, Reyburn HT, Pazmany L, Chiu I, Mandelboim O, Strominger JL. 1997. Impaired spontaneous endocytosis of HLA-G. *Eur. J. Immunol.* 27(10):2714–19
75. Park B, Lee S, Kim E, Chang S, Jin M, Ahn K. 2001. The truncated cytoplasmic tail of HLA-G serves a quality-control function in post-ER compartments. *Immunity* 15(2):213–24
76. Walpole NG, Kjer-Nielsen L, Kostenko L, McCluskey J, Brooks AG, et al. 2010. The structure and stability of the monomorphic HLA-G are influenced by the nature of the bound peptide. *J. Mol. Biol.* 397(2):467–80
77. Apps R, Gardner L, Moffett A. 2008. A critical look at HLA-G. *Trends Immunol.* 29(7):313–21
78. Loke YW, King A, Burrows T, Gardner L, Bowen M, et al. 1997. Evaluation of trophoblast HLA-G antigen with a specific monoclonal antibody. *Tissue Antigens* 50(2):135–46
79. Blaschitz A, Juch H, Volz A, Hutter H, Daxboeck C, et al. 2005. The soluble pool of HLA-G produced by human trophoblasts does not include detectable levels of the intron 4-containing HLA-G5 and HLA-G6 isoforms. *Mol. Hum. Reprod.* 11(10):699–710
80. Clements CS, Kjer-Nielsen L, Kostenko L, Hoare HL, Dunstone MA, et al. 2005. Crystal structure of HLA-G: a nonclassical MHC class I molecule expressed at the fetal-maternal interface. *PNAS* 102(9):3360–65
81. Lee N, Malacko AR, Ishitani A, Chen MC, Bajorath J, et al. 1995. The membrane-bound and soluble forms of HLA-G bind identical sets of endogenous peptides but differ with respect to TAP association. *Immunity* 3(5):591–600
82. Gonen-Gross T, Achdout H, Arnon TI, Gazit R, Stern N, et al. 2005. The CD85J/leukocyte inhibitory receptor-1 distinguishes between conformed and β2-microglobulin-free HLA-G molecules. *J. Immunol.* 175(8):4866–74
83. Boyson JE, Erskine R, Whitman MC, Chiu M, Lau JM, et al. 2002. Disulfide bond-mediated dimerization of HLA-G on the cell surface. *PNAS* 99(25):16180–85
84. Gonen-Gross T, Achdout H, Gazit R, Hanna J, Mizrahi S, et al. 2003. Complexes of HLA-G protein on the cell surface are important for leukocyte Ig-like receptor-1 function. *J. Immunol.* 171(3):1343–51
85. Hirayasu K, Arase H. 2015. Functional and genetic diversity of leukocyte immunoglobulin-like receptor and implication for disease associations. *J. Hum. Genet.* 60(11):703–8

86. Apps R, Gardner L, Sharkey AM, Holmes N, Moffett A. 2007. A homodimeric complex of HLA-G on normal trophoblast cells modulates antigen-presenting cells via LILRB1. *Eur. J. Immunol.* 37(7):1924–37
87. Shiroishi M, Kuroki K, Rasubala L, Tsumoto K, Kumagai I, et al. 2006. Structural basis for recognition of the nonclassical MHC molecule HLA-G by the leukocyte Ig-like receptor B2 (LILRB2/LIR2/ILT4/CD85d). *PNAS* 103(44):16412–17
88. Shiroishi M, Kuroki K, Ose T, Rasubala L, Shiratori I, et al. 2006. Efficient leukocyte Ig-like receptor signaling and crystal structure of disulfide-linked HLA-G dimer. *J. Biol. Chem.* 281(15):10439–47
89. Kuroki K, Matsubara H, Kanda R, Miyashita N, Shiroishi M, et al. 2019. Structural and functional basis for LILRB immune checkpoint receptor recognition of HLA-G isoforms. *J. Immunol.* 203(12):3386–94
90. Li C, Houser BL, Nicotra ML, Strominger JL. 2009. HLA-G homodimer-induced cytokine secretion through HLA-G receptors on human decidual macrophages and natural killer cells. *PNAS* 106(14):5767–72
91. Fu B, Zhou Y, Ni X, Tong X, Xu X, et al. 2017. Natural killer cells promote fetal development through the secretion of growth-promoting factors. *Immunity* 47(6):1100–13.e6
92. Marlin R, Duriez M, Berkane N, de Truchis C, Madec Y, et al. 2012. Dynamic shift from CD85j/ILT-2 to NKG2D NK receptor expression pattern on human decidual NK during the first trimester of pregnancy. *PLOS ONE* 7(1):e30017
93. Ristich V, Liang S, Zhang W, Wu J, Horuzsko A. 2005. Tolerization of dendritic cells by HLA-G. *Eur. J. Immunol.* 35(4):1133–42
94. Wu J, Horuzsko A. 2009. Expression and function of immunoglobulin-like transcripts on tolerogenic dendritic cells. *Hum. Immunol.* 70(5):353–56
95. Hanna J, Goldman-Wohl D, Hamani Y, Avraham I, Greenfield C, et al. 2006. Decidual NK cells regulate key developmental processes at the human fetal-maternal interface. *Nat. Med.* 12(9):1065–74
96. Goodridge JP, Witt CS, Christiansen FT, Warren HS. 2003. KIR2DL4 (CD158d) genotype influences expression and function in NK cells. *J. Immunol.* 171(4):1768–74
97. Witt CS, Martin A, Christiansen FT. 2000. Detection of *KIR2DL4* alleles by sequencing and SSCP reveals a common allele with a shortened cytoplasmic tail. *Tissue Antigens* 56(3):248–57
98. Faure M, Long EO. 2002. KIR2DL4 (CD158d), an NK cell-activating receptor with inhibitory potential. *J. Immunol.* 168(12):6208–14
99. Cantoni C, Falco M, Pessino A, Moretta A, Moretta L, Biassoni R. 1999. P49, a putative HLA-G1 specific inhibitory NK receptor belonging to the immunoglobulin superfamily. *J. Reprod. Immunol.* 43(2):157–65
100. Ponte M, Cantoni C, Biassoni R, Tradori-Cappai A, Bentivoglio G, et al. 1999. Inhibitory receptors sensing HLA-G1 molecules in pregnancy: Decidua-associated natural killer cells express LIR-1 and CD94/NKG2A and acquire p49, an HLA-G1-specific receptor. *PNAS* 96(10):5674–79
101. Yan WH, Fan LA. 2005. Residues Met^{76} and Gln^{79} in HLA-G α1 domain involved in KIR2DL4 recognition. *Cell Res.* 15(3):176–82
102. Moradi S, Berry R, Pymm P, Hitchen C, Beckham SA, et al. 2015. The structure of the atypical killer cell immunoglobulin-like receptor, KIR2DL4. *J. Biol. Chem.* 290(16):10460–71
103. Clements CS, Kjer-Nielsen L, McCluskey J, Rossjohn J. 2007. Structural studies on HLA-G: implications for ligand and receptor binding. *Hum. Immunol.* 68(4):220–26
104. Rajagopalan S, Long EO. 1999. A human histocompatibility leukocyte antigen (HLA)-G-specific receptor expressed on all natural killer cells. *J. Exp. Med.* 189(7):1093–100
105. Rajagopalan S, Bryceson YT, Kuppusamy SP, Geraghty DE, Van Der Meer A, et al. 2006. Activation of NK cells by an endocytosed receptor for soluble HLA-G. *PLOS Biol.* 4(1):e9
106. Rajagopalan S, Long EO. 2012. KIR2DL4 (CD158d): an activation receptor for HLA-G. *Front. Immunol.* 3:258
107. Moffett A, Shreeve N. 2022. Local immune recognition of trophoblast in early human pregnancy: controversies and questions. *Nat. Rev. Immunol.* **https://doi.org/10.1038/s41577-022-00777-2**
108. Verma S, King A, Loke YW. 1997. Expression of killer cell inhibitory receptors on human uterine natural killer cells. *Eur. J. Immunol.* 27(4):979–83
109. Manaster I, Mizrahi S, Goldman-Wohl D, Sela HY, Stern-Ginossar N, et al. 2008. Endometrial NK cells are special immature cells that await pregnancy. *J. Immunol.* 181(3):1869–76

110. Mcgrath E, Ryan EJ, Lynch L, Golden-Mason L, Mooney E, et al. 2009. Changes in endometrial natural killer cell expression of CD94, CD158a and CD158b are associated with infertility. *Am. J. Reprod. Immunol.* 61(4):265–76
111. Sharkey AM, Xiong S, Kennedy PR, Gardner L, Farrell LE, et al. 2015. Tissue-specific education of decidual NK cells. *J. Immunol.* 195(7):3026–32
112. Ivarsson MA, Stiglund N, Marquardt N, Westgren M, Gidlöf S, Björkström NK. 2017. Composition and dynamics of the uterine NK cell KIR repertoire in menstrual blood. *Mucosal Immunol.* 10(2):322–31
113. Suryawanshi H, Morozov P, Straus A, Sahasrabudhe N, Max KEA, et al. 2018. A single-cell survey of the human first-trimester placenta and decidua. *Sci. Adv.* 4(10):eaau4788
114. Garcia-Alonso L, Handfield LF, Roberts K, Nikolakopoulou K, Fernando RC, et al. 2021. Mapping the temporal and spatial dynamics of the human endometrium in vivo and in vitro. *Nat. Genet.* 53(12):1698–711
115. Whettlock EM, Von Woon E, Cuff AO, Browne B, Johnson MR, Male V. 2022. Dynamic changes in uterine NK cell subset frequency and function over the menstrual cycle and pregnancy. *Front. Immunol.* 13:880438
116. Pique-Regi R, Romero R, Tarca AL, Sendler ED, Xu Y, et al. 2019. Single cell transcriptional signatures of the human placenta in term and preterm parturition. *eLife* 8:e52004
117. Vazquez J, Chasman DA, Lopez GE, Tyler CT, Ong IM, Stanic AK. 2020. Transcriptional and functional programming of decidual innate lymphoid cells. *Front. Immunol.* 10:3065
118. Guo C, Cai P, Jin L, Sha Q, Yu Q, et al. 2021. Single-cell profiling of the human decidual immune microenvironment in patients with recurrent pregnancy loss. *Cell Discov.* 7:1
119. Zhou Y, Fu B, Xu X, Zhang J, Tong X, et al. 2020. PBX1 expression in uterine natural killer cells drives fetal growth. *Sci. Transl. Med.* 12(537):eaax1798
120. Chen P, Zhou L, Chen J, Lu Y, Cao C, et al. 2021. The immune atlas of human deciduas with unexplained recurrent pregnancy loss. *Front. Immunol.* 12:689019
121. Wu Z, Wang M, Liang G, Jin P, Wang P, et al. 2021. Pro-inflammatory signature in decidua of recurrent pregnancy loss regardless of embryonic chromosomal abnormalities. *Front. Immunol.* 12:772729
122. Strunz B, Bister J, Jönsson H, Filipovic I, Crona-Guterstam Y, et al. 2021. Continuous human uterine NK cell differentiation in response to endometrial regeneration and pregnancy. *Sci. Immunol.* 6(56):eabb7800
123. Krop J, van der Zwan A, Ijsselsteijn ME, Kapsenberg H, Luk SJ, et al. 2022. Imaging mass cytometry reveals the prominent role of myeloid cells at the maternal-fetal interface. *iScience* 25(7):104648
124. Soares MJ, Varberg KM, Iqbal K. 2018. Hemochorial placentation: development, function, and adaptations. *Biol. Reprod.* 99(1):196–211
125. Brosens I, Pijnenborg R, Vercruysse L, Romero R. 2011. The "Great Obstetrical Syndromes" are associated with disorders of deep placentation. *Am. J. Obstet. Gynecol.* 204(3):193–201
126. Duffy JMN, Cairns AE, Magee LA, von Dadelszen P, van 't Hooft J, et al. 2020. Standardising definitions for the pre-eclampsia core outcome set: a consensus development study. *Pregnancy Hypertens.* 21:208–17
127. Hiby SE, Walker JJ, O'Shaughnessy KM, Redman CWG, Carrington M, et al. 2004. Combinations of maternal KIR and fetal HLA-C genes influence the risk of preeclampsia and reproductive success. *J. Exp. Med.* 200(8):957–65
128. Nakimuli A, Chazara O, Hiby SE, Farrell L, Tukwasibwe S, et al. 2015. A KIR B centromeric region present in Africans but not Europeans protects pregnant women from pre-eclampsia. *PNAS* 112(3):845–50
129. Kelemu T, Erlandsson L, Seifu D, Hansson E, Abebe M, et al. 2020. Polymorphism in killer cell immunoglobulin-like receptors and human leukocyte antigen-c and predisposition to preeclampsia in Ethiopian pregnant women population. *J. Reprod. Immunol.* 141:103169
130. Huhn O, Chazara O, Ivarsson MA, Retière C, Venkatesan TC, et al. 2018. High-resolution genetic and phenotypic analysis of KIR2DL1 alleles and their association with pre-eclampsia. *J. Immunol.* 201(9):2593–601. Erratum. 2019. *J. Immunol.* 202(6):1904
131. Xiong S, Sharkey AM, Kennedy PR, Gardner L, Farrell LE, et al. 2013. Maternal uterine NK cell-activating receptor KIR2DS1 enhances placentation. *J. Clin. Investig.* 123(10):4264–72

132. Hiby SE, Apps R, Sharkey AM, Farrell LE, Gardner L, et al. 2010. Maternal activating KIRs protect against human reproductive failure mediated by fetal HLA-C2. *J. Clin. Investig.* 120(11):4102–10
133. Hiby SE, Apps R, Chazara O, Farrell LE, Magnus P, et al. 2014. Maternal KIR in combination with paternal HLA-C2 regulate human birth weight. *J. Immunol.* 192(11):5069–73
134. Clark MM, Chazara O, Sobel EM, Gjessing HK, Magnus P, et al. 2016. Human birth weight and reproductive immunology: testing for interactions between maternal and offspring KIR and HLA-C genes. *Hum. Hered.* 81(4):181–93
135. Blokhuis JH, Hilton HG, Guethlein LA, Norman PJ, Nemat-Gorgani N, et al. 2017. KIR2DS5 allotypes that recognize the C2 epitope of HLA-C are common among Africans and absent from Europeans. *Immunity Inflamm. Dis.* 5(4):461–68
136. Varla-Leftherioti M, Spyropoulou-Vlachou M, Niokou D, Keramitsoglou T, Darlamitsou A, et al. 2003. Natural killer (NK) cell receptors' repertoire in couples with recurrent spontaneous abortions. *Am. J. Reprod. Immunol.* 49(3):183–91
137. Witt CS, Goodridge J, Gerbase-DeLima MG, Daher S, Christiansen FT. 2004. Maternal KIR repertoire is not associated with recurrent spontaneous abortion. *Hum. Reprod.* 19(11):2653–57
138. Akbari S, Shahsavar F, Karami R, Yari F, Anbari K, Ahmadi SAY. 2020. Recurrent spontaneous abortion (RSA) and maternal KIR genes: a comprehensive meta-analysis. *JBRA Assist. Reprod.* 24(2):197–213
139. Faridi RM, Das V, Tripthi G, Talwar S, Parveen F, Agrawal S. 2009. Influence of activating and inhibitory killer immunoglobulin-like receptors on predisposition to recurrent miscarriages. *Hum. Reprod.* 24(7):1758–64
140. Hiby SE, Regan L, Lo W, Farrell L, Carrington M, Moffett A. 2008. Association of maternal killer-cell immunoglobulin-like receptors and parental HLA-C genotypes with recurrent miscarriage. *Hum. Reprod.* 23(4):972–76
141. Faridi RM, Agrawal S. 2011. Killer immunoglobulin-like receptors (KIRs) and HLA-C allorecognition patterns implicative of dominant activation of natural killer cells contribute to recurrent miscarriages. *Hum. Reprod.* 26(2):491–97
142. Vargas RG, Bompeixe EP, França PP, Marques de Moraes M, da Graça Bicalho M. 2009. Activating killer cell immunoglobulin-like receptor genes' association with recurrent miscarriage. *Am. J. Reprod. Immunol.* 62(1):34–43
143. Nowak I, Malinowski A, Tchórzewski H, Barcz E, Wilczyński JR, et al. 2009. Frequencies of killer immunoglobulin-like receptor genotypes influence susceptibility to spontaneous abortion. *J. Appl. Genet.* 50(4):391–98
144. Su N, Wang H, Zhang B, Kang Y, Guo Q, et al. 2018. Maternal natural killer cell immunoglobulin receptor genes and human leukocyte antigen-C ligands influence recurrent spontaneous abortion in the Han Chinese population. *Exp. Ther. Med.* 15(1):327–37
145. Mansour L, Alkhuriji A, Babay ZA, Alqadheeb S, Al-Khulaifi F, et al. 2020. Association of killer immunoglobulin-like receptor and human leukocyte antigen class I ligand with recurrent abortion in Saudi women. *Genet. Test. Mol. Biomarkers* 24(2):78–84
146. Alecsandru D, Barrio A, Garrido N, Aparicio P, Pellicer A, et al. 2020. Parental human leukocyte antigen-C allotypes are predictive of live birth rate and risk of poor placentation in assisted reproductive treatment. *Fertil. Steril.* 114(4):809–17
147. Wilczyńska K, Wiśniewski A, Malinowski A, Barcz E, Wilczyński JR, et al. 2019. *ERAP*, *KIR* and *HLA-C* gene interaction in susceptibility to recurrent spontaneous abortion in the Polish population. *Hum. Immunol.* 80(5):344–48
148. Long W, Shi Z, Fan S, Liu L, Lu Y, et al. 2015. Association of maternal KIR and fetal HLA-C genes with the risk of preeclampsia in the Chinese Han population. *Placenta* 36(4):433–37
149. Larsen TG, Hackmon R, Geraghty DE, Hviid TVF. 2019. Fetal human leukocyte antigen-C and maternal killer-cell immunoglobulin-like receptors in cases of severe preeclampsia. *Placenta* 75:27–33
150. Saito S, Takeda Y, Sakai M, Nakabayahi M, Hayakawa S. 2006. The incidence of pre-eclampsia among couples consisting of Japanese women and Caucasian men. *J. Reprod. Immunol.* 70(1–2):93–98
151. Moffett A, Hiby S, Carrington M. 2006. Letter in response to article by Saito et al. *J. Reprod. Immunol.* 71(2):132–33

152. Chazara O, Moffett A. 2015. Association of maternal KIR and fetal HLA-C genes with the risk of preeclampsia in the Chinese Han population, Long et al. *Placenta* 36(8):967
153. Moffett A, Hiby S. 2009. Influence of activating and inhibitory killer immunoglobulin-like receptors on predisposition to recurrent miscarriages. *Hum. Reprod.* 24(8):2048–49
154. De Oliveira LG, Lash GE, Murray-Dunning C, Bulmer JN, Innes BA, et al. 2010. Role of interleukin 8 in uterine natural killer cell regulation of extravillous trophoblast cell invasion. *Placenta* 31(7):595–601
155. Wallace AE, Host AJ, Whitley GS, Cartwright JE. 2013. Decidual natural killer cell interactions with trophoblasts are impaired in pregnancies at increased risk of preeclampsia. *Am. J. Pathol.* 183(6):1853–61
156. Apps R, Sharkey A, Gardner L, Male V, Kennedy P, et al. 2011. Ex vivo functional responses to HLA-G differ between blood and decidual NK cells. *Mol. Hum. Reprod.* 17(9):577–86
157. Kennedy PR, Chazara O, Gardner L, Ivarsson MA, Farrell LE, et al. 2016. Activating KIR2DS4 is expressed by uterine NK cells and contributes to successful pregnancy. *J. Immunol.* 197(11):4292–300
158. Abbas Y, Oefner CM, Polacheck WJ, Gardner L, Farrell L, et al. 2017. A microfluidics assay to study invasion of human placental trophoblast cells. *J. R. Soc. Interface* 14(130):20170131
159. Zhang SM, Tian FJ, Zeng WH, Ma XL, Ren J-B, Lin Y. 2018. XCL1-XCR1 pathway promotes trophoblast invasion at maternal-fetal interface by inducing MMP-2/MMP-9 activity. *Am. J. Reprod. Immunol.* 80(3):e12990
160. Pijnenborg R, Vercruysse L, Hanssens M. 2006. The uterine spiral arteries in human pregnancy: facts and controversies. *Placenta* 27(9–10):939–58
161. Kam EPY, Gardner L, Loke YW, King A. 1999. The role of trophoblast in the physiological change in decidual spiral arteries. *Hum. Reprod.* 14(8):2131–38
162. Craven CM, Morgan T, Ward K. 1998. Decidual spiral artery remodelling begins before cellular interaction with cytotrophoblasts. *Placenta* 19(4):241–52
163. Gambino LS, Wrefordm NG, Bertram JF, Dockery P, Lederman F, Rogers PAW. 2002. Angiogenesis occurs by vessel elongation in proliferative phase human endometrium. *Hum. Reprod.* 17(5):1199–206
164. Smith SD, Dunk CE, Aplin JD, Harris LK, Jones RL. 2009. Evidence for immune cell involvement in decidual spiral arteriole remodeling in early human pregnancy. *Am. J. Pathol.* 174(5):1959–71
165. Hazan AD, Smith SD, Jones RL, Whittle W, Lye SJ, Dunk CE. 2010. Vascular-leukocyte interactions: mechanisms of human decidual spiral artery remodeling in vitro. *Am. J. Pathol.* 177(2):1017–30
166. Robson A, Harris LK, Innes BA, Lash GE, Aljunaidy MM, et al. 2012. Uterine natural killer cells initiate spiral artery remodeling in human pregnancy. *FASEB J.* 26(12):4876–85
167. Fraser R, Whitley GSJ, Thilaganathan B, Cartwright JE. 2015. Decidual natural killer cells regulate vessel stability: implications for impaired spiral artery remodelling. *J. Reprod. Immunol.* 110:54–60
168. Lash GE, Schiessl B, Kirkley M, Innes BA, Cooper A, et al. 2006. Expression of angiogenic growth factors by uterine natural killer cells during early pregnancy. *J. Leukoc. Biol.* 80(3):572–80
169. Turco MY, Gardner L, Kay RG, Hamilton RS, Prater M, et al. 2018. Trophoblast organoids as a model for maternal-fetal interactions during human placentation. *Nature* 564(7735):263–67
170. Haider S, Meinhardt G, Saleh L, Kunihs V, Gamperl M, et al. 2018. Self-renewing trophoblast organoids recapitulate the developmental program of the early human placenta. *Stem Cell Rep.* 11(2):537–51
171. Okae H, Toh H, Sato T, Hiura H, Takahashi S, et al. 2018. Derivation of human trophoblast stem cells. *Cell Stem Cell* 22(1):50–63.e6
172. Megli CJ, Coyne CB. 2022. Infections at the maternal-fetal interface: an overview of pathogenesis and defence. *Nat. Rev. Microbiol.* 20(2):67–82
173. Quinnan GV, Kirmani N, Rook AH, Manischewitz JF, Jackson L, et al. 1982. Cytotoxic T cells in cytomegalovirus infection: HLA-restricted T-lymphocyte and non-T-lymphocyte cytotoxic responses correlate with recovery from cytomegalovirus infection in bone-marrow-transplant recipients. *N. Engl. J. Med.* 307(1):7–13
174. Biron CA, Byron KS, Sullivan JL. 1989. Severe herpesvirus infections in an adolescent without natural killer cells. *N. Engl. J. Med.* 320(26):1731–35
175. Cottineau J, Kottemann MC, Lach FP, Kang YH, Vély F, et al. 2017. Inherited GINS1 deficiency underlies growth retardation along with neutropenia and NK cell deficiency. *J. Clin. Investig.* 127(5):1991–2006

176. Enders G, Daiminger A, Bäder U, Exler S, Enders M. 2011. Intrauterine transmission and clinical outcome of 248 pregnancies with primary cytomegalovirus infection in relation to gestational age. *J. Clin. Virol.* 52(3):244–46
177. Siewiera J, El Costa H, Tabiasco J, Berrebi A, Cartron G, et al. 2013. Human cytomegalovirus infection elicits new decidual natural killer cell effector functions. *PLOS Pathog.* 9(4):e1003257
178. Crespo ÂC, Strominger JL, Tilburgs T. 2016. Expression of KIR2DS1 by decidual natural killer cells increases their ability to control placental HCMV infection. *PNAS* 113(52):15072–77
179. Hammer Q, Rückert T, Borst EM, Dunst J, Haubner A, et al. 2018. Peptide-specific recognition of human cytomegalovirus strains controls adaptive natural killer cells. *Nat. Immunol.* 19(5):453–63
180. Feyaerts D, van der Meer A, Joosten I, van der Molen RG. 2019. Selective expansion and CMV-dependency in pregnancy trained human endometrial NK cells. *Cell. Mol. Immunol.* 16(4):410–11
181. de Mendonça Vieira R, Meagher A, Crespo ÂC, Kshirsagar SK, Iyer V, et al. 2020. Human term pregnancy decidual NK cells generate distinct cytotoxic responses. *J. Immunol.* 204(12):3149–59
182. Sen Santara S, Angela AC, Mulik S, Ovies C, Boulenouar S, et al. 2021. Decidual NK cells kill Zika virus-infected trophoblasts. *PNAS* 118(47):e2115410118
183. Crespo ÂC, Mulik S, Dotiwala F, Ansara JA, Sen Santara S, et al. 2020. Decidual NK cells transfer granulysin to selectively kill bacteria in trophoblasts. *Cell* 182(5):1125–39.e18
184. Male V. 2022. SARS-CoV-2 infection and COVID-19 vaccination in pregnancy. *Nat. Rev. Immunol.* 22(5):277–82
185. Lu-Culligan A, Chavan AR, Vijayakumar P, Irshaid L, Courchaine EM, et al. 2021. Maternal respiratory SARS-CoV-2 infection in pregnancy is associated with a robust inflammatory response at the maternal-fetal interface. *Medicine* 2(5):591–610.e10
186. Garcia-Flores V, Romero R, Xu Y, Theis KR, Arenas-Hernandez M, et al. 2022. Maternal-fetal immune responses in pregnant women infected with SARS-CoV-2. *Nat. Commun.* 13(1):320
187. Keskin DB, Allan DSJ, Rybalov B, Andzelm MM, Stern JNH, et al. 2007. TGFβ promotes conversion of $CD16^+$ peripheral blood NK cells into $CD16^-$ NK cells with similarities to decidual NK cells. *PNAS* 104(9):3378–83
188. Lynch L, Golden-Mason L, Eogan M, O'Herlihy C, O'Farrelly C. 2007. Cells with haematopoietic stem cell phenotype in adult human endometrium: relevance to infertility? *Hum. Reprod.* 22(4):919–26
189. Vacca P, Vitale C, Montaldo E, Conte R, Cantoni C, et al. 2011. $CD34^+$ hematopoietic precursors are present in human decidua and differentiate into natural killer cells upon interaction with stromal cells. *PNAS* 108(6):2402–7
190. Male V, Hughes T, McClory S, Colucci F, Caligiuri MA, Moffett A. 2010. Immature NK cells, capable of producing IL-22, are present in human uterine mucosa. *J. Immunol.* 185(7):3913–18
191. Taylor HS. 2004. Endometrial cells derived from donor stem cells in bone marrow transplant recipients. *JAMA* 292(1):81–85
192. Sentman CL, Meadows SK, Wira CR, Eriksson M. 2004. Recruitment of uterine NK cells: induction of CXC chemokine ligands 10 and 11 in human endometrium by estradiol and progesterone. *J. Immunol.* 173(11):6760–66
193. Carlino C, Stabile H, Morrone S, Bulla R, Soriani A, et al. 2008. Recruitment of circulating NK cells through decidual tissues: a possible mechanism controlling NK cell accumulation in the uterus during early pregnancy. *Blood* 111(6):3108–15
194. Chantakru S, Wang W-C, van den Heuvel M, Bashar S, Simpson A, et al. 2003. Coordinate regulation of lymphocyte-endothelial interactions by pregnancy-associated hormones. *J. Immunol.* 171(8):4011–19
195. Yamaguchi T, Kitaya K, Daikoku N, Yasuo T, Fushiki S, Honjo H. 2006. Potential selectin L ligands involved in selective recruitment of peripheral blood $CD16^-$ natural killer cells into human endometrium. *Biol. Reprod.* 74(1):35–40
196. Höglund P, Brodin P. 2010. Current perspectives of natural killer cell education by MHC class I molecules. *Nat. Rev. Immunol.* 10(10):724–34
197. 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. Erratum. 2017. *Nat. Commun.* 8:15444

198. Shreeve N, Depierreux D, Hawkes D, Traherne JA, Sovio U, et al. 2021. The CD94/NKG2A inhibitory receptor educates uterine NK cells to optimize pregnancy outcomes in humans and mice. *Immunity* 54(6):1231–44.e4
199. Wang X, Peng H, Tian Z. 2019. Innate lymphoid cell memory. *Cell. Mol. Immunol.* 16(5):423–29
200. Goldman-Wohl D, Gamliel M, Mandelboim O, Yagel S. 2019. Learning from experience: cellular and molecular bases for improved outcome in subsequent pregnancies. *Am. J. Obstet. Gynecol.* 221(3):183–93
201. Sojka DK. 2020. Uterine natural killer cell heterogeneity: lessons from mouse models. *Front. Immunol.* 11:290
202. Huhn O, Zhao X, Esposito L, Moffett A, Colucci F, Sharkey AM. 2021. How do uterine natural killer and innate lymphoid cells contribute to successful pregnancy? *Front. Immunol.* 12:607669
203. Filipovic I, Chiossone L, Vacca P, Hamilton RS, Ingegnere T, et al. 2018. Molecular definition of group 1 innate lymphoid cells in the mouse uterus. *Nat. Commun.* 9(1):4492
204. Bulmer JN, Morrison L, Longfellow M, Ritson A, Pace D. 1991. Granulated lymphocytes in human endometrium: histochemical and immunohistochemical studies. *Hum. Reprod.* 6(6):791–98
205. Kaur G, Porter CBM, Ashenberg O, Lee J, Riesenfeld SJ, et al. 2022. Mouse fetal growth restriction through parental and fetal immune gene variation and intercellular communications cascade. *Nat. Commun.* 13(1):4398
206. Abbas Y, Turco MY, Burton GJ, Moffett A. 2020. Investigation of human trophoblast invasion in vitro. *Hum. Reprod. Update* 26(4):501–13
207. Moffett A, Chazara O, Colucci F, Johnson MH. 2016. Variation of maternal KIR and fetal HLA-C genes in reproductive failure: too early for clinical intervention. *Reprod. Biomed. Online* 33(6):763–69

Annual Review of Immunology
Volume 41, 2023

Contents

Errata

An online log of corrections to *Annual Review of Immunology* articles may be found at http://www.annualreviews.org/errata/immunol