The role of uterine NK cells in the etiology of preeclampsia

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Abstract

Preeclampsia is a leading cause of maternal and fetal mortality and morbidity, complicating 2-8% of all pregnancies worldwide. Although the exact etiology of preeclampsia is unknown, it is widely accepted that insufficient placentation through defects in spiral artery remodelling is involved. Uterine natural killer cells (uNK cells) are maternal leukocytes which have several important roles in spiral artery remodelling. As both uNK cells and preeclampsia are related to arterial remodelling, they are most likely also related to each other. In this essay I have elaborated on the role of uNK cells in the etiology of preeclampsia. For arterial remodelling, invasion of extravillous trophoblast into maternal spiral arteries is essential. Furthermore, cross-talk between the uNK cells, extravillous trophoblasts and vascular cells is essential. Cross-talk occurs either through natural killer receptor-ligand interactions or soluble factors. Several studies have indicated that altered cross-talk contributes to preeclampsia. They reported that secreted factors of uNK cells isolated out of high RI pregnancies show reduced endothelial cell activation, ECM destruction and vascular apoptosis. Furthermore, secreted factors of these cells also failed to successfully recruit EVTs. In addition, genetic studies have reported that the combination of maternal AA KIRs with fetal HLA-C2s increases the risk of preeclampsia. To summarize, insufficient arterial remodelling associated with preeclampsia appears to be the result of an altered activation state of uNK cells. Alterations in the secretion state of uNK cells affect the initial stages of remodelling but also trophoblast recruitment and invasion into the spiral arteries. Furthermore, uNK cell – EVT receptor interactions are also involved.

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Introduction

Preeclampsia is a leading cause of maternal and fetal mortality and morbidity, complicating 2-8% of all pregnancies worldwide (1). Preeclampsia is defined by the onset of hypertension along with proteinuria, during the second half of pregnancy and is characterized by a systemic inflammatory response and generalized vascular dysfunction of the maternal body (2). These processes can affect multiple organ systems, e.g. the central nervous system, renal system, respiratory system, coagulation system and/or liver. In addition, placental involvement means that the fetus is also at risk (1,3).

Generally, the outcome for preeclampsia is good. However, the disorder may progress into rare but potential fatal complications as eclampsia (seizures), HELPP syndrome (haemolysis, elevated liver enzymes and low platelets), disseminated intravascular coagulation (DIS) and placenta abruption or infarction (1). If these complications are left untreated they ultimately result in the death of either mother or child or both of them. The only known treatment for preeclampsia is the delivery of the placenta, suggesting a leading role for the placenta in the disease etiology (2).

The placenta is essential for pregnancy, as it is the organ which connects the mother and fetus. The major function of the placenta is to provide a system for the exchange of nutrients, waste and respiratory gasses between maternal and fetal blood. Other important functions are the secretion of hormones and the protection of the fetus (4,5).

The placenta is a discoid structure and consists out of the fetal chorionic plate and the maternal decidua **(Fig.1)**, the specialized uterine mucosa (endometrium) which is present during pregnancy. The cavity between these structures, called the intervillous space, contains multiple protrusions of fetal villous tree structures. Maternal blood originating from uterine spiral arteries fills the intervillous space, bathing the fetal villi in blood. The villi surface is thin and highly vascularized by fetal capillaries, making the distance between both circulations is short enough to allow for maternal-fetal exchange (6). In addition, the fetal villi can be divided in floating and anchoring villi. Anchoring villi extend from the chorionic plate to the decidua and provide support. Branching off from these anchoring villi are floating villi, which are involved in maternal-fetal exchange (7,8).

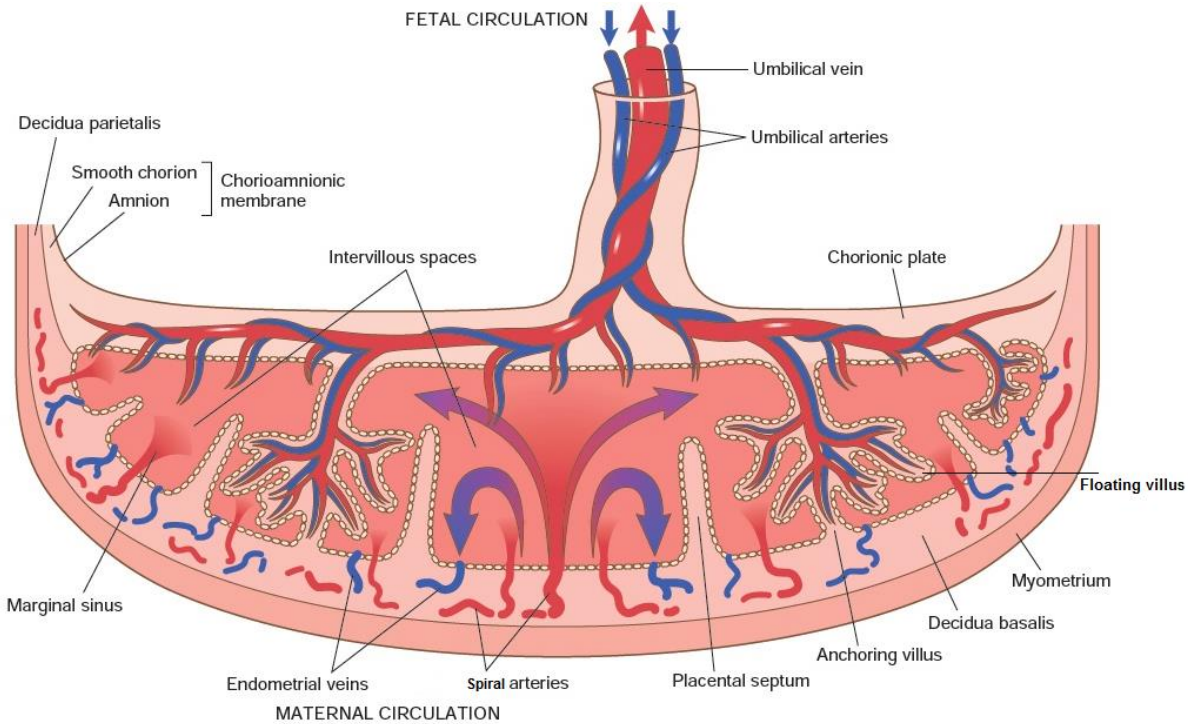

Figure 1. Anatomy of the placenta. The placenta is composed out of the fetal chorionic plate, villi (anchoring and floating) and maternal decidua. The chorionic villi are in contact with maternal blood that enters the intervillous spaces through spiral arteries (72).

The development of the placenta takes place directly after the implantation of the blastocyst, the fluid filled spherical structure of a fertilized ovum five days into the development. The structure consists out of an inner cell mass, which is called the embryoblast and will develop into the embryo, and an outer cell mass, called the trophoblast. The trophoblast cells differentiate in highly proliferative cytotrophoblast cells **(Fig.1)**, which will proliferate and fuse together to form a highly invasive syncytiotrophoblast (ST) outer layer. The ST leads the invasion of the blastocyst into the decidua. As the blastocyst is implanted the cytotrophoblasts and ST contribute to the formation of the floating villi. At the top of some floating villi the cytotrophoblasts penetrate the ST outer layer to attach to the decidua, forming the anchoring villi. Subsequently, these cytotrophoblast invade the decidua becoming extravillous cytotrophoblasts (EVTs) (7,8).

These EVTs play an important role in the establishment of adequate perfusion of the placenta. The EVTs penetrate one third of the myometrium, and subsequently invade the maternal spiral arteries to remodel them. Spiral arteries are small maternal arteries which supply blood to the endometrium during the secretory phase of the menstrual cycle. However, remodelling of these arteries is necessary to meet the requirements of the placenta and fetus during pregnancy. Remodelling involves EVT mediated degradation and replacement of muscular media and endothelial cells **(Fig.)**. As a result, the arteries are transformed from narrow, high resistance, low flow vessels to dilated, low resistance, high flow vessels that lack maternal vasomotor control (9).

Although the EVTs are very important for arterial remodelling, the initial stages take place without EVTs. Based upon histological research, arterial remodelling has been divided in four stages **(Fig.2)**. The first stage consists of activation and dilatation of muscular media and endothelial cells. No EVTs are present at this stage. In stage two maternal leukocytes infiltrate the vascular wall and mediate disruption and disorganization of the vascular layers. This process continues in stage three, but is distinguished by the new presence of EVTs. In the final stage the spiral arteries are completely remodelled, the EVTs have completely replaced the arterial wall (9,10). So, maternal leukocytes are along with the EVTs involved in placentation.

During implantation and placentation at least 40% of the cells in the decidua are maternal leukocytes (11). The most abundant leukocyte is the uterine natural killer cell (uNK cell), which make up for more than 70% of the resident leukocytes (12). uNK cells are a special subtype of natural killer cells, with several unique characteristics. They are characterized by a low cytotoxic capacities, and a good capacity to secrete cytokines, chemokines and angiogenic factors (13). uNK cells have several roles in the regulation of spiral artery remodelling, in both EVT independent and dependent stages (10).

Arterial remodelling is a complex process with multiple steps, defects in each of these steps can contribute to insufficient remodelling. Although the exact etiology of preeclampsia is unknown, it is widely accepted that insufficient placentation, through defects in remodelling are involved **(Fig. 3)** (3). Because uNK cells are important in arterial remodelling, they are probably also involved when remodelling is insufficient. Therefore, in this essay I am going to discuss the role of uNK cells in the etiology of preeclampsia.

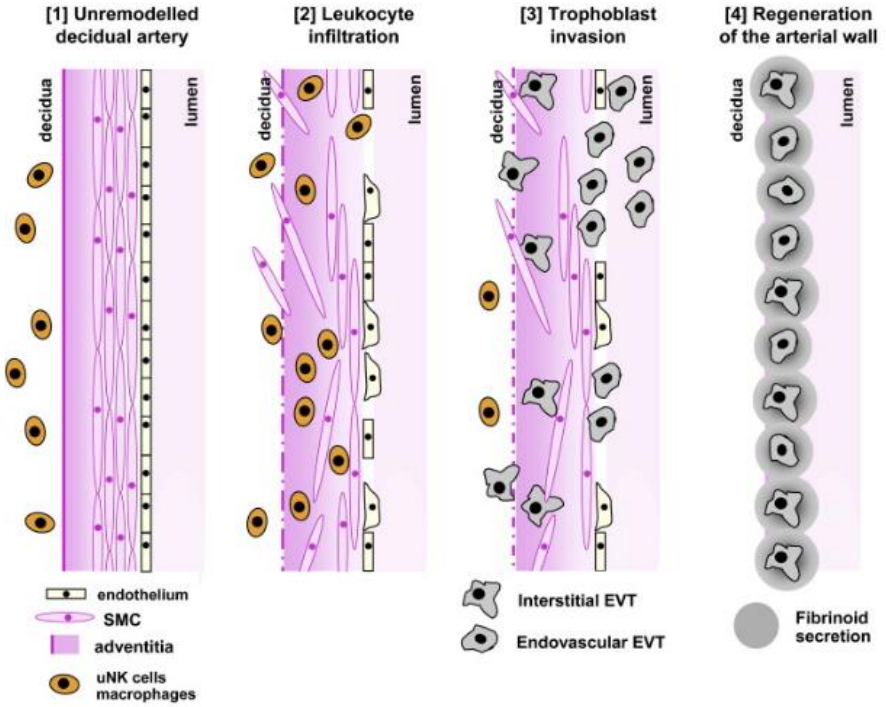

Figure 2. The four stages of spiral artery remodelling. 1) the unremodelled decidual artery **2)** leukocyte infiltration **3)** trophoblast invasion **4)** regeneration of the arterial wall (9)**.**

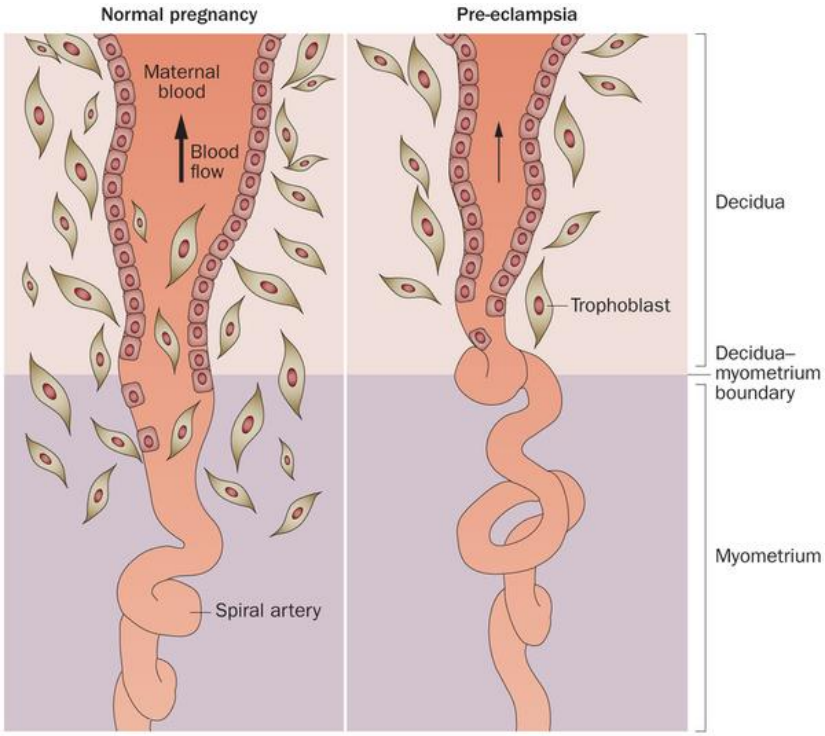

Figure 3. Spiral artery remodelling. Successful **r**emodelling in normal pregnancy (left) and failure in preeclampsia (right) (14).

Chapter 1: Normal pregnancy

1.1 Placentation

After fertilization, the zygote undergoes mitotic cell divisions while it migrates from the fallopian tube towards the uterus. At day 5 the structure enters the uterus and is called the blastocyst **(Fig.4A)**. The blastocyst consists out of an inner cell mass, which will develop into the embryo, and an outer layer, called the trophoblast. Trophoblast cells are the earliest cells to differentiate from the zygote, and play a leading role in implantation of the blastocyst and the development of the placenta (7,8)

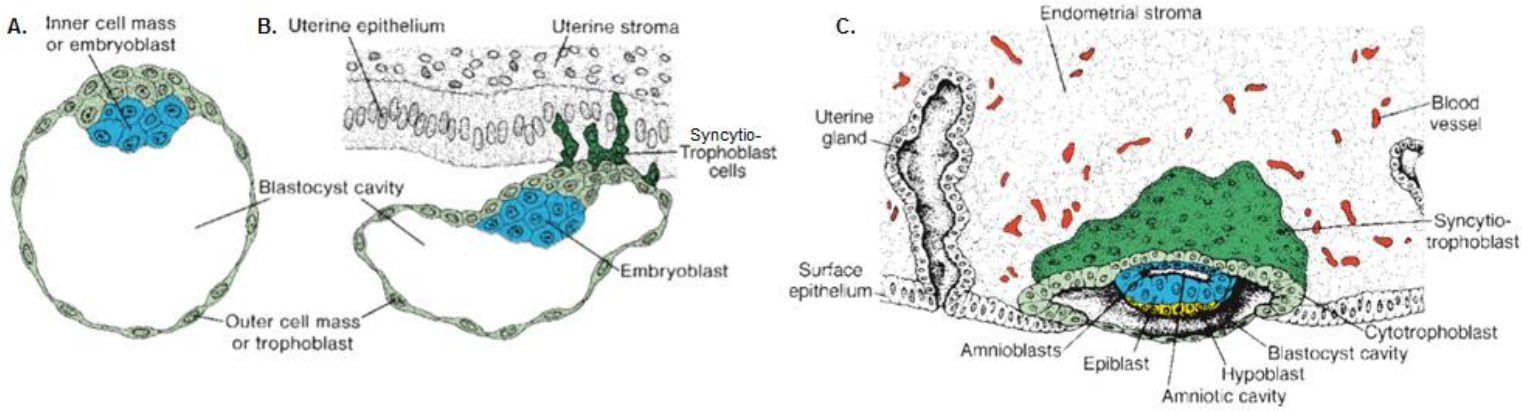

Figure 4. A) Human blastocyst with inner (blue) and outer (green) cell mass at day 4.5. **B)** Schematic representation of the penetration of the uterine epithelium by syncytiotrophoblast (dark green) cells at day 6 and **C)** day 7.5. Adapted from (8).

To prepare the uterus for implantation and placentation the endometrium undergoes decidualization. This reaction starts in the late secretory phase of the menstrual cycle under influence of progesterone, which is secreted by the corpus luteum (follicular remnant after ovulation). Decidualization is characterized by an enlargement of stromal cells due to the accumulation of glycogen and lipids, an increase of glandular epithelial secretion and oedema. The endometrium is now called the decidua (7,8).

At the beginning of implantation fetal trophoblast cells differentiate into multinucleated cytotrophoblasts. These cytotrophoblasts proliferate, aggregate and fuse to form a highly invasive mononucleated ST outer layer (15). The ST adheres to the uterine wall and penetrate the epithelium to invade the decidual stroma **(Fig.4B and C)**. As implantation progresses vacuoles appear in the syncytium, which form lacunae when they fuse together **(Fig. 6A)**. As the ST penetrates further into the decidua they erode maternal capillaries **(Fig. 6B)**. Blood of these capillaries fills the lacunae, forming the intervillous space, which is the beginning of uteroplacental circulation **(Fig.6C and D)** (8,16).

The next step in placental development is the formation of floating villous structures (also called villous trophoblast) **(Fig.6C and D)**. At first, cytotrophoblasts penetrate into the syncytium and form columns surrounded by ST, the primary villi **(Fig.5 A)**. Subsequently, fetal mesenchymal cells penetrate into the core of the columns, changing them into secondary villi **(Fig.5B)**. The definitive placental villi are formed as the fetal mesenchymal cells differentiate into connective tissue and vascular cells **(Fig.5C)**. These vascular cells connect with embryonal circulation, and thereby indirectly connect the placenta and the embryo. The villous structures are in direct contact with maternal blood as they float in the intervillous space. Therefore, the ST forms a structural and biomolecular barrier between the fetal and maternal circulation. In addition, the ST produces most of the placental hormones (8,16).

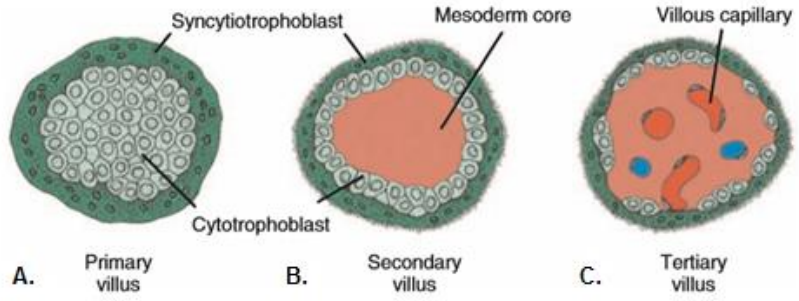

Figure 5. Developmental stages of the floating villi. Transverse section of **A)** a primary villus **B)** secondary villus and **C)** tertiary villus (adapted from (8)).

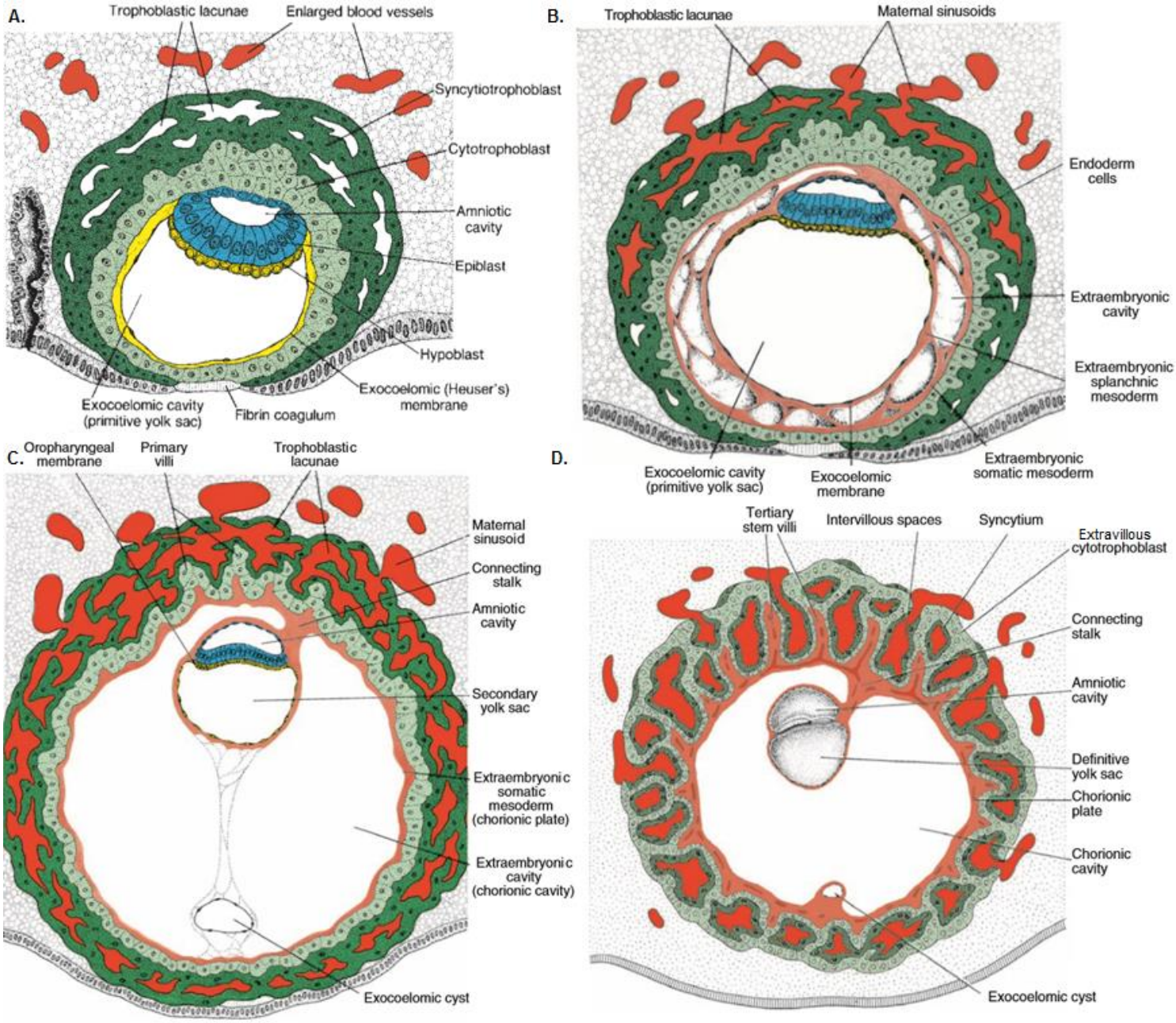

Figure 6. Major stages in implantation and placentation. A blastocyst at **A)** 9-days, **B)** A 12-days, **C)** A 13-days and. **D)** the end of the third week (Adapted from (8)).

During the first 20 weeks of pregnancy, cytotrophoblasts detach from the floating villi to aggregate into columns that attach to the decidua (anchoring villi) **(Fig.7 and 8)**. These cytotrophoblasts are called the extravillous cytotrophoblasts (EVTs). Individual EVTs at the end of the columns migrate through the decidua to the inner third of the myometrium (interstitial EVT), or invade maternal spiral arteries to remodel them (endovascular EVT). During spiral artery remodelling EVTs contribute to the degradation and replacement of vascular smooth muscle cells (VSMC) and endothelial cell layers to create large diameter vessels with low resistance that lack maternal vasomotor control (17–19). This modification prevents the high velocity of the maternal blood to potentially damage the trophoblast villi, compress the fetal vessels in the villi and cause oxidative stress. Furthermore, the adaptations to the spiral arteries are necessary to meet the demand for blood from the placenta as pregnancy progresses (16).

Although the EVTs are crucial for vascular remodelling, initial stages of remodelling occur in the absence of EVTs **(Fig.2)**. At first the arteries show decidualization-associated signs of endothelial cell activation and vascular smooth muscle cell (VSMC) hypertrophy (20). Subsequently maternal leukocytes (macrophage and uNK cells) infiltrate the vascular wall and initiate disorganization and disruption of VSMCs and endothelial cell layers (10,20). The next stage apprehends substantial loss of the VSMCs and endothelial cells, combined with the presence of EVTs. In the fully remodelled arteries the VSMC layer is completely absent, fibrinoid deposition is present and endothelial cells are replaced by endovascular EVTs (9,10).

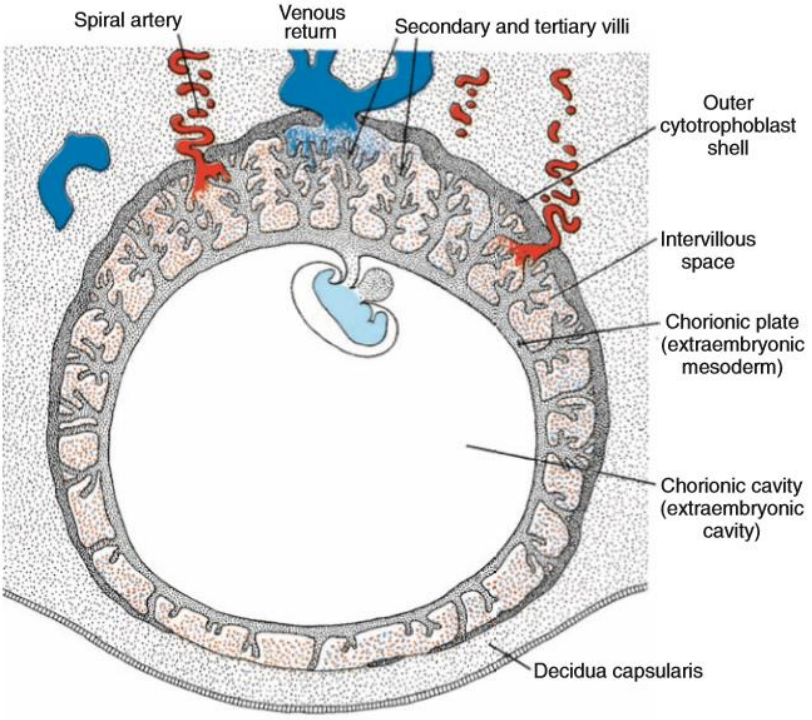

Figure 7. Human embryonal development. Human embryo at the beginning of the second month (8).

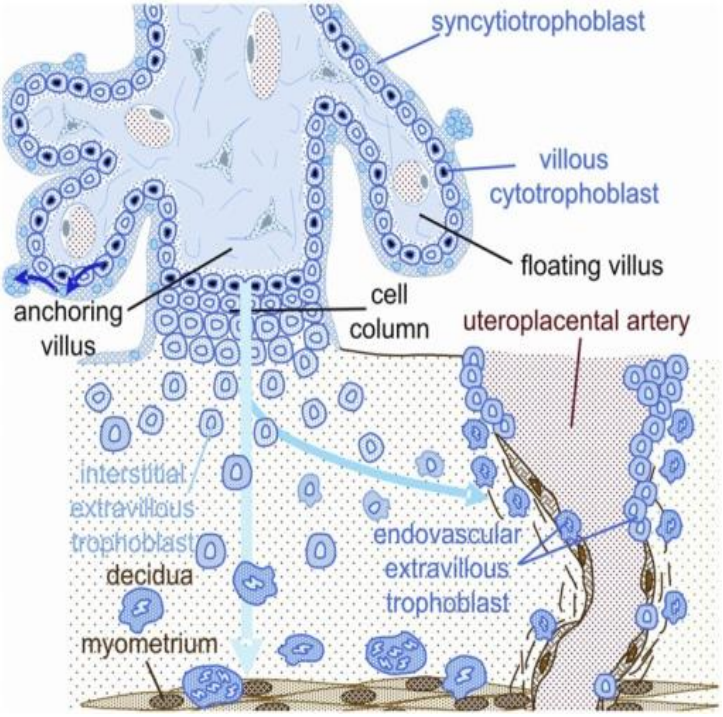

Figure 8. Schematic representation of trophoblast invasion. Fetal cells (blue) invade into the maternal decidua, myometrium and spiral arteries (brown) (73).

1.2 Uterine NK cells

Natural killer (NK) cells are large lymphocytes that are an important part of the innate immune system. They are best known for their cellular cytotoxic capacities and the secretion of pro-inflammatory cytokines and chemokines. Once they detect an infected or tumour cell their immediate response is to kill the cell, which is why they are called natural killer cells. Even though the killing of these cells is their main purpose, they do not harm healthy cells. This is prevented because their activity is determined by the integration of signals derived from inhibitory or activating receptors (21,22). NK cells can be divided into several subtypes, each phenotypically and functionally different (table 1) (21,23). In this essay I will focus on the type of NK cells which are abundantly present in the uterus during pregnancy, the uterine NK cells (uNKs).

Immature uNK cells are already present in the endometrium during each menstrual cycle. With the onset of decidualization cell numbers increase and during the first trimester more than 70% of the leukocytes in the decidua are uNK cells. The cell numbers remain high throughout the second trimester, but starts to decline after 20 weeks of gestation and reaches normal endometrium numbers at term (12). Monocytes/macrophages, T-cells and dendritic cells are also present in the decidua during the first trimester, but with lower cell numbers (11).

Table 1. Differences between uNK and pNK cells (21,22)			
NK-subtype	$CD56^{bright}CD16^{-}$ uNK	$CD56^{bright}CD16^{-}$ pNK (10%)	$CD56^{dim}CD16^{+}$ pNK (90%)
Cytotoxicity	Granular, severely reduced cytotoxicity	Contains no granules	Granular, severely cytotoxic
KIR receptor expression	Yes	No	Yes
Cytokine production	Yes	Responds to nonspecific stimuli	Low
Other differences	Produces a lot of proangiogenic factors		

Characteristics and origin

uNK cells are characterized by poor cytotoxicity and a good capacity to secrete cytokines, chemokines and angiogenic factors (13). Although the uNK cells are not cytotoxic, they have prominent cytoplasmic granules containing perforin, granzymes and granulysin (24,25). It has been reported that uNK cells are unable to polarize the microtubule organizing centre (MTOC) into forming mature activating synapses and release the cytotoxic granules (26). It is still unknown which mechanism prevents polarization of the MTOC. In addition, the presence of ligands which trigger inhibitory receptors also contribute to cytotoxic repression (27,28).

The exact origin of uNK cells is still unclear. Several studies have reported that the uNK cells are derived from peripheral blood NK cells (pNK cells). These pNK cells are recruited from the circulation, and the decidual microenvironment modifies their phenotype and function (29–31). Since immature uNK cells are already present in the endometrium during each menstrual cycle, other studies proposed that that they are generated *in utero* from progenitors/precursors (21,32). Despite the ambiguity of their origin, terminal differentiation and maintenance of uNK cells is mediated *in utero* by interleukin-15 (IL-15), which is produced by decidual stromal cells (DSC) and macrophage (33).

uNK cell activity

uNK cell activity is regulated by integrated signals from inhibitory and activating surface receptors. The most important receptor families for uNK cells are the killer cell Ig-like receptors (KIR), type C-lectin receptors (CD94/NKG2) and leukocyte Ig-like receptors (LIR) (**Table 2**) (34–36). Ligands for these receptors are adhesion molecules, HLA-class I molecules, cytokines and chemokines. The HLA-class I molecules are of a particular interest as the EVTs express non-classical class I molecules HLA-E and G, and the classical polymorphic HLA-C (37–39).

Table 2. Inhibitory and activating uNK cell receptors (21,22)			
Inhibitory receptors	**Ligand**	**Activating receptors**	**Ligand**
KIR2DL1/2/3* KIR2DL4	HLA-C HLA-G	KIR2DS1/2/3/4/5 KIR3DS1	HLA-C
CD94/NKG2A/B	HLA-E	CD94/NKG2C/E/H NKG2D**	HLA-E MIC and UL16BPs
LIR	HLA-G		

* KIRs names are based on the number of their extracellular Ig-like domain (2D or 3D) and the length of their cytoplasmic tail long (L) or short (S). ** NKG2D is a homodimer, while all the other NKG2D subtypes are heterodimers with CD94. MIC = MHC class I chain-related antigens. UL16BPs = UL16 binding proteins.

HLA-C molecules are the dominant ligand for KIRs. KIRs are transmembrane glycoproteins encoded by highly polymorphic genes, which are part of the leukocyte receptor complex on chromosome 19. Two basic KIR haplotypes have been distinguished, type A and B. The A haplotype consists of 7 genes, which code for inhibitory receptors (except for activating KIR2DS4). The B haplotype has additional genes (up to 12), encoding mainly for activating receptors (35). The maternal KIR genotype can be AA (0-1 activating KIR), AB (1-6 activating KIRs) or BB (3-10 activating KIRs)(40).

KIR binding specificity of HLA-C proteins is determined by two allotype groups, which have a different amino acid at position 80 of the α-domain (39). Group C1 interacts with inhibitory KIR2DL2 and 3, while group C2 interacts with inhibitory KIR2DL1 and activating KIR2DS1 (Table 3) (41). Each pregnancy is characterized by different combinations of maternal KIRs and fetal HLA-C variants, resulting in differential activation of the uNK cells (39).

Table 3. Overview of uNK KIR presence, interactions and effects. (35,40)

HLA-C allotype – KIR interactions		**Receptor effect on uNK cells**	**Maternal KIRs present based upon genotype.**		
C1	C2		AA	BB	AB
KIR2DL2	KIR2DL1	Inhibitory	X	X	X
KIR2DL3	KIR2DS1	Activating		X	X

The CD94/NKG2 C-type lectin receptors are a heterodimer of CD94 and a NKG2 subunit (42). Seven NKG2 subunits (A, B, C, D, E, F and H) have been identified. The genes coding for both CD94 and the NKG2 subtypes are clustered on the natural killer receptor complex (NKC) on chromosome 12 (43). The most discussed receptor is the inhibitory receptor CD94/NKG2A, which is present on approximately 90% of the uNK cells (27). The CD94/NKG2A heterodimer recognizes HLA-E as a ligand (44). HLA-E – CD94/NKG2A interactions are involved in immune tolerance of the semi-allogeneic fetus, by the inhibition of cytotoxic activity (27).

LIRs are expressed by a minority of the uNK cells (38). Just like KIRs, their genes are a part of the leukocyte receptor complex on chromosome 19. LIRs bind to all HLA type I molecules but have a particular high affinity for HLA-G, which are only expressed by EVTs and slightly polymorphic. The HLA-G receptor is also recognized by inhibitory KIR2DL4 (45). HLA-G – LIR interactions are involved in immune tolerance of the semi-allogeneic fetus, by the inhibition of cytotoxic activity (28,46).

Placentation

During placentation, uNK cells at the maternal-fetal interface secrete a lot of soluble factors that regulate the recruitment of EVTs by chemotaxis. Among these soluble factors are proangiogenic factors, chemokines and cytokines, including IL-8, C-C motif chemokine ligand 5 (CCL5) and interferon-inducible-protein-10 (IP-10 or CXCL10) (13,47). Moreover, EVTs also produce a variety of chemokines and cytokines with the potential to coordinate uNK cells (47,48).

As mentioned earlier, EVTs express the HLA type I molecule HLA-C. Studies have reported that uNK cell activation through HLA-C KIR interaction is essential for EVTs invasion into the spiral arteries (49,50). Cross-talk between uNK cells and EVTs through either interaction of NKRs and their concomitant ligands or the secretion of soluble factors contributes directly or indirectly to EVTs recruitment and invasion into the spiral arteries (13,49).

The precise role of uNK cells in EVT independent remodelling is not completely known. It has been proposed that in early pregnancy uNK cells infiltrate unremodelled spiral arteries and secrete angiopoietin-1 (Ang-1), Ang-2, vascular endothelial growth factor C (VEGF-C), Interferon-γ (INF-γ), Fas-ligand (FasL) and metalloproteinases (MMP) to modulate cells and cellular interactions (cell-cell and cell-extra cellular matrix (ECM)), resulting in the disorganization and disruption of VSMCs and endothelial layers. This enables the EVTs to penetrate the arteries, colonize the wall and cause further disruption. Eventually, the EVTs degrade the remaining ECM, VSMCs and replace the endothelial cell layer (10,51,52).

Chapter 2: Preeclampsia

2.1 Clinical features, epidemiology, risk factors and etiology

Preeclampsia is a pregnancy-specific disorder and one of the major causes for maternal and fetal morbidity and mortality. Globally it affects 2-8 % of the pregnancies (1). Preeclampsia is characterized by the onset of hypertension (blood pressure ≥ 140/90 mm Hg on 2 occasions ≥ 4 h apart) and proteinuria (≥ 300 mg/24 h) in the last 20 weeks of pregnancy (criteria differs among international societies) (2). Depending on the degree of hypertension and the involvement of clinical symptoms preeclampsia is classified as mild or severe (table 4) (53). Some cases of preeclampsia present with atypical symptoms, making it difficult to confirm diagnosis (3).

Preeclampsia is a heterogeneous disorder and is divided in 2 subtypes depending on the time of disease onset. Early-onset preeclampsia develops before 34 weeks of gestation and late-onset develops at 34 weeks or later (54). Early-onset preeclampsia is associated with a higher risk for the development of life-threatening complications and adverse effects for the fetus. Life-threatening complications associated with preeclampsia are; the progression to eclampsia, placental abruption, acute kidney injury and the development of HELLP. Adverse fetal effects are uterine growth restriction and prematurity (53–55).

Delivery of the fetus and the placenta is the only known treatment for preeclampsia. It is recommended to induce labour in woman above 37 weeks of gestation with mild preeclampsia, and above 34 weeks when the disorder is severe. Before this, woman with both mild and severe preeclampsia are best served by hospitalisation to monitor for disease worsening or complications. The fetus is left alone but watched closely, called expective management, which allows for the development of the fetus so that the risk for complications due to prematurity (delivery <37 weeks) are reduced. Induction is recommended in women with severe preeclampsia at any gestational age above 23 weeks as the fetus is not viable (56). Although the preeclamptic symptoms disappear after delivery, both mother and fetus may develop long-term complications (57).

The risk for preeclampsia is higher in women with a prior history or family history of preeclampsia, an age above 40, multiple gestation, null parity, pre-existing disorders (diabetes mellitus, obesity and chronic hypertension) and certain ethnicities (African-American and Filipino) (2,3,53). Smoking during pregnancy reduces the risk of preeclampsia (58).

Although the exact cause of preeclampsia remains unknown, there is a consensus that the placenta has a key role in its etiology, as the removal of the placenta ends the disease (3). In preeclampsia, maternal spiral arteries often show a lack of physiological change through deficient EVT invasion **(Fig.3)** (59). The insufficient arterial remodelling leads towards reduced placental perfusion which causes placental ischemia, oxidative stress and inflammation. This is followed by the release of syncytiotrophoblastic factors (e.g. anti-angiogenic factors and pro-inflammatory cytokines) into the intervillous space. These factors induce an enhanced maternal systemic inflammatory response and generalized vascular dysfunction, resulting in the clinical symptoms of preeclampsia (60,61).

It has been proposed that both subtypes of preeclampsia have a differential etiology (54). The etiology of early-onset preeclampsia is more associated with abnormal placentation, whereas late-onset preeclampsia is secondary to maternal microvascular diseases, or has a genetic component (61). The etiological distinction is supported by the fact that early-onset preeclampsia is associated with fetal growth restriction and inadequate and incomplete trophoblast invasion of spiral arteries. Late-onset preeclampsia is associated with normal offspring, and normal or slightly altered spiral arties (61). Morphological studies have shown that late-onset placentas are similar to ones in normal pregnancies, while early-onset placentas are abnormal (62). Furthermore, differences in the levels of anti-angiogenic factors have been found. The level of soluble fms-like tyrosine kinase 1 (sFlt-1) and placental growth factor are higher in early-onset preeclampsia (63,64).

Table 4. Severe features of preeclampsia*
• Blood pressure ≥ 160/110 mm Hg on 2 occasion ≥ 4 h apart • Thrombocytopenia (platelet count < 100.00/µL) • Impaired liver function as indicated by elevated blood concentration of liver enzymes (serum AST or ALT > 2 times normal • Severe persistent right upper quadrant or epigastric pain as a sign of liver distention • Progressive renal insufficiently (serum creatinine > 1.1 mg/dL or doubling of serum creatinine in the absence of renal disease • Pulmonary oedema • New-onset cerebral or visual disturbances (e.g. blurred vision, scotomata, severe headache, altered mental status)
*According to the American college of obstetricians and gynaecologists (53).

2.2 Preeclampsia and uterine NK cells

EVT (in)dependent remodelling **-** In early onset preeclampsia defective EVTs invasion contributes to insufficient arterial remodelling. As explained in the previous chapter uNK cells are involved in arterial remodelling in several ways. They recruit the EVTs to the spiral arteries, and play a role in both EVT independent and dependent remodelling. uNK cells interact with the spiral arteries and invading EVTs through the secretion of soluble factors, as well as the direct interaction of their inhibitory and activating receptors and concomitant ligands (10,13,39,49).

Studying the potential contribution of uNK cells to preeclampsia during the first trimester of pregnancy is difficult, because at that moment the outcome of the pregnancy is still unknown. Therefore, uterine artery Doppler ultrasound resistance index (RI), an indirect measure of spiral artery remodelling, has been developed. Doppler ultrasound RI measures the resistance to maternal blood flow in uterine arteries. High uterine artery RI is associated with poor arterial remodelling, indicating a higher risk of early onset preeclampsia (65,66). Several studies have compared uNK cells isolated from high RI pregnancies (9-14 weeks of pregnancy), to uNK cells isolated from normal RI pregnancies (age-matched controls).

One study cultured conditioned medium (CM) from either normal RI uNK cells or high RI uNK cells with a SGHEC-7 endothelial cell line for 24 hours. Intracellular adhesion molecule 1 (ICAM-1) expression was determined as measure for activation. The high RI uNK cells were less capable to activate the endothelial cells. Furthermore, secreted factors from either high RI uNK cells or normal RI uNK cells were cultured with structures of SGHEC-7 cells invaded in Matrigel. Matrigel is similar to the ECM composition of uterine vessels (67). The endothelial cell–Matrigel structures were disrupted by secreted factors from normal RI uNK cells, while the secreted factors from high RI uNK cells were less capable of disrupting these structures (51).

Another study cultured normal RI uNK cells or high RI uNK in a co-culture with either VSMCs or endothelial cells. The normal RI uNK cells induced apoptosis in both vascular cells. They showed that the pro-apoptotic factor FasL secreted by the normal RI uNK cells contributed to the vascular apoptosis. Conversely, the high RI uNK cells had a decreased FasL secretions, what resulted in impaired vascular apoptosis (65).

The above mentioned studies suggest that uNK cells from high RI pregnancies have an altered secretory profile, resulting in reduced endothelial activation, disruption of the ECM and vascular cell apoptosis. As these processes are all involved in the initial stage of spiral artery remodelling, failure might contribute to preeclampsia (51,65).

EVTs are crucial for the completion of spiral artery remodelling. uNK cells secrete factors that regulate the recruitment of EVTs by chemotaxis. One study reported that high RI uNK cells are less capable to chemically attract EVTs (SGHPL-4 cells, an EVTs cell line were used) and inducing the EVT outgrowth from placental villi. However, the expression of factors IL-6, IL-8 and IP-10, previously

described as important in EVT recruitment, was not different from normal RI uNK cells (13,68). This kindled the investigation of the downstream signalling pathways activated by uNK cell secreted factors in EVTs, which showed that, in response to CM from normal RI uNK cells, the classical mitogen-activated protein kinase signalling pathway (P42/44 MAPK or ERK1/2) and phosphatidylinositol 3-kinase (PI3K)-Akt signalling pathways were more phosphorylated and thereby more activated (47).

These results indicate also that uNK cells from high RI pregnancies have an altered expression of secreted factors, which reduces their ability to regulate the recruitment of EVTs and may contribute to preeclampsia. This is confirmed by a later study, which investigated the secretion of angiogenic factors endostatin and angiogenin by isolated uNK cells. They showed that high RI uNK cells secrete higher levels of both angiogenic factors (69).

NKR – HLA-C interactions **-** Fetal EVT HLA-C receptors are ligands for maternal uNK killer like-Ig receptors (KIRs). Both the genes encoding for the uNK KIRs and the HLA-C molecules are highly polymorphic. The maternal KIRs have two haplotypes, A and B. The A type has 6 inhibitory and 1 activating receptor. The B type has up to 12 additional receptors, mainly activating (table 2 and 3) (35). The maternal KIR genotype can be AA (0-1 activating KIR), AB (1-6 activating KIRs) or BB (3-10 activating KIRs) (40).

The HLA-C receptors can be distinguished in two allotypes, C1 and C2. C1 interacts predominantly with 2 inhibitory KIRs (KIR2DL2 and 3), while C2 interacts with both an inhibitory (KIR2DL1) and an activating (KIR2DS1) KIR. The C2 allotype binds strong and more specifically with KIR2DL1 than C1 with KIR2DL2 and 3 (40). In addition, fetal EVTs express both the maternal and paternal C allele on its cell surface (41).

It has been reported that the combination of maternal AA KIRs (mainly inhibitory KIRs) with fetal HLA-C2 increases the risk of preeclampsia. As this reaction results into strong inhibition of the uNK cells, it has been suggested that they are no longer able to adequately regulate EVTs invasion into spiral arteries. The combination of maternal AA KIRs and fetal C1 has no effect on pregnancy outcome (49).

Interestingly, the increased risk is only present when the fetus possesses more C2 genes than the mother (Genotype: fetal C2/C1 with maternal C1/C1 or fetal C2/C2 and maternal C2/C1 or C1/C1), or when the only C2 of the fetus is paternal. Therefore, it has been suggested that uNK cells are calibrated to maternal C2 during their development (70). Also, the presence of paternal C2 appears to be detrimental. Conversely, the KIR B haplotype (both inhibitory and activating receptors) in combination with fetal C2 appears to be protective from preeclampsia (39).

In addition, uNK cells isolated from high RI pregnancies have a different receptor repertoire compared to uNK cells isolated from normal RI pregnancies. The high RI uNK cells have reduced KIR2DL/S1,3,5 and LILRB1 expression, ligands for HLA-C and HLA-G on EVTs. These results implicate that uNK cells in preeclampsia have altered interactions with EVTs due to decreased receptor expression, what might have an impact on spiral artery remodelling (71).

Conclusion

The process of arterial remodelling is essential for the establishment of adequate perfusion of the placenta and is mediated by uNK cells. Insufficient spiral artery remodelling and subsequent abnormal placentation are associated with early onset preeclampsia. As both uNK cells and preeclampsia are related to arterial remodelling, they are most likely also related to each other. In this essay I have elaborated on the role of uNK cells in the etiology of preeclampsia.

In the first trimester of a healthy pregnancy, uNK cells have important roles in uterine spiral artery remodelling. uNK cells are involved in both EVT independent (Stage I and II) and dependent (III and IV) remodelling. Recruitment of EVTs, via chemotaxis, is also mediated by uNK cells. To successfully remodel the arteries, cross-talk between the uNK cells, EVTs and vascular cells is essential. Cross-talk occurs either through NKR-ligand interactions or soluble factors (10,13,47).

Several studies have indicated that altered cross-talk contributes to preeclampsia. They reported that secreted factors of uNK cells isolated out of high RI pregnancies show reduced endothelial cell activation, ECM destruction and vascular apoptosis (51,65). Furthermore, secreted factors of these cells also failed to successfully recruit EVTs (47,69). The exact changes leading to this altered uNK secretion state are yet unknown. It would be interesting to know if these alterations are a response to changes in the uterine environment or if they have a genetic component.

In addition, genetic studies have reported that the combination of maternal AA KIRs with fetal HLA-C2s increases the risk of preeclampsia. These interactions result into strong inhibition of the uNK cells. Hence, the uNK cells are unable to support invasion of EVTs and maternal spiral artery remodelling. Since 30% of the preeclamptic pregnancies have the increased risk KIR AA genotype, not all cases of pre-eclampsia are explained by KIR AA-HLA-C interactions (39,49).

To summarize, insufficient arterial remodelling associated with preeclampsia appears to be the result of an altered activation state of uNK cells. Alterations in the secretion state of uNK cells affect the initial stages of remodelling but also trophoblast recruitment and invasion into the spiral arteries. Furthermore, uNK cell – EVT receptor interactions are also involved.

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