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Review

The role of NK cells and HLA system in preeclampsia

Kalinderis Michail[1], Kalinderi Kallirhoe[2], Papanikolaou Alexis[3]

[1] Department of Obstetrics and Gynecology, Princess Royal University hospital, Orpington, United Kingdom
[2] Department of General Biology, Aristotle University of Thessaloniki, Medical School, Thessaloniki, Greece
[3] 1st Department of Obstetrics and Gynecology, Aristotle University of Thessaloniki, Medical School, Papageorgiou hospital, Thessaloniki, Greece

Correspondence
Papanikolaou Alexis, 1st Department of Obstetrics and Gynecology, Aristotle University of Thessaloniki, Medical School, Papageorgiou hospital, Thessaloniki, Greece
E - mail: papalex@med.auth.gr

Abstract

Preeclampsia is a pregnancy - specific syndrome associated with increased maternal and fetal morbidity and mortality. The development of preeclampsia occurs in two stages including reduced placental invasion, followed by the activation of endothelial cells and the release of various factors leading to the clinical manifestations of this syndrome. The initial trigger for the incomplete trophoblast invasion in the maternal decidua is still unknown, however the role of the immune system is believed to be of great importance. According to recent data, the pathogenesis of preeclampsia may arise from the interaction between the killer immunoglobulin - like receptors (KIR) of the natural killer (NK) cells of a pregnant woman and the major histocompatibility complex (MHC) of the developing embryo. This review analyses current data regarding the possible association of the NK - MHC system with preeclampsia.

Keywords: preeclampsia; NK cells; KIR receptors; HLA - C2 gene

Preeclampsia is a multisystem syndrome of unknown etiology, characterized by new - onset of hypertension (blood pressure≥ 140/90mmHg) after the 20th week of gestation and proteinuria (≥ 300mg/24h) resolving after delivery of the placenta. Nowadays, preeclampsia complicates approximately 2 - 8% of all pregnancies and remains the greatest cause of maternal and fetal morbidity and mortality[1]. Although the exact pathogenic mechanisms of the syndrome still remain unidentified, preeclampsia is believed to develop in two stages. More specifically, during normal pregnancy and placentation the invasion of cytotrophoblast cells in the spiral arteries induce the remodeling of these maternal vessels into high - capacitance and low - resistance vessels which serve the increased need of blood flow during pregnancy. On the contrary, in preeclampsia the cytotrophoblast endovascular invasion is shallow, resulting in incomplete uteroplacental perfusion and finally placental ischemia. The subsequent activation of endothelial cells and the release of multiple factors contribute to the clinical manifestations of preeclampsia[2 - 5].

The initial trigger for the incomplete trophoblast invasion that characterizes preeclampsia remains currently unknown, however the role of the immune

system in this procedure is believed to be of crucial importance. During the invasion of trophoblast cells in the decidua, the fetal cells come in contact with the cells of the mother. The non - rejection of the fetus indicates the pivotal role of the mother's immune system, as well as the importance of immunological adjustment for the development of pregnancy. The immunological background of preeclampsia is supported by a wealth of epidemiological data, such as the fact that preeclampsia occurs more often during the first pregnancy, whereas its incidence is decreased in subsequent pregnancies. Moreover, a previous miscarriage can act protectively against preeclampsia. However, with a different partner the risk of preeclampsia is the same as in primiparous women. It has also been observed that prolonged sperm exposure within a stable relation reduces the risk of preeclampsia, while the use of condom increases the risk of preeclampsia[6 - 9].

Until now, the immunological interplay between the mother and the fetus during pregnancy was attributed to interactions between paternal antigens and maternal T lymphocytes. However, current research is mainly focused on the emerging role of natural killer (NK) cells of the mother and not of T lymphocytes. More specifically, according to recent data, the implantation, acceptance and normal development of pregnancy seems to largely depend on the interactions between the NK cells in the maternal decidua and the major histocompatibility complex (MHC) expressed in the fetus. This review describes current knowledge on the MHC system and on NK cells and their receptors, focusing on their possible role in the development of preeclampsia.

Major histocompatibility complex

MHC is a gene cluster, which contains a large number of different genes which are grouped into 3 classes, accordin.g to the construction and function of their products. In particular, the genes of this cluster are located in the short arm of chromosome 6 and are divided into 3 classes: MHC - I, MHC - II and MHC - III. MHC - I is expressed in all nucleated cells of the body. Cytotoxic T cells recognize foreign peptides always in association with class I MHC proteins (MHC - restricted antigen recognition). MHC - II molecules are found in antigen presenting cells (APCs), e.g. B lymphocytes, monocytes, macrophages, Kupffer cells, astrocytes and Langerhans cells. MHC - II molecules recognize and activate T helper cells. MHC - III molecules encode for complement proteins, cytokines and heat shock proteins[10,11].

Each of the above 3 classes contains many loci, while each of these loci on chromosome 6 can have many alleles. Each individual carries on chromosome 6 only one of the alleles of a MHC gene. MHC alleles are closely linked together and are inherited from each parent as haplotypes, i.e. as a set of alleles. Therefore, each individual inherits one haplotype from the mother and one from the father. These two haplotypes, one from each parent, constitute the genotype.

In humans, the MHC complex is referred to as Human Leucocyte Antigen (HLA) referring to proteins encoded by MHC genes which are expressed on the cell membrane of leukocytes. Each antigen of MHC is characterized by: 1) the MHC or HLA symbol that indicates the histocompatibility complex 2) a letter which represents the locus (MHC or HLA - A, - DR, - DQ) 3) a number that characterizes the antigen (MHC or HLA - A1, - DR4, - DQ5). So, each class of MHC genes includes: 1) MHC - I: a) classical [classical I (class Ia)] genes: HLA - A, HLA - B, HLA - C, b) non - classical [non classical (class Ib)] genes: HLA - E, HLA - F, HLA - G. 2) MHC - II: genetic locus HLA - DP, HLA - DQ, HLA - DR. The MHC - DP locus consists of two A genes, MHC - DPA1 and MHC - DPA2 and two B genes, MHC - DPB1 and MHC - DPB2. The MHC - DQ locus consists of two A genes, MHC - DQA1 and MHC - DQA2 and three B genes, MHC - DQB1, MHC - DQB2 and MHC - DQB3. The MHC - DR locus, comprises an A gene (DRA) and more than one B genes (DRB). 3) MHC - III: It is the densest region of the MHC gene, with one gene every 20kb of DNA, including genes of the classical and alternative complement pathway and genes encoding the tumor necrosis factor a and b[12].

The role of the MHC complex is very important

Table 1. Inhibitory and activating genes of KIR A and KIR B haplotypes

KIR	Inhibitory genes	Activating genes	Inhibitory / activating genes
A Haplotype	KIR3DL3 KIR2DL3 KIR2DL1 KIR3DL1 KIR3DL2	KIR2DS4	KIR2DL4
B Haplotype	KIR2DL2 KIR2DL5	KIR2DS2 KIR2DS3 KIR2DS5 KIR2DS1 KIR3DS1	-

during pregnancy, as it determines the non - rejection of the fetus from the mother's immune system. More specifically, the trophoblast does not express class I (HLA - A and HLA - B) and class II (HLA - DP, HLA - DQ, HLA - DR) molecules, thereby avoiding the activation of the immune system against the maternal placenta. Alternatively, it expresses HLA - C class Ia and HLA - E, HLA - F, HLA - G class Ib molecules, facilitating the avoidance of the immune response against the fetus. The reaction of these molecules with the NK cells in the uteroplacental surface appears to be of crucial importance for the recognition of the fetus and the development of a normal, uncomplicated pregnancy.

NK cells - KIR receptors

NK cells were first described in 1975, as a class of lymphocytes that has cytotoxic activity against leukemia cells. Today, NK cells are not confined to a lytic action, but play a key role during pregnancy, affecting the process of trophoblast invasion[13 - 15]. More specifically, during pregnancy a number of immune cells are identified in the uteroplacental surface. In the early stages of pregnancy, about 10% of these are T lymphocytes, 20% are macrophages, 2% are dendritic cells and up to 70% decidual NK (dNK) cells which are different from NK cells in the peripheral blood. With the development of pregnancy and as syncytiotrophoblast cells come in contact with the maternal blood, a second interaction phase, prevailing after the 20 weeks of pregnancy, arises in which T and B lymphocytes, monocytes, dendritic cells, NK cells and granulocytes are present[16].

NK cells of the endometrium (uterine natural killer - uNK or decidual natural killer - dNK cells), in contrast to peripheral blood NK cells (peripheral natural killer cells - pNK) which are CD56dim, are CD56bright[17]. Moreover, unlike the cytotoxic action of pNK, uNK cells have mainly inflammatory and regulatory roles, secreting cytokines (IL - 8 and IP - 10) and angiogenic factors (Vascular endothelial growth factor - VEGF and Placental growth factor - PlGF) that promote trophoblast invasion and affect the remodeling of spiral arteries. It has been found that the dysfunction of uNK cells leads to pregnancy disorders such as preeclampsia, indicating uNK cells as "guards" of pregnancy. The function of NK cells is regulated by specific receptors via the production of activating or inhibitory signals. The NK cell receptors are encoded by two structurally distinct families of molecules: 1) KIR receptors (killer immunoglobulin - like receptors, KIRs) and 2) CD94 - NKG2 receptors (lectin - like CD94: NKG2 heterodimers). Of these, KIR receptors are the ones that

Table 2. Basic ligants of the KIR receptor-HLA system

HLA-C		HLA-B	HLA-A	HLA-G
HLA-C1	HLA-C2			
KIR2DL2 KIR2DL3	KIR2DL1 KIR2DS1	KIR3DL1	KIR3DL2	KIR2DL4

Table 3. The risk of preeclampsia depending on the combination of maternal KIR haplotype and fetal HLA-C genotype

Maternal KIR haplotype	Fetal HLA-C genotype		
	C1/C1	C1/C2	C2/C2
A/A	↓	↑	↑
A/B	↓	↓	↓
B/B	↓	↓	↓

have recently been found to have an important role in preeclampsia[18].

KIR receptors are membrane glycoproteins which are expressed on NK cells and certain T lymphocytes and are encoded by a gene cluster on chromosome 19q13.4[19]. KIR proteins have 2 or 3 extracellular immunoglobulin structural regions (2D or 3D, respectively) and a long or short intracellular structural region (L or S, respectively). KIR proteins with an 'L' cytoplasmic tail have an inhibitory activity, whereas KIR proteins with an 'S' cytoplasmic tail have an activating effect. The letter 'P' represents pseudogenes. The last digit indicates the number of the gene encoding a protein with a particular structure. Each KIR receptor of NK cells consists of two haplotypes, A and B, depending on the number and type of genes that it contains (Table 1). The A haplotype contains a fixed number of genes from which 5 have inhibitory activity (KIR2DL1, 2DL3, 3DL1, 3DL2 and 3DL3), one has activating ability (KIR2DS4) and one has dual action (KIR2DL4) (Table 1). The A haplotype is more frequently found in Caucasians and is generally considered to have inhibitory activity. The B haplotype is characterized by greater diversity in terms of the number of genes and alleles that it contains. From these genes, most (2DS1, 2DS2, 2DS3, 2DS5 and 3DS1) have activating ability, while only two (2DL2, 2DL5) have inhibitory activity (Table 1). The B haplotype is therefore considered as the activating haplotype of KIR receptors and is highly polymorphic between different ethnicities. The KIR3DL3, KIR2DL4, KIR3DL2 and KIR3DP1 genes are common between the two haplotypes[18 - 20]. So, the potential KIR genotypes are AA for individuals homozygous for the A haplotype, AB for heterozygous individuals, carrying both the A and the B haplotype, and BB for individuals homozygous for the B haplotype.

NK cells, HLA system and immunobiology of preeclampsia

The immune cells that reside at the interface between the placenta and uterus are thought to play many important roles in pregnancy. For many years the main interest was focused on T lymphocytes, however the role of NK cells is nowadays in the frontline of scientific research regarding pregnancy outcome. Actually, the percentage of T lymphocytes in the uteroplacental surface is small, while NK cells account for up to 70% of the leukocytes at this site. Moreover, the extravillous trophoblast has no specific ligands for T lymphocytes, such as HLA class II molecules or HLA - A and HLA - B molecules of class I[21]. On the contrary, invading trophoblast cells express an unusual and unique combination of three class I molecules, HLA - G, HLA - E and HLA - C. Of these, only HLA - C is polymorphic, but is also the main ligand for NK cells. So, the KIR receptors of NK cells, which are also characterized by their variability, are bound to HLA - C molecules. Thus, there is a highly polymorphic system, containing the KIR receptor from the mother's side and HLA - C from the side of fetal trophoblast, which appears to play a crucial role during pregnancy, affecting trophoblast invasion and the development of preeclampsia[22].

A number of genetic studies have focused on the possible contribution of the genetic variability of the KIR - HLA - C system to the development of preeclampsia[20]. HLA - C alleles are classified into two groups according to the amino acid present at position 80 of the corresponding protein. The C1 group corresponds to the asparagine amino acid and is connected with the inhibitory receptors, KIR2DL2 and KIR2DL3, while the C2 group corresponds to the amino acid lysine and is linked to the inhibitory receptor, KIR2DL1, and the activating receptor, KIR2DS1. Recently, the KIR2DL2 receptor, and less the KIR2DL3 receptor, have been found to be weakly associated with HLA - C2. The KIR3DL1 receptor is linked to HLA - B, the KIR3DL2 receptor is linked to HLA - A and the KIR2DL4 receptor to HLA - G (Table 2).

In a pioneer study of Hiby et al, preeclampsia was found to occur more often in women with the KIR AA genotype, characterized by an inhibitory activity, and at least one C223. In contrast, the maternal KIR genotype does not appear to have an important role in cases where the embryo is homozygous for C1 (Table 3).

One possible explanation is that C2 compared to C1 binds more strongly to its receptor. Thus, when C1 is linked to the KIR2DL2 and KIR2DL3 receptors, the produced inhibitory signal for the uNK cells is milder, thus the procedure of trophoblast invasion remains unaffected. On the contrary, when C2 is linked to the KIR2DL1 receptor, the generated inhibitory signal for the uNK cells is much stronger, influencing trophoblast invasion, rendering it incomplete[24]. Thus, in normal pregnancy the activation of uNK cells via interaction with HLA - C molecules of the extravillous trophoblast remains intact, as well as the procedure of placentation and of spiral arteries remodeling. On the other hand, in case of insufficient activation of the uNK cells, the remodeling of spiral arteries is poor leading to an increased risk of preeclampsia.

But what are the protective genes in the KIR B haplotype when the fetus expresses HLA - C2? Recent studies show that the frequency of KIR genes in the telomeric end of the B haplotype is decreased in cases of preeclampsia. The presence of the activating gene, KIR2DS1, in the telomeric end of the B haplotype appears to act protectively, reducing the very strong inhibitory effect resulting from the binding of HLA - C2 to the inhibitory receptor, KIR2DL1[25]. Interestingly, in a recent study Xiong et al demonstrated that the protective effect of KIR2DS1 may be due to activation of dNK cells and production of proteins that aid trophoblast migration, such as granulocyte macrophage - colony stimulating factor (GM - CSF), leading to enchanced placentation[26]. So, the presence of HLA - C2 in the fetus leads to a strong inhibitory signal which in order to be compensated, requires the presence of an activating KIR gene, such as KIR2DS1, that promotes the production of proteins beneficial of placentation.

The possible effect of specific maternal KIR and fe-

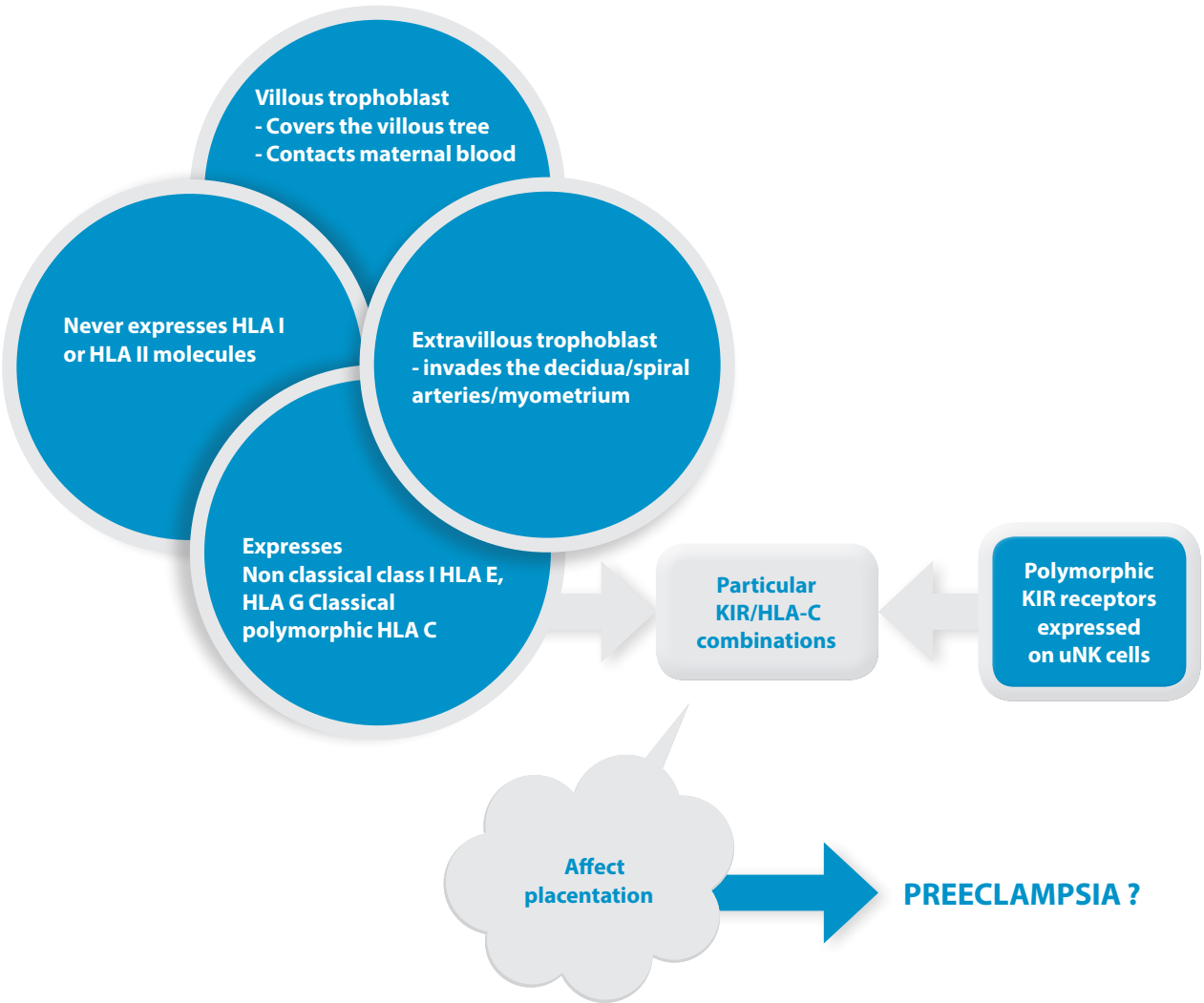

Figure 1: The KIR/HLA system and preeclampsia

tal HLA - C genes on preeclampsia could also be sufficient to explain the correlation of this syndrome with paternal antigen specificity. According to this theory, the mother that has the KIR AA genotype and lacks the protective B haplotype may have a successful first pregnancy with her partner if the fetus is HLA - C1. However, if a subsequent partner offers an HLA - C2 gene in the fetus then this second pregnancy has increased chances of developing preeclampsia. In contrast, women with KIR AA genotype who lack the protective B haplotype may develop preeclampsia in the first pregnancy, if their partner transmits HLA - C2 genes to the fetus. If a second partner is HLA - C1 homozygous, the subsequent pregnancy should be normal. However, if the mother has an HLA - C2 gene, there is always the risk of a KIR/HLA - C2 mismatching. Unfortunately, these women may have repeated pregnancies complicated with preeclampsia. Also, in a pregnancy resulting from oocyte donation, every woman without the protective B haplotype seems to be in increased danger, if there is an HLA - C2 gene either in the oocyte or in the sperm donor[25]. Moreover, males who are homozygous for the HLA - C2 gene could be considered as "dangerous males" as they would definitely offer one HLA - C2 gene in the fetus[27]. In a recent study, women with KIR AA genotype and fewer HLA - C2 genes compared with the fetus or if the

HLA - C2 gene in the fetus was inherited from the father, were characterized by a much higher risk of preeclampsia[28,29].

KIR and HLA genes are in different chromosomes and are inherited independently. Given the association of the HLA - C2 and KIR AA genotype with reproductive failure, including preeclampsia, it would be expected that the combinations of these genes should be depleted by natural selection. Interestingly, studies in different populations have shown that there is an outstanding reversal of the relationship of the gene frequencies of KIR AA and HLA - C2 in a wide range of ethnicities, so as not to appear together in high frequencies. Notably, Afro - Caribbeans, a population that is characterized by the highest frequency of the two genes, is a population known to be at higher risk for preeclampsia. Moreover, Asian males are considered as "low - risk fathers" because they have a low frequency of HLA - C2[32]. It seems that the need for reproductive success acts selective pressure on these two gene systems. However, these genes are not completely depleted, probably due to balancing selective pressures that retain both the KIR AA genotype and the HLA - C2 genotype. Possibly, these genotypes provide protection against other diseases or participate in the process of reproduction via other, hitherto unknown, positive mechanisms.

Conclusion

During pregnancy there are two areas of interaction between the mother's immune system and the fetal placenta; the villous trophoblast, which comes in contact with the maternal blood through the intravillous space, and the extravillous trophoblast, including all the trophoblast cells which infiltrate the uterine wall and the spiral arteries. The villous trophoblast comes in contact with the whole maternal immune system (systemic immune system), while the extravillous trophoblast comes directly in contact with the maternal tissues, locally reacting with the immune cells of the uterus mucosa. The villous trophoblast never expresses HLA I or HLA II molecules and is considered as immunologically inactive, while the extravillous trophoblast cells express HLA - G, HLA - E and HLA - C molecules (Figure 1). The HLA molecules of the fetus are under the constant control of the KIR receptors expressed on NK cells of a pregnant woman. The variability of the maternal KIR and fetal HLA - C system is believed to be of pivotal importance during pregnancy, affecting the process of trophoblast invasion, leading to pregnancy disorders, such as preeclampsia.

Conflict of interest

All authors declare no conflict of interest.

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