

## NK Cells and Trophoblasts: Partners in Pregnancy

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**In placental mammals, viviparity—the production of living young within the mother’s body—evolved under the auspices of the immune system. Elements of immunity were incorporated, giving pregnancy a mildly inflammatory character. Formation of the placenta, the organ that feeds the fetus, involves a cooperation between maternal natural killer (NK) cells and fetal trophoblast cells that remodels the blood supply. Recent research reveals that this process and human reproductive success are influenced by polymorphic HLA-C ligands and their killer cell immunoglobulin-like receptors (KIR).**

*Placental Reproduction Incorporates Innate Immune Mechanisms.* The first mammals were not viviparous but oviparous. They laid eggs, as is still done by the duck-billed platypus and both the long- and short-beaked echidnas (1). At the time of ancestral mammals, ~220 million years ago, the fundamentals of immunity as we know it were already in place (2). It was within this context that mammalian viviparity and placental reproduction began to evolve ~100 million years ago (3, 4) and use aspects of the immune system. Resisting infection and reproducing its kind are absolute essentials if a new species is to compete and survive for any length of time. The competition is such (all species lose eventually) that successful immune and reproductive systems maintain their edge by adapting to changing circumstance. Consequently, these two systems vary widely among extant mammals (5, 6, 7). In particular, human reproduction can seem bizarre and inexplicably divergent from that of other species (8).

In its broadest sense, the immune system’s job is to demolish, disinfect, and rebuild damaged tissues (9). Reproduction involves similar processes, which are driven by hormones rather than microbial trespass. In women of child-bearing age who are not pregnant, the outermost part of the epithelial lining of the uterus (the endometrium) is pulled apart and refashioned on a regular basis—the 28-d menstrual cycle (10). During the 9-d proliferative phase preceding ovulation, the endometrium becomes built up,

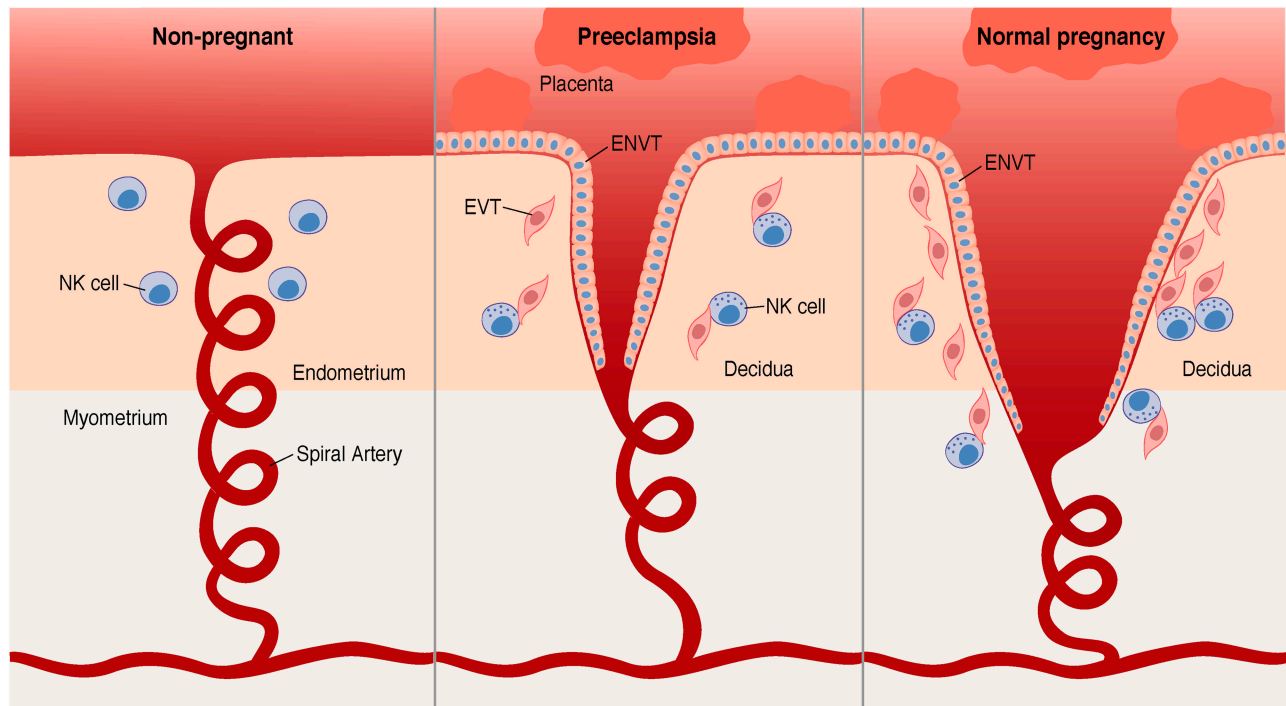
richly vascularized, and infiltrated with small, agranular NK cells (11, 12). Ovulation marks the beginning of the 14-d secretory phase in which the NK cells proliferate and differentiate. In this phase, an embryo can implant, but in its absence NK cells die off and the outer endometrium sheds during the 5-d period of bleeding known as menstruation.

Upon implantation of an embryo, the menstrual cycle is broken and pregnancy commences. The implanted embryo, the blastocyst, comprises an inner cell mass, which develops into the fetus, and an outer trophoblast layer that forms the placenta. By tapping into the maternal circulation, the placenta serves the fetus with food procured from the mother’s blood. Obtaining adequate blood supply necessitates some remodeling of blood vessels in the outer endometrium, which in the process becomes a much changed tissue called the decidua. The work involves an expanded and activated population of maternal NK cells in the decidua and extravillous trophoblast cells. The latter are directed to move into the decidua, where they strip off the muscular wall of the spiral arteries and supplant the endothelial cells that line these vessels (Fig. 1) (10).

Human spiral arteries are remodeled to a much greater depth than those of other species—a process that is only completed by weeks 16–20 of pregnancy (13). The once muscular vessels are converted into large flaccid conduits to meet the escalating demands of the human fetus, particularly those of its exceptionally large brain (8, 14). Although the decidual NK cell population dwindles in the second half of pregnancy—and by birth is gone—during this period the maternal–fetal interaction creates a state of mild systemic inflammation, as typified by activated vascular endothelium, leukocytes, and plasma proteins (15). Metabolic changes occur, including insulin resistance, that could serve to divert nutrients away from the mother and toward the growing fetus. Thus, cellular and molecular components of innate immunity are seen to be active throughout the duration of pregnancy: NK cells in the first half and inflammation in the second half.

*Uterine NK Cells Differ from Those in the Blood.* NK cells constitute 50–90% of the leukocytes in the decidua, a tissue in which B and T cells are rare. Two types of peripheral blood NK cells are distinguished: those with low CD56 expression specialize in cytotoxicity, whereas those with high CD56 favor cytokine secretion (16). Uterine NK cells express the highest amounts of CD56 and are also skewed

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**Figure 1.** In pregnancy, the spiral arteries are remodeled by extravillous trophoblast cells and NK cells. The left panel shows the nonpregnant endometrium in the secretory phase of the menstrual cycle just before menstruation. The right panel shows the endometrium in the second half of normal pregnancy when the spiral arteries are remodeled to a depth that penetrates the myometrium. The middle panel shows the situation in preeclampsia where the extent and depth of remodeling is less than in normal pregnancy. These vascular changes are effected by extravillous trophoblast cells (EVT) with the help of activated NK cells. In the process, the enlarged vessels become lined with endovascular trophoblast cells (ENVT) (10).

toward cytokine secretion (17). Analysis of the transcription of 10,000 genes revealed 278 whose expression differed by more than threefold in uterine NK cells compared with blood NK cells (18). Most of these genes were up-regulated in the uterine NK cells. It is still unknown whether these differences arise from differentiation that occurs within the decidua or from expansion there of rare precursors from the peripheral blood with atypical phenotype. Either way the extent of the differences points to uterine NK cells having functions specific to pregnancy.

Evidence from several quarters supports the view that decidual NK cells cooperate with extravillous trophoblasts to remodel the spiral arteries. Of the various forms of trophoblast, only the extravillous trophoblasts express MHC class I molecules, and those they express—HLA-C, E and G—are all good ligands for NK cell receptors (19). HLA-G is expressed only by extravillous trophoblasts and medullary epithelial cells of the thymus (20), whereas HLA-C and HLA-E have ubiquitous distribution. HLA-G is the LILRB1 ligand (21), HLA-E is the ligand for CD94-NKG2A/C (22), and HLA-C ligands engage several members of the KIR family (23). These ligand–receptor pairs provide candidates for controlling the interaction of decidual NK cells with extravillous trophoblast cells, liaisons which are clearly visible under the microscope (Fig. 1).

*Combinations of Maternal KIR and Fetal HLA-C Affect Preeclampsia.* Preeclampsia is a disorder specifically of human reproduction that becomes manifest during the second

half of 5–10% of pregnancies (24). It can be caused by incomplete remodeling of the spiral arteries, which leads to high maternal blood pressure and elevated concentration of urinary protein. Although originating in the placenta, the effects of preeclampsia become systemic: perturbation of maternal circulation and poor fetal growth (15). In the absence of care or intervention, preeclampsia can deteriorate into eclampsia, which threatens vital maternal organs and can lead to death for both mother and child. If only one of them dies, it is usually the mother. Worldwide, most pregnancy-associated mortality is due to preeclampsia and eclampsia. Convulsions typify eclampsia, hence the name which derives from eklampsia, the Greek word for a sudden flash or development (25). The only known cure for preeclampsia is to deliver the fetus, the physician's challenge being to balance the needs of both mother and child in deciding when to intervene with either drug-induced labor or a caesarian section (24).

Hearsay and clinical observation pointed to the possibility that polymorphisms in both maternal and paternal genes contribute to preeclampsia. Maternally, preeclampsia runs in families, and some men seem prone to fathering preeclamptic pregnancy (10). In this issue, Hiby et al. implicate HLA-C on fetal trophoblast cells and KIR on uterine NK cells as factors affecting preeclampsia (26). These particular trophoblast ligands and NK cell receptors were selected for study because they were known to have extensive polymorphism of immunological importance. Hiby et al. compared a panel

of 200 pregnant women with preeclampsia and 201 women with normal pregnancies. For these women and their babies *KIR* and *HLA-C* genotypes were determined. Although *HLA-C* and *KIR* are both exceedingly diverse, there is hierarchy to their variability, which allows the two systems to be simplified in functionally sensible ways.

The *KIR* locus consists of 7–15 closely packed genes, which form numerous haplotypes that differ in both gene content and allele combination. *KIR* haplotypes form two groups, designated *A* and *B* based on the relative content of genes encoding inhibitory and activating *KIR* (27). The simpler group, *A* haplotypes, contains mainly genes for inhibitory *KIR*, whereas the more complicated group, *B* haplotypes, has additional genes encoding activating *KIR*. Hiby et al. found preeclampsia to be more prevalent in women who are homozygous for group *A* *KIR* haplotypes (*AA*) than women who are either heterozygous (*AB*) or homozygous for group *B* haplotypes (*BB*) (26). In short, an absence of activating *KIR* favors preeclampsia.

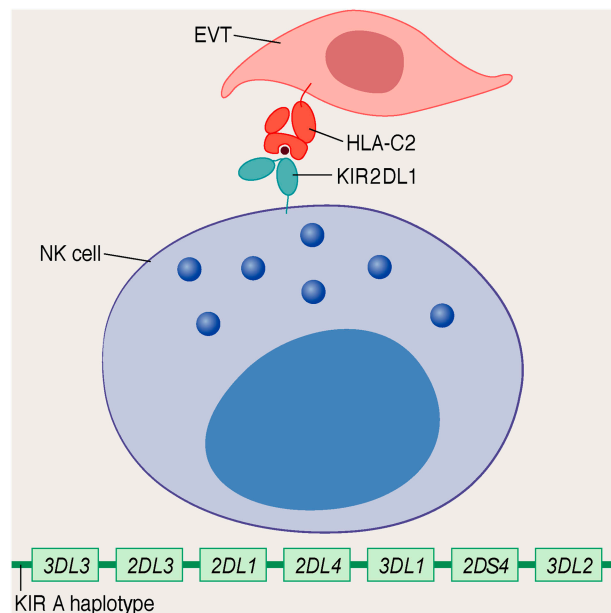
*HLA-C* allotypes form two groups according to *KIR* specificity and the residue present at position 80 in the amino-acid sequence (28). The *C1* group allotypes are

ligands for the inhibitory *KIR2DL2* and *KIR2DL3* and have asparagine at position 80; the *C2* group allotypes are ligands for the inhibitory *KIR2DL1* and the activating *KIR2DS1* and have lysine at position 80. Of the two, *C2* is a stronger ligand than *C1* (29). Hiby et al. found that the increased prevalence of preeclampsia in *AA* *KIR* women was entirely due to pregnancies where the fetus genotype is either homozygous *C2* or heterozygous *C1C2*. Thus, it is the combination of maternal *AA* *KIR* genotype and fetal *C2* genotype which most frequently leads to preeclampsia (Fig. 2) (26).

For the interaction between extravillous trophoblast and uterine NK cells, the combination of fetal *HLA-C2* and maternal *AA* *KIR* genotype is the one expected to deliver the strongest inhibitory signals to NK cells. *C2* binds more tightly to its cognate *KIR* than *C1*, and maternal NK cells of *AA* genotype can express the inhibitory *C2* receptor (*KIR2DL1*) but not the activating *KIR* that could provide compensatory activating signals. When comparing disease status and maternal *KIR* genotype for all the pregnancies involving a *C2* fetus, Hiby et al. found that activating *KIR* decrease the likelihood of preeclampsia and that their effects are cumulative (26). These correlations all point to overly inhibited uterine NK cells causing trophoblast cells to prematurely cease the remodeling of maternal blood vessels, thereby increasing the probability for preeclampsia. This model also ties in nicely with studies of pregnant NK cell-defective mice, revealing impaired modification of the spiral arteries that is remedied by administration of interferon  $\gamma$ , a prominent NK cell cytokine (30, 31). Conversely, insufficient inhibition of decidual NK cells may favor spontaneous abortion (32).

The association of *KIR* and *HLA-C* with preeclampsia is not a simple all-or-nothing phenomenon like the type seen when a unique gene function is completely missing. Only a minority of pregnancies with *C2* fetuses and *AA* mothers lead to preeclampsia, and not all preeclamptic pregnancies have this genotype combination. Hiby et al. suggest that, quantitatively, there exists a spectrum of predisposition to preeclampsia due to different *HLA* class I–*KIR* combinations, of which only the most prominent reached statistical significance in their study (26). This genetic complexity echoes the complications confronted clinically in defining and diagnosing preeclampsia, which can be considered not so much a disease or disorder but “simply the extreme end of a continuum of characteristics common to all pregnancies” (15).

**Natural Selection by Preeclampsia.** In the absence of successful medical intervention, preeclampsia is a significant cause of mortality for pregnant women and their young. This is seen in developing countries today (33) and would, historically, have been universally true. In human populations, deaths from preeclampsia are predicted to reduce the frequency of *C2* *HLA-C*, group *A* *KIR*, or both factors. Hiby et al. found evidence for such selection in the *HLA-C* and *KIR* frequencies of modern human populations. Overall, they observed an inverse correlation between the fre-



**Figure 2.** Preeclampsia is associated with uterine NK cells of *KIR* genotype *AA* and the presence of *HLA-C2* on the surface of extravillous trophoblast cells. An extravillous trophoblast (EVT) is shown interacting with a maternal uterine NK cell. EVT are the only trophoblast cells that express MHC class I. The *HLA-C2* molecule is shown interacting with its cognate inhibitory receptor *KIR2DL1*. The inhibition due to this ligand–receptor interaction favors preeclampsia in the absence of compensating activating *KIR*. The functional *KIR* genes of a group *A* haplotype are shown in order below the NK cell. The *KIR* nomenclature give the number of a receptor’s extracellular domains (2D or 3D) the length of the cytoplasmic tail (long or short) and a final number distinguishing receptors with similar structure. Long-tailed *KIR* inhibit and short-tailed *KIR* activate, except *KIR2DL4* which does both. *HLA-C2* refers to any one of the *HLA-C* allotypes that has lysine at position 80. Many other ligand–receptor pairs that contribute to the cell–cell interaction are not depicted.

quencies of *C2* and the *AA KIR* genotype. Populations with the highest frequency of *AA KIR*—Japanese and Koreans—have the lowest *C2* frequencies, whereas populations with the lowest frequency of *AA KIR*—Aboriginal Australians and Asian Indians—have the highest *C2* frequencies. This striking correlation indicates that selection for reproductive success has shaped the polymorphisms of *HLA class I* and *KIR* and could be solely responsible for the emergence and maintenance of the balanced polymorphisms between the *C1* and *C2* groups of *HLA-C* alleles and the group *A* and group *B KIR* haplotypes, all of which are rather new, having originated during the evolution of the great apes and humans (34, 35). In this scenario, some mortality from preeclampsia is the price paid for increasing brain size and the competitive advantages that brings.

*A Peaceful Paradigm for Reproductive Immunology.* The cells and molecules of the immune system are commonly depicted as the armies and weaponry with which a mammalian host engages in epic struggle with a fiendish microbial empire. In reproductive immunology such martial metaphor has also been a guiding light, with the twist that the conflict is between mother and fetus. The fetus is perceived as invading the mother and the mother rebuked for not rejecting its allogeneic advances. Apart from the obvious problem with this thesis—that fetal rejection is a trait unlikely to be passed on—its implication, that the fetus has an adversarial relationship with the mother's immune system, is inherently unlikely, not least because mammalian viviparity and placentation began evolving long after the immune system was put in place. This evolution, which was very successful, would not have involved sudden confrontations of the type experienced by a laboratory mouse when it is given an MHC-incompatible skin graft. A more sensible paradigm is now emerging, one in which cells and molecules of immunity are part and parcel of the reproductive process and used in a constructive manner. With this the relationship between uterine NK cells and extravillous trophoblast cells appears as cooperation, a partnership in pregnancy, rather than trench warfare between defenders of the mother country and fetal invaders in search of blood.

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