Commentary

NK Cells and Trophoblasts: Partners in Pregnancy

Peter Parham

Department of Structural Biology and Department of Microbiology and Immunology, Stanford University School of Medicine, Stanford, CA 94305

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 remolds 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 placentation began to evolve ~100 million years ago (3, 4) and use aspects of the immune system. Resisting infection and reproducing its kind are absolutes 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 different from that of other species (8).

In its broadest sense, the immune system’s job is to demolish, disinfest, 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 preceeding 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 tropoblast 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 decidua 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 cytosis, whereas those with high CD56 favor cytokine secretion (16). Uterine NK cells express the highest amounts of CD56 and are also skewed...
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 upregulated 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 decidinal NK cells cooperate with extracellular trophoblasts to remodel the spiral arteries. Of the various forms of trophoblast, only the extracellular 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 extracellular 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 decidinal NK cells with extracellular 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 eklampsis, 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 cesarean 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

---

**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 (ENV) (10).
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 KIR2DL1 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 γ, 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-
frequencies 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 paradigm in mind, 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.

I thank Laurent Abi-Rached for helpful and stimulating discussion on questions of evolution.

Submitted: 15 September 2004
Accepted: 23 September 2004

References

1. Rothchild, I. 2003. The yolkless egg and the evolution of eutherian viviparity. Biol. Reprod. 68:337–357.
2. Kasahara, M., T. Suzuki, and L. Du Pasquier. 2004. On the origins of the adaptive immune system: novel insights from invertebrates and cold-blooded vertebrates. Trends Immunol. 25:105–111.
3. Ji, Q., C.-I. Yuan, J.R. Wible, J.-P. Zhang, and J.A. Georgl. 2002. The earliest known eutherian mammal. Nature. 416: 816–822.
4. Weil, A. 2002. Mammalian evolution: onwards and upwards. Nature. 416:798–799.
5. Pijnenborg, R., and L. Vercruysse. 2004. Thomas Huxley and the rat placenta in the early debates on evolution. Placenta. 25:233–237.
6. Carter, A.M., and A.C. Enders. 2004. Comparative aspects of trophoblast development and placentation. Reprod. Biol. Endocrinol. 2:46.
7. Mestas, J., and C.C. Hughes. 2004. Of mice and not men: differences between mouse and human immunology. J. Immunol. 172:2731–2738.
8. Robillard, P.-Y., T.C. Hulsey, G.A. Dekker, and G. Chaoout. 2003. Preeclampsia and human reproduction. An essay of a long term reflection. J. Reprod. Immunol. 59:93–100.
9. Matzinger, P. 2002. The danger model: a renewed sense of self. Science. 296:301–305.
10. Moffett-King, A. 2002. Natural killer cells and pregnancy. 2002. Nat. Rev. Immunol. 2:656–663.
11. Bulmer, J.N., D. Hollings, and A. Ritson. 1987. Immunoy-tochemical evidence that endometrial stromal granulocytes are granulated lymphocytes. J. Pathol. 153:281–288.
12. King, A., C. Birkby, and Y.W. Loke. 1989. Early human decidual cells exhibit NK activity against the K562 cell line but not against first trimester trophoblast. Cell. Immunol. 118:337–344.
13. Naicker, T., S.M. Khedum, J. Moodley, and R. Pijnenborg. 2003. Quantitative analysis of trophoblast invasion in preeclampsia. Acta Obstet. Gynecol. Scand. 82:722–729.
14. Martin, R.D. 1996. Scaling of the mammalian brain: the maternal energy hypothesis. Neur. Physiol. Sci. 11:149–156.
15. Redman, C.W.G., and I.L. Sargent. 2003. Pre-eclampsia, the placenta and the maternal systemic inflammatory response—a review. Placenta. 24(Suppl A):S21–S27.
16. Cooper, M.A., T.A. Feinhinger, S.C. Tumer, K.S. Chen, B.A. Ghaheri, T. Ghayur, W.E. Carson, and M.A. Caliguiri. 2001. Human natural killer cells: a unique innate immunoregulatory role for the CD56 bright subset. Blood. 97:3146–3151.
17. King, A., N. Balendran, P. Wooding, N.P. Carter, and Y.W. Loke. 1991. CD3+ leukocytes present in the human uterus during early placentation: phenotypic and morphologic characterization of the CD56+ population. Dev. Immunol. 1:169–190.
18. Koopman, L.A., H.D. Kopcow, B. Rybalov, J.E. Boyson, J.S. Orange, F. Schatz, R. Masch, C.J. Lockwood, A.D. Schachter, P.J. Park, and J.L. Strominger. 2003. HLA-G: pre-eclampsia, immunity and vascular events. Acta Obstet. Gynecol. 82:722–729.
19. Trundley, A., and A. Moffett. Human uterine leukocytes and pregnancy 2004. Tissue Antigens 63: 1–12.
20. Le Bouteiller, P., N. Pizzato, A. Barakonyi, and C. Solier. 2003. HLA-G: pre-eclampsia, immunity and vascular events. J. Reprod. Immunol. 59:219–234.
21. Chapman, T.L., A.P. Heikama, A.P. West, and P.J. Bjorkman. 2000. Crystal structure and ligand binding properties of the D1D2 region of the inhibitory receptor LIR-1 (ILT2). Immunity. 13:727–736.
22. King, A., D.S. Allan, M. Bowen, S. Joseph, S. Verma, S.E. Hiby, A.J. McMichael, Y.W. Loke, and V.M. Braud. 2000. HLA-E is expressed on trophoblast and interacts with CD94:NKG2 receptors on decidual NK cells. Eur. J. Immunol. 30:1623–1631.
23. Vilches, C., and P. Parham. 2002. KIR: diverse, rapidly evolving receptors of innate and adaptive immunity. Annu. Rev. Immunol. 20:217–251.
24. Walker, J.J. 2000. Pre-eclampsia. *Lancet.* 356:1260–1265.
25. Fisher, S.J. 2004. The placental problem: linking abnormal cytotrophoblast differentiation to the maternal symptoms of preeclampsia. *Reprod. Biol. Endocrinol.* 2:53.
26. Hiby, S.E., J.J. Walker, K.M. O’Shaughnessy, C.W.G. Redman, M. Carrington, J. Trowsdale, and A. Moffett. 2004. Combinations of maternal KIR and fetal HLA-C genes influence the risk of preeclampsia and reproductive success. *J. Exp. Med.* 200:957–965.
27. Uhrberg, M., N.M. Valiante, B.P. Shum, H.G. Shilling, K. Lienert Weidenbach, B. Corliss, D. Tyan, L.L. Lanier, and P. Parham. 1997. Human diversity in killer cell inhibitory receptor genes. *Immunity.* 7:753–763.
28. Colonna, M., G. Borsellino, M. Falco, G.B. Ferrara, and J.L. Strominger. 1993. HLA-C is the inhibitory ligand that determines dominant resistance to lysis by NK1- and NK2-specific natural killer cells. *Proc. Natl. Acad. Sci. USA.* 90:12000–12004.
29. Winter, C.C., J.E. Gumperz, P. Parham, E.O. Long, N., and Wagmann 1998. Direct binding and functional transfer of NK cells inhibitory receptors reveal novel patterns of HLA-C allotype recognition. *J. Immunol.* 161:571–577.
30. Ashkar, A.A., J.P. Di Santo, and B.A. Croy. 2000. Interferon gamma contributes to initiation of uterine vascular modification, decidual integrity and uterine natural killer cell maturation during normal murine pregnancy. *J. Exp. Med.* 192:259–270.
31. Croy, B.A., H. He, S. Esadeg, Q. Wei, D. McCartney, J. Zheng, A. Borzychowski, A.A. Ashkar, G.P. Black, S. S. Evans, et al. 2003. Uterine natural killer cells: insights into their cellular and molecular biology from mouse modelling. *Reproduction.* 126:149–160.
32. Varla-Leftherioti, M., M. Spyropoulou-Vlachou, D. Nikou, T. Keramitsoglou, A. Darlamitsou, C. Tsekoura, M. Papadimitropoulos, V. Lepage, C. Balafoutas, and C. Stavropoulos-Giokas. 2003. Natural killer (NK) cell receptors’ repertoire in couples with recurrent spontaneous abortions. *Am. J. Reprod. Immunol.* 49:183–191.
33. Duley, L. 1992. Maternal mortality associated with hypertensive disorders of pregnancy in Africa, Asia, Latin America and the Caribbean. *Br. J. Obstet. Gynaecol.* 99:547–553.
34. Adams, E.J., and P. Parham. 2001. Species-specific evolution of MHC class I genes in the higher primates. *Immunol. Rev.* 183:41–64.
35. Rajalingam, R., P. Parham, and L. Abi-Rached. 2004. Domain shuffling has been the main mechanism forming new hominoid KIR. *J. Immunol.* 172:356–369.