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Commentary

The mother–child union: The case of missing-self and protection of the fetus

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The body's immune system is well known for its capacity to distinguish between self, i.e., the body's normal tissues, and foreign invaders, such as microorganisms (1). This self versus non-self discrimination is best illustrated by the clinical outcomes of solid tissue transplantation. Donor grafts that are matched to the recipient's "tissue types" result in successful transplantation, whereas mismatched transplants are rejected. Over the years, immunologists have carefully dissected the intricacies of rejection that is regulated by highly polymorphic (many genes, multiple alleles) molecules encoded in a genetic region termed the major histocompatibility complex (MHC) or HLA complex in humans. Transplant rejection is primarily mediated by T lymphocytes that are also responsible for cell-mediated immunity. T cells can recognize molecules encoded within the MHC, such as MHC class I or class II molecules, and can recognize and be stimulated by foreign MHC molecules, such as on the transplanted tissue, resulting in rejection. In inbred animals, classic transplantation laws were defined by studies of skin grafting. Generally, transplants between two different, MHC-mismatched inbred strains are rejected, whereas MHC-matched transplants are accepted. An F_1 hybrid offspring produced by mating of the two inbred strains codominantly expresses MHC alleles from both parents. The F_1 hybrid is capable of accepting a transplant from either parent, but each parent rejects F_1 hybrid tissue (because it has "foreign" MHC molecules from the other parent). These clear-cut laws even guide clinical transplantation of solid organs in human patients.

One obvious violation of the classic transplantation paradigms is the case of a mother's successful ability to nurture a fetus in the womb. This maternal–fetal "tolerance" is such a notable exception that it has stymied most attempts to explain it. Among the possibilities are that the womb is an immunologically privileged site, protected from the immune system, or that maternal immune responses are immunosuppressed during pregnancy (2, 3). However, studies over the last few years have rejuvenated excitement that maternal–fetal tolerance may be due to a specific and direct interaction (or lack thereof) between fetal and maternal cells. Recently, Rouas-Freiss *et al.* (4) provided new evidence involving an MHC class I-like molecule termed HLA-G and natural killer (NK) cells, adding significantly to an emerging literature that implicates these seemingly unrelated molecules and cells.

Generally, fetal trophoblast cells are in contact with the maternal circulation and should be subjected to attack by circulating maternal T cells if paternal MHC molecules are perceived as foreign. However, the fetal cells normally do not express "classical" MHC class I molecules, or class Ia molecules, termed HLA-A, -B, and -C in humans (5) [although there is evidence for low level expression of HLA-C (6)]. Moreover, trophoblast cells do not express MHC class II molecules. Intuitively, this should suffice to protect the fetus from immune destruction from T cells, except for the NK cell. Recent studies, summarized previously in the *Proceedings* (7), have shown that NK cells differ from other lymphocytes in their self versus non-self discriminatory mechanisms. Initially described in terms of capacity to kill tumors, NK cells appear capable of detecting "missing-self" because they are chronically inhibited from activation (killing or cytokine production) by MHC class I molecules, normally ubiquitously expressed on tissues (8). In the absence of MHC class I on a target cell, the NK cells are released from inhibition and kill the target. Thus, fetal trophoblast cells should be extremely susceptible to killing by maternal NK cells (or even fetal NK cells, should they be mature enough) because the trophoblasts lack expression of the classical MHC class I molecules.

Indeed, there is now ample evidence that maternally derived NK cells accumulate at the sites of implantation and in the uterine mucosa (decidua) during pregnancy in essentially all placental species studied thus far, whereas few T cells are present (9). Described in rodent anatomical studies as uterine granulated metrial gland cells (10), these decidual cells closely resemble conventional NK cells found in lymphoid tissues in terms of bone marrow derivation, cell surface molecule expression, and functional activities (killing, cytokine response, and production) (11, 12), except that decidual NK cells generally do not express CD16, which is present on most peripheral NK cells (13). Moreover, the uterine NK cells appear to be activated because they also express molecules generally found on conventional NK cells only after activation (9, 13, 14). Most studies therefore support the thesis that decidual and conventional NK cells are quite similar, except for anatomical distribution and probable activation. If NK cells accumulate in the area of fetal cells that lack MHC class I molecules, how are they prevented from killing the fetus?

NK cells express receptors that are specific for certain MHC class I molecules, and upon engagement, these receptors deliver inhibitory signals that block activation through other receptors or pathways (7). The NK cell receptors fall into two general categories based on structure: lectin-like molecules that are type II integral membrane proteins, i.e., the Ly-49 family in mice, and the CD94/NKG2 molecules in humans (15, 16). The second type of MHC receptors consists of Ig-superfamily type I transmembrane proteins, such as the killer inhibitory receptors (KIR) in humans (17–19), and possibly, gp49B1 in mice (20, 21). Regardless of structure, the receptors appear to inhibit NK cell activation by recruitment of the tyrosine phosphatase, SHP-1, to a phosphorylated tyrosine in the immunoreceptor tyrosine-based inhibitory motif, present in the cytoplasmic domains of both types of inhibitory receptors (22–24).

Although further studies are required, decidual NK cells express MHC class I inhibitory receptors (9, 25, 26). Despite this, specificity and expression analyses have revealed that both

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types of inhibitory receptors are expressed on overlapping "subsets" of NK cells (7). Targets expressing individual MHC class I molecules are not killed by NK cells with inhibitory receptors specific for that MHC molecule but the targets remain susceptible to killing by other NK cells. Although an individual NK cell receptor has "promiscuous" specificity for more than one MHC class I molecule, to date, no one MHC class I molecule has been found to inhibit all NK cells. Thus, even if maternal NK cells use their NK cell receptors to recognize MHC class I, it was difficult to understand how all of these NK cells could be turned off in the maternal–fetal environment.

The answers to these issues appear to be resident in ongoing studies of an interesting, "non-classical" MHC class I, or class Ib, molecule known as HLA-G (27, 28). A relatively nonpolymorphic molecule, it appears to be expressed only in fetal tissues, particularly in trophoblast cells at the maternal–fetal interface (29, 30). Although mRNA can be detected more broadly by reverse transcriptase–PCR, the results of this very sensitive assay have not been verified by protein expression studies (31). HLA-G molecules appear to resemble classical HLA class I molecules in terms of intracellular assembly with β_2-microglobulin (β_2m) and peptide fragments (32, 33). However, the capacity for peptide presentation may not be relevant to their apparent role in maternal–fetal interactions.

Recent reports indicate that HLA-G molecules are also capable of inhibiting NK cells, and Rouas-Freiss *et al.* (4) provide new information that may be especially important in understanding maternal–fetal interactions. Transfection of HLA-G cDNA into an NK-sensitive target was performed, and expression levels were comparable to HLA-G expression on a choriocarcinoma (malignant trophoblast) cell line that presumably reflects the expression of HLA-G on the surface of normal trophoblast cells (34). Most surprisingly, and extending the observations of other groups (11, 35), the HLA-G transfectant was completely protected from killing by polyclonal NK cell populations from 20 different donors. Inhibition was reversed by anti-HLA-G-specific mAbs. Recently, Pazmany *et al.* (36) also described studies on two distinct human NK cell clones. Regardless of clonal specificity for conventional MHC class I (HLA-C) alleles, they found that HLA-G expression inhibited NK cell activity. This inhibition was due to an apparent interaction with two distinct Ig-superfamily-type NK cell receptors, with otherwise clear specificity for either of two dimorphic allelic groups of HLA-C molecules. However, in another study, this interaction was confined to a different KIR molecule, NKAT3 (37). Inasmuch as HLA-G transfectants that expressed relatively low levels of HLA-G were examined, the latter results (37) may indicate a higher affinity for NKAT3, normally specific for HLA-B alleles, so the other KIR may be active only when HLA-G is expressed at normal levels. Interestingly, Rouas-Freiss *et al.* (4) also found a cell line (YT2C2) with NK-like activity that was inhibited by HLA-G expression but did not apparently express the HLA-C-specific KIR receptors. Whether YT2C2 expresses an HLA-B-specific receptor, like NKAT3, is not yet known. Because it currently appears that CD94 associates with all known human lectin-like NK cell receptors and is expressed on all NK cells, including the vast majority of decidual NK cells (26), the lectin-like receptors were attractive candidate receptors for HLA-G in YT2C2. However, YT2C2 does not express CD94, excluding this possibility, but it is unknown if YT2C2 expresses NKG2 molecules, whether NKG2 could pair with molecules other than CD94, or even if the lectin-like receptors bind HLA-G. In addition, YT2C2 may express yet-to-be-defined NK cell receptors. Nevertheless, these studies are consistent with the proposal by Rouas-Freiss *et al.* (4) that HLA-G molecules may represent "public" or universal ligands for receptors on all NK cells. (The NK cell field has now come full circle from a previous underappreciation for subsets with specificity for only certain MHC class I molecules to the possibility that one MHC-like molecule could inhibit all NK cells.) Presumably, regardless of the maternal repertoire of NK cell receptors, or the expression of individual receptors on NK cell subsets, HLA-G expression by the fetus should be sufficient to protect it from any maternal NK cell attack. Although the role of a soluble HLA-G molecule is unknown in this context (38), missing-self receptors may therefore regard HLA-G-expressing fetal tissues as displaying self.

HLA-G expression therefore begins to explain protection from maternal NK cells but it does not explain the situation in mice for several reasons. Until recently, no HLA-G homologue had been identified in mice. However, Sipes *et al.* (39) described the cloning of a mouse MHC class Ib molecule that resembles HLA-G in tissue distribution, perhaps with even tighter restriction to mouse fetal tissues. By reverse transcriptase–PCR analysis, this MHC molecule is expressed only in blastocyst and placenta. Although certain mouse strains lack a functional gene, it is otherwise identical in other strains examined, implying that a conserved function exists and that there may be additional related molecules yet to be described. If the blastocyst MHC molecule is related to HLA-G and to fetal protection, the capacity to genetically alter expression of this molecule in mice predicts the development of extremely interesting investigations that should provide significant functional insight into HLA-G and blastocyst MHC by providing tests of their role in preventing fetal destruction. Moreover, there will be benefit from further analysis of mice transgenic for HLA-G that display faithful recapitulation of HLA-G expression in trophoblasts (40).

Another consideration arises when examining mice with targeted mutations in genes for molecules known to be important in the normal expression of MHC class I. For example, classical MHC class I molecules are synthesized in the endoplasmic reticulum, where they assemble with β_2m and peptides (41). The latter are apparently translocated from the cytoplasm and across the ER membrane by transporters of antigen processing (TAP). In cells with deficiencies in β_2m or TAP expression, MHC class I expression is nearly absent. Such cells are extremely susceptible to lysis by NK cells (42, 43). Likewise, targeted mutations in β_2m or TAP genes results in animals with significant loss of normal MHC class I expression (44, 45), and their cells are susceptible to natural killing (46). Because HLA-G and blastocyst MHC molecules resemble MHC class I molecules, such disruption may result in marked decrease in trophoblast expression of these molecules and fetal susceptibility. Indeed, embryonic cells may not normally express TAP genes (47), and these cells are quite susceptible to killing by IL-2-activated NK cells (48). Yet, β_2m or TAP-deficient animals apparently develop normally.

It is not currently known, however, if HLA-G and related molecules require all aspects of conventional MHC class I assembly pathways for expression. For example, reasonable levels of HLA-G can be expressed on cells that lack TAP expression (32). Moreover, alternatively spliced HLA-G transcripts have been described (49). Notably, one transcript (HLA-G2) lacks the α2 domain that is required for formation of the peptide binding cleft in which MHC class I-associated peptides reside. Rouas-Freiss *et al.* (4) expressed HLA-G2 by transfection and show reactivity of this molecule with an HLA-G-specific mAb and W6/32, which generally detects surface HLA class I molecules in association with β_2-m. Although this result should be confirmed by immunoprecipitation analysis, HLA-G2 expression inhibited NK cell killing. Inasmuch as HLA-G2 molecules lack the peptide binding domain, its capacity to inhibit strongly suggests that HLA-G molecules need not bind peptides to inhibit NK cells. Moreover, the three-dimensional structure of the α1 domain is unknown, but it presumably folds into an α-helix that may be specifically recognized by NK cell receptors because these

receptors recognize α1 domains in classical HLA class I molecules (50–52). It is also possible that HLA-G2 molecules form dimeric structures resembling MHC class II molecules. It is not yet known whether any or all of these issues apply to the mouse blastocyst MHC molecule.

Finally, what are the NK cells doing in the uterine decidua? Are they preventing other maternal immune responses, such as T cell immigration and activation? Further analysis is required to provide experimental evidence to address the issues and to substantiate the speculations raised here. Nevertheless, the stage is now set to further analyze a previously perplexing area of immunology, the union between mother and child.

Note Added in Proof. CD94/NKG2A receptors can also interact with HLA-G and inhibit human NK cell activation (K. Soderstrom, J. H. Phillips, and L. L. Lanier, unpublished observations presented at the Keystone Symposium on Tolerance and Autoimmunity, April 13–19, 1997, Keystone, CO).

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