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Commentary

Natural killer cells fertile with receptors for HLA-G?

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Natural killer (NK) cells are lymphocytes involved in the innate immune response against certain microbial and parasitic infections (1, 2). These cells comprise ≈5–20% of peripheral blood lymphocytes and are present in spleen, liver, bone marrow, and thymus at a lower frequency and are rare in lymphatics and lymph nodes. However, in the mucosal decidual tissues of the maternal uterus, NK cells are the most abundant class of lymphocyte, representing up to 95% of all lymphocytes. Initially identified by their distinct morphology, NK cells in the placenta were designated ''granulated metrial gland cells'' in rodents (3) and ''uterine granular lymphocytes'' in humans (4, 5). Why do NK cells preferentially accumulate in the decidua? No clear rationale has emerged to explain this phenomenon. Given the established role of NK cells in innate immunity, protection of the fetus against transplacental infections is a good bet, but nonimmune functions involving fetal sustenance have also been proposed (6).

With an abundance of maternal decidual NK cells, what prevents recognition and elimination of fetal tissues as ''nonself''? This issue is of particular relevance because NK cells have been shown to mediate rejection of parental bone-marrow allografts in F_1 recipients, demonstrating that NK cells are capable of alloantigen recognition and elimination of cells perceived as nonself (7). Therefore, why do maternal NK cells in the decidua tolerate the semiallogeneic fetal tissues? Several explanations are possible. First and foremost, fetal tissues may evade detection by maternal NK cells simply by failing to express antigens that evoke an NK cell attack. Alternatively, fetal tissues in contact with maternal lymphocytes may actively turn off or suppress the effector function of the mother's immune defenses. In this issue of the *Proceedings*, Ponte *et al.* (8) report that NK cells in pregnant women preferentially up-regulate expression of their inhibitory NK cell receptors that bind to HLA-G, an MHC class I molecule that is predominantly expressed by fetal extravillous trophoblasts.

Kärre and colleagues (9) initially observed that NK cells can detect and eliminate tumors that lack expression of MHC class I antigens. This led to the discovery and characterization of an abundance of cell-surface receptors that are capable of recognizing MHC class I molecules, resulting in the inactivation of NK cell effector function on encountering potential target cells bearing self class I ligands (reviewed in refs. 10–12). In humans, three structurally distinct families of NK cell receptors that bind MHC class I molecules have been identified. The killer cell Ig-like receptors (KIR) are encoded by a family of about 10 genes located on human chromosome 19q13.4. Many of these receptors are responsible for the recognition of polymorphic epitopes present in the α1 domain of HLA-B and HLA-C molecules (13–16). KIR bind to the trimolecular class I complexes (heavy chain, β2-microglobulin, and bound peptide), and the composition of the peptide can influence this interaction (17, 18). The CD94/NKG2 NK cell receptor is a member of the C type lectin superfamily, is present on human chromosome 12p12–p13, and is responsible for recognition of the nonpolymorphic MHC class I molecule HLA-E (19–21). Whereas the CD94/NKG2 receptor also recognizes a trimolecular complex of HLA-E with β2-microglobulin and bound peptide, an unusual feature of HLA-E is that it preferentially and perhaps exclusively binds peptides that are derived from the leader segments of certain other class I proteins, including leader peptides from certain alleles of HLA-A, -B, -C, and -G. Without these leader peptides, HLA-E cannot be expressed on the cell surface (22, 23). Another family of receptors for MHC class I molecules that are predominantly expressed on myeloid cells, but also a subset of NK cells, are the ILT (also called LIR) molecules (24, 25). The ILT receptors are encoded by genes of the Ig superfamily present on human chromosome 19q13.3 just centromeric of the *KIR* genes (24, 25). ILT2 and ILT4 are receptors for MHC class I, but unlike KIR, are quite promiscuous in their ligand specificity, binding HLA-A, -B, -C, and -G ligands (25–29). All of these inhibitory NK cell receptors, the KIR, NKG2A, and ILT2, possess an immunoreceptor tyrosine-based inhibitory motif (ITIM) sequence in their cytoplasmic domains that is responsible for inactivation of NK cell effector function. On ligand binding, the tyrosine in the ITIM is phosphorylated and subsequently recruits a cytoplasmic protein tyrosine phosphatase, e.g., SHP-1 or SHP-2, that is responsible for inhibition of NK cell activity (12).

Most tissues, excluding the central nervous system, constitutively express MHC class I molecules. However, human fetal extravillous trophoblasts, in contact with material tissues, lack substantial expression of the conventional MHC class I molecules HLA-A and HLA-B and instead express another class I gene designated HLA-G (30) and possibly low levels of HLA-C (31). Unlike HLA-A and -B, HLA-G demonstrates limited polymorphism (32) and is largely restricted in expression to fetal tissues (30, 33–36); although there have been reports of HLA-G transcripts in other anatomical sites (37–39), including thymic epithelial cells, where it may be important for establishing T cell tolerance (39). Although highly restricted in tissue distribution, HLA-G shares many features with the conventional MHC class I molecules. It is expressed on the cell surface as a trimolecular complex, comprised of an HLA-G heavy chain that is noncovalently associated with β2-microglobulin (30) and an ≈9-aa peptide in the antigen-binding groove (40, 41). Surface expression of HLA-G requires functional TAP, and the bound peptides are indistinguishable from peptides bound to classical class I molecules (40, 41). Both membrane-bound and soluble HLA-G proteins have been detected (41–43).

In 1994, Chumbley *et al.* (44) first reported that transfection of HLA-G into a B lymphoblastoid cell line protected these target cells from lysis by decidual NK cells. While the ability of HLA-G to inhibit NK cell-mediated lysis has been reproduced by many laboratories (8, 45–52), the NK cell receptors responsible for HLA-G recognition have remained a controversial issue. Initial studies suggested that the KIR molecules recognizing HLA-Bw4 (53) or HLA-C (51, 52) cross-reacted with HLA-G; however, these findings have not been confirmed

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in subsequent work by several independent laboratories (48, 49, 54, 55). Many or most of the NK cell clones recognizing target cells expressing HLA-G may in fact not express any receptors that interact directly with HLA-G itself but instead may rely on the ability of the HLA-G leader fragments to bind to the HLA-E peptide-binding groove and permit cell-surface expression of HLA-E, which in turn is recognized by the inhibitory CD94/NKG2A receptor (48, 49, 54, 56, 57). In contrast, the ILT2 (also called LIR-1) receptor directly binds to HLA-G (26, 58) and on the minor subset of NK cells expressing ILT2 this receptor inhibits lysis of target cells expressing HLA-G (26, 29, 49).

Until recently, it seemed that NK cell recognition of HLA-G by peripheral blood or decidual NK cells could be explained in most situations by the CD94/NKG2A receptor interacting with HLA-E containing an HLA-G-derived leader peptide in the antigen-binding groove or in a minority of cases direct HLA-G recognition by ILT2 on an NK cell subset (refs. 48 and 49; A. King, Y. W. Loke, and V. M. Braud, personal communication). However, Ponte *et al.* (8) in this issue and Rajagopalan *et al.* (55) now implicate a receptor encoded by the KIR family in HLA-G recognition. This molecule, originally designated KIR103 (59), [referred to hereafter as KIR2DL4 (60)], differs from other KIR in both its structure and distribution. All other KIR molecules either contain a relatively long cytoplasmic domain with two ITIM sequences responsible for the receptor's inhibitory function and lack a charged amino acid in the transmembrane or have short cytoplasmic regions lacking ITIM and possess a lysine in the transmembrane that permits association with DAP12, an adaptor protein with an immunoreceptor tyrosine-based activation motif (ITAM) that allows these KIR isoforms to activate NK cell function. By contrast, KIR2DL4 has a single ITIM in the cytoplasmic region as well as a lysine in the transmembrane (59). Rajagopalan *et al.* (55) have shown by genetic transfer that the KIR2DL4 molecule inhibits NK cell lysis of target cells expressing HLA-G. Furthermore, both Rajagopalan *et al.* (55) and Cantoni *et al.* (61) have demonstrated direct binding of KIR2DL4 fusion proteins to HLA-G, confirming the specificity of this interaction. The most provocative finding in the study by Ponte *et al.* (8) is the report that KIR2DL4 is expressed on a substantial proportion of NK cells in the decidua during the first trimester of pregnancy and on essentially all NK cells obtained from the placenta at term, but KIR2DL4 was not detected on the circulating peripheral blood NK cells in the mother. Remarkably, a comparison of the peripheral blood NK cells from pregnant and nonpregnant women indicated an up-regulation in the cell surface expression of CD94/NKG2A and ILT2 (LIR-1) receptors in the pregnant women. These results suggest a pregnancy-induced hormonal regulation of the NK cell receptors that are implicated in recognition of HLA-G on fetal trophoblasts. Previous studies have also shown a difference in the antigen phenotype between maternal peripheral blood and decidua-derived NK cells (45, 62).

Despite convincing evidence that KIR2DL4 can recognize HLA-G, several aspects remain unclear. First, previous studies have shown that unlike other KIR molecules that are arrayed on overlapping subsets of NK cells, KIR2DL4 transcripts are present in all NK cell clones, including NK cells from male donors (63). Consistent with ubiquitous transcription of this gene, Rajagopalan *et al.* (55) reported that all peripheral blood NK cells express KIR2DL4 on the cell surface. This discrepancy between the results of Rajagopalan *et al.* (55) and Ponte *et al.* (8) regarding expression of KIR2DL4 needs to be resolved to interpret the potential significance of this receptor in pregnancy. If indeed all NK cells express KIR2DL4, then this raises a further paradox. Many NK cell clones lacking CD94/NKG2A and ILT2 are able to kill HLA-G bearing target cells, apparently because they are unable to detect HLA-G. If these NK clones express KIR2DL4, why doesn't it work? Whether the unusual structural features of KIR2DL4 account for this phenomenon should be explored.

The issue of whether HLA-G exists for the protection of the fetus from maternal NK cells should also be viewed in a broader biological context. As yet, there is no evidence in other nonprimate species for an HLA-G structural homolog or another MHC class I molecule with preferential expression by trophoblasts or fetal tissues. Furthermore, a human with a homozygous *HLA-G*-null mutation did not suffer any dire consequences during fetal development (64). Finally, it should be noted that fetal mice with homozygous disrupted β2-microglobulin genes are not rejected by their heterozygous +/− mothers. Therefore, whereas the colocalization of HLA-G on human fetal trophoblasts and the abundance of maternal NK cells in the decidua is a provocative observation, as yet it is uncertain whether this represents guilty by association or a symbiotic physiological interaction.

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