

## Role of NK cells and HLA-G in endometriosis

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### 1. ABSTRACT

Impaired natural killer (NK) activity in women with endometriosis is thought to promote implantation and progression of endometrial tissue, in accord with Sampson's hypothesis. However, the mechanisms responsible for decreased NK cell activity and the antigens recognized by NK cells are not clear. We focused on human leukocyte antigen (HLA)-G, a ligand of NK receptors, expression and its menstrual cycle changes by eutopic endometrium. Interestingly, HLA-G expression was identified on eutopic endometrium only in the menstrual phase but not in the proliferative or secretory phases. Furthermore, HLA-G expressing cells were also detected in peritoneal fluid during the menstrual period. During retrograde menstruation, HLA-G expressing endometrial tissue may enter the peritoneal cavity, and may be reduced by immunosurveillance system. Although peritoneal NK cells play an important role in this system, impairment of NK cytotoxicity via HLA-G may allow peritoneal endometrial cell survival and implantation. In this review, we discuss the pathogenesis of endometriosis from the viewpoint of intraperitoneal immune interaction between NK cell receptors and HLA-G that can enter into peritoneal cavity from eutopic endometrium through retrograde menstruation.

### 2. INTRODUCTION

Endometriosis is a condition in which foci of hormonally responsive endometrial tissue including epithelial and stromal components, are present at ectopic sites, particularly in the peritoneal cavity. A menstrual reflux/implantation theory was proposed by Sampson (1), and an epithelial metaplasia hypothesis (2) has also been proposed. However, the pathogenesis of endometriosis is still not clearly understood.

Since the 1990's, the host immune response in the peritoneal cavity has received increasing attention with respect to the initiation and progression of endometriosis (3-8). Cells in the peritoneal fluid (PF) are mostly macrophages, accompanied by other cells such as lymphocytes, natural killer (NK) cells, and mesothelial cells. In addition, endometrial components deposited by retrograde menstruation can be identified in most women during menstruation (9-11).

Decreased NK activity in the peripheral blood (PB) and PF in women with endometriosis was first

reported by Oosterlynck (3,4); this decrease is thought to promote implantation of endometrial tissue as a tissue graft (9). However, the cause of decreased NK cell activity and the endometrial antigens to which NK cells can respond are not clear.

Since the identification in 1995 of the killer cell immunoglobulin-like receptor (KIR) on NK cells (12, 13), a number of KIR families that can recognize self-antigens and regulate cytotoxicity have been identified (14). In women with endometriosis, inhibitory-motif KIR2DL1<sup>+</sup> NK cells were increased in the PB and PF (15-17), suggesting a possible cause of decreased NK activity in endometriosis. The ligand recognized by KIR2DL1 is the classical human leukocyte antigen (HLA)-C (HLA class Ia).

Another HLA, HLA-G was identified as the ligand of another type of KIR, KIR2DL4 (18). HLA-G, considered as non-classical HLA (HLA class Ib), is strongly expressed by the placental trophoblast, and is thought to be involved in the maintenance of pregnancy (19-24). Recently, HLA-G has also been identified on cells sustaining stress and damage (25), and is expressed on the glandular epithelium of peritoneal endometriotic tissue (26). Although HLA-G is thought to be a specific antigen expressed on the epithelium of endometriotic tissue, the origin of HLA-G is unknown.

During retrograde menstruation, antigens expressed by eutopic endometrial tissue may enter the peritoneal cavity (9-11). Retrograde menstruation is backflow of menstrual discharge from the uterine cavity through the Fallopian tubes into the peritoneal cavity, and has been identified as a physiological phenomenon observed in women with patent tubes (9-11).

Blood was found in the peritoneal fluid in 90% of women with patent tubes at laparoscopy during the perimenstrual period irrespective of whether endometriosis was present or not (27). However, if the Fallopian tubes were occluded, then evidence of blood in the pelvis was found in only 15% of patients; indicating that retrograde menstruation is a very common physiological event in all menstruating women with patent tubes (27).

The peritoneal cavity may have a physiological system that clears displaced tissue and debris from the peritoneal cavity. Peritoneal NK cells may play an important role in this system. Endometrial HLA-G expression during the menstrual period and entry into the peritoneal cavity may induce a local immune response with immunocompetent cells, including KIR2DL4-expressing NK cells. Decreased NK cell activity may allow endometrial cell survival and ectopic implantation.

In this review, we describe HLA-G antigen expression by the eutopic endometrium and its receptor expression on NK cells, and we also discuss the relation

of their interaction and the pathogenesis of endometriosis.

### 3. NATURAL KILLER (NK) CELLS AND ENDOMETRIOSIS

#### 3.1. NK cells

The NK cell is a cytotoxic lymphocyte that constitutes a major component of the innate immune system. Because NK cells can attack target cells without requiring antigen sensitization, they are called “natural killers” (28). These cells participate in the host defenses, including against infection (29) and tumors (30), as well as tissue grafting rejection (31), but can also adversely affect pregnancies (32).

NK cells are included in the large granular lymphocytes (LGL), and they usually express the cell surface markers CD16 (Fc gamma RIII) (33) and CD56 (neural cell adhesion molecule: NCAM) (34) in humans. In addition to killing target cells, NK cells secrete cytokines such as the antiviral cytokine interferon (IFN)- $\gamma$  (35, 36) and the inflammatory and antitumor cytokine tumor necrosis factor (TNF)- $\alpha$  (37).

NK cell activity is assessed by cytotoxicity against the NK sensitive chronic myelogenous leukemia cell line K562 (38, 39). NK cells do not require activation in order to kill target cells that are missing the major histocompatibility complex (MHC) class I. Kärre *et al.* (40, 41) proposed a “missing self” hypothesis, in which NK cells are toxic to target cells that do not express MHC determinants characteristic for “self”, while toxicity against target cells that express these determinants is inhibited.

During the past decade, NK cells have been shown to have activating receptors, that activate the NK cell when it binds to a target cell (28), and also inhibitory receptors that transmit an inhibitory signal if they encounter class I MHC molecules on a cell surface. The “missing self” hypothesis was supported by the identification of KIRs on NK cells that recognize self-determinants in MHC class I (12, 13), inhibiting NK cytotoxicity against target cells bearing these determinants.

#### 3.2. Activation of NK cells

Because of their strong cytotoxic activity and the potential for autoreactivity, NK cell activity is strictly regulated by certain factors. Cytokines play a crucial role in NK cell regulation. Cytokines involved in NK activation include interleukin (IL)-2 (35), IL-12 (42), IL-15 (43), IL-18 (44), IL-21 (45), IFN- $\gamma$  (35), granulocyte-macrophage colony-stimulating factor (GM-CSF) (46).

NK cells, as well as macrophages and several other cell types, express the Fc receptor (FcR) molecule (Fc gamma RIII: CD16/Leu-11 antigen), an activating receptor that can bind the Fc portion of antibodies (33). This binding allows NK cells to lyse target cells through antibody dependent cellular cytotoxicity (ADCC) (47, 48). NK cells are fundamentally different from T cells in lineage and non-self recognition (40, 41).

Other than the Fc receptor, NK cells express several different activating and inhibitory receptors, which maintain the balance of positive and negative signals for the cytolytic mechanism. The KIR family recognizes and binds its ligand, the classical and non-classical MHC class I molecules (12, 13). Though KIR has both activation and inhibitory motifs in cytoplasm, a lot of these receptors mediate inhibition of NK cell cytotoxicity (12, 13). Beside the KIR, the non-classical MHC I molecule HLA-E is recognized by the lectin-like CD94/NKG2 receptor family containing both activation and inhibitory forms (49-51). Thus, an individual NK cell can simultaneously express both activating and inhibitory receptors.

### 3.3. NK cells and endometriosis

In 1991, Oosterlynck *et al.* reported decreased NK cell activity in the PB against autologous endometrium in women with endometriosis (3). This is the first report of a relationship between the host immune system and the onset and development of endometriosis. Impaired NK cell activity in women with endometriosis is thought to promote implantation of endometrial tissue as a tissue graft, in accord with Sampson's hypothesis (1). Subsequently, decreased NK cell activity in PF from women with endometriosis was also reported (4).

This unique immune response, with decreased NK cell activity and its positive correlation with the severity of the disease in both the PB and PF, may lead to a common consensus concerning the pathogenesis of endometriosis (3-8).

Concerning NK cell function, decreased NK cell activity is not caused by a quantitative defect, but by a qualitative problem (52). Although a significant decrease of NK cytotoxicity was seen in the PF of women with endometriosis, no difference was seen in the proportion of NK cells in both the PB and PF between women with and without endometriosis (53). Decreased NK cytotoxicity in PB and PF of women with endometriosis was due to a functional deficit, not a quantitative deficit, of NK cells (52, 53).

After removal of endometriotic lesions, decreased NK cell activity and impaired cytotoxicity from autologous and heterologous lymphocytes against the endometrium remained unchanged, and that cytotoxicity was still significantly decreased compared to women without endometriosis. These results suggest a primary deficiency in NK cell activity in women with endometriosis, and correlate to the high relapse rate following treatment (54).

The mechanisms responsible for decreased NK cell activity, and also the antigens recognized by NK cells in endometriosis, are not clearly understood. The persistence of endometrial cells in the peritoneal cavity of women with endometriosis (55) is mainly due to decreased NK cell activity, but is also partially due to resistance of the endometrium to NK cytotoxicity (56-58).

The "missing self" hypothesis was supported by the identification of KIRs on NK cells that recognize self-determinants in the MHC class I (39, 40), inhibiting NK cytotoxicity against target cells bearing these determinants.

In women with endometriosis, inhibitory motif killer immunoglobulin-like receptor on NK cells in the PB as well as in the PF was significantly increased in comparison with women without endometriosis (15-17), indicating decreased NK cell activity and cytotoxic reaction to endometriotic cells.

## 4. NK CELL RECEPTORS

### 4.1. Killer-cell immunoglobulin-like receptor (KIR)

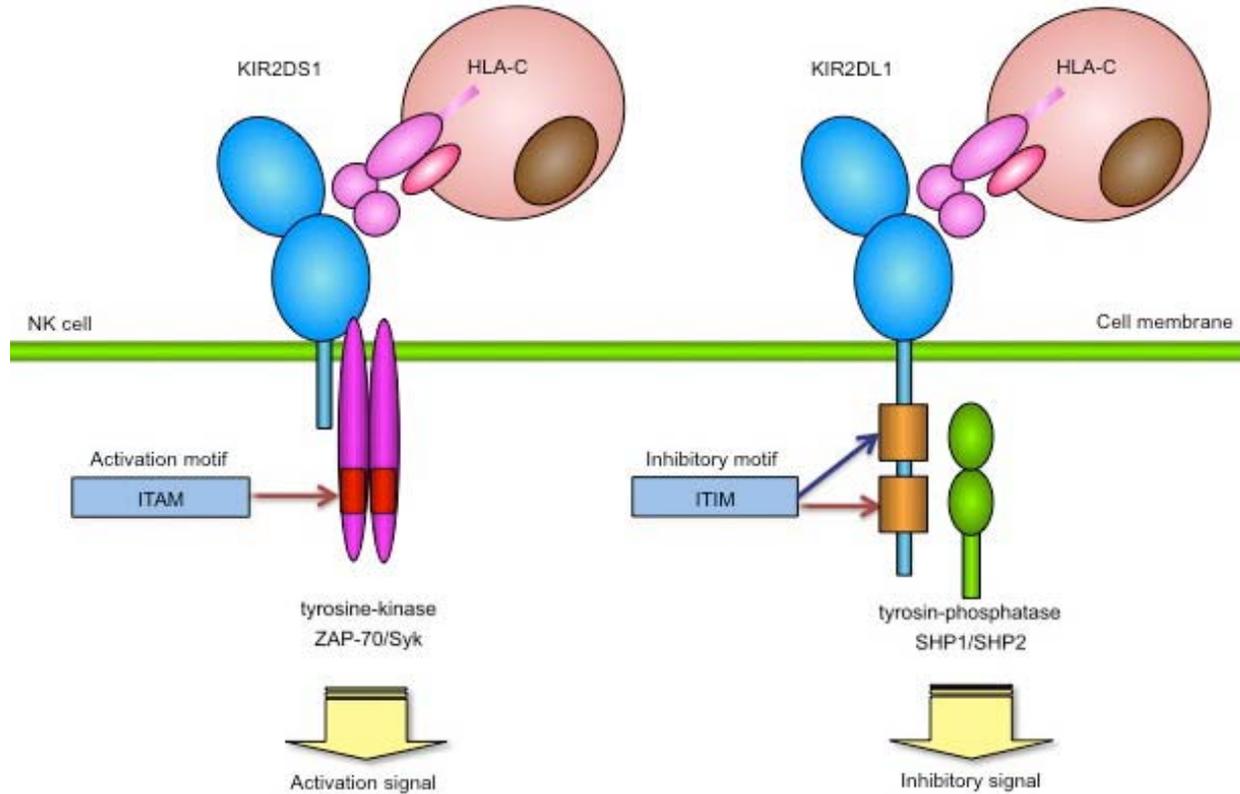
In 1995, phenotypic and functional analysis of a large number of NK clones showed that clones expressing activating p50 molecules consistently co-expressed inhibitory p58 receptors for HLA class I-C alleles (12). A novel family of KIRs has been defined for transmembrane glycoproteins expressed by NK cells and subsets of T cells.

The KIR genes are polymorphic and highly homologous, and they are identified in a cluster on chromosome 19q13.4 (12). The KIR proteins are classified by the number of immunoglobulin (Ig)-like extracellular domain (2D or 3D) receptors, and by whether they have a long (L) or short (S) cytoplasmic tail. KIR proteins with a long cytoplasmic domain elicit inhibitory signals upon ligand binding *via* an immunoreceptor tyrosine-based inhibitory motif (ITIM) after binding with tyrosinophosphatase SHP1/SHP2, while KIR proteins with a short cytoplasmic domain that lacks the ITIM instead associate with the tyrosine kinase-binding protein ZAP-70/Syk to switch activating signals *via* an immunoreceptor tyrosine-based activation motif (ITAM) (59-61) (Figure 1).

Most KIRs are inhibitory, indicating that their recognition of MHC suppresses the cytotoxic activity of their NK cells. Only a limited number of KIRs have the ability to activate cells. The ligands for several KIR proteins are subsets of both classical HLA class Ia (HLA-A, B, and C) and also non-classical HLA class Ib (HLA-G) (62, 63). KIR proteins are therefore considered to play an important role in the regulation of the immune response.

Recently, KIR2DL4 has become the focus of attention due to its distinctive cytotoxicity within the KIR family. KIR2DL4 is an unusual member of the KIR family expressed in all NK cells and some T cells. KIRs are composed of intracytoplasmic ITIM and ITAM domains, by which they regulate cytotoxicity (60, 61). KIR2DL4 includes both wild (10A) and mutant (9A) receptor types (64,65). KIR2DL4 can originally activate the cytotoxicity of NK cells, despite the presence of an ITIM in its cytoplasmic tail (10A) (66). Mutant forms of KIR2DL4 (9A) have been engineered that lack either the tyrosine in the ITIM or an arginine-tyrosine motif in the transmembrane region that is required for the activation signal.

KIR2DL4 has been shown to specifically recognize HLA-G (67), and exhibits "activation" potential so that ITIM does not influence its activating function (64, 66, 68). Yan *et al.* demonstrated that residues Met 76 and Gln79 in the HLA-G alpha 1 domains are involved in KIR2DL4 recognition, and induce cytotoxicity. KIR2DL4 showed high cytotoxicity against wild type HLA-G



**Figure 1.** CD158a proteins are classified by extracellular 2 domain KIR receptors with a long or short cytoplasmic tail. KIR proteins with a long cytoplasmic domain transduce inhibitory signals upon ligand binding via ITIM after binding tyrosin-phosphatase SHP1/SHP2, while KIR proteins with a short cytoplasmic domain that lacks the ITIM instead associate with the tyrosine kinase-binding protein ZAP-70/Syk to elicit activating signals via ITAM. NK: natural killer; KIR: killer immunoglobulin-like receptor; ITIM: immunoreceptor tyrosine-based inhibitory motif; ITAM: immunoreceptor tyrosine-based activation motif

expressing K562 cells, as compared to mutant HLA-G expressing K562 cells. These results indicate that KIR2LL4 is activated and retained cytotoxicity to HLA-G expressing cells as its target (67).

Other than KIR2DL4, ILT-2, which also belongs to the Ig-super family receptors and has four extracellular Ig domains and four ITIMs in its intracytoplasmic tail, and ILT-4, which is selectively expressed in monocytes, macrophages and dendritic cells (DCs), can bind to both classical HLA class Ia molecules and non-classical HLA class Ib molecules HLA-G. ILT-2 and ILT-4 are both inhibitory members, having variable numbers of cytoplasmic ITIM domains (69).

#### 4.1.1. KIR2DL1/KIR3DS1 and endometriosis

Next we will discuss the cause of decreased NK cell activity in terms of the inhibitory NK receptor KIR in women with endometriosis.

The percentage of cells expressing KIR2DL1 among NK cells in the PF and PB was significantly higher in women with endometriosis than in controls; suggesting KIR2DL1 plays a role in NK cell suppression in endometriosis (15). The increased percentage of KIR2DL1<sup>+</sup>

NK cells in the PB of women with endometriosis was undiminished by laparoscopic surgery or gonadotropin releasing hormone (GnRH) agonist treatment. This overexpression may be the primary event, and represents a risk factor for development of endometriosis and its recurrence after treatment (16). ITIM-KIR expression by PB-NK cells was significantly greater than ITAM-KIR expression in both women with endometriosis and controls. However, ITIM-KIR expression by PB-NK cells was significantly higher in women with endometriosis than in controls. (17). In women with endometriosis, expression of ICAM-1 by peritoneal macrophages was significantly lower, and expression of KIR by NK cells in the PF and PB significantly higher, than in controls (70). Furthermore, increased ITIM-KIR2DL1 expression by NK cells, and decreased HLA-ABC and -DR expression by macrophages, suggest decreased functional activation in women with endometriosis (72). As described above, increased KIR2DL1 expression by NK cells may represent a risk factor for the pathogenesis of endometriosis.

According to KIR genotype investigation, the frequency of KIR3DS1 (ITAM) and the inhibitory KIRs/HLA class I combination genotypes was significantly higher in patients with endometriosis than in controls (72).

These results suggest that polymorphism in KIRs may be associated with a susceptibility to endometriosis.

### 4.1.2. KIR2DL4 and endometriosis

KIR2DL4 expressing NK cells are identified both in the PB and PF (73). HLA-G, a ligand for KIR2DL4, ILT-2, and ILT-4 were expressed by eutopic endometrium only in the menstrual phase and not in the proliferative or secretory phases (73). HLA-G expressing cells were detected in the PF during the menstrual phase. During retrograde menstruation, eutopic endometrial cells bearing HLA-G may enter the peritoneal cavity and react locally with KIR2DL4.

According to the innate immunological function of KIR2DL4 toward HLA-G, KIR2DL4 expressing NK cells in the PF may be dominantly cytotoxic for HLA-G expressing endometrial cells that enter the peritoneal cavity during retrograde menstruation. 9A/10A polymorphism would control the response of peritoneal NK cells against membrane-bound HLA-G on endometrial cells. Furthermore, inhibitory ILT-2 and ILT-4 receptors may affect the local immune response, together with KIR2DL4. Impaired and decreased cytotoxicity to peritoneal endometrial cells may therefore allow endometrial cell survival, and result in ectopic implantation of peritoneal endometriosis.

### 4.2. CD94/NKG2A

CD94/NKG2A is a C-type lectin receptor family composed of the CD94 chain covalently associated with a member of the NKG2 family (74, 75). The ligand of CD94/NKG2A is the non-classical HLA Ib molecule, HLA-E. After binding of CD94 with NKG2A that contains ITIM (76), the CD94/NKG2A heterodimer constitutes an inhibitory receptor, whereas the association of CD94 with other NKG2 lacking ITIM (NKG2C) may not constitute an inhibitory receptor (77). Valés-Gómez *et al.* showed that the inhibitory CD94/NKG2A receptor has a higher binding affinity for HLA-E than the activating CD94/NKG2C receptor, indicating that HLA-E can dominantly inhibit NK cell-mediated lysis by interacting with CD94/NKG2A receptors (78).

The HLA-E and CD94/NKG2A interaction plays a central role in the innate immune system. In particular, in early pregnancy, the overall effect of CD94/NKG2A interaction with HLA-E is the inhibition of cytotoxicity by decidual NK cells and the maintenance of pregnancy (79).

#### 4.2.1. CD94/NKG2A and endometriosis

Regardless of its interesting inhibitory function, little has been reported concerning CD94/NKG2A and endometriosis. Galandrini *et al.* reported that in women with stage III and IV endometriosis, the percentage of CD94/NKG2A<sup>+</sup> peritoneal NK cells was significantly higher than in the control group (80). Simultaneously, HLA-E, the CD94/NKG2A ligand, was identified in endometriotic tissue (80). Target cells bearing HLA-E were resistant to NK cell-mediated cytotoxicity in a CD94/NKG2A-dependent manner. Increased expression of CD94/NKG2A in peritoneal NK cells may mediate the

resistance of endometriotic tissue to NK cell cytotoxicity, thereby contributing to the progression of endometriosis (80).

### 4.3. Immunoglobulin-like transcript (ILT)

The immunoglobulin-like transcript (ILT) gene family includes up to 11 members in humans. The extracellular portion includes at least two, and usually four, immunoglobulin domains. ILT-2 through -5 are all inhibitory members, having variable numbers of cytoplasmic ITIM domains (69). ILT1, ILT7 and ILT8 contain a short cytoplasmic tail and a charged amino acid residue in the transmembrane domain that delivers an activating signal through the cytoplasmic ITAM of the associated common  $\gamma$  chain of the Fc receptor (FcR $\gamma$ ) (69). ILT family proteins are expressed predominantly on NK cells, T cells, and monocytes, and have been shown to bind both classical HLA class Ia (HLA-A and -B) and non-classical HLA class Ib (HLA-G1, -E and -F) molecules (69).

#### 4.3.1 ILT and endometriosis

ILT-2 and -4 can bind HLA-G with a 3 to 4-fold higher affinity than to classical MHC class Ia (81), suggesting that ILT/HLA-G recognition may play a dominant role in the regulation of NK cell, T cell, and monocyte activation. Furthermore, ILT2 and ILT4 effectively compete with CD8 for MHC class I binding, raising the possibility that ILT2 modulates CD8<sup>+</sup> T cell activation by blocking CD8 binding as well as by recruiting inhibitory molecules through its ITIM (81). Thus, HLA-G exerts inhibitory functions via ILT2 and ILT4 inhibitory receptors.

Although ILT is thought to play a role in the pathogenesis of endometriosis, surprisingly there have been no reports described the interaction between ILT and endometriosis.

## 5. LIGANDS FOR NK RECEPTORS ON ENDOMETRIAL CELLS

### 5.1. HLA class Ia

In 1990, HLA class I molecules were identified in human endometrial and endocervical epithelial cells (85). This finding suggests that KIRs expressed on NK cells can respond via their ligands HLA class I on eutopic endometrium (15-17). Progesterone also induces HLA class I mRNA expression in endometrial cultured cells in the secretory phase (87).

In women with endometriosis, significantly higher expression of HLA class I than in controls, both in the glandular and stromal cells, was observed (82). Furthermore, women with endometriosis had a significantly higher expression of HLA class I molecules in eutopic endometrial cells than controls. This is a possible explanation for their higher resistance to NK cytolysis (82).

According to our investigations, HLA-C expressing endometrial cells may enter the peritoneal cavity through retrograde menstruation and react locally with peritoneal NK cells expressing KIR2DL1 (15-17).

Impaired KIR2DL1 on peritoneal NK cells may allow endometrial cell survival and ectopic implantation in the peritoneal cavity, favoring the onset and progression of endometriosis.

### 5.2. HLA-E (HLA class Ib)

HLA-E is an HLA class Ib histocompatibility antigen. This class I molecule is a heterodimer comprising a heavy chain and a light chain ( $\beta$ -2 microglobulin) (83). The heavy chain is anchored in the membrane. HLA-E binds a restricted subset of peptides derived from the leader peptides of other HLA class I molecules. The heavy chain is approximately 45 kDa and its gene contains 8 exons. Exon one encodes the leader peptide, exons 2 and 3 encode the  $\alpha$ 1 and  $\alpha$ 2 domains, which both bind the peptide, exon 4 encodes the  $\alpha$ 3 domain, exon 5 encodes the transmembrane region, and exons 6 and 7 encode the cytoplasmic tail (83).

HLA-E is one of a family of molecules known as HLA class Ib, and has a very specialized role in cell recognition by NK cells. HLA-E is very highly conserved, and presents a small repertoire of peptides of various origins (84). NK cells recognize the HLA-E peptide complex using the heterodimeric inhibitory receptor CD94/NKG2A or the activating receptor CD94/NKG2C. When CD94/NKG2A is stimulated, it produces an inhibitory effect on the cytotoxic activity of the NK cell to prevent cell lysis (78).

#### 5.2.1. HLA-E and endometriosis

In women with stage III and IV endometriosis, the percentage of CD94/NKG2A<sup>+</sup> peritoneal NK cells was significantly higher than in the control group (80). HLA-E, the CD94/NKG2A ligand, was detected in endometriotic tissues. Target cells bearing HLA-E were resistant to NK cell-mediated cytotoxicity in a CD94/NKG2A-dependent manner. Increased expression of CD94/NKG2A in peritoneal NK cells may mediate the resistance of endometriotic tissue against NK cell cytotoxicity, thus contributing to the progression of endometriosis (80).

### 5.3. HLA-G (HLA class Ib)

HLA-G is a HLA non-classical class I heavy chain paralogue. This HLA class I molecule is a heterodimer comprising a heavy chain and a light chain ( $\beta$ -2 microglobulin). The heavy chain is anchored in the membrane (85).

A member of the HLA class Ib group, HLA-G, is alternatively spliced. Seven alternatively spliced transcripts have been identified, of which four are predicted to encode membrane bound, and three soluble proteins (85).

#### 5.3.1. Structural features of HLA-G

The heavy chain is approximately 45 kDa and its gene contains 8 exons. Exon 1 encodes the leader peptide, exons 2 and 3 encode the  $\alpha$ 1 and  $\alpha$ 2 domain, which both bind the peptide, exon 4 encodes the  $\alpha$ 3 domain, exon 5 encodes the transmembrane region, and exon 6 encodes the cytoplasmic tail (86, 87).

HLA-G encodes multiple isoforms as a result of alternative splicing. The full-length isoform HLA-G1 is

structurally similar to other class I genes, except for the truncated cytoplasmic tail. The G2 isoform results from the removal of exon 3 and homodimerizes to form an HLA class II-like structure (86, 87). These two isoforms are expressed as soluble proteins (HLA-G5 and -G6, respectively) due to the inclusion of intron 4 sequences in the mature mRNA, resulting in secreted proteins with an additional 21 amino acids (encoded by intron 4 sequences) following the 3 domain (86, 87). HLA-G3 results from the removal of exons 3 and 4. HLA-G4 and -G7 mRNAs are not abundant in the placenta. Exon 4 (encoding the 3 domain) is spliced out of the HLA-G4 transcript; the HLA-G7 transcript includes exon 2 and part of intron 2, and is predicted to encode a small soluble isoform (86, 87).

#### 5.3.2. Receptors for HLA-G

HLA-G appears to be recognized mainly by ILT-2 and -4 receptors, which are expressed on T and B lymphocytes, as well as on NK cells and monocytes, and inhibits the activating signals received by these cells. Other than ILT, HLA-G also can react with KIR2DL4 expressed on NK cells and T cells. Although ILT2, ILT3, ILT4, and KIR2DL4 expression is up-regulated by HLA-G in antigen-presenting cells, NK cells, and T cells (88), the question of whether the HLA-G/KIR2DL4 interaction indicates activation or inhibition for NK cells is complicated and controversial.

#### 5.3.3. Function of HLA-G

Since it was first identified on placental trophoblasts, HLA-G is thought to contribute to the maintenance of pregnancy (86, 89, 90, 91). Other than the trophoblast under pathological conditions, HLA-G is expressed in response to inflammatory conditions (92), various stimuli including stress (25), and cytokines such as IL-10 and IFNs (93), by allografts (94), by tumors (25, 95, 96, 97) and in the course of HIV infection (98).

#### 5.3.4. HLA-G and NK receptors

While HLA-G can interact with both ILTs (81, 99, 100) and KIR2DL4 (67), ILTs rather than KIR2DL4 are thought to contribute to the maintenance of pregnancy (101). HLA-G, strongly expressed on placental trophoblasts, may have the advantage of requiring inhibitory receptors ILT on NK cells as its ligand (81).

ILT-2 and -4 can bind HLA-G with a 3 to 4-fold higher affinity than to classical HLA class Ia (81), suggesting that ILT/HLA-G recognition may play a dominant role in the inhibitory regulation of NK, T, and myelomonocytic cell activation. This may be an advantage in inhibiting KIR2DL4 activation with HLA-G interaction.

Interaction of the inhibitory receptor ILT-2 with HLA-G has been demonstrated to inhibit the polarization of NK cell lytic granules and microtubules. Even though the activation receptor CD2 is accumulated at the NK cell synapse, the ILT2/HLA-G interaction efficiently inhibited intracellular calcium mobilization and IFN- $\gamma$  polarized production of NK cells (102).

## NK cells/HLA-G in endometriosis

KIR2DL4 features both activating and inhibitory receptors, raising the question of what type of signals are transmitted by this receptor under certain conditions (62). KIR2DL4 functions as an activating receptor with the unique and autonomous ability to induce rapid IFN- $\gamma$  secretion, but not cytotoxicity, by resting NK cells in the absence of cytokines such as IL-2 and IL-12 (103). Although KIR2DL4 can bind and react with membrane-bound HLA-G (67), biochemical analysis of KIR2DL4 binding to HLA-G monomers and dimers yielded only negative results, raising questions as to the validity of HLA-G/KIR2DL4 interaction (104). The KIR2DL4 and HLA-G interaction is therefore complex and controversial.

Hofmeister *et al.* demonstrated that HLA-G regulates the immune response by binding different receptors expressed on NK cells, T cells and myeloid cells. HLA-G1 can inhibit NK and T-cell mediated lysis of target cells by its interaction with the inhibitory receptors ILT2 and ILT4. However, engaging KIR2DL4 triggers different reactions depending on the activation status of effector cells (105).

Yan *et al.* demonstrated that residue Met76, Gln79 mutated to Ala76, 79 in the  $\alpha 1$  domain of HLA-G protein could affect the binding affinity between KIR2DL4 and HLA-G. The KIR2DL4-transfected NK-92 cells showed a different cytolytic ability to the wild type HLA-G and mutant type HLA-G transfected K562 targets (67). Furthermore, when KIR2DL4-transfected NK-92 cells were used as effectors against target K562 cells or HLA-G transfectants, NK-92 cells showed a strong cytolytic effect to HLA-G-transfected K562 cells via KIR2DL4 when compared with that by K562 cells (76).

### 5.3.5. HLA-G and endometriosis

Concerning HLA-G expression, Hornung *et al.* demonstrated for the first time that HLA-G was not expressed in eutopic endometrium or endometriotic tissue (106). HLA-G protein was not detectable in PF specimens from patients with endometriosis or controls. Moreover, ectopic and normal endometrial tissues and stromal cells did not express HLA-G in the proliferative phase. They concluded that immune cell inhibition in endometriosis must be mediated by factors other than HLA-G.

Furthermore, HLA-G expression was identified in the glandular epithelium of peritoneal endometriotic implants, but not in the eutopic endometrium (26). Both HLA-G protein and gene transcripts were localized in the glandular epithelium of peritoneal endometriotic lesions, but were absent in stromal cells and normal endometrium in both the proliferative and secretory phases, regardless of evidence of endometriosis. They concluded that differential expression of HLA-G suggests that peritoneal inflammation or cellular stress may up-regulate mechanisms to promote ectopic endometrial cell survival.

According to our recent findings, KIR2DL4 expressing NK cells are identified both in the PB and PF. Interestingly, HLA-G, a ligand for KIR2DL4, was expressed by eutopic endometrium only in the menstrual

phase, and not in the proliferative or secretory phases (73). HLA-G- expressing cells were detected in the PF during the menstrual phase (73). Epithelial cells bearing HLA-G may enter the peritoneal cavity during retrograde menstruation, allowing the antigen to react locally with KIR2DL4.

### 5.3.6. Soluble HLA-G and endometriosis

An increased amount of soluble HLA-G (sHLA-G) molecules in the PB is found in different neoplastic diseases. HLA-G expression in cancer cells has been shown to be important for the escape from immunosurveillance by host T cells and NK cells (25, 95, 96, 97).

Soluble HLA-G has been demonstrated to induce apoptosis of activated CD8<sup>+</sup> T cells (107, 108) and to modulate NK cells (109), whereas membrane-bound HLA-G proteins have been shown to inhibit both NK cell and T-cell mediated cytotoxicity, to suppress proliferation of allo-specific CD4<sup>+</sup> T cells (110).

Soluble HLA-G was detected in PF from women with endometriosis and from controls. These results indicate that sHLA-G accumulates in the PF. The source of these molecules may be mainly PF macrophages and other peritoneal cells (111).

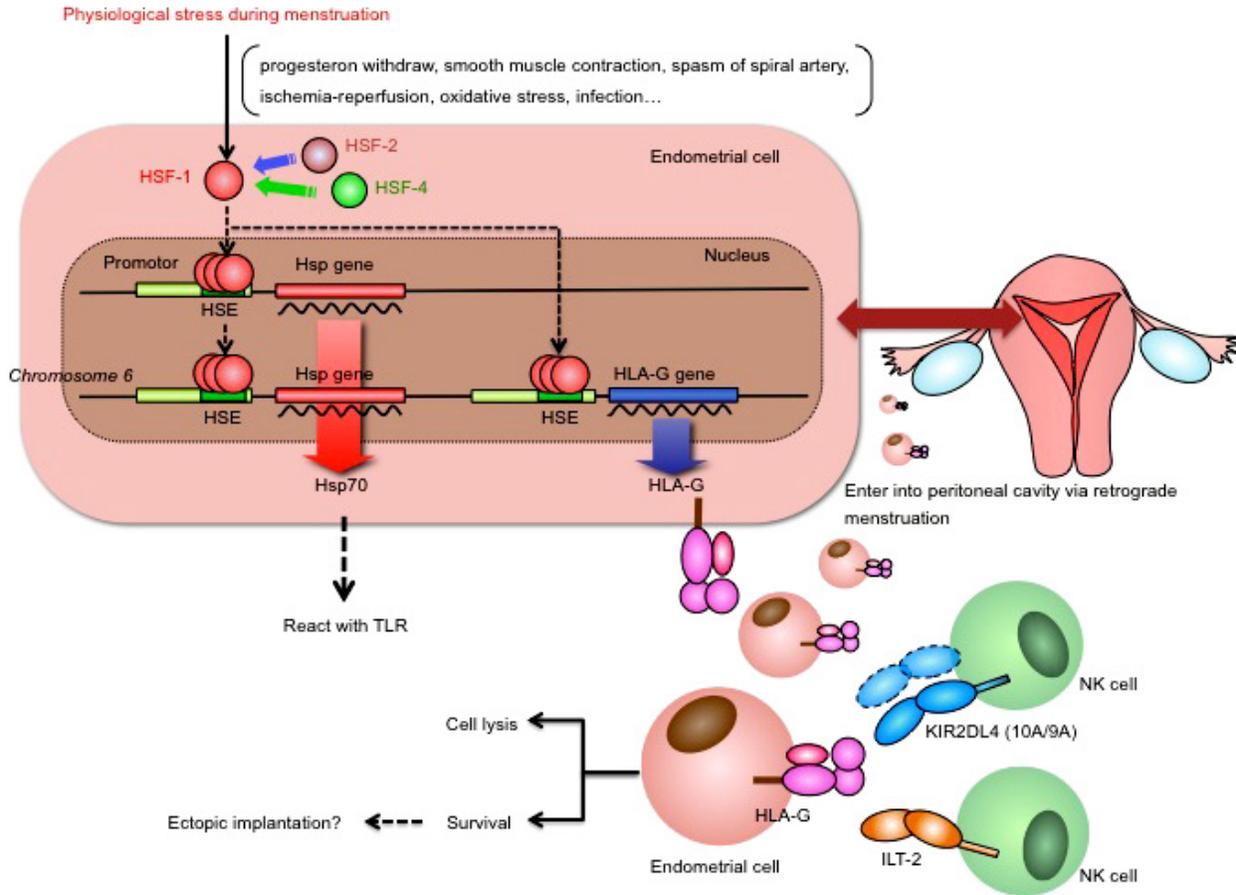
Serum sHLA-G concentration levels in patients with deep endometriosis were comparable to those seen in patients with ovarian cancer. Serum sHLA-G levels should provide important information regarding the degree of disturbance of immune system regulation in both ectopic endometrial cells and the cancer cell suppressive microenvironment (112).

### 5.3.7. HLA-G and stress reaction in the eutopic endometrium

In 2000, Ibrahim *et al.* showed that stress, including heat shock and arsenite treatment, induced increased levels of the different HLA-G alternative transcripts, without affecting other MHC class I molecules such as HLA-A, -B, -E, and -F transcripts (25).

A heat shock element (HSE) that binds to the heat shock factor 1 (HSF1) trimer in stress conditions was further identified within the HLA-G promoter. When stress such as heat shock, bacterial infection, or poisons (including arsenites) affects cells, HSF-1, usually regulated by HSF-2 and -4, in the cytoplasm translocates into the nucleus, turns into a trimer, and binds with the HSE located in the promoter regions of the heat shock protein (Hsp)-s and HLA-G genes (57). This binding resulted in hsp70 and HLA-G induction (25).

Considering the ability of HLA-G to modulate the function of immunocompetent cells, a new feature of HLA-G as a signal regulating the immune response to stress to escape from the host immunosurveillance system was hypothesized. We demonstrated that HLA-G is found in eutopic endometrial cells during menstruation (73). HLA-G expression by the eutopic endometrium during menstruation reflects the stressful intrauterine environment.



**Figure 2.** Eutopic endometrial cells express HLA-G in addition to Hsp70 in response to physiological stress reactions during menstruation, and enter the peritoneal cavity *via* retrograde menstruation. In the peritoneal cavity, endometrial cells may react with surrounding NK cells through HLA-G binding with their receptors, KIR2DL4 and/or ILT-2 (our hypothesis). HLA: human leukocyte antigen; Hsp: heat shock protein; NK: natural killer; KIR: killer-cell immunoglobulin-like receptor; ILT: immunoglobulin-like transcript

In the usual menstrual cycle, a decrease in the serum progesterone level induces uterine smooth muscle contraction and spiral artery spasms, resulting in menstruation (113). Smooth muscle contraction and spiral artery spasms may induce oxidative stress *via* ischemia-reperfusion injury (114). Tissue breakdown during menstruation may be a direct cause of tissue stress reaction. Transient bacterial invasion and inflammation may also occur in the eutopic endometrium during menstruation. Increased expression of Hsp70 and HLA-G in the endometrium during menstruation may result from such “physiological” stress. Hsp70 and HLA-G expressing endometrial cells in retrograde menstruation may react with immunocompetent cells in the PF and finally disappear from the peritoneal cavity. However, an impaired and aberrant immunocompetent cell reaction to Hsp70 or HLA-G in the PF may lead to endometrial cell survival and implantation, and the development of endometriosis. HLA-G expression in peritoneal endometriosis may be the result of stress in the peritoneal cavity and escape from the immunosurveillance system (26). Recently, it has been

suggested that in women with endometriosis, stress proteins, such as Hsp70, or bacterial endotoxin may induce pelvic inflammation *via* Toll-like receptor 4 (119,120) (Figure 2). We presume that there is a vicious cycle between stress reaction and inflammation in pelvic environment to induce HLA-G/KIR2DL4-mediated escape of NK cytotoxicity resulting in survival of endometrial cells.

## 6. CONCLUSIONS

In this review, we demonstrated that HLA-G is expressed in the eutopic endometrium during menstruation, suggesting the existence of physiological stress during this phase of the menstrual cycle. HLA-G expressing endometrial cells may enter into the peritoneal cavity through retrograde menstruation and react with immunocompetent cells, in particular NK cells. In the peritoneal cavity, although HLA-G can react with both inhibitory and activation NK receptors, in healthy women, HLA-G expressing endometrial cells may be cleared from the peritoneal cavity by the intraperitoneal immunosurveillance system.

Impairment of the physiological immunosurveillance system, in particular NK cell function, may favor endometrial cell survival and implantation, and trigger the onset of endometriosis.

Further studies of endometrial cell antigens during menstruation and their immunocompetent responder cells retaining specific ligands in the PF may contribute to a better understanding of the pathogenesis of endometriosis.

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**NK cells/HLA-G in endometriosis**

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