HLA-G in Human Early Pregnancy: Control of Uterine Immune Cell Activation and Likely Vascular Remodeling

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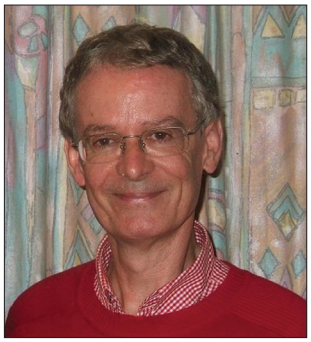

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Despite a number of controversies, the functional importance of human leukocyte antigen G (HLA-G) in early human pregnancy is now sustained by a large amount of sound data. Membrane-bound and soluble HLA-G isoforms, either as β2-microglobulin–free or –associated as monomers or dimers, are expressed by different trophoblast subpopulations, the only fetal-derived cells that are directly in contact with maternal cells (maternal–fetal interfaces). Trophoblast HLA-G is the specific ligand of multiple cellular receptors present in maternal immune and non-immune cells, including CD8, leukocyte immunoglobulin-like receptor (LILR) B1, LILRB2, killer cell immunoglobulin-like receptor (KIR) 2DL4, and possibly CD160. Trophoblast HLA-G specific engagement of these cellular receptors triggers either inhibitory or activating signals in decidual $CD8^+$ T cells, $CD4^+$ T cells, natural killer (NK) cells, macrophages, dendritic cells, or endothelial cells. Such HLA-G–receptor specific interactions first contribute to limit potentially harmful maternal anti-paternal immune response by impairment of decidual NK cell cytotoxicity, inhibition of $CD4^+$ and $CD8^+$ T-cell and B-cell proliferation, and induction of apoptosis of activated $CD8^+$ T cells. Second, these HLA-G specific interactions contribute to stimulate placental development through secretion of angiogenic factors by decidual NK cells and macrophages, and to provide a protective effect for the outcome of pregnancy by the secretion of interleukin (IL)-4 by decidual trophoblast antigen-specific $CD4^+$ T cells. *(Biomed J 2015;38:32-38)*

Key words: decidua, human leukocyte antigen G, killer cell immunoglobulin-like receptor 2DL4, leukocyte immunoglobulin-like receptor B1, natural killer cell, trophoblast

Since its gene discovery in the eighties,[1,2] human leukocyte antigen G (HLA-G) has been the subject of hundreds of reports dealing with its unique structural features among the other HLA class I molecules, low polymorphism, gene conservation, restricted tissue distribution, and functional properties. This article will give an updated overview on the role of HLA-G at the maternal–fetal interface during human pregnancy, leaving an open discussion on some points of disagreement.[3-8] The reader may also refer to previous excellent reviews on the subject.[9-13]

HLA-G is expressed by fetal-derived trophoblast

Seven alternatively spliced transcripts of HLA-G can be generated from a single mRNA [Figure 1]. This results in the translation of four so-called membrane-bound isoforms (full length HLA-G1, and HLA-G2, -G3, and -G4 smaller forms), as well as three soluble isoforms (HLA-G5, -G6, and -G7).[13-16] Due to a stop codon in intron 4, these latter forms have neither transmembrane nor cytoplasmic translated domains. As a result, these secreted isoforms exhibit a small tail corresponding to the translated part of intron 4. HLA-G2, -G3, and -G4 truncated isoforms were shown to be sequestered in the endoplasmic reticulum, making their stable expression at the cell surface very unlikely.[17-19] Only membrane-bound HLA-G1 and soluble HLA-G5 are associated non-covalently with the β2-microglobulin (β2m) through the α1 and α3 domains. β2m-free HLA-G heavy chains have also been described.[20-22] HLA-G possesses two unique cysteine residues in the α1 (Cys

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42) and α2 (Cys 147) domains that can form disulfide bonds leading to HLA-G dimers or trimers.[23-26] This formation of disulfide bonds is a unique characteristic of HLA-G. In HLA-G, positions 195 and 197 of the α3 domain are occupied by phenylalanine and tyrosine residues, respectively, compared with serine and histidine in the other HLA class I molecules.[24] This contributes for the higher affinity of HLA-G binding to leukocyte immunoglobulin-like receptor (LILR) B1 receptor compared with the other HLA class I molecules.[27]

In early human gestation, distinct subpopulations of trophoblast cells have been identified.[28] These include villous and extravillous cytotrophoblast. Two-layered villous trophoblast covers the floating and anchoring chorionic villi, providing the barrier through which metabolic exchange between mother and fetus occurs. The inner villous cytotrophoblast sits on basement membrane covered by the outer syncytiotrophoblast. One of the roles of extravillous trophoblast is to invade the decidua basalis (placental bed) and decidual spiral arteries to favor uterine vascular remodeling, converting them to high-conductance vessels with larger diameters.[29] Maternal blood of the intervillous space (delivered by the spiral arteries) with syncytiotrophoblast and maternal decidua infiltrated with extravillous cytotrophoblast, both represent sites of contact between maternal and fetal cells.[28,30,31] Abundant maternal immune cells populate the decidua basalis in early pregnancy. The dominant populations are the decidual natural killer (dNK) cells, and macrophages. Decidual CD4+ T cells, CD8+ T cells and dendritic cells are more variable in numbers, whereas B cells and NKT cells are rare.[11,30,32] There is a common agreement that membrane-bound HLA-G1 isoform is strongly expressed at the cell surface of extravillous cytotrophoblast infiltrating the decidua basalis (interstitial trophoblast, placental bed giant cells) as well as the chorion membrane in the decidua parietalis.[33,34] The expression of HLA-G by interstitial extravillous trophoblast increases as it migrates toward the vasculature.[35] HLA-G1 is also expressed on these extravillous cytotrophoblast cells which migrate into portions of spiral arteries close to the intervillous space in early pregnant uterus (endovascular trophoblast) and replace endothelial cells.[6,36] Such invasion of trophoblast into the spiral arteries is essential to provide the blood supply to the growing fetus. The presence of HLA-G1 dimer has been reported at the cell surface of first-trimester extravillous cytotrophoblast.[37] Free heavy chain HLA-G5 homodimer has also been detected in villous cytotrophoblast.[21] The HLA-G2 and HLA-G6 isoforms were found in extravillous cytotrophoblast cells, including those forming the trophoblastic shell distal to the villous.[12,38] Although this subject is still controversial,[5,13] I belong to those groups of authors who provided conclusive experimental evidence that soluble HLA-G5 is detectable in both extravillous and villous cytotrophoblast as well as in the chorion membrane and syncytiotrophoblast.[6,9,12,21,39-41] However, as suggested by Loke and King,[42] one cannot exclude that localization of HLA-G protein in syncytiotrophoblast might result from an initial transcription in the villous cytotrophoblast and subsequent storage in the overlying syncytial layer. Rhesus monkey Mamu-AG, which shares a number of features with HLA-G, including similar spliced and intron 4–retaining soluble forms, is likely a functional homolog of HLA-G.[43] Indeed, pattern of localization of these soluble Mamu-AG forms is very similar to that described in human placenta, that is, extravillous cytotrophoblast as well as syncytiotrophoblast and some villous trophoblast.[43]

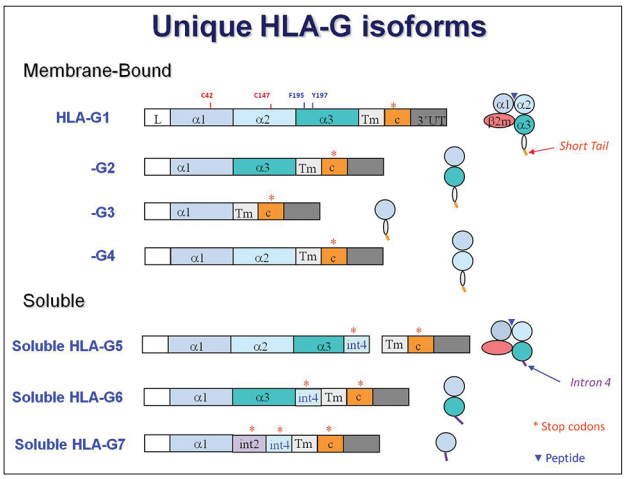

Figure 1: Schematic diagram of HLA-G transcriptional isoforms described to date. HLA-G1 encodes a full-length membrane-bound molecule associated with β2-microglobulin. HLA-G2, -G3, and -G4 forms encode shorter molecules that may not be stably expressed at the cell surface.[17,18] HLA-G5, -G6, and -G7 encode soluble forms that lack the transmembrane and cytoplasmic domains. L: Exon 1 encoding the leader sequence; α1, α2, α3: Exons 2, 3, and 4 encoding the α1, α2, and α3 external domains, respectively; Tm: Exon 5 encoding the transmembrane domain; C: Exons 6 and 7 encoding the cytoplasmic domain; 3′UT: Exon 8 encoding the 3′ untranslated region. The asterisks indicate the presence of a stop codon. Small triangles represent bound peptides. C42 and C147 are the cysteines forming intermolecular disulfide bonds. F195 and Y197 are the residues that allow a higher affinity of HLA-G to LILRB1.

HLA-G is the specific ligand of multiple receptors expressed by immune and nonimmune cells

Multiple HLA-G cellular receptors have been reported to date [Figure 2]. They include the T-cell co-receptor CD8, LILRB1 (also called ILT2), LILRB2 (also called ILT4), killer cell immunoglobulin-like receptor (KIR) 2DL4, and CD160.[10,12] Both LILRB1 and LILRB2 are expressed by a wide variety of immune cells including macrophages and dendritic cells.[6,44] LILRB1 is also present in the subsets of NK cells, CD8+ and CD4+ T cells, as well as B cells.[6] KIR2DL4 is expressed by a subpopulation of dNK cells.[45-47] CD160 is present on some activated endothelial cells,[48]

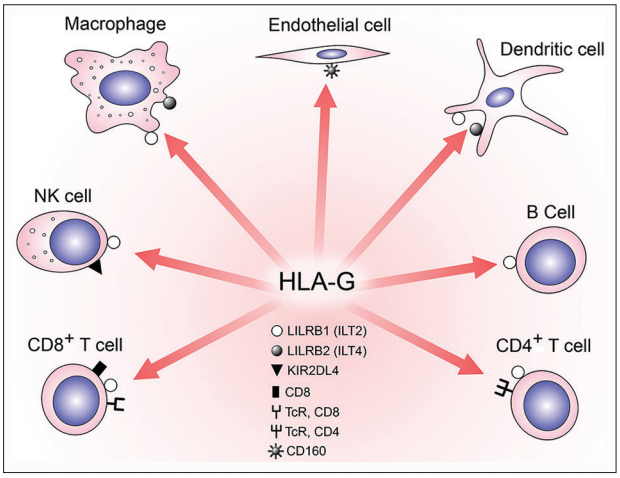

Figure 2: Multiple receptors for HLA-G are expressed on immune and other cells. Many types of cells that participate in immune responses express receptors for HLA-G, including $CD4^+$ and $CD8^+$ T cells, B cells, NK cells, macrophages, and dendritic cells. HLA-G also binds to some activated endothelial cells expressing CD160. Reprinted from Hunt JS, Morales PJ, Pace JL, Fazleabas AT, Langat DK. A commentary on gestational programming and functions of HLA-G in pregnancy. Placenta 2007;28 Suppl A:S57-63. Copyright 2014, with permission from Elsevier.

$CD4^+$ and $CD8^+$ T-cell subsets,[49] and the $CD56^{dim}$ $CD16^+$ major NK cell subset in circulating blood.[50,51] In the pregnant uterus, CD8, LILRB1, LILRB2, and KIR2DL4 receptors expressed by maternal decidual cells can interact with trophoblast HLA-G specific ligand. Homologs of these receptors for HLA-G have been found in rhesus monkey decidual leukocytes.[43] Functional validation of the HLA-G–receptor interaction was demonstrated in a study using HLA-G–knock-down first-trimester extravillous trophoblast.[52] Peripheral blood (PB) NK cells were able to kill the HLA-G knock out (KO)-extravillous trophoblast compared with HLA-G expressing trophoblast control target cells. These data indicated that interactions between cell surface HLA-G and the LILRB1 and KIR2DL4 expressed by some PB-NK and dNK cell subpopulations are functionally important.[52]

HLA-G controls local maternal immune response at the maternal–fetal interface

Soluble HLA-G exerts immunosuppressive functions toward decidual $CD8^+$ and $CD4^+$ T cells

HLA-G specifically binds CD8.[53] Our group[54] and Contini *et al*.[55] have provided evidence that soluble HLA-G5 induces apoptosis of activated $CD8^+$ T cells via specific ligation to CD8 and activation of the Fas/FasL pathway. We further found that HLA-G5 purified from human villous trophoblast supernatant exerted the same apoptotic effect on activated $CD8^+$ T cells after different times of culture.[40] These results suggested that the paucity of $CD8^+$ T cells observed in the decidua basalis of first-trimester pregnancy[56] was the result of soluble HLA-G5 apoptotic function. Such functional properties of soluble HLA-G are likely to contribute to the immunosuppressive state at the syncytiotrophoblast border as purified villous trophoblast differentiates *in vitro* in syncytiotrophoblast, secreting functional HLA-G5 together with hCG.[40] Moreover, both soluble HLA-G5 and HLA-G6 were also shown to decrease CD8 expression.[38] Another study indicated that soluble HLA-G5 dimers inhibited allorecognition by reducing proliferation of both $CD4^+$ and $CD8^+$ T cells.[57] Soluble HLA-G produced by trophoblast may thus be considered as an immunosuppressive molecule toward decidual $CD4^+$ and $CD8^+$ activated T cells.

LILRB1 and LILRB2 specific interaction with HLA-G trophoblast ligand modulates the effector functions of decidual NK cells and macrophages

LILRB1 and LILRB2 recognize all HLA class I molecules. However, their affinity to bind HLA-G is much higher than for other HLA class I molecules.[53] The disulfide-linked homodimeric complex of β2m-associated HLA-G expressed at the cell surface of trophoblast dramatically increases the LILRB1 binding and signaling.[37] LILRB1 is expressed by a dNK cell subset present in both the decidua basalis and decidua parietalis.[8] Specific ligation of membrane-bound HLA-G to LILRB1 present in this dNK subpopulation has functional consequences in terms of dNK impairment of cytotoxicity[58] and secretion of interleukin (IL)-6, IL-8, and tumor necrosis factor alpha (TNF-α) pro-inflammatory cytokines.[59,60] Engagement of LILRB1 by HLA-G homodimer on decidual macrophages also resulted in up-regulation of the same pro-inflammatory transcripts and proteins, but the amount of cytokines secreted was much larger than that produced by dNK.[60] Dimers of β2m-free HLA-G5 and HLA-G6 bind to LILRB2 with good avidity,[27] suggesting that these soluble forms of HLA-G may bind to decidual macrophages or dendritic cells expressing this receptor.[13]

Successful pregnancy in humans has been associated with production of IL-4 by T cells at the maternal–fetal interface.[61] Our recent report provided an array of data which strongly suggest that soluble HLA-G5 produced by trophoblast could be responsible for the production of IL-4 by decidual T cell.[62] Using both *in vitro* and *in vivo* experiments, the authors have demonstrated that HLA-G5 down-regulated IL-12 secretion by decidual macrophages, but increased IL-4 production by decidual $CD4^+$ T cells.[62] They further demonstrated that such HLA-G5–mediated regulation of decidual cytokine production correlated with the down-modulation of LILRB1 expression on decidual macrophages and increased levels of the same receptor on decidual $CD4^+$ T cells.[62]

Binding of HLA-G to LILRB1 inhibits B cell functions

LILRB1 is also expressed by B cells [Figure 2]. A recent report indicated that HLA-G blocked B cell proliferation, differentiation, and immunoglobulin release in both T-cell–dependent and –independent models of B cell activation.[63] Although very few B cells are present at the maternal–fetal interface, one can think that HLA-G may contribute to prevent unwanted local B cell effector functions.

HLA-G promotes HLA-E cell surface expression in extravillous cytotrophoblast and subsequent dNK cell activation

HLA-G indirectly controls NK cell activation through the provision of peptides that stabilize HLA-E trophoblast expression. HLA-G indeed provides a leader sequence signal nonapeptide (VMAPRTLFL) which preferentially binds to HLA-E peptide-binding groove with high affinity.[64,65] Both HLA-E and HLA-G are expressed on extravillous cytotrophoblast,[66] and most dNK cells express high levels of CD94/NKG2A inhibitory receptor in healthy pregnant uterus.[13,45,67] Thus, engagement of CD94/NKG2A receptor present on dNK cells by its HLA-E specific ligand is likely to negatively control the cytotoxic function of dNK cells in healthy pregnancy.[13,45,68] In contrast, interaction between trophoblast HLA-E and CD94/NKG2C activating receptor on dNK cells is very likely to occur in case of uterine viral infection to eliminate infected decidual cells.[69]

Therefore, both HLA-G expressing extravillous cytotrophoblast invading the decidua basalis and villous trophoblast interact with different maternal immune cell receptors which contribute to regulate local maternal immunity.

Trophoblast HLA-G is likely to contribute to regulate vascular uterine remodeling in early pregnancy

HLA-G is also the ligand of KIR2DL4,[70] which interacts with the antigen-binding cleft of HLA-G.[71] Several reports indicate that KIR2DL4 is expressed by dNK cells.[45,46,60] Additional findings show that KIR2DL4 is transcribed in dNK cells to a higher degree than in PB-NK.[46] Despite some recent controversy,[7] sound experimental data provided by Rajagopalan,[72] Rajagopalan and Long,[70] and Rajagopalan *et al*.[73] argue for the physiological relevance of KIR2DL4–HLA-G interaction during pregnancy. Specific engagement of KIR2DL4 by soluble HLA-G induces the secretion of pro-inflammatory and pro-angiogenic factors that are needed for uterine vascular growth and remodeling in the early weeks of gestation.[70,72] Microarray of the transcriptional production of pro-angiogenic and pro-inflammatory molecules by resting PB-NK induced by KIR2DL4 agonist monoclonal antibody[74] confirmed these latter findings. Furthermore, it has been demonstrated that KIR2DL4 mediates a new mode of signaling after ligation of its soluble HLA-G specific ligand. Soluble HLA-G is bound and endocytosed by KIR2DL4 into Rab5 early endosomes.[74,75] Signaling at these early endosomes involves Akt phosphorylation at S473 by DNA-PKcs that is associated with KIR2DL4. This triggers activation of the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) pathway, leading to the transcription of genes encoding pro-inflammatory and pro-angiogenic factors.[75] Such endosomal KIR2DL4-induced signaling pathway was considered as a senescence signature.[76] Moreover, the dNK cell transcriptome revealed a strong senescence signature.[76] The secretome of KIR2DL4-stimulated senescent NK was shown to trigger *in vitro* vascular permeability of the endothelium and endothelial cell tube formation.[76] Cross-linking with anti-KIR2DL4 monoclonal antibody on dNK cells resulted in up-regulation of IL-6, IL-8, and TNF-α cytokines.[60] Such properties are likely to play a role in promoting vascularization and uterine vascular remodeling at the maternal–fetal interface sites expressing HLA-G. To my knowledge, whether KIR2DL4/soluble HLA-G proteins are present in the dNK cell endosomes *in situ* is as yet unknown.

Soluble HLA-G was shown to inhibit growth factor–induced motility and invasion of extravillous trophoblast, suggesting that it may play a role in the control of trophoblast invasive properties and spiral artery remodeling.[77]

Using several *in vitro* experiments, our group has reported that soluble HLA-G5 inhibited angiogenesis via interaction with the CD160 receptor expressed on some activated endothelial cells.[48] Using *in vivo* animal models of ocular or tumoral neoangiogenesis, we further demonstrated that an agonist monoclonal antibody against CD160 had the same anti-angiogenic effect as the HLA-G5 physiological ligand.[78] Whether HLA-G5 produced by endovascular trophoblast negatively regulates in some ways local uterine angiogenesis during early pregnancy through interaction with CD160 remains to be determined.

Conclusions and outlook

As outlined by Hunt and Petroff in a recent lecture,[9] "a novel concept of pregnancy is that controlling the activities of the highly responsive uterine immune cells is achieved primarily, if not exclusively, by the unusual fetal cells that make up the placenta, the trophoblastic lineage." An array of accumulating evidence strongly indicates that membrane-bound HLA-G1 and soluble HLA-G5 expressed by trophoblast specifically interact during early pregnancy with multiple cellular receptors expressed by decidual allogenic immune and non-immune maternal cells. In healthy

pregnancy, such specific receptor–HLA-G ligand interactions trigger a unique cytokine and angiogenic factor production that is likely to control spiral artery remodeling and subsequent placental development and reproductive outcome. Such HLA-G interactions also contribute to prevent unwanted localized maternal allogenic immune reaction against paternal antigens. Functional studies on HLA-G in pathologic pregnancies may bring useful information. Indeed defective HLA-G expression has been associated with preeclampsia.[79,80] Low levels of HLA-G may be related to shallow trophoblast invasion of maternal spiral arteries. One report similarly described a decreased expression of HLA-G in extravillous cytotrophoblast of term placenta infected with *Plasmodium falciparum*.[81]

However, some controversies dealing with HLA-G functionality still persist. One report, for instance, concluded that no functional effects of HLA-G were found on freshly isolated first-trimester dNK cells.[8] A clue to definitely prove the beneficial role of HLA-G in the successful outcome of pregnancy may arise from future experimental studies using appropriate, standardized reagents, including recombinant β2m-associated or -free HLA-G isoforms, HLA-G monomers, dimers, or trimers, as well as specific monoclonal antibodies to these different forms of HLA-G, with well-defined epitope mapping. Other needs are functional assays that better reflect the *in vivo* situation. It is particularly important to avoid non-specific *in vitro* activation of purified decidual cells. Using negative magnetic-activated cell sorting (MACS) purification of decidual cells might thus be preferable. When designing experiments, it might also be wise to use freshly purified decidual cells and/or trophoblast cell subpopulations from the same early decidua.

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