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MULTI-AUTHOR REVIEW

The importance of HLA-G expression in embryos, trophoblast cells, and embryonic stem cells

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Abstract The nonclassical HLA-G molecule is a trophoblast-specific molecule present in almost every pregnancy. It differs from classical HLA class I molecules by the low degree of allelic variants and the high diversity of protein structures. HLA-G is reported to be a tolerogenic molecule that acts on cells of both innate and adaptive immunity. At the maternal–fetal interface HLA-G seems to be responsible largely for the reprogramming of local maternal immune response. This review will focus on the HLA-G gene expression profile in pregnancy, in preimplantation embryos, and in human embryonic stem cells with emphasis on the structural diversity of the HLA-G protein and its potential functional and diagnostic implications.

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Introduction

During human pregnancy, the immune environment has to conduct a very delicate and deliberate business at the maternal–fetal interface. The maternal immune system is able to recognize and to eliminate alloantigens derived from bacteria or virus, but it is also able to tolerate genetically different fetal cells, especially extravillous trophoblast cells invading the maternal decidua or entering the spiral arteries. Although these fetal cells are exposed to maternal immune cells, they do not trigger any immune reaction.

To establish and to maintain an immune privileged site where the embryo can survive and grow despite its genetic dissimilarities, the fetal and the maternal immune systems have to cooperate jointly. Of several mechanisms that are operative in pregnancy, the unique HLA status of trophoblast cells seems to be the most crucial factor in regulating an alloimmune response by the mother. Trophoblast cells do not express most of the polymorphic classical HLA molecules (HLA-A, HLA-B, HLA-DR, HLA-DQ, and HLA-DP). Trophoblast cells do preferentially express HLA-G molecules as well as small amounts of HLA-C, HLA-E, and HLA-F [1]. The human leukocyte antigen-G (HLA-G) belongs to the family of the nonclassical HLA class I molecules. In contrast to classical HLA class I molecules, HLA-G displays a low degree of polymorphism, an unusually large variety of molecular structures due to alternative splicing of the primary transcript, and a restricted expression profile under physiological

conditions. Four membrane-anchored (HLA-G1, HLA-G2, HLA-G3, HLA-G4) and three secreted (HLA-G5, HLA-G6, HLA-G7) isoforms are described [2–4]. Besides the secreted HLA-G molecules, soluble forms of HLA-G can also be generated by mechanisms of shedding or proteolytic cleavage of membrane-anchored molecules [5]. Among all isoforms, the HLA-G1 and HLA-G5 molecules are the most abundantly expressed ones. These molecules represent the full extracellular length molecules with three α-domains noncovalently associated with β2-microglobulin (β2-m), whereas other isoforms lack one or two α-domains. As classical class I, HLA-G1/5 molecules are able to bind peptides consisting of nine amino acids [6]. The diversity of peptides presented by HLA-G1/5, however, is very restricted compared to classical class I molecules [1, 7, 8].

With regard to function, the presence of HLA-G molecules is able to suppress the cellular and humoral immune response being triggered by the classical HLA class I (HLA-A, B, C) or class II (HLA-DR, DQ, DP) molecules. HLA-G molecules (1) inhibit the proliferation and cytotoxic functions of T cells and natural killer (NK) cells [9–17], (2) drive T cells into an immunosuppressive profile or provoke the generation of regulatory T cells [18–21], (3) induce the apoptosis of endothelial cells [22], (4) inhibit the differentiation of antigen-presenting cells [20–22], (5) alter the cytokine profile towards a Th2 polarization [23], and (6) up-regulate inhibitory receptors on all kinds of effectors cells [24]. These immune-suppressive functions are mediated by the interaction of HLA-G either with LILRB (leukocyte immunoglobulin-like receptor, subfamily B)1 [ILT- (immunoglobulin-like transcript-)2/CD85j] or LILRB2 (ILT-4/CD85d), CD8 α-chain, killer immunoglobulin-like receptor 2DL4 (KIR2DL4/CD158d), and CD160. As secreted/shed HLA-G (sHLA-G) and membrane-anchored molecules exhibit the same receptor specificity, both HLA-G and its soluble counterparts are potent regulator molecules for the innate and acquired cellular immune response. In line with its strong immune tolerogenic functionality, HLA-G is strongly expressed during pregnancy but an ectopic expression is also found in thymus, cornea, and erythroid cells as well as in blood cells [25–29] and in nonphysiological situations such as transplantation, cancer, infections, and autoimmunity [30, 31].

Indeed, HLA-G was the first HLA class I molecule being identified on trophoblast cells [32]. The following extensive studies demonstrate that HLA-G is present in almost every pregnancy. So far, no pregnancy in which all of the proteins derived from the HLA-G gene are absent has been reported [33]. Thus, HLA-G seems largely to be responsible for the reprogramming of local maternal immune response. However, how can this molecule on the one hand induce tolerance in certain places and on the other hand allow immune activation to produce proinflammatory and proangiogenic factors elsewhere? This review will focus on HLA-G gene expression in pregnancy, in preimplantation embryos, and in human embryonic stem cells with emphasis on the structural diversity of the HLA-G protein, its detection, and potential functional as well as diagnostic implications.

Expression of HLA-G molecules by trophoblast cells and the functional implication

Physiological conditions

The tissue most involved in immune regulation at the fetal–maternal interface is the placenta. It is comprised of cells of both maternal and fetal origin. The precursor cells of the human placenta are the fetal trophoblasts that first appear 4 days after fertilization as the outer layer of the blastocyst. These early blastocyst trophoblasts then differentiate into all the other cell types found in the human placenta. The placental trophoblasts are critical for a successful pregnancy through their actions mediating implantation, immune protection, vascular remodelling, and delivery. All these critical steps are regulated by a complex interplay between trophoblasts and endometrium. The cytotrophoblasts and syncytiotrophoblasts form a villous structure; from around 8 weeks after fertilization the cytotrophoblast will break through syncytiotrophoblast shell and invade into the decidua. The extravillous cytotrophoblasts reach the spiral arteries, which are converted from narrow to wide vessels allowing a much greater flow of maternal blood around the villi. The villous trophoblasts are the barrier between maternal and fetal circulation with a potent immunoinhibitory effect. Trophoblast cells maintain a tolerogenic status at the fetal–maternal interface expressing immunosuppressant soluble molecules such as transforming growth factor-B1 [34], interleukin-10 [35], indolemine 2, 3-dioxygenase [36], programmed death ligand (PDL)1 [37], and HLA-G [1]. The expression of HLA-G antigens by trophoblasts is of major importance in protecting the fetus from the semiallogeneic response of the mother. In fact, both membrane-bound and soluble HLA-G isoforms are expressed by extravillous cytotrophoblast cells (CTBs) [32]. HLA-G molecules are able to control uNK cell cytotoxicity by interacting with the inhibitory receptors KIR2DL4 [13], LILRB1, and LILRLB2 [15, 38]. The ability of HLA-G molecules to protect trophoblast cells from NK cell lysis is demonstrated by HLA-G-targeting, small interfering RNA in JEG-3 choriocarcinoma-derived cell lines, which become more susceptible to uNK cytolysis [39, 40]. Interestingly, HLA-G isoforms have a different expression profile from the villi into the deciduas. Studies on the expression of HLA-G clearly demonstrate that (1)

HLA-G3, HLA-G4, and HLA-G7 mRNAs are rarely present in placentas, (2) HLA-G1, b2m-associated HLA-G5, and HLA-G2/G6 are present only at the leading edge of trophoblast columns and in decidual CTBs, (3) HLA-G5 is ubiquitous in trophoblast subpopulations and may be produced as either free heavy chain (from CTB precursor stem cells and placental CTB cells) [41] or associated with b2m (by extravillous CTB cells). Of note, HLA-G5 molecules synthesized in placental villous CTB are b2m-free probably because of the low levels of b2m mRNA present in this cell type, whereas extravillous CTBs exhibit the cell surface homodimeric b2m-associated HLA-G1 expression [42]. In particular the HLA-G1 disulphide-linked homodimer has an increased avidity for the LILRB1 receptor expressed by decidual myelomonocytic cells, while the HLA-G5 free heavy chain binds strongly to LILRB2 [41]. These different affinities might modulate the invasion of trophoblasts in maternal deciduas and the functions of local maternal immune responses in the uterus. In fact, there is also a different expression of LILRB receptors in placenta cells. In the villous placenta, LIRB1 is restricted to stromal cells, which are composed primarily of fibroblasts and macrophages. In contrast, LILRB2 is detected in the placenta vascular smooth muscle. The pattern of expression of these two receptors suggests a specific immunological and vascular function for HLA-G binding during pregnancy [43]. On the basis of the specific receptor interactions, HLA-G molecules are able to control the maternal immune system and the remodelling of uterine vascularization inhibiting endothelial cell proliferation, migration, and tubule formation by secreted HLA-G molecules by extravillous CTBs [1, 24, 44, 45]. Furthermore, sHLA-G takes part in the angiogenesis process regulating growth factor-stimulated trophoblast motility and invasion [46], endothelial cell activity via the inhibition of FGF2 (fibroblast growth factor2) or VEGF (vascular endothelial growth factor)-induced angiogenesis, and the activation of endothelial cell apoptosis by interaction with the glycosylphosphatidylinositol (GPI)-anchored CD160 receptor expressed in endothelial cells [22]. The variable expression of HLA-G isoforms and LILRBs is of extreme interest as they can interact differently, maintaining a controlled inhibition while permitting the formation of an appropriate microenvironment necessary for a viable maternal–fetal interface [47].

The CTB expression of HLA-G is regulated by different environmental and genetic factors. It is known that HLA-G mRNA transcription is up-regulated by hormones (i.e., progesterone) [48] and interferons (IFNs) [49]. Interleukin-10 up-regulates both HLA-G [50, 51] and its receptors LILRB1 and LILRB2 [52]. Hypoxia induces the expression of all HLA-G transcripts in term CTBs [53]; epidermal growth factor (EGF), a trophoblast growth and syncytialization factor, enhances protein expression of HLA-G5 [41]. Genetic polymorphisms at the 5′ upstream regulatory region (URR) and at the 3′ untranslated region (UTR) of the *HLA-G* gene seem to influence the production of HLA-G molecules. In particular a 14 base pair (14 bp) insertion/deletion (ins/del) polymorphism in the 3′ UTR (exon 8) [54] influences mRNA stability [55]. Polymorphisms in the 5′ URR regulate the transcription of the *HLA-G* gene [56]. Histone acetylation is involved in HLA-G expression in trophoblast cells. Increased histone acetylation in *HLA-G* promoter chromatin is significantly enhanced in JEG-3 cells coinciding with a higher HLA-G expression [57]. Intriguing results have also been obtained for allele-specific miRNA (microRNA) targets in the *HLA-G* gene, which might play a relevant role in the HLA-G expression pattern. In particular, miR-513a-5p, miR-518c*, miR-1262 and miR-92a-1*, miR-92a-2*, miR-661, miR-1224-5p, and miR-433 miRNAs influence one or more of the +3003, +3010, +3027, and +3035 SNPs in the 3′ UTR. The miR-2110, miR-93, miR-508-5p, miR-331-5p, miR-616, miR-513b, and miR-589* miRNAs target the 14 bp fragment region, and miR-148a, miR-19a*, miR-152, mir-148b, and miR-218-2 influence the +3142 C/G polymorphism [58].

Pathological conditions

Complications during pregnancy may affect the woman, the fetus, or both. Miscarriage, recurrent spontaneous abortion, and pre-eclampsia account for the most frequent pregnancy complications. Miscarriage is the spontaneous end of a pregnancy when the embryo or fetus is still unable to survive, generally in humans before 20 weeks of gestation. Miscarriages can occur for many reasons, including genetic, uterine, or hormonal abnormalities, reproductive tract infections, and tissue rejection [59]. Recurrent spontaneous abortion (RSA) is defined as three or more pregnancy losses. The aetiology is often unclear and may be multifactorial (genetics, anatomical, endocrine, placental anomalies, hormonal problems, infection, smoking and alcohol consumption, exposure to environmental factors, coagulation, and immunoregulatory protein defects). Pre-eclampsia is characterized by hypertension during pregnancy in association with proteinuria and affects at least 5–8% of all pregnancies. Preeclampsia may develop from 20 weeks gestation on and its progress differs among patients. It seems to be caused by a hypoxic placenta that leads to an immune reaction characterized by inflammatory mediators from the placenta acting on the vascular endothelium. Disregulation of the immunological control at the fetal–maternal interface seems to play a role in these pregnancy complications. The lack of an established immunological tolerance in pregnancy results in an

immune response against paternal antigens expressed by the fetus at the placenta, causing severe health problems for both the fetus and the mother.

Taking into consideration the important role of HLA-G molecules in controlling the tolerogenic condition at the fetal–maternal interface, several studies have evaluated the possible aberrant expression of these antigens during these pathological conditions of pregnancy. The importance of HLA-G production by placental trophoblasts is evident in preeclampsia and in unexplained RSA. There is a reduced expression of both HLA-G mRNA and protein in comparison with control placentas [60–63]. These observations are sustained also by the role of the HLA-G-murine orthologue Qa2 molecule during pregnancy [64, 65]. Qa2 is a nonclassical class Ib MHC protein and is the product of the preimplantation embryo development (*Ped*) gene. The lack of embryonic expression of Qa2 results in a range of disadvantageous phenotypes, including lower preimplantation cleavage rate, lower survival to term, and lower birth weight compared with embryos showing the *Ped* gene [66]. The absence of HLA-G/Qa2 expression by fetal cells can unbalance the regulation of the pregnancy process leading to severe complications.

Not only the HLA-G molecules expressed at the fetal–maternal interface seem to be important to control pregnancy outcome but also sHLA-G molecules present in the plasma of the mother. In pregnant women, sHLA-G levels in plasma samples increase during the first trimester, with a peak in the third month, in comparison with nonpregnant women [67, 68]. On the contrary sHLA-G plasma levels decrease during the third trimester [69, 70] while they have an impressive boost at delivery [71] probably deriving from the shedding of placental membrane-bound HLA-G1 molecules. Soluble HLA-G molecules could derive from both the placenta and the maternal immune cells, as suggested by the longitudinal study of 45 volunteers during normal pregnancy, where the authors demonstrated that monocyte-derived dendritic cells obtained from pregnant women during the first and third trimester secrete more sHLA-G than those obtained from nonpregnant women [67]. During pregnancy complications, there is a documented variation in the levels of sHLA-G in women's plasma samples. Compared to uncomplicated pregnancies, women with placental abruption have sHLA-G levels less than one-third those of the control pregnancies ($p < 0.0001$). Interestingly, pregnant women with low sHLA-G plasma levels are characterized by a relative risk of 7.12 of developing placental abruption [72]. Women who experienced miscarriage have very low or undetectable plasmatic sHLA-G levels in the second month of pregnancy [67]. sHLA-G levels in plasma from women who subsequently develop preeclampsia and spontaneous abortion are lower than those in control pregnant women, in the first, second [70, 73, 74], and third trimesters [69, 75].

It can be assumed that there is a defect in both maternal and fetal HLA-G secretion during complicated pregnancies. Interestingly, the lower secretion of HLA-G by the placenta could be influenced by the significantly increased release of microparticles through the syncytiotrophoblast membrane into the maternal circulation in preeclampsia [76]. The analysis of these microparticles (MPs) in maternal plasma has shown increased levels of HLA-G-positive MPs in term preeclamptic women compared to control pregnant women [77]. This observation indicates a dysfunctional extravillous CTB turn-over in preeclampsia that could reduce HLA-G secretion. The lower secretion of HLA-G by maternal immune cells could be influenced by *HLA-G* gene polymorphisms, which affect mRNA stability. In particular the HLA-G 14 bp ins allele decreases mRNA stability [78] and protein production [2, 51, 79–81]. RSA is associated with the presence of an increased frequency of women heterozygous [82, 83] or homozygous for the 14 bp ins allele [OR 2.7; 95% confidencial interval (CI) = 1.1–6.5; $p = 0.03$] [80, 84] in comparison with controls. Hviid et al. [84] have shown more RSA women carrying the G*0106 allele (16%), characterized by the presence of the 14 bp insertion, compared to controls (2%). The HLA-G 14 bp ins/del polymorphism seems also to affect the fetal HLA-G expression as independent studies have reported fetuses carrying the homozygous genotype for the 14 bp ins allele with a significantly increased risk of preeclampsia [85–88]. In addition the 5′ URR region seems to be implicated in pathological pregnancies. The −725C/G polymorphism is associated with fetal loss and an increased risk for miscarriage (OR 2.76, 95% CI 1.08–7.09; $P = 0.035$). This polymorphism flanks a binding site for the transcription factor IRF-1 (interferon rergulatory factor) and creates a methylated CpG dinucleotide, which could alter the conformation of DNA and affect IRF-1 binding and subsequently *HLA-G* transcription [89]. However, some studies failed to demonstrate a clear association between *HLA-G* gene polymorphisms and pregnancy complications [90–94], indicating that several mechanisms could influence HLA-G expression and subsequent pregnancy outcome. The presence of different HLA-G isoforms has to be taken into consideration in all of these studies as some polymorphisms could affect the stability of only some transcripts and preserve other isoforms that could sustain the pregnancy immune regulation. The identification of healthy subjects homozygous for HLA-G*01:05 N, a null allele that presents a single base deletion, preventing translation of both membrane-bound (HLA-G1) and full-length soluble isoforms (HLA-G5) as well as of the spliced HLA-G4 isoform, suggests that the other HLA-G isoforms produced by this allele are able to

overcome the absence of HLA-G1 and HLA-G5 molecules and protect cells expressing these isoforms from NK cell cytolysis, as demonstrated by Le Discorde et al. [95].

Much work remains to be done before all the different patterns of expression in trophoblast cells during physiological and pathological conditions can be explained. This analysis is of extreme importance for the comprehension of HLA-G modulation in physiological conditions, with the objective of clarifying the aberrant HLA-G expression in pathological conditions and helping in the identification of the biological tools to sustain HLA-G expression where it has a beneficial effect (pregnancy, transplantation, autoimmune, and inflammatory diseases) or to counteract HLA-G modulation where it is detrimental (tumor, viral infections).

In pregnancies, HLA-G molecules could already play a role in clinical practice. Most pregnancy complications are controversial in terms of diagnosis and treatment. As an example, preeclampsia can mimic and can be confused with many other diseases, and none of the signs are specific. The lower levels of sHLA-G detected in maternal plasma and the *HLA-G* polymorphism association could assist clinicians in an accurate and reliable diagnosis. In this context, it is of importance that, in the majority of studies, sHLA-G molecules were detected by monoclonal antibodies recognizing HLA-G molecules in association with β2-m according to the ELISA formats established and validated by the international "Wet Workshop for Quantification of Soluble HLA-G" in Essen (Germany) in 2004 [96] or by the commercially available ELISA from EXBIO Praha. Thus, the detection and quantification of soluble HLA-G molecules that are not associated with β2-m, e.g., HLA-G6, remain to be analyzed in pregnancy. These isoforms are of special interest as they are reported to interact preferentially with LILRB2 but not with the LILRB1 receptor. Moreover, the *HLA-G* genetic background of the mother could be an a priori sign of an increased risk of complication during pregnancy. These women could be identified and proposed for a more strict follow-up. It is noteworthy that with an appropriate and timely treatment, the success rate is approximately 80%. Therefore, the use of HLA-G as a biological and genetic marker could improve the management of pregnant women. The ability to control HLA-G expression in pathological pregnancies and in women with a high risk of pregnancy complications could be a tool to cure and prevent these conditions with a deep impact not only for the individual but also for society. The possibility of reducing the incidence of these complications and of increasing fertility with the use of a biological molecule that is normally expressed during pregnancy, such as HLA-G, could reduce the use of medical procedures to achieve successful pregnancies, which is still connected with high health risks.

HLA-G expression in preimplantation embryos and its clinical importance

Preimplantation development is the first critical step in early human development followed by a successful implantation of the embryo. It covers the time period from before fertilization to implantation including the final maturation of oocyte, fertilization, oocyte to zygote transition, cell proliferation, and differentiation and formation of blastocysts. Important questions include whether HLA-G is also expressed during preimplantation development and whether HLA-G can be used as a marker for the prediction of a successful implantation of the embryo after assisted reproduction techniques? HLA-G mRNA has indeed been demonstrated during preimplantation development from unfertilized oocytes and early embryos from the two-cell cleavage stage onwards to the blastocyst. HLA-G expression was found to be variable among equal stage embryos even in embryos from the same parents [97]. The probability of detection increases with the developmental stage, from 35% in cleavage stage embryos to 100% in morulas and blastocysts [98]. β2-m mRNA has been demonstrated in 86% of blastocysts, in half of them it was found together with HLA-G. The presence of HLA-G mRNA in blastocysts has been found to be significantly associated with a higher cleavage rate [97] and with pregnancy after transfer of sibling embryos from the same cycle [99]. Importantly, different culture conditions or infertility treatments [in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI) of eggs] did not influence HLA-G mRNA expression [99]. The analysis of different HLA-G mRNA splice variants also revealed a different expression pattern in equal stage embryos [98]. HLA-G3 and HLA-G4 mRNA have been reported to be most predominant in all preimplantation stages and are detected more frequently than HLA-G1. HLA-G2, HLA-G5, and HLA-G6 were the least represented. HLA-G5 could only be detected in a pool of cleavage stage embryos (not in a single embryo extract), in 20% of morulas (1/5), and in blastocysts (5/25); HLA-G1 was found in 40% of eight-cell cleavage stage embryos (2/5), in 60% of morulas (3/5), and in 80% of blastocysts (20/25). Since HLA-G mRNA quantities per embryo were low and PCR reactions clearly differed in sensitivity—higher for HLA-G3 and G4 and lower for HLA-G5 and G6—isoform differentiation in preimplantation embryos is yet inconclusive.

HLA-G1/G5 protein expression has been detected in human preimplantation embryos in association with β2-m [97–101]. The most evolved preimplantation stage expressing HLA-G1/G5 was the hatching blastocyst that makes contact with the endometrium at the moment of implantation [100]. Similarly to HLA-G mRNA, HLA-G1/G5 protein expression differs among equal stage embryos and

increases with developmental stage. HLA-G1/G5 protein has been found in 75% of cleavage stage embryos (15/20), in 100% of morulas (3/3), and in 100% of blastocysts (5/5) [98]. Lower prevalences have been reported in poor quality embryos: 58% in arrested cleavage stage embryos and 77% in day 6 blastocysts [100]. HLA-G1/G5 protein is present in chromosomally abnormal embryos. Theoretically HLA-G expression in embryos may also differ among individuals due to different HLA-G alleles or promoter polymorphism [102, 103]. HLA-G expression in blastocysts is nowadays considered to be restricted to trophoblast (TE) cells evolving towards placenta and to be absent in the inner cell mass (ICM), which is evolving into the fetus [97, 98, 100]. Because HLA-G1/G5 is expressed during the early stages of preimplantation development, the differentiation into TE or ICM would imply suppression of HLA-G1/G5 expression in ICM cells. A preliminary report at the 2009 HLA-G Congress in Paris (results submitted for publication) shows however that HLA-G1/G5 is expressed in early ICM cells and becomes down-regulated during hatching [101]. Methodological differences such as the use of protease for removal of zona pellucida, immunosurgery for ICM isolation, and distinct monoclonal antibodies may explain previous negative results.

sHLA-G molecules are definitely present in culture supernatants of preimplantation embryos. Due to the low sHLA-G concentration in embryo cultures (EC), the HLA-G immunodetection method and sample treatment strongly determine the reliability of the sHLA-G measurement in embryo culture supernatants. Technical difficulties have led to very controversial results in the past [100, 104–118] but are being resolved. sHLA-G in embryo culture supernatants is mostly measured by sandwich ELISA or ALBIA (addressable laser bead immunoassay) techniques. Generally HLA-G antibodies are used that recognize the HLA-G1:β2-m or HLA-G5:β2-m complex; a few studies have been performed using antibodies (mab 4H84 or MEM-G/1) recognizing β2-m free heavy chains of all HLA-G isoforms [70, 115]. In the past, sHLA-G immunoassays differed in standardization, detection limit, and reproducibility. Impurity of the HLA-G standard material could result in overestimation of the sHLA-G concentration. Absolute amounts of 2–756 ng of sHLA-G have been reported in the supernatants of single or multiple embryo cultures [119], while the daily protein release of a human embryo is estimated to be 150 pg and its total protein content to be 50 ng [120]. The estimated amount of sHLA-G in embryo culture supernatants is more likely situated in the picogram range. If an ELISA is used with a detection limit of 1–10 ng/mL, most samples will fluctuate around the detection limit, where reproducibility is low. However, for the ALBIA technique a detection limit of 300 pg/mL and a coefficient of variation of 11.6% within one assay and 17.9% among different assays have recently been described [110, 111]. Thus, sensitive and reproducible techniques (ALBIA as well as certain ELISA) for the detection of sHLA-G in embryo culture supernatant have successfully been developed by research laboratories [110–112, 117], but equally performing commercial systems are not available yet.

In addition to technical difficulties, differences in the preservation of the culture supernatants between collection and sHLA-G measurement as well as differences in ART procedures may also explain inconsistent sHLA-G detection across centers/studies. sHLA-G in embryo culture supernatant is present as two isoforms: secreted HLA-G5 and shed HLA-G1 resulting from proteolytically cleaved membrane-bound HLA-G1. After collection, embryo culture supernatants have to be handled with care and deep-frozen immediately since it is rather uncertain whether the embryo can provide a high affinity peptide to form a stable HLA-G heavy chain:β2-m complex. Therefore, it is very likely that sHLA-G molecules derived from embryos are unstable molecules, prone to degradation if not frozen immediately. Furthermore, the origin of sHLA-G in embryo culture supernatant is still unknown. sHLA-G has been demonstrated in follicular fluid, originating from the oocyte, granulosa, or contaminating blood cells [112, 121]. Carry-over of follicular fluid or cells into the embryo culture supernatant may differ among centers and among embryos obtained after ICSI or IVF, due to different procedures for washing and oocyte denudation. In one study the percentage of sHLA-G-positive embryo cultures was indeed found to be significantly higher in IVF cycles [117], where in general fewer washing steps of the oocyte were performed compared to ICSI.

Recently, a German multi-center study [110], in which 29 ART centers with 4,212 EC from 2,364 cycles were included, demonstrated that the proportion of sHLA-G-positive EC increases with the cleavage stages from 11% for zygotes to 21% in two- to nine-cell embryos and 30% in embryos larger than ten cells. The low proportion of sHLA-G-positive embryo supernatants derived from zygotes gives reasonable doubt that contamination from oocyte retrieval is the major source of sHLA-G. In the early stages of embryos, sHLA-G in the culture supernatant may originate from translational activation of oocyte mRNA stores and consequently reflect oocyte rather than embryo quality, but after embryonic genome activation between the four- and eight-cell stage, it might only be produced by the embryo. In the former case, it is useful to measure sHLA-G in the follicle or very early during preimplantation development (day 1); in the latter case sHLA-G produced by the embryo should be analyzed from day 2 to 3 onwards. In contrast to blastocyst culture medium collected at day 5, small amounts of sHLA-G produced early will make high demands on the sensitivity of the

techniques. The early HLA-G synthesis by the embryo may, on the other hand, be more relevant for embryo quality than the higher absolute amount of HLA-G detected at day 5. Thus, the ideal moment for collection of embryo culture supernatant has yet to be determined.

Besides collection time, many variations in embryo cultures exist among the diverse ART centers, such as single versus multiple embryo culture, composition and volume of culture medium, proportion of culture medium versus total volume analyzed in the immunoassay. Considering these issues, the German multi-center study revealed that only the cleavage stage of the embryo is an independent factor that influences the sHLA-G detection whereas culture volume and duration, and the number of embryos in culture and the cryopreservation status of fertilized oocytes were found not to significantly affect the detection of sHLA-G. Nevertheless, another study including three ART centers with 1,405 EC from 355 cycles suggested that the composition of the embryo culture medium may influence HLA-G detection either by an effect on the embryo or by interfering with the immunodetection technique [117].

sHLA-G in the culture supernatant of preimplantation embryos is being investigated as a noninvasive marker for the selection of embryos with implantation potential. Choosing the best embryo becomes increasingly important, considering the tendency to reduce the number of transferred embryos in order to prevent multiple pregnancies. Today the embryologists' decision is based on morphological criteria such as developmental rate, cell number, and degree of fragmentation [122]. However, many approaches to improve embryo selection are being studied, such as the gene expression profile of cumulus cells [123, 124], embryo metabolism [125–127], and peptide markers in follicular fluid or embryo culture supernatant, amongst them sHLA-G. The presence of sHLA-G in embryo culture supernatant has been shown by meta-analysis to increase the probability of clinical pregnancy from 45 to 72%, despite differences in ART and sHLA-G detection among centers [104]. The German multi-center study revealed that sHLA-G testing would improve the overall pregnancy rate from 30 to 40% [110]. The true predictive value of sHLA-G in embryo culture supernatant for embryo implantation can only be elucidated by additional studies linking a single HLA-G-producing embryo with pregnancy, by combining standardized ART, single embryo culture and single embryo transfer with a sensitive standardized immunoassay for sHLA-G following standardized pre-analytical procedures preventing HLA-G degradation. The first step in that direction was performed by the German multi-center study [110], in which the sHLA-G status of embryos could clearly be associated with pregnancy after single embryo transfer; sHLA-G testing doubled the probability of pregnancy rate to 26%. Furthermore, this study provided significant evidence that the morphological scoring system is still the best strategy for the selection of embryos, but that the sHLA-G status of the embryo might be considered as a second parameter if a choice has to be made between embryos of morphologically equal quality. If these results are reproduced by other studies and if the functionally relevant HLA-G protein structure that plays a crucial role in embryo implantation with its corresponding receptors is identified, then HLA-G should definitely be taken into consideration as a marker for the development of new strategies in embryo selection. For scientific and diagnostic reasons it is extremely important to figure out why certain embryos express and other embryos do not express HLA-G molecules in their supernatants.

HLA-G expression in human embryonic stem cells and its functional implication

HLA-G-expressing cells seem to have immuno-privileged properties in autoimmunity, transplantation, and tumor progression [30, 31]. In addition, the immunosuppressive properties of adult bone-marrow-derived multipotent mesenchymal stem cells (MSC) have, besides IDO and PGE2, recently been determined to secrete HLA-G5, which inhibited NK cell-mediated cytotoxicity and IFN-α,-γ secretion, suppressed allogeneic T cell proliferation, and induced the expansion of $CD4^{+}CD^{25high}FOXP3^{+}$ regulatory T cells [128, 129].

Recent studies have suggested that human embryonic stem cells (hESC) are also immune-privileged cells [130]. These pluripotent cells are derived from the inner cell mass of blastocysts. They are extremely promising for transplantation and treatment of degenerative diseases because they have the capacity to propagate indefinitely in culture without losing pluripotency and they can be induced to differentiate into any cell type of the body. Their pluripotency is demonstrated when they can be induced to differentiate into cells representing the three germ layers (ectoderm, mesoderm, endoderm) in vitro (embryoid bodies) [131] and in vivo (teratomas in muscles or testis of SCID mice) [132]. However, their capacity to form teratomas should be followed with caution because benign tumors may eventually develop into malignant cancers. Major concern has been raised because after injecting undifferentiated hESC into engrafted human fetal tissue in SCID mice, primitive undifferentiated tumors rather than teratomas have been found [133].

For transplantation purposes, hESC are specifically directed in vitro towards a highly specialized cell type. However, undifferentiated hESC may contaminate the transplant and induce malignancy. Besides safety issues

such as tumor development and the risk of transmitting pathogens from cell culture [134, 135], the immune response induced by hESC and the differentiated cells in the recipient are a major concern. Are hESC able to escape the immune system? HESC express HLA class I molecules at a very low level that increases moderately after differentiation but reaches the level of somatic cells in the presence of IFN-α and IFN-γ [136]. They have no potential for antigen presentation because their gene expression signature lacks T cell activation genes and HLA class II or costimulatory molecules are not expressed in their membrane [130, 136, 137]. Therefore, hESC should not be able to induce a robust immune response by direct antigen presentation as reported by Li [138], who showed that hESC fail to stimulate proliferation of alloreactive T cells and inhibit third-party allogeneic dendritic cell-mediated T cell proliferation. However, when indirectly presented by professional antigen presenting cells (APC) such as dendritic cells (DC) syngeneic to the responder T cells, they were shown to induce T cell proliferation as well as human fibroblasts [137]. Moreover, hESC were acutely rejected in immunologically competent mice, strongly indicating that they are not immune-privileged during direct and indirect antigen presentation.

HESC are resistant to NK-cell-mediated cytotoxicity and less susceptible than somatic cells to cytotoxic T cell lysis, although this can occur in cases of very efficient peptide-loading and in the presence of IFN-α, -γ [130]. Are these immune-suppressive features of hECS cells due to HLA-G expression? HLA-G expression in hESC has been investigated at the protein and RNA level with inconclusive results. Protein studies have been performed with immunofluorescence, either using flow cytometry [136] or microscopy [139]. The HLA-G protein was not detected by flow cytometry on trypsinized hESC in conditions that did reveal total HLA class I, HLA-E, and β2-m. Since enzymatic digestion of adherent cells or immunophenotyping conditions optimized for highly expressing transfectants are known to underestimate weakly expressed proteins, these results have to be interpreted with care. In hESC-derived mesenchymal progenitor cells, a low HLA-G expression on the cell surface membrane (tenfold below the one of classical HLA-I proteins) could be demonstrated using the MEM-G/1 antibody. Here it has to be pointed out that the MEM-G/1 antibody recognizes all HLA-G isoforms not associated with β2-m [140].

In hESC colonies driven into trophoblast differentiation by 5-day cultures on MatrigelT^{TM} in the presence of bone morphogenetic protein-4 (BMP-4), HLA-G was found to be variably expressed in the peripheral cells adapting trophoblast morphology, expressing human chorionic gonadotropin (hCG), stage-specific embryonic antigen-1 (SSEA-1), and cytokeratin-7 and cytokeratin-8 without the stem cell marker octamer binding transcription factor-4 (Oct-4) also designated as POU5F1. Here again an antibody was used, recognizing all HLA-G isoforms not associated with β2-m. The central core cells remained morphologically undifferentiated, expressing POU5F1 but not HLA-G, except for some regions where HLA-G staining penetrated the core region. Unfortunately these cells were not further characterized, nor were undifferentiated hESC examined before Matrigel^{TM}/BMP-4 culture [139, 141]. Trophoblast cells originating from differentiated hESCs offer perspectives for the development of an in vitro implantation model.

HLA-G mRNA expression has been reported in hESC using semiquantitative oligonucleotide array profiling on amplified RNA [139]. Variable HLA-G expression levels were found in seven tested hESC lines. These observations were confirmed by real-time PCR as recently presented at the 5th International Conference on HLA-G in Paris (submitted for publication; see also [101]). Finding HLA-G in hESC cells is not totally unexpected because it also has been found in ICM cells of early human blastocysts, the source of the pluripotent hESC cells. The HLA-G structure and role in immunotolerance and tumor development in hESCs will have to be investigated in further studies.

Concluding remarks

The expression profile of certain HLA-G structures during preimplantation development and pregnancy suggests their special task in reprogramming local maternal immune tolerance towards the fetus. Thus, one important step forward will be to investigate the relationship between the environmental conditions and the location of certain HLA-G structures, and HLA-G's functional consequences in pregnancy. At the same time, the different protein structures of HLA-G pose a great challenge for their specific detection. Thus, new methods have to be developed allowing the identification of certain HLA-G structures by antibodies and receptors. The results obtained will improve our knowledge of the role of HLA-G during pregnancy and sustain its potential use in clinical practice both as a potential biomarker and as a "natural" immunoregulatory molecule during pathological conditions.

References

1. Ishitani A, Sageshima N, Lee N, Dorofeeva N, Hatake K, Marquardt H, Geraghty DE (2003) Protein expression and peptide binding suggest unique and interacting functional roles for HLA-E, F, and G in maternal–placental immune recognition. J Immunol 171:1376–1384

2. Fujii T, Ishitani A, Geraghty DE (1994) A soluble form of the HLA-G antigen is encoded by a messenger ribonucleic acid containing intron 4. J Immunol 153:5516–5524
3. Ishitani A, Geraghty DE (1992) Alternative splicing of HLA-G transcripts yields proteins with primary structures resembling both class I and class II antigens. Proc Natl Acad Sci USA 89:3947–3951
4. Paul P et al (2000) Identification of HLA-G7 as a new splice variant of the HLA-G mRNA and expression of soluble HLA-G5, -G6, and -G7 transcripts in human transfected cells. Hum Immunol 61:1138–1149
5. Park GM, Lee S, Park B, Kim E, Shin J, Cho K, Ahn K (2004) Soluble HLA-G generated by proteolytic shedding inhibits NK-mediated cell lysis. Biochem Biophys Res Commun 313:606–611
6. Diehl M, Munz C, Keilholz W, Stevanovic S, Holmes N, Loke YW, Rammensee HG (1996) Non-classical HLA-G molecules are classical peptide presenters. Curr Biol 6:305–314
7. Lee N, Malacko AR, Ishitani A, Chen MC, Bajorath J, Marquardt H, Geraghty DE (1995) The membrane-bound and soluble forms of HLA-G bind identical sets of endogenous peptides but differ with respect to TAP association. Immunity 3:591–600
8. Walpole NG, Kjer-Nielsen L, Kostenko L, McCluskey J, Brooks AG, Rossjohn J, Clements CS (2010) The structure and stability of the monomorphic HLA-G are influenced by the nature of the bound peptide. J Mol Biol 397:467–480
9. Bahri R et al (2006) Soluble HLA-G inhibits cell cycle progression in human alloreactive T lymphocytes. J Immunol 176:1331–1339
10. Dorling A, Monk NJ, Lechler RI (2000) HLA-G inhibits the transendothelial migration of human NK cells. Eur J Immunol 30:586–593
11. Fournel S, Aguerre-Girr M, Huc X, Lenfant F, Alam A, Toubert A, Bensussan A, Le Bouteiller P (2000) Cutting edge: soluble HLA-G1 triggers CD95/CD95 ligand-mediated apoptosis in activated CD8+ cells by interacting with CD8. J Immunol 164:6100–6104
12. Le Rond S, Azema C, Krawice-Radanne I, Durrbach A, Guettier C, Carosella ED, Rouas-Freiss N (2006) Evidence to support the role of HLA-G5 in allograft acceptance through induction of immunosuppressive/regulatory T cells. J Immunol 176:3266–3276
13. Rajagopalan S, Bryceson YT, Kuppusamy SP, Geraghty DE, van der Meer A, Joosten I, Long EO (2006) Activation of NK cells by an endocytosed receptor for soluble HLA-G. PLoS Biol 4:e9
14. Rajagopalan S, Fu J, Long EO (2001) Cutting edge: induction of IFN-gamma production but not cytotoxicity by the killer cell Ig-like receptor KIR2DL4 (CD158d) in resting NK cells. J Immunol 167:1877–1881
15. Rouas-Freiss N, Goncalves RM, Menier C, Dausset J, Carosella ED (1997) Direct evidence to support the role of HLA-G in protecting the fetus from maternal uterine natural killer cytolysis. Proc Natl Acad Sci USA 94:11520–11525
16. Rouas-Freiss N, Marchal RE, Kirszenbaum M, Dausset J, Carosella ED (1997) The alpha1 domain of HLA-G1 and HLA-G2 inhibits cytotoxicity induced by natural killer cells: is HLA-G the public ligand for natural killer cell inhibitory receptors? Proc Natl Acad Sci USA 94:5249–5254
17. Yan WH, Fan LA (2005) Residues Met76 and Gln79 in HLA-G alpha1 domain involve in KIR2DL4 recognition. Cell Res 15:176–182
18. Gros F, Cabillic F, Toutirais O, Maux AL, Sebti Y, Amiot L (2008) Soluble HLA-G molecules impair natural killer/dendritic cell crosstalk via inhibition of dendritic cells. Eur J Immunol 38:742–749
19. LeMaoult J, Krawice-Radanne I, Dausset J, Carosella ED (2004) HLA-G1-expressing antigen-presenting cells induce immunosuppressive CD4+ T cells. Proc Natl Acad Sci USA 101:7064–7069
20. Liang S, Ristich V, Arase H, Dausset J, Carosella ED, Horuzsko A (2008) Modulation of dendritic cell differentiation by HLA-G and ILT4 requires the IL-6–STAT3 signaling pathway. Proc Natl Acad Sci USA 105:8357–8362
21. Ristich V, Liang S, Zhang W, Wu J, Horuzsko A (2005) Tolerization of dendritic cells by HLA-G. Eur J Immunol 35:1133–1142
22. Fons PCS, Cartwright JE, Lenfant F, L′Faqihi F, Giustiniani J, Herault JP, Gueguen G, Bono F, Savi P, Aguerre-Girr M, Fournel S, Malecaze F, Bensussan A, Plouët J, Le Bouteiller P (2006) Soluble HLA-G1 inhibits angiogenesis through an apoptotic pathway and by direct binding to CD160 receptor expressed by endothelial cells. Blood 108:2608–2615
23. Kanai T, Fujii T, Kozuma S, Yamashita T, Miki A, Kikuchi A, Taketani Y (2001) Soluble HLA-G influences the release of cytokines from allogeneic peripheral blood mononuclear cells in culture. Mol Hum Reprod 7:195–200
24. LeMaoult J, Zafaranloo K, Le Danff C, Carosella ED (2005) HLA-G up-regulates ILT2, ILT3, ILT4, and KIR2DL4 in antigen presenting cells, NK cells, and T cells. FASEB J 19:662–664
25. Amiot L, Onno M, Renard I, Drenou B, Guillaudeux T, Le Bouteiller P, Fauchet R (1996) HLA-G transcription studies during the different stages of normal and malignant hematopoiesis. Tissue Antigens 48:609–614
26. Le Discorde M, Moreau P, Sabatier P, Legeais JM, Carosella ED (2003) Expression of HLA-G in human cornea, an immune-privileged tissue. Hum Immunol 64:1039–1044
27. Mallet V, Fournel S, Schmitt C, Campan A, Lenfant F, Le Bouteiller P (1999) Primary cultured human thymic epithelial cells express both membrane-bound and soluble HLA-G translated products. J Reprod Immunol 43:225–234
28. McMaster MT et al (1995) Human placental HLA-G expression is restricted to differentiated cytotrophoblasts. J Immunol 154:3771–3778
29. Menier C, Rabreau M, Challier JC, Le Discorde M, Carosella ED, Rouas-Freiss N (2004) Erythroblasts secrete the nonclassical HLA-G molecule from primitive to definitive hematopoiesis. Blood 104:3153–3160
30. Carosella ED, Favier B, Rouas-Freiss N, Moreau P, Lemaoult J (2008) Beyond the increasing complexity of the immunomodulatory HLA-G molecule. Blood 111:4862–4870
31. Carosella ED, Moreau P, Lemaoult J, Rouas-Freiss N (2008) HLA-G: from biology to clinical benefits. Trends Immunol 29:125–132
32. Ellis SA, Sargent IL, Redman CW, McMichael AJ (1986) Evidence for a novel HLA antigen found on human extravillous trophoblast and a choriocarcinoma cell line. Immunology 59:595–601
33. Hunt JS, Langat DL (2009) HLA-G: a human pregnancy-related immunomodulator. Curr Opin Pharmacol 9:462–469
34. Xuan YH, Choi YL, Shin YK, Ahn GH, Kim KH, Kim WJ, Lee HC, Kim SH (2007) Expression of TGF-beta signaling proteins in normal placenta and gestational trophoblastic disease. Histol Histopathol 22:227–234
35. Pang ZJ, Zhou JG, Huang LP (2008) Interleukin-10 may participate in regulating trophoblast invasion in human placentae throughout gestation. Am J Reprod Immunol 60:19–25
36. Munn DH, Zhou M, Attwood JT, Bondarev I, Conway SJ, Marshall B, Brown C, Mellor AL (1998) Prevention of allogeneic fetal rejection by tryptophan catabolism. Science 281: 1191–1193

37. Guleria I et al (2005) A critical role for the programmed death ligand 1 in fetomaternal tolerance. J Exp Med 202:231–237
38. Brown D, Trowsdale J, Allen R (2004) The LILR family: modulators of innate and adaptive immune pathways in health and disease. Tissue Antigens 64:215–225
39. Chen JH, Yao YQ, Hou KB, Lei YF, Yin W (2007) Construction of eukaryotic expression plasmid expressing siRNA targeting HLA-G gene and detection of its specific downregulation effect. Xi Bao Yu Fen Zi Mian Yi Xue Za Zhi 23:409–412
40. Sun LL, Han Y, Chen JH, Yao YQ (2008) Down-regulation of HLA-G boosted natural killer cell-mediated cytolysis in JEG-3 cells cultured in vitro. Fertil Steril 90:2398–2405
41. Morales PJ, Pace JL, Platt JS, Langat DK, Hunt JS (2007) Synthesis of beta(2)-microglobulin-free, disulphide-linked HLA-G5 homodimers in human placental villous cytotrophoblast cells. Immunology 122:179–188
42. Apps R, Gardner L, Sharkey AM, Holmes N, Moffett A (2007) A homodimeric complex of HLA-G on normal trophoblast cells modulates antigen-presenting cells via LILRB1. Eur J Immunol 37:1924–1937
43. McIntire RH, Sifers T, Platt JS, Ganacias KG, Langat DK, Hunt JS (2008) Novel HLA-G-binding leukocyte immunoglobulin-like receptor (lilr) expression patterns in human placentas and umbilical cords. Placenta 29:631–638
44. Morales PJ, Pace JL, Platt JS, Phillips TA, Morgan K, Fazleabas AT, Hunt JS (2003) Placental cell expression of HLA-G2 isoforms is limited to the invasive trophoblast phenotype. J Immunol 171:6215–6224
45. Solier C, Aguerre-Girr M, Lenfant F, Campan A, Berrebi A, Rebmann V, Grosse-Wilde H, Le Bouteiller P (2002) Secretion of pro-apoptotic intron 4-retaining soluble HLA-G1 by human villous trophoblast. Eur J Immunol 32:3576–3586
46. McCormick J, Whitley GS, Le Bouteiller P, Cartwright JE (2009) Soluble HLA-G regulates motility and invasion of the trophoblast-derived cell line SGHPL-4. Hum Reprod 24:1339–1345
47. Gonen-Gross T et al (2010) Inhibitory NK receptor recognition of HLA-G: regulation by contact residues and by cell specific expression at the fetal–maternal interface. PLoS One 5:e8941
48. Yie SM, Li LH, Li GM, Xiao R, Librach CL (2006) Progesterone enhances HLA-G gene expression in JEG-3 choriocarcinoma cells and human cytotrophoblasts in vitro. Hum Reprod 21:46–51
49. Lefebvre S et al (2001) A specific interferon (IFN)-stimulated response element of the distal HLA-G promoter binds IFN-regulatory factor 1 and mediates enhancement of this nonclassical class I gene by IFN-beta. J Biol Chem 276:6133–6139
50. Moreau P, Adrian-Cabestre F, Menier C, Guiard V, Gourand L, Dausset J, Carosella ED, Paul P (1999) IL-10 selectively induces HLA-G expression in human trophoblasts and monocytes. Int Immunol 11:803–811
51. Rizzo R, Hviid TV, Stignani M, Balboni A, Grappa MT, Melchiorri L, Baricordi OR (2005) The HLA-G genotype is associated with IL-10 levels in activated PBMCs. Immunogenetics 57:172–181
52. Anderson KJ, Allen RL (2009) Regulation of T-cell immunity by leucocyte immunoglobulin-like receptors: innate immune receptors for self on antigen-presenting cells. Immunology 127:8–17
53. Nagamatsu T et al (2004) Hypoxia does not reduce HLA-G expression on extravillous cytotrophoblasts. J Reprod Immunol 63:85–95
54. Humphrey KE, Harrison GA, Cooper DW, Wilton AN, Brennecke SP, Trudinger BJ (1995) HLA-G deletion polymorphism and pre-eclampsia/eclampsia. Br J Obstet Gynaecol 102:707–710
55. Bohjanen PR, Petryniak B, June CH, Thompson CB, Lindsten T (1991) An inducible cytoplasmic factor (AU-B) binds selectively to AUUUA multimers in the 3′ untranslated region of lymphokine mRNA. Mol Cell Biol 11:3288–3295
56. Hviid TV, Sorensen S, Morling N (1999) Polymorphism in the regulatory region located more than 1.1 kilobases 5' to the start site of transcription, the promoter region, and exon 1 of the HLA-G gene. Hum Immunol 60:1237–1244
57. Holling TM, Bergevoet MW, Wierda RJ, van Eggermond MC, van den Elsen PJ (2009) Genetic and epigenetic control of the major histocompatibility complex class Ib gene HLA-G in trophoblast cell lines. Ann NY Acad Sci 1173:538–544
58. Castelli EC et al (2009) In silico analysis of microRNAS targeting the HLA-G 3′ untranslated region alleles and haplotypes. Hum Immunol 70:1020–1025
59. Gracia CR, Sammel MD, Chittams J, Hummel AC, Shaunik A, Barnhart KT (2005) Risk factors for spontaneous abortion in early symptomatic first-trimester pregnancies. Obstet Gynecol 106:993–999
60. Hara N, Fujii T, Yamashita T, Kozuma S, Okai T, Taketani Y (1996) Altered expression of human leukocyte antigen G (HLA-G) on extravillous trophoblasts in preeclampsia: immunohistological demonstration with anti-HLA-G specific antibody "87G" and anti-cytokeratin antibody "CAM5.2". Am J Reprod Immunol 36:349–358
61. Lim KH, Zhou Y, Janatpour M, McMaster M, Bass K, Chun SH, Fisher SJ (1997) Human cytotrophoblast differentiation/invasion is abnormal in preeclampsia. Am J Pathol 151:1809–1818
62. Peng B, Zhang L, Xing AY, Hu M, Liu SY (2008) The expression of human leukocyte antigen G and E on human first trimester placenta and its relationship with recurrent spontaneous abortion. Sichuan Da Xue Xue Bao Yi Xue Ban 39:976–979
63. Yie SM, Li LH, Li YM, Librach C (2004) HLA-G protein concentrations in maternal serum and placenta tissue are decreased in preeclampsia. Fertil Steril 83:30–36
64. Comiskey M, Domino KE, Warner CM (2007) HLA-G is found in lipid rafts and can act as a signaling molecule. Hum Immunol 68:1–11
65. Comiskey M, Goldstein CY, De Fazio SR, Mammolenti M, Newmark JA, Warner CM (2003) Evidence that HLA-G is the functional homolog of mouse Qa-2, the Ped gene product. Hum Immunol 64:999–1004
66. McElhinny AS, Exley GE, Warner CM (2000) Painting Qa-2 onto Ped slow preimplantation embryos increases the rate of cleavage. Am J Reprod Immunol 44:52–58
67. Alegre E, Diaz-Lagares A, Lemaoult J, Lopez-Moratalla N, Carosella ED, Gonzalez A (2007) Maternal antigen presenting cells are a source of plasmatic HLA-G during pregnancy: longitudinal study during pregnancy. Hum Immunol 68:661–667
68. Hunt JS, Jadhav L, Chu W, Geraghty DE, Ober C (2000) Soluble HLA-G circulates in maternal blood during pregnancy. Am J Obstet Gynecol 183:682–688
69. Hackmon R, Hallak M, Krup M, Weitzman D, Sheiner E, Kaplan B, Weinstein Y (2004) HLA-G antigen and parturition: maternal serum, fetal serum and amniotic fluid levels during pregnancy. Fetal Diagn Ther 19:404–409
70. Yie SM, Taylor RN, Librach C (2005) Low plasma HLA-G protein concentrations in early gestation indicate the development of pre-eclampsia later in pregnancy. Am J Obstet Gynecol 193:204–208
71. Rizzo R, Stignani M, Amoudruz P, Nilsson C, Melchiorri L, Baricordi O, Sverremark-Ekstrom E (2009) Allergic women have reduced sHLA-G plasma levels at delivery. Am J Reprod Immunol 61:368–376
72. Steinborn A, Rebmann V, Scharf A, Sohn C, Grosse-Wilde H (2003) Placental abruption is associated with decreased maternal plasma levels of soluble HLA-G. J Clin Immunol 23:307–314
73. Borzychowski AM, Sargent IL, Redman CW (2006) Inflammation and pre-eclampsia. Semin Fetal Neonatal Med 11:309–316

74. Steinborn A, Varkonyi T, Scharf A, Bahlmann F, Klee A, Sohn C (2007) Early detection of decreased soluble HLA-G levels in the maternal circulation predicts the occurrence of preeclampsia and intrauterine growth retardation during further course of pregnancy. Am J Reprod Immunol 57:277–286
75. Pfeiffer KA, Rebmann V, Passler M, van der Ven K, van der Ven H, Krebs D, Grosse-Wilde H (2000) Soluble HLA levels in early pregnancy after in vitro fertilization. Hum Immunol 61:559–564
76. Ishihara N, Matsuo H, Murakoshi H, Laoag-Fernandez JB, Samoto T, Maruo T (2002) Increased apoptosis in the syncytiotrophoblast in human term placentas complicated by either preeclampsia or intrauterine growth retardation. Am J Obstet Gynecol 186:158–166
77. Orozco AF, Jorgez CJ, Ramos-Perez WD, Popek EJ, Yu X, Kozinetz CA, Bischoff FZ, Lewis DE (2009) Placental release of distinct DNA-associated micro-particles into maternal circulation: reflective of gestation time and preeclampsia. Placenta 30:891–897
78. Rousseau P, Le Discorde M, Mouillot G, Marcou C, Carosella ED, Moreau P (2003) The 14 bp deletion–insertion polymorphism in the 3′ UT region of the HLA-G gene influences HLA-G mRNA stability. Hum Immunol 64:1005–1010
79. Hiby SE, King A, Sharkey A, Loke YW (1999) Molecular studies of trophoblast HLA-G: polymorphism, isoforms, imprinting and expression in preimplantation embryo. Tissue Antigens 53:1–13
80. Hviid TV, Hylenius S, Lindhard A, Christiansen OB (2004) Association between human leukocyte antigen-G genotype and success of in vitro fertilization and pregnancy outcome. Tissue Antigens 64:66–69
81. Hviid TV, Hylenius S, Rorbye C, Nielsen LG (2003) HLA-G allelic variants are associated with differences in the HLA-G mRNA isoform profile and HLA-G mRNA levels. Immunogenetics 55:63–79
82. Abbas A, Tripathi P, Naik S, Agrawal S (2004) Analysis of human leukocyte antigen (HLA)-G polymorphism in normal women and in women with recurrent spontaneous abortions. Eur J Immunogenet 31:275–278
83. Tripathi P, Abbas A, Naik S, Agrawal S (2004) Role of 14-bp deletion in the HLA-G gene in the maintenance of pregnancy. Tissue Antigens 64:706–710
84. Hviid TV, Hylenius S, Hoegh AM, Kruse C, Christiansen OB (2002) HLA-G polymorphisms in couples with recurrent spontaneous abortions. Tissue Antigens 60:122–132
85. Hylenius S, Andersen AM, Melbye M, Hviid TV (2004) Association between HLA-G genotype and risk of pre-eclampsia: a case-control study using family triads. Mol Hum Reprod 10:237–246
86. Larsen MH, Hylenius S, Andersen AM, Hviid TV (2010) The 3′-untranslated region of the HLA-G gene in relation to pre-eclampsia: revisited. Tissue Antigens 75:253–261
87. Moreau P et al (2008) HLA-G gene polymorphism in human placentas: possible association of G*0106 allele with pre-eclampsia and miscarriage. Biol Reprod 79:459–467
88. O′Brien M, McCarthy T, Jenkins D, Paul P, Dausset J, Carosella ED, Moreau P (2001) Altered HLA-G transcription in pre-eclampsia is associated with allele specific inheritance: possible role of the HLA-G gene in susceptibility to the disease. Cell Mol Life Sci 58:1943–1949
89. Ober C, Aldrich CL, Chervoneva I, Billstrand C, Rahimov F, Gray HL, Hyslop T (2003) Variation in the HLA-G promoter region influences miscarriage rates. Am J Hum Genet 72:1425–1435
90. Aruna M, Sudheer PS, Andal S, Tarakeswari S, Reddy AG, Thangaraj K, Singh L, Reddy BM (2010) HLA-G polymorphism patterns show lack of detectable association with recurrent spontaneous abortion. Tissue Antigens 76(3):216–222
91. Iversen AC et al (2008) The HLA-G 14 bp gene polymorphism and decidual HLA-G 14 bp gene expression in pre-eclamptic and normal pregnancies. J Reprod Immunol 78:158–165
92. Lin A, Yan WH, Dai MZ, Chen XJ, Li BL, Chen BG, Fan LA (2006) Maternal human leukocyte antigen-G polymorphism is not associated with pre-eclampsia in a Chinese Han population. Tissue Antigens 68:311–316
93. Penzes M, Rajczy K, Gyodi E, Reti M, Feher E, Petranyi G (1999) HLA-G gene polymorphism in the normal population and in recurrent spontaneous abortion in Hungary. Transplant Proc 31:1832–1833
94. Yamashita T et al (1999) Analysis of human leukocyte antigen-G polymorphism including intron 4 in Japanese couples with habitual abortion. Am J Reprod Immunol 41:159–163
95. Le Discorde M, Le Danff C, Moreau P, Rouas-Freiss N, Carosella ED (2005) HLA-G*0105N null allele encodes functional HLA-G isoforms. Biol Reprod 73:280–288
96. Rebmann V, LeMaoult J, Rouas-Freiss N, Carosella ED, Grosse-Wilde H (2007) Quantification and identification of soluble HLA-G isoforms. Tissue Antigens 69(Suppl 1):143–149
97. Jurisicova A, Casper RF, MacLusky NJ, Mills GB, Librach CL (1996) HLA-G expression during preimplantation human embryo development. Proc Natl Acad Sci USA 93:161–165
98. Yao YQ, Barlow DH, Sargent IL (2005) Differential expression of alternatively spliced transcripts of HLA-G in human preimplantation embryos and inner cell masses. J Immunol 175:8379–8385
99. Jurisicova A, Casper RF, MacLusky NJ, Librach CL (1996) Embryonic human leukocyte antigen-G expression: possible implications for human preimplantation development. Fertil Steril 65:997–1002
100. Shaikly VR, Morrison IE, Taranissi M, Noble CV, Withey AD, Cherry RJ, Blois SM, Fernandez N (2008) Analysis of HLA-G in maternal plasma, follicular fluid, and preimplantation embryos reveal an asymmetric pattern of expression. J Immunol 180:4330–4337
101. Verloes A, de Velde HV, Mateizel I, Cauffman G, De Waele M, Devroey P, LeMaoult J, Vercammen M (2009) HLA-G: expression in human embryonic stem cells. Tissue Antigens 74:83
102. Hviid TV, Moller C, Sorensen S, Morling N (1998) Co-dominant expression of the HLA-G gene and various forms of alternatively spliced HLA-G mRNA in human first trimester trophoblast. Hum Immunol 59:87–98
103. Ober C et al (1998) HLA-G1 protein expression is not essential for fetal survival. Placenta 19:127–132
104. Vercammen MJ, Verloes A, Van de Velde H, Haentjens P (2008) Accuracy of soluble human leukocyte antigen-G for predicting pregnancy among women undergoing infertility treatment: meta-analysis. Hum Reprod Update 14:209–218
105. Criscuoli L et al (2005) Lack of histocompatibility leukocyte antigen-G expression in early embryos is not related to germinal defects or impairment of interleukin-10 production by embryos. Gynecol Endocrinol 20:264–269
106. Desai N, Filipovits J, Goldfarb J (2006) Secretion of soluble HLA-G by day 3 human embryos associated with higher pregnancy and implantation rates: assay of culture media using a new ELISA kit. Reprod Biomed Online 13:272–277
107. Fisch JD, Keskintepe L, Ginsburg M, Adamowicz M, Sher G (2007) Graduated Embryo Score and soluble human leukocyte antigen-G expression improve assisted reproductive technology outcomes and suggest a basis for elective single-embryo transfer. Fertil Steril 87:757–763

108. Fuzzi B et al (2002) HLA-G expression in early embryos is a fundamental prerequisite for the obtainment of pregnancy. Eur J Immunol 32:311–315
109. Noci I et al (2005) Embryonic soluble HLA-G as a marker of developmental potential in embryos. Hum Reprod 20:138–146
110. Rebmann V, Switala M, Eue I, Grosse-Wilde H (2010) Soluble HLA-G is an independent factor for the prediction of pregnancy outcome after ART: a German multi-centre study. Hum Reprod 25(7):1691–1698
111. Rebmann V, Switala M, Eue I, Schwahn E, Merzenich M, Grosse-Wilde H (2007) Rapid evaluation of soluble HLA-G levels in supernatants of in vitro fertilized embryos. Hum Immunol 68:251–258
112. Rizzo R et al (2007) Soluble HLA-G molecules in follicular fluid: a tool for oocyte selection in IVF? J Reprod Immunol 74:133–142
113. Sageshima N et al (2007) Soluble HLA-G is absent from human embryo cultures: a reassessment of sHLA-G detection methods. J Reprod Immunol 75:11–22
114. Sher G, Keskintepe L, Batzofin J, Fisch J, Acacio B, Ahlering P, Ginsburg M (2005) Influence of early ICSI-derived embryo sHLA-G expression on pregnancy and implantation rates: a prospective study. Hum Reprod 20:1359–1363
115. Sher G, Keskintepe L, Fisch JD, Acacio BA, Ahlering P, Batzofin J, Ginsburg M (2005) Soluble human leukocyte antigen G expression in phase I culture media at 46 hours after fertilization predicts pregnancy and implantation from day 3 embryo transfer. Fertil Steril 83:1410–1413
116. Sher G, Keskintepe L, Nouriani M, Roussev R, Batzofin J (2004) Expression of sHLA-G in supernatants of individually cultured 46-h embryos: a potentially valuable indicator of 'embryo competency' and IVF outcome. Reprod Biomed Online 9:74–78
117. Tabiasco J et al (2009) Soluble HLA-G in IVF/ICSI embryo culture supernatants does not always predict implantation success: a multicentre study. Reprod Biomed Online 18:374–381
118. Yie SM, Balakier H, Motamedi G, Librach CL (2005) Secretion of human leukocyte antigen-G by human embryos is associated with a higher in vitro fertilization pregnancy rate. Fertil Steril 83:30–36
119. Vercammen M, Verloes A, Haentjens P, Van de Velde H (2009) Can soluble human leucocyte antigen-G predict successful pregnancy in assisted reproductive technology? Curr Opin Obstet Gynecol 21:285–290
120. Menezo Y, Elder K, Viville S (2006) Soluble HLA-G release by the human embryo: an interesting artefact? Reprod Biomed Online 13:763–764
121. Rizzo R et al (2009) Production of sHLA-G molecules by in vitro matured cumulus-oocyte complex. Int J Mol Med 24:523–530
122. della Ragione T, Verheyen G, Papanikolaou EG, Van Landuyt L, Devroey P, Van Steirteghem A (2007) Developmental stage on day-5 and fragmentation rate on day-3 can influence the implantation potential of top-quality blastocysts in IVF cycles with single embryo transfer. Reprod Biol Endocrinol 5:2
123. Assou S et al (2008) A non-invasive test for assessing embryo potential by gene expression profiles of human cumulus cells: a proof of concept study. Mol Hum Reprod 14:711–719
124. McKenzie LJ, Pangas SA, Carson SA, Kovanci E, Cisneros P, Buster JE, Amato P, Matzuk MM (2004) Human cumulus granulosa cell gene expression: a predictor of fertilization and embryo selection in women undergoing IVF. Hum Reprod 19:2869–2874
125. Botros L, Sakkas D, Seli E (2008) Metabolomics and its application for non-invasive embryo assessment in IVF. Mol Hum Reprod 14:679–690
126. Leese HJ, Baumann CG, Brison DR, McEvoy TG, Sturmey RG (2008) Metabolism of the viable mammalian embryo: quietness revisited. Mol Hum Reprod 14:667–672
127. Singh AP, Castranio T, Scott G, Guo D, Harris MA, Ray M, Harris SE, Mishina Y (2008) Influences of reduced expression of maternal bone morphogenetic protein 2 on mouse embryonic development. Sex Dev 2:134–141
128. Selmani Z, Naji A, Gaiffe E, Obert L, Tiberghien P, Rouas-Freiss N, Carosella ED, Deschaseaux F (2009) HLA-G is a crucial immunosuppressive molecule secreted by adult human mesenchymal stem cells. Transplantation 87:S62–S66
129. Selmani Z et al (2008) Human leukocyte antigen-G5 secretion by human mesenchymal stem cells is required to suppress T lymphocyte and natural killer function and to induce CD4+ CD25highFOXP3+ regulatory T cells. Stem Cells 26:212–222
130. Drukker M (2006) Immunogenicity of embryonic stem cells and their progeny. Methods Enzymol 420:391–409
131. Itskovitz-Eldor J, Schuldiner M, Karsenti D, Eden A, Yanuka O, Amit M, Soreq H, Benvenisty N (2000) Differentiation of human embryonic stem cells into embryoid bodies compromising the three embryonic germ layers. Mol Med 6:88–95
132. Thomson JA, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS, Jones JM (1998) Embryonic stem cell lines derived from human blastocysts. Science 282:1145–1147
133. Shih CC, Forman SJ, Chu P, Slovak M (2007) Human embryonic stem cells are prone to generate primitive, undifferentiated tumors in engrafted human fetal tissues in severe combined immunodeficient mice. Stem Cells Dev 16:893–902
134. Gertow K et al (2004) Organized development from human embryonic stem cells after injection into immunodeficient mice. Stem Cells Dev 13:421–435
135. Heins N et al (2004) Derivation, characterization, and differentiation of human embryonic stem cells. Stem Cells 22:367–376
136. Drukker M et al (2002) Characterization of the expression of MHC proteins in human embryonic stem cells. Proc Natl Acad Sci USA 99:9864–9869
137. Grinnemo KH et al (2006) Human embryonic stem cells are immunogenic in allogeneic and xenogeneic settings. Reprod Biomed Online 13:712–724
138. Li L et al (2004) Human embryonic stem cells possess immune-privileged properties. Stem Cells 22:448–456
139. Das P, Ezashi T, Schulz LC, Westfall SD, Livingston KA, Roberts RM (2007) Effects of fgf2 and oxygen in the bmp4-driven differentiation of trophoblast from human embryonic stem cells. Stem Cell Res 1:61–74
140. Yen BL, Chang CJ, Liu KJ, Chen YC, Hu HI, Bai CH, Yen ML (2009) Brief report—human embryonic stem cell-derived mesenchymal progenitors possess strong immunosuppressive effects toward natural killer cells as well as T lymphocytes. Stem Cells 27:451–456
141. Schulz LC, Ezashi T, Das P, Westfall SD, Livingston KA, Roberts RM (2008) Human embryonic stem cells as models for trophoblast differentiation. Placenta 29(Suppl A):S10–S16