Cell. Mol. Life Sci. (2011) 68:397–404
DOI 10.1007/s00018-010-0581-6

Cellular and Molecular Life Sciences

MULTI-AUTHOR REVIEW

HLA-G in organ transplantation: towards clinical applications

Frederic Deschaseaux · Diego Delgado · Vito Pistoia · Massimo Giuliani · Fabio Morandi · Antoine Durrbach

Received: 21 October 2010 / Accepted: 22 October 2010 / Published online: 20 November 2010
© Springer Basel AG 2010

Abstract HLA-G plays a particular role during pregnancy in which its expression at the feto–maternal barrier participates into the tolerance of the allogenic foetus. HLA-G has also been demonstrated to be expressed in some transplanted patients, suggesting that it regulates the allogenic response. In vitro data indicate that HLA-G modulates NK cells, T cells, and DC maturation through its interactions with various inhibitory receptors. In this paper, we will review the data reporting the HLA-G involvement of HLA-G in human organ transplantation, then factors that can modulate HLA-G, and finally the use of HLA-G as a therapeutic tool in organ transplantation.

Keywords HLA-G · Mesenchymal stem cells · Immunoregulation

F. Deschaseaux
Etablissement Français du Sang, EA3855 Tours, France

D. Delgado
Heart Transplant Program, Peter Munk Cardiac Centre, Toronto General Hospital, University Health Network, Toronto, ON, Canada

V. Pistoia · F. Morandi
Department of Neurosciences, Ophthalmology and Genetics, University of Genoa, Genoa, Italy

M. Giuliani · A. Durrbach (✉)
INSERM U1014, Département de Néphrologie, Hôpital du Kremlin-Bicêtre, IFRNT, Université Paris XI, 78 rue du Général Leclerc, 94270 Le Kremlin-Bicêtre, France
e-mail: antoine.durrbach@vjf.inserm.fr

Introduction

HLA-G has a restricted expression in physiological conditions but can be upregulated in various situations suggesting different levels of regulation. During pregnancy, its expression at the feto–maternal barrier suggests a particular regulation by progesterone as demonstrated in vitro [1–4]. HLA-G is also expressed by amnion epithelial cells [5, 6],and erythroid and endothelial cells of fetal blood vessels in the placenta [7, 8], as well as in thymus [9], cornea [10], pancreas [11] and nail matrix [12]. HLA-G belongs to the non-classical class-I molecule family and is composed by a three alpha domain which bind to b2-microglobulin. An alternative splicing of the primary transcript generates the membrane-bound isoforms HLA-G1 (complete molecule), HLA-G2 (minus α2 domain), HLA-G3 (minus α2 α3 domains), HLA-G4 (minus α3 domain) and the soluble isoforms HLA-G5 (soluble HLA-G1), HLA-G6 (soluble HLA-G2) and HLA-G7 (soluble HLA-G3) (17–39 kDa) [13, 14]. HLA-G molecule has been shown to inhibit NK cells, T cells, and DC maturation in vitro. It exerts its inhibitory functions through interactions with KIR2DL4 (CD158d) expressed on NK cells [15], ILT-2 (LILRB1/CD85j) expressed on all immune cell subsets and ILT-4 (LILRB2/CD85d) expressed on myeloid lineages [16–18]. The HLA-G inhibitory effect can occur by cell-to-cell contact, cell-to-cell-dependent uptake of HLA-G (trogocytosis) [19, 20], or through the release of soluble forms or the shedding of membrane bound HLA-G.

Some variations in the level of expression of its transcript and the identification of polymorphisms have led to the identification of some differences among patients associated with HLA-G expression. In addition to its role in the feto–maternal graft tolerance [21], several reports have

also indicated that HLA-G can also be involved in the acceptance of allogenic organ transplantation. In this paper, we will first describe the data reporting the involvement of HLA-G in human organ transplantation, then factors that can modulate HLA-G, and finally the use of HLA-G as a therapeutic tool in organ transplantation [22].

Involvement of HLA-G in transplantation

Because of its immunosuppressive properties, HLA-G has been studied in the context of transplantation [23]. In two retrospective single center studies, membrane-bound and soluble HLA-G expression was assessed in heart transplant patients. HLA-G was detected in myocardial biopsies and serum samples of approximately 20% of the heart transplant population [24, 25]. This group of patients, compared to the HLA-G-negative group, had significantly fewer episodes of acute rejection. It was found that only 11% of patients with recurrent moderate to severe rejection (rejecting group) showed HLA-G expression in biopsy specimens. However, 86% of patients who never experienced acute cellular rejection (non-rejecting group) were HLA-G-positive. Additionally, whereas 15 cases of graft vasculopathy were detected in HLA-G negative patients, none of the nine HLA-G-positive patients experienced graft vasculopathy. These results suggest that HLA-G expression may diminish the host immune response thereby reducing acute rejection and coronary disease post-transplantation, and that the presence of HLA-G is durable after transplantation.

In addition, a recent study, conducted on cultured human aortic and coronary artery endothelial cells to assess the expression of HLA-G [26], shows that different stressors, including hypoxia/reoxygenation, interferon-γ, tumor necrosis factor-α, interleukin-10, immunosuppressive agents or progressive doses of progesterone, could modulate HLA-G expression. HLA-G levels in culture supernatants were detected and quantified. Interestingly, only progesterone induced HLA-G expression in a dose-dependent manner. This finding was reproduced in coronary artery smooth muscle cells. This important finding confirms that myocardial cells and also endothelial cells are capable of expressing HLA-G.

Similar results have been observed in other solid organ transplants. Creput et al. [27, 28] have shown that 14 out of 41 liver and 5 out of 9 kidney transplant biopsies expressed HLA-G. Liver biliary and renal tubular epithelial cells, which are often susceptible target cells of acute rejection, may express HLA-G for the first time when transplanted. Intriguingly, it was found that, when a liver allograft is simultaneously transplanted with a kidney, the former protects the latter from acute rejection, as 32.5% of kidney recipients compared to only 6% of combined kidney and liver recipients developed acute kidney graft rejection. There is a strong inverse correlation between HLA-G expression in the transplanted liver and hepatic graft rejection; specifically, HLA-G expression was shown to significantly decrease graft rejection. More importantly, there were no cases of acute or chronic rejection of the kidney graft in patients who expressed HLA-G in their liver graft. In addition, HLA-G expression correlates with the presence in the blood of a population of $CD3^{+}CD4^{low}$ and $CD3^{+}CD8^{low}$ suppressor T cell subsets involved in transplant acceptance [29].

Similarly, in 64 lung transplant recipients, HLA-G expression was detected in bronchial epithelial cells from stable patients, but never in patients with acute rejection or with bronchiolitis obliterans syndrome, which is considered a form of chronic rejection [30].

Soluble HLA-G levels have also been shown to correlate with better graft function following transplantation. In heart transplant patients, acute rejection is significantly less frequent in patients with increased HLA-G plasma levels [24]. In renal transplant patients, there is an inverse relationship between soluble HLA-G levels and both organ failure from chronic rejection and the production of anti-HLA antibodies, indicating inhibition of the humoral response [31–35]. Similarly, HLA-G was associated with a lower incidence of C4d deposit in heart transplant suggesting a reduced risk for patients to develop an humoral rejection [36].

Factors influencing the expression of HLA-G

Genetic polymorphisms

Polymorphisms in the 3UT region have been extensively studied. The presence of 14 bp of 'exon 8' correlates with low levels of mRNA expression [37, 38] and is associated with the out-splicing of the first 92 bp of exon 8 [39]. These transcripts were shown to be more stable than the complete RNA [40]. In human heart transplantation, the 14-bp polymorphism of the HLA-G gene influenced the expression of soluble HLA-G and is associated with low rejection rates [41]. These novel findings indicate the potential use of HLA-G polymorphisms as a genetic indicator to guide immune therapy in heart transplant recipients. Seven SNPs have also been identified in this region of the gene. The +3142 C/G SNP which might be associated with some micro-RNA participates in the regulation of HLA-G expression. An extensive analysis of the promoter has also led to the identification of at least 29 SNPs [42, 43] which can be involved in the regulatory expression of HLA-G and which might explain some variabilities in HLA-G expression in human.

Cytokines, growth factors and hormones

HLA-G has also been demonstrated to be produced in physiopathological conditions during organ transplantation, inflammation, viral infections and by tumor cells [22]. These pathological expressions suggested that several levels of regulation, not only related to the genetic control but also associated with micro-environmental factors, can take place. Several cytokines (both anti-inflammatory (IL-10, IL-4, IL-5, IL-6) and pro-inflammatory [tumor necrosis factor (TNF)α, IL-1β, IL-2], and transforming growth factors (TGF-β), or growth factors such as granulocyte macrophage colony-stimulating factor (GM-CSF), granulocyte colony-stimulating factor (G-CSF), colony-stimulating factor (CSF-1), LIF and epidermal growth factor (EGF) have been found to upregulate HLA-G. In the context of allogenic transplantation, IFNs, IL10, and TGFβ have been associated with the expression of HLA-G [44, 45]. Despite a non-conserved HLA class I ISRE in the proximal promoter of HLA-G, several investigations have revealed that the HLA-G gene is responsive to up-regulation following treatment with IFN-α, IFN-β and IFN-γ [44, 46–48] in several cell types such as trophoblast cell lines, blood cells (monocytes and macrophage cell lines) and glioblastoma cell lines. The mechanisms of action of IL10 and TGFβ are not fully understood but both play an important role since they increase HLA-G and could participate in the regulation of the allogenic response [49].

IL-10 was demonstrated to increase both HLA-G mRNA and cell surface protein expression by monocytes [45], mesenchymal stem cells [50], renal cell carcinoma (RCC) cell lines [51] and human decidua stroma cells [52], and also soluble HLA-G protein by mononuclear cells from patients suffering from non-Hodgkin lymphomas (T-NHL) [53], acute myeloblastic leukaemia (AML) and acute lymphoblastic leukaemia (B-ALL) [52]. Moreover, a reduced placental IL-10 production was observed in pre-eclampsia [54] and correlates with a low level of expression of HLA-G transcription [38, 55]. On the other hand, the IL-10 homolog produced by cytomegalovirus (CMV) can up-regulate HLA-G protein expression at the monocyte cell surface and is supposed to participate in the mechanisms of escape of CMV to the immune system [56]. Whatever the mechanisms action of HLA-G, the effect of both IL-10 and IFN treatment requires basal transcriptional activity to enhance HLA-G gene expression [57].

Hypoxia/ischemia

Hypoxia is a common feature in organ transplantation during organ procurement, starvation and re-implantation. Hypoxia is also present in physiological conditions in which HLA-G is produced. For example, hypoxia followed by normoxia regulates the trophoblastic invasion and the vascular remodeling of the uterine tissues [58]. In low oxygen-containing conditions, cells express the hypoxia-inducible factor (HIF), which activates transcription of many genes controlling glycolysis, glucose transport, cell survival and death, cell adhesion, angiogenesis and erythropoiesis [59, 60]. Hypoxia, induced by iron chelator desferrioxamine, is associated with a 16-fold HLA-G gene transcription in melanoma cell lines [61]. The computer search analysis has identified a putative consensus of hypoxia response element located in HLA-G promoter. Whether this HRE is functional is still unknown [61]. As for the effect of hypoxia on HLA-G cell surface expression, results differ depending on the cell type or culture conditions [62]. Its role during ischemia reperfusion in different transplanted organs has to be defined.

Impact of immunosuppressive molecules on HLA-G expression

Excepted for steroids and Belatacept, the role of the different molecules used remains unknown. HLA-G is regulated by progesterone and to a lesser extent by steroids that slightly increase transcription of HLA-G in trophoblast explants [63, 64]. It remains to be demonstrated whether in vivo steroids have an effect on the expression of HLA-G. The low level of HLA-G in kidney recipients who were frequently receiving steroids would suggest that low doses of steroid will have a low impact on HLA-G expression. Conversely, it appears recently that patients treated with Belatacept had a high concentration of soluble HLA-G. In vitro analysis has shown that dendritic cells in a context of an allogenic reaction are able to synthesize and secrete HLA-G in the presence of Belatacept [65, 66]. The secretion is maintained in the presence of Belatacept indicating that HLA-G participates in the allogenic graft acceptance.

In heart transplant patients receiving two different antiproliferative agents—everolimus (RAD) and mycophenolate mofetil (MMF) [67]—in association with cyclosporine (CsA), high levels of soluble HLA-G were measured among patients receiving RAD with 78% expressing high levels of plasma HLA-G) compared to 25% for patients receiving MMF. CsA did not appear to influence HLA-G expression, as there was no correlation between HLA-G levels and blood CsA area under the time-concentration curve ($r^2 = 0.19$, $p = 0.25$). This study suggests that RAD but not MMF is associated with HLA-G expression in heart transplant patients. Nonetheless, soluble HLA-G expression appears to have a constant pattern of expression independent of the type of antiproliferative agent used.

Mechanism of action of HLA-G on the allogenic response

Both HLA-G membrane bound form (namely HLA-G1) and soluble HLA-G (namely HLA-G5) can impair the allogenic response [68, 69]. Despite them displaying a classI structure, HLA-G does not inhibit immune responses through TCR interactions but rather through its interaction with inhibitory receptors expressed by lymphocytes and antigen-presenting cells [70, 71]. To date, three HLA-G receptors have been identified: the killer immunoglobulin-like receptor KIR2DL4/CD158d [15], and the leukocyte immunoglobulin (Ig)-like receptors, LILRB1/ILT-2/CD85j and LILRB2/ILT-4/CD85d [72]. KIR2DL4 expression is restricted to NK cells, and ILT-4 is myeloid lineage-specific, while ILT-2 is expressed by monocytes, dendritic cells, T cells, B cells and NK cells. With such a large distribution of HLA-G-receptors, various immune functions may be altered by HLA-G. On dendritic cells (DC), HLA-G has been demonstrated to impair DC maturation [73]. In T cells, HLA-G has been demonstrated to impair cytotoxic activities of NK and CD8 cells [74, 75], and to induce apoptosis of a subset of stimulated CD8 cells through the activation of Fas pathway [76–78], whereas we have demonstrated that HLA-G is associated with the proliferation and expansion of T lymphocytes via their inhibition of their cell cycle [79]. Interestingly, we have also demonstrated that this inhibition is associated with the acquisition of suppressive functions of T cells, which are independent of the expression of Fox-P3 transcription factor but are associated with low levels of expression in CD4 and CD8 co-receptors at the cell surface of T cells [80]. This phenotype has been observed ($CD3^+CD4^{low}$ and $CD3^+CD8^{low}$ T cells) in vitro and in vivo in transplanted patients. The down-modulation of CD4 and CD8 surface expression on HLA-G-driven T cells results from both transcriptional and post-translational regulation, and their lowering is supposed to alter the synapse formation [29].

In addition, these $CD3^+CD4^{low}$ and $CD3^+$ $CD8^{low}$ T cells show an increased representation of $CD45RA^+$ cells and exhibit a TH2 phenotype secreting high amounts of IL-4, IL-10 and IL-13, and having a low production of IFN-γ [81]. Secreted cytokines have the potential functions to increase HLA-G production and to participate in the generation of suppressor T cells [82].

HLA-G as a potential tool for transplantation

Because of its direct or indirect (via the expansion of regulatory T cells) immunoregulatory properties, HLA-G has been proposed as a therapeutic biotherapy for allogenic transplantation. Two particular approaches have been investigated:

- The use of a recombinant molecule. The soluble fraction of HLA-G has been demonstrated to impair allogenic response [70]. Its potential as a biotherapy has been evaluated by the group of Horuzsko who have injected multimeric recombinant HLA-G molecules aggregated with microbeads into mice having skin transplantation [83]. The single injection of HLA-G was associated with a delayed occurrence of acute rejection of the skin indicating that HLA-G is a potential tool to prevent acute rejection.
- The use of amniotic HLA-G secreting cells. Recent developments have involved HLA-G producing cells to participate in tissue repair and/or immunomodulation. Cardiomyocytes derived from amniotic cells have been expended in vitro and then infused in rat having myocardiac infarction [84]. Those cells improved cardiac functions and reduced myocardiac fibrotic scare. Interestingly, these cells that still expressed HLA-G and injected in an xenogenic context (human to rat) did not require any immunosuppression to be active and favor the local expansion of regulatory T cells (Fox P3 positive).

Mesenchymal stem cells secreting HLA-G

Bone marrow mesenchymal stem cells (also referred to as multipotent stromal cells; MSCs) have also been demonstrated to produce HLA-G. MSCs are non-hematopoietic bone marrow adult stem cells [85] with a phenotype characterized by negative staining for hematopoietic markers (CD45, CD14) and positive for other markers such as CD73, CD90, CD105, CD146 and CD166 [86]. MSCs can be induced in vitro and in vivo to differentiate into various mesenchymal tissues such as bone, cartilage, muscle, tendon, adipose tissue, and hematopoiesis-supporting stroma [87, 88]. This was demonstrated by several clinical investigations in patients treated for hematological diseases by co-transplantation of hematopoietic stem cells and MSCs [89] and for the treatment of osteogenesis imperfecta in children in which allogenic MSCs have been injected in utero [90] or in children. Five of six patients showed engraftment in bone and bone marrow stroma and accelerated growth velocity during the first 6 months post-infusion as compared with the 6 months preceding the infusions [91]. Although MSCs are hypoimmunogeneic cells since they do not express HLA-DR and co-stimulatory molecules, e.g., CD80, CD83, they exert a wide spectrum of immunomodulatory activities that target cells of both the innate and adaptive immunity and include inhibition of lymphocyte proliferation to alloantigens [92]. The low

immunogenicity of MSCs are in part due to their low expression of HLA class I-peptide complexes on the cell surface which is associated with a weak intracellular expression of some the antigen processing machinery (APM) components such the chaperone ERp57 and immunoprotesosomal components LMP7 and LMP10, which remained at a low level even in the presence of IFNγ [93]. Their immunoregulatory capacities [92] are due to the synthesis of indoleamine 2,3 dioxygenase (IDO), IL10, TGFβ, prostaglandin E2 and HLA-G, suggesting that they can be used for inducing tolerance in allografting [94]. Notably, clinical studies have been conducted to measure the capacity of MSCs to inhibit graft-versus-host disease (GvHD) after allogenic hematopoietic stem cell transplantation. In their pioneering study, Le Blanc et al. [95] injected allogenic MSCs into a patient with refractory, acute GvHD. Two productions of MSCs were infused to finally generate complete suppression of GvHD. To date, several clinical investigations have been conducted, and data have confirmed the immunosuppression potential of MSCs [89]. Among mechanisms involved in such immunomodulations, the cell–cell contacts seem to be crucial since they exhibit the strongest inhibition effects when compared to the non-contact conditions. We have shown that human MSCs mainly secreted HLA-G1 and -G5 isoforms and that those molecules contributed to inhibition of T cell proliferation in allogenic conditions and to suppression of NK cell lysis toward target cells through KIR2DL4 [50]. In association with the APM modification in MSCs, HLA-G and also the ability of MSCs to express PI-9, a physiological antagonist of Granzyme-B, would participate in the natural resistance of these cells to killing activities of NK cells [93]. In addition, we have shown that contact between MSCs and allogenic T cells increased HLA-G5 in supernatants, generating full immunosuppression in association with IL10 acting together as an amplification feedback loop to expand functional $CD4^{+}CD25^{high}$ FoxP3+regulatory T cells [50]. Therefore, after their transplantation in allogenic conditions, MSCs, through HLA-G, could inhibit activated immune cells but could also induce tolerance by increasing specific regulatory T cells.

In addition to bone marrow, MSCs can be isolated from other types of tissues such as placenta, cord blood, adipose tissue and fetal tissue [96, 97]. Recently, data have shon that MSCs deriving either from adipose tissue or from deciduas or from cord blood were able to express HLA-G molecules [98]. A study using cord blood-derived MSCs also showed that cells were able to secrete HLA-G even if MSCs were expanded in vitro within medium containing 5% platelet lysate used as a substitute for calf serum [99]. These culture conditions are crucial for the production of MSCs at the clinical level, and these cells consistently demonstrated immunosuppression capacities on T and NK cells. Moreover, cord blood-derived MSCs expressed mainly soluble HLA-G suggesting that such an isoform is preferentially expressed by MSCs whatever the tissue origin. However, we observed that long-term culture of MSCs could decrease the expression of HLA-G [50]. Such observations have been reported with other types of cells. Indeed, most cell lines, known to secrete HLA-G, lost their expression with time which indicates that microenvironmental factors are likely to play a role in supporting HLA-G expression [100]. This point is crucial and could therefore interest cell therapy centers capable of producing MSCs for their clinical use. These are in accordance with in vivo data in rat showing that MSCs are able to delay graft rejection but without inducing graft tolerance. The maintenance of immunosuppressive properties of MSCs and/or their survival has to be determined, as well the associated role of current immunosuppressive drugs to maintained their immunosuppressive functions.

Conclusion

In allogenic transplantation, HLA-G has been associated with a lower risk for the development of acute and chronic rejection. HLA-G has an important role in inhibiting the activation of allogenic T cells and also in impairing effector cells such as CD8 T cells and NK cells. In contrast, it favors the development of regulatory T Cells. HLA-G appears as an interesting marker of the good evolution of allogenic transplantation. It also constitutes an interesting tool to regulate the allogenic response. Future development will evaluate its ability to prevent allogenic activation alone as a recombinant molecule or in association with immunosuppressive drugs. In addition, promising strategies will be to use stem cells that produce HLA-G and or regulatory molecules for the prevention of acute and chronic rejection and to dissect efficient association toward this goal.

References

1. 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
2. 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
3. Kovats S, Main EK, Librach C, Stubblebine M, Fisher SJ, DeMars R (1990) A class I antigen, HLA-G, expressed in human trophoblasts. Science 248:220–223
4. Yie SM, Xiao R, Librach CL (2006) Progesterone regulates HLA-G gene expression through a novel progesterone response element. Hum Reprod 21:2538–2544

5. Houlihan JM, Biro PA, Harper HM, Jenkinson HJ, Holmes CH (1995) The human amnion is a site of MHC class Ib expression: evidence for the expression of HLA-E and HLA-G. J Immunol 154:5665–5674
6. Hammer A, Hutter H, Blaschitz A, Mahnert W, Hartmann M, Uchanska-Ziegler B, Ziegler A, Dohr G (1997) Amnion epithelial cells, in contrast to trophoblast cells, express all classical HLA class I molecules together with HLA-G. Am J Reprod Immunol 37:161–171
7. 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
8. Blaschitz A, Lenfant F, Mallet V, Hartmann M, Bensussan A, Geraghty DE, Le Bouteiller P, Dohr G (1997) Endothelial cells in chorionic fetal vessels of first trimester placenta express HLA-G. Eur J Immunol 27:3380–3388
9. Crisa L, McMaster MT, Ishii JK, Fisher SJ, Salomon DR (1997) Identification of a thymic epithelial cell subset sharing expression of the class Ib HLA-G molecule with fetal trophoblasts. J Exp Med 186:289–298
10. 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
11. Cirulli V et al (2006) The class I HLA repertoire of pancreatic islets comprises the nonclassical class Ib antigen HLA-G. Diabetes 55:1214–1222
12. Ito T et al (2005) Immunology of the human nail apparatus: the nail matrix is a site of relative immune privilege. J Invest Dermatol 125:1139–1148
13. 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
14. Kirszenbaum M, Moreau P, Gluckman E, Dausset J, Carosella E (1994) An alternatively spliced form of HLA-G mRNA in human trophoblasts and evidence for the presence of HLA-G transcript in adult lymphocytes. Proc Natl Acad Sci USA 91:4209–4213
15. Rajagopalan S, Long EO (1999) A human histocompatibility leukocyte antigen (HLA)-G-specific receptor expressed on all natural killer cells. J Exp Med 189:1093–1100
16. Colonna M, Navarro F, Bellon T, Llano M, Samaridis J, Angman L, Cella M, Lopez-Botet M (1997) A common inhibitory receptor for major histocompatibility complex class I molecules on human lymphoid and myelomonocytic cells. J Exp Med 186:1809–1818
17. Colonna M et al (1998) Human myelomonocytic cells express an inhibitory receptor for classical and nonclassical MHC class I molecules. J Immunol 160:3096–3100
18. Shiroishi M et al (2003) Human inhibitory receptors Ig-like transcript 2 (ILT2) and ILT4 compete with CD8 for MHC class I binding and bind preferentially to HLA-G. Proc Natl Acad Sci USA 100:8856–8861
19. LeMaoult J, Caumartin J, Daouya M, Favier B, Le Rond S, Gonzalez A, Carosella ED (2007) Immune regulation by pretenders: cell-to-cell transfers of HLA-G make effector T cells act as regulatory cells. Blood 109:2040–2048
20. Caumartin J, Favier B, Daouya M, Guillard C, Moreau P, Carosella ED, LeMaoult J (2007) Trogocytosis-based generation of suppressive NK cells. EMBO J 26:1423–1433
21. 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
22. Carosella ED, Moreau P, Lemaoult J, Rouas-Freiss N (2008) HLA-G: from biology to clinical benefits. Trends Immunol 29:125–132
23. Rouas-Freiss N, Naji A, Durrbach A, Carosella ED (2007) Tolerogenic functions of human leukocyte antigen G: from pregnancy to organ and cell transplantation. Transplantation 84:S21–S25
24. Lila N et al (2002) Human leukocyte antigen-G expression after heart transplantation is associated with a reduced incidence of rejection. Circulation 105:1949–1954
25. Lila N, Carpentier A, Amrein C, Khalil-Daher I, Dausset J, Carosella ED (2000) Implication of HLA-G molecule in heart-graft acceptance. Lancet 355:2138
26. Sheshgiri R, Rao V, Tumiati LC, Xiao R, Prodger JL, Badiwala M, Librach C, Delgado DH (2008) Progesterone induces human leukocyte antigen-g expression in vascular endothelial and smooth muscle cells. Circulation 118:S58–S64
27. Créput C et al (2003) HLA-G expression in biliary epithelial cells is associated with allograft acceptance in liver-kidney transplantation. J Hepatol 39:587–594
28. Creput C, Le Friec G, Bahri R, Amiot L, Charpentier B, Carosella E, Rouas-Freiss N, Durrbach A (2003) Detection of HLA-G in serum and graft biopsy associated with fewer acute rejections following combined liver-kidney transplantation: possible implications for monitoring patients. Hum Immunol 64:1033–1038
29. Naji A et al (2007) $CD3^{+}CD4^{low}$ and $CD3^{+}CD8^{low}$ are induced by HLA-G: novel human peripheral blood suppressor T cell subsets involved in transplant acceptance. Blood 110:3936–3948
30. Brugiere O et al (2009) Immunohistochemical study of HLA-G expression in lung transplant recipients. Am J Transplant 9:1427–1438
31. Lila N, Amrein C, Guillemain R, Chevalier P, Fabiani JN, Carpentier A (2007) Soluble human leukocyte antigen-G: a new strategy for monitoring acute and chronic rejections after heart transplantation. J Heart Lung Transplant 26:421–422
32. Luque J et al (2006) sHLA-G levels in the monitoring of immunosuppressive therapy and rejection following heart transplantation. Transpl Immunol 17:70–73
33. Luque J et al (2006) Soluble HLA-G in heart transplantation: their relationship to rejection episodes and immunosuppressive therapy. Hum Immunol 67:257–263
34. Qiu J, Terasaki PI, Miller J, Mizutani K, Cai J, Carosella ED (2006) Soluble HLA-G expression and renal graft acceptance. Am J Transplant 6:2152–2156
35. Rebmann V, Bartsch D, Wunsch A, Mollenbeck P, Golda T, Viebahn R, Grosse-Wilde H (2009) Soluble total human leukocyte antigen class I and human leukocyte antigen-G molecules in kidney and kidney/pancreas transplantation. Hum Immunol 70:995–999
36. Sheshgiri R, Rao V, Mociornita A, Ross HJ, Delgado DH (2010) Association between HLA-G expression and C4d staining in cardiac transplantation. Transplantation 89:480–481
37. 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
38. 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
39. 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

40. 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
41. Torres MI, Luque J, Lorite P, Isla-Tejera B, Palomeque T, Aumente MD, Arizon J, Pena J (2009) 14-Base pair polymorphism of human leukocyte antigen-G as genetic determinant in heart transplantation and cyclosporine therapy monitoring. Hum Immunol 70:830–835
42. Tan Z, Shon AM, Ober C (2005) Evidence of balancing selection at the HLA-G promoter region. Hum Mol Genet 14:3619–3628
43. Hviid TV, Rizzo R, Christiansen OB, Melchiorri L, Lindhard A, Baricordi OR (2004) HLA-G and IL-10 in serum in relation to HLA-G genotype and polymorphisms. Immunogenetics 56:135–141
44. Yang Y, Geraghty DE, Hunt JS (1995) Cytokine regulation of HLA-G expression in human trophoblast cell lines. J Reprod Immunol 29:179–195
45. 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
46. 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
47. Chu W, Gao J, Murphy WJ, Hunt JS (1999) A candidate interferon-gamma activated site (GAS element) in the HLA-G promoter does not bind nuclear proteins. Hum Immunol 60:1113–1118
48. Chu W, Yang Y, Geraghty DE, Hunt JS (1999) Interferons enhance HLA-G mRNA and protein in transfected mouse fibroblasts. J Reprod Immunol 42:1–15
49. Cadet P, Rady PL, Tyring SK, Yandell RB, Hughes TK (1995) Interleukin-10 messenger ribonucleic acid in human placenta: implications of a role for interleukin-10 in fetal allograft protection. Am J Obstet Gynecol 173:25–29
50. 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 $CD4^{+}CD25^{high}$ FOXP3+regulatory T cells. Stem Cells 26:212–222
51. Dunker K, Schlaf G, Bukur J, Altermann WW, Handke D, Seliger B (2008) Expression and regulation of non-classical HLA-G in renal cell carcinoma. Tissue Antigens 72:137–148
52. Blanco O, Tirado I, Munoz-Fernandez R, Abadia-Molina AC, Garcia-Pacheco JM, Pena J, Olivares EG (2008) Human decidual stromal cells express HLA-G: effects of cytokines and decidualization. Hum Reprod 23:144–152
53. Sebti Y et al (2003) Soluble HLA-G molecules are increased in lymphoproliferative disorders. Hum Immunol 64:1093–1101
54. Makris A, Xu B, Yu B, Thornton C, Hennessy A (2006) Placental deficiency of interleukin-10 (IL-10) in preeclampsia and its relationship to an IL10 promoter polymorphism. Placenta 27:445–451
55. Goldman-Wohl DS, Ariel I, Greenfield C, Hanoch J, Yagel S (2000) HLA-G expression in extravillous trophoblasts is an intrinsic property of cell differentiation: a lesson learned from ectopic pregnancies. Mol Hum Reprod 6:535–540
56. Spencer JV, Lockridge KM, Barry PA, Lin G, Tsang M, Penfold ME, Schall TJ (2002) Potent immunosuppressive activities of cytomegalovirus-encoded interleukin-10. J Virol 76:1285–1292
57. Moreau P, Flajollet S, Carosella ED (2009) Non-classical transcriptional regulation of HLA-G: an update. J Cell Mol Med 13:2973–2989
58. Genbacev O, Zhou Y, Ludlow JW, Fisher SJ (1997) Regulation of human placental development by oxygen tension. Science 277:1669–1672
59. Brahimi-Horn MC, Chiche J, Pouyssegur J (2007) Hypoxia and cancer. J Mol Med 85:1301–1307
60. Mazure NM et al (2004) HIF-1: master and commander of the hypoxic world. A pharmacological approach to its regulation by siRNAs. Biochem Pharmacol 68:971–980
61. Chang CC, Ferrone S (2003) HLA-G in melanoma: can the current controversies be solved? Semin Cancer Biol 13:361–369
62. Mouillot G, Marcou C, Zidi I, Guillard C, Sangrouber D, Carosella ED, Moreau P (2007) Hypoxia modulates HLA-G gene expression in tumor cells. Hum Immunol 68:277–285
63. Moreau P et al (2001) Glucocorticoid hormones upregulate levels of HLA-G transcripts in trophoblasts. Transplant Proc 33:2277–2280
64. Sheshgiri R et al (2008) Myocardial HLA-G reliably indicates a low risk of acute cellular rejection in heart transplant recipients. J Heart Lung Transplant 27:522–527
65. Chavez H, Beaudreuil S, Abbed K, Taoufic Y, Kriaa F, Charpentier B, Durrbach A (2007) Absence of CD4CD25 regulatory T cell expansion in renal transplanted patients treated in vivo with Belatacept mediated CD28-CD80/86 blockade. Transpl Immunol 17:243–248
66. Bahri R, Naji A, Menier C, Charpentier B, Carosella ED, Rouas-Freiss N, Durrbach A (2009) Dendritic cells secrete the immunosuppressive HLA-G molecule upon CTLA4-Ig treatment: implication in human renal transplant acceptance. J Immunol 183:7054–7062
67. Sheshgiri R, Gustafsson F, Sheedy J, Rao V, Ross HJ, Delgado DH (2009) Everolimus but not mycophenolate mofetil therapy is associated with soluble HLA-G expression in heart transplant patients. J Heart Lung Transplant 28:1193–1197
68. Naji A, Durrbach A, Carosella ED, Rouas-Freiss N (2007) Soluble HLA-G and HLA-G1 expressing antigen-presenting cells inhibit T-cell alloproliferation through ILT-2/ILT-4/FasL-mediated pathways. Hum Immunol 68:233–239
69. Riteau B, Menier C, Khalil-Daher I, Sedlik C, Dausset J, Rouas-Freiss N, Carosella ED (1999) HLA-G inhibits the allogeneic proliferative response. J Reprod Immunol 43:203–211
70. Lila N, Rouas-Freiss N, Dausset J, Carpentier A, Carosella ED (2001) Soluble HLA-G protein secreted by allo-specific CD4+T cells suppresses the allo-proliferative response: a CD4+T cell regulatory mechanism. Proc Natl Acad Sci USA 98:12150–12155
71. Riteau B, Rouas-Freiss N, Menier C, Paul P, Dausset J, Carosella ED (2001) HLA-G2, -G3, and -G4 isoforms expressed as nonmature cell surface glycoproteins inhibit NK and antigen-specific CTL cytolysis. J Immunol 166:5018–5026
72. Colonna M et al (1998) Human myelomonocytic cells express an inhibitory receptor for classical and nonclassical MHC class I molecules. J Immunol 160:3096–3100
73. Liang S, Baibakov B, Horuzsko A (2002) HLA-G inhibits the functions of murine dendritic cells via the PIR-B immune inhibitory receptor. Eur J Immunol 32:2418–2426
74. Le Gal FA et al (1999) HLA-G-mediated inhibition of antigen-specific cytotoxic T lymphocytes. Int Immunol 11:1351–1356
75. Riteau B, Menier C, Khalil-Daher I, Martinozzi S, Pla M, Dausset J, Carosella ED, Rouas-Freiss N (2001) HLA-G1 co-expression boosts the HLA class I-mediated NK lysis inhibition. Int Immunol 13:193–201
76. Contini P, Ghio M, Poggi A, Filaci G, Indiveri F, Ferrone S, Puppo F (2003) Soluble HLA-A,-B,-C and -G molecules induce apoptosis in T and NK CD8+cells and inhibit cytotoxic T cell activity through CD8 ligation. Eur J Immunol 33:125–134

77. 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
78. Lindemann M, Rebmann V, Ottinger HD, Schmolke K, Kreuzfelder E, Grosse-Wilde H (2004) rhG-CSF effect on mixed lymphocyte cultures and circulating soluble HLA antigen levels in volunteer stem cell donors. Exp Hematol 32:1103–1109
79. Bahri R et al (2006) Soluble HLA-G inhibits cell cycle progression in human alloreactive T lymphocytes. J Immunol 176: 1331–1339
80. 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
81. Suarez A, Mozo L, Gutierrez C (2002) Generation of CD4(+)CD45RA(+) effector T cells by stimulation in the presence of cyclic adenosine 5′-monophosphate-elevating agents. J Immunol 169:1159–1167
82. Wan YY, Flavell RA (2006) The roles for cytokines in the generation and maintenance of regulatory T cells. Immunol Rev 212:114–130
83. Horuzsko A, Lenfant F, Munn DH, Mellor AL (2001) Maturation of antigen-presenting cells is compromised in HLA-G transgenic mice. Int Immunol 13:385–394
84. Tsuji H et al (2010) Xenografted human amniotic membrane-derived mesenchymal stem cells are immunologically tolerated and transdifferentiated into cardiomyocytes. Circ Res 106:1613–1623
85. Lo Celso C et al (2009) Live-animal tracking of individual haematopoietic stem/progenitor cells in their niche. Nature 457:92–96
86. Pittenger MF et al (1999) Multilineage potential of adult human mesenchymal stem cells. Science 284:143–147
87. Deschaseaux F, Pontikoglou C, Sensebe L (2010) Bone regeneration: the stem/progenitor cells point of view. J Cell Mol Med 14:103–115
88. Deschaseaux F, Sensebe L, Heymann D (2009) Mechanisms of bone repair and regeneration. Trends Mol Med 15:417–429
89. Kode JA, Mukherjee S, Joglekar MV, Hardikar AA (2009) Mesenchymal stem cells: immunobiology and role in immunomodulation and tissue regeneration. Cytotherapy 11:377–391
90. Le Blanc K et al (2005) Fetal mesenchymal stem-cell engraftment in bone after in utero transplantation in a patient with severe osteogenesis imperfecta. Transplantation 79:1607–1614
91. Horwitz EM, Gordon PL, Koo WK, Marx JC, Neel MD, McNall RY, Muul L, Hofmann T (2002) Isolated allogeneic bone marrow-derived mesenchymal cells engraft and stimulate growth in children with osteogenesis imperfecta: Implications for cell therapy of bone. Proc Natl Acad Sci USA 99:8932–8937
92. Uccelli A, Moretta L, Pistoia V (2008) Mesenchymal stem cells in health and disease. Nat Rev Immunol 8:726–736
93. Morandi F et al (2008) Immunogenicity of human mesenchymal stem cells in HLA-class I-restricted T-cell responses against viral or tumor-associated antigens. Stem Cells 26:1275–1287
94. Le Blanc K, Ringden O (2005) Immunobiology of human mesenchymal stem cells and future use in hematopoietic stem cell transplantation. Biol Blood Marrow Transplant 11:321–334
95. Le Blanc K, Rasmusson I, Sundberg B, Gotherstrom C, Hassan M, Uzunel M, Ringden O (2004) Treatment of severe acute graft-versus-host disease with third party haploidentical mesenchymal stem cells. Lancet 363:1439–1441
96. Secco M et al (2008) Multipotent stem cells from umbilical cord: cord is richer than blood!. Stem Cells 26:146–150
97. Yen BL et al (2005) Isolation of multipotent cells from human term placenta. Stem Cells 23:3–9
98. Ivanova-Todorova E et al (2009) HLA-G expression is up-regulated by progesterone in mesenchymal stem cells. Am J Reprod Immunol 62:25–33
99. Avanzini MA et al (2009) Generation of mesenchymal stromal cells in the presence of platelet lysate: a phenotypic and functional comparison of umbilical cord blood- and bone marrow-derived progenitors. Haematologica 94:1649–1660
100. LeMaoult J, Le Discorde M, Rouas-Freiss N, Moreau P, Menier C, McCluskey J, Carosella ED (2003) Biology and functions of human leukocyte antigen-G in health and sickness. Tissue Antigens 62:273–284