Human Immunology 77 (2016) 711–719

Contents lists available at ScienceDirect

journal homepage: www.elsevier.com/locate/humimm

Mouse models for studies of HLA-G functions in basic science and pre-clinical research

Anh Thu Nguyen-Lefebvre [a], Ashwin Ajith [a], Vera Portik-Dobos [a], Daniel D. Horuzsko [a], Laura L. Mulloy [b], Anatolij Horuzsko [a,b,*]

[a] Molecular Oncology and Biomarkers Program, Georgia Regents University Cancer Center, 1140 Laney Walker Blvd, Augusta, GA 30912, USA
[b] Department of Medicine, Georgia Regents University, 1120 15th Street, Augusta, GA 30912, USA

ARTICLE INFO

Article history:
Received 20 November 2015
Revised 27 January 2016
Accepted 10 February 2016
Available online 13 April 2016

Keywords:
HLA-G
Transgenic mice
Humanized mice
Transplantation

ABSTRACT

HLA-G was described originally as a tolerogenic molecule that allows the semiallogeneic fetus to escape from recognition by the maternal immune response.

This review will discuss different steps in the study of *HLA-G* expression and functions *in vivo*, starting with analyses of expression of the *HLA-G* gene and its receptors in transgenic mice, and continuing with applications of HLA-G and its receptors in prevention of allograft rejection, transplantation tolerance, and controlling the development of infection. Humanized mouse models have been discussed for developing *in vivo* studies of HLA-G in physiological and pathological conditions. Collectively, animal models provide an opportunity to evaluate the importance of the interaction between HLA-G and its receptors in terms of its ability to regulate immune responses during maternal-fetal tolerance, survival of allografts, tumor-escape mechanisms, and development of infections when both HLA-G and its receptors are expressed. In addition, *in vivo* studies on HLA-G also offer novel approaches to achieve a reproducible transplantation tolerance and to develop personalized medicine to prevent allograft rejection.

1. Introduction

HLA-G, a non-classical HLA class I molecule, was identified for the first time in 1986 by the group of McMichael by studying choriocarcinoma cells [1]. The HLA-G molecule differs from classical HLA class I molecules by its genetic diversity, expression, structure and functions. The gene encoding HLA-G molecules displays a low level of polymorphism [2,3] and restrictively express in physiological conditions in cytotrophoblast cells [4], amniotic fluid [5], adult thymic medulla [6], adult cornea epithelial cells [7] and stem cells [8–10].

The gene encoding HLA-G molecules is composed of 8 exons separated by 7 introns. Alternative splicing of the HLA-G primary transcript generates 7 isoforms; 4 of them are membrane-bound proteins (HLA-G1, G2, G3 and G4) and 3 of them are soluble proteins (HLA-G5, G6 and G7) [11].

In some pathological conditions, expression of the *HLA-G* gene could be induced by non-rejected allograft [12,13], lesion-infiltrating antigen presenting cells (APCs) during inflammatory diseases [14,15], and tumor tissues and their tumor infiltrating APCs [16–18]. However, its tolerogenic function can be favorable or detrimental for the patient [19] when HLA-G proteins bind to its inhibitory and activating receptors, immunoglobulin-like transcript-2 (ILT2/C85j/LILRB1) and transcript-4 (ILT4/CD85d/LILRB2), killer cell immunoglobulin-like receptor (KIR2DL4/CD158d), CD160, CD4 and CD8. Lymphoid and myeloid cells express ILT2 receptor, whereas ILT4 is expressed by myeloid cells and KIR2DL4 by NK (natural killer) cells and some $CD8^+$ T cells [20–23]. It was reported that interactions between HLA-G proteins and its receptors could affect different immune responses

Abbreviations: APC, antigen presenting cell; DI, double transgenic mice expressing $h\beta_2m$ and *CD8*; GFP, green fluorescence protein; HCMV, human cytomegalovirus; iNOS2, induced nitric oxide synthase 2; NFAT, nuclear factor of activated T-cells; NF-κB, nuclear factor kappa B; NSG, non-obese diabetic, severe combined immunodeficiency, gamma; PD-1, programmed cell death 1; Ped, preimplantation embryonic development; (PIR)-B, paired immunoglobulin-like receptor B; TRI, triple transgenic mice expressing *HLA-G*, $h\beta_2m$ and *CD8*; ZAP-70, zeta-chain-associated protein kinase.

* Corresponding author at: Molecular Oncology and Biomarkers Program, Georgia Regents University Cancer Center, Department Medicine, Georgia Regents University, USA.
E-mail address: ahoruzsko@gru.edu (A. Horuzsko).

http://dx.doi.org/10.1016/j.humimm.2016.02.012
0198-8859/

including T cell proliferation, NK cell and $CD8^+$ T cell cytotoxicity and dendritic cell (DC) maturation [19–26]. In contrast, during pregnancy, its tolerogenic function at the maternal-fetal interface protects the fetus from destruction by its mother's immune system [4].

In order to better understand the functions of HLA-G and the mechanisms induced by the interaction of HLA-G complexes with its receptors *in vivo*, a mouse model was developed. Clinical and translational research in recent years has been heavily influenced by the development of animal models that can approximately replicate the human pathological condition under study. Research animals such as rodents provide scientists with a complex biological system composed of cells, tissues and organs in comparison to the controlled and sterile *in vitro* approach. In relevance of the 105 Noble prizes awarded for Physiology or Medicine, 91 were directly dependent on animal research. As such, research on non-human primates within the US comprises only around 1% while the majority (95%) of animal research is conducted on rodents. Insights gained from mouse experiments can be correlated to the human system since both share up to 95% of their genomes and have around 200 common disease conditions [27]. In this scenario, a mouse with a specific pathological condition can serve as a model for the human patient with the same disease. In addition, the mouse is amenable to extensive genetic modification, making it possible to produce new strains that can accurately mimic the human disease. Lastly, *via* inbreeding and accurate mating setups, it is possible to maintain a specific strain of mice indefinitely. These advantages make mouse models a cost-effective and efficient tool for biomedical research.

This review reports different steps in the study of *HLA-G* expression and functions *in vivo*, including creation of transgenic mice containing HLA-G and its receptors, then continuing with humanized mouse models that were developed to study HLA-G in physiological and pathological conditions.

2. Mice expressing HLA-G molecules

The *HLA-G* gene was discovered with studies that aimed to understand how the maternal immune system displays immune tolerance against the fetus during the pregnancy process. In 1953, Medawar [28] suggested that the fetus could be protected from maternal immune cells by a specific *HLA* gene profile, since proteins encoded by the *HLA* gene family exhibit an important function in the regulation of the immune response. In the 1990s, work performed by McMichael and DeMars' groups showed that trophoblast-derived choriocarcinoma cells and freshly harvested villous cytotrophoblast cells from first trimester placenta express the *HLA-G* gene [4,29].

In order to determine whether human HLA-G can be expressed in extra-embryonic tissues and to further understand regulation of the *HLA-G* gene transcription, the first *HLA-G* transgenic mouse model was created in Orr's laboratory in 1993 [30]. *HLA-G* transgenic mouse lines containing different constructs of the *HLA-G* gene (HLA-G coding region and diverse 5′ upstream regulatory elements) were generated. Then they analyzed the expression of *HLA-G* transcripts in different tissues of these transgenic mice: thymus, spleen, lung, kidney, brain, testicle, blood and extra-embryonic tissues. It was shown that the 5′ upstream regions of *HLA-G* are important for its pattern expression, and especially, a 250 bp fragment located 1.1 kb upstream of the translation start site was shown to be essential for efficient extraembryonic expression. Moreover, they identified that *HLA-G* mRNA levels were highest in the spleen and thymus tissues, moderate in the lung, kidney and testicle tissues, and finally very low in the brain. These data are typical of the pattern of MHC class I expression in human and in mice [31,32]. *HLA-G* pattern expression during the pregnancy process showed a dramatic increase between p.c.d. (post-coitus day) 12.5 and 16.5. Furthermore, the expression of *HLA-G* was higher in the placenta/parietal yolk sac samples than in the visceral yolk sac/amnion samples. One year later, data provided by Horuzsko and co-workers confirmed and demonstrated that transcription of the *HLA-G* gene begins very soon after implantation of mouse embryos and persists in some tissues from adult *HLA-G* transgenic mouse [33]. After embryos implant, transcription of the *HLA-G* transgene in mice starts at p.c.d. 5.5 in the uterine wall, in cells derived from embryos until term at day 19 (Fig. 1A). These data underline that the regulation of *HLA-G* expression during early post-implantation development is similar in mice and humans. This transcriptional regulation could imply an interaction between *cis*-acting regulatory elements in the *HLA-G* DNA included in the transgene constructs and *trans*-acting factors present in murine cells. Analysis realized in adult mice confirmed data obtained previously by Orr's group, consisting of the presence of *HLA-G* transcripts in thymus, spleen, liver and testicles but not in uterus of female mice. In contrast to the previous study, no *HLA-G* transcript was detectable from brain, heart or kidney (Fig. 1B).

The fact that the *HLA-G* gene expresses during the pregnancy could suggest that HLA-G proteins are able to modulate maternal T cell responses directed against MHC genes expressed by the fetus cells. Lee and co-workers reported that HLA-G proteins extracted and purified from human lymphoblastoid cells formed a complex with short nonameric peptides derived from diverse proteins [34]. This finding suggests the existence of a human T cell repertoire that might be capable to recognize these complexes. To further study the role of HLA-G associated to polypeptides in the modulation of T cell response, triple transgenic (TRI) mice were generated [35]. These transgenic mice express human *HLA-G*, human *β_2-microglobulin* (*$h\beta_2m$*) to permit the expression of HLA-G on the cell surface, as well as human *CD8α* (*hCD8α*) in anticipation that this molecule could facilitate the interaction between murine thymocytes or T cells and murine cells that express HLA-G and hβ_2m (Fig. 2A) [35]. At the same time, they generated double transgenic (DI) mice expressing only *hCD2/hCD8α* and *$h\beta_2m$* (Fig. 2B). Engraftment of tail skin from TRI mice into normal mice provoked rapid rejection of skin grafts. This data showed that transgene expression in TRI mice induced a strong immune response leading to graft rejection. Interestingly, when tissues from DI mice were engrafted into normal mice, only 25% of grafts were rejected (Fig. 2C). Together, these results demonstrated that HLA-G is a strong transplantation antigen in mice and could provoke graft rejection. To further understand the mechanisms implicating the function of HLA-G in the transplant rejection, TRI mice were immunized with the peptide RHPKYKTEL (G1), which strongly binds to HLA-G protein. Immunized mice expressed specific cytotoxic T cells, which recognized HLA-G-peptide complex and lysed the cells expressing these complexes. Others results obtained from Lenfant and co-workers showed that HLA-G tetrameric complex binds to other peptides, for example, 5 peptides derived from the human cytomegalovirus (HCMV) pp65 protein, and generates a specific population of cytotoxic T cells in TRI mice that are able to kill human cell lines infected with HCMV [36]. Also, HLA-G proteins are able to present peptide to murine T cells and induce them to differentiate into effector cytotoxic T cells.

In 2003, Warner group identified, for the first time, a functional homolog of human HLA-G in mouse: Qa-2 molecules encoded by the *Ped* gene (preimplantation embryonic development) [37]. The structures and features of human HLA-G and mouse Qa-2 are very close. Both of them expressed membrane-bound isoforms (HLA-G1, 2, 3 and 4 in human and GPI-linked in mouse) and soluble isoforms (HLA-G5, G6 and G7 in human and S1 Qa-2 and S2 Qa-2 in mouse). All HLA-G membrane-bound isoforms and GPI-linked

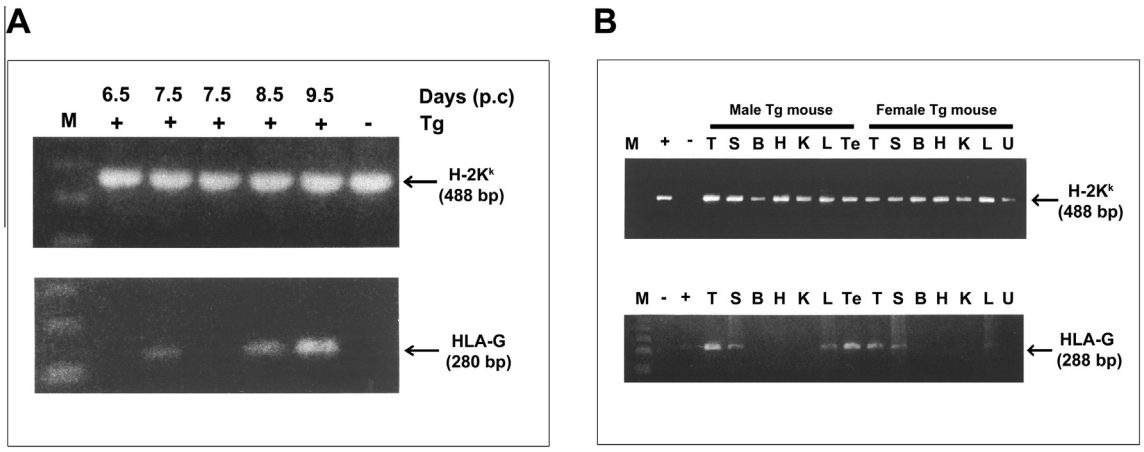
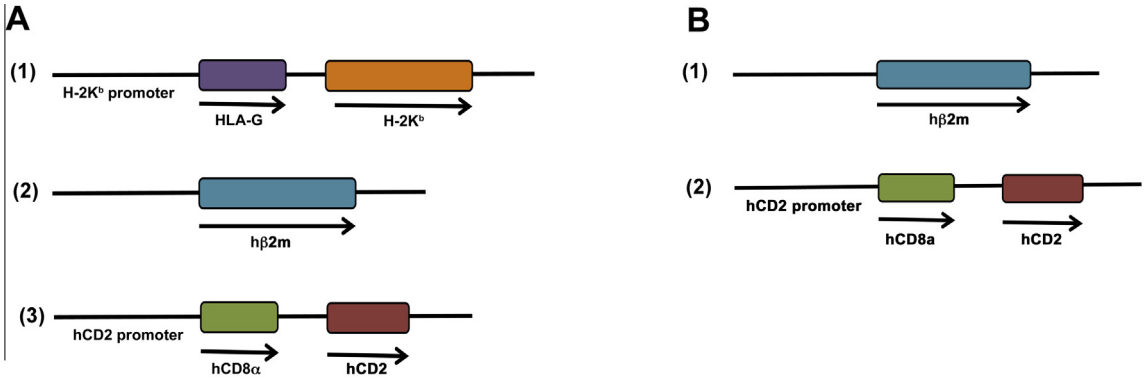


Fig. 1. Detection of HLA-G RNA during embryonic development of HLA-G transgenic mice and in tissues from adult HLA-G transgenic mice. (A) RNA samples prepared from HLA-G transgenic (Tg+) or control (Tg−) embryos at various times postcoitus (p.c) were analyzed by RT-PCR to detect transcripts of HLA-G and H-2K^k, which was used as housekeeping gene. (B) RNA samples isolated from tissues of male and female HLA-G adult transgenic mice were analyzed by RT-PCR to detect transcripts of HLA-G and H-2K^k. Tissues analyzed were: thymus (T), spleen (S), brain (B), heart (H), kidney (K), liver (L), testicles (Te) and uterus (U). Data from Horuzsko et al. [33] *HLA-G transcription of HLA-G transgenes commences shortly after implantation during embryonic development in mice.*



C

Donor mouse	Recipient mouse	No. of mice with rejection/total	Graft survival times (days)	Range
TRI	CBA/Ca	17/17	10, 10, 10, 11, 13, 13, 13, 13, 13, 14, 15, 15, 15, 16, 18, 24, 32	10-32
DI	CBA/Ca	2/8	16, 18, >120, >120, >120, >120, >120, >120	–
TRI	DI	7/7	19, 21, 21, 23, 25, 25, 25	19-25
CBK[a]	CBA/Ca	7/7	10, 11, 11, 16, 18, 24, 24	10-24

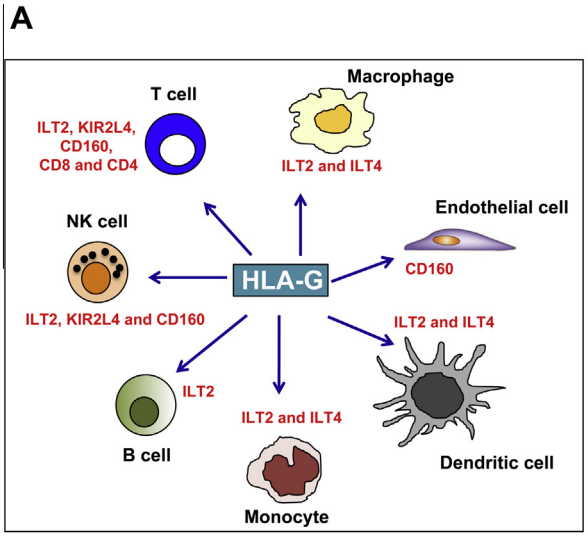
[a]Skin grafts mismatched at a single murine MHC class I gene (H-2K^b, CBK grafts on CBA recipients).

Fig. 2. Generation of triple, double transgenic mice and rejection of transgenic skin transplant. (A) Triple transgenic mice (TRI) carried 1) the recombinant *H-2K^b/HLA-G* gene, 2) *hβ2m* gene and 3) *hCD8α* cDNA linked to an expression cassette derived from the *hCD2* gene. (B) Double transgenic mice (DI) expressed 1) *hβ2m* gene and 2) *hCD8α* cDNA linked to an expression cassette derived from the *hCD2* gene. (C) Rejection of TRI transgenic skin transplant by normal (CBA/Ca) and DI mice (from Horuzsko et al. [35] *HLA-G functions as a restriction element and a transplantation antigen in mice*).

localized in lipid rafts on the cell membranes, and they might have the ability to induce signaling transduction *via* proteins present in lipid rafts [38]. Moreover the Qa-2 positive and negative-expressing mice are available from Jackson Laboratory.

3. Mice expressing HLA-G receptors

At the present time, 6 inhibitory and activating receptors which recognized HLA-G proteins or complexes containing HLA-G were identified: ILT2, ILT4, KIR2DL4, CD160, CD4 and CD8. ILT2 is expressed by B cells, some T cells, some NK cells and all monocytes/DCs and macrophages [21]; ILT4 is expressed by myeloid cells, DCs and macrophages [22]; KIR2DL4 is expressed by peripheral and decidual NK cells and some T cells [23]; CD160 is expressed by endothelial cells, T cells and NK cells [39–41]; CD4 and CD8 are expressed by T cells [42] (Fig. 3A and B). In order to discover the mechanisms and signaling pathways that are induced by the interaction of HLA-G with its receptors, transgenic mice expressing ILT2 and ILT4 were generated [43]. Horuzsko's group generated transgenic mice expressing the human *ILT4* gene under control of the mouse *CD11c* promoter [43]. These transgenic mice expressed ILT4 only on DCs, since in human it was known that



B

Receptor	HLA-G binding domain
CD8	α3
ILT2	α3 + β2m
ILT4	α3
KIR2DL4	α1

Fig. 3. HLA-G and its receptors. (A) Different cell types express HLA-G inhibitory and activating receptors. ILT2 receptors were shown on the cell surface of T cells, NK cells, B cells, monocytes, dendritic cells and macrophages; ILT4 on monocyte, dendritic cell and macrophage cell surface; KIR2DL4 on T cell and NK cell surface; CD8 and CD4 on T cell surface; and CD160 on endothelial cell, T cell and NK cell surface. (B) HLA-G subunits α1 and α3 display an important role in the interaction between HLA-G with its receptors. Data in table modified from Amiot et al. [78]. *Biology of the immunomodulatory molecule HLA-G in human liver diseases.*

the inhibitory receptors ILT2 and ILT4 are expressed predominantly on myeloid DCs [44]. Results obtained by the same group showed that HLA-G tetrameric complexes are able to generate tolerogenic DCs with a decrease in expression of MHC class II genes and co-stimulatory molecules [45]. ILT4 transgenic mice, with the B6 background, behave very closely to the control mice: ILT4 transgenic mice were born at the expected rate, bred well and were phenotypically normal. Analysis of tissues (thymus, spleen, liver, brain, kidney, stomach, intestine and skin) revealed no major abnormalities [43]. DCs isolated from the bone marrow of ILT4 transgenic mice or normal mice were exposed to HLA-G tetrameric complexes and tested for their ability to present the murine MHC class II-binding peptide to the cell hybridoma DO11.10. ILT4-expressing DCs display decreased ability to present peptide to T cells. The HLA-G/ILT4 interaction on DCs seems to affect the efficiency of antigen presentation by MHC class II. The data demonstrated that the induction of tolerogenic DCs by HLA-G is due to the inhibition of maturation and differentiation of myeloid DCs. Immature DCs are defective for the assembly and transport of MHC class II molecules to the cell surface. Indeed, during the maturation process, DCs increase their surface expression of MHC class II complexes. This increase is due to a dramatic change in localization of MHC class II molecules, which move from the endosomal structures onto the plasma membrane in mature DCs [46].

It has been reported that HLA-G molecules could form dimeric complexes [11,47], and its efficiency to bind to its receptors depend on its ability to polymerize. Chemical analysis showed that dimerization of HLA-G molecules occurs *via* a disulfide bond, due to the cysteine residue localized in position 42 of the α1 domain [47–51]. M8 cells were transfected with *HLA-G1* through *HLA-G6,* and total proteins were extracted from the transfected cells. Western blot analysis under reducing and non-reducing conditions demonstrated the presence of HLA-G1, G2, G4, G5 and G6 dimers/homodimers [11]. Using the ILT2-mediated signaling and NFAT-GFP (nuclear factor of activated T-cells-green fluorescence protein) reporter system, Liang and co-workers found that HLA-G5 dimeric and HLA-G1 tetrameric complexes are potent to induce the most efficient ILT-mediated inhibitory signaling [52]. Using ILT4 transgenic mice, they showed that in the presence of HLA-G5 dimers and HLA-G1 tetramers, a fewer number of activated/mature DCs was observed in lymph nodes. In contrast, the presence of HLA-G5 monomers could induce more efficiently activated/mature DCs. DCs isolated from HLA-G5 dimers and HLA-G1 tetramers injected into ILT4 transgenic mice regulate positively the transcription of interleukin 6 (*Il6*), which displays an important function in the down-regulation of expression of MHC class II molecules. Moreover, the treatment of ILT4-expressing DCs with HLA-G1 tetramers induces phosphorylation of STAT3 molecules (Signal transducer and activator of transcription 3). Kitamura and co-workers discovered that the IL-6/STAT3 signaling pathway controls the intracellular MHC class II αβ dimer level through cathepsin S activity in DCs [53]. One question remains to be clarified: how the interaction of HLA-G with ILT4 could induce expression of the *Il6* gene? The answer to this question came from data obtained from Horuzsko's group, which showed that the interaction between HLA-G and ILT4 induces the recruitment and activation of SHP-1 and SHP-2 phosphatases [52]. SHP-1, and especially SHP-2, modulate the NF-κB pathway (nuclear factor kappa B) in a MAPK (mitogen-activated protein kinase)-independent fashion in induction of *Il6* transcription (Fig. 4A).

Skin allograft experiments were performed on ILT4 transgenic mice to study the role of HLA-G in graft survival. Injection of tetrameric HLA-G complexes into normal mice (B6 background) marginally prolonged the allograft survival due to the interaction of HLA-G with the murine paired immunoglobulin-like receptor (PIR)-B [54]. However, the effect of HLA-G was more impressive when a skin allograft was performed on ILT4 transgenic mice that received HLA-G tetrameric complex injection. The presence of HLA-G tetrameric complexes prolonged significantly the allograft survival, and 75% of the grafts survived over 40 days (Fig. 5A). Furthermore, analysis of $CD4^+$ and $CD8^+$ cells from spleens of ILT4 transgenic mice showed decreased synthesis of IL-2. These data demonstrated that the interaction of HLA-G with its ILT4 receptor modulates the immune response of allogeneic T cells *in vivo* and prolongs skin allograft survival.

ILT2 transgenic mice, with the B6 background, were generated, with the expression of *ILT2* under the control of the human *CD2* promoter [55]. ILT2 transgenic mice expressed high levels of ILT2 on the majority of the peripheral T and B cells, and on almost all NK and NKT cells and thymocytes. The pattern of *ILT2* expression

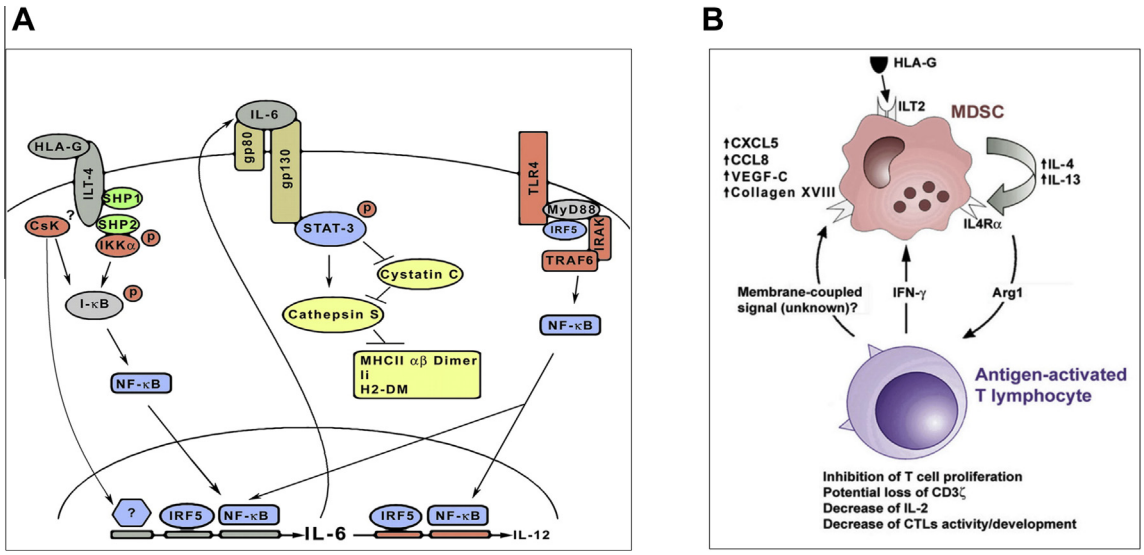


Fig. 4. Signaling mechanisms induced by HLA-G and its receptors. (A) Proposed signaling pathways induced by HLA-G-ILT4 causing arrest of maturation/activation of DCs. HLA-G induces phosphorylation of its receptor ILT4 and recruitment of SHP-1 and SHP-2 phosphatases. SHP-2 enhances activation of NF-κB and downstream IL-6 production. IL-6 induces STAT3 phosphorylation, which decreases cystatin C level, the endogenous inhibitor of cathepsins, and enhanced cathepsin S activities. Cathepsin S decreases intracellular MHC class II αβ dimer levels, invariant chain (Ii), and H2-DM molecule levels in DCs (from Liang et al. [52]. *Modulation of dendritic cell differentiation by HLA-G and ILT4 requires the IL-6-STAT3 signaling pathway*). (B) Potential mechanisms of enhancing the immunosuppressive activities of MDSCs by HLA-G and ILT2. Interaction between HLA-G with ILT2 on MDSCs increases secretion of IL-4 and IL-13, and expression of IL-4Rα, which enhance production and activation of Arginase I. Arginase I inhibits T cell proliferation, decreases expression of IL-2 in T cells, causing potential loss of CD3ζ chain, decreases cytotoxic T lymphocytes (CTL) development and activity. In addition, release of IFN-γ from antigen-activated T cells promotes the activation of MDSCs with enhance expression of chemokine ligands CXCL5 and CCL8 to augment their suppressive effect on antigen-activated T cells (from Zhang et al. [61]. *Human inhibitory receptor ILT2 amplifies CD11b$^+$Gr1$^+$ myeloid-derived suppressor cells that promote long-term survival of allograft*).

in transgenic mice is similar to that observed in humans [56,57]. However, deeper analysis showed 1) the number of thymocytes in ILT2 transgenic mice was lower compared to control mice; 2) the number of double negative (DN, CD4$^-$ CD8$^-$) cells was increased; and 3) the number of double positive (DP, CD4$^+$ CD8$^+$) and single positive (SP CD4$^+$ or SP CD8$^+$) cells was reduced. These observations indicate that thymic hypocellularity in ILT2 transgenic mice may be due to a defect at the double negative stage of development. Furthermore, these transgenic mice exhibited an accumulation of cells in the DN3 stage, which consists in the time point that the TCRβ (T cell receptor beta) locus is rearranged by a RAG-dependent mechanism in order to form the pre-TCR complex. Interruption of this complex leads to the arrest of cells in DN3 stage [58]. Liang and co-workers demonstrated that the expression of ILT2 on mature T cells down-regulates ZAP-70 and CD3ζ phosphorylation and also inhibits the early TCR-mediated T cell activation process. They identified at least one of the murine MHC class I molecules, H-2D^b, that binds to the human ILT2 *in vivo* [55].

Using ILT2 transgenic mice for skin graft survival analysis, Horuzsko's group found that a skin graft from a fully allogeneic donor was prolonged in ILT2 transgenic mice (Fig. 5B) and a skin graft from bm1 donors, carrying the MHC class I alloantigen, was prolonged or accepted (Fig. 5C). The same experiments were performed with allogeneic MHC class II-disparate allografts on ILT2 transgenic mice; the data showed that expression of ILT2 increased prolongation and acceptance of the skin graft (Fig. 5D). They also detected increased expression of PD-1 (programmed cell death 1) receptor in these ILT2 mice. This receptor is expressed by activated, but not unstimulated T cells, and plays an important function in maintenance of peripheral tolerance to self-antigens and prevention of allograft rejection [59]. Furthermore, comparisons between CD11b$^+$Gr-1$^+$ cells isolated from ILT2 transgenic mice and non-transgenic mice pointed to different mechanisms used by these cells in the presence of HLA-G to prolong allograft survival. CD11b$^+$Gr-1$^+$ cells were identified as a heterogeneous population of myeloid-derived cells including immature DCs, macrophages, granulocytes, and other myeloid cells at early stages of differentiation. These cells were shown to exhibit suppressive immune responses and to express ILT2 receptor, and were named myeloid-derived suppressor cells (MDSCs) [60]. In healthy, non-transgenic mice, these cells were found in the bone marrow, peripheral blood and spleen. In the absence of HLA-G, the number of MDSCs in peripheral blood mononuclear cells (PBMCs) was higher in ILT2 transgenic mice compared to non-transgenic mice (6.6% *vs* 3.4%). The same data concerning MDSC number were obtained by analyzing the spleen (3.6% *vs* 1.7%) [61]. It was reported by Horuzsko's group that ILT2 has at least one natural murine ligand, H-2D^b, and that the interaction between H-2D^b and ILT2 requires the presence of β2m [55]. By using ILT2$^+$/β2m$^-$ and β2$^-$ transgenic mice, Zhang and co-workers showed: 1) in the absence of HLA-G, the number of MDSCs is similar between β2m$^-$ and ILT2$^+$/β2m$^-$ mice in PBMCs; and 2) in the presence of HLA-G, this number increased more than 2.5-fold in ILT2$^+$/β2m$^-$ compared to β2m$^-$ mice (3.6% *vs* 9.4%). These results demonstrated the ability of HLA-G to induce the expansion of MDSCs *in vivo* [61]. In order to better understand the mechanisms used by MDSCs to prolong allograft survival when HLA-G binds on its receptor ILT2 *in vivo*, allogeneic skin grafts were performed on normal mice and ILT2 transgenic mice. Graft-induced CD11b$^+$Gr-1$^+$ cells from these two groups were analyzed. Gene analysis showed that CD11b$^+$Gr-1$^+$ cells from ILT2 mice exhibit lower expression of MHC class I genes and CD11c. In contrast, higher expression of IL4-Rα and its ligands IL-4 and IL-13 was detected on MDSCs from ILT2 mice. IL4-Rα was known to play an important role in the suppressive activity of MDSCs. Moreover, the transcriptional level of *Arginase I* was increased 3.8-fold in MDSCs from ILT2 mice compared to control mice. Arginase I and iNOS2 are the key enzymes that are produced by myeloid cells and play a crucial role in the immunosuppressive function of MDSCs on T cells. To determine the suppressive potential of graft-induced MDSCs isolated from

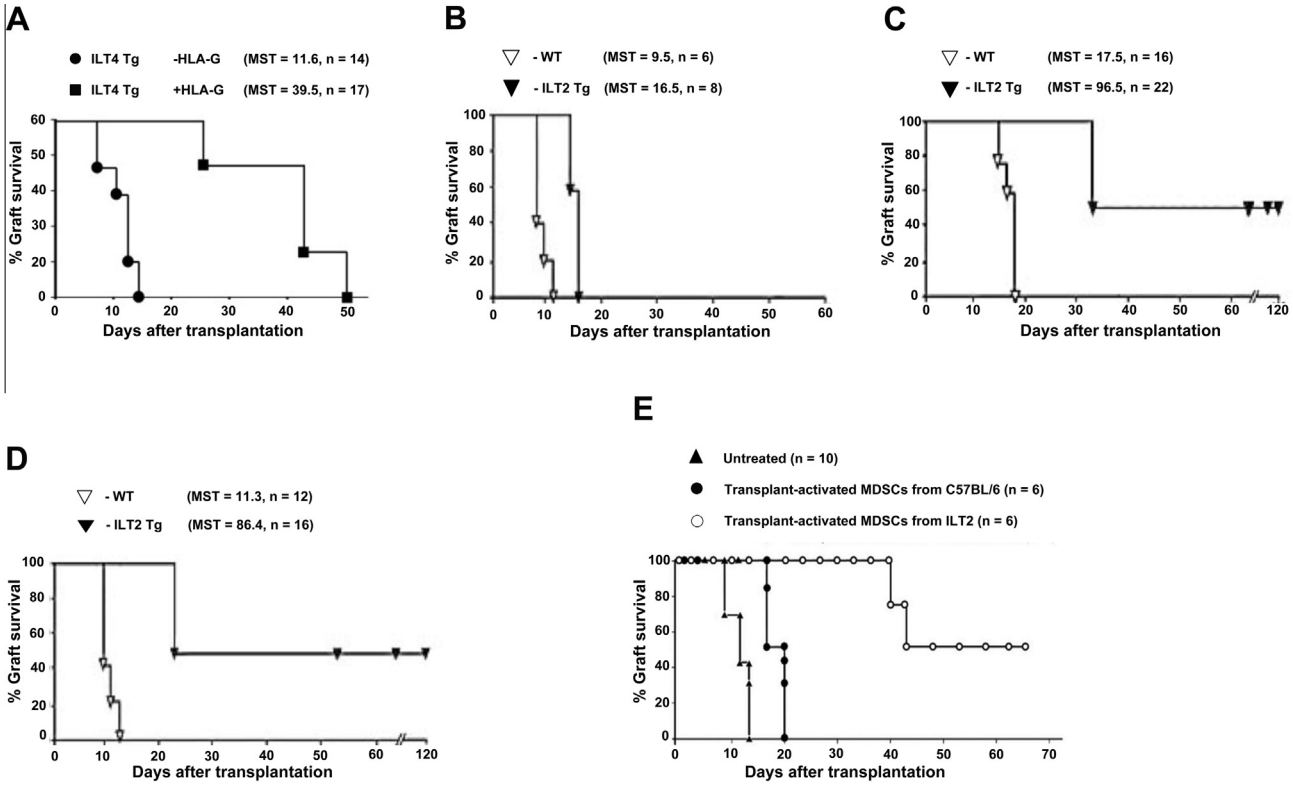


Fig. 5. Skin allograft survival prolonged by the interaction of HLA-G and its receptors. (A) ILT4 transgenic mice received a skin graft from MHC class II-mismatched donor mice. Two days before grafting, one group of mice received HLA-G1. The presence of HLA-G1 increases significantly the survival of the graft in the ILT4 recipient mice. The results shown are the mean survival time (MST) (from Ristich et al. [79] *Mechanisms of prolongation of allograft survival by HLA-G/ILT4-modified dendritic cells*). (B) Fully allogeneic skin graft survival was prolonged on ILT2 transgenic mice compared to non-transgenic mice. (C) Skin graft survival was prolonged or accepted on ILT2 transgenic mice that received an allograft from MHC class I-mismatched mutant mice. (D) Skin graft survival was prolonged or accepted on ILT2 transgenic mice that received an allograft from MHC class II-mismatched mutant mice (B, C, D from Liang et al. [55] *Human ILT2 receptor associates with murine MHC class I molecules in vivo and impairs T cell function*). (E) Prolongation of allograft survival on B6 mice treated with MDSCs isolated from ILT2 mice (from Zhang et al. [61] *Human inhibitory receptor ILT2 amplifies CD11b$^+$Gr1$^+$ myeloid-derived suppressor cells that promote long-term survival of allografts*).

ILT2 or control mice, these two groups of MDSCs were co-cultured with T cells (Fig. 4B). MDSCs isolated from ILT2 mice induce reduced T cell proliferation and inhibit production and secretion of IL-2 by T cells (Fig. 5B). Previous data obtained by the same group proved that skin allografts performed on ILT2 mice exhibited long-term survival or acceptance [55]. To dissect the role of MDSCs in this process, graft-derived CD11b$^+$Gr-1$^+$ cells from ILT2 mice were adoptively transferred into normal mice, the recipient of the allogeneic skin. 2×10^5 of these cells prolonged the allogeneic graft survival in normal mice (Fig. 5E). Data obtained by Zhang and co-workers indicate that the binding of HLA-G to its ILT2 receptor on MDSCs favors the expansion of these cells and increases their immunosuppressive function on T cells in order to delay or avoid the rejection of an allogeneic graft.

HLA-G proteins seems to display an important function in the tolerization of the immune system *via* the interaction with its receptors present on DCs, B cells, T cells, NK, NKT, and myeloid cells. However, the question concerning how HLA-G binds to its receptors in order to induce the tolerization effect remains unclear. Carosella's group generated different recombinant HLA-G proteins that mimic the complexes formed by HLA-G1 with β2m (B2m-HLA-G1s-Fc), HLA-G5 with β2m (B2m-HLA-G5), HLA-G subunit α with mouse IgG Fc (Alpha1-Fc) and only HLA-G subunit α (Alpha1-peptide). They demonstrated *in vivo* that these complexes could exist as monomers, dimers or multimers [62]. Other data support these observations since ILT2 and ILT4 receptors exhibit higher affinity for HLA-G multimers than the monomeric structures [49]. All of the HLA-G recombinant proteins display a positive effect by increasing the graft survival even if the recombinant B2m-HLA-G5 is more efficient than B2m-HLA-G1s-Fc, and Alpha1-Fc is more efficient than Alpha1-peptide. It was reported that subunits α1 and α3 are very important for the function of HLA-G since they serve as binding sites to β2m, ILT2 and ILT4 (Fig. 3B). Dimeric (α1-α3) synthetic polypeptides were generated and were tested *in vivo*. Results showed that a single dose injection of this polypeptide before skin graft transplantation increases significantly the graft survival [62].

Generation of *HLA-G* transgenic mice and ILT2, ILT4 transgenic mice allowed a better understanding of the mechanisms induced by the interaction between HLA-G and its receptors concerning the tolerization process. Recombinant and synthetic HLA-G polypeptides brought to light that HLA-G dimeric complexes are more efficient than monomers and that especially subunits α1 and α3 are essential for its tolerization function in graft survival.

4. Humanized mouse models

In vivo analysis of complex biological processes can provide a definitive understanding of their mechanistic functions. In this aspect, mouse models mimicking human biological systems have been invaluable for research. Yet, often these models are not capable of recapitulating the human physiological system, and research conducted in the former cannot be directly applied to the latter. Herein, humanized mouse models have been developed as an integral research tool for the study of human cells and tissues [63]. These mouse models are defined as immunodeficient mice

Table 1
Engraftment sources for humanized mouse models.

Model	Engraftment source	Application
PBMC humanized mice (Human Peripheral Blood Lymphocyte)	Engraftment of human mature immune cells	Study of human mature immune system
$CD34^+$ humanized mice	Human $CD34^+$ hematopoietic stem cell engraftment	Study of human naive immune system and hematopoiesis
BLT mice	Human fetal liver and thymus engraftment	Study of human naive immune system with minimal GVHD

engrafted with human hematopoietic cells or as mice that can transgenically express human genes. Development of such models has been dependent on a systemic progression of genetic modification on immunodeficient mice, amongst them the humanized NOD (non-obese diabetic) scid (severe combined immunodeficiency) gamma (NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl}/SzJ) (NSG) mice is one of the most adaptable strains for research [27,64]. The NSG mouse has shown the highest rates of successful engraftment and generation of human B and T cells. The strain was developed by the research team of Leonard D. Shultz at Jackson Laboratory and was affectionately called "Lenny's mouse" [65]. The mouse is a non-obese diabetic model harboring the Prkdc^{scid} mutation and is homozygous for a targeted mutation in the *Il2r gamma* chain locus. The *Prkdc* (Protein kinase, DNA-activated, catalytic polypeptide) gene encodes the protein kinase responsible for repairing DNA double-strand breaks occurring during V(D)J recombination. A loss of function in this gene causes the recombination events to be inhibited, leading to sub-optimal expression of T cell receptors and immunoglobulin genes. As a result, the mice lack mature T and B cells [66]. The IL-2R gamma chain serves as an integral receptor for numerous cytokines and impairs NK cell development. Most advantageously, the loss in signaling across this receptor significantly increased the life span of the strain, while most of the NOD-scid mice died due to thymic lymphomas, a phenomenon abrogated by the *Il2r gamma* mutation [67]. In combination, the NSG mice showed altered antigen expression, imperfect myeloid lineage differentiation, defective NK cell activity and immature T and B cell responses, showing the best engraftment rates amongst all other humanized mouse models [68]. As a result the NSG mouse has become the most versatile model for clinical and biomedical research [69,70].

There are multiple technical approaches for the engraftment of a functional human immune system within the NSG model. Two fundamental steps include: a) myeloablation of host bone marrow, which provides a niche for the donor stem cells; and b) enrichment of the successfully engrafted cells [71]. The source for repopulating the immune system of the NSG mice can be: 1) fetal cells; 2) human peripheral blood leukocytes; and 3) human stem cells [72]. In the first approach, human fetal thymus and lymph nodes are surgically transplanted onto the mice. Since the fetal cells are immature and incapable of self- *vs* non self-recognition, expanded donor human T cells do not elicit the graft-versus-host reaction (GVHR), which is a distinct advantage to the model. However, this route requires advanced surgical skills and technical expertise. The second technique makes use of human peripheral blood leukocytes. The cells themselves can be injected either intravenously or through the intrafemoral route. By using mature cells, this model provides a fast and efficient means of studying the human immune system *in vivo*. The third approach involves direct injection of enriched human hematopoietic stem cells (HSCs). Their sources include umbilical cord blood, bone marrow, fetal liver and peripheral blood [67]. The initial 12 weeks, post engraftment, during which expansion of the human immune system takes place,

Table 2
The most appropriate NSG humanized mouse models for research applications.

Model	Nature	Application
NOD.Cg-*B2m*^{tm1Unc} *Prkdc*^{scid} *Il2rg*^{tm1Wjl}/SzJ	Triple mutant: *Scid* mutation + interleukin 2 receptor gamma chain (*Il2rg*) deficiency + beta-2 microglobulin (*B2m*) deficiency	Useful for study of *in vivo* mechanisms of xenogeneic GVHD and assess therapeutic agents
NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl} Tg(HLA-A/H2-D/B2m)1Dvs/SzJ	Immunodeficient mice that express human HLA class 1 heavy and light chains	Supports active maturation of human T cells with HSC engraftment and amenable for basic immune research
NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl} Tg(HLA-A2.1)1Enge/SzJ	Triple mutant: *Scid* mutation + interleukin 2 receptor gamma chain (*Il2rg*) deficiency + expression of human *HLA-A2.1* MHC class I	Study immune responses to Epstein-Barr virus for vaccine development
NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl} *H2-Ab1*^{tm1Gru} Tg(HLA-DRB1)31Dmz/SzJ	Compound mutant: *Scid* mutation + interleukin 2 receptor gamma chain (*Il2rg*) deficiency + murine *MHC* class II deficiency + expression of human leukocyte antigen *DR4* gene	Targeting $CD4^+$ T cells in transplantation studies with absence of xeno-GVHD complications
NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl} Tg(HLA-DRA*0101,HLA-DRB1*0101)1Dmz/GckRolyJ	Triple mutant: *Scid* mutation + interleukin 2 receptor gamma chain (*Il2rg*) deficiency + expression of human/mouse chimeric *MHC* Class II transgene	Application in transplantation and vaccine research
NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl} Tg(PGK1-KITLG*220)441Daw/SzJ	Kit ligand (*SCF*) transgenic that does not require preconditioning irradiation	Cytokine transgenic mice applicable in study of human mast cell development and allergic responses
NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl} Tg(CMV-IL3, CSF2, KITLG)1Eav/MloySzJ	Classical immunodeficient strain with transgenic expression of several human cytokines for human immune cell expansion post engraftment	Useful for a variety of transplantation studies

will be critical, with the mice being acutely vulnerable to pathogens. After this period, human CD45$^+$ cells can be detected in the peripheral blood of the NSG mice *via* flow cytometry [70]. Ideally, human immune cells should occupy at least 35% of the overall blood population, denoting successful engraftment and repopulation in the mice (Table 1).

In the field of transplantation research, the NSG humanized mouse has become the go-to model for studying GVHR as well as for testing T regulatory cells (Tregs) as a potential therapy for minimizing rejection [73]. Xenograft studies using the NSG model have demonstrated the action of human Tregs in modulating transplantation rejection and have provided insights into the pathological nature of graft-versus-host disease (GVHD) [64,67]. In the tumorigenesis field, NSG mice have become the standard model for studies on primary human acute myeloid leukemia (AML) [74]. The strain has been integral for defining the nature of human tumor stem cells phenotypically and functionally. Application of the NSG model in tumor studies has been impactful in understanding the features of antitumor immune responses, providing new approaches for cancer immunotherapy [75,76]. These studies have demonstrated the ability of human breast carcinomas to generate a tumor-promoting microenvironment responsible for inducing crosstalk between DCs and CD4$^+$ T_H2 (T helper 2 cells) [66]. In addition, since the NSG mice provide successful generation of human T cells, this model has been impactful for the study of drugs that target such a cell population, *e.g.*, teplizumab. In retrospect, the NSG mouse strain has proven to be a versatile strain for the study of the human immune system and disease condition in mice (Table 2).

In the field of HLA-G research, humanized mouse models can advance our current knowledge of this non-classical MHC molecule. Our laboratory is focusing on the investigation of the effects of different HLA-G molecules on modulation of the function of immune cells using NSG humanized models. We have also developed methods and humanized models to control human allograft rejection using classical and modified immunosuppressive therapies. This will allow clinicians to optimize and immunologically "personalize" the treatment of allograft rejection. Presently, the available NSG models for other HLA molecules, such as the NSG-HLA-A2/HHD model, have been impactful for the *in vivo* examination of human immune responses [77]. Such humanized mice possessing HLA transgenes have been integral in investigating type 1 diabetes, multiple sclerosis and rheumatoid arthritis, all of which are autoimmune disorders associated with the HLA family of molecules.

5. Concluding remarks and future directions

Although impactful, there are yet debilitating limitations for the NSG model, the most crucial being the limited development of lymph nodes. Other problems, such as a lack of the human HLA immune system have been overcome with transgenes for this family of molecules [27]. However, complex issues such as the limited migration of human immune cells into non-lymphoid mouse organs are difficult to overcome. Transgenic expression of human cytokines and appropriate adhesion molecules can be used to solve such trafficking issues [63,66]. The greatest obstacle still remaining is the inability of mature human myeloid cells to migrate into the murine system due to anemia, making the establishment of the model difficult and time consuming. However, even with such challenges, the advantages posed by the NSG model outweigh their defects. With more strains of immunodeficient mice still in development, the humanized mouse models have become an essential tool for biomedical research providing valuable insights into the human immune system. In the future, it will be advantageous to generate new NSG-HLA-G mice, for example NOD.Cg-*Prkdc*^{scid}-*Il2rg*^{tm1Wjl}Tg(HLA-G/H2-D/B2M)1Dvs/SzJ, to study functions of HLA-G in several other models of human diseases.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

The authors thank Dr. Rhea-Beth Markowitz for critically reading the manuscript. This work was supported by the Carlos and Marguerite Mason Trust.

References

[1] S.A. Ellis, I.L. Sargent, C.W. Redman, A.J. McMichael, Evidence for a novel HLA antigen found on human extravillous trophoblast and a choriocarcinoma cell line, Immunology 59 (1986) 595.
[2] M. Kirszenbaum, S. Djoulah, J. Hors, I. Le Gall, E.B. de Oliveira, S. Prost, et al., HLA-G gene polymorphism segregation within CEPH reference families, Hum. Immunol. 53 (1997) 140.
[3] M. Kirszenbaum, S. Djoulah, J. Hors, S. Prost, J. Dausset, E.D. Carosella, Polymorphism of HLA-G gene and protein, J. Reprod. Immunol. 43 (1999) 105.
[4] S. Kovats, E.K. Main, C. Librach, M. Stubblebine, S.J. Fisher, R. DeMars, A class I antigen, HLA-G, expressed in human trophoblasts, Science 248 (1990) 220.
[5] R. Hackmon, M. Hallak, M. Krup, D. Weitzman, E. Sheiner, B. Kaplan, et al., HLA-G antigen and parturition: maternal serum, fetal serum and amniotic fluid levels during pregnancy, Fetal Diagn. Ther. 19 (2004) 404.
[6] V. Mallet, A. Blaschitz, L. Crisa, C. Schmitt, S. Fournel, A. King, et al., HLA-G in the human thymus: a subpopulation of medullary epithelial but not CD83(+) dendritic cells expresses HLA-G as a membrane-bound and soluble protein, Int. Immunol. 11 (1999) 889.
[7] M. Le Discorde, P. Moreau, P. Sabatier, J.M. Legeais, E.D. Carosella, Expression of HLA-G in human cornea, an immune-privileged tissue, Hum. Immunol. 64 (2003) 1039.
[8] Z. Selmani, A. Naji, I. Zidi, B. Favier, E. Gaiffe, L. Obert, et al., 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 (2008) 212.
[9] A. Verloes, H. Van de Velde, J. LeMaoult, I. Mateizel, G. Cauffman, P.A. Horn, et al., HLA-G expression in human embryonic stem cells and preimplantation embryos, J. Immunol. 186 (2011) 2663.
[10] C. Menier, M. Rabreau, J.C. Challier, M. Le Discorde, E.D. Carosella, N. Rouas-Freiss, Erythroblasts secrete the nonclassical HLA-G molecule from primitive to definitive hematopoiesis, Blood 104 (2004) 3153.
[11] K.Y. HoWangYin, M. Loustau, J. Wu, E. Alegre, M. Daouya, J. Caumartin, et al., Multimeric structures of HLA-G isoforms function through differential binding to LILRB receptors, Cell. Mol. Life Sci. 69 (2012) 4041.
[12] N. Lila, A. Carpentier, C. Amrein, I. Khalil-Daher, J. Dausset, E.D. Carosella, Implication of HLA-G molecule in heart-graft acceptance, Lancet 355 (2000) 2138.
[13] N. Rouas-Freiss, J. LeMaoult, P. Moreau, J. Dausset, E.D. Carosella, HLA-G in transplantation: a relevant molecule for inhibition of graft rejection?, Am J. Transplant. 3 (2003) 11.
[14] H. Wiendl, L. Behrens, S. Maier, M.A. Johnson, E.H. Weiss, R. Hohlfeld, Muscle fibers in inflammatory myopathies and cultured myoblasts express the nonclassical major histocompatibility antigen HLA-G, Ann. Neurol. 48 (2000) 679.
[15] S. Aractingi, N. Briand, C. Le Danff, M. Viguier, H. Bachelez, L. Michel, et al., HLA-G and NK receptor are expressed in psoriatic skin: a possible pathway for regulating infiltrating T cells?, Am J. Pathol. 159 (2001) 71.
[16] P. Paul, N. Rouas-Freiss, I. Khalil-Daher, P. Moreau, B. Riteau, F.A. Le Gal, et al., HLA-G expression in melanoma: a way for tumor cells to escape from immunosurveillance, Proc. Natl. Acad. Sci. U.S.A. 95 (1998) 4510.
[17] F. Morandi, I. Levreri, P. Bocca, B. Galleni, L. Raffaghello, S. Ferrone, et al., Human neuroblastoma cells trigger an immunosuppressive program in monocytes by stimulating soluble HLA-G release, Cancer Res. 67 (2007) 6433.
[18] L. Kren, K. Muckova, E. Lzicarova, M. Sova, V. Vybihal, T. Svoboda, et al., Production of immune-modulatory nonclassical molecules HLA-G and HLA-E by tumor infiltrating ameboid microglia/macrophages in glioblastomas: a role in innate immunity?, J Neuroimmunol. 220 (2010) 131.
[19] E.D. Carosella, P. Moreau, J. LeMaoult, N. Rouas-Freiss, HLA-G: from biology to clinical benefits, Trends Immunol. 29 (2008) 125.
[20] C. Cantoni, S. Verdiani, M. Falco, A. Pessino, M. Cilli, R. Conte, et al., P49, a putative HLA class I-specific inhibitory NK receptor belonging to the immunoglobulin superfamily, Eur. J. Immunol. 28 (1998) 1980.
[21] M. Colonna, F. Navarro, T. Bellon, M. Llano, P. Garcia, J. Samaridis, et al., A common inhibitory receptor for major histocompatibility complex class I molecules on human lymphoid and myelomonocytic cells, J. Exp. Med. 186 (1997) 1809.

[22] M. Colonna, J. Samaridis, M. Cella, L. Angman, R.L. Allen, C.A. O'Callaghan, et al., Human myelomonocytic cells express an inhibitory receptor for classical and nonclassical MHC class I molecules, J. Immunol. 160 (1998) 3096.
[23] S. Rajagopalan, E.O. Long, A human histocompatibility leukocyte antigen (HLA)-G-specific receptor expressed on all natural killer cells, J. Exp. Med. 189 (1999) 1093.
[24] C. Menier, N. Rouas-Freiss, B. Favier, J. LeMaoult, P. Moreau, E.D. Carosella, Recent advances on the non-classical major histocompatibility complex class I HLA-G molecule, Tissue Antigens 75 (2010) 201.
[25] E.D. Carosella, B. Favier, N. Rouas-Freiss, P. Moreau, J. LeMaoult, Beyond the increasing complexity of the immunomodulatory HLA-G molecule, Blood 111 (2008) 4862.
[26] N. Rouas-Freiss, P. Moreau, J. LeMaoult, E.D. Carosella, The dual role of HLA-G in cancer, J. Immunol. Res. 2014 (2014) 359748.
[27] L.D. Shultz, F. Ishikawa, D.L. Greiner, Humanized mice in translational biomedical research, Nat. Rev. Immunol. 7 (2007) 118.
[28] P. Medawar, Some immunological and endocrinological problems raised by the evolution of viviparity in vertebrates, Symp. Soc. Exp. Biol. (1952).
[29] S.A. Ellis, M.S. Palmer, A.J. McMichael, Human trophoblast and the choriocarcinoma cell line BeWo express a truncated HLA Class I molecule, J. Immunol. 144 (1990) 731.
[30] C.M. Schmidt, R.G. Ehlenfeldt, M.C. Athanasiou, L.A. Duvick, H. Heinrichs, C.S. David, et al., Extraembryonic expression of the human MHC class I gene HLA-G in transgenic mice. Evidence for a positive regulatory region located 1 kilobase 5′ to the start site of transcription, J. Immunol. 151 (1993) 2633.
[31] B. David-Watine, A. Israel, P. Kourilsky, The regulation and expression of MHC class I genes, Immunol. Today 11 (1990) 286.
[32] D.S. Singer, J.E. Maguire, Regulation of the expression of class I MHC genes, Crit. Rev. Immunol. 10 (1990) 235.
[33] A. Horuzsko, P.D. Tomlinson, T. Strachan, A.L. Mellor, Transcription of HLA-G transgenes commences shortly after implantation during embryonic development in mice, Immunology 83 (1994) 324.
[34] N. Lee, A.R. Malacko, A. Ishitani, M.C. Chen, J. Bajorath, H. Marquardt, et al., The membrane-bound and soluble forms of HLA-G bind identical sets of endogenous peptides but differ with respect to TAP association, Immunity 3 (1995) 591.
[35] A. Horuzsko, J. Antoniou, P. Tomlinson, V. Portik-Dobos, A.L. Mellor, HLA-G functions as a restriction element and a transplantation antigen in mice, Int. Immunol. 9 (1997) 645.
[36] F. Lenfant, N. Pizzato, S. Liang, C. Davrinche, P. Le Bouteiller, A. Horuzsko, Induction of HLA-G-restricted human cytomegalovirus pp65 (UL83)-specific cytotoxic T lymphocytes in HLA-G transgenic mice, J. Gen. Virol. 84 (2003) 307.
[37] M. Comiskey, C.Y. Goldstein, S.R. De Fazio, M. Mammolenti, J.A. Newmark, C.M. Warner, Evidence that HLA-G is the functional homolog of mouse Qa-2, the Ped gene product, Hum. Immunol. 64 (2003) 999.
[38] M. Comiskey, K.E. Domino, C.M. Warner, HLA-G is found in lipid rafts and can act as a signaling molecule, Hum. Immunol. 68 (2007) 1.
[39] P. Fons, S. Chabot, J.E. Cartwright, F. Lenfant, F. L'Faqihi, J. Giustiniani, et al., Soluble HLA-G1 inhibits angiogenesis through an apoptotic pathway and by direct binding to CD160 receptor expressed by endothelial cells, Blood 108 (2006) 2608.
[40] C. Pombo, E.J. Wherry, E. Gostick, D.A. Price, M.R. Betts, Elevated expression of CD160 and 2B4 defines a cytolytic HIV-specific CD8+ T-Cell population in elite controllers, J. Infect. Dis. 212 (2015) 1376.
[41] T.C. Tu, N.K. Brown, T.J. Kim, J. Wroblewska, X. Yang, X. Guo, et al., CD160 is essential for NK-mediated IFN-gamma production, J. Exp. Med. 212 (2015) 415.
[42] P. Le Bouteiller, HLA-G in human early pregnancy: control of uterine immune cell activation and likely vascular remodeling, Biomed. J. 38 (2015) 32.
[43] V. Ristich, S. Liang, W. Zhang, J. Wu, A. Horuzsko, Tolerization of dendritic cells by HLA-G, Eur. J. Immunol. 35 (2005) 1133.
[44] L. Borges, D. Cosman, LIRs/ILTs/MIRs, inhibitory and stimulatory Ig-superfamily receptors expressed in myeloid and lymphoid cells, Cytokine Growth Factor Rev. 11 (2000) 209.
[45] S. Liang, A. Horuzsko, Mobilizing dendritic cells for tolerance by engagement of immune inhibitory receptors for HLA-G, Hum. Immunol. 64 (2003) 1025.
[46] J.A. Villadangos, P. Schnorrer, N.S. Wilson, Control of MHC class II antigen presentation in dendritic cells: a balance between creative and destructive forces, Immunol. Rev. 207 (2005) 191.
[47] J.E. Boyson, R. Erskine, M.C. Whitman, M. Chiu, J.M. Lau, L.A. Koopman, et al., Disulfide bond-mediated dimerization of HLA-G on the cell surface, Proc. Natl. Acad. Sci. U.S.A. 99 (2002) 16180.
[48] T. Gonen-Gross, H. Achdout, T.I. Arnon, R. Gazit, N. Stern, V. Horejsi, et al., The CD85J/leukocyte inhibitory receptor-1 distinguishes between conformed and beta 2-microglobulin-free HLA-G molecules, J. Immunol. 175 (2005) 4866.
[49] M. Shiroishi, K. Kuroki, T. Ose, L. Rasubala, I. Shiratori, H. Arase, et al., Efficient leukocyte Ig-like receptor signaling and crystal structure of disulfide-linked HLA-G dimer, J. Biol. Chem. 281 (2006) 10439.
[50] R. Apps, L. Gardner, A.M. Sharkey, N. Holmes, A. Moffett, A homodimeric complex of HLA-G on normal trophoblast cells modulates antigen-presenting cells via LILRB1, Eur. J. Immunol. 37 (2007) 1924.
[51] T. Gonen-Gross, H. Achdout, R. Gazit, J. Hanna, S. Mizrahi, G. Markel, et al., Complexes of HLA-G protein on the cell surface are important for leukocyte Ig-like receptor-1 function, J. Immunol. 171 (2003) 1343.
[52] S. Liang, V. Ristich, H. Arase, J. Dausset, E.D. Carosella, A. Horuzsko, Modulation of dendritic cell differentiation by HLA-G and ILT4 requires the IL-6-STAT3 signaling pathway, Proc. Natl. Acad. Sci. U.S.A. 105 (2008) 8357.
[53] H. Kitamura, H. Kamon, S. Sawa, S.J. Park, N. Katunuma, K. Ishihara, et al., IL-6-STAT3 controls intracellular MHC class II alphabeta dimer level through cathepsin S activity in dendritic cells, Immunity 23 (2005) 491.
[54] S. Liang, B. Baibakov, A. Horuzsko, HLA-G inhibits the functions of murine dendritic cells via the PIR-B immune inhibitory receptor, Eur. J. Immunol. 32 (2002) 2418.
[55] S. Liang, W. Zhang, A. Horuzsko, Human ILT2 receptor associates with murine MHC class I molecules in vivo and impairs T cell function, Eur. J. Immunol. 36 (2006) 2457.
[56] D. Saverino, M. Fabbi, F. Ghiotto, A. Merlo, S. Bruno, D. Zarcone, et al., The CD85/LIR-1/ILT2 inhibitory receptor is expressed by all human T lymphocytes and down-regulates their functions, J. Immunol. 165 (2000) 3742.
[57] D. Belkin, M. Torkar, C. Chang, R. Barten, M. Tolaini, A. Haude, et al., Killer cell Ig-like receptor and leukocyte Ig-like receptor transgenic mice exhibit tissue- and cell-specific transgene expression, J. Immunol. 171 (2003) 3056.
[58] E.H. Palacios, A. Weiss, Function of the Src-family kinases, Lck and Fyn, in T-cell development and activation, Oncogene 23 (2004) 7990.
[59] S.E. Sandner, M.R. Clarkson, A.D. Salama, A. Sanchez-Fueyo, C. Domenig, A. Habicht, et al., Role of the programmed death-1 pathway in regulation of alloimmune responses in vivo, J. Immunol. 174 (2005) 3408.
[60] E. Peranzoni, S. Zilio, I. Marigo, L. Dolcetti, P. Zanovello, S. Mandruzzato, et al., Myeloid-derived suppressor cell heterogeneity and subset definition, Curr. Opin. Immunol. 22 (2010) 238.
[61] W. Zhang, S. Liang, J. Wu, A. Horuzsko, Human inhibitory receptor immunoglobulin-like transcript 2 amplifies CD11b+Gr1+ myeloid-derived suppressor cells that promote long-term survival of allografts, Transplantation 86 (2008) 1125.
[62] J. LeMaoult, M. Daouya, J. Wu, M. Loustau, A. Horuzsko, E.D. Carosella, Synthetic HLA-G proteins for therapeutic use in transplantation, FASEB J. 27 (2013) 3643.
[63] L.D. Shultz, M.A. Brehm, J.V. Garcia-Martinez, D.L. Greiner, Humanized mice for immune system investigation: progress, promise and challenges, Nat. Rev. Immunol. 12 (2012) 786.
[64] W.J. Racki, L. Covassin, M. Brehm, S. Pino, R. Ignotz, R. Dunn, et al., NOD-scid IL2rgamma(null) mouse model of human skin transplantation and allograft rejection, Transplantation 89 (2010) 527.
[65] P.M. Conn, J.V. Parker, The animal research war, FASEB J. 22 (2008) 1294.
[66] L.D. Shultz, M.A. Brehm, S. Bavari, D.L. Greiner, Humanized mice as a preclinical tool for infectious disease and biomedical research, Ann. N. Y. Acad. Sci. 1245 (2011) 50.
[67] M. Hogenes, M. Huibers, C. Kroone, R. de Weger, Humanized mouse models in transplantation research, Transplant. Rev. (Orlando) 28 (2014) 103.
[68] F. Ishikawa, M. Yasukawa, B. Lyons, S. Yoshida, T. Miyamoto, G. Yoshimoto, et al., Development of functional human blood and immune systems in NOD/SCID/IL2 receptor gamma chain(null) mice, Blood 106 (2005) 1565.
[69] JAX Mice, The Jackson Laboratory, Clinical and Research Services, 2015.
[70] M.A. Brehm, A. Cuthbert, C. Yang, D.M. Miller, P. DiIorio, J. Laning, et al., Parameters for establishing humanized mouse models to study human immunity: analysis of human hematopoietic stem cell engraftment in three immunodeficient strains of mice bearing the IL2rgamma(null) mutation, Clin. Immunol. 135 (2010) 84.
[71] T. Strowig, A. Rongvaux, C. Rathinam, H. Takizawa, C. Borsotti, W. Philbrick, et al., Transgenic expression of human signal regulatory protein alpha in Rag2−/−gamma(c)−/− mice improves engraftment of human hematopoietic cells in humanized mice, Proc. Natl. Acad. Sci. U.S.A. 108 (2011) 13218.
[72] T. Pearson, D.L. Greiner, L.D. Shultz, Creation of "humanized" mice to study human immunity, Curr. Protoc. Immunol. (2008) (Chapter 15: Unit 15 21).
[73] M.A. King, L. Covassin, M.A. Brehm, W. Racki, T. Pearson, J. Leif, et al., Human peripheral blood leucocyte non-obese diabetic-severe combined immunodeficiency interleukin-2 receptor gamma chain gene mouse model of xenogeneic graft-versus-host-like disease and the role of host major histocompatibility complex, Clin. Exp. Immunol. 157 (2009) 104.
[74] Y. Saito, N. Uchida, S. Tanaka, N. Suzuki, M. Tomizawa-Murasawa, A. Sone, et al., Induction of cell cycle entry eliminates human leukemia stem cells in a mouse model of AML, Nat. Biotechnol. 28 (2010) 275.
[75] N.K. Vudattu, F. Waldron-Lynch, L.A. Truman, S. Deng, P. Preston-Hurlburt, R. Torres, et al., Humanized mice as a model for aberrant responses in human T cell immunotherapy, J. Immunol. 193 (2014) 587.
[76] G. Dranoff, Experimental mouse tumour models: what can be learnt about human cancer immunology?, Nat Rev. Immunol. 12 (2012) 61.
[77] L.D. Shultz, Y. Saito, Y. Najima, S. Tanaka, T. Ochi, M. Tomizawa, et al., Generation of functional human T-cell subsets with HLA-restricted immune responses in HLA class I expressing NOD/SCID/IL2r gamma(null) humanized mice, Proc. Natl. Acad. Sci. U.S.A. 107 (2010) 13022.
[78] L. Amiot, N. Vu, M. Samson, Biology of the immunomodulatory molecule HLA-G in human liver diseases, J. Hepatol. 62 (2015) 1430.
[79] V. Ristich, W. Zhang, S. Liang, A. Horuzsko, Mechanisms of prolongation of allograft survival by HLA-G/ILT4-modified dendritic cells, Hum. Immunol. 68 (2007) 264.