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