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Distinctive Immunological Functions of HLA-G

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1. Introduction

HLA-G is a non-classical HLA class Ib molecule belonging to the Major Histocompatibility Complex (MHC) located on the short arm of chromosome 6. The tissue-restricted distribution of HLA-G, the low polymorphism in the coding region, the fact that HLA-G primary transcript is alternatively spliced in seven isoforms, and the inhibitory action on immune cells, constitute four hallmarks of HLA-G, which distinguish it from other HLA class I molecules (Carosella et al., 1999). In healthy conditions, a basal level of HLA-G gene transcription is observed in most cells and tissues. However, translation into HLA-G protein is restricted to trophoblasts at the fetal-maternal interface (Carosella et al., 2003), and in adults, to thymic epitelial, cornea, mesenchymal stem cells (MSCs), nail matrix, pancreatic-β cells, erythroid and endothelial precursors. HLA-G can be also neo-expressed in pathological conditions including malignant transformation, viral infections, inflammatory and autoimmune diseases, and allogeneic transplantation (Carosella, 2011).

HLA-G locus is very low polymorphic and, due to its structure, HLA-G molecule can recognize and present only a restricted peptide repertoire compared to classical HLA class I molecules (Clements et al., 2005). These peculiarities render HLA-G exclusively oriented towards immune inhibition and tolerance. In the late ninety, Rouas-Freiss et al. showed that HLA-G expressing trophoblasts were protected from maternal NK cell-mediated cytolysis, indicating for the first time HLA-G as a key molecule in fetal-maternal tolerance (Rouas-Freiss et al., 1997). From this first observation, several groups have worked to define the mode of action of HLA-G and in which settings it is involved in promoting tolerance. It is now generally accepted that HLA-G is a unique molecule with several immuno-modulatory properties: it plays an important role not only in fetal-maternal tolerance, but also in modulating immune responses and promoting and maintaining peripheral tolerance in healthy and pathological conditions. HLA-G can indeed act on both the innate and the adaptive branches of immunity and regulate short- and long-term immune-responses.

2. HLA-G molecule

The alternative splicing of HLA-G primary transcript results in seven different isoforms, four of which are membrane bound (HLA-G1 to –G4) and three are soluble (HLA-G5 to –G7) forms (Fujii et al., 1994; Ishitani and Geraghty, 1992) (Fig. 1A).

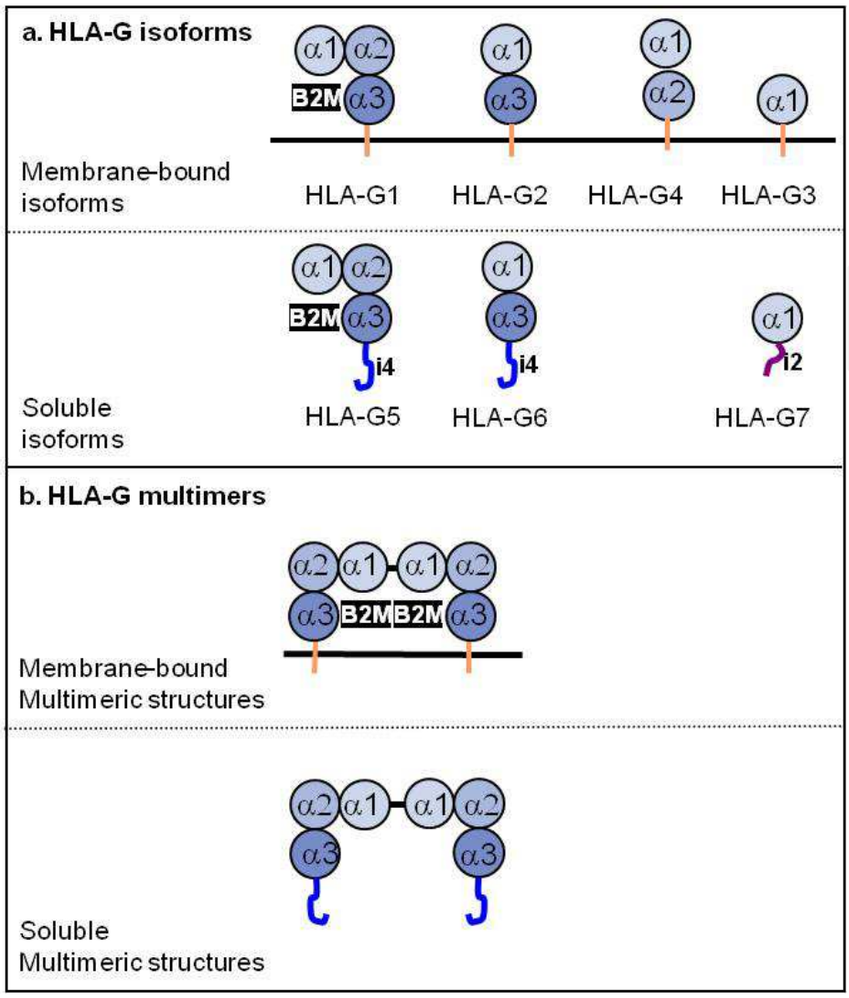


Fig. 1. HLA-G isoforms, monomers and dimers

A. The alternative splicing of a unique primary transcript yields 7 protein isoforms: truncated isoforms are generated by excision of one or two exons encoding globular (α) domains, whereas translation of intron 4 (i4) or intron 2 (i2) yields soluble isoforms that lack the transmembrane domain. **B**. HLA-G molecules can form homodimers through the generation of Cys42-Cys42 disulfide bonds.

In addition, a soluble HLA-G1 isoform (shed HLA-G1) can be generated by the membrane HLA-G1 proteolytic cleavage that is dependent on metalloproteinase activity (Park et al., 2004), and is regulated by Nitric Oxide (NO) concentration (Diaz-Lagares et al., 2009) and Tumor Necrosis Factor (TNF)-α/NFkB pathway activation (Zidi et al., 2006). Soluble and membrane-bound HLA-G isoforms have similar functions. HLA-G1, HLA-G5, and shed HLA-G1 are the most described isoforms in healthy tissues (Paul et al., 2000) and their structure is similar to that of classical HLA class I molecules: a heavy chain of three globular domains non-covalently associated with β2-microglobulin (β2m) and a nona-peptide (Clements et al., 2005). The other HLA-G isoforms contain the α1 domain but lack one or two of the other globular domains, and are not associated with β2m and the peptide. The

presence of the α3 domain is important to HLA-G functions since it represents a binding site for HLA-G receptors (Clements et al., 2005; Clements et al., 2007).

HLA-G possesses two unique cysteine residues, in position 42 of the α1 domain and in position 147 of the α2 domain. Through these residues, HLA-G may dimerize by intra-molecular disulfide bonds (Boyson et al., 2002). Membrane-bound or soluble HLA-G dimers were detected both *in vitro* and *in vivo* (Apps et al., 2007; Boyson et al., 2002; Gonen-Gross et al., 2005) (Fig. 1B). Dimerization of HLA-G is one of its key features since dimers bind to HLA-G receptors with higher affinity and slower dissociation rates compared to monomers (Shiroishi et al., 2006a) and, as a consequence, dimers, but not monomers, carry most of the HLA-G functions (Gonen-Gross et al., 2003; Li et al., 2009).

HLA-G acts through three inhibitory receptors: immunoglobulin-like transcript (ILT)2 (CD85j/LILRB1), ILT4 (CD85d/LILRB2), the killer cell immunoglobulin-like receptor (KIR)2DL4 (CD158d), and the co-receptor CD8 (Colonna et al., 1997; Colonna et al., 1998; Rajagopalan and Long, 1999) (Fig. 2).

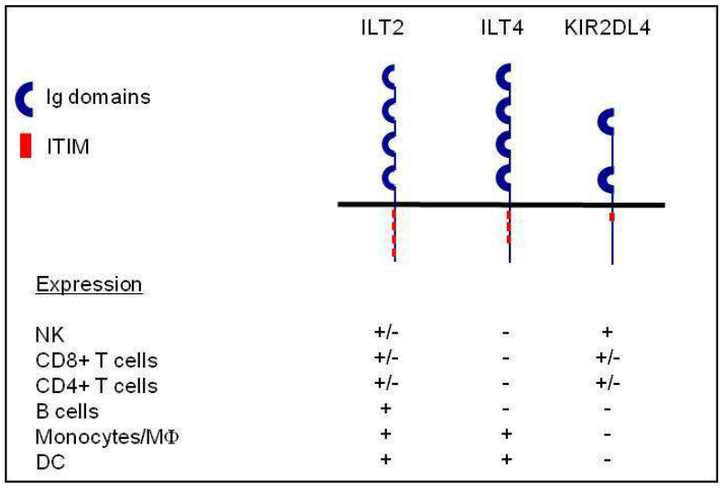


Fig. 2. HLA-G receptors

Inhibitory receptors known to bind HLA-G. Basic structural organization and expression patterns are shown. The HLA-G structural configuration that these receptors are known to bind are indicated as '+' (reported binding) and '–' (reported absence of binding or minor binding).

ILT2 is expressed by B cells, some T cells (both $CD4^+$ and $CD8^+$), a sub-population of NK cells, and all monocytes/Dendritic Cells (DCs) (Colonna et al., 1997), whereas ILT4 is only expressed by mono/macrophages and DCs (Colonna et al., 1998). KIR2DL4 is expressed by some $CD8^+$ T and NK cells (Goodridge et al., 2003).

Even if ILT2 and ILT4 recognize other HLA Class I molecules, HLA-G is their ligand of highest affinity and they bind HLA-G dimers even more strongly (Shiroishi et al., 2006a; Shiroishi et al., 2003). ILT2 and ILT4 can recognize different HLA-G structures: ILT2 binds only β2m-associated HLA-G1/G5 isoforms, whereas ILT4 also recognizes their β2m-free counterparts (Gonen-Gross et al., 2005; Shiroishi et al., 2006b). Finally, significantly higher expression of ILT2 was necessary for efficient HLA-G tetramer binding, suggesting that this

interaction may have relatively lower affinity compared to that of ILT4 (Allan et al., 1999). Interestingly, HLA-G can directly influence the expression rate of its receptors both at mRNA level and at the protein level (LeMaoult et al., 2005). Thus, HLA-G is unique in its ability to be expressed in different isoforms and to act through inhibitory receptors.

3. Mechanisms underlying HLA-G expression

Even if theoretically any tissue might express HLA-G, its physiological expression is restricted to few tissues such as trophoblasts, thymic epithelium, cornea, MSC and pancreatic β-cells (Carosella, 2011). Nonetheless, in pathological conditions, HLA-G expression can be induced and/or up-regulated. So far the reason why HLA-G can be expressed in some, but not in all tissues has not been fully elucidated. However, several factors such as immune-modulatory cytokines and hormones, were described to potentially affect transcriptional mechanisms responsible for HLA-G expression (Moreau et al., 2009). In contrast to classical HLA class I molecules, HLA-G expression is not influenced by the transcription factor NF-*k*B (Gobin et al., 1998) or by classical pro-inflammatory cytokines such as Interferon (IFN)-γ (Gobin et al., 1999), since responsive elements for IFN-γ are missed in HLA-G promoter (Gobin et al., 1999; Steimle et al., 1995). In contrast, HLA-G transcriptional rate is increased by the presence of a number of anti-inflammatory cytokines including IFN-β (Lefebvre et al., 2001), Interleukin (IL)-4, IL-5, IL-6 (Deschaseaux et al., 2011), and IL-10 (Moreau et al., 1999) and can be modulated by Granulocyte Macrophage Colony-Stimulating Factor (GM-CSF) (Onno et al., 2000; Yang et al., 1996), Transforming Growth Factor (TGF)-β, or Granulocyte Colony-Stimulating Factor (G-CSF).

In addition to cytokines, hormones like glucocorticoids (dexamethasone) and progesterone were shown to increase the secretion of both HLA-G5 and HLA–G6 by trophoblasts (Akhter et al., 2011; Moreau et al., 2001; Yie et al., 2006a; Yie et al., 2006b). HLA-G expression can be also influenced by Adenosine Triphosphate (ATP) and by the tryptophan catalayzing enzyme indoleamine 2 3-dioxygenase (IDO). ATP acts as a potent inhibitor of HLA-G1 and HLA–G5 production from LPS-activated PBMCs *via* down-regulation of IL-10 (Rizzo et al., 2009). IDO, known to be involved in the generation of tolerogenic microenvironments by tryptophan depletion and in the generation of T regulatory (Treg) cells (Chung et al., 2009; Munn et al., 2002), was shown to differentially modulate HLA-G expression in monocytes and myeloid DCs. IDO blocked HLA-G cell-surface expression on monocytes (Gonzalez-Hernandez et al., 2005), but it induced HLA-G expression and shedding in myeloid DCs (Lopez et al., 2006). Overall, a number of immune mediators can influence HLA-G expression. It has to be taken in account that these molecules can be up-regulated in conditions, such as inflammation, in which HLA-G expression is needed to control or dampen the immune response.

In addition to soluble factors, it has been recently reported that "transient" HLA-G expression can be acquired *via* trogocytosis, a cell-to-cell contact-dependent uptake of membranes and associated molecules from one cell by another (reviewed in (Davis, 2007; LeMaoult et al., 2007a). Trogocytosis of HLA-G was shown for activated $CD4^+$ and $CD8^+$ T cells (LeMaoult et al., 2007b), activated NK cells (Caumartin et al., 2007), and monocytes (HoWangYin et al., 2011). For $CD4^+$ T cells, the most important consequence of HLA-G acquisition, is that the newly-expressing cells became transiently tolerogenic (LeMaoult et al., 2007a), as discussed below. Thus, trogocytosis generates HLA-G positive cells, which

display, but do not express HLA-G. The ability of different cells to transiently acquire HLA-G expression broadens the immuno-modulatory activity of HLA-G.

4. HLA-G immunological function

The functions of HLA-G are exclusively oriented towards immune inhibition and tolerance. HLA-G exerts its inhibitory functions on several immune cells through direct binding to the inhibitory receptors ILT2, ILT4, and KIR2DL4 (Shiroishi et al., 2006b).

4.1 Short-term immuno-modulation mediated by HLA-G

HLA-G:ILT2 interaction modulates NK cell activity (Rouas-Freiss et al., 1997). HLA-G-expressing target cells are indeed resistant to the lysis mediated by NK cells (Riteau et al., 2001). Although the mechanisms underlying this effect are not completely elucidated, it has been recently defined that HLA-G:ILT2 interaction disrupts the immunological synapse (IS), a supramolecular structure responsible for both T and NK cell activation and function (Favier et al., 2010), leading to inhibition of NK cytolysis. HLA-G binds to KIR2DL4 on NK cells (Shiroishi et al., 2006b). However, it still remains unclear which are the effects mediated by this interaction. Ligation of KIR2DL4 activates cytokine production but not cytotoxicity (Rajagopalan et al., 2001), and it has been recently proposed that activation of KIR2DL4 by soluble HLA-G activates a pro-inflammatory/pro-angigenic responses, consistent with a role in promoting vascularization during early pregnancy (Rajagopalan et al., 2006). HLA-G can also bind CD8, which is expressed by a subpopulation of NK cells leading to Fas ligand expression, and induction of apoptosis (Contini et al., 2003).

The interaction between HLA-G and $CD8^+$ T cells impairs the antigen-specific cytotoxic T lymphocyte (CTLs) activity (Le Gal et al., 1999). HLA-G has a dose-dependent effect on the generation of allo-CTL responses, while it seems to not affect pre-existing allo-CTLs (Kapasi et al., 2000). Furthermore, during later stages of T cell activation, HLA-G:CD8 interaction promotes TCR-independent apoptosis of $CD8^+$ T cells through the same mechanisms described for NK cells (Fournel et al., 2000).

During the early phase of $CD4^+$ T cell activation, HLA-G:ILT2 interaction induces a cell cycle blockade at the G1 phase (Bahri et al., 2006), and inhibits allo-reactive T-cell proliferation (Naji et al., 2007a). Interestingly, it has been proposed that HLA-G isoforms have different impact on the cytokine secretion profile of $CD4^+$ T cells. HLA-G1 was indeed shown to promote T helper (h)2 polarization of naïve T cells (Kanai et al., 2001b; Kapasi et al., 2000) whereas HLA-G5 induces tumor necrosis factor (TNF)-α, IFN-γ, and IL-10 (Kanai et al., 2001a). Morandi et al. recently described that HLA-G5 has an additional effect on $CD4^+$ T cells since it down-regulates expression and function of CCR2, CXCR3, and CXCR4 on different subsets of activated $CD4^+$ T cells, impairing their migratory capability (Morandi et al., 2010).

The immunological functions of HLA-G on B cells are poorly described. It has been shown that in patients who underwent renal transplantation, serum levels of soluble HLA-G are negatively associated with anti-HLA antibodies and with graft rejection, suggesting that soluble HLA-G may possibly act to inhibit the immune humoral responses against HLA (Qiu et al., 2006). Further investigation is needed to better highlight the role of HLA-G in modulating B cell responses.

Myeloid antigen presenting cells (APCs) express both ILT2 and ILT4, which render them targets of HLA-G-mediated modulation. In the presence of HLA-G, myeloid APCs fail to stimulate allo-proliferative T cell responses *in vitro* (LeMaoult et al., 2004) and reduce NK-mediated cytotoxicity (Gros et al., 2008). Moreover, HLA-G inhibits the up-regulation of HLA Class II, CD80 and CD86 on myeloid DCs in response to LPS or allo-activation signals *in vitro* (Ristich et al., 2007) (Gros et al., 2008). Thus, HLA-G inhibits the functions and differentiation of myeloid APCs, leading to improper T lymphocyte activation and to impair NK cytotoxic activity. However, HLA-G does not block myeloid APCs functions, but induces them to differentiate into tolerogenic cells. Several reports indeed show that HLA-G treatment up-regulates the expression of cytokines, chemokines, and chemokine receptors by myeloid APCs (Apps et al., 2007; Gros et al., 2008; Li et al., 2009; Liang et al., 2008). The first evidence that HLA-G promotes the induction of tolerogenic APCs comes from studies conducted by the group of Horuzsko demonstrating that HLA-G treatment of monocyte-derived DCs that highly express ILT2 and ILT4 results in cells with tolerogenic-like phenotype and the potential to induce T-cell anergy (Ristich et al., 2005). Results obtained in a murine model support this notion by showing that HLA-G inhibited maturation of DCs (Horuzsko et al., 2001) and that in ILT4 transgenic mice, the HLA-G:ILT4 interaction impaired DC maturation *in vivo*, leading to delayed skin allograft rejection (Liang et al., 2002). Thereby, HLA-G by interacting with ILT receptors present on myeloid APCs induces their differentiation into regulatory cells.

4.2 Long-term tolerogenic properties of HLA-G

Myeloid APCs are not only the cells more sensitive to HLA-G-mediated modulation, but they also commonly express HLA-G. Myeloid APCs may express all HLA-G isoforms (Le Friec et al., 2004), but cell-surface HLA-G1 and secreted HLA-G5 have been described the most. In pathological contexts including transplantation, cancer, viral infections, and inflammatory diseases, the expression of HLA-G on myeloid cells can be up-regulated (Carosella et al., 2008). Recently, in liver-transplanted patients, the presence of HLA-G-expressing myeloid DCs has been correlated with tolerance and graft acceptance (Castellaneta et al., 2011). Similarly, in cancer (reviewed in (Amiot et al., 2011) as well as in viral infections (reviewed in (Fainardi et al., 2011), myeloid APCs expressing HLA-G were reported, and often correlated with poor clinical outcomes. Myeloid APCs not only express membrane-bound HLA-G1 but they may also secrete or shed HLA-G molecules, contributing to the generation of a tolerogenic microenvironment. Such a microenvironment may alter the functions not only of lymphocytes, but also of the HLA-G-expressing myeloid APCs themselves, in a tolerogenic feedback loop. HLA-G can indeed directly promote its expression and the expression of its receptors on myeloid APCs (LeMaoult et al., 2005). Thus, myeloid APCs may be viewed as suppressor cells capable of inhibiting other immune effectors, and also of generating regulatory cells including Treg cells.

Treg cells are critical players for preservation of immune homeostasis and for establishment and maintenance of peripheral tolerance. Treg cells belonging to different T cell subsets, including $CD4^+$ and $CD8^+$, NKT, and γδ T cells. The best characterized Treg populations are the forkhead box P3 (FOXP3)-expressing, $CD4^+CD25^+$ Tregs ($FOXP3^+$ Tregs) (Sakaguchi et al., 2010), and the $CD4^+$ IL-10-producing Tr1 cells (Akdis et al., 2004; Barrat et al., 2002; Groux et al., 1997; Roncarolo et al., 2006). $FOXP3^+$ Tregs and Tr1 cells

are distinct populations of Treg cells that play a non-redundant role in maintaining tolerance, and are distinguished from one another by their distinct phenotype and cytokine profiling. FOXP3+ Treg cells are characterized by the constitutively-high expression of CD25 and the transcription factor FOXP3 (Sakaguchi, 2005), and their development and function is strictly dependent on FOXP3 (Bacchetta et al., 2007; Gavin et al., 2007). Tr1 cells can be induced in the periphery upon chronic antigen (Ag) stimulation in the presence of IL-10 (Roncarolo et al., 2006), and are currently identified by their unique cytokine profile consisting of high levels of IL-10, TGF-β, and low levels of IL-2 and IFN-γ and the absence of IL-4 after stimulation (Bacchetta et al., 1994; Groux et al., 1997). Once activated through their specific TCR, Tr1 cells secrete IL-10 and TGF-β that directly inhibit effector T cell proliferation and expression of HLA class II and co-stimulatory molecules on APCs, which indirectly suppress effector T-cell activation. More recently, we demonstrated that Tr1 cells specifically kill myeloid cells *via* a Granzyme B-dependent mechanism (Magnani et al., 2011).

HLA-G-expressing APCs were shown to be capable of priming naïve T cells to become Treg cells. Indeed, APC lines over-expressing membrane-bound HLA-G1 induced the differentiation of $CD4^+$ and $CD8^+$ T cells able to inhibit allogeneic responses (LeMaoult et al., 2004). Resulting HLA-G-induced Treg cells included $CD4^{low}$ and $CD8^{low}$ T cells that suppress *via* soluble factors (Naji et al., 2007a; Naji et al., 2007b). These results are in agreement with those obtained in patients who received a combined liver-kidney transplant, in which high plasma levels of HLA-G5 correlated with an increased percentage of suppressor T cells (Le Rond et al., 2004; Naji et al., 2007b). They are also in line with results showing that high HLA-G5 plasma levels in the peripheral blood of stem cell-transplanted patients are associated with the expansion in peripheral blood of $CD4^+CD25^+CD152^+$ T cells with suppressive activity (Le Maux et al., 2008).

We recently identified and characterized a new subset of human DCs that arises in the presence of IL-10 and endogenously expresses cell-surface HLA-G (Gregori et al., 2010). DC-10 are characterized by the outstanding ability to produce IL-10 and by the expression of high levels of membrane-bound HLA-G1 and other tolerogenic signaling molecules such as ILT2, ILT3, and ILT4 (Fig. 3).

DC-10 are potent inducers of adaptive allo-specific Tr1 cells (Gregori et al., 2010). Furthermore, allergen-specific Tr1 cells can be generated *in vitro* by stimulating human T cells with autologous tolerogenic DC-10 pulsed with allergen (Pacciani et al., 2010). Interestingly, the expression of membrane-bound HLA-G1 and that of its receptors are up-regulated by IL-10 on both DC-10 and T cells, and the expression of high levels of membrane-bound HLA-G1, ILT4, and IL-10 by DC-10 is critical to the generation of Tr1 cells by DC-10 (Rossetti et al., 2011).

5. HLA-G expressing regulatory cells

In addition to myeloid cells that can constitutively or transiently express HLA-G, T cells or other immune cells can express or secrete HLA-G.

A subpopulation of $CD4^+$ HLA-$G1^+$ and $CD8^+$ HLA-$G1^+$ T cells has been described (Feger et al., 2007). They represent a small subset of peripheral blood cells in healthy individuals, but

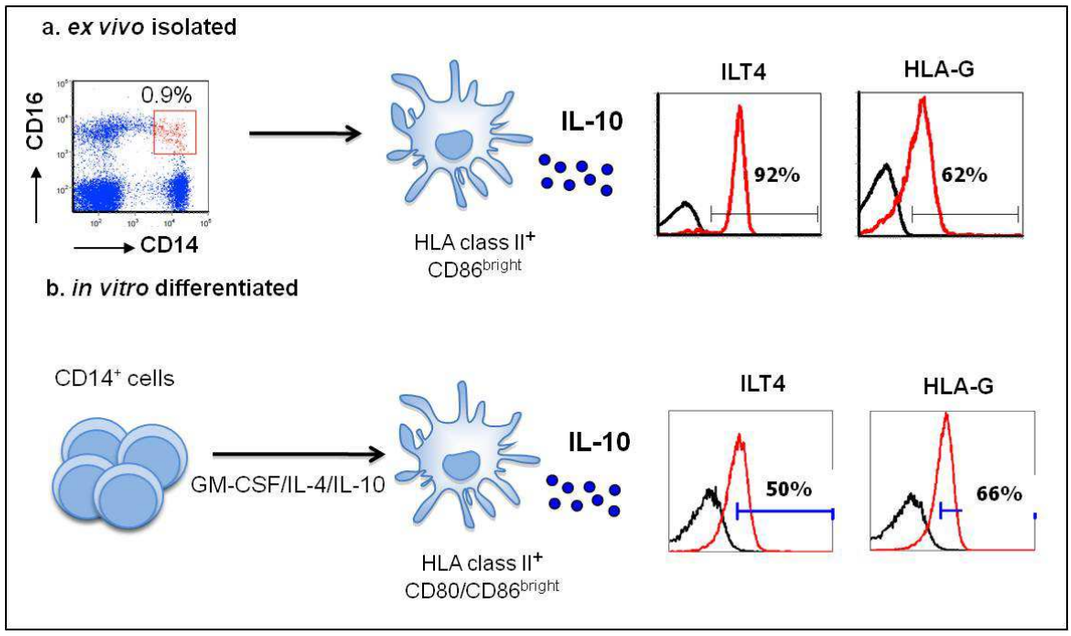


Fig. 3. A unique subset of human tolerogenic DC: DC-10

A. DC-10 are present *in vivo* and characterized by the expression of the surface markers CD14, CD16, CD83. They also express high levels of HLA-G1 and its receptor ILT4. **B.** DC-10 can be differentiated *in vitro* starting from the CD14+ fraction of PBMCs cultured for 7 days in the presence of GM-CSF, IL-4 and IL-10.

are increased at sites of inflammation, such as in the central nervous system of patients with neuro-inflammatory disorders and in muscle tissue in patients with idiopathic myositis (Feger et al., 2007; Huang et al., 2009a; Wiendl et al., 2005). $CD4^+$ $HLA\text{-}G1^+$ T cells represent a population of naturally occurring Treg cells distinct from nTreg cells, since they lack CD25 and FOXP3 expression. $HLA\text{-}G^+$ Treg cells showed reduced proliferation in response to allogeneic (mDCs) and polyclonal stimulation (αCD3/CD28), and have a cytokine profiling different from Tr1 cells. $CD4^+$ $HLA\text{-}G^+$ and $CD8^+$ $HLA\text{-}G^+$ Treg cells inhibit the proliferation of autologous $HLA\text{-}G^-$ T cells through HLA-G1/sHLA-G1 (Feger et al., 2007) and soluble factors, including IL-10 (Huang et al., 2009b). A population of induced $HLA\text{-}G^+$ T cells has been also described, these allo-specific $CD4^+$ and $CD8^+$ T cells were shown to express HLA-G1 and to secrete HLA-G5 (Le Rond et al., 2004; Lila et al., 2001). Moreover, we demonstrated that up-regulation of HLA-G on $CD4^+$ T cells is critically required for DC-10 mediated induction of adoptive Tr1 cells (Gregori et al., 2010).

Adult bone marrow MSCs can also express HLA-G and have been included in the list of regulatory cells. MSCs are multipotent cells that play an important role in tissue regeneration and also have strong immuno-modulatory properties (reviewed in (Uccelli et al., 2008)). MSCs express low levels of HLA Class I molecules, do not express HLA class II or co-stimulatory molecules, and therefore do not induce T-cell activation. It has been demonstrated that MSCs constitutively express HLA-G5 (Selmani et al., 2009). Even though HLA-G expression decrease during MSCs expansion *in vitro*, recent studies clearly identified it as a key contributor to MSCs immune-tolerogenic functions (Nasef et al., 2007; Rizzo et al.,

2008; Selmani et al., 2008). In addition to a direct suppressive function through HLA-G5, MSCs can induce *via* a cell-to-cell contact mechanism $CD4^+$ $CD25^{high}$ $Foxp3^+$ Treg cells (Selmani et al., 2008).

6. HLA-G and its polymorphisms

Very few polymorphisms have been identified in the coding region of the HLA-G locus. The heavy chain encoding region exhibits 33 Single Nucleotide Polymorphisms (SNPs) but only 13 amino acid variations are observed. This peculiarity is important in determining the biological function of HLA-G, since a reduced variability leads to a limited peptide repertoire and presentation capability (Clements et al., 2005), and can influence the polymerization rate. Moreover, the 5′ Up-stream Regulatory Region (5′ URR), containing the promoter, and the 3′ untranslated region (UTR) exhibit numerous nucleotide variations that may influence HLA-G expression (Donadi et al., 2011; Larsen and Hviid, 2009) and consequently its tissue distribution in healthy and pathological conditions (Fig. 4).

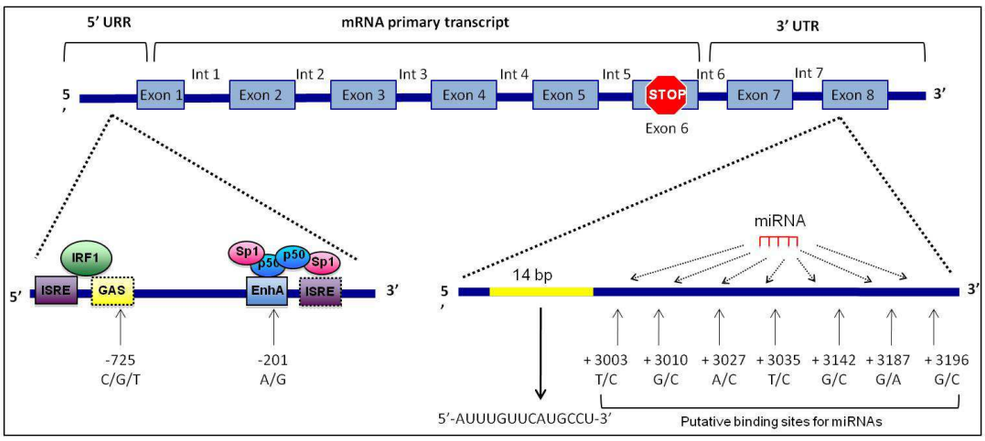


Fig. 4. HLA-G locus and its polymorphisms

Schematic representation of HLA-G locus. The major polymorphisms observed at the 5′URR and at 3′UTR regions are indicated. URR: Up-stream regulatory region; UTR: Untraslated region.

6.1 5′ up-stream regulatory region (URR)

Because of the presence of regulatory elements, polymorphisms at the 5′ URR might have a relevant impact in the regulation of HLA-G expression (Fig. 4). The region up-stream from the transcriptional start site of HLA-G contains 27 different polymorphisms but only 13 of them are within or very close to known transcription factor binding sites or regulatory elements (Hviid et al., 1999; Ober et al., 2003; Tan et al., 2005). Interestingly, all the 27 polymorphisms are in strong linkage disequilibrium (LD) and define 13 unique promoter haplotypes (Ober et al., 2003; Tan et al., 2005). Among the 5′ URR polymorphisms, only the -725C/G/T single nucleotide polymorphism (SNP) has been associated with HLA-G expression. Ober et al. demonstrated that the presence of G in position -725 significantly increases HLA-G transcription rate in JEG-3 cell line (Ober et al., 2006) and, when it is present in both parents, it is significantly associated with fetal loss rate (Ober et al., 2003).

An additional interesting SNP located in 5′ URR is the -201 A/G SNP since it resides into the enhancer A region (Hviid et al., 1999). However, it still remains to be clarified if this polymorphism has any impact on the expression of certain HLA-G alleles.

6.2 3'untraslated region (UTR)

The exon 7 of the HLA-G locus is always absent in the mature messenger(m)RNA. Moreover, due to the presence of a stop codon in the exon 6 of HLA-G, the exon 8 is not translated. Thus, exon 8 is considered the 3′ UTR of the mature RNA. This region contains several regulatory elements (Kuersten and Goodwin, 2003), including polyadenylation signals, AU-rich elements (Alvarez et al., 2009), and several polymorphic sites that may potentially influence mRNA stability, turnover, mobility and splicing pattern. 3′UTR polymorphisms that can influence HLA-G expression are: i) the insertion (INS) or deletion (DEL) of a 14bp fragment (14bp INS/DEL) that has been associated with mRNA stability; ii) the SNP at position +3142, which may be a target for certain microRNAs (miRNAs), small RNA sequences which once bound to a complementary mRNA lead to its translational repression or degradation and gene silencing (Bartel, 2009); iii) additional six SNPs landed in putative binding sites for miRNAs (Castelli et al., 2009) (Fig.4).

The first identified 3′UTR HLA-G polymorphism is the 14bp INS/DEL and has been studied the most. The insertion of the 14bp fragment, results in the formation of a cryptic breakpoint in the mRNA that loose the first 92bp of exon 8 (Hviid et al., 2003). Rousseau et al. demonstrated that HLA-G transcript with the 92bp deleted seems to be more stable than the complete mRNA fragment generated by the 14bp DEL (Rousseau et al., 2003), suggesting that 14bp INS might be associated with high levels of HLA-G expression. Nonetheless, several groups have demonstrated that 14 bp INS/INS genotype is associated with lower serum and plasma level of sHLA-G1/HLA-G5 compared to those observed in 14bp INS/DEL and 14bp DEL/DEL genotypes (Chen et al., 2008; Hviid et al., 2004; Hviid et al., 2006), an observation that constitutes the "14bp paradox". Consistently, conflicting results have been obtained in different studies concerning the association of the 14bp HLA-G genotypes with autoimmune diseases, pathological pregnancy, recurrent spontaneous abortions, and pre-eclampsia. Notably, the presence of 14bp DEL has been found to be predictive for the incidence of GvHD after unrelated (La Nasa et al., 2007) and HLA-identical sibling (Sizzano et al., in press) Human Stem Cell Transplantation (HSCT) for beta-thalassemia.

Several SNPs at 3′UTR of HLA-G locus have been identified (Castelli et al., 2009). Among them, the +3142 C/G SNP has been proposed to be critically involved in HLA-G regulation since it is associated with asthma (Tan et al., 2005). The G variant of a G/C SNP at position +3142 of the 3′UTR has been hypothesized to increase the affinity of the resulting mRNA for miR-148a, miR-148b and miR-152 (Veit and Chies, 2009). Among these miRNAs, only miR-152 has been demonstrated responsible for HLA-G post-transcriptional regulation. Indeed, over-expression of miR-152 in JEG-3 cell lines resulted in decreasing HLA-G expression and increased susceptibility to NK cell–mediated cytolysis (Zhu et al., 2010).

In silico analysis of the 3′UTR of the HLA-G locus revealed the presence of additional six SNPs that are landed in putative binding sites for miRNAs (Castelli et al., 2009). Interestingly the 14bp INS/DEL and the 7 SNPs arrange in different combinations to generate eight distinct haplotypes of 3′UTR HLA-G region (Castelli et al., 2010) (Table 1).

Therefore, it has been hypothesized that the expression of HLA-G might be influenced by a combination of specific polymorphisms. Our group is currently investigating the impact of the HLA-G 3'UTR haplotypes on HLA-G expression. Preliminary results indicate that different 3'UTR HLA-G haplotypes are associated with variable levels of HLA-G1 expression on myeloid APCs.

Haplotype	14-bp	+3003	+3010	+3027	+3035	+3142
UTR-1	Del	T	G	C	C	C
UTR-2	Ins	T	C	C	C	G
UTR-3	Del	T	C	C	C	G
UTR-4	Del	C	G	C	C	C
UTR-5	Ins	T	C	C	T	G
UTR-6	Del	T	G	C	C	C
UTR-7	Ins	T	C	A	T	G
UTR-8	Ins	T	G	C	C	G

Table 1. 3'UTR HLA-G haplotypes

7. Conclusions and perspectives

Since the discovery of HLA-G, research has established its role in modulating immune responses and inducing tolerance. While a great deal of progress has been made in understanding the mechanisms underlying immune modulation by HLA-G, several questions remain to be clarified. First, HLA-G can promote the differentiation of regulatory cells and HLA-G-expressing cells act as regulatory cells, however additional studies are required to better define their role in promoting tolerance. Second, although it is generally accepted that 5' URR and 3' UTR are involved in HLA-G expression, further investigation are needed to elucidate the molecular mechanisms involved in HLA-G regulation. Third, 3'UTR HLA-G haplotypes have been identified, future studies are warranted to define whether they can influence the transcriptional rate of HLA-G isoforms and their functions. Answering these questions will not only bring us closer to understanding how HLA-G function, but also how to exploit or modulate its suppressive activity for targeted therapy against a wide variety of diseases.

8. Acknowledgement

This work was supported by Telethon Foundation, the Associazione Italiana per la Ricerca sul Cancro (AIRC) IG 8978, and the Italian Ministry of Health.

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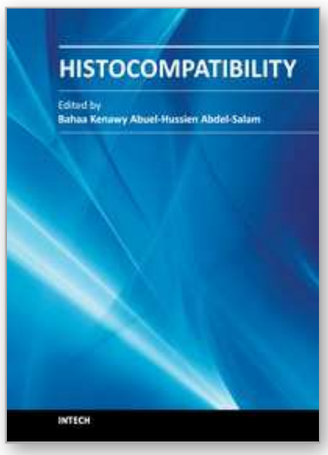
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Histocompatibility

Edited by Dr. Bahaa Abdel-Salam

ISBN 978-953-51-0589-3
Hard cover, 188 pages
Publisher InTech
Published online 02, May, 2012
Published in print edition May, 2012

This book presents some recent researches related to histocompatibility for scientists interested in this field. It includes 10 chapters, in different topics, prepared by Sundararajulu Panneerchelvam and Mohd Nor Norazmi; Giada Amodio and Silvia Gregori; Adema Ribic; Bahaa K. A. Abdel-Salam; Kai-Fu Tang; Roberto Biassoni, Irene Vanni and Elisabetta Ugolotti; Wei-Cheng Yang, Lien-Siang Chou and Jer-Ming Hu; Shatrah Othman and Rohana Yusof; Masahiro Hirayama, Eiichi Azuma and Yoshihiro Komada; Gustav Roder, Linda Geironson, Elna Follin, Camilla Thuring and Kajsa Paulsson.

How to reference

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Giada Amodio and Silvia Gregori (2012). Distinctive Immunological Functions of HLA-G, Histocompatibility, Dr. Bahaa Abdel-Salam (Ed.), ISBN: 978-953-51-0589-3, InTech, Available from: http://www.intechopen.com/books/histocompatibility/distinctive-immunological-functions-of-hla-g

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