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MULTI-AUTHOR REVIEW

The role of HLA-G in immunity and hematopoiesis

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Abstract The non-classical HLA class I molecule HLA-G was initially shown to play a major role in feto–maternal tolerance. Since this discovery, it has been established that HLA-G is a tolerogenic molecule which participates to the control of the immune response. In this review, we summarize the recent advances on (1) the multiple structures of HLA-G, which are closely associated with their role in the inhibition of NK cell cytotoxicity, (2) the factors that regulate the expression of HLA-G and its receptors, (3) the mechanism of action of HLA-G at the immunological synapse and through trogocytosis, and (4) the generation of suppressive cells through HLA-G. Moreover, we also review recent findings on the non-immunological functions of HLA-G in erythropoiesis and angiogenesis.

Keywords HLA-G · ILT · Dimers · Immunological synapse · Trogocytosis · Regulatory cells · Haematopoiesis · Erythroid progenitors · Angiogenesis

Abbreviations

HLA-G	Human leukocyte antigen G
NK cell	Natural killer cell
IS	Immunological synapse
ILT	Immunoglobulin-like transcript
B2M	β2-Microglobulin

Introduction

The expression of the human leukocyte antigen (HLA)-G molecule was initially described at the fetal–maternal interface on cytotrophoblasts. There, it was shown to contribute to the protection of the fetus from the mother's immune system [1]. Further studies have shown that HLA-G expression was not restricted to fetal tissues. Indeed, it was reported that HLA-G is also expressed by adult thymic epithelial cells, erythroblasts, pancreatic islets and mesenchymal stem cells. The restricted expression pattern of HLA-G, its low polymorphism and inhibitory action on immune cell functions, and also the fact that *HLA-G* primary transcript is alternatively spliced leading to seven protein isoforms (four membrane-bound: HLA-G1–HLA-G4; and three soluble: HLA-G5–HLA-G7), constitute four remarkable characteristics that currently distinguish HLA-G from other HLA class I molecules [2].

Besides its restricted expression in healthy tissues, HLA-G expression can be induced in numerous pathological conditions where its tolerogenic function can be either favorable or detrimental for the patient, depending on the nature of the pathology [3]. For example, induction of

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HLA-G expression in allotransplanted patients is correlated with a better allograft acceptance, while in cancer it is associated with an advanced grade of the tumor [4]. In these contexts, HLA-G expression is tightly regulated by micro-environmental factors both at transcriptional and post-transcriptional levels.

The tolerogenic function of HLA-G is mediated through direct binding with inhibitory receptors immunoglobulin-like transcript-2 and -4 (ILT2, ILT4 also known as LILRB1 and LILRB2), and killer immunoglobulin-like receptor (KIR)2DL4, whose expression is tightly regulated. These receptors are differentially expressed by immune cells. While ILT2 inhibitory receptor is expressed by lymphoid and myeloid cells, ILT4 is solely expressed by myeloid cells and KIR2DL4 only by NK cells and some $CD8^+$ T cells [5–8]. The effect of HLA-G interaction with these inhibitory receptors depends on its multimerization state and has been well reported to affect diverse immune responses including T cell proliferation, NK cell and $CD8^+$ T cell cytotoxicity and dendritic cell maturation [3, 9]. Moreover, HLA-G has been shown to induce different subsets of suppressive/regulatory cells.

Besides its role on the immune system, HLA-G is also involved early in embryo development by favoring its implantation, and in the initial steps of hematopoiesis and angiogenesis [10].

In this review, we report the latest advances of HLA-G-mediated tolerance at both molecular and cellular levels, and the functions of HLA-G unrelated to the immune response.

The multiple structures of HLA-G

HLA-G has multiple shapes: (1) it can already be expressed as seven different isoforms due to alternative splicing of its primary transcript, (2) the structure of HLA-G1 and HLA-G5 isoforms is similar to that of classical HLA class I molecules and can be found as heterotrimers (heavy chain, β2M, peptide) or as free heavy chain [11], and (3) membrane-bound isoforms of HLA-G can be shed by proteolytic cleavage, giving rise to soluble HLA-G isoforms that may differ from the secreted ones [12]. Considering the possibility that all isoforms may be shed, as is HLA-G1, this brings the number of HLA-G possible isoforms/structures to 14, of which 12 are already published [11–18]. These are the simplest HLA-G structures and, until recently, they were the basis for HLA-G research. Since the demonstration that HLA-G commonly forms homomultimers [19, 20] which carry most if not all of its inhibitory functions, things have changed and the identification, characterization, and use of possible HLA-G structures currently motivate active research.

The first crystal structure of HLA-G was published in 2005 [21] and was that of an HLA-G1/β2M/peptide heterotrimeric complex, also called an HLA-G1 monomer. This report confirmed that the HLA-G1 monomer globally resembles classical HLA class I molecules, but differs at the level of its peptide binding groove and its alpha-3 domain. The peptide binding groove of HLA-G resembles that of HLA-E, with an extensive network of contacts that constrains the repertoire of peptides that HLA-G can present. However, the HLA-G alpha-3 domain structure differs from and is more hydrophobic than those of classical HLA class I molecules. The authors hypothesized that this may be the basis for the higher affinity of HLA-G for LILRB1/ILT2.

Dimers of HLA-G molecules were first evidenced in 2002, including at the surface of transfected cells [19]. Dimerization of HLA-G was shown to happen mainly because of a unique cysteine residue at position 42, which allowed the formation of a disulfide bond between two HLA-G molecules. Another free cysteine, Cys147, may also participate in the formation of Cys147–Cys42 dimers, but with less efficiency. Crystal structures of HLA-G1 dimers [22] showed that they are made up of molecules joined head-to-tail by Cys42–Cys42 disulfide bonds, and that dimerization does not induce significant structural changes on the main frames of the monomers. However, HLA-G1 homodimers had an oblique orientation that exposed the receptor-binding sites of the alpha-3 domain upwards and made it more accessible. From these data, the authors also hypothesized that membrane-bound and soluble HLA-G homodimers should only differ slightly. Such soluble HLA-G5 homodimers were later evidenced in vitro [23]. Finally, HLA-G1 homodimers were found at the cell surface of naturally expressing cells in vitro [13, 23] and at the surface of human extravillous trophoblast cells in vivo [23], providing their physiological relevance. Dimerization does not concern only HLA-G1/G5 properly folded and associated with β2M. Indeed, HLA-G HLA-G1/G5 free heavy chain (fHC) dimers [13, 16] and HLA-G2 dimers [24] were evidenced. HLA-G5 fHC homodimers were even detected in vivo and seem to be the main HLA-G5 structure produced by human villous trophoblast cells [16].

All these various structures would actually not count for much if monomers and dimers had equivalent functions, but in the case of HLA-G, it seems that direct inhibitory function depends on multimerization [20]. Because HLA-G dimers take an oblique orientation, the ILT2 and ILT4 binding sites of the alpha-3 domains are more accessible. Consequently, HLA-G dimers can bind two ILT receptors, and with a higher affinity and slower dissociation rates than monomers (K_d of monomers vs dimers were calculated at 3.5 μM vs ~6.7 nM for ILT2, and 15 μM vs 750 nM for ILT4) [22]. In accordance with this, biochemical data showed that ILT2 bound mostly cell surface dimers in vitro [20, 23] and in vivo [23], that dimers signaled through ILT2 much more efficiently than monomers [22], and that

the inhibitory function of HLA-G is mostly due to dimers, not monomers [20, 23].

All these reports are turning points in the field of HLA-G research: thus far, only 14 monomeric structures were really taken into account, out of which only two are routinely tested by ELISA, but since all HLA-G isoforms possess the Cys42 responsible for dimerization, all translated isoforms could theoretically form dimers, as well as shed ones and β2M-free ones. This would increase the number of possible dimeric structures. Furthermore, one can count HLA-G homotrimers, and also hypothesize that, since Cys42 is common to all monomers, heterodimers of various HLA-G monomers might exist and function.

This sudden diversification of HLA-G possible shapes has added to the complexity of HLA-G in vivo regulation, titration, and physiopathological significance. Indeed, there is much data relating to the functions of HLA-G1 and HLA-G5, but very few that concern HLA-G isoforms; furthermore, most of these data were obtained using in vitro systems and cells transfected with HLA-G1 or HLA-G5 cDNA-containing plasmids, thus biasing the experimental systems towards the most classical HLA class I-like of the HLA-G molecules. The same bias now applies to in vivo HLA-G. Indeed, in order to establish HLA-G relevance in vivo, ELISA tests were set up because they constitute a relatively high throughput methodology, and the most used ones were set up using antibodies that detect only β2M-associated HLA-G1 and HLA-G5 [25]. In truth, these ELISA methods did allow fast titration of HLA-G1/G5 in plasma samples, and were the means by which HLA-G physiopathological significance was eventually established, particularly in the contexts of transplantation and in vitro fertilization (see the corresponding reviews in this special issue). Their downside is their restricted specificity for some HLA-G structures. To cite but one example: the presence of HLA-G in the plasma of heart-transplanted patients and its pathological significance was established by immunoprecipitation and showed that two of five patients in a first study [26], and two or nine patientsin a second study [27], expressed HLA-G6 and not HLA-G5. Had β2-microglobulin-associated HLA-G1/G5-specific ELISA been used, these patients would have been labeled "HLA-G negative", and the significance of the correlation between HLA-G presence in the plasma and the reduced incidence of rejection would have been decreased.

Factors regulating the expression of HLA-G and its receptors

HLA-G exerts its function in selective sites whose microenvironment will be permissive for its expression. Besides expression at the fetal–maternal interface, HLA-G proteins can be found after organ transplantation, malignant transformation, viral infections and inflammatory diseases [28]. In these situations, HLA-G may be expressed by the tumor, virus-infected or grafted cells, and also by infiltrating immune cells. Such selective spatio-temporal expression of HLA-G is directly linked to the capacity of the injured cells and tissues to respond to microenvironmental factors including: stress [29], nutrient deprivation [30], hypoxia [31], hormones such as progesterone [32], and cytokines such as GM-CSF [33], IFNs [34], IL-10 [35, 36], TNF-α [37], TGF-β [33], and LIF [38]. These factors regulate HLA-G expression at either transcriptional or post-transcriptional level (for a complete review, see [39]).

Recent studies have shed new light on factors regulating HLA-G gene and protein expression. Among these factors, endogenous as well as exogenous (i.e., drugs) agents have been characterized. Studies carried out on the association between HLA-G and allograft acceptance have permitted the identification of IFN-γ, TNF-α, and, for the first time, epidermal growth factor (EGF), as factors up-regulating *HLA-G* in primary cultured bronchial epithelial cells in vitro [40]. This result provides mechanistic basis for HLA-G expression in accepted lung transplants [40]. EGF is present in the lung allograft microenvironment where it plays an important role in the regulation of airway inflammation and fibrosis [41]. Whether EGF-mediated HLA-G synthesis in bronchial epithelial cells participates in the control of the inflammatory and remodeling response following lung transplantation remains to be defined. HLA-G was also found to confer protection from acute rejection and cardiac allograft vasculopathy in heart transplant recipients [27, 42]. In this context, progesterone, a steroid hormone, induces HLA-G expression in vascular endothelial and smooth muscle cells in vitro [43]. Since allograft vasculopathy remains a major complication post-heart transplant, induction of HLA-G in vascular endothelial and smooth muscle cells might represent a novel therapeutic strategy to protect against heart graft rejection.

Like HLA-G, progesterone is expressed in high amounts from the trophoblast during pregnancy along with which they both exert immunomodulatory activities [1, 44]. At the maternal–fetal interface, these molecules are in contact with mesenchymal stem cells (MSC) whose inhibitory properties towards immune effectors have been well characterized [36, 45]. A recent study showed that expression of HLA-G by human MSC isolated from adipose tissue, bone marrow or decidua, is up-regulated by progesterone at both mRNA and protein levels [46]. Hence, MSC appear as newly detected targets of progesterone that are likely involved in the immune interactions at the maternal–fetal interface where these cells may contribute to both tissue remodeling and maintenance of maternal–fetal tolerance. The effect of progesterone on HLA-G

expression was recently supported in breast cancer. Indeed, with regard to oncology, HLA-G expression has been detected in a variety of human neoplastic diseases in situ, including breast cancer [47]. Around two-thirds of women with breast cancer have estrogen/progesterone receptor (ER/PgR)-positive disease. The selective estrogen modulator tamoxifen has been offered as a standard first-line endocrine therapy for ER-positive cancer patients for several decades [48]. RU486, a progesterone antagonist, has also been shown as a growth inhibitory agent [49]. In this context, HLA-G mRNA and protein expression was enhanced in breast cancer cells in vitro upon estradiol/progesterone treatment while reduced by their antagonists, tamoxifen and RU486 [50]. These findings represent an additional mechanism of action for the two anti-cancer drugs, in that they may improve host immune status by inhibiting HLA-G expression in cancer cells thereby blocking HLA-G-mediated tumor escape from immunosurveillance.

The influence of exogenous factors that regulate HLA-G expression in patients is receiving increased attention. In this regard, soluble HLA-G plasma levels were found to be enhanced after cyclosporine or tacrolimus administration in some heart transplant patients [51]. The effect of the immunosuppressive therapy on HLA-G expression was supported by recent results showing augmented HLA-G plasma levels in kidney transplant patients treated with the CTLA4-Ig fusion protein (Belatacept) [52]. This therapeutic agent is associated with better graft function and survival. Definition of the mechanism by which such therapy promotes HLA-G expression showed that dendritic cells secrete soluble HLA-G upon CTLA4-Ig treatment. CTLA4-Ig-treated DC acquire tolerogenic properties as they suppress T cell proliferation through soluble HLA-G release [52].

The expression of HLA-G inhibitory receptors can also be regulated. Of note, HLA-G is able by itself to control the expression of its own receptors on APC, T cells and NK cells [53]. Interestingly, HLA-G and its receptors ILT2 and ILT4 were found up-regulated in the course of the same pathologies including HIV infection [54, 55], breast carcinoma [47], and skin inflammatory diseases [56, 57], suggesting that common factors may influence both expressions. Accordingly, tryptophan deprivation through indoleaminedioxygenase (IDO) activity was described as inducing HLA-G as well as ILT4 on DC [58, 59]. Functionally, tryptophan-deprived DC show a reduced capacity to stimulate T cells, while increasing the induction of $CD4^+CD25^+FOXP3^+$ T regulatory cells [58, 59].

Cyclosporin A (CsA) is a calcineurin inhibitor and rapamycin (RAPA), an inhibitor of the mammalian target of rapamycin (mTOR) which are conventionally used to treat transplant rejection through their capacity to affect T cell alloproliferation by limiting IL-2 synthesis or IL-2 receptor-mediated signaling. These agents were also found to modify the alloimmune response by interfering with DC function. Indeed, analysis of the influence of these immunosuppressants on ILT2, ILT3, ILT4 and HLA-G expression during DC differentiation from monocytes showed that RAPA, unlike CsA, down-regulates the costimulatory molecules CD40, CD80, and CD86 as well as the inhibitory receptors on DC [60]. RAPA produced no effect on HLA-G compared to controls. These RAPA-treated DC exhibited tolerogenic properties as evidenced by their low allostimulatory capacity but did not increase the numbers of $FOXP3^+$ regulatory T (Tr) cells [60]. This result contrasts with the increased expression of ILT3 and ILT4 on DC treated with IFN-α and IL-10 conferring tolerogenic properties to DC as defined by inhibition of allogeneic T cell proliferation and generation of allospecific T regulatory cells [61]. Notably, both IFN-α and IL-10 are also known as HLA-G inducers [35, 39]. Induction of ILT expression on non-professional APC mainly endothelial cells, represents a promising platform to induce tolerance towards allografts without compromising the recipient's immune system. Indeed, endothelial cells represent the first barrier between the donor organ and the recipient immune system. Expression of ILT3 and ILT4 has been demonstrated in endothelial cells in vitro and in vivo, resulting in modulated endothelium-dependent T cell activation [62].

Niflumic acid, a member of non-steroidal anti-inflammatory agents renders DC tolerogenic through up-regulation of ILT3 and ILT4 [63]. These results indicate that, in addition to its anti-inflammatory actions, niflumic acid exerts immunosuppressive properties which may be therapeutically useful in controlling chronic immune and/or inflammatory diseases. Whether niflumic acid modulates HLA-G expression remains unknown.

DC and monocytes are activated by numerous stimuli among which are microbial components such as lipopolysaccharide (LPS) binding to toll-like receptor (TLR). Bacterial LPS induces the release of ATP which participates in the inflammatory response as a proinflammatory mediator by activating the inflammasome complex, inducing IL-1 secretion and cell damaging agents such as oxygen radicals, cationic proteins and metalloproteases. A recent study has shown that extracellular ATP can also act as a proinflammatory mediator by inhibiting production of tolerogenic molecules such as IL-10 and HLA-G in monocytes [64].

Mechanisms of tolerance induced through HLA-G at the immunological synapse

During NK/target cell interaction, the integration of the signals delivered by target cell ligands to NK cell receptors

is accompanied by a specific molecular reorganization of NK cell surface receptors and cytoplasmic components. This supra-molecular structure has been named NK cell immunological synapse (IS) [65]. The NK cell IS can be either inhibitory or activating depending on the nature of the ligand/receptor interactions involved [66].

Even though it is well established that HLA-G expression by tumor cells protects them from NK cell cytolytic activity, it was not known how the NK cell synapse was organized during this process. In a recent report, the molecular events taking place at the NK cell IS upon interaction between HLA-G1 and ILT2 was investigated.

In this study, it was shown that the expression of HLA-G1 by tumor target cells inhibits cytoskeleton reorganization at the NK cell IS [67]. Indeed, stimulation of ILT2 through HLA-G1 binding led to the inhibition of both filamentous actin (F-actin) accumulation and microtubule-organizing center (MTOC) polarization at the NK cell IS. Such a default of cytoskeleton reorganization was associated with an impairment of NK cell lytic granules polarization toward target cells. Time-lapse video microscopy experiments showed that, in conjugates formed between NK cells and target cells negative for HLA-G1, lytic granules rapidly polarize to the area of contact whereas they remain diffuse in the cytosol of NK cells conjugated with target cells expressing HLA-G1 isoform at their surface.

These results are in agreement with previous studies showing a relationship between cytoskeleton rearrangements at the NK cell IS and efficient delivery of NK cell lytic granules at the area of contact [66, 68]. Even though the molecular signaling components relying on actin cytoskeleton and MTOC to lytic granules movement are not yet fully characterized, it has been clearly evidenced that cytoskeletal rearrangements in NK cells depend on Vav protein family through the activation of Rac, Cdc42 and Rho GTPases [69, 70]. A new actin cytoskeleton remodeling factor named HS1 has been recently identified as an upstream component involved in the reorganization of NK cell cytoskeleton [71]. In this regard, HS1 may represent a potent target of SHP-1 and/or SHP-2 phosphatases of which recruitment and activation would be induced through ILT2 upon its interaction with HLA-G1. However, biochemical studies will be required to determine if phosphatases activated through ILT2/HLA-G1 interaction act directly on HS1 activity.

A peculiar feature of this NK cell inhibitory IS was that it occurred in the absence of both ILT2 and HLA-G1 accumulation at the contact site. Previous studies have shown that, in the "classical" inhibitory NK cell IS, NK cell inhibitory receptors and HLA molecules accumulate at the area of contact during their interaction [65, 72, 73]. Even though the localization of ILT2 and HLA-G1 at the NK cell inhibitory IS was analyzed at different time points, their accumulation at the area of contact was never observed. These results are in agreement with previous observations on intercellular transfer of HLA-G1 from target cell to NK cell by trogocytosis [74]. Indeed, analysis of HLA-G1 transfer in NK/target cell conjugates by confocal microscopy never revealed any accumulation of HLA-G1 at the NK cell synapse, prior, during or after its transfer from target cell to NK cell. Since surface plasmon resonance studies have revealed that HLA-G1 shows a 50-fold higher avidity for ILT2 than classical HLA class I molecule, it might therefore be possible that the ILT2/HLA-G interaction does not require accumulation at the NK cell IS to deliver an efficient inhibitory signal [75].

Two studies have reported for the first time that CD2 or 2B4 activatory receptors accumulated at the inhibitory NK cell IS [67, 76]. One hypothesis to explain the accumulation of some activatory receptors at the inhibitory NK cell IS is that it could constitute a mechanism that facilitates inhibition by maintaining in close proximity to activatory receptors with the tyrosine phosphatases SHP-1 and SHP-2 recruited by ILT2. However, the molecular mechanism leading to the accumulation of activating receptors at the inhibitory NK cell synapse despite the absence of F-actin reorganization is a question which remains to be elucidated.

The soluble HLA-G5 isoform has been shown to be secreted by several cancer types and to correlate with metastasis and advanced grade [3, 77]. In this regard, experimental data indicate that the NK cell cytotoxic activity was inhibited by tumor target cells expressing soluble HLA-G5. However, soluble HLA-G5 alone was not able to inhibit NK cell cytotoxic activity. Therefore, the effect of HLA-G5 secreted by tumor target cells on NK cell IS structure in NK/target cells (HLA-G5$^+$ or HLA-G$^-$) conjugates was investigated.

In NK cell/target cells, HLA-G5$^+$ conjugates, both F-actin accumulation and lytic granules polarization toward target cells, were impaired. By contrast, in NK/target cell HLA-G$^-$ conjugates F-actin and lytic granules were found accumulated at the NK cell IS [78]. These observations could result from the fact that HLA-G5 inhibitory action requires NK/tumor target cell conjugate formation in order to be efficient. Indeed, by such a mechanism, the concentration of HLA-G5 secreted in the interstice of the NK/tumor target cell conjugates might be locally higher, thus allowing efficient inhibition of NK cell cytotoxic function through inhibitory receptor interaction. Confocal microscopy analysis and three-dimensional reconstruction of tumor target cells indicated that HLA-G5 localization was evenly distributed in the cytoplasm of tumor target cells even after 30 min of conjugate with NK cells. Despite the homogeneous distribution of HLA-G5, the polarization of NK cell

perforin lytic granules toward the target cell was inhibited. These results suggest that the release of HLA-G5 by the target cell is not specifically polarized toward the NK cell but that it can nevertheless reach concentrations in the interstice that are sufficiently high to block the functions of the effector cells and protect the target cell NK cell-mediated cytotoxicity.

Various studies have reported that HLA-G molecules were able to form dimers through a cystein present in position 42 of the alpha-1 domain and that dimers of HLA-G are much more efficient than monomers in inhibiting NK cell cytotoxicity [19, 20]. It has been determined by western blot analysis in non-reducing conditions that HLA-G5-positive target cells, used for inhibitory NK cell IS studies, produced monomers and dimers in equal proportions. These data are in agreement with previous reports and suggest that the inhibition of NK cytotoxic activity induced by tumor target cells might be mediated mainly through secreted HLA-G5 dimers rather than monomers. However, engineering of tumor target cells secreting only HLA-G5 monomers (i.e., mutated on cystein 42) will be helpful to determine if dimerization of secreted HLA-G5 is necessary to induce NK cell inhibitory IS formation.

The inhibition of F-actin reorganization through ILT2 triggering was initially reported on T cells [79]. In addition, $CD8^+$ T cell cytolytic function has been shown to be inhibited upon interaction of ILT2 with HLA-G1 expressed by tumor target cells [80]. Since $CD8^+$ T cell cytolytic activity and its IS structure are linked to antigen concentration, it would be interesting to determine if the strength of the inhibitory signal delivered by ILT2/HLA-G1 interaction modify the structure of $CD8^+$ T cell IS equally at low and high concentrations of antigen.

HLA-G1 mechanism of action through trogocytosis

In 2002, a report by Wiendl et al. [81] stated that "Few HLA-G-positive cells are sufficient to inhibit alloreactive lysis of HLA-G-negative glioma cells". In this study, the capability of allogeneic PBMC to lyse mixtures of HLA-G-positive and HLA-G-negative glioma cells was investigated. It was reported that significant inhibition of lysis was observed when 10% or more of the target cells expressed HLA-G [81]. This means that HLA-G-positive glioma cells protected HLA-G-negative glioma cells from destruction. However, this is inconsistent with the simple "HLA-G equals shield" mode of action, in which effector cells are inhibited by the engagement of HLA-G molecules expressed by the target cells. Indeed, had this model been strictly true, only HLA-G-positive glioma cells would have been protected.

Recent reports have resolved this issue, by evidencing intercellular transfers of functional membrane-bound HLA-G, which constitutes a novel mode of action for this molecule.

Inter-cell exchange of membrane-bound antigens was first demonstrated in 1973 in the context of LPS transfer between lymphocytes [82], and since then, various mechanisms have been described for it. One can cite uptake of shed antigens, uptake of apoptotic cell antigens, uptake of exosomes, nanotube formation, and trogocytosis (for a review of each of these various mechanisms, see [83]). Trogocytosis (reviewed in [83, 84]) is a mechanism of fast, cell-to-cell contact-dependent uptake of membranes and associated molecules from one cell by another. Trogocytosis has been demonstrated for α/β T cells [85–87], γ/δ T cells [88], B cells [89], NK cells [90], antigen-presenting cells [91], and tumor cells [92]. Molecules whose transfers have been studied the most include but are not restricted to MHC-I, MHC-II, CD54, CD80, CD86, HLA-G, Fc receptors and inhibitory receptors. Regardless of the cell types involved, the main parameters of trogocytosis are (1) a dependence on cell-to-cell contact, (2) fast transfer kinetics in the order of minutes, (3) transfer of membrane patches containing intact membrane-bound molecules and proper orientation on the new cell, and (4) limited lifetime of the acquired proteins at the surface of the acquirer cell. Functionally, acquisition of large membrane patches by one cell from another may temporarily endow the acceptor cell with some functions of the donor cells. Indeed, (1) $CD8^+$ T cells which acquired their cognate MHC class I + peptide ligands became susceptible to "fratricide" antigen-specific cytolysis [86, 87, 93], and (2) T cells which acquired HLA-DR and CD80 could stimulate resting T cells in an antigen-specific manner, and thus behave as APC themselves [94–96].

In three recent studies [74, 97, 98], it was shown that activated $CD4^+$ and $CD8^+$ T cells, activated NK cells, and monocytes can acquire HLA-G1-containing membrane fragments from HLA-G1-expressing antigen-presenting cells (APCs) and tumor cells. For these three cell subsets, acquisition of HLA-G1 was cell-contact dependent, fast (a few minutes), temporary (a few hours), and concerned not only HLA-G1, but all molecules contained within the transferred membrane patch. The interaction of HLA-G with one of its receptors was not required for trogocytosis, and the molecules responsible for HLA-G transfer remain undetermined. Functionally, the consequences of HLA-G acquisition clearly differed for lymphocytes and monocytes. Concerning lymphocytes, upon acquisition of HLA-G1-containing membranes, effector $CD4^+$ T cells stopped proliferating, stopped responding to stimulation, and behaved as regulatory T cells capable of inhibiting the reactivity of autologous T cells in vitro [84]. Similarly,

upon acquisition of HLA-G1-containing membranes from tumor cells, effector NK cells stopped proliferating, stopped being cytotoxic towards legitimate targets, and behaved as regulatory cells capable of inhibiting the cytotoxic functions of other NK cells [74]. This immediate functional inversion from effector cell to regulatory cell was directly due to acquired cell-surface HLA-G1 as shown by specific blocking of HLA-G1/ILT2 interactions. In summary, effector cells of the lymphocyte lineage that acquired HLA-G1 by trogocytosis acted as HLA-G^+ regulatory cells through membrane-bound HLA-G1 for a limited amount of time.

Monocytes did not behave as did lymphocytes upon acquisition of HLA-G1-containing membranes: even though their capability to acquire membranes by trogocytosis was greater than that of lymphocytes, and even though in a few cases monocytes behaved as regulatory cells after they acquired HLA-G, in 90% of the cases, acquisition of HLA-G by monocytes had no impact on their function. This was not related to differences in acquired HLA-G structural features, but to a faster membrane internalization rate in monocytes than in lymphocytes, which caused acquired HLA-G to be removed from the monocytes surface in <2 h, compared to 24 for lymphocytes. This reduced lifetime of acquired HLA-G at the monocytes cell surface may be insufficient for a function to be observed.

Through trogocytosis, a few HLA-G1-expressing tumor cells might thus protect a comparatively larger area by: (1) quickly increasing locally the number of regulatory HLA-G1-positive cells without time-consuming and potentially hazardous unlocking of HLA-G expression; and (2) spreading HLA-G1 presence to a larger area than that covered by the few HLA-G1-expressing cells; this would (3) block the function of any effector cell in that area; and (4) dampen/stop the local reaction, preventing damage to tissues in the vicinity of HLA-G1-expressing cells. Trogocytosis of HLA-G by lymphocytes might therefore explain how a few HLA-G-positive cells are sufficient to inhibit alloreactive lysis of HLA-G-negative glioma cells [81].

Trogocytosis of HLA-G1 represents a new mechanism of immune regulation through the direct and immediate conversion of effector cells into temporary regulatory cells in situ, providing local immune suppression without affecting the overall reactivity of the immune system. Such a mechanism might occur in all contexts in which HLA-G is membrane bound, and would be particularly significant in those where HLA-G expression is heterogenous and/or scarce, including pregnancy, transplantation, oncology, and autoimmunity. Depending on the situation, the generation of a tolerogenic milieu through trogocytosis of HLA-G and emergency immune suppression might therefore prove beneficial, or deleterious.

Induction of suppressor/regulatory cells through HLA-G

Regulatory T (Tr) cells are critical players for preservation of immune homeostasis and for establishment and maintenance of peripheral tolerance. Tr cells belonging to different T cell subsets, including $CD4^+$ and $CD8^+$, NKT, and $\gamma\delta$ T cells, have been described. The best-defined Tr cells are the natural occurring $CD4^+$ Tr (nTr) cells and the adaptive $CD4^+$ IL-10-producing type 1 regulatory T (Tr1) cells. nTr cells arise from the thymus and their suppressor function is strictly dependent on high expression of the transcription factor FOXP3 (forkhead box 3) [99, 100]. Several markers, including CD25, CTLA-4, and GITR, are constitutively expressed by human nTr cells; however, none of them, including FOXP3, can be used as a bona fide marker of human nTr cells [101], as they are up-regulated in activated effector human T cells. Recently, it has been shown that GARP, an orphan toll-like receptor, which is selectively expressed only by activated human nTr cells, but not in activated effector T cells, represents a bona fide marker of human nTr cells [102, 103]. nTr cells suppress T cell responses mainly by contact-dependent mechanisms [104], which are still not completely elucidated. Adaptive IL-10-producing Tr1 cells are induced in the periphery upon chronic antigen (Ag) stimulation in the presence of IL-10 [105]. Tr1 cells are defined by the ability to produce high levels of IL-10 and TGF-β, low amounts of IFN-γ and IL-2, and detectable levels of IL-5, in the absence of IL-4 [106–108]. A variety of membrane-bound markers have been described to identify Tr1 cells, including ICOS [109, 110], or CD18 [111]. However, none of these markers has been confirmed in different experimental systems and, therefore, so far no molecular marker that specifies Tr1 cells has been found. Tr1 cells are induced in an Ag-specific manner; therefore, in contrast to other Tr cells, they are Ag-specific. Once activated through their specific TCR, Tr1 cells secrete IL-10 and TGF-β that directly inhibit effector T cells proliferation and expression of MHC class II and costimulatory molecules on antigen-presenting cells, which indirectly suppress effector T cells activation. IL-10-producing Tr1-like cells generated with CD3/CD46 cross-linking [112] or classical Tr1 cells (unpublished observations) can suppress T cell responses via perforin and granzyme B.

Increasing evidence indicates that long-term immunomodulatory effects of HLA-G are associated to its ability to induce suppressor cells [113, 114]. Focusing on $CD4^+$ T cells, several populations of Tr cells expressing HLA-G or induced by HLA-G have been reported. However, it has been difficult to determine whether these cells are related to one of the well-characterized Tr cells. In this section, we will try to classify HLA-G-related $CD4^+$ Tr cells according

to their phenotype and mechanisms of suppression. Moreover, we will attempt to establish whether the mode of Tr cell induction and/or the HLA-G isoforms involved in their generation might influence their phenotype and function.

HLA-G-expressing Tr cells

HLA-G1-expressing T cells, although at low frequency, emerge from allostimulated T cells after mixed lymphocyte reaction (MLR) in vitro, and in patients who received a combined liver–kidney transplant in vivo [115]. However, it was not clear whether those cells were pre-existing cells or were induced from naïve T cells upon exposure to Ag. Only recently, it has become evident that a population of $CD4^+$ T cells expressing membrane-bound HLA-G1 ($CD4^+HLA\text{-}G^+$ T cells) is present in both peripheral blood and thymus of healthy individuals [116], and that it is enriched at sites of inflammation [116, 117]. $CD4^+HLA\text{-}G^+$ T cells are Tr cells that secrete HLA-G5 [116] and IL-10, but not TGF-β [118], and, although they seems to be of thymic origin, they do not express FOXP3 [116]. Notably, $CD4^+HLA\text{-}G^+$ Tr cells in inflamed tissues express high levels of ICOS [117], a marker associated with IL-10-producing T cells in peripheral blood [110]. $CD4^+HLA\text{-}G^+$ Tr cells have been proposed to preferentially migrate into inflamed central nervous system (CNS) due to the selective expression of CCR5 [117]. However, it cannot be excluded that $CD4^+HLA\text{-}G^+$ Tr cells present in CNS are de novo induced from the $HLA\text{-}G^-$ T cells. Suppression mediated by $CD4^+HLA\text{-}G^+$ Tr cells does not require cell-to-cell contact, and is dependent on HLA-G [116], and on IL-10, but not TGF-β [118]. Thus, although $CD4^+HLA\text{-}G^+$ Tr cells are derived from the thymus, they do not express FOXP3 and suppress via a mechanism that is dependent on soluble mediators.

HLA-G-induced Tr cells

T cells primed with HLA-G1-transfected KG1a or LCL (HLA-G1-expressing APC) differentiate in vitro into suppressor T cells, called HLA-G-induced Tr cells [119–121]. HLA-G-induced Tr cells are characterized by low expression of CD4 (or CD8), and do not express high levels of CD25 and FOXP3 [121]. HLA-G-induced Tr cells suppress T cell responses via soluble factors, including IL-10, but not HLA-G [121]. Thus, HLA-G-induced Tr cells are functionally similar to adaptive IL-10-producing Tr1 cells, but are still distinct from them as they are phenotypically different, are not anergic, and are not induced in an Ag-specific manner. Recently, a novel subset of human tolerogenic DC, termed DC-10, which express high levels of membrane-bound HLA-G1 and of other tolerogenic molecules including ILT2, ILT3, ILT4 has been described [114, 122]. Stimulation of $CD4^+$ T cells with allogeneic DC-10 promotes the induction of a population of anergic T cells, which contains a significant proportion of allo-specific IL-10-producing Tr1 cells. Suppression mediated by Tr1 cells induced by DC-10 is Ag-specific and it is dependent on IL-10 and TGF-β[122].

HLA-G5, which is released in biologic fluids, not only inhibits allogeneic T-cell proliferation but also induces suppressor T cells both in vitro [120] and in vivo [121, 123]. High levels of HLA-G5 in plasma of patients who received a combined liver–kidney transplant correlate with high plasma levels of IL-10, increased percentage of suppressor T cells, and graft survival [121]. Likewise, high HLA-G5 levels in plasma of patients transplanted with peripheral blood stem cell are associated with expansion in peripheral blood of $CD4^+CD25^+CD152^+$ T cells with suppressive activity, and no acute Graft versus Host Disease [124]. Addition of HLA-G5 during T cell priming promotes the induction of a population of Tr cells that are superimposable to those generated with HLA-G1-expressing APC [121]. In this system, HLA-G5 was used to prime T cells in the presence of APC that, by providing additional signaling to T cells (see later), favor the induction of Tr cells which, although mediating suppression via IL-10, are distinct from bona fide Tr1 cells. Moreover, HLA-G5 secreted by MSCs, when added as third party irradiated cells in MLR, inhibits allo-proliferation of T cells and endorses the generation of $CD4^+CD25^+FOXP3^+$ suppressor cells [36]. Based on these findings, we might conclude that HLA-G5 induces populations of Tr cells that are distinct in their phenotype and mode of action depending on the microenvironment in which they are generated. In the presence of APC, HLA-G5 promotes the differentiation of suppressor cells that inhibit T-cell responses via IL-10, whereas in the presence of MSCs, which produce other soluble factors such as TGF-β, PGE-2, and HGF, HLA-G5 induces a population of Tr cells which resemble nTr cells. Similarly to HLA-G5, shed soluble HLA-G1 (sHLA-G1) inhibits allogeneic T-cells responses in vitro [121], and induces the differentiation of a population of suppressor T cells (unpublished observations). Shed sHLA-G1-induced Tr cells express CD25 but not FOXP3, produce low levels of IL-10, and IL-4, and do not suppress T cell-mediated responses via IL-10. Thus, shed sHLA-G1-induced Tr cells are distinct from Tr1 cells.

HLA-G is not only directly, but also indirectly, involved in Tr cell induction [125–127]. Treatment of DC isolated from human ILT4-transgenic mice with HLA-G1 tetramers results in a population of tolerogenic DC, called HLA-G-modified DC [125–127], that promote the induction of suppressor T cells responsible for inhibition of skin graft rejection [128]. The resulting suppressor T cells are $CD25^+CD152^+$, and secrete significant amounts of IL-10

with the concomitant reduced ability to secrete IFN-γ and IL-2 [129]. Treatment of human monocyte-derived DC with HLA-G1 tetramers prevents their maturation and renders them able to induce the generation of human $CD4^+CD25^+CD152^+$ suppressor T cells and of IL-10-producing $CD8^+CD28^-$ Tr cells [129].

The mode of HLA-G-related Tr cells induction determines their fate

Notably, depending on the mode of Tr cell induction, the HLA-G-related Tr cells are different. This can be explained by several factors including: HLA-G isoforms that induce Tr cells (membrane-bound HLA-G1 vs soluble HLA-Gs), the different nature of the Tr-inducers cells (professional vs non-professional APC), the microenvironment in which Tr cells are generated (presence vs absence of high levels of IL-10), and the ILT-mediated signaling involved in Tr cell generation (ILT2-mediated- vs ILT4-mediated-signaling pathway).

The tolerogenic functions of HLA-G1 and HLA-G5 are highly dependent on their form (monomer or dimer) [130]. HLA-G is expressed in disulfide-linked dimer form at the cell surface [131], and, to lesser extent, in solution [132]. HLA-G dimers exhibits higher affinity to ILTs than the monomers, resulting in enhanced ILT-mediated inhibitory signaling [22, 132]. Not only HLA-G dimers but also higher HLA-G molecular complexes, such as HLA-G1 tetramers, induce strong ILT2-mediated signaling in vitro in an NFAT-GFP reporter system, and ILT4-mediated inhibition of DC maturation in vivo in ILT4-transgenic mice more than HLA-G5 monomers [133]. Thus, the overall biological effects of HLA-G seem to be influenced by its form. This characteristic could also account for the phenotypical and functional differences of HLA-G-related Tr cells. HLA-G5 derived from MSCs, promotes the induction of Tr cells, which require cell-to-cell contact to exert their suppressive activity, and are phenotypically similar to nTr cells [36]. Shed sHLA-G1 allows the induction of Tr cells which are distinct from classical Tr1 cells. Conversely, HLA-G1-expressing APC primed T cells to become long-term unresponsive, which, once irradiated and used as third party suppressor cells, inhibit proliferation of T cells [119], via soluble factors, including IL-10 [121]. Similarly, allogeneic DC-10, which express high levels of membrane-bound HLA-G1, promote the differentiation of classical Tr1 cells, which suppress T-cell responses in a cytokine-dependent manner [114]. Therefore, it can be postulated that HLA-G5 and shed sHLA-G1, which are likely to remain in the monomeric form to a greater extent than membrane-bound HLA-G1, allow the induction of Tr cells that suppress via an IL-10-independent mechanism. Conversely, membrane-bound HLA-G1, having the unique ability to generate high molecular complexes on the cell surface, seems to favor the induction of Tr cells which are likely related to adaptive IL-10-producing Tr1 cells. Nevertheless, it has to be taken into account that Tr cells induced by HLA-G1-expressing APC are distinct from classical Tr1 cells as they need to be irradiated to exert their suppressive activity, and are not-Ag specific. Thus, additional molecules are required, in conjunction with membrane-bound HLA-G1, to promote classical Tr1 cells.

DC-10, which are powerful inducers of classical Tr1 cells, express, in addition to membrane-bound HLA-G1, ILT2, ILT3, and ILT4. Interaction between membrane-bound HLA-G1 and ILT4 on tolerogenic DC-10 and their ligands, ILT2 and membrane-bound HLA-G1 on T cells are concomitantly necessary for Tr1 cell induction [114, 122]. Importantly, differentiation of Tr1 cells by DC-10 is dependent on IL-10. Autocrine production of IL-10 by DC-10 sustains the expression of membrane-bound HLA-G1 and ILT4 on DC and up-regulates the expression of membrane-bound HLA-G1 on T cells. Interactions between membrane-bound HLA-G1 and ILT4 on tolerogenic DC together with HLA-G1-mediated signaling in T cells represent crucial requirements for IL-10 production by T cells and generation of suppressor Tr1 cells. Tr cells generated with HLA-G1-expressing APC suppress T-cell responses via IL-10; nevertheless, they are distinct from classical IL-10-producing Tr1 cells, being not anergic and not Ag-specific. This discrepancy can be partially explained by the limited ILT4-mediated signaling during Tr cell induction by HLA-G1-expressing APC.

HLA-Gs mediate the induction of Tr cells by interacting with ILT2 on T cells, which provide an inhibitory signal to T cells that prevents T-cell activation and allows the induction of suppressor T cells. The inability of suppressor T cells generated with soluble HLA-Gs, in the absence of professional APC, to secrete IL-10 can be explained by the lower HLA-G1-mediated signaling in T cells and by the absence of ILT4-mediated signaling in APC. During DC-10-mediated Tr1 cell induction, inhibition of the interaction between HLA-G1 and ILT2 has no consequences on Tr1 cell differentiation, indicating that ILT2/HLA-G1 pathway is not involved in the induction of Tr1 cells. However, it cannot be excluded that ILT2/HLA-G1 interaction may provide an additional inhibitory signal on T cells that, in a microenvironment containing high levels of IL-10, synergizes with the signal mediated by ILT4/HLA-G1 interaction to promote T-cell anergy and induction of Tr1 cells. In line with this concept are results obtained with HLA-G1-expressing APC in which a limited increased in IL-10 [121] is probably not sufficient to support the up-regulation of membrane-bound HLA-G1 on T cells. Thus, to favor the generation of classical IL-10-producing Tr1

cells are essential different factors: Ag-stimulation in the presence of APC expressing membrane-bound HLA-G1 and ILT4, high levels of IL-10 in the microenvironment, ILT4-mediated signaling on APC, and HLA-G1-mediated signaling (in conjunction with ILT2-mediated signaling) on T cells.

Thus, the different phenotype and functions of HLA-G-related Tr cells are dependent on several factors, including the HLA-G isoforms that influence their forms (momomers vs multimeric complexes), the presence of APC, and the microenvironment in which HLA-G-related Tr cells are induced.

Non-immunological functions of HLA-G in erythropoiesis and angiogenesis

HLA-G was first described on trophoblast cells, which form the external cell layers of the placenta [134–136]. The lack of classical class I and II MHC antigens on trophoblast, the only fetal tissue in direct contact with the mother, was a part of the explanation of fetal evasion from semi-allograft rejection. In addition, the observation that HLA-G was present on invasive trophoblast suggested that this non-classical class I MHC molecule could have a role in the maternal/fetal immune response. Indeed, HLA-G expression by invasive cytotrophoblast cells was shown to inhibit uterine blood mononuclear cell cytotoxicity [1]. The immunosuppressive properties of HLA-G were over the years extensively characterized [80, 137–142]. Beside its role in the adaptations of the maternal immune system to pregnancy, HLA-G could also be involved at the very early stages of pregnancy since it is expressed in unfertilized oocytes and in blastocysts, in the trophectoderm cells that give rise to trophoblast [143–145]. The correlation of HLA-G expression with cleavage rate suggested that this molecule might play an important role in human pre-embryo development. HLA-G expression in pre-implantation embryos was further confirmed and the most recent studies have associated high levels of soluble forms of HLA-G from embryo culture supernatants with higher pregnancy and implantation rate [146].

Given that HLA-G is emerging as being involved early in the human embryo development, it was hypothesized that this molecule could also be present in the initial steps of primitive hematopoiesis and endothelial vascular formation [147].

At that time, HLA-G expression on both hematopoietic and endothelial cells had been considered in the context of a common mesodermal precursor to these two lineages, the hemangioblast [148]. This hypothesis was originally issued because the hematopoietic and endothelial lineages are produced simultaneously at the same anatomical site. The most recent studies confirm the existence in vivo of the hemangioblast, and new findings support the generation of hematopoietic progenitors through an intermediate hemogenic endothelium [149–151].

In vertebrate organisms, there are two successive waves of hematopoiesis which take place at distinct times and sites in the embryo and which generate different blood cell precursors in terms of self-renewal capability and differentiation. The primitive hematopoiesis refers to the generation of the first hematopoietic precursors which originate from the extraembryonic yolk sac and which only lead to short-time erythroid reconstitution. The definitive hematopoiesis begins in the aorta-gonad-mesonephros region and produces the hematopoietic stem cells, the multipotent precursors able to self-renew and to differentiate in definitive erythroid, myeloid and lymphoid lineages. The HLA-G5 soluble protein isoform was identified in the erythropoietic lineage in all organs sustaining primitive to definitive erythropoiesis, i.e., yolk sac, aorta-gonad-mesonephros, liver, spleen and bone marrow [147]. This protein isoform was observed during the entire course of embryo and fetal development in hematopoietic organs but also in children and adult bone marrow. Since HLA-G5 was secreted from the erythroid progenitor stage to the reticulocyte, it was wondered whether this protein was involved in the proliferation, maturation, or both of the erythroid precursors [147].

Regulation of erythropoiesis occurs through erythropoietin (EPO) whose binding to EPO receptor (EPOR) activates the JAK (Janus kinase)-STAT pathway. It was shown that HLA-G5 inhibits the proliferation of normal erythoid cells through G1 cell cycle arrest and not through apoptosis [152]. Accordingly, HLA-G5 was previously shown to decrease the proliferation of alloreactive T cells by reducing cell cycle progression [153]. The mechanism by which HLA-G5 down-modulates erythroid cell growth occurs through dephosphorylation of the JAK2 upstream signaling protein and the STAT-3 and STAT-5 downstream ones. HLA-G5 did not interact with EPOR but rather acted through the recruitment of a phosphatase [152]. Of note, neither ILT2 nor ILT4 inhibitory receptors were expressed on the erythroid lineage. Clinical relevance was provided by analyzing the role of HLA-G5 in patients suffering from polycythemia vera (PV), a myeloproliferative disorder (MPD) characterized by erythroid lineage overproduction. Most of the PV patients and half the patients with the two main others MPD, namely essential thrombocythemia and idiopathic myelofibrosis, were described as carrying an acquired activating mutation in the gene encoding JAK2, i.e., JAK2 V617F, which confers constitutive activity to the kinase with enhanced downstream signaling [154–157]. Interestingly, HLA-G5 had the same inhibitory effects on JAK2 V617F-induced EPOR signaling and prevented the

EPO-independent growth of the erythroid progenitors from polycythemia vera patients [152]. Thus, HLA-G5 represents a new parameter to be considered in therapeutic approaches to polycythemia vera. Indeed, HLA-G5 may allow reduction of the increased erythroid cell proliferation, the critical clinical manifestation of PV. At that time, the only treatment which led a high molecular response was IFN-α [158], whose use in cancer immunotherapy induces high HLA-G5 serum levels [159, 160]. It still remains to determine whether therapeutic benefit following IFN-α treatment occurs by enhancing HLA-G5 expression in PV patients. Since then, additional mutations were identified in the JAK-STAT pathway in some patients with JAK2 V617F(−) MPD [161]. Actually, it would be interesting to investigate whether HLA-G5 also target these mutated proteins and to test the therapeutic value of HLA-G5 in a murine model.

Based on the existence of the hemangioblast, soluble HLA-G protein (sHLA-G) was tracked in the endothelial cells from embryo to adult. Soluble HLA-G protein was localized in the early embryo in the developing vessels from the chorionic villi and the juxta-allantoid part of the yolk sac [147]. Accordingly, the endothelial cells of fetal chorionic vessels of first trimester were shown to express HLA-G [162]. HLA-G protein was present in the endothelial cells of the fetal capillaries present in the mesenchymal core of the chorionic villi, but in none of the other surrounding cellular components. By contrast, HLA-G was never observed in the maternal endothelial cells present in spiral arteries of the decidua parietalis. In agreement with these observations, sHLA-G was not identified in endothelial cells lining mature vessels [147]. Unlike erythroid cells, it could not be determined which sHLA-G isoform was expressed in endothelial cells from developing vessels. Indeed, human umbilical vein endothelial cells (HUVEC) did not express HLA-G, even upon HLA-G-inducing factors such as interferon γ (IFN-γ), probably due to the mature stage of these cells.

Considering the immunological role of HLA-G, many studies have demonstrated that HLA-G is a good prognostic factor for engraftment, not only in the context of pregnancy in which the fetus can be considered a semi-allogeneic transplant but also in allogeneic transplants (heart, liver–kidney, kidney and lung transplants) [27, 40, 52, 163]. Moreover, high HLA-G levels were recently associated with poor prognosis in various cancers [4, 77, 164, 165]. In these three contexts, there is a neo-angiogenesis. In addition, erythroid cells, but not other mature hematopoietic cells, secrete angiogenic factors [166]. It was therefore hypothesized that HLA-G5 may have a pro-angiogenic effect [147]. To test it, the proliferation and the migration of HUVEC in presence of HLA-G5 were analyzed. No effect was observed (unpublished observations). We also engrafted HLA-G5-transfected cells on chick chorioallantoic membrane (CAM), the CAM assay allowing us to quantitatively evaluate the pro-angiogenic effect of a protein. No neo-vascularization was observed on the chick eggs (unpublished observations). Drawing a parallel with the effect of HLA-G on erythroid cells and regarding our results, we had thought that HLA-G could have an opposite role on angiogenesis. This was confirmed recently when the HLA-G5 protein was shown to inhibit fibroblast growth factor-2 (FGF-2)-induced capillary-like tubule formation in vitro and in vivo using a rabbit corneal neovascularization model [167]. The anti-angiogenic effect of HLA-G5 did not occur through its binding to known HLA-G inhibitory receptors, which are absent on endothelial cells, but through the By55/CD160 receptor, a glycosylphosphatidylinositol (GPI)-anchored receptor expressed by endothelial cells. The mechanism by which HLA-G5 exerts this effect is by inducing apoptosis of confluent and proliferating endothelial cells. To explain the benefit of its action on endothelial cells for successful fetal engraftment in maternal decidua, the authors hypothesized that, during early pregnancy, HLA-G5 secreted by endovascular trophoblasts might replace vascular cells of the maternal spiral arteries by inducing their apoptosis, thus transforming them into high-conductance vessels. However, by inhibiting vascularization, HLA-G5 would have an anti-tumoral role which may counterbalance its involvement as a tumor escape mechanism from antitumor response.

The expression of HLA-G in both erythroid and endothelial progenitors in time and space is in accordance with their common origin, the hemangioblast. Interestingly, upon progesterone treatment, HLA-G is induced in smooth muscle cells [43], for which in vitro studies suggested that they are also progeny of the hemangioblast [168]. Moreover, since it inhibits malignant erythropoietic clones in polycythemia vera and angiogenesis, HLA-G5 may represent an attractive therapeutic tool for preventing malignant erythroid cell growth.

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