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MULTI-AUTHOR REVIEW

Emerging topics and new perspectives on HLA-G

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Abstract Following the Fifth International Conference on non-classical HLA-G antigens (HLA-G), held in Paris in July 2009, we selected some topics which focus on emerging aspects in the setting of HLA-G functions. In particular, HLA-G molecules could play a role in: (1) various inflammatory disorders, such as multiple sclerosis, intracerebral hemorrhage, gastrointestinal, skin and rheumatic diseases, and asthma, where they may act as immunoregulatory factors; (2) the mechanisms to escape immune surveillance utilized by several viruses, such as human cytomegalovirus, herpes simplex virus type 1, rabies virus, hepatitis C virus, influenza virus type A and human immunodeficiency virus 1 (HIV-1); and (3) cytokine/chemokine network and stem cell transplantation, since they seem to modulate cell migration by the downregulation of chemokine receptor expression and mesenchymal stem cell activity blocking of effector cell functions and the generation of regulatory T cells. However, the immunomodulatory circuits mediated by HLA-G proteins still remain to be clarified.

Keywords HLA-G · Inflammation · Cytokine/chemokine network · Viral infection · Stem cells

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Introduction

Human Leukocyte Antigen-G (HLA-G) are class Ib HLA antigens structurally related to class Ia HLA-A, -B, and -C (HLA-I) products because they are immunologically functional peptides presenting heterodimeric glycoproteins non-covalently associated with β_2-microglobulin which are linked to the Major Histocompatibility Complex (MHC) locus on chromosome 6 [1, 2]. HLA-G molecules are classified as non-classical HLA-I proteins due to the following characteristics: (1) their limited allelic polymorphism [3]; (2) their peculiar expression pattern characterized by the generation of seven protein isoforms which include four membrane bound (G1, G2, G3, and G4) and three soluble (G5, G6, G7) proteins [4]; and (3) their restricted tissue distribution [5]. Among the different HLA-G isoforms, membrane-bound HLA-G1 and soluble HLA-G5 (HLA-G5) are those most expressed and studied [1]. HLA-G5 proteins are directly secreted in this form and represent the soluble counterparts of membrane-bound HLA-G1. However, HLA-G1 can be proteolytically released as soluble molecules (sHLA-G1) after shedding from the cell surface. Therefore, sHLA-G1 and HLA-G5 are considered the most

important functionally active soluble HLA-G isoforms [6]. Under physiological circumstances, the expression of HLA-G molecules is mainly detected in placental trophoblast cells [7], but also in thymus [8], cornea [9], nail matrix [10], pancreas [11], and erythroid and endothelial precursors [12]. However, a cell surface HLA-G ectopic expression can also be detected on monocytes [13] and in several pathological conditions such as transplantation, tumors, viral infections and autoimmune diseases [1, 2]. It is currently accepted that HLA-G antigens may act as immunomodulatory molecules, since they maintain tolerance at the feto–maternal interface [14], favor graft tolerance [15], decrease inflammatory and immune responses [16], and support tumor [17] and virally infected cells immune escape [18]. The immunosuppressive properties of both membrane-bound and soluble HLA-G proteins are probably due to their ability in: (1) inhibiting the activity and mediating apoptosis of cytotoxic $CD8^+$ T cells and NK cells [19–21]; (2) inhibiting proliferation of $CD4^+$ T cells and driving them into an immunosuppressive profile [22, 23]; (3) inhibiting antigen presenting cells and B cells differentiation [24, 25]; (4) promoting a shift of Th1/Th2 balance toward Th2 polarization [26, 27]; and (5) inducing regulatory T cells [28]. These effects depend on interactions of HLA-G molecules with their specific inhibitory receptors ILT-2 (LILRB1/CD85j), ILT-4 (LILRB2/CD85d) and KIR2DL4 (CD158d) expressed by immune cells [29]. In addition, an anti-inflammatory cytokine, such as interleukin (IL)-10, up-regulates HLA-G expression, which in turn increases IL-10 secretion [13]. Based on these observations, in recent years, the involvement of HLA-G molecules in inflammation and immunomodulation has received increasing attention. In particular, it has recently become clear that HLA-G antigens could play a crucial role in various inflammatory disorders, such as multiple sclerosis, intracerebral hemorrhage, gastrointestinal, skin and rheumatic diseases and asthma. A growing body of evidence also suggests an implication of these proteins in the cytokine/chemokine network, viral infections and stem cell functions. These emerging data are attractive since a better understanding of the exact mechanisms underlying the interactions between HLA-G molecules and different immune events may help us to open new avenues in identifying more efficient therapeutic strategies in several autoimmune and infectious conditions, and in regenerative medicine. Therefore, these important aspects of HLA-G action will be systematically reviewed in the following sections.

Inflammation

HLA-G structures were previously shown to act as regulatory factors of immune responses operating in several inflammatory conditions [30]. The influence of these HLA-G properties has been recently identified in some neurological disorders and better clarified in a group of systemic manifestations.

HLA-G and multiple sclerosis

Multiple sclerosis (MS) is a chronic inflammatory demyelinating and neurodegenerative disease of the central nervous system (CNS) with an unknown etiology that is widely considered to be autoimmune in nature [31]. MS usually affects young adults, and women more frequently than men, and is clinically characterized by the dissemination in space and time of relapses which refer to the occurrence of neurological symptoms and signs [32]. About 80% of MS patients start with a relapsing–remitting (RR) course that, over time, transforms into a secondary progressive (SP) course. In a smaller group of patients (20%), MS begins with a primary progressive (PP) [32]. However, according to the recent proposed criteria [33], the diagnosis of MS requires the detection of multi-focal lesions in the periventricular white matter on T2-weighted magnetic resonance imaging (MRI) scans with or without Gadolinium (Gd) enhancement on T1-weighted MRI scans. In this setting, clinical evidence of disease activity is considered as the presence of relapse at neurological examination, whereas MRI appearance of disease activity is defined as the occurrence of lesions with Gd enhancement on T1-weighted MRI scans. Epidemiological studies indicate that exposure to an environmental factor, such as an infectious agent, in combination with genetic predisposition, could be implicated in MS pathogenesis [31]. MS is currently believed to be mediated by autoreactive $CD4^+$ T helper 1 (Th1) cells which traffick across the blood–brain barrier (BBB) and migrate into the CNS after activation [31]. These cells orchestrate a combined attack of both innate and acquired immune responses directed against myelin proteins consisting of a cooperation among monocytes, macrophages (innate immune system), B cells, $CD4^+$ T cells and $CD8^+$ T cells (acquired immune system) and resulting in CNS inflammation leading to myelin damage and axonal loss. It is generally assumed that the initiation of this coordinate immune reaction takes place in the periphery due to failure of self-tolerance [31]. Brain antigens can be recognized as non-self by a dysfunction of regulatory immune cells (“autoimmune hypothesis”) or by a reaction with proteins released from the CNS after primary degeneration (“degeneration hypothesis”) or infection (“infection hypothesis”) [31]. The first demonstration of the involvement of HLA-G in MS autoimmunity was achieved about 7 years ago [34]. In this study, the presence of CSF detectable levels of sHLA-G in RRMS patients and, occasionally, in other inflammatory neurological disorders (OIND) and non-inflammatory

neurological disorders (NIND) was reported, for the first time. In addition, CSF levels of sHLA-G were higher in RRMS than in controls and increased, in association with IL-10 values, in RRMS patients without than in those with MRI evidence of disease activity. Of note, in RRMS patients, CSF concentrations of sHLA-G and IL-10 were positively correlated with inactive MRI disease and CSF IL-10 titers were more elevated in patients with than in those without CSF measurable levels of sHLA-G. These data suggested that CSF sHLA-G levels may modulate MS disease activity acting as anti-inflammatory molecules under the control of IL-10 CSF levels which may enhance sHLA-G production. The existence of high CSF concentrations of sHLA-G in MS patients and their association with clinical and MRI stable disease were repeatedly confirmed in subsequent investigations in which: (1) an intrathecal production of sHLA-G was more frequent in MS than in inflammatory and non-inflammatory controls and predominated in clinically and MRI inactive compared to clinically and MRI inactive MS [35]; (2) sHLA-G concentrations reciprocally fluctuated in CSF and serum of MS patients because they were decreased in serum of clinically stable MS and increased in CSF of MRI inactive MS [35]; (3) CSF levels of HLA-G5 and not those of sHLA-G1 isoforms were more increased in MS than controls and in MS patients without MRI appearance of disease activity than in those with MRI Gd-enhancing lesions [36]; and (4) CSF values of sHLA-G and anti-apoptotic sFas molecules were inversely correlated in MS patients with no evidence of MRI disease activity, since CSF concentrations of sFas were lower in MS than in controls and in MRI inactive than in MRI active MS [37]. Moreover, in MS patients undergoing interferon (IFN)-β 1b therapy, serum sHLA-I levels were elevated after the first month of treatment and higher in responder than in non-responder in the first trimester of therapy, suggesting that the favorable effect of IFN-β 1b could be mediated by an increase in serum sHLA-I concentrations during the first 3 months of treatment [38]. Taken together, these findings seem to indicate that high CSF levels of sHLA-G antigens are strongly associated with the MS recovery phase, since they probably operate as anti-inflammatory molecules leading to the downregulation of the MS brain inflammatory response. In particular, the resolution of MS autoimmunity promoted by sHLA-G molecules may be due to immunosuppressive properties of the HLA-G5 isoform. Data coming from other reports have recently reinforced the hypothesis that this may play a role in MS pathogenesis. First, HLA-G derived from peripheral blood monocytes of MS patients was able to suppress Th1 and Th2 cytokine production and proliferation of $CD4^+$ T cells, whereas RNA and protein HLA-G expression on peripheral blood monocytes (PBMCs) were lower in MS patients than in healthy donors and increased after the first month of treatment with IFN-β [39]. These results strengthened the assumption that an association between up-regulation of HLA-G antigens and immunomodulatory effects of IFN-β probably exists. Second, immunohistochemical expression of HLA-G and its inhibitory receptors (ILT-2 and ILT-4) was strongly up-regulated within and around MS lesions where microglia, macrophages and endothelial cells were recognized as the cellular sources [40]. Furthermore, protein HLA-G expression on monocytes was high on cultured human microglial cells after activation with Th1 pro-inflammatory cytokines and elevated on monocytes in CSF of MS patients. Third, a novel subpopulation of naturally occurring $CD4^+$ and $CD8^+$ regulatory T cells expressing HLA-G (HLA-G^{pos} T_{reg}) was recently described in peripheral blood of healthy persons [41]. This subset was of thymic origin, present in small amounts, expressed HLA-G1 on their cell surface, exerted their suppressive functions through the secretion of HLA-G5 and the shedding of sHLA-G1, and was also detected in CSF of MS patients, where it was more frequent than in peripheral blood and slightly more represented in MS patients with than in those without relapse. Further studies demonstrated that IL-10 contributed to mediate the suppression activity of $CD4^+$ HLA-G^{pos} T_{reg} [42] which were highly represented in CSF and inflammatory brain lesions of MS patients as activated central memory T cells capable of migrating from the periphery to the intratecal compartment due to the expression of CCR5 [43]. Collectively, these observations provide evidence that sHLA-G antigens, and in particular HLA-G5 isoform, are likely involved in the resolution of MS autoimmunity acting as anti-inflammatory molecules, and suggest that HLA-G^{pos} T_{reg} could play a role in the development of a CNS immunosuppressive microenvironment at the sites of inflammation in MS. However, the exact mechanisms operating in the complex immunomodulatory circuit having sHLA-G proteins as key mediators still remain to be clarified. Therefore, it can currently only be hypothesized that, in MS, macrophages and HLA-G^{pos} T_{reg} infiltrating the brain, endothelial cells and microglia could promote the suppression of $CD4^+$ Th1 cell pro-inflammatory responses and $CD8^+$ T and NK cell cytotoxicity via release of HLA-G5. These effects would be mediated by the interactions between sHLA-G molecules and their receptors resulting in the inhibition of proliferation of $CD4^+$ Th1 cells and the apoptotic elimination of activated $CD8^+$ T and NK cells invading the brain by using the Fas/FasL-dependent pathway. In addition, IL-10 secreted by astrocytes may increase the production of HLA-G5 molecules, which in turn, stimulate IL-10 synthesis. The final outcome would consist in a deviation of the Th1/Th2 balance toward Th2 directions leading to the formation of an anti-inflammatory intrathecal

milieu responsible for the termination of neuroinflammation related to remission of the disease (Fig. 1, panel B) [44].

HLA-G and intracerebral hemorrhage

Spontaneous intracerebral hemorrhage (SICH) is a bleeding-into-brain parenchyma due to spontaneous rupture of small arteries or arterioles damaged by chronic hypertension or amyloid angiopathy and constitutes about 10–20% of all strokes, and represents the form of stroke with greater mortality and disability [45, 46]. Two main components can be identified in SICH [45, 46]: (1) the hemorrhagic core that represents the site of bleeding located in central part of the hematoma; and (2) the perihematomal area that constitutes the rim of edematous brain tissue surrounding the hemorrhagic core placed in the periphery of hematoma. SICH is not a monophasic event, but a dynamic phenomenon characterized by a typical temporal progression including different stages (acute, subacute and chronic) [46]. The prognosis of SICH is principally dependent on the extent and evolution of the primary and secondary brain damage [45, 46]. Primary brain injury occurs in the hemorrhagic core where the extravasated blood causes a direct brain tissue destruction and hematoma formation is associated to mass effect. Conversely, secondary injury takes place in the perihematomal area and is related to tissue compression due to edema development. The current lack of a beneficial therapy in SICH [46] reflects the limited understanding of its pathogenesis concerning, in particular, the exact mechanisms implicated in the appearance and evolution of perihematomal edema. In this setting, it is commonly presumed that a strong inflammatory response could contribute to secondary injury promoted by edema reaction that develops in the region peripheral to hematoma [47]. A recent study [48] reported, for the first time, that serum sHLA-G and sHLA-G1 levels showed a characteristic time course after hematoma appearance, since they increased at 48 h and then decreased at 7 days when compared to corresponding baseline values observed at 24 h after bleeding. Conversely, no significant changes in serum concentrations of HLA-G5 were found over time after SICH. In addition, serum sHLA-G and sHLA-G1 values and perihematomal edema volume were positively correlated at 24 h and at 48 h after SICH. These preliminary results argued in favor of a potential implication of sHLA-G in ongoing inflammatory response associated to perihematomal edema development taking place in the

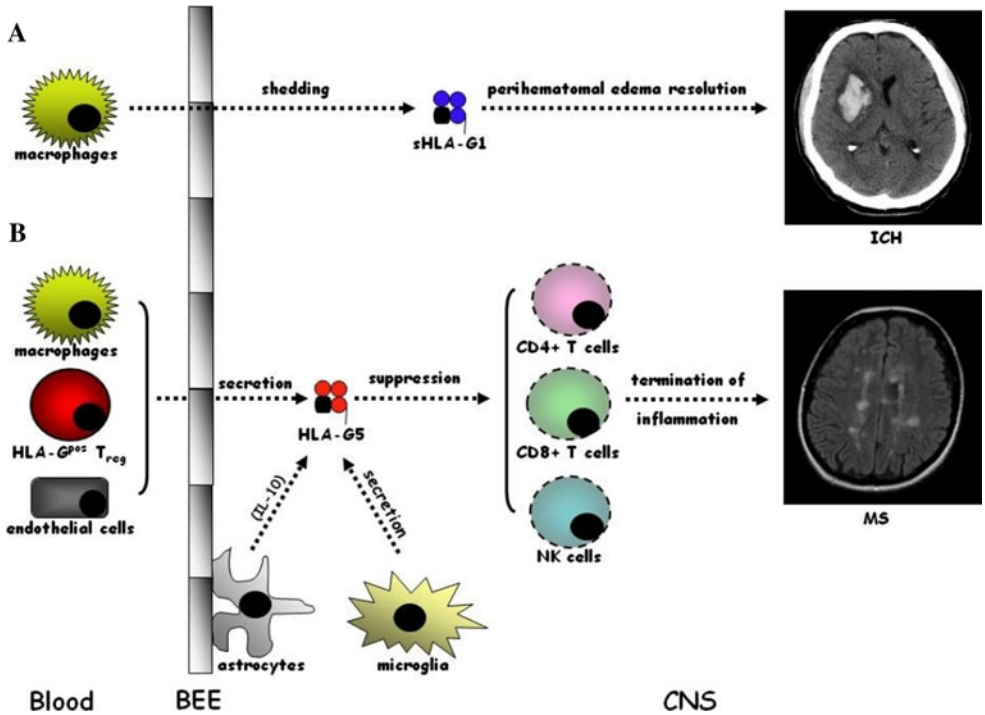

Fig. 1 Schematic representation showing the potential role of sHLA-G in intracerebral hemorrhage (ICH, **a**) and multiple sclerosis (MS, **b**). **a** The shedding of sHLA-G1 by macrophages migrating into the brain during ICH may contribute in perihematomal edema resolution. **b** The secretion of HLA-G5 by macrophages and HLA-G^{pos} T$_{reg}$ infiltrating the central nervous system (CNS) across the blood-brain barrier (BEE), endothelial cells and microglia, sustained by a IL-10 release by astrocytes, may promote the suppression of CD4$^+$ Th1 cell activity and the apoptotic removal of CD8$^+$ T cells and NK cells that favour the formation of an anti-inflammatory intrathecal microenvironment leading to the termination of MS inflammation

transition from acute to subacute phases after SICH. Therefore, it is tempting to speculate that this supposed anti-inflammatory property of sHLA-G molecules in SICH may be due to the shedding of sHLA-G1 isoform by HLA-G expressing macrophages migrating into the CNS (Fig. 1, panel A) [1, 40]. The demonstration that the migration of leukocytes into the hematoma peaks at 2–3 days after onset [47] and, given the competence of HLA-G in suppressing immune cells through Fas/FasL-dependent apoptotic mechanisms, the detection of an inverse correlation between serum levels of sFas and perihematomal edema size associated with increased concentrations of FasL in perihemorrhagic region [49] seems partially to support this speculation.

HLA-G and gastrointestinal diseases

The immuno-tolerance dysfunction and the deregulation of the immune system, as far as the altered balance of pro- and anti-inflammatory cytokines in inflammatory bowel disease, propose a possible role of HLA-G molecules in the pathogenesis and progression of Crohn's disease (CD) and ulcerative colitis (UC) [50]. Torres et al. [51] firstly demonstrated, by immunohistochemistry, the presence of HLA-G molecules in the inflamed mucosa biopsies of UC patients, characterized by a modified Th2 response, in spite of the absence of these molecules in CD intestinal biopsies, associated with a Th1 cytokine pattern. In 2008, Rizzo et al. [52] observed, by an in vitro model, a clear difference in the production of soluble HLA-G (sHLA-G) molecules between CD and UC patients. LPS-stimulated PBMCs from UC patients secreted lower sHLA-G levels, associated with lower IL-10 production, compared to CD LPS-stimulated PBMCs, whereas the presence of sHLA-G molecules and IL-10 in unstimulated PBMC supernatants of CD patients seems inadequate to counteract the chronic inflammatory condition. The authors proposed this in vitro test as a useful diagnostic tool able to distinguish UC from CD patients at an early stage. The discrepancy between the deficit in sHLA-G production by UC PBMCs and the expression in gastrointestinal biopsies could be explained by the different specific microenvironment. HLA-G gene is characterized by the presence of an insertion/deletion of a 14-bp sequence polymorphism at the 3' untranslated region; the insertion of 14 bp increases the instability of the HLA-G mRNA causing lower protein production [53]. Glass et al. [54] showed an increased frequency of both the insertion of a 14-bp allele and the heterozygous genotype (+14/−14 bp) in UC patients when compared to CD subjects, in particular in those CD cases positive for ileocecal resection, suggesting a potential effect of HLA-G gene as a disease-modulating gene. Recently, Torres et al. [55] showed increased serum concentration of sHLA-G and the presence of sHLA-G molecules in biopsies of intestinal mucosa of coeliac patients, totally absent in healthy biopsies. Coeliac disease is an autoimmune disorder characterized by an anomalous innate immune response at the gut level. These data could suggest HLA-G as possible tool for the diagnosis of IBD and coeliac disease.

HLA-G and skin diseases

The data obtained on HLA-G modulation in skin cancer and inflammatory diseases show a dual role of HLA-G molecules in this site [56]. HLA-G molecules were found in psoriatic lesional plaques [57], a chronic autoimmune skin disease characterized by T cells infiltration. A recent work by Borghi et al. [58] found significant lower plasmatic levels of both sHLA-G and IL-10 in psoriatic patients compared to healthy subjects, suggesting a favorable condition for the development of inflammation. Moreover, the presence of HLA-G molecules in biopsies from atopic dermatitis was provided by Khosrotehrani et al. [59], mainly in association with a favorable prognosis. The modulation of HLA-G molecules at transcriptional and translational levels was also observed in epidermal cells of pemphigus vulgaris (PV) patients, an autoimmune blistering disease of the skin and mucous membranes [60]. Moreover, Gazit et al. [61] found an increased presence of the deletion allele of the HLA-G 14-bp polymorphism in Jewish PV patients. Advances in the understanding of the clinical relevance of HLA-G might be a key for future therapeutic tools in the treatment skin inflammatory disease and tumors.

HLA-G and rheumatoid diseases

Verbruggen et al. [62] found, for the first time in 2006, significantly lower sHLA-G levels in rheumatoid arthritis (RA) patients, compared to healthy subjects. The impaired sHLA-G expression could contribute to the development of RA, a chronic inflammatory and autoimmune disease characterized by cartilage and joint destruction, invasion of the joints by immune cells and an unbalanced Th1 microenvironment [63]. Rizzo et al. [64] demonstrated a pharmacogenetic role of the HLA-G 14-bp polymorphism in the clinical response of RA patients to methotrexate (MTX). The authors observed an increased production of IL-10 and sHLA-G after PBMCs in vitro culture with MTX. They also found a significant association between the presence of the −14/−14 bp genotype and a good response to MTX therapy suggesting this HLA-G polymorphism as a possible therapy marker in the early phases of the disease. Moreover, Veit et al. [65] showed an increased frequency of the 14-bp sequence deletion allele and of the −14/−14 bp genotype in juvenile idiopathic

arthritis (JIA) females, while no significant differences were observed between healthy subjects and RA patients. They suggested the HLA-G 14 bp polymorphism as a possible factor of distinction between these two diseases. Rosado et al. [66] was first to report the presence of higher levels of HLA-G, compared to healthy subjects, in patients with systemic lupus erythematosus (SLE), a chronic autoimmune inflammatory disease. Rizzo et al. [67] found significant lower concentrations of sHLA-G in the plasma of mild untreated SLE patients than in healthy controls and a significant increased frequency of the 14-bp sequence insertion allele and of the +14/+14-bp genotype. Moreover, Veit et al. [68] observed an excess of heterozygous 14-bp genotypes, in particular in subjects with a milder disease, while Wu et al. [69] found a similar distribution of the 14-bp genotype between SLE Han Chinese patients and healthy subjects. These conflicting results could be due to environmental and/or ethnicity-specific factors as far as to the heterogeneous clinical feature and mostly to the therapeutic treatment of the investigated patients. Otherwise, these data propose HLA-G as a candidate gene for association to rheumatic diseases and suggest a careful analysis of HLA-G regulation and allelic polymorphisms.

HLA-G and asthma

A possible involvement of HLA-G molecules in the Th2-polarized immune response typical of allergic disease such as asthma has been recently proposed [70, 71]. Nicolae et al. [72] suggested HLA-G as a novel asthma and hyper-responsiveness susceptibility gene and demonstrated, by immunohistochemistry, the expression of HLA-G in bronchial epithelial cells that could be involved in the local inflammatory response to allergens. Moreover, Tan et al. [73] reported a single nucleotide polymorphism in the HLA-G gene able to influence the targeting of three microRNA that negatively regulate gene expression and contribute to the risk of asthma development. Rizzo et al. [70] demonstrated a defective production of sHLA-G molecules by PBMCs from asthmatic patients after in vitro stimulation with LPS, due to low IL-10 levels. The low HLA-G secretion could influence the persistence of the chronic airway asthmatic inflammation. Conversely, Tahan et al. reported higher levels of plasmatic sHLA-G in asthmatic children [71] suggesting a role of these antigens in atopy. These different results could probably be due to the different specimens analyzed. The production of anti-inflammatory HLA-G molecules might contribute to the modulation of the immune response. Further, a recent work by Mapp et al. observed the influence of workplace exposure to isocyanates on sHLA-G production and IL-10 secretion and occupational non-allergic asthma development [74]. An overview about the main current literature on the association between HLA-G and gastrointestinal, skin and rheumatoid diseases and asthma is reported in Table 1.

Overall, these findings indicate that HLA-G molecules display a broader range of action in different inflammatory diseases in which these antigens seem to play opposite roles, because their expression can be both beneficial and detrimental also depending on the site where they exert their functions.

Cytokines/chemokines

The modulation of cytokines and chemokines has a crucial impact on several physiological and/or pathological conditions due to their central role in orchestrating different events associated with immune responses. Therefore, the recent demonstration that HLA-G molecules could be involved in these regulatory mechanisms shed a new light on cytokine/chemokine network.

HLA-G and cytokines: an overview

Cytokines are small proteins secreted by different cell types of the immune system upon antigen stimulation. Immune responses are mediated and regulated by the interaction of cytokines with different receptors: (1) class I (hematopoietin), (2) class II (interferons), (3) immunoglobulin superfamily receptors, (4) TNF receptors, and (5) chemokine receptors. Engagement of these receptors leads to activation of JAK/STAT proteins, and finally to gene transcription modulation. Recently, several observations have suggested that secretion of different cytokines in different cell populations of the immune system may be modulated by HLA-G molecules. HLA-G5 interact with inhibitory receptor Ig-like transcript (ILT) 4 on dendritic cells (DC), dampening their maturation and activation. Moreover, ILT4 engaged by soluble (s)HLA-G1/G5 recruit SHP-2 phosphatase and modulate NF-kB pathway, leading to increase of mRNA levels and secretion of IL-6 in DC [75]. Up-regulation of IL-6 in DC leads to the development of immunosuppressive/tolerogenic DC. Moreover, knock-out mice for IL-6 had increased numbers of mature DC, demonstrating that endogenous IL-6 inhibits DC maturation. IL-6 modulate gene transcription predominantly by phosphorylation and activation of Stat3 [76]. sHLA-G molecules modulate the maternal immune response against fetal tissues; in this respect, modulation of cytokines secretion in NK cells and macrophages at the maternal–fetal interface may be relevant. sHLA-G stimulate the release of pro-angiogenic factors and cytokines, leading to the establishment of a microenvironment that allows the implant of the fetus. HLA-G molecules induce the release

Table 1 Summary of the main studies on the association between HLA-G and gastrointestinal, skin, and rheumatoid diseases and asthma

Study	Disease	Technical approach	Samples	Results
HLA-G and gastrointestinal disease				
Torres et al., 2004 [51]	Ulcerative colitis (UC); Crohn's disease (CD)	Immunohistochemistry	Inflamed mucosa biopsies	Presence of HLA-G molecules in UC, absence in CD
Glas et al., 2007 [54]	Ulcerative colitis; Crohn's disease	Genotyping by PCR	DNA	Increased frequency of +14-bp allele and +14/−14-genotype in UC patients compared to CD
Rizzo et al., 2008 [52]	Ulcerative colitis; Crohn's disease	In vitro culture, ELISA	PBMC culture supernatants	Lower sHLA-G and IL-10 levels in UC LPS-stimulated PBMCs compared to CD LPS-stimulated PBMCs. Production of HLA-G and IL-10 in unstimulated PBMCs from CD patients
Torres et al., 2006 [55]	Coeliac disease	Immunohistochemistry, ELISA	Biopsies of intestinal mucosa, serum	Serum and intestinal expression of HLA-G in relationship with clinical conditions
HLA-G and skin				
Aractingi et al., 2001 [57]	Psoriasis	RT-PCR, Immunohistochemistry	Lesional plaque	Presence of specific RNAs for HLA-G1 and HLA-G5 in psoriasis but not controls. Presence of soluble HLA-G5 transcripts
Borghi et al., 2008 [58]	Psoriasis	ELISA	Plasma	Lower plasmatic levels of sHLA-G and IL-10 in psoriatic patients compared to healthy subjects
Khosrotehrani et al., 2001 [59]	Atopic dermatitis	Immunohistochemistry, Immunofluorescence	Skin biopsies	Expression of HLA-G antigen mainly associated to a favorable prognosis
Gazit et al., 2004 [61]	Pemphigus vulgaris	PCR	DNA	Increased presence of −14/−14 genotype of the HLA-G 14-bp polymorphism in Jewish patients
Yari et al., 2008 [60]	Pemphigus vulgaris	RT-PCR	Epidermal cells	Modulation of HLA-G molecules at transcriptional and translational levels
HLA-G and rheumatoid disease				
Verbruggen et al., 2006 [62]	Rheumatoid arthritis (RA)	ELISA	Plasma	Presence of lower levels of sHLA-G in RA compared to healthy subjects
Rizzo et al., 2006 [64]	Rheumatoid arthritis	In vitro culture, ELISA, PCR	PBMC culture supernatants, DNA	Increased production of IL-10 and sHLA-G after PBMCs in vitro culture with MTX. Significant association between −14 and −14 HLA-G 14-bp polymorphism genotype and a good response to MTX therapy

Table 1 continued

Study	Disease	Technical approach	Samples	Results
Veit et al., 2008 [65]	Juvenile idipathic arthritis (JIA)	PCR	DNA	Increased frequency of the −14-bp allele and −14/−14-bp genotype of the HLA-G 14-bp polymorphism in JIA females compared to healthy females
Rosado et al., 2008 [66]	Systemic lupus erythematosus (SLE)	Flow cytometry, immunohistochemistry, ELISA	PBMCs, serum, cutaneous biopsies	Presence of higher levels of HLA-G and IL-10 in SLE patients compared to healthy subjects
Rizzo et al., 2008 [67]	Systemic lupus erythematosus	ELISA, PCR	Plasma, DNA	Presence of lower concentration of sHLA-G in mild untreated SLE patients than in healthy controls. Increased frequency of +14-bp allele and of +14/+14 genotype of the 14-bp polymorphism in SLE subjects
Veit et al., 2009 [68]	Systemic lupus erythematosus	PCR	DNA	Increase of heterozygous 14-bp genotype, in particular in subjects with a milder disease
Wu et al., 2009 [69]	Systemic lupus erythematosus	PCR	DNA	Similar distribution of the 14-bp genotype between SLE Han Chinese patients and healthy subjects
HLA-G in asthma				
Nicolae et al., 2005 [72]	Asthma	Positional candidate gene study, immunohistochemistry	DNA, bronchial epithelial cells	HLA-G as a novel asthma and hyperreponsiveness susceptibility gene. Expression of HLA-G in bronchial epithelia cells
Rizzo et al., 2005 [70]	Asthma	In vitro culture, ELISA	PBMC culture supernatants	Defective production of sHLA-G molecules by PBMCs from asthmatic patients after in vitro stimulation with LPS
Tahan et al., 2006 [71]	Asthma	ELISA	Plasma	Higher levels of plasmatic sHLA-G in asthmatic children
Tan et al., 2007 [73]	Asthma	Luciferase assay, ELISA	DNA, bronchial epithelial cells	Single nucleotide polymorphism in the HLA-G gene able to influence the targeting of three micro-RNA that negatively regulate gene expression. Expression of sHLA-G in asthmatic bronchial epithelial cells
Mapp et al., 2009 [74]	Occupational asthma	ELISA	Plasma	sHLA-G production and IL-10 secretion are influenced by workplace exposure to isocyanates

of pro-inflammatory cytokines, i.e., IL-6, IL-8, TNF-α in decidual, but not peripheral, $CD56^+$ NK cells, and in both peripheral and decidual $CD14^+$ monocytes, by interacting with KIR2DL4 receptor [77]. The modulation of cytokine release was higher in decidual counterparts of NK cells and macrophages, and appears to be related to the induction vascular remodeling of the uterine spiral arteries. Similar effects of sHLA-G were observed on resting peripheral blood NK cells [78]. sHLA-G molecules induce release of IL-6, IL-8 and TNF-α, plus IL-23, IL-1-β, and IFN-γ. Moreover, two chemokines, i.e., MIP-3-α and MIP-3-β, are increased. The authors demonstrated that agonistic antibodies against KIR2DL4 are more effective than sHLA-G in the induction of cytokine release by NK cells. These evidences are reinforced by data obtained on uterine NK cells [79]. When the latter cells are stimulated with IL-15 in the presence of sHLA-G, secretion of Th-1 type cytokines, i.e., TNF-α and IFN-γ, is increased. The engagement of different receptors by sHLA-G is related to different modulation of cytokine production. In fact, the induction of pro-inflammatory cytokines in NK cells and macrophages have been associated with engagement of KIR2DL4 receptor, whereas interaction of sHLA-G with ILT2/CD85j receptor dampens the release of pro-inflammatory cytokines. NK cell lines expressing ILT2 receptor and incubated with $HLA\text{-}G^+$ cell lines display decreased levels of IFN-γ (both mRNA and protein). The same results were obtained with $ILT2^+/KIR3DL1^-$ peripheral blood NK and T cells. IFN-γ release upon activation was strongly inhibited in both cell populations in the presence of $HLA\text{-}G^+$ cell lines [80]. HLA-G molecules may also induce the secretion of anti-inflammatory cytokines. HLA-G5/G6 increased the secretion of TGF-β1 by myelomonocytic cell line U937, suggesting that anti-inflammatory cytokines may be secreted by maternal macrophages exposed to HLA-G5/G6 secreted by cytotrophoblast cells [81]. A strong correlation between sHLA-G and the immunosuppressive Th2-related cytokine IL-10 has been reported. IL-10 is secreted by human cytotrophoblast cells [82] and acts as a strong inducer of sHLA-G release by monocytes and human trophoblast cells [13]. Moreover, IL-10 induces up-regulation of HLA-G in most of human cancer cells, providing them an immune escape mechanism [83]. Finally, serum levels of IL-10 and sHLA-G appear to be mutually related [70]. Recently, it has been demonstrated that sHLA-G can induce novel immunosuppressor $CD3^+CD4^{low}$ or $CD3^+CD8^{low}$ T cell subsets, whose inhibitory function is related to IL-10 secretion [84], consistent with data obtained on $HLA\text{-}G^+/CD4^+$ regulatory T cells [42]. A correlation between sHLA-G and Th2-related cytokine is also suggested by studies that describe increased levels of sHLA-G in sera from patients with allergic diseases [85]. Moreover, allergic patients treated with sublingual immunotherapy (SLIT), that induce a shift toward Th1 polarization and increase serum levels of IFN-γ, display decreased serum levels of sHLA-G [86].

HLA-G and cytokines in mesenchymal stem cells functions

Human mesenchymal stem cells or mesenchymal stromal cells (MSC) are a rare population of multipotent cells of the bone marrow. These cells can differentiate in osteoblast, chondroblast, adipocytes, stromacytes or neurons, and these

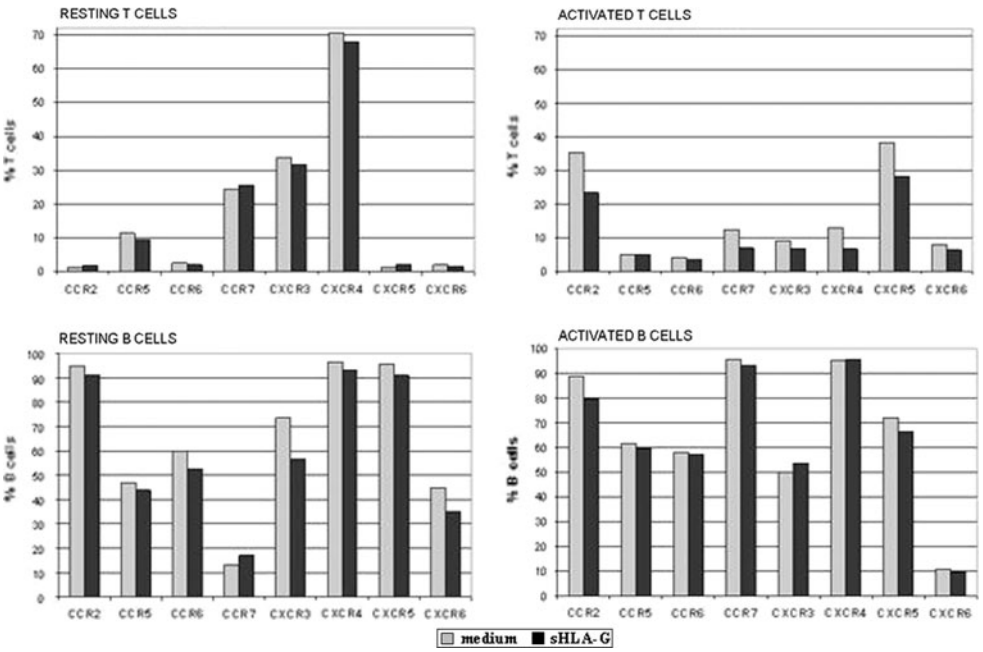

Fig. 2 Flow cytometry analysis of the expression of chemokine receptors in the presence (*black bars*) or absence (*grey bar*) of sHLA-G (100 ng/ml) in activated and resting T and B cells

features make them attractive for regenerative medicine [87–89]. MSC exert immunomodulatory properties by cell contact or by soluble factors, such as prostaglandin E2, TGF-β1, IL-10 and indoleamine 2, 3-dioxygenase [90]. MSC can inhibit allogeneic T cell proliferation [91], B cell functions [92], DC maturation [93], and NK-mediated cytotoxicity [94]. Moreover, MSC expand $CD4^+/CD25^+/FOXP3^+$ regulatory T cells [91]. Some immunoregulatory functions of MSC are related to sHLA-G. Release of sHLA-G by MSC protects themselves from NK-mediated cytolysis and dampens IFN-γ release by NK cells [95, 96]. Again, HLA-G and IL-10 are strongly related. MSC treated with IL-10 secrete higher levels of sHLA-G, and such effect was dose-dependent. Moreover, cells co-cultured with MSC display higher levels of IL-10 secretion, and such effect was reverted by adding blocking antibodies against sHLA-G [96]. IL-10 and sHLA-G are present in culture supernatants when MSC are co-cultured with PBMNC in the presence of PHA. Blocking experiments with anti-IL-10 and/or anti-sHLA-G confirm a crucial role of both molecules in MSC-mediated immunosuppression [97]. Finally, cells co-cultured with MSC display higher levels of IL-10 and TGF-β transcripts and higher expression of HLA-G [98].

HLA-G and chemotaxis: rationale and preliminary evidences

Chemotaxis is crucial for the recirculation of immune effector cells between peripheral blood, secondary lymphoid organs and inflamed tissues, and is mediated by the interaction between chemotactic cytokines (chemokines) and G protein-coupled chemokine receptors. Several papers have reported modulation of chemokine release by HLA-G in different cell populations [78]. However, so far, no information is available on a possible modulation of chemotaxis performed by HLA-G. Migration of NK cells, but not of T cells, across porcine endothelial cells is inhibited when the latter cells are transfected with HLA-G1, whereas the migration was unaffected when endothelial cells are transfected with HLA-A2 [99]. These data suggest that migration of NK cells across the placenta may be modulated by HLA-G. Recently, it has been demonstrated that trophoblast-derived SGHPL-4 cell lines transfected with sHLA-G display a decreased level of motility and invasion capacity in vitro after stimulation with hepatocyte growth factor [100]. These data suggested an autocrine inhibition of trophoblast invasion, mediated by sHLA-G secretion. Taken together, these findings suggested that HLA-G molecules may affect cell motility and migration of different cell subsets, with an undefined mechanism. Morandi and associates tested whether sHLA-G modulation of cell migration involves the modulation of chemokine receptor expression, performing a preliminary experiment on peripheral blood mononuclear cells. Cells were cultured in vitro with medium alone (resting cells) or activated with specific *stimuli* (anti-CD3 mAb or anti-Ig mAb plus CpG for T or B cells, respectively), in the presence or absence of sHLA-G. The expression of chemokine receptors was analyzed by flow cytometry, gating on $CD3^+$ T cells or $CD19^+$ B cells. Bars indicated percent of positive cells (Fig. 2). The expression of some chemokine receptors, i.e., CCR2, CCR7, CXCR3, CXCR4, and CXCR5 was reduced by sHLA-G in activated but not in resting T cells. Such effect appears to be less relevant in resting or activated B cells than in T cells.

Taken together, these data suggest that HLA-G antigens are able to modulate cytokine and chemokine secretion-mediating immune responses, MSC functions and chemotaxis. In these circumstances, HLA-G proteins may play a key role in determining the final outcome of cytokine and chemokine actions.

Viral infections

Host immune defense mechanisms are efficient at eliminating most viral infections. However, some viruses have developed multiple strategies for subverting host immune defenses, thus facilitating their spread in the host [101]. One such important mechanism involves certain viral-induced changes in the levels and distribution of classical (Ia) and non-classical (Ib) HLA class I antigens that protect the host infected cells by blocking the host's immune defenses. Protection from attack by cytotoxic T lymphocytes (CTL) is primarily mediated by down-regulation of the classical HLA class I antigens (A and B) [101]. Virus-infected cells are protected against attack by NK cells by the non-classical HLA class I, HLA-G, when this molecule is expressed in virus-infected cells. This molecule, both on the cell surface and in soluble form, is also capable of suppressing the functions of various immune cells, including T regulatory lymphocytes and dendritic cells, providing a long-term immunosuppression function [102]. It may, therefore, be that the diminished immune function induced by HLA-G in the host sometimes leads to an advantage for virus progression by helping viruses subvert the host's antiviral defenses [103]. In what follows, the most important evidence is discussed regarding the implication of HLA-G in viral immune defense as one of the strategies adopted by escaping viruses.

HLA-G in human immunodeficiency virus (HIV-1) infection

Human immunodeficiency virus type 1 (HIV-1) infection is associated with severe and progressive loss of the immune

Table 2 HLA-G expression on the surface in viral infected cells

Virus	Cell type	Study type	HLA-G changes	References
CMV	Human peripheral blood monocytes from CMV infected individuals	In vivo	Increase	Yan et al., 2009 [115]
	Macrophages and astrocytoma stimulated with monocytes CMV infected	In vitro	Increase	Onno et al., 2000 [18]
	Alveolar macrophages stimulated with inflammatory citokynes	Ex vivo	Increase	Onno et al., 2000 [18]
	Astrocytoma transfected with CMV	In vitro	Decrease	Pizzato et al., 2003 [122]
	Astrocytoma transfected with CMV	In vitro	Decrease	Barel et al., 2003 [123]
RABV	Neurons, neuroblastoma and astrocytoma infected with RABV	In vitro	Increase	Lafon et al., 2005 [119]
	Neurons and astrocytes infected with RABV	In vitro	Increase	Megret et al., 2007 [124]
HSV-1	Neurons, neuroblastoma and astrocytoma infected with HSV-1	In vitro	Increase	Lafon et al., 2005 [119]
	Neurons culture infected with HSV-1	In vitro	Increase	Megret et al., 2007 [124]
HIV-1	Peripheral blood $CD8^+$ lymphocytes from HAART treated patients	In vivo	Increase	Lozano et al., 2009 [125]
	Peripheral blood monocytes from HAART treated patients	In vivo	Increase	Cabello et al., 2003 [105]
	Macrophages and gliomas infected with HIV-1	In vivo	Decrease	Derrien et al., 2004 [126]
IAV	Alveolar epithelial cells infected with IEV	In vitro	Increase	LeBouder et al., 2009 [121]

Increment of the levels of sHLA-G has also been observed in human cytomegalovirus infection by Yan [115], Lafon [119] and in HIV infection by Donaghy [127] and Lajoie [128]

CMV Cytomegalovirus, *RABV* rabies virus, *HVS-1* herpes virus simplex, *IAV* influenza virus A, *HIV* human immunodeficiency virus

function in infected persons, leading to high risk of opportunistic infections and malignancies. It is known that HIV-1 protects infected cells from CTL and NK recognition and lyses by classical HLA-A and B down-regulation and non-classical HLA-G molecule up-regulation, respectively. Since, the immunoregulatory ability of HLA-G has become known, the involvement of this molecule in the progression of HIV-1 infection has been widely examined. Many authors, including members of Peña's group, have studied the role of HLA-G in HIV-1 infection in order to better understand the possible implication of this molecule in the escape of HIV-1. Studies have focused on the expression of HLA-G in monocytes, which are relevant as reservoirs of HIV-1, and in lymphocytes, which are known to be more susceptible to be infected by HIV-1. HLA-G in monocytes obtained from HIV-1 seropositive patients was analyzed in our laboratory, and we found that most monocytes express HLA-G, although in healthy individuals, only a small proportion of them express this molecule [104]. Peña and coworkers subsequently examined the possibility that this might be one consequence of highly active antiretroviral therapy (HAART) administered to such patients, and we found a greater proportion of monocytes expressing HLA-G in patients undergoing HAART compared to those not receiving any treatment [105] (Table 2). As HAART has different components, including NRTIs, NNRTIs, and IPs, the same group subsequently studied the effect of each of them on changes in HLA-G level, finding that NRTIs are the main contributor to the rise in HLA-G level [106]. This was confirmed by the fact that the numbers of monocytes expressing HLA-G gradually diminished after NRTIs were discontinued. The cause of this change in the surface expression of HLA-G is currently a topic of discussion. HIV Nef is involved in down-regulating surface classical HLA class I antigens by interacting with their cytoplasmic domain [107], but HIV Nef may not be able to interact with the HLA-G molecule, which has a truncated domain [108]. Besides the inability of viral Nef to down-regulate HLA-G, other changes, such as in IL-10, which is normally elevated in HIV infection and has the ability to up-regulate expression of HLA-G [13], may indirectly influence the expression of this molecule. Peña and colleagues also studied the expression of HLA-G in T cells obtained from HIV-1 seropositive individuals and found that a higher proportion of them expressed HLA-G [104]. Later, they found that the majority of them were $CD8^+HLA\text{-}G^+$ with the regulatory T phenotype previously described in healthy individuals by the group of Feger [41]. HLA-G polymorphism in HIV infection has also been studied and various HLA-G alleles have been associated with different levels of risk of HIV infection. An association of G-0105N with protection and G-010108 with susceptibility to HIV infection has thus been found [109, 110]. On the other hand, a greater susceptibility to HIV infection is associated with the presence of G-010108 alone or together with G-010401 [109]. G-010108 has a substitution at codon 57 (G–A) without changes in amino acid sequence [111]. In an attempt to elucidate possible immunological mechanisms that would protect children born to HIV-1-infected mothers, Aikhionbare's group [112] presented evidence that dissimilarities in HLA-G DNA sequence variants could influence the transmission of HIV-1 from infected mothers to their infants.

HLA-G in human cytomelovirus (HCMV) infection

HCMV is a herpes virus that causes widespread, persistent human infection in a delicate balance between the progression of the virus and the defenses of the host [113]. Although HCMV did not usually cause clinical pathology in immunocompetent individuals, it was capable of inducing serious complications when the host immune system is immature or suppressed, such as occur in the fetus and neonates, and in immunodeficient patients. It is known that HCMV has evolved a number of independent strategies to evade the immune system [114]. Thus, it has been recently described how HCMV has developed various mechanisms to impede NK cell recognition by inducing expression of HLA-G by the host cells. Specifically, the group of Onno [18] have reported that HLA-G is produced during viral reactivation in macrophages and astrocytoma cells after allogenic stimulation with monocytes latently infected by HCMV. The group of Yan [115] have also reported that the percentage of HLA-G-positive monocytes and plasma-soluble HLA-G levels in patients with active HCMV infection were both dramatically higher than in healthy individuals. On the other hand, Pizzato et al. [108] have demonstrated down-regulation of HLA-G in astrocytoma cells. The up-regulation observed in HLA-G is probably related to IL-10, which increased in HCMV infection [115]. One particular issue is the analysis of HLA-G in trophoblasts in HCMV infection, as these are a major target of HCMV infection in pregnancy [116]. Such infections cause birth defects, as well as spontaneous fetal loss. It has long been known that HCMV produces unique short (US) viral glycoproteins, and information on the down-regulation of HLA-G by some of these proteins has been documented in detail for choriocarcinoma [117], although other mechanisms may participate in this phenomenon. Interestingly, when the studies of HLA-G expression after HCMV infection were performed on mielomonocytic cells, more powerful effects up-regulating HLA-G were found than when these studies were done on trophoblast-related cells, where an opposite effect, HLA-G down-regulation, was observed. Further studies are therefore required to better understand such differences.

HLA-G in herpes virus simplex (HSV) and rabies virus (RABV) infections

The ability of certain neurotropic viruses to induce the formation of HLA-G in infected neurons, thus conferring protection against NK cells [118], has recently been demonstrated. Confirming this, Lefon [119] thus showed that both neuronotropic viruses, such as HSV-1 and RABV, are capable of triggering up-regulation in active infected neurons to express HLA-G1 and produce HLA-G5 soluble isoforms. However, RABV primarily up-regulates HLA-G1, in contrast to HSV-1 which changes HLA-G5, showing that RABV and HSV-1 can evade the host immune response totally (RABV) or partially (HSV-1) regulating HLA-G. The causes of these effects are not well known, although they are probably mediated by soluble components, such as IFN-β or IFN-γ, which have demonstrated the ability to up-regulate HLA-G in uninfected cells.

HLA-G in hepatitis C virus (HCV) and influenza A virus (IAV) infections

There is also some evidence that HCV and IAV viruses use HLA-G as a strategy to evade the immune system. Thus, Cordero's group [120] have recently demonstrated that HLA-G polymorphism influences susceptibility to HCV infection in certain infectious diseases, which suggests a possible involvement of the HLA-G molecule in response to infections. LeBouder et al. [121] have also shown that several strains of IAV differentially up-regulate HLA-G expression in alveolar epithelial cells, and that the severity of the infection may therefore be related to the capacity of different strains of the virus to up-regulate HLA-G. A summary of viral infected cells expressing HLA-G is listed in Table 2.

In summary, one of the main mechanisms of virus evasion is the induction of changes in levels of the classical HLA-G proteins. This enables the virus to prevent infected cells from being recognized and attacked by CTL and NK cells.

HLA-G and the new perspectives in stem cell therapy for regenerative medicine

Regenerative medicine using stem cell technology is emerging as a promising therapy to replace or repair damaged tissues [129–132]. However, their successful use remains closely dependent on the fate and acceptance of these cells after transplantation. Besides the multiple characteristics that support their use in regenerative medicine, efficient translation of stem cell therapy to the clinic still requires a better understanding of their immunological properties and interaction with the host immune system. Several in vitro and in vivo studies have demonstrated that mesenchymal stem cells can modulate the function of the immune system and exhibit immunosuppressive properties [91, 93, 94, 133–135]. Mesenchymal stem cells have been proposed for the control of graft rejection [136], graft versus host disease [137] and autoimmune disorders [138]. However, the exact underlying molecular mechanisms involved in these properties remains incompletely characterized. Compared to embryonic stem cells, adult stem cells have gained increasing interest for regenerative medicine

thanks to their self-renewal potential, multipotency and the lack of ethical restrictions related to their use. Recently, scientific reports have highlighted the involvement of HLA-G as a key molecule in the immunomodulatory properties of mesenchymal stem cells. The aim of this section is to briefly review the current knowledge on the expression of HLA-G by adult stem cells, its modulation, and involvement in tolerance induction in allogeneic cell transplantations. HLA-G may play a role in solid organ acceptance [139], and increased HLA-G plasmatic levels measured after hematopoietic stem cells transplantation are correlated with better graft acceptance, suggesting the involvement of HLA-G molecules in allogeneic graft tolerance induction [140]. In vitro studies have confirmed this hypothesis by demonstrating the constitutive expression and secretion of HLA-G5 by human mesenchymal stem cells (MSC) and its direct implication in MSC immunomodulatory effect [96, 141]. Indeed, using a blocking antibody directed against HLA-G, Selmani et al. have demonstrated the requirement of HLA-G5 for bone marrow (BM)-MSC to induce the suppression of the NK lytic activity, the inhibition of allogeneic T cell response, and the expansion of $CD4^+$ $CD25^{high}$ $FOXP3^+$ regulatory T cells. Interestingly, HLA-G expression on MSC can be modulated by several agents such as progesterone [142], IFN-γ [143], and cell-to-cell contact with alloactivated T cells or IL-10 [96]. This latter cytokine up-regulates HLA-G5 in bone-marrow (BM)-MSC, but also seems to be involved in a positive feedback loop with HLA-G5 as suggested by the decrease of IL-10 BM-MSC production induced after HLA-G5 blockade. Because MSC harvested from various tissues present heterogeneous subpopulations, some studies have recently been focused on the identification of a potential MSC marker associated with a significant suppressive effect. Both Stro-1 and CD90 could be good candidates as one study showed that a Stro-1-enriched population of MSC presents a stronger inhibitory effect in vitro than other MSC and another linked the level of CD90 expression to MSC inhibitory ability [144, 145]. In both cases, the enhanced inhibition was correlated with an increased HLA-G gene expression. To date, the implication of HLA-G in the immunomodulatory effect of MSC was mainly documented in BM-derived MSC. Few data are currently available for other MSC like those from the human umbilical cord matrix in which the expression of HLA-G has only been described at the mRNA level as demonstrated by RT-PCR [146, 147]. A standard comparative evaluation of HLA-G expression in MSC isolated from other tissues is mandatory to allow a better understanding of the role of such molecule in stem cell transplantation dedicated to solid and non-solid tissues. HLA-G expression and function profile should be studied during the differentiation process of these MSC both in vitro and in situ, in order to understand its ontogenetic expression profile. HLA-G expression and function should also be evaluated when the transplanted stem cells were isolated from the same tissue or from different ones. All these data will help to determine the exact role of HLA-G in modulating the immunological properties of stem cells and to open up new perspectives in the field of stem cell transplantation. Indeed, in transplanted patients, the HLA-G plasma level could be a new prognostic indicator of graft tolerance. The monitoring of HLA-G could be an indicator to adapt the immune suppressive treatment and thereby avoid their deleterious side effects. The identification of a MSC specific marker of strong immunosuppressive effect or an agent inducing HLA-G up-regulation might lead to enhanced MSC inhibitory capacity by the selection of a specific subpopulation or the pre-incubation of MSC with drugs before their clinical use. The development of 'tolerogenic' MSC through the modulation of HLA-G expression could allow successful allogeneic stem cell transplantation when autologous infusion is not recommended according to the primary disease or the instable recipient status. Furthermore, the co-transplantation of stem cells expressing HLA-G could be used as a new immunosuppressive strategy to induce donor-specific tolerance in solid organ or cell transplantation. As very few HLA-G-expressing cells are necessary to exert a significant inhibitory effect [148], the secretion of HLA-G by the engrafted MSC could sustain a tolerogenic microenvironment aimed at protecting recipients from graft rejection. However, the question of the safety of HLA-G-expressing cells remains to be addressed before their clinical use.

Concluding remarks

The accumulating evidence reported above suggests a potential tolerogenic role for HLA-G antigens in inflammation. In fact, HLA-G proteins seem to be involved in the termination of neuroinflammation related to remission of MS where their anti-inflammatory functions could be attributable to immunomodulatory properties of HLA-G5 isoform that is probably secreted into the brain by resident CNS cells and migrating immune cells. In addition, the association between the time course of fluctuations in serum levels of sHLA-G and perihematomal edema temporal evolution indicates that sHLA-G molecules may also exert anti-inflammatory properties in SICH through the shedding of sHLA-G1 isoform by macrophages migrating into the brain. Furthermore, HLA-G molecules could be implicated in the regulation of the immune system during other inflammatory, autoimmune and allergic conditions, such as gastrointestinal, skin and rheumatic diseases and asthma, in which HLA-G antigens seem to have a positive

role in down-modulating the immune response. In particular, in these disorders, HLA-G proteins could directly interact with immune cells or control the balance between Th1 and Th2 cytokines. On the other hand, it has recently become clear that cytokine and chemokine secretion may be modulated by HLA-G in different cell populations and in various physiological and/or pathological conditions. These mechanisms may have a role in HLA-G-induced immune suppression or may be related to HLA-G-induced release of pro-angiogenic factors during pregnancy. Moreover, motility and invasion capacity of different cell types can be dampened by HLA-G molecules. Preliminary data also suggest that cell migration can be modulated by sHLA-G through the down-regulation of chemokine receptor expression. Also of relevance is the demonstration that inducing changes in the levels of HLA-G in infected cells is a strategy used by several types of viruses to evade the immune system. In particular, HCMV, HSV-1, RABV, HCV, IAV, and HIV-1 seem to promote a HLA-G up-regulation to prevent infected cells from being recognized and attacked by the CTL and NK cells. In certain virus infections, HLA-G polymorphism also influences susceptibility to infection. Finally, stem cell transplantation is emerging as a promising therapy in regenerative medicine based on the longevity, self-renewal and multipotency properties of stem cells. Recently, research has demonstrated the constitutive expression of HLA-G on MSC and the relevant implication of HLA-G in MSC immunomodulatory effect through the blocking of effector cell functions and the generation of regulatory T cells. However, further studies are required to clarify whether: (1) HLA-G antigens are actually implicated in the pathogenesis of CNS and systemic inflammatory diseases or merely represent an indirect manifestation of an inflammatory immune activation taking place within the brain and the periphery, respectively; (2) cell migration can be effectively regulated by HLA-G proteins through the down-regulation of chemokine receptor expression; (3) HLA-G antigens can really contribute to the inhibition of immune functions by certain viruses, and thus provide an advantage for the development of infection by subverting their hosts' antiviral defenses; and (4) HLA-G expressing MSC have truly an immunomodulatory activity, are safe after injection and, thus, are therapeutically beneficial. In this way, the comprehension of the specific role of these molecules in the development and progression of inflammatory and viral disorders, as well as in tolerance induction during cell transplantation, and an improvement in our knowledge on the mechanisms of action of HLA-G that could lead to useful disease-modifying therapies able to modulate HLA-G production, may justify the use of HLA-G molecules as a marker of inflammation, viral infection and drug treatment and open up new perspectives for stem cell transplantation.

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References

1. Carosella ED, Moreau P, LeMaoult J, Rouas-Freiss N (2008) HLA-G: from biology to clinical benefits. Trends Immunol 3:125–132
2. Carosella ED, Favier B, Rouas-Freiss N, Moreau P, LeMaoult J (2008) Beyond the increasing complexity of the immunomodulatory HLA-G molecules. Blood 111:4862–4870
3. Ishitani A, Gerarthy DE (1992) Alternative splicing of HLA-G transcripts yields proteins with primary structures resembling both class I and class II antigens. Proc Natl Acad Sci USA 89:3947–3951
4. Paul P, Cabestre FA, Ibrahim EC, Lefevbre S, Khalil-Daher I, Vazeux G, Quiles RM, Bermond F, Dausset J, Carosella ED (2000) Identification of HLA-G7 as a new slice variant of the HLA-G mRNA and expression of soluble HL-G5, -G6, and -G7 transcripts in human transfected cells. Hum Immunol 61:1138–1149
5. Kovats S, Main EK, Librach C, Stubblebine M, Fisher SJ, DeMars R (1990) A class I antigen, HL-G, expressed in human trophoblasts. Science 144:220–223
6. LeMaoult J, Le Discorde M, Rouas-Freiss N, Moreau P, Menier C, McCluskey J, Carosella ED (2003) Biology and functions of human leukocyte antigen-G in health and sickness. Tissue Antigens 62:273–284
7. Ellis SA, Palmer LS, McMichael AJ (1990) Human trophoblast and the choriocarcinoma cell line BeWo express a truncated HLA class I molecule. J Immunol 144:731–735
8. Mallet V, Blaschitz A, Crisa L, Schmitt C, Fournel S, King A, Loke YW, Dohr G, Le Bouteiller P (1999) HLA-G in the human thymus: a subpopulation of medullary epithelial but not CD83(+) dendritic cells expresses HLA-G as a membrane-bound and soluble protein. Int Immunol 11:889–898
9. Le Discorde M, Moreau P, Sabatier P, Legeais JM, Carosella ED (2003) Expression of HLA-G in human cornea, an immune-privileged tissue. Hum Immunol 64:1039–1044
10. Ito T, Ito N, Saathoff M, Stampachiacchiere B, Bettermann A, Bulfone-Paus S, Takigawa M, Nickoloff BJ, Paus R (2005) Immunology of the human nail apparatus: the nail matrix is a site of relative immune privilege. J Invest Dermatol 125:1139–1148
11. Cirulli V, Zalatan J, McMaster M, Prinsen R, Salomon DR, Ricordi C, Torbett BE, Meda P, Crisa L (2006) The class I HLA repertoire of pancreatic islets comprises the nonclassical class Ib antigen HLA-G. Diabetes 55:1214–1222
12. Menier C, Rabreau M, Challier JC, Le Discorde M, Carosella ED, Rouas-Freiss N (2004) Erythroblasts secrete the nonclassical HLA-G molecule from primitive to definitive hematopoiesis. Blood 104:3153–3160
13. Moreau P, Adrian-Cabestre F, Menier C, Guiard V, Gourand L, Dausset J, Carosella ED, Paul P (1999) IL-10 selectively induces

HLA-G expression in human trophoblasts and monocytes. Int Immunol 11:803–811
14. Rizzo R, Melchiorri L, Stignani M, Baricordi OR (2007) HLA-G expression is a fundamental prerequisite to pregnancy. Hum Immunol 68:244–250
15. Lila N, Amrein C, Guillemain R, Chevalier P, Latremouille C, Fabiani JN, Dausset J, Carosella ED, Carpentier A (2002) Human leukocyte antigen-G expression after heart transplantation is associated with a reduced incidence of rejection. Circulation 105:1949–1954
16. Pistoia V, Morandi F, Wang X, Ferrone S (2007) Soluble HLA-G: are they clinically relevant? Semin Cancer Biol 17:469–479
17. Paul P, Rouas-Freiss N, Khalil-Daher I, Moreau P, Riteau B, Le Gal FA, Avril MF, Dausset J, Guillet JG, Carosella ED (1998) HLA-G expression in melanoma: a way for tumor cells to escape from immunosurveillance. Proc Natl Acad Sci USA 95:4510–4515
18. Onno M, Pangault C, Le Friec G, Guilloux V, Andre P, Fauchet R (2000) Modulation of HLA-G antigens expression by human cytomegalovirus: specific induction in activated macrophages harboring human cytomegalovirus infection. J Immunol 164:6426–6434
19. Rouas-Freiss N, Marchal RE, Kirszenbaum M, Dausset J, Carosella ED (1997) The α_1 domain of HLA-G1 and HLA-G2 inhibits cytotoxicity induced by natural killer cells: is HLA-G the public ligand for natural killer cell inhibitory receptors? Proc Natl Acad Sci USA 94:5249–5254
20. Fournel S, Aguerre-Gir M, Huc X, Lenfant F, Alam A, Toubert A, Bensussan A, Le Bouteiller P (2000) Cutting edge: soluble HLA-G1 triggers CD95/CD 95 ligand-mediated apoptosis in activated $CD8^+$ cells by interacting CD8. J Immunol 164:6100–6104
21. Contini P, Ghio M, Poggi A, Filaci G, Indiveri F, Ferrone S, Puppo F (2003) Soluble HLA-A, -B, -C, and -G molecules induce apoptosis in T and NK $CD8^+$ cells and inhibit cytotoxic T cell activity through CD8 ligation. Eur J Immunol 33:125–134
22. Lila N, Rouas-Freiss N, Dausset J, Carpentier A, Carosella ED (2001) Soluble HLA-G protein secreted by allo-specific $CD4^+$ T cells suppresses the allo-proliferative response: a $CD4^+$ T cell regulatory mechanism. Proc Natl Acad Sci USA 98:12150–12155
23. Le Rond S, Azèma C, Krawice-Radanne I, Durrbach A, Guettier C, Carosella ED, Rouas-Freiss N (2006) Evidence to support the role of HLA-G5 in allograft acceptance through induction of immunosuppressive/regulatory T cells. J Immunol 176:3266–3276
24. Horuzsko A, Lenfant F, Munn DH, Mellor AL (2001) Maturation of antigen-presenting cells is compromised in HLA-G transgenic mice. Int Immunol 13:385–394
25. Colonna M, Navarro F, Bellón T, Llano M, García P, Samaridis J, Angman L, Cella M, López-Botet M (1997) A common inhibitory receptor for major histocompatibility complex class I molecules on human lymphoid and myelomonocytic cells. J Exp Med 186:1809–1818
26. Kapasi K, Albert SE, Yie S, Zavazava N, Librach CL (2000) HLA-G has a concentration-dependent effect on the generation of an allo-CTL response. Immunology 101:191–200
27. Kanai T, Fujii T, Kozuma S, Yamashita T, Miki A, Kikuchi A, Taketani Y (2001) Soluble HLA-G influences the release of cytokines from allogeneic peripheral blood mononuclear cells in culture. Mol Hum Reprod 7:195–200
28. LeMaoult J, Caumartin J, Daouya M, Favier B, Le Rond S, Gonzalez A, Carosella ED (2007) Immune regulation by pretenders: cell-to-cell transfers of HLA-G make effector T cells act as regulatory cells. Blood 109:2040–2048
29. LeMaoult J, Zafaranloo K, Le Danff C, Carosella ED (2005) HLA-G up-regulates ILT2, ILT3, ILT4, and KIR2DL4 in antigen presenting cells, NK cells, and T cells. FASEB J 19:662–664
30. Carosella ED, Moreau P, Aractingi S, Rouas-Freiss N (2001) HLA-G: a shield against inflammatory aggression. Trends Immunol 22:553–555
31. Sospedra M, Martin R (2005) Immunology of multiple sclerosis. Annu Rev Immunol 23:683–747
32. Compston A, Coles A (2008) Multiple sclerosis. Lancet 372:1502–1517
33. McDonald WI, Compston A, Edan G, Goodkin D, Hartung HP, Lublin FD, McFarland HF, Paty DW, Polman CH, Reingold SC, Sandberg-Wollheim M, Sibley W, Thompson A, van den Noort S, Weinshenker BY, Wolinsky JS (2001) Recommended diagnostic criteria for multiple sclerosis: guidelines from the international panel on the diagnosis of multiple sclerosis. Ann Neurol 50:121–127
34. Fainardi E, Rizzo R, Melchiorri L, Vaghi L, Castellazzi M, Marzola A, Govoni V, Paolino E, Tola MR, Granieri E, Baricordi OR (2003) Presence of detectable levels of soluble HLA-G molecules in CSF of relapsing-remitting multiple sclerosis: relationship with CSF soluble HLA-I and IL-10 concentrations and MRI findings. J Neuroimmunol 142:149–158
35. Fainardi E, Rizzo R, Melchiorri L, Castellazzi M, Paolino E, Tola MR, Granieri E, Baricordi OR (2006) Intrathecal synthesis of soluble HLA-G and HLA-I molecules are reciprocally associated to clinical and MRI activity in patients with multiple sclerosis. Mult Scler 12:2–12
36. Fainardi E, Rizzo R, Melchiorri L, Stignani M, Castellazzi M, Caniatti ML, Baldi E, Tola MR, Granieri E, Baricordi OR (2007) Soluble HLA-G molecules are released as HLA-G5 and not as soluble HLA-G1 isoforms in CSF of patients with relapsing-remitting multiple sclerosis. J Neuroimmunol 192:219–225
37. Fainardi E, Rizzo R, Melchiorri L, Stignani M, Castellazzi M, Tamborino C, Paolino E, Tola MR, Granieri E, Baricordi OR (2008) CSF levels of soluble HLA-G and Fas molecules are inversely associated to MRI evidence of disease activity in patients with relapsing remitting multiple sclerosis. Mult Scler 14:446–454
38. Fainardi E, Rizzo R, Melchiorri L, Castellazzi M, Govoni V, Caniatti L, Paolino E, Tola MR, Granieri E, Baricordi OR (2004) Beneficial effect of interferon-β 1b treatment in patients with relapsing-remitting multiple sclerosis is associated with an increase in serum levels of soluble HLA-I molecules during the first 3 months of therapy. J Neuroimmunol 148:206–211
39. Mitsdoerffer M, Schreiner B, Kieseier B, Neuhaus O, Dichgans J, Hartung H-P, Weller M, Wiendl H (2005) Monocyte-derived HLA-G acts as a strong inhibitor of autologous CD4 T cell activation and is upregulated by interferon-β in vitro and in vivo: rationale for the therapy of multiple sclerosis. J Neuroimmunol 159:155–164
40. Wiendl H, Feger U, Mittelbronn M, Jack C, Schreiner B, Stadelmann C, Antel J, Brueck W, Meyermann R, Bar-Or A, Kieseier BC, Weller M (2005) Expression of the immune-tolerogenic major histocompatibility molecule HLA-G in multiple sclerosis: implications for CNS immunity. Brain 128:2689–2704
41. Feger U, Tolosa E, Huang YH, Waschbisch A, Biedermann T, Melms A, Wiendl H (2007) HLA-G expression defines a novel regulatory T cell subset present in human peripheral blood and sites of inflammation. Blood 110:568–577
42. Huang YH, Zozulya AL, Weidenfeller C, Schwab N, Wiendl H (2009) T cell suppression by naturally occurring HLA-G-expressing regulatory $CD4^+$ T cells is IL-10 dependent and reversible. J Leukoc Biol 86:273–281

43. Huang YH, Zozulya AL, Weidenfeller C, Metz I, Buck D, Toyka KV, Brück W, Wiendl H (2009) Specific central nervous system recruitment of HLA-G (+) regulatory T cells in multiple sclerosis. Ann Neurol 66:171–183
44. Fainardi E, Rizzo R, Castellazzi M, Stignani M, Granieri E, Baricordi OR (2009) Potential role of soluble human leukocyte antigen-G molecules in multiple sclerosis. Hum Immunol 70:981–987
45. Mayer SA, Rincon F (2005) Treatment of intracerebral haemorrhage. Lancet Neurol 4:662–672
46. Xi G, Keep RF, Hoff JT (2006) Mechanisms of brain injury after intracerebral haemorrhage. Lancet Neurol 5:53–63
47. Wang J, Doré S (2007) Inflammation after intracerebral hemorrhage. J Cereb Blood Flow Metab 27:894–908
48. Fainardi E, Rizzo R, Lupato A, Ramponi V, De Santis G, Garofano F, Stignani M, Tamborino C, Castellazzi M, Casetta I, Baricordi OR (2010) Timing of serum soluble HLA-G levels in acute and subacute phases after spontaneous intracerebral hemorrhage. Acta Neurochir 106(Suppl):141–145
49. Delgado P, Cuadrado E, Rosell A, Alvarez-Sabín J, Ortega-Aznar A, Hernández-Guillamón M, Penalba A, Molina CA, Montaner J (2008) Fas system activation in perihematomal areas after spontaneous intracerebral hemorrhage. Stroke 39:1730–1734
50. Wen Z, Fiocchi C (2004) Inflammatory bowel disease: autoimmune or immune-mediated pathogenesis? Clin Dev Immunol 11:195–204
51. Torres MI, Le Discorde M, Lorite P, Ríos A, Gassull MA, Gil A, Maldonado J, Dausset J, Carosella ED (2004) Expression of HLA-G in inflammatory bowel disease provides a potential way to distinguish between ulcerative colitis and Crohn's disease. Int Immunol 16:579–583
52. Rizzo R, Melchiorri L, Simone L, Stignani M, Marzola A, Gullini S, Baricordi OR (2008) Different production of soluble HLA-G antigens by peripheral blood mononuclear cells in ulcerative colitis and Crohn's disease: a noninvasive diagnostic tool? Inflamm Bowel Dis 14:100–105
53. Hviid TV, Hylenius S, Rorbye C, Nielsen LG (2003) HLA-G allelic variants are associated with differences in the HLA-G mRNA isoform profile and HLA-G mRNA levels. Immunogenetics 55:63–79
54. Glas J, Török HP, Tonenchi L, Wetzke M, Beynon V, Teshome MY, Cotofana S, Schiemann U, Griga T, Klein W, Epplen JT, Folwaczny C, Folwaczny M, Mussack T, Weiss EH (2007) The 14 bp deletion polymorphism in the HLA-G gene displays significant differences between ulcerative colitis and Crohn's disease and is associated with ileocecal resection in Crohn's disease. Int Immunol 19:621–626
55. Torres MI, López-Casado MA, Luque J, Peña J, Ríos A (2006) New advances in coeliac disease: serum and intestinal expression of HLA-G. Int Immunol 18:713–718
56. Urosevic M (2007) HLA-G in the skin–friend or foe? Semin Cancer Biol 17:480–484
57. Aractingi S, Briand N, Le Danff C, Viguier M, Bachelez H, Michel L, Dubertret L, Carosella ED (2001) HLA-G and NK receptor are expressed in psoriatic skin: a possible pathway for regulating infiltrating T cells? Am J Pathol 159:71–77
58. Borghi A, Fogli E, Stignani M, Melchiorri L, Altieri E, Baricordi O, Rizzo R, Virgili A (2008) Soluble human leukocyte antigen-G and interleukin-10 levels in plasma of psoriatic patients: preliminary study on a possible correlation between generalized immune status, treatments and disease. Arch Dermatol Res 300:551–559
59. Khosrotehrani K, Le Danff C, Reynaud-Mendel B, Dubertret L, Carosella ED, Aractingi S (2001) HLA-G expression in atopic dermatitis. J Invest Dermatol 117:750–752
60. Yari F, Zavaran Hosseini A, Nemat Gorgani M, Khorramizadeh MR, Mansouri P, Kazemnejad A (2008) Expression of HLA-G in the skin of patients with pemphigus vulgaris. Iran J Allergy Asthma Immunol 7:7–12
61. Gazit E, Slomov Y, Goldberg I, Brenner S, Loewenthal R (2004) HLA-G is associated with pemphigus vulgaris in Jewish patients. Hum Immunol 65:39–46
62. Verbruggen LA, Rebmann V, Demanet C, De Cock S, Grosse-Wilde H (2006) Soluble HLA-G in rheumatoid arthritis. Hum Immunol 67:561–567
63. Feuchtenberger M, Müller S, Roll P, Waschbisch A, Schäfer A, Kneitz C, Wiendl H, Tony HP (2008) Frequency of regulatory T cells is not affected by transient B cell depletion using anti-CD20 antibodies in rheumatoid arthritis. Open Rheumatol J 2:81–88
64. Rizzo R, Rubini M, Govoni M, Padovan M, Melchiorri L, Stignani M, Carturan S, Ferretti S, Trotta F, Baricordi OR (2006) HLA-G 14 bp polymorphism regulates the methotrexate response in rheumatoid arthritis. Pharmacogenet Genomics 16:615–623
65. Veit TD, Vianna P, Scheibel I, Brenol CV, Brenol JC, Xavier RM, Delgado-Cañedo A, Gutierrez JE, Brandalize AP, Schuler-Faccini L, Chies JA (2008) Association of the HLA-G 14 bp insertion/deletion polymorphism with juvenile idiopathic arthritis and rheumatoid arthritis. Tissue Antigens 71:440–446 Erratum in: Tissue Antigens 72, 185
66. Rosado S, Perez-Chacon G, Mellor-Pita S, Sanchez-Vegazo I, Bellas-Menendez C, Citores MJ, Losada-Fernandez I, Martin-Donaire T, Rebolleda N, Perez-Aciego P (2008) Expression of human leukocyte antigen-G in systemic lupus erythematosus. Hum Immunol 69:9–15
67. Rizzo R, Hviid TV, Govoni M, Padovan M, Rubini M, Melchiorri L, Stignani M, Carturan S, Grappa MT, Fotinidi M, Ferretti S, Voss A, Laustrup H, Junker P, Trotta F, Baricordi OR (2008) HLA-G genotype and HLA-G expression in systemic lupus erythematosus: HLA-G as a putative susceptibility gene in systemic lupus erythematosus. Tissue Antigens 71:520–529
68. Veit TD, Cordero EA, Mucenic T, Monticielo OA, Brenol JC, Xavier RM, Delgado-Cañedo A, Chies JA (2009) Association of the HLA-G 14 bp polymorphism with systemic lupus erythematosus. Lupus 18:424–430
69. Wu FX, Wu LJ, Luo XY, Tang Z, Yang MH, Xie CM, Liu NT, Zhou JG, Guan JL, Yuan GH (2009) Lack of association between HLA-G 14 bp polymorphism and systemic lupus erythematosus in a Han Chinese population. Lupus 18:1259–1266
70. Rizzo R, Mapp CE, Melchiorri L, Maestrelli P, Visentin A, Ferretti S, Bononi I, Miotto D, Baricordi OR (2005) Defective production of soluble HLA-G molecules by peripheral blood monocytes in patients with asthma. J Allergy Clin Immunol 115:508–513
71. Tahan F, Patiroglu T (2006) Plasma soluble human leukocyte antigen G levels in asthmatic children. Int Arch Allergy Immunol 141:213–216
72. Nicolae D, Cox NJ, Lester LA, Schneider D, Tan Z, Billstrand C, Kuldanek S, Donfack J, Kogut P, Patel NM, Goodenbour J, Howard T, Wolf R, Koppelman GH, White SR, Parry R, Postma DS, Meyers D, Bleecker ER, Hunt JS, Solway J, Ober C (2005) Fine mapping and positional candidate studies identify HLA-G as an asthma susceptibility gene on chromosome 6p21. Am J Hum Genet 76:349–357
73. Tan Z, Randall G, Fan J, Camoretti-Mercado B, Brockman-Schneider R, Pan L, Solway J, Gern JE, Lemanske RF, Nicolae D, Ober C (2007) Allele-specific targeting of microRNAs to HLA-G and risk of asthma. Am J Hum Genet 81:829–834
74. Mapp CE, Ferrazzoni S, Rizzo R, Miotto D, Stignani M, Boschetto P, Maestrelli P, Baricordi OR (2009) Soluble human

leucocyte antigen-G and interleukin-10 levels in isocyanate-induced asthma. Clin Exp Allergy 39:812–819
75. Liang S, Ristich V, Arase H, Dausset J, Carosella ED, Horuzsko A (2008) Modulation of dendritic cell differentiation by HLA-G and ILT4 requires the IL-6–STAT3 signaling pathway. Proc Natl Acad Sci USA 105:8357–8362
76. Park SJ, Nakagawa T, Kitamura H, Atsumi T, Kamon H, Sawa S, Kamimura D, Ueda N, Iwakura Y, Ishihara K, Murakami M, Hirano T (2004) IL-6 regulates in vivo dendritic cell differentiation through STAT3 activation. J Immunol 173:3844–3854
77. Li C, Houser BL, Nicotra ML, Strominger JL (2009) HLA-G homodimer-induced cytokine secretion through HLA-G receptors on human decidual macrophages and natural killer cells. Proc Natl Acad Sci USA 106:5767–5772
78. Rajagopalan S, Bryceson YT, Kuppusamy SP, Geraghty DE, van der Meer A, Joosten I, Long EO (2006) Activation of NK cells by an endocytosed receptor for soluble HLA-G. PLoS Biol 4:e9
79. van der Meer A, Lukassen HG, van Cranenbroek B, Weiss EH, Braat DD, van Lierop MJ, Joosten I (2007) Soluble HLA-G promotes Th1-type cytokine production by cytokine-activated uterine and peripheral natural killer cells. Mol Hum Reprod 13:123–133
80. Morel E, Bellon T (2008) HLA class I molecules regulate IFN-gamma production induced in NK cells by target cells, viral products, or immature dendritic cells through the inhibitory receptor ILT2/CD85j. J Immunol 181:2368–2381
81. McIntire RH, Morales PJ, Petroff MG, Colonna M, Hunt JS (2004) Recombinant HLA-G5 and -G6 drive U937 myelomonocytic cell production of TGF-beta1. J Leukoc Biol 76:1220–1228
82. Roth I, Corry DB, Locksley RM, Abrams JS, Litton MJ, Fisher SJ (1996) Human placental cytotrophoblasts produce the immunosuppressive cytokine interleukin 10. J Exp Med 184:539–548
83. Urosevic M, Dummer R (2003) HLA-G and IL-10 expression in human cancer-different stories with the same message. Semin Cancer Biol 13:337–342
84. Naji A, Le Rond S, Durrbach A, Krawice-Radanne I, Creput C, Daouya M, Caumartin J, LeMaoult J, Carosella ED, Rouas-Freiss N (2007) $CD3^+$ $CD4^{low}$ and $CD3^+$ $CD8^{low}$ are induced by HLA-G: novel human peripheral blood suppressor T cell subsets involved in transplant acceptance. Blood 110:3936–4398
85. Ciprandi G, Contini P, Murdaca G, DeAmici M, Gallina AM, Puppo F (2009) Soluble serum HLA-G and HLA-A, -B, -C molecules in patients with seasonal allergic rhinitis exposed to pollens. Int Immunopharmacol 9:1058–1062
86. Ciprandi G, Contini P, Fenoglio D, Sormani MP, Negrini S, Puppo F, Indiveri F (2008) Relationship between soluble HLA-G and HLA-A, -B, -C serum levels, and interferon-gamma production after sublingual immunotherapy in patients with allergic rhinitis. Hum Immunol 69:409–413
87. Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, Simonetti DW, Craig S, Marshak DR (1999) Multilineage potential of adult human mesenchymal stem cells. Science 284:143–147
88. Uccelli A, Moretta L, Pistoia V (2006) Immunoregulatory function of mesenchymal stem cells. Eur J Immunol 36:2566–2573
89. Uccelli A, Moretta L, Pistoia V (2008) Mesenchymal stem cells in health and disease. Nat Rev Immunol 8:726–736
90. Uccelli A, Pistoia V, Moretta L (2007) Mesenchymal stem cells: a new strategy for immunosuppression? Trends Immunol 28:219–226
91. Aggarwal S, Pittenger MF (2005) Human mesenchymal stem cells modulate allogeneic immune cell responses. Blood 105:1815–1822
92. Corcione A, Benvenuto F, Ferretti E, Giunti D, Cappiello V, Cazzanti F, Risso M, Gualandi F, Mancardi GL, Pistoia V, Uccelli A (2006) Human mesenchymal stem cells modulate B cell functions. Blood 107:367–372
93. Jiang XX, Zhang Y, Liu B, Zhang SX, Wu Y, Yu XD, Mao N (2005) Human mesenchymal stem cells inhibit differentiation and function of monocyte-derived dendritic cells. Blood 105:4120–4126
94. Spaggiari GM, Capobianco A, Becchetti S, Mingari MC, Moretta L (2006) Mesenchymal stem cell-natural killer cell interactions: evidence that activated NK cells are capable of killing MSCs, whereas MSCs can inhibit IL-2-induced NK cell proliferation. Blood 107:1484–1490
95. Morandi F, Raffaghello L, Bianchi G, Meloni F, Salis A, Millo E, Ferrone S, Barnaba V, Pistoia V (2008) Immunogenicity of human mesenchymal stem cells in HLA-class I-restricted T cell responses against viral or tumor-associated antigens. Stem Cells 26:1275–1287
96. Selmani Z, Naji A, Zidi I, Favier B, Gaiffe E, Obert L, Borg C, Saas P, Tiberghien P, Rouas-Freiss N, Carosella ED, Deschaseaux F (2008) Human leukocyte antigen-G5 secretion by human mesenchymal stem cells is required to suppress T lymphocyte and natural killer function and to induce $CD4^+$ CD25high $FOXP3^+$ regulatory T cells. Stem Cells 26:212–222
97. Rizzo R, Campioni D, Stignani M, Melchiorri L, Bagnara GP, Bonsi L, Alviano F, Lanzoni G, Moretti S, Cuneo A, Lanza F, Baricordi OR (2008) A functional role for soluble HLA-G antigens in immune modulation mediated by mesenchymal stromal cells. Cytotherapy 10:364–375
98. Nasef A, Chapel A, Mazurier C, Bouchet S, Lopez M, Mathieu N, Sensebe L, Zhang Y, Gorin NC, Thierry D, Fouillard L (2007) Identification of IL-10 and TGF-beta transcripts involved in the inhibition of T-lymphocyte proliferation during cell contact with human mesenchymal stem cells. Gene Expr 13:217–226
99. Dorling A, Monk N, Lechler R (2000) HLA-G inhibits the transendothelial cell migration of human NK cells: a strategy for inhibiting xenograft rejection. Transplant Proc 32:938
100. McCormick J, Whitley GS, Le Bouteiller P, Cartwright JE (2009) Soluble HLA-G regulates motility and invasion of the trophoblast-derived cell line SGHPL-4. Hum Reprod 24:1339–1345
101. Alcami A, Koszinowski UH (2000) Viral mechanisms of immune evasion. Trends Microbiol 8:410–418
102. Carosella ED, HoWangYin KY, Favier B, LeMaoult J (2008) HLA-G-dependent suppressor cells: diverse by nature, function, and significance. Hum Immunol 69:700–707
103. Tripathi P, Agrawal S (2007) The role of human leukocyte antigen E and G in HIV infection. AIDS 21:1395–1404
104. Lozano JM, Gonzalez R, Kindelan JM, Rouas-Freiss N, Caballos R, Dausset J, Carosella ED, Pena J (2002) Monocytes and T-lymphocytes in HIV-1-positive patients express HLA-G molecule. AIDS 16:347–351
105. Cabello A, Rivero A, Garcia MJ, Lozano JM, Torre-Cisneros J, Gonzalez R, Duenas G, Galiani MD, Camacho A, Santamaria M, Solana R, Montero C, Kindelan JM, Pena J (2003) HAART induces the expression of HLA-G on peripheral monocytes in HIV-1 infected individuals. Hum Immunol 64:1045–1049
106. Rivero A, Lozano JM, Gonzalez R, Garcia-Jurado G, Camacho A, Torres-Cisneros J, Pena J (2007) Nucleoside reverse transcriptase inhibitors are able and protease inhibitors unable to induce the tolerogenic molecule HLA-G1 on monocytes from HIV-1 infected patients. Hum Immunol 68:303–306
107. Adnan S, Balamurugan A, Trocha A, Bennett MS, Ng HL, Ali A, Brander C, Yang OO (2006) Nef interference with HIV-1-specific CTL antiviral activity is epitope specific. Blood 108:3414–3419

108. Pizzato N, Derrien M, Lenfant F (2004) The short cytoplasmic tail of HLA-G determines its resistance to HIV-1 Nef-mediated cell surface downregulation. Hum Immunol 65:1389–1396
109. Matte C, Lajoie J, Lacaille J, Zijenah LS, Ward BJ, Roger M (2004) Functionally active HLA-G polymorphisms are associated with the risk of heterosexual HIV-1 infection in African women. AIDS 18:427–431
110. Lajoie J, Hargrove J, Zijenah LS, Humphrey JH, Ward BJ, Roger M (2006) Genetic variants in nonclassical major histocompatibility complex class I human leukocyte antigen (HLA)-E and HLA-G molecules are associated with susceptibility to heterosexual acquisition of HIV-1. J Infect Dis 193:298–301
111. Clements CS, Kjer-Nielsen L, Kostenko L, Hoare HL, Dunstone MA, Moses E, Freed K, Brooks AG, Rossjohn J, McCluskey J (2005) Crystal structure of HLA-G: a nonclassical MHC class I molecule expressed at the fetal-maternal interface. Proc Natl Acad Sci USA 102:3360–3365
112. Aikhionbare FO, Kumaresan K, Shamsa F, Bond VC (2006) HLA-G DNA sequence variants and risk of perinatal HIV-1 transmission. AIDS Res Ther 3:28
113. Soderberg-Naucler C, Nelson JY (1999) Human cytomegalovirus latency and reactivation—a delicate balance between the virus and its host's immune system. Intervirology 42:314–321
114. Powers C, DeFilippis V, Malouli D, Fruh K (2008) Cytomegalovirus immune evasion. Curr Top Microbiol Immunol 325:333–359
115. Yan WH, Lin A, Chen BG, Chen SY (2009) Induction of both membrane-bound and soluble HLA-G expression in active human cytomegalovirus infection. J Infect Dis 200:820–826
116. Fisher S, Genbacev O, Maidji E, Pereira L (2000) Human cytomegalovirus infection of placental cytotrophoblasts in vitro and in utero: implications for transmission and pathogenesis. J Virol 74:6808–6820
117. Jun Y, Kim E, Jin M, Sung HC, Han H, Geraghty DE, Ahn K (2000) Human cytomegalovirus gene products US3 and US6 down-regulate trophoblast class I MHC molecules. J Immunol 164:805–811
118. Wiendl H (2007) HLA-G in the nervous system. Hum Immunol 68:286–293
119. Lafon M, Prehaud C, Megret F, Lafage M, Mouillot G, Roa M, Moreau P, Rouas-Freiss N, Carosella ED (2005) Modulation of HLA-G expression in human neural cells after neurotropic viral infections. J Virol 79:15226–15237
120. Cordero EA, Veit TD, da Silva MA, Jacques SM, Silla LM, Chies JA (2009) HLA-G polymorphism influences the susceptibility to HCV infection in sickle cell disease patients. Tissue Antigens 74:308–313
121. LeBouder F, Khoufache K, Menier C, Mandouri Y, Keffous M, Lejal N, Krawice-Radanne I, Carosella ED, Rouas-Freiss N, Riteau B (2009) Immunosuppressive HLA-G molecule is upregulated in alveolar epithelial cells after influenza A virus infection. Hum Immunol 70:1016–1019
122. Pizzato N, Garmy-Susini B, Le Bouteiller P, Lenfant F (2003) Down-regulation of HLA-G1 cell surface expression in human cytomegalovirus infected cells. Am J Reprod Immunol 50:328–333
123. Barel MT, Pizzato N, van Leeuwen D, Bouteiller PL, Wiertz EJ, Lenfant F (2003) Amino acid composition of alpha1/alpha2 domains and cytoplasmic tail of MHC class I molecules determine their susceptibility to human cytomegalovirus US11-mediated down-regulation. Eur J Immunol 33:1707–1716
124. Megret F, Prehaud C, Lafage M, Moreau P, Rouas-Freiss N, Carosella ED, Lafon M (2007) Modulation of HLA-G and HLA-E expression in human neuronal cells after rabies virus or herpes virus simplex type 1 infections. Hum Immunol 68:294–302
125. Lozano JM, Gonzalez R, Luque J, Frias M, Rivero A, Pena J (2009) $CD8^+$ $HLA\text{-}G^+$ regulatory T cells are expanded in HIV-1-infected patients. Viral Immunol 22:463–465
126. Derrien M, Pizzato N, Dolcini G, Menu E, Chaouat G, Lenfant F, Barre-Sinoussi F, Bouteiller PL (2004) Human immunodeficiency virus-1 downregulates cell surface expression of the non-classical major histocompatibility class I molecule HLA-G1. J Gen Virol 85:1945–1954
127. Donaghy L, Gros F, Amiot L, Mary C, Maillard A, Guiguen C, Gangneux JP (2007) Elevated levels of soluble non-classical major histocompatibility class I molecule human leucocyte antigen (HLA)-G in the blood of HIV-infected patients with or without visceral leishmaniasis. Clin Exp Immunol 147:236–240
128. Lajoie J, Fontaine J, Tremblay C, Routy JP, Poudrier J, Roger M (2009) Persistence of high levels of blood soluble human leukocyte antigen-G is associated with rapid progression of HIV infection. AIDS 23:1437–1440
129. Barry FP, Murphy JM (2004) Mesenchymal stem cells: clinical applications and biological characterization. Int J Biochem Cell Biol 36:568–584
130. Lysy PA, Campard D, Smets F, Malaise J, Mourad M, Najimi M, Sokal EM (2008) Persistence of a chimerical phenotype after hepatocyte differentiation of human bone marrow mesenchymal stem cells. Cell Prolif 41:36–58
131. Najimi M, Khuu DN, Lysy PA, Jazouli N, Abarca J, Sempoux C, Sokal EM (2007) Adult derived human liver mesenchymal-like cells as a potential progenitors reservoir of hepatocytes? Cell Transplant 16:717–728
132. Campard D, Lysy PA, Najimi M, Sokal EM (2008) Native umbilical cord matrix stem cells express hepatic markers and differentiate into hepatocyte-like cells. Gastroenterology 134:833–848
133. Bartholomew A, Sturgeon C, Siatskas M, Ferrer K, McIntosh K, Patil S, Hardy W, Devine S, Ucker D, Deans R, Moseley A, Hoffman R (2002) Mesenchymal stem cells suppress lymphocyte proliferation in vitro and prolong skin graft survival in vivo. Exp Hematol 30:42–48
134. Le Blanc K, Rasmusson I, Sundberg B, Gotherstrom C, Hassan M, Uzunel M, Ringden O (2004) Treatment of severe acute graft-versus-host disease with third party haploidentical mesenchymal stem cells. Lancet 363:1439–1441
135. Beyth S, Borovsky Z, Mevorach D, Liebergall M, Gazit Z, Aslan H, Galun E, Rachmilewitz J (2005) Human mesenchymal stem cells alter antigen-presenting cell maturation and induce T cell unresponsiveness. Blood 105:2214–2219
136. Crop MJ, Baan CC, Korevaar SS, Ijzermans JN, Alwayn IP, Weimar W, Hoogduijn MJ (2009) Donor-derived mesenchymal stem cells suppress alloreactivity of kidney transplant patients. Transplantation 87:896–906
137. Le Blanc K, Frassoni F, Ball L, Locatelli F, Roelofs H, Lewis I, Lanino E, Sundberg B, Bernardo ME, Remberger M, Dini G, Egeler RM, Bacigalupo A, Fibbe W, Ringdén O, Developmental Committee of the European Group for Blood, Marrow Transplantation (2008) Mesenchymal stem cells for treatment of steroid-resistant, severe, acute graft-versus-host disease: a phase II study. Lancet 371:1579–1586
138. Hügle T, Daikeler T (2010) Stem cell transplantation for autoimmune diseases. Haematologica 95:185–188
139. Menier C, Rouas-Freiss N, Favier B, LeMaoult J, Moreau P, Carosella ED (2009) Recent advances on the non-classical major histocompatibility complex class I HLA-G molecule. Tissue Antigens 75:201–206
140. Le Maux A, Noël G, Birebent B, Grosset J-M, Vu N, De Guibert S, Bernard M, Semana G, Amiot L (2008) Soluble human leucocyte antigen-G molecules in peripheral blood haematopoietic stem cell transplantation: a specific role to prevent acute

graft-versus-host disease and a link with regulatory T cells. Clin Exp Immunol 152:50–56
141. Nasef A, Mathieu N, Chapel A, Frickm J, Francois S, Mazurier C, Boutarfa A, Bouchet S, Gorin NC, Thierry D, Fouillard L (2007) Immunosuppressive effects of mesenchymal stem cells: involvement of HLA-G. Transplantation 84:231–237
142. Ivanova-Todorova E, Mourdjeva M, Kyurkchiev D, Bochev I, Stoyanova E, Dimitrov R, Timeva T, Yunakova M, Bukarev D, Shterev A, Tivchev P, Kyurkiev S (2009) HLA-G expression is up-regulated by progesterone in mesenchymal stem cells. Am J Reprod Immunol 62:25–33
143. Blanco O, Tirado I, Munoz-Fernandez R, Abadia-Molina AC, Garcia-Pacheco JM, Pena J, Olivares EG (2008) Human decidual stromal cells express HLA-G: effects of cytokines and decidualization. Hum Reprod 23:144–152
144. Nasef A, Zhang YZ, Mazurier C, Bouchet S, Bensidhoum M, Francois S, Gorin C, Lopez M, Thierry D, Fouillard L, Chapel A (2009) Selected stro-1-enriched bone marrow stromal cells display a major suppressive effect on lymphocyte proliferation. Int J Lab Hem 31:9–19
145. Campioni D, Rizzo R, Stignani M, Melchiorri L, Ferrari L, Moretti S, Russo A, Bagnara GP, Bonsi L, Alviano F, Lanzoni G, Cuneo A, Baricordi OR, Lanza F (2009) A decreased positivity for CD90 on human mesenchymal stromal cells is associated with a loss of immunosuppressive activity by MSCs. Cytometry B Clin Cytom 76:225–230
146. Weiss ML, Anderson C, Medicetty S, Seshareddy KB, Weiss RJ, VanderWerff I, Troyer D, McIntosh KR (2008) Immune properties of human umbilical cord warthon's jelly-derived cells. Stem cells 26:2865–2874
147. La Rocca G, Anzalone R, Corrao S, Magno F, Loria T, Lo Iacono M, Di Stefano A, Giannuzzi P, Marasa L, Cappello F, Zummo G, Farina F (2009) Isolation and characterization of Oct-4^+/HLA-G^+ mesenchymal stem cells from human umbilical cord matrix: differentiation potential and detection of new markers. Histochem Cell Biol 131:267–282
148. Wiendl H, Mitsdoerffer M, Hofmeister V, Wischhusen J, Bornemann A, Meyermann R, Weiss EH, Melms A, Weller M (2002) A functional role of HLA-G expression in human gliomas: an alternative strategy of immune escape. J Immunol 168:4772–4780