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REVIEW

Impact of HLA-G analysis in prevention, diagnosis and treatment of pathological conditions

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Abstract

Human leukocyte antigen-G (HLA-G) is a non-classical HLA class I molecule that differs from classical HLA class I molecules by low polymorphism and tissue distribution. HLA-G is a tolerogenic molecule with an immune-modulatory and anti-inflammatory function on both innate and adaptative immunity. This peculiar characteristic of HLA-G has led to investigations of its role in pathological conditions in order to define possible uses in diagnosis, prevention and treatment. In recent years, HLA-G has been shown to have an important implication in different inflammatory and autoimmune diseases, pregnancy complications, tumor development and aggressiveness, and susceptibility to viral infections. In fact, HLA-G molecules have been reported to alternate at both genetic and protein level in different disease situations, supporting its crucial role in pathological conditions. Specific pathologies show altered levels of soluble (s)HLA-G and different *HLA-G* gene polymorphisms seem to correlate with disease. This review aims to update scientific knowledge on the contribution of HLA-G in managing pathological conditions.

Key words: Human leukocyte antigen-G; Pathology; Prognosis; Diagnosis; Treatment; Marker

Core tip: Human leukocyte antigen-G (HLA-G) is a tolerogenic molecule. HLA-G has been shown to have important implications in different pathological conditions where it is reported to alternate at both protein and genetic level. The peculiar immunoregulatory function of HLA-G and its dysregulation in different diseases have led to investigation of its role in pathological conditions in order to define possible uses in diagnosis, prevention and treatment. This review aims to update scientific knowledge on the contribution of HLA-G in managing pathological conditions.

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INTRODUCTION

Diagnosis and prevention of diseases is mainly based on the identification of specific biological markers and drug targets. In view of this, the possibility of easy and fast identification of molecules, for example in biological fluids, seems to be even more necessary.

In recent years, different studies have demonstrated that human leukocyte antigen-G (HLA-G), a non-classical class I molecule, could fulfil this necessity[1-3]. In fact, HLA-G expression and levels in biological fluids, cells and tissues in different pathological conditions have been shown. Several authors reported that the level of soluble

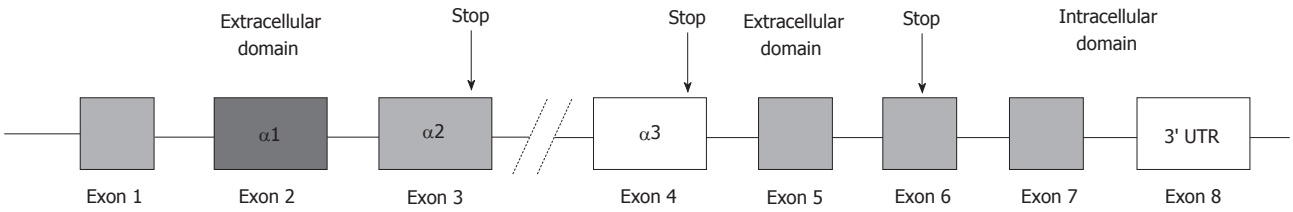

Figure 1 Human leukocyte antigen-G gene. UTR: Untranslated region.

HLA-G and gene polymorphisms correlate with disease outcome and the therapeutic success of treatment[4-6].

HLA-G MOLECULE

HLA-G is a major histocompatibility complex class I antigen encoded by a gene on chromosome 6p21. It differs from classical HLA class I molecules by its restricted tissue distribution and limited polymorphism in the coding region. To date, 50 alleles (IMGT HLA database, August 2013) and 16 proteins are known. The gene structure of HLA-G is homologous to other HLA class I (Ia) genes consisting of 7 introns and 8 exons coding the heavy chain of the molecule. Exon 1 encodes the peptide signal, while exons 2, 3 and 4 encode the extracellular α1, α2 and α3 domains, respectively. Exons 5 and 6 encode the transmembrane and cytoplasmic domains of the heavy chain. Exon 7 is always absent from mature mRNA due to the stop codon in exon 6; exon 8 is not translated (Figure 1). Seven HLA-G isoforms exist due to mRNA alternative splicing and differential association with β2-microglobulin; two of these are found on the cell surface and in biological fluids: Membrane-bound G1 and soluble G5, which lacks the trans-membrane and intracellular domains of membrane-bound G1 (Figure 1)[7]. HLA-G possesses an unpaired cysteine residue at position 42 on an external loop of the peptide binding groove that enables the dimerisation[8,9]. HLA-G monomers are recognized by the inhibitory receptors LILRB1 and LILRB2 and by KIR2DL4[10]. LILR receptors have a greater affinity for the dimeric form that increases the signaling transduction, especially in natural killer (NK) cells[11,12]. The interaction of HLA-G molecules with inhibitory receptors induces apoptosis of activated Crohn's disease ($CD8^+$) T cells[11], modulates the activity of NK cells[13,14] and of dendritic cells (DC)[15,16], blocks allo-cytotoxic T lymphocyte response[17] and induces expansion of suppressor T cell populations, such as $CD4^+CD25^+FoxP3^+$ regulatory T (Treg) cells[18,19]. Moreover, HLA-G is expressed at high levels on DC-10 cells, human DCs with tolerogenic activity and an outstanding ability to produce interleukin (IL)-10[16]. Interestingly, the expression of membrane-bound HLA-G1 and that of its receptors is up-regulated by IL-10 on DC-10 and the expression of high levels of membrane-bound HLA-G1, ILT4 and IL-10 by DC-10 is critical to the generation of allergen-specific Tr1 cells by DC-10[16].

The HLA-G production is controlled by several polymorphisms, both in the promoter and in the 3' untranslated region (3' UTR), modifying the affinity of gene targeted sequences for transcriptional or post-transcriptional factors, respectively[20].

Twenty-nine single nucleotide polymorphisms (SNPs) have been identified in the HLA-G promoter region which may be involved in the regulation of HLA-G expression, considering that many of these polymorphisms are within or close to known or putative regulatory elements. The HLA-G 5' upstream regulatory region (URR) is unique among the *HLA* genes[21] and is unresponsive to NF-κB[22] and interferon (IFN)-γ[23] due to the presence of a modified enhancer A and a deleted interferon-stimulated response element (ISRE). A locus control region located -1.2 kb from exon 1 exhibits a binding site for CREB1 factor, which also binds to two additional cAMP response elements at -934 and -770 positions from the ATG. In addition, a binding site ISRE for interferon response factor-1 is located at the -744 base pair (bp) position[24] and is involved in HLA-G transactivation following IFN-β treatment[24]. The HLA-G promoter also contains a heat shock element at the -459/-454 position that binds heat shock factor-1[25] and a progesterone receptor binding site at -37 bp from ATG[26]. Several promoter region polymorphisms coincide with or are close to known or putative regulatory elements and thus may affect the binding of HLA-G regulatory factors[27]. The -725 C > G/T SNP is very close to ISRE, in which the -725 G allele is associated with a significantly higher expression level compared with the others[28]. The polymorphic sites at the 5' URR are frequently in linkage disequilibrium with the polymorphic sites identified at the 3' UTR, some of them influencing alternative splicing and mRNA stability.

A 14 bp insertion/deletion (INS/DEL) polymorphism (rs66554220) in exon 8 involves mRNA stability and expression[29,30]. In particular, the DEL allele stabilizes the mRNA with a consequent higher HLA-G expression[30,31]. The presence of an adenine at position +3187 modifies an AU-rich motif in the HLA-G mRNA and decreases its stability[32]. One SNP C > G at the +3142 bp position (rs1063320) affects the expression of the HLA-G locus by increasing the affinity of this region for the microRNAs (miR)-148a, miR-148b and miR-152, therefore decreasing the mRNA availability by mRNA degradation and translation suppression[33]. The influence of the +3142G allele has been demonstrated by a functional study in which HLA-G high-expressing JEG-3 choriocarcinoma-derived cells have been transfected with miR-148a, decreasing soluble HLA-G levels. The

discordant results obtained by Manaster *et al*[34], who have reported the lack of +3142 C > G effect on the miRNA control of membrane HLA-G expression, prompt further considerations on the relationship between this polymorphism and membrane HLA-G expression. Other SNPs are identified as implicated in miRNA interaction. In particular, +3003, +3010, +3027 and +3035 SNPs are targets for miR-513a-5p, miR-518c*, miR-1262 and miR-92a-1*, miR-92a-2*, miR-661, miR-1224-5p and miR-433 miRNAs[35]. The miR-2110, miR-93, miR-508-5p, miR-331-5p, miR-616, miR-513b, and miR-589* miRNAs target the 14bp INS/DEL fragment region and miR-148a, miR-19a*, miR-152, mir-148b, and miR-218-2 target the +3142 C/G polymorphism.

HLA-G is a stress-inducible gene; heat shock, hypoxia and arsenite increase different HLA-G alternative transcripts[25,36,37]. The indoleamine 2,3-dioxygenase, an enzyme which metabolizes tryptophan, induces HLA-G expression during monocyte differentiation into DCs[38]. The anti-inflammatory and immunosuppressive IL-10 has been correlated with concomitant HLA-G expression[30,39]. Transactivation of HLA-G transcription has also been demonstrated by leukemia inhibitory factor[40], progesterone[26] and methotrexate[41] cell exposure. Furthermore, IFN-α, -β and -γ enhance HLA-G cell-surface expression by tumors or monocytes[42,43]. HLA-G expression could be acquired by trogocytosis, where a "donor" cell that expresses membrane HLA-G exchanges membrane parts containing HLA-G with a "recipient" cell that is not expressing HLA-G molecules. In this particular situation, "recipient" cells will acquire and make use of membrane HLA-G molecules from a "donor" HLA-G positive cell without the activation of HLA-G gene. Trogocytosis of antigen presenting cell HLA-G1 by T cells in humans makes T cells unresponsive[44]. It has been shown that HLA-G1 can be acquired by NK cells from tumor cells. NK cells that acquire HLA-G1 stop proliferating, are no longer cytotoxic and behave like suppressor cells capable of inhibiting other NK-cell functions[14].

HLA-G's role in immune-tolerance was discovered studying its expression in trophoblast cells at the fetus-maternal interface[45]. The importance of HLA-G production by placental trophoblasts is evident in pre-eclampsia and unexplained recurrent spontaneous abortion (RSA). Several studies have found an aberrant or reduced expression of both HLA-G mRNA and protein in pathological compared with control placentas[46-48], with a possible implication in fetal protection and vascular events.

HLA-G expression has been documented in a few tissues during physiological conditions, such as cornea, thymus, erythroid and endothelial precursors[49-51], and in a variable percentage of serum/plasma samples from healthy subjects[52] where the main producers are activated CD14 positive monocytes[53]. A modified expression of HLA-G molecules has been observed during "no-physiological" conditions, such as viral infection[54-57], cancer[58,59], transplantation[60-64], inflammatory and autoimmune diseases[65,66].

Thus, a growing body of evidence has indicated HLA-G as a suitable key factor in different pathologies. In fact, the immune-modulation by HLA-G may exhibit two distinct effects in pathological conditions: It could be protective in inflammatory and autoimmune diseases[2,65-67], or on the other hand it could be dangerous, for example in tumors or infectious diseases[54-56,58,59]. Based on this evidence, the role of HLA-G in inflammatory and autoimmune diseases has gained considerable clinical interest for the possibility of exploiting it as a molecular biomarker and a therapeutic target.

HLA-G AND PATHOLOGICAL CONDITIONS: PERSPECTIVES IN PREVENTION, DIAGNOSIS AND TREATMENT

Given the immunomodulatory nature of HLA-G molecule, it could be considered a good reference parameter for prevention, diagnosis and treatment in autoimmune and inflammatory diseases.

HLA-G has been analyzed in different pathologies. In this review, we focus on the importance of HLA-G analysis in common and debilitating pathologies characterized by a dysregulation in host immune system in which HLA-G plays a central role.

HLA-G impact in rheumatic disease

Rheumatic disease is a general term used to describe numerous conditions that affect the joints [rheumatoid arthritis (RA)], connective tissues [scleroderma, systemic lupus erythematosus (SLE)] and vessels (vasculitis). Rheumatic diseases are inflammatory and autoimmune diseases, the second most common cause of disability after musculoskeletal injuries. RA (OMIM, #180300) is caused by the immune system attacking synovial cells and treatments include disease modifying anti-rheumatic drugs (DMARDs) and, more recently, biological agents. An important goal of RA therapy has shifted to initiate treatment early and aggressively to achieve remission or low disease activity as quickly as possible. This "treat-to-target" concept has been shown to maximize long-term healthy life[68,69].

Interestingly, RA patients present with an abnormal regulatory network in the immune response, which includes *HLA-G* gene[70]. Serum sHLA-G protein concentration is significantly lower in RA[71] patients than in controls. The decreased sHLA-G concentrations may lead to a chronic activation of inflammatory cells and contribute to the development of the disease. The evaluation of sHLA-G molecules at the specific inflammation site of the synovia reported higher levels of sHLA-G in RA[72] patients. The release of HLA-G in the inflamed synovium may be related to the recruitment of activated HLA-G positive immune cells and the local production by activated synovial fibroblasts[73] that could interact with immune inhibitory receptors and maintain a chronic inflammatory response. These data suggest that there is

a different production of HLA-G molecules on the basis of the local and systemic environments, characterized by different molecular factors and cell types. Interestingly, a recent work confirmed the role of HLA-G molecules in RA. The authors used an intracutaneous treatment of HLA-G monomer or dimer molecules in collagen-induced arthritis model mice. These molecules produced excellent anti-inflammatory effects with a single, local administration[74]. Notably, the dimer exhibited higher immunosuppressive effects than the monomer due to the higher dimer affinity for PIR-B, the mouse homolog of the LILRBs. The HLA-G 14 bp INS/DEL polymorphism has been evaluated as a pharmacogenetic marker of MTX therapy[41]. The authors showed an increase of the 14 bp DEL/DEL genotype in the responder group, characterized by a reduction in disease activity score (DAS28) measured before and after six months of treatment with MTX. In contrast to this study, there are two researches with negative results: (1) 130 RA patients responsive to MTX did not show a significant difference in 14 bp DEL/INS allelic and genotypic distribution (DAS28 < 3.2)[75]; and (2) 186 RA patients, previously untreated with MTX, were prospectively followed up and considered as responders with a DAS28 of up to 2.4 after six months of treatment[76]. No significant association between HLA-G 14 bp INS/DEL and MTX efficacy was observed. Comparing these studies, the discordant results may reflect population differences in gene expression that could influence the power of association studies and lead to different levels of association. In addition, the different doses of MTX and the different cut-off used for RA therapy response definition could affect the results obtained.

Rizzo *et al*[2] evaluated the possible role of HLA-G molecules as biomarkers for RA treatment in a follow-up study. Twenty-three early RA (ERA) patients were analyzed during a 12 mo follow-up disease treatment for sHLA-G levels in plasma samples, mHLA-G and ILT2 expression on peripheral blood CD14 positive cells, and typed HLA-G 14 bp DEL/INS polymorphism. Interestingly, the authors observed that ERA patients with low sHLA-G and membrane HLA-G expression suffered a more severe disease. In fact, sHLA-G levels inversely correlated with DAS28 and ultrasonographic power Doppler scores, used to define the severity and progression of the disease. Interestingly, sHLA-G up-modulation is evident after 3 mo of DMARDs therapy, while a significant reduction in tumor necrosis factor-α levels is evident after 9 mo therapy when a clear amelioration of the disease is evident, with a high specificity for HLA-G detection in EA condition. Moreover, the implication of the HLA-G 14 bp INS/DEL polymorphism is confirmed as the presence of the DEL allele characterizes the patients with a significant improvement in disease status.

SLE (OMIM, #601744) is a systemic autoimmune disease of the connective tissue that can affect any part of the body. Rosado *et al*[77] and Chen *et al*[78] showed higher sHLA-G and IL-10 levels in SLE patients in comparison with healthy controls, while Rizzo *et al*[66] observed lower sHLA-G concentrations in SLE patients. The differences in sHLA-G levels in these two papers could be due to the difference in the analyzed samples (serum or plasma) since it is known that the highest sHLA-G levels are recovered from plasma samples compared with serum collected from the same subjects because of a trapping phenomenon during clot formation that could subtract sHLA-G from the serum[79]. As a proof, Monsiváis-Urenda *et al*[80] evidenced a diminished expression of HLA-G in monocytes and in mature CD83 positive DCs from SLE patients compared with healthy controls. In addition, monocytes from SLE patients showed a decreased induction of HLA-G expression in response to IL-10. Finally, lymphocytes from SLE patients displayed a lower acquisition of HLA-G (by trogocytosis) from autologous monocytes compared to controls. Interestingly, ILT-2 receptor expression is increased on lymphocytes from SLE patients, in particular, in CD3 positive cells, CD19 positive cells, CD56 positive cells and related to IL-10 and anti-DNA antibodies[78]. These results confirm the presence of a HLA-G impaired expression in patients with SLE and a possible role in the pathogenesis. Using a SNP mapping approach, HLA-G gene is reported to be a novel independent locus with SLE interaction[81]. In particular, HLA-G 14 bp INS/DEL polymorphism and HLA-G +3142 C > G SNP were analyzed in a SLE population. SLE patients showed a higher frequency of 14 bp INS allele and 14 bp INS/INS genotype[66]. Moreover, 14 bp INS/INS patients presented the highest disease activity[82]. On the contrary, the evaluation of HLA-G 14 bp INS/DEL polymorphism in a SLE Brazilian population failed to present an association[83], while the +3142 G allele was found to be associated with SLE susceptibility[84]. The +3142 G allele and the +3142 GG genotype frequencies are increased among SLE patients compared with controls[85]. These data support the role of HLA-G molecules in the control of the SLE condition and in particular several results sustain the lower HLA-G expression as a risk factor for SLE development.

HLA-G impact in central nervous system inflammatory diseases

Multiple sclerosis (MS) (OMIM, #126200) is a chronic inflammatory demyelinating and neurodegenerative disease of the central nervous system (CNS) with unknown etiology that is widely considered to be autoimmune in nature[86]. The presence in CSF of detectable sHLA-G levels in relapsing-remitting MS (RRMS) patients and, occasionally, in other inflammatory neurological disorders and non-inflammatory neurological disorders was reported for the first time by Fainardi and coauthors[87]. In addition, sHLA-G levels in CSF are higher in RRMS than in controls and increased, in association with IL-10 values, in RRMS patients without than in those with magnetic resonance imaging (MRI) evidence of disease activity[88]. The importance of sHLA-G level evaluation as a biomarker for MS is confirmed[89]. Of note, in RRMS

patients, CSF concentrations of sHLA-G and IL-10 are positively correlated with inactive MRI disease and CSF IL-10 titers are more elevated in patients with than in those without CSF measurable levels of sHLA-G. These data suggest 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 together with the influence due to HLA-G polymorphisms[67]. The existence of high CSF concentrations of sHLA-G in MS patients and their association with clinical and MRI stable disease have been repeatedly confirmed in subsequent investigations in which: (1) An intrathecal production of sHLA-G is more frequent in MS than in inflammatory and non-inflammatory controls and predominated in clinically and MRI inactive compared to clinically and MRI active MS[88]; (2) sHLA-G concentrations reciprocally fluctuate in CSF and serum of MS patients because they are decreased in the serum of clinically stable MS and increased in CSF of MRI inactive MS[65]; (3) CSF levels of HLA-G5 and not those of sHLA-G1 isoforms are increased in MS compared to controls and in MS patients without MRI appearance of disease activity than in those with MRI Gd-enhancing lesions[90]; and (4) CSF values of sHLA-G and antiapoptotic sFas molecules are inversely correlated in MS patients with no evidence of MRI disease activity since CSF concentrations of sFas are lower in MS than in controls and in MRI inactive than in MRI active MS[90]. Interestingly, HLA-G and its inhibitory receptors (ILT-2 and ILT-4) are strongly up-regulated within and around MS lesions where microglia, macrophages and endothelial cells are recognized as the cellular sources[91]. Furthermore, protein HLA-G expression is higher on cultured human MS microglial cells after activation with Th1 proinflammatory cytokines and a novel subpopulation of naturally occurring CD4 positive and CD8 positive Treg cells expressing HLA-G (HLA-Gpos Treg) has been recently described in peripheral blood of MS patients with relapse[92].

Further studies demonstrated that IL-10 contributes to mediating the suppressive activity of CD4 positive HLA-G^{pos} T_{reg}[93] which are highly represented in CSF and inflammatory brain lesions of MS patients as activated central memory T cells capable of migrating from the periphery to intrathecal compartment due to the expression of CCR5[94]. These results strengthen the assumption of an association between HLA-G antigens and MS.

Collectively, these observations provide evidence that HLA-G antigens are likely to be involved in the resolution of MS autoimmunity acting as anti-inflammatory molecules and suggest that HLA-G positive Treg could play a role in the development of a CNS immunosuppressive microenvironment at the sites of inflammation in MS.

HLA-G impact in other inflammatory and autoimmune diseases

HLA-G proves to also be an important biological marker in other pathologies, for example, gastrointestinal and allergic diseases and diabetes.

Inflammatory bowel disease (OMIM, #266600) is the general term for CD and ulcerative colitis (UC), two chronic inflammatory disorders of the intestine which have different clinical, morphological and immunological characteristics.

Torres *et al*[95] studied intestinal samples of UC and CD patients and, by using an immunohistochemistry technique, demonstrated that while UC intestinal cells presented with HLA-G on their surface, CD intestinal biopsies did not. This result combined with high levels of IL-10 found in the lamina propria of the colon of UC patients suggested that HLA-G can regulate the mucosal immune responses in UC. The distribution of the 14 bp INS/DEL polymorphism in UC and CD was investigated by Glas *et al*[96]. They observed an increase of both 14 bp DEL/INS and 14 bp INS/INS genotypes and a consequent decrease of the high producer genotype (14 bp DEL/DEL) in UC subjects in comparison with CD patients. Also, Rizzo *et al*[97] found a different HLA-G expression in UC and CD patients. Non activated peripheral blood mononuclear cells from CD patients spontaneously secrete sHLA-G, while those from UC patients and healthy donors do not. Furthermore, after stimulation with LPS, both cells from CD and healthy subjects show sHLA-G production, while this does not happen in UC patients. This defective production in UC patients seems to be due to an altered secretion of IL-10 in response to inflammation. The different HLA-G expression profiles in UC and CD patients sustain the different etiopathogenesis at the origin of these two diseases. This hypothesis is sustained by the different modulation of HLA-G observed in the two pathologies after therapy[98]. On the basis of this evidence, it is possible to propose sHLA-G and IL-10 levels as diagnostic parameters to facilitate the diagnosis of UC and CD patients.

Asthma (OMIM, #600807) is a chronic disease affecting approximately 300 million people worldwide, with 180000 deaths resulting annually from severe asthma attacks. Asthma is characterized by chronic inflammation in the airway, which consequently narrows more easily in response to a variety of triggers than the airway of a healthy individual. Nicolae *et al*[99] suggested the role of HLA-G as a potential asthma and bronchial hyperresponsiveness (BHR) susceptibility gene. In particular, susceptibility varies depending on whether the mother has asthma or BHR. A G/G genotype at SNP -964G/A in the promoter region was associated with asthma in the offspring of mothers with either asthma or BHR, whereas the A/A genotype was associated with asthma in the offspring of asthma- and BHR-free mothers. Tan *et al*[33] discovered an association between +3142 C > G (rs1063320) and asthma. HLA-G5 is expressed by airway epithelium and is present in the bronchoalveolar lavage fluid from asthmatic patients[100,101]. In addition to the local presence in airways, sHLA-G may also be found in asthmatic subjects outside the lung. The plasma sHLA-G

levels are higher in atopic asthmatic children than in both non-atopic, asthmatic and non-atopic, non-asthmatic children[101]. The 14 bp INS/DEL polymorphism has no impact on plasma sHLA-G levels in the atopic, asthmatic children. Thus, circulating HLA-G may be important as a biomarker and could potentially modulate immune function more broadly, while the local abundance in airways may have a more direct relationship with immune modulation in the mucosa. There is also *in vitro* evidence that the presence of HLA-G may be different in an asthma condition in comparison with physiological status. sHLA-G expression by peripheral blood mononuclear cells is reduced in asthmatic patients[102] while it is increased in asthma induced by isocyanates[103]. This different behavior may represent differences in biological roles in different disease contexts. A loss of HLA-G could reduce immunosuppression and perpetuate inflammation, whereas increased HLA-G in asthma could be an attempt to reassert immunosuppression. Interestingly, HLA-G is differentially expressed during the lung development[104], suggesting a potential role in lung inflammation induction and chronicization.

Allergic rhinitis (AR) (OMIM, #607154) is characterized by a Th2 polarized immune response. sHLA-G molecules are increased in sera of patients with pollen-induced AR studied outside the pollen season[105], during the pollen season[106] and in perennial AR patients[107]. Interestingly, sublingual immunotherapy (SLIT) for AR is able to reduce sHLA-G serum levels in pollen allergic patients[108,109], suggesting a clinical implication as a biomarker of response to SLIT. Interestingly, children with AR have significantly higher levels of sHLA-G molecules than normal controls or children with allergic asthma[110].

HLA-G impact in pathological pregnancies

During human pregnancy, the maternal immune system recognizes and eliminates alloantigens derived from bacteria or virus, but it tolerates genetically different fetal cells, especially extravillous trophoblast cells invading the maternal decidua or entering the spiral arteries. The expression of HLA-G antigens by trophoblasts is of major importance in protecting the fetus from the semiallogeneic response of the mother[111].

The lack of an established immunological tolerance in pregnancy results in an immune response against paternal antigens expressed by the fetus at the placenta, causing severe health problems for both the fetus and the mother. Complications during pregnancy may affect the woman, the fetus, or both. Miscarriage, RSA and pre-eclampsia account for the most frequent pregnancy complications[112] and the dysregulation of the immunological control at the fetal-maternal interface seems to play a role in these pregnancy complications.

Interestingly, there is a reduced expression of both HLA-G mRNA and protein in pathological compared with control placentas[46-48,113]. In pregnant women, there is a peak of sHLA-G levels in plasma samples in the first trimester that is not evidenced in complicated pregnancies[114,115]. In particular, pregnant women with low sHLA-G plasma levels are characterized by a relative risk of 7.12 of developing placental abruption[116].

The lower secretion of HLA-G by maternal immune cells seems to be in part influenced by HLA-G gene polymorphisms, affecting mRNA stability. In particular, the HLA-G 14 bp ins allele decreases mRNA stability[29,117] and protein production[30,39,118-120]. The HLA-G 14 bp INS/DEL polymorphism seems to affect the fetal HLA-G expression as independent studies have reported fetuses carrying the homozygous genotype for the 14 bp INS allele with a significantly increased risk of pre-eclampsia[121-124]. In addition, the 5' URR seems to be implicated in pathological pregnancies[125]. The confirmed role of HLA-G molecules during pregnancy suggests a potential use in clinical practice. Most pregnancy complications are controversial in terms of diagnosis and treatment. As an example, pre-eclampsia can mimic and be confused with many other diseases and none of the signs are specific. The lower levels of sHLA-G detected in maternal plasma and the HLA-G polymorphism association could assist clinicians in an accurate and reliable diagnosis. Moreover, the HLA-G genetic background of the mother could be an a priori sign of an increased risk of complication during pregnancy. These women could be identified and proposed for a stricter follow-up. It is noteworthy that with an appropriate and timely treatment, the success rate is approximately 80%. Therefore, the use of HLA-G as a biological and genetic marker could improve the management of pregnant women. Moreover, the ability to control HLA-G expression in pathological pregnancies and in women with a high risk of pregnancy complications and infertility could be a tool to cure and prevent these conditions with a deep impact, not only for the individual but also for society.

Until now, more than 15000 embryo culture supernatants have been evaluated for sHLA-G expression, with a positive correlation with embryo implantation rate and pregnancy outcome[126]. However further research is needed to investigate HLA-G in assisted reproductive technologies, but recent studies suggest that sHLA-G is a good candidate as a valuable non-invasive embryo marker to improve pregnancy outcome[127]. Three aspects should be taken into consideration: (1) The recognition of a common sHLA-G detection protocol; (2) The necessity to identify a standardized range for positivity; and (3) The comprehension of the factors involved in the differential expression of sHLA-G between equal stage embryos originating from the same woman.

HLA-G impact in tumors

A high frequency of HLA-G surface expression and increased sHLA-G serum levels has been detected in both hematological and solid tumors. HLA-G and sHLA-G expression correlates with a poor clinical outcome in tumor patients, suggesting a role in the immune escape mechanism of tumors. The frequency of HLA-G expression varies between different types of cancer and even between

different studies in the same type of tumor, probably due to the criteria of patient selection and the methodology used. In hematological malignancies, HLA-G expression was documented with a higher frequency in acute myeloid leukemia cases[128], B and T acute lymphoid leukemia and chronic B lymphocyte leukemia[1,129].

HLA-G expression is frequent in choriocarcinoma[45,130,131], breast[132-135], endometrial[136], and ovarian cancers[137]. In digestive tumors, HLA-G expression was described in esophageal squamous cell carcinoma[138], colorectal cancer[139,140], gastric cancer[19], and liver cancer[141]. In relation to increased membrane HLA-G expression in cancer, higher circulating sHLA-G concentrations were described in patients suffering from different types of cancer[142,143].

These data suggest that HLA-G levels might be used as a diagnostic tool to distinguish between malignant and benign tumors and during disease follow-up. Moreover, HLA-G might serve as a possible marker for tumor sensitivity to chemotherapy and as a prognostic marker for advanced disease stage and clinical outcome. HLA-G assay, either in biological fluids or in biopsies, may have a clinical value in diagnosis, staging, or prognosis of cancer, but prospective validation studies should be conducted in order to use it as a biomarker.

Indeed, it would be important to suppress its immune-suppressive expression in cancer. HLA-G blockade in those tumors that express it remains an attractive therapeutic strategy against cancer. Targeting HLA-G-expressing cancer cells would be also important for maximize the efficacy of anticancer therapies. An experimental approach to target HLA-G-expressing cells in a renal cell carcinoma model was the use of HLA-G- derived peptides based on the binding motif to the HLA-A24[144]. HLA-G peptides induced a cytotoxic attack against HLA-G-expressing HLA-A24 tumor cells, suggesting that HLA-G-mediated suppression can be overcome using peptide-derived immunotherapy.

HLA-G impact in viral infections

Host immune defence mechanisms are efficient at eliminating most viral infections. However, some viruses have developed multiple strategies for subverting host immune defences, thus facilitating their spread in the host[145]. Virus-infected cells are protected against attack by NK cells by HLA-G, providing a long-term immunosuppression function. It may be, therefore, 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 defences[146].

Human immunodeficiency virus type 1 (HIV-1) infection is associated with severe and progressive loss of the immune function in infected persons. It is known that HIV-1 protects infected cells from T lymphocytes and NK cell 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. Studies have focused on the expression of HLA-G in monocytes, which are relevant as reservoirs of HIV-1, and in lymphocytes, which are more susceptible to be infected by HIV-1. Monocytes obtained from HIV-1 seropositive patients expressed HLA-G, although only a small proportion of healthy individuals express this molecule[147]. This might be a consequence of highly active antiretroviral therapy (HAART) since a greater proportion of monocytes expressing HLA-G was observed in patients undergoing HAART compared to untreated[148]. T cells obtained from HIV-1 seropositive individuals were found to express HLA-G at a higher proportion[149] and behave as HLA-G$^+$ Treg.

Human cytomegalovirus (HCMV) is a herpes virus causing widespread, persistent human infection in a delicate balance between the progression of the virus and the defences of the host[150]. HCMV has evolved a number of independent strategies to evade the immune system. HLA-G is produced during viral reactivation in macrophages and astrocytoma cells[56] and the percentage of HLA-G-positive monocytes and sHLA-G levels in patients with active HCMV infection were both dramatically higher than in healthy individuals[151]. The up-regulation observed in HLA-G is probably related to a virus-encoded homologue of human IL-10 (cmvIL-10)[151], which prevents NK cell recognition of infected cells.

Evidence also supports a role of HLA-G in human papilloma virus (HPV) infections. In fact, HLA-G may play a role in mediating HPV infection risk[152] and facilitate cervical cancer development[153].

The ability of specific neurotropic viruses to induce the formation of HLA-G in infected neurons, thus conferring protection against NK cells, was demonstrated. For example, herpes simplex virus-1 and Rhabdovirus[154], trigger the expression and up-regulation of membrane and soluble HLA-G molecules in actively infected neurons.

There is also some evidence that HCV and HBV viruses use HLA-G as a strategy to evade the immune response[155-158].

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. The main challenge would be to block HLA-G up-modulation by viral infection in order to allow the recognition by immune cells.

CONCLUSION

This review has underlined the importance of HLA-G molecules in pathological conditions.

The literature data suggest that HLA-G could be implicated in both risk and disease chronicization where this antigen is characterized by an impaired expression depending on the different disease environment.

In fact, HLA-G proteins seem to be involved in the

Table 1 Summary of the main studies on human leukocyte antigen G and pathological conditions

Topics	HLA-G genetics and polymorphism	Protein	Ref.
Autoimmune and inflammatory pathologies			
Rheumatoid arthritis		Lower plasma sHLA-G levels than in controls	71
		Higher sHLA-G levels in the synovia	73
		Plasma level of sHLA-G correlates with disease activity parameters	2
	Increase in 14 bp DEL/DEL genotype frequency in responsive patients to MTX treatment		41
	Increase in 14 bp DEL allele frequency in patients with improved disease status		2
Systemic lupus erythematosus		Higher level of sHLA-G and IL-10 in plasma that in controls	77,78
	Higher frequency of 14 bp INS allele and 14 bp INS/INS genotype than in controls	Lower concentration of sHLA-G in serum than in controls	66
		Decrease in HLA-G expression in monocytes and DCs	80
	14 bp INS/INS genotype is associated to the highest disease activity		82
	+3142 G allele and +3142 GG genotype are more frequent in SLE and associated to SLE susceptibility		84,85
Multiple sclerosis		sHLA-G levels in MS CSF are higher than in controls	87,88
	sHLA-G levels in MS could be influenced by HLA-G 14 bp and +3142 C < G polymorphisms		67
		sHLA-G level are increased in serum of CFS of MRI inactive MS	65,90
		HLA-G expression in monocytes is lower than in controls	91
		Presence of HLA-Gpos Treg cells in peripherial blood	92
Inflammatory bowel disease Crohn's disease and ulcerative colitis		HLA-G is present on UC intestinal cells but not in CD biopsies	95
	14 bp INS/DEL and 14 bp INS/INS are increased in UC in comparison with CD patients		96
		PBMCs from CD patients secrete spontaneously sHLA-G	97
		Different modulation of HLA-G by therapy in UC and CD	98
Asthma	-964 G < A and +3142 C < G SNPs are associated with asthma	Expression of HLA-G in airway epithelium and airway system	99,100
		sHLA-G plasma levels are higher in atopic asthmatic children	101
		sHLA-G secretion is increased in asthma induced by isocyanates	103
Allergic rhinitis		Higher sHLA-G serum levels than controls	106-110
Pathological pregnancy		Decreased HLA-G expression in placenta than uncomplicated pregnancies	46-48,112,113
Pre-eclampsia	Increased 14 bp INS/INS genotype frequency than uncomplicated pregnancies		120-125
Tumors		Increased HLA-G expression in tumor cells	1,45,128-143
		Higher sHLA-G serum levels than controls	142
Viral infection		Increased HLA-G expression in viral infected cells	145
HIV-1		Increased HLA-G expression in infected monocytes and T cells	147
HCMV		Increased HLA-G expression in infected monocytes	56
		Increased sHLA-G serum levels than controls	151

sHLA-G: Soluble human leukocyte antigen G; IL-10: Interleukine-10; SNPs: Single nucleotide polymorphisms; RA: Rheumatoid arthritis; SLE: Systemic lupus erythematosus; MS: Multiple sclerosis; IBD: Inflammatory bowel disease; CD: Crohn's disease; UC: Ulcerative colitis; AR: Allergic rhinitis; HCMV: Human cytomegalovirus; HIV-1: Human immunodeficiency virus-1; MRI: Magnetic resonance imaging; HLA-G: Human leukocyte antigen G; INS/DEL: Insertion/deletion; CFS: Chronic fatigue syndrome.

regulation of the immune system during autoimmune and allergic conditions, such as gastrointestinal, skin, neurological, rheumatic diseases, in pathological pregnancies and in the immune escape mechanisms during viral infections and tumor transformation. In particular, in these disorders, HLA-G proteins could directly interact with immune cells or control the balance between Th1 and Th2 cytokines. In fact, a disequilibrium in this setting would maintain an inflammatory and immune-deregulated condition.

The comprehension of the specific role and mechanisms of action of HLA-G antigens in the development and progression of inflammatory and autoimmune disorders could justify the use of HLA-G molecules as a marker of inflammation and drug treatment and open up new therapeutic perspectives. Moreover, the definition of the role of HLA-G genetic polymorphisms as risk and pharmacogenetic markers could sustain the clinical relevance of HLA-G typing in the laboratory routine. In particular, the possibility to use simple, non-invasive and standardized tools for HLA-G analysis makes it quickly transferable to the health care system practice. These could help in pathology outcome prediction and support treatment decisions.

As reported in Table 1, there are still contrasting results that need to be taken into consideration. The present challenge is to confirm whether HLA-G molecules have a potential role in prevention and diagnosis of pathological conditions. The perspective to identify pharmacological strategies to control the HLA-G production would represent a concrete possibility to improve the control of inflammation and to guide the therapeutic approach. In fact, the possible use of HLA-G as a therapeutic target is of extreme interest.

The ability to modulate HLA-G molecules on the cell surface and to administer HLA-G molecules[74] seems to be at the basis of these cell therapies, suggesting the importance of further studies on HLA-G role in pathological conditions and the possibility of having a controlled modification of the HLA-G level according to disease status and pregnancy complications.

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