Can HLA-G predict disease course in rheumatoid arthritis patients?

Rheumatoid arthritis remains a major clinical problem, with many patients having persistent systemic inflammatory disease, resulting in progressive erosive joint damage and high levels of disability. The goal of rheumatoid arthritis therapy has shifted to initiate treatment early and aggressively to achieve remission, or low disease activity as quickly as possible. To achieve this 'treat-to-target' concept, it is necessary to identify new biomarkers for disease progression and treatment follow-up, and to identify new therapeutic targets. The focus of this review is to summarize current research regarding the expression of HLA-G molecules in rheumatoid arthritis and the possible implications for the future management of the disease.

KEYWORDS: biomarker = disease-modifying antirheumatic drugs = early arthrtitis = HLA-G = rheumatoid arthritis

Rheumatoid arthritis (RA; Online Mendelian Inheritance in Man [OMIM], #180300 [201]) is a chronic systemic inflammatory autoimmune disease causing symmetrical polyarthritis of the large and small joints. It affects 0.5-1.0% of the general population in the developed world, mainly between the ages of 30-50 years, and it is 2.5-times more common in women than men. It is clinically characterized by joint pain, stiffness and swelling due to synovial inflammation, and effusion [1]. Patients may develop extra-articular symptoms including fever, fatigue, anemia, interstitial lung involvement, vasculitis, nodules and osteoporosis, and show an elevation of acute-phase reactants such as C-reactive protein and erythrocyte sedimentation rate [2]. The disease can be a fluctuating or progressive course, which can result in joint destruction, deformity, disability and premature death.

The etiology of RA is not fully understood but is believed to result from interactions between genetic, infectious and environmental factors, where a triggering event, possibly an autoimmune or infectious response, initiates joint inflammation, causing a complex immune response that eventually leads to RA complications. Proinflammatory cytokines such as TNF- α , IL-1 and IL-6 play an important role in the pathogenesis of RA.

Managing RA

RA treatments include disease-modifying antirheumatic drugs (DMARDs) and more recently, biological agents. Traditionally, first-line treatment incorporates conventional DMARDs that counteract inflammatory processes and slow disease progression [3]. These include methotrexate (MTX) as an 'anchor drug', leflunomide, sulfasalazine and hydroxychloroquine. Nowadays, for patients who do not respond to conventional DMARDs, biological agents are available and include TNF- α inhibitors (infliximab, adalimumab, golimumab, certolizumab and etanercept), IL-1 inhibitors (anakinra), selective modulators of T-cell activation (abatacept), CD20 B-cell depleting agent (rituximab) and IL-6 inhibitors (tocilizumab), as well as small-molecule inhibitors (tofacitinib) [4].

The past decade has seen a tremendous amount of change in the field of rheumatology. The treatment of RA is undergoing steady change as new medications are approved and new regimens are attempted. Patients can now be diagnosed, treated and expect a high quality of life, with less joint damage and fewer disabilities. 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 shown to maximize long-term healthy life [5].

The recent revision in RA classification criteria and updated recommendations for treatments are useful to diagnose RA patients at an earlier point in the disease course [6]. The concept of achieving tight control of RA and treating-to-target has been well established and utilizes early diagnosis and aggressive treatment. Regular monitoring requires the availability of new markers for disease progression and treatment follow-up, in order to improve the efficacy of the RA management, thus reducing the clinical and social costs. This review summarizes current research regarding the Roberta Rizzo^{*1}, Daria Bortolotti¹, Olavio R Baricordi¹, Ilaria Farina², Melissa Padovan² & Marcello Govoni² ¹Department of Medical Sciences, Section of Microbiology & Medical

enetics, University of Ferrara, errara, Luigi Borsari, 6-44121 Ferrara, Italy Department of Medical Sciences, ection of Rheumatology, University errara. Ferrara. Italy

uthor for corresponden

- el.: +39 053 245 5382 ax: +39 053 297 4470
- ax: +39 053 297 447(



ISSN 1758-4272

627

expression of HLA-G molecules in RA and their possible implications for the future management of the disease (TABLE 1).

HLA-G antigen

HLA-G is a MHC class I antigen encoded by a gene on chromosome 6p21. It differs from classical HLA class I molecules due to limited polymorphisms in the coding region, 50 alleles (IMGT HLA database [202]) and 16 proteins and a restricted tissue distribution. Seven HLA-G isoforms exist owing to mRNA alternative splicing and differential association with β2-microglobulin. Two isoforms are found on cell surfaces and in biological fluids; membrane-bound G1 and soluble G5, which lacks the transmembrane 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, but leukocyte Ig-like receptors present a greater affinity for the dimeric form [9]. The interaction of HLA-G molecules with inhibitory receptors induces apoptosis of activated CD8⁺ T cells [10], modulates the activity of NK cells [11,12] and dendritic cells (DC) [13,14], blocks allocytotoxic T-lymphocyte responses [8,15] and induces expansion of suppressor T-cell populations, such as CD4⁺, CD25⁺, FOX-P3⁺ and Treg cells [16,17]. Moreover, HLA-G is expressed at high levels on DC-10 cells, human DCs with tolerogenic activity and an outstanding ability to produce IL-10 [14]. Interestingly, the expression of membrane-bound HLA-G1 and that of its receptors is upregulated 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 [14].

The HLA-G production is controlled by several polymorphisms both in the promoter and in the 3' untranslated region modifying the affinity of gene-targeted sequences for transcriptional or post-transcriptional factors, respectively [18].

In total, 29 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 is unique

Bone marrow-derived mononuclear cells	Cono expression		
patients	profiles by DNA microarray	<i>HLA-G</i> gene is upregulated in RA patients	[66]
Blood samples from 106 juvenile idiopathic arthritis patients, 265 RA patients, 356 healthy adults and 85 healthy children	PCR	Increased frequency of the 14 bp DEL allele in juvenile idiopathic arthritis patients	[68]
Plasma samples from 106 RA patients (80 women and 26 men) and control plasma samples were obtained from 56 healthy men and 48 women	ELISA	sHLA-G levels are lower in RA patients in comparison with controls. sHLA-G levels increase in correlation with disease activity and are affected by the presence of disease-predisposing HLA	[70]
Peripheral blood mononuclear cells from healthy individuals and non-MTX-treated RA patients	ELISA and PCR	MTX induces the sHLA-G molecules. A significant association is observed between the highest levels of sHLA-G and the 14 bp DEL/DEL genotype	[39]
Genomic DNA	GWAS	HLA-G is a gene associated with RA	[67]
Plasma sample and peripheral blood mononuclear cells from 23 MTX-treated ERA patients	ELISA, flow cytometry, PCR	sHLA-G upregulation is evident after a 3 month course of disease-modifying antirheumatic drugs, with a positive follow-up	[77]
Collagen-induced arthritis model mice	Intracutaneous treatment with HLA-G monomer or dimer molecules	HLA-G monomer or dimer molecules have produced excellent anti-inflammatory effects with a single, local administration	[74]
	Blood samples from 106 juvenile idiopathic arthritis patients, 265 RA patients, 356 healthy adults and 85 healthy children Plasma samples from 106 RA patients (80 women and 26 men) and control plasma samples were obtained from 56 healthy men and 48 women Peripheral blood mononuclear cells from healthy individuals and non-MTX-treated RA patients Genomic DNA Plasma sample and peripheral blood mononuclear cells from 23 MTX-treated ERA patients Collagen-induced arthritis model mice	Blood samples from 106 juvenile idiopathic arthritis patients, 265 RA patients, 356 healthy adults and 85 healthy childrenPCRPlasma samples from 106 RA patients (80 women and 26 men) and control plasma samples were obtained from 56 healthy men and 48 womenELISAPeripheral blood mononuclear cells from healthy individuals and non-MTX-treated RA patientsELISA and PCRGenomic DNAGWASPlasma sample and peripheral blood mononuclear cells from 23 MTX-treated ERA patientsELISA, flow cytometry, PCRCollagen-induced arthritis model mice theumatoid arthritis; GWAS:Genome-wide association studies; MTX: MethotrexaIntracutaneous treatment with HLA-G monomer or dimer molecules	Blood samples from 106 juvenile idiopathic arthritis patients, 265 RA patients, 356 healthy adults and 85 healthy childrenPCRIncreased frequency of the 14 bp DEL allele in juvenile idiopathic arthritis patientsPlasma samples from 106 RA patients (80 women and 26 men) and control plasma samples were obtained from 56 healthy men and 48 womenELISASHLA-G levels are lower in RA patients in cromparison with controls. sHLA-G levels increase in correlation with disease activity and are affected by the presence of disease-predisposing HLAPeripheral blood mononuclear cells from healthy individuals and non-MTX-treated RA patientsELISA and PCRMTX induces the sHLA-G molecules. A significant association is observed between the highest levels of sHLA-G and the 14 bp DEL/DEL genotypeGenomic DNAGWASHLA-G is a gene associated with RAPlasma sample and peripheral blood mononuclear cells from 23 MTX-treated ERA patientsELISA, flow cytometry, PCRSHLA-G upregulation is evident after a 3 month course of disease-modifying artirheumatic drugs, with a positive follow-upCollagen-induced arthritis model mice treatment with HLA-G monomer or dimer moleculesIntracutaneous treatment with HLA-G monomer or dimer moleculesheumatoid arthritis; GWAS: Genome-wide association studies; MTX: Methotrexate; RA: Rheumatoid arthritis; sHLA-G: Serum HLA-G



Figure 1. HLA-G isoforms and conformations. Membrane and soluble HLA-G isoforms are reported as monomeric and dimeric conformation. Data taken from [100–103].

among the HLA genes [19] and is unresponsive to NF- κ B [20] and IFN- γ [21], owing to the presence of a modified enhancer A and a deleted interferon sequence responsive element. A locus control region located -1.2 kb from exon 1 exhibits a binding site for CREB1 factor, which also binds to two additional cyclic AMP response elements at -934 and -770 positions from the initiation codon ATG. In addition, a binding site interferon sequence responsive element for IRF-1 is located at the -744 Base pair (bp) position [22] and is involved in HLA-G transactivation following IFN- β treatment [22]. The *HLA-G* promoter also contains a heat shock element at the -459/-454 position that binds HSF-1 [23] and a progesterone receptor binding site at -37 bp from ATG [24]. 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 [25]. The -725C>G/T SNP is very close to interferon sequence responsive element, in which the -725G allele is associated with a significantly higher expression level compared with the other alleles [26]. The polymorphic sites at the 5' upstream regulatory region are frequently in linkage disequilibrium with the polymorphic sites identified at the 3' untranslated region, some of which influencing alternative splicing and mRNA stability.

A 14 bp insertion/deletion (INS/DEL) polymorphism (rs66554220) in exon 8 involves mRNA stability and expression [27,28]. In particular the DEL alleles stabilize the mRNA with a consequent higher HLA-G expression [28,29]. The presence of adenine at position +3187, modifies an AU-rich motif in the HLA-G mRNA; decreasing its stability [30]. One SNP C>G at the +3142 bp position (rs1063320) has been explored by Tan and coauthors [31]. The presence of a guanine at the +3142 position may influence the expression of the HLA-G locus by increasing the affinity of this region for the miRNAs: miR-148a, miR-148b and miR-152; therefore, decreasing the mRNA availability by mRNA degradation and translation suppression. 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 contrasting results obtained by Manaster and coauthors [32], who have reported the absence of +3142C>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 influenced by miR-513a-5p, miR-518c*, miR-1262 and miR-92a-1*, miR-92a-2*, miR-661, miR-1224-5p and miR-433 miRNAs [33]. The miR-2110,

miR-93, miR-508–5p, miR-331–5p, miR-616, miR-513b and miR-589* miRNAs target the 14 bp INS/DEL fragment region, and miR-148a, miR-19a*, miR-152, miR-148b and miR-218-2 also influence the +3142 C/G polymorphism.

HLA-G is a stress-inducible gene: heat shock, hypoxia and arsenite increase different HLA-G alternative transcripts [34,35]. The indolamine 2,3-dioxygenase, an enzyme which metabolizes tryptophan, induces HLA-G expression during monocyte differentiation into dendritic cells [36]. The anti-inflammatory and immunosuppressive IL-10 has been correlated with concomitant HLA-G expression [28,37]. Transactivation of HLA-G transcription has also been demonstrated by leukemia inhibitory factor [38], progesterone [24] and MTX [39] cell exposure. Furthermore, IFN- α , IFN- β and IFN- γ enhance HLA-G cell-surface expression by tumors or monocytes [40,41]. 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 transduction into protein. Trogocyotsis of HLA-G1 molecules expressed by antigen-presenting cells and transferred to T cells in humans, makes T cells unresponsive [42]. It has been demonstrated 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 therefore behave like suppressor cells capable of inhibiting other NK-cell functions [43].

The role of *HLA-G* in immune-tolerance has been discovered by studying its expression in trophoblast cells at the fetus–maternal interface [44]. The importance of HLA-G production by placental trophoblasts is evident in pre-eclampsia and unexplained recurrent spontaneous abortion. Several studies have found an aberrant or reduced transcription and protein expression of HLA-G in pathological placentas in comparison with control samples [45–47] with a possible implication in fetal protection and vascular events.

HLA-G expression has been documented in a number of tissues, such as cornea, thymus, erythroid and endothelial precursors, during physiological processes [48–50] and in a variable percentage of serum/plasma samples from healthy subjects [51], where the main producers are activated CD14⁺ monocytes [52]. A modified expression of HLA-G molecules have been observed during 'nophysiological' conditions, such as viral infection [53–56], cancer [57–58], transplantation [59–63], and inflammatory and autoimmune diseases [64,65]. Recently, the role of HLA-G molecules in inflammation has gained a scientific and clinical interest as a proposed molecular biomarker and a possible therapeutic target.

HLA-G in RA

Bone marrow mononuclear cells from RA patients present an abnormal regulatory networks in the immune response [66]. Gene-expression profiles in bone marrow-derived RA mononuclear cells have shown 1910 downregulated and 764 upregulated genes, which include the HLA-G gene. A confirmation was obtained by Bakir-Gungor and Sezerman [67] using genome-wide association studies (GWAS), where they identified *HLA-G* as a gene associated with RA. To understand the role of HLA-G in RA, several studies on both gene polymorphisms and protein expression have been conducted. The genetic analysis of the HLA-G 14 bp INS/DEL polymorphism and two-promoter SNPs (rs1736936, -1202T/C and rs2735022, -586C/T) in HLA-G gene reported no allelic and genotypic differences in RA patients subdivided according to disease characteristics and development [68,69]. On the contrary, the analysis of patients with juvenile idiopathic arthritis (JIA; OMIM, #604302 [201]), the most common form of persistent arthritis in children, have shown a significant correlation between the 14 bp DEL allele and JIA susceptibility in females when compared with controls of the same gender [68]. These data exclude any implication of HLA-G genetic background in RA, although they do support a possible role in JIA. These observations support the presence of different physiopathogenic pathways between RA and JIA. Moreover, RA and JIA present different HLA associations, demonstrating that the immunogenetic factors involved in susceptibility to these two diseases are different. At a protein level, serum HLA-G (sHLA-G) concentration is significantly lower in both RA [70] and JIA [71] patients than in controls. The similar decrease in sHLA-G concentrations may lead to a chronic activation of inflammatory cells and contribute to the development of these two diseases. We can hypothesize that low levels of sHLA-G are not able to maintain an anti-inflammatory and immuneregulated systemic environment, which could worsen the disease development. Interestingly, even though JIA susceptibility in females correlates with the high producer 14 bp DEL allele, the secretion of HLA-G is limited in the serum. These data suggest a stronger implication of protein transduction mechanisms compared with a genetic background. The evaluation of sHLA-G molecules at the specific inflammation site of the synovia, reported higher levels of sHLA-G in both RA and JIA patients [71,72]. The release of HLA-G into 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], which could interact with immuneinhibitory receptors and maintain a chronic inflammatory response. Interestingly, in a group of RA patients with HLA specificities associated with the disease (HLA-DRB1*01, HLA-DRB1*04 and HLA-DRB1*10), the sHLA-G levels are higher and positively correlated with disease activity parameters, such as C-reactive protein and the number of swollen joints [70]. The highest sHLA-G concentrations may represent the inability to control inflammation and confirm the upregulation of HLA-G gene transcription observed by gene-expression profile analysis [66]. 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. Notably, further research is needed to evaluate the role of HLA-G molecules in these different compartments and in RA development.

Interestingly, a recent work has confirmed the role of HLA-G molecules in RA. The authors made an intracutaneous treatment of collageninduced arthritis mouse model with HLA-G monomer or dimer molecules. These molecules were able to produce excellent anti-inflammatory effects with a single, local administration [74]. Notably, the dimer has exhibited higher immunosuppressive effects in comparison with the monomer conformation owing to the higher affinity of the dimers for paired immunoglobulin-like receptor B, the mouse homolog of the LILRBs. These data should be confirmed by further experiments, but support the use of HLA-G dimers as a useful anti-RA agent, in small amounts with minimal side effects.

HLA-G as a marker of RA treatment

HLA-G expression in inflammatory and autoimmune diseases is a relatively new area of investigation. The specific role of HLA-G molecules in the control of inflammation and immune response suggest an implication in both risk and disease chronicization, where this antigen is characterized by an impaired expression, depending on the different disease environment.

The *HLA-G* 14 bp INS/DEL polymorphism has been evaluated as pharmacogenetic marker

of RA therapy. MTX, the major DMARD, is implicated in the increased production of IL-10 in patients with RA, which correlates with better therapeutic response [39]. IL-10 is one of the most efficient inducers of HLA-G secretion by peripheral blood monocytes, activated by lipopolysaccharides and with the highest levels in the 14 bp DEL/DEL genotype [37]. The analysis of the HLA-G 14 bp INS/DEL polymorphism in 156 MTX-treated RA patients has demonstrated an increase of the 14 bp DEL/DEL genotype in the responder group, characterized by a reduction in disease activity score in 28 joints (DAS28) measured before and after 6 months of treatment with MTX [38]. In contrast to this study, there are two studies with negative results: in one study, 130 RA patients have presented no significant difference in 14 bp DEL/INS allelic and genotypic distribution in patients responsive to MTX (DAS28: <3.2) [75]; in the second study, 186 RA patients, who had never been treated with MTX, were prospectively followed and were considered to be responders, with a DAS28 of up to 2.4 after 6 months of treatment [76]. No significant association between HLA-G 14 bp INS/DEL and MTX efficacy has been observed. Comparing these studies, the opposite results may reflect population differences in gene expression, which could influence the power-of-association studies and lead to different levels of association. In addition, the different doses of MTX and the different cutoffs used for RA therapy response definition could affect the results obtained.

Interestingly, Rizzo and coauthors [77] have evaluated the possible role of HLA-G molecules as biomarkers for RA treatment in a follow-up study. In total, 23 early RA (ERA) patients were analyzed during a 12-month follow-up of disease treatment for sHLA-G levels in plasma samples, mHLA-G and ILT2 expression on peripheral blood CD14⁺ cells and typed for *HLA-G* 14 bp DEL/INS polymorphism. The authors have 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 upregulation is evident after 3 months of DMARD therapy, while a significant reduction in TNF- α levels is evident after 9 months of therapy, when a clear amelioration of the disease is evident, with a high specificity for HLA-G detection in EA condition. In fact, the presence of sHLA-G has been observed in 100% of ERA patients, in comparison with 8% of subjects with other

arthropathies and 23% of controls. The receiver operating characteristic curve analysis reports an accuracy of 87.9%, sensitivity of 100% and specificity of 81.4%. In particular, the coevaluation of sHLA-G and ACPA positivities provides a test with an accuracy of 84.8%, a sensitivity of 73.9% and specificity of 91.7%, suggesting the use of both these molecules in the definition of ERA patients and treatment follow-up. 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.

HLA-G impact in other rheumatic diseases

The expression of HLA-G molecules has also been evaluated in other rheumatic diseases, such as scleroderma, systemic lupus erythematosus (SLE), Kawasaki disease, Behcet's disease and sarcoidosis.

Scleroderma (SSc; OMIM, #18175 [201]) is an autoimmune rheumatic disease of the connective tissue. Based on the extent of cutaneous involvement SSc can be classified as limited SSc, involving acral skin, or diffuse SSc if skin involvement extends more proximally including abdomen, trunk and face. SSc is characterized by alterations of the microvasculature, disturbances of the immune system and by massive deposits of collagen and other matrix substances in the connective tissue. The course, and even the initial events in the pathogenesis of SSc, are still poorly understood. The presence of inflammatory infiltrates, mainly CD4+ T cells, around blood vessels and at sites of active connective tissue formation suggests their pathogenetic role, together with an increased secretion of Th1 cytokines [78]. The skin biopsies from patients with SSc with a longer survival, lower frequency of vascular cutaneous ulcers, telangiectasias and inflammatory polyarthralgia, present HLA-G molecule expression, suggesting a role in immune control [79].

SLE (OMIM, #601744 [201]) is a systemic autoimmune disease of the connective tissue that can affect any part of the body. The immune response is mainly characterized by Th2-cell predominance. Rosaso and coauthors [80] and Chen and coauthors [81] have demonstrated higher levels of sHLA-G and IL-10 in SLE patients in comparison with healthy controls, while Rizzo and coauthors [82] have 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) [83]. As proof, Monsiváis-Urenda and coauthors [84] have provided evidence of a diminished expression of HLA-G in monocytes and in mature CD83+ dendritic cells from SLE patients compared with healthy controls. In addition, monocytes from SLE patients have shown a diminished induction of HLA-G expression in response to IL-10. Finally, lymphocytes from SLE patients have displayed a lower acquisition of HLA-G (by trogocytosis) from autologous monocytes compared with controls. Interestingly, ILT-2 receptor expression is increased on lymphocytes from SLE patients, in particular in CD3⁺, CD19⁺, CD56⁺ cells and related to IL-10 and anti-DNA antibodies [81]. These results confirm the presence of a HLA-G impaired expression in patients with SLE and a possible role in pathogenesis. Using SNP mapping approach, the HLA-G gene is reported as a novel independent locus with SLE interaction [85]. In particular, the HLA-G 14 bp INS/DEL polymorphism and the HLA-G +3142C>G SNP have been analyzed in the SLE population. SLE patients have shown a higher frequency of 14 bp INS allele and 14 bp INS/INS genotype [82]. Moreover, 14 bp INS/INS patients have presented the highest disease activity [86]. On the contrary, the evaluation of HLA-G 14 bp INS/DEL polymorphism in a SLE Brazilian population has failed to present an association [87]. The +3142G allele and the +3142GG genotype frequencies are increased among SLE patients as compared with controls [88]. These data sustain the role of HLA-G molecules in the control of SLEs; in particular several results sustain lower HLA-G expression as a risk factor for SLE development.

Behçet's syndrome (OMIM, # 109650 [201]) is a rare, immune-mediated, systemic vasculitis with mucous membrane ulceration and ocular involvements. The *HLA-G*010101* allele is associated with a reduced risk of Behçet's syndrome while *HLA-G*010102*, *G*0105N* alleles and the 14 bp INS/DEL polymorphism are associated with an increased risk of Behçet's syndrome [89,90].

Kawasaki disease (OMIM, # 611775 [201]) is an acute, self-limited vasculitis that effects infants and children. Without treatment, approximately 15–25% of patients with Kawasaki disease will develop coronary artery aneurysms, making this disease the leading cause of acquired heart disease among children in developed countries. Although an infectious agent is highly suspected, the etiology of the disease is largely unknown. However, it has been established that inflammation is a central feature of Kawasaki disease. Several lines of evidence suggest that genetic and immunological factors play important roles in disease susceptibility and outcomes. Interestingly, nonsynonymous SNP (C\A) of the *HLA-G* gene (rs12722477, Leu134Ile) is significantly associated with Kawasaki disease [91].

Sarcoidosis togliere is a systemic inflammatory granulomatous disease associated with an accumulation of CD4⁺ T cells and a Th1 immune response. The etiology is unknown but at the molecular level several studies have shown HLA associations (e.g., *HLA-DRB1*1101*) [92]. Overall, 47 patients with sarcoidosis have been analyzed for different *HLA-G* alleles/polymorphisms [93]. The 14 bp INS allele has been observed more often in sarcoidosis patients than in controls. Only rare and weak expression of *HLA-G* has been observed in granulomas from sarcoidosis patients, supporting the genetic results.

Conclusion

Joint damage occurs early in the course of RA and approximately 75% of patients with ERA develop erosive changes within the first 2 years of disease. Controlled clinical trials have shown that an early aggressive therapeutic approach with DMARDs can slow or even stop the progression of damage in RA, therefore an early diagnosis is of extreme importance. Besides physical examination, which still remains the gold standard in identifying the presence of arthritis, musculoskeletal ultrasonography has been proven to be helpful in detecting joint inflammation, especially at an early and subclinical stage [94]. Moreover, power Doppler technique helps today to distinguish active from inactive synovitis [95].

The reviewed literature seems to sustain a direct role of HLA-G molecules in RA and other rheumatic diseases. In particular, HLA-G could create an immune-regulatory environment that is fundamental for disease activity control. A disequilibrium in this setting would maintain an inflammatory and immune-dysregulated condition, typical for RA disease. Although several studies have indicated the association between HLA-G and RA as previously revised by Brenol *et al.* [96], these results should be validated by further scientific investigations, before a clinical use of HLA-G as a reliable biomarker for RA treatment is proposed.

Indeed, the use of sHLA-G as a biomarker to evaluate early prognosis and disease activity in ERA patients is of extreme interest. The identification of significant biomarkers aimed to monitor patient therapy and to avoid under/ overtreatment is of outstanding importance to assess and predict ERA evolution. Rheumatoid factor and ACPA, when present, are well recognized negative prognostic factors for joint damage progression but their predictive value is still limited [97]. For this reason new prognostic and reliable biomarkers are urgently needed. The evaluation of HLA-G levels in plasma samples from ERA patients, in combination with other biomarkers, such as ACPA, increases the specificity of DMARD treatment follow-up and disease progression, which is the main target in biomarker identification. The use of accessible plasma samples for sHLA-G quantitation would be an important factor in order to obtain a readyto-use system for clinical protocols. It will be important to identify a cutoff value of sHLA-G levels to monitor treatment follow-up and disease progression, and bring back the considerations made on average concentrations obtained from a cohort of patients to the single individual. In this context, the availability of new, reproducible and standardized biomarkers is of interest in ERA clinical management. Moreover, since HLA-G molecules have a role in other rheumatic diseases, we could hypothesize to transfer the knowledge acquired with RA studies in other clinical contexts.

Future perspective

The understanding of the specific role and mechanisms of action of HLA-G molecules in the development and progression of RA could justify the use of HLA-G molecules as a marker of inflammation and drug treatment, and open up new therapeutic perspectives for RA patients. A possible interaction in the complex RA molecular pathways is reported in FIGURE 2. HLA-G could be hypothesized to act as an immune-modulator balancing B-cell auto-antibody production and Treg dysregulation. The identification of pharmacological strategies, with the aim of controlling HLA-G production, could be a possible method in improving the control of inflammation, guiding a therapeutic approach. In fact, the possible use of HLA-G as a therapeutic target is of extreme interest. These considerations are sustained by the recent results on HLA-G dimer use to control collagen-induced arthritis in murine models [74] and the role of HLA-G molecules in the control of the immune-regulatory functions of mesenchymal stem cells derived from human umbilical cord (HUCSC) [98]. Recently, human umbilical cord stem cells have been shown to have a therapeutic potential for cartilage repair in RA [99]. The efficacy of HUCSCs is connected with the expression of HLA-G molecules that guarantee the immune regulatory functions of the treatment. Interestingly, TNF- α is able to downmodulate HLA-G expression and



Figure 2. Schematic representation of the possible implication of HLA-G molecules in the complex rheumatoid arthritis molecular pathways. The autoimmune response in rheumatoid arthritis is triggered by APCs recognized by T cells. This interaction induces Th1 and Th17 cell shift and the production of IL-6 and TNF by APCs that activate B-cell differentiation in plasma cells, secreting auto-antibodies. Th1-cell secretion of IL-6 and IFN- γ and Th17-cell production of IL-17 activate macrophage secretion of IL-12 that maintains the Th1-cell activation, and IL-1 and TNF production. These two cytokines activate synovial fibroblasts secretion of IL-6 that promote osteoclast and condrocyte survival, as well as differentiation and activation with consequent joint damage. The production of IL-10 and HLA-G molecules by macrophages and osteblasts interferes with plasma cell activation, Th1 differentiation and induces the formation of Tregs.

APC: Antigen-presenting cell; BCR: B-cell receptor; FcR: Fc receptor; sHLA: Serum HLA-G; TCR: T-cell receptor.

HUCSC-dependent immune suppression. The administration of a TNF inhibitor seems to be a potential effective therapy for ameliorating HUCSC efficacy in RA control, maintaining HLA-G expression. For this, the ability to modulate HLA-G molecules on cell surface seems to be at the basis of these cell therapies, suggesting the importance of further study on HLA-G role in RA and the possibility to have a controlled modification of the HLA-G level according to disease status.

Moreover, the confirmation of the role of HLA-G molecules and genetic polymorphisms as risk and pharmacogenetic markers in RA disease could improve the laboratory routine analysis for RA management. In particular, the possibility to use simple, noninvasive and standardized [78] tools for HLA-G analysis makes it quickly transferable to the healthcare system practice. These could help in RA outcome prediction and support the clinicians in treatment decisions.

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received orpending, or royalties.

No writing assistance was utilized in the production of this manuscript.

Executive summary

Managing rheumatoid arthritis

- Rheumatoid arthritis (RA) treatments include disease-modifying antirheumatic drugs and, more recently, biological agents.
- An important goal of RA therapy has shifted to initiate treatment early and aggressively to achieve remission as quickly as possible.
- Regular monitoring requires the availability of new markers for disease progression and treatment follow-up, in order to improve the efficacy of the RA management, thus reducing the clinical and social costs.

HLA-G antigen

- HLA-G are immune regulatory molecules.
- HLA-G expression is controlled by specific gene polymorphisms.
- HLA-G expression is induced by different molecules such as methotrexate.

HLA-G in RA

- Serum HLA-G concentration is significantly lower in RA than in controls.
- The intracutaneous treatment of collagen-induced arthritis model mice with HLA-G monomer or dimer molecules has produced excellent anti-inflammatory effects with a single, local administration.

HLA-G as a marker of RA treatment

- An increase of the 14 bp DEL/DEL genotype has been observed in the responder group, characterized by a reduction in disease activity score in 28 joints measured before and after 6 months of treatment with methotrexate.
- HLA-G upregulation is evident after 3 months of disease-modifying antirheumatic drug therapy when a clear amelioration of the disease is evident.
- The presence of the 14 bp DEL allele characterizes the patients with a significant improvement in disease status.

Conclusion

- The reviewed literature seems to corroborate a direct role of HLA-G molecules in RA.
- HLA-G could create an immune-regulatory environment that is fundamental for disease activity control. A disequilibrium in this setting would maintain an inflammatory and immune-dysregulated condition, typical of RA disease.
- The evaluation of HLA-G levels in plasma samples from EA patients, in combination with other biomarkers as anticyclic citrullinated peptide, increases the specificity of disease-modifying antirheumatic drug treatment follow-up and disease progression.
- The confirmation of the role of HLA-G molecules and genetic polymorphisms as risk and pharmacogenetic markers in RA could improve the laboratory routine analysis for RA management.
- The identification of a cutoff value of serum HLA-G levels to monitor treatment follow-up and disease progression will bring back the considerations made on average concentrations obtained from a cohort of patients to the single individual.
- The ability to modulate HLA-G molecules according to disease status seems to be a potential new therapeutic target in RA.

References

Papers of special note have been highlighted as: • of interest

- of considerable interest
- Majithia V, Geraci SA. Rheumatoid arthritis: diagnosis and management. *Am. J. Med.* 120(11), 936–939 (2007).
- 2 Firestein GS. Evolving concepts of rheumatoid arthritis. *Nature* 423(6937), 356–361 (2003).
- 3 Smolen JS, Aletaha D, Koeller M, Weisman MH, Emery P. New therapies of rheumatoid arthritis. *Lancet* 370(9602), 1861–1874 (2007).
- 4 Choy EH, Kavanaugh AF, Jones SA. The problem of choice: current biologic agents and future prospects in RA. *Nat. Rev. Rheumatol.* 9, 154–163 (2013).
- 5 Smolen JS, Aletaha D, Bijlsma JW *et al.*; T2T Expert Committee. Treating rheumatoid arthritis to target: recommendations of an international task force. *Ann. Rheum. Dis.* 69, 631–637 (2010).
- Treat-to-target strategy for rheumatoid arthritis (RA) management.

- 6 Aletaha D, Neogi T, Silman AJ et al. Rheumatoid arthritis classification criteria: an American College of Rheumatology/ European League Against Rheumatism collaborative initiative. Arthritis Rheum. 62, 2569–2581 (2010).
- 7 Ishitani A, Geraghty D. Alternative splicing of *HLA-G* transcripts yields proteins with primary structures resembling both class I and class II antigens. *Proc. Nat. Acad. Sci. USA* 89 (9), 3947–3951 (1992).
- Apps R, Gardner L, Sharkey AM, Holmes N, Moffett A. A homodimeric complex of HLA-G on normal trophoblast cells modulates antigen-presenting cells via LILRB1. *Eur. J. Immunol.* 37(7), 1924–1937 (2007).
- 9 Favier B, HoWangYin KY, Wu J et al. Tolerogenic function of dimeric forms of HLA-G recombinant proteins: a comparative study in vivo. PLoS ONE 6(7), e21011 (2011).
- Shiroishi M, Tsumoto K, Amano K et al. Human inhibitory receptors Ig-like transcript 2 (ILT2) and ILT4 compete with CD8 for MHC class I binding and bind preferentially

to HLA-G. *Proc. Natl Acad. Sci. USA* 100(15), 8856–8861 (2003).

- 11 Marchal-Bras-Goncalves R, Rouas-Freiss N, Connan F *et al.* A soluble HLA-G protein that inhibits natural killer cell-mediated cytotoxicity. *Transplant Proc.* 33(3), 2355–2359 (2001).
- 12 Caumartin J, Favier B, Daouya M et al. Trogocytosis-based generation of suppressive NK cells. EMBO J. 26(5), 1423–1433 (2007).
- 13 Liang S, Ristich V, Arase H, Dausset J, Carosella ED, Horuzsko A. Modulation of dendritic cell differentiation by HLA-G and ILT4 requires the IL-6 – STAT3 signaling pathway. *Proc. Natl Acad. Sci. USA* 105(24), 8357–8362 (2008).
- 14 Gregori S, Tomasoni D, Pacciani V et al. Differentiation of type 1 T regulatory cells (Tr1) by tolerogenic DC-10 requires the IL-10-dependent ILT4/HLA-G pathway. Blood 116(6), 935–944 (2010).
- 15 Kapasi K, Albert SE, Yie S, Zavazava N, Librach CL. HLA-G has a concentration-

dependent effect on the generation of an allo-CTL response. *Immunology* 101(2), 191–200 (2000).

- 16 Naji A, Le Rond S, Durrbach A et al. CD3⁺ CD4low and CD3⁺ CD8low are induced by HLA-G: novel human peripheral blood suppressor T-cell subsets involved in transplant acceptance. *Blood* 110(12), 3936–3948 (2007).
- 17 Du L, Xiao X, Wang C *et al.* Human leukocyte antigen-G is closely associated with tumor immune escape in gastric cancer by increasing local regulatory T cells. *Cancer Sci.* 102 (7), 1272–1280 (2011).
- 18 Donadi EA, Castelli EC, Arnaiz-Villena A, Roger M, Rey D, Moreau P. Implications of the polymorphism of *HLA-G* on its function, regulation, evolution and disease association. *Cell. Mol. Life Sci.* 68(3), 369–395 (2011).
- Solier C, Mallet V, Lenfant F, Bertrand A, Huchenq A, Le Bouteiller P. *HLA-G* unique promoter region: functional implications. *Immunogenetics*. 53(8), 617–625 (2001).
- 20 Gobin SJ, Keijsers V, Cheong C, van Zutphen M, Van den Elsen PJ. Transcriptional regulation of *HLA-G. Transplant Proc.* 31(4), 1857–1859 (1999).
- 21 Gobin SJ, van Zutphen M, Woltman AM, van den Elsen PJ. Transactivation of classical and nonclassical HLA class I genes through the IFN-stimulated response element. *J. Immunol.* 163(3), 1428–1434 (1999).
- 22 Lefebvre S, Berrih-Aknin S, Adrian F *et al.* A specific interferon (IFN)-stimulated response element of the distal HLA-G promoter binds IFN-regulatory factor 1 and mediates enhancement of this nonclassical class I gene by IFN-beta. *J. Biol. Chem.* 276(9), 6133–6139 (2001).
- 23 Ibrahim EC, Morange M, Dausset J, Carosella ED, Paul P. Heat shock and arsenite induce expression of the nonclassical class I histocompatibility *HLA-G* gene in tumor cell lines. *Cell. Stress Chaperones* 5(3), 207–218 (2000).
- 24 Yie SM, Xiao R, Librach CL. Progesterone regulates *HLA-G* gene expression through a novel progesterone response element. *Hum. Reprod.* 21(10), 2538–2544 (2006).
- 25 Castelli EC, Mendes-Junior CT, Veiga-Castelli LC, Roger M, Moreau P, Donadi EA. A comprehensive study of polymorphic sites along the *HLA-G* gene: implication for gene regulation and evolution. *Mol. Biol. Evol.* 28(11), 3069–3086 (2011).
- 26 Ober C, Billstrand C, Kuldanek S, Tan Z. The miscarriage-associated *HLA-G -725G* allele influences transcription rates in JEG-3 cells. *Hum. Reprod.* 21(7), 1743–1748 (2006).

- 27 Hviid TV, Hylenius S, Rorbye C, Nielsen LG. HLA-G allelic variants are associated with differences in the *HLA-G* mRNA isoform profile and *HLA-G* mRNA levels. *Immunogenetics* 55(2), 63–79 (2003).
- 28 Hviid TV, Rizzo R, Christiansen OB, Melchiorri L, Lindhard A, Baricordi OR. HLA-G and IL-10 in plasma in relation to *HLA-G* genotype and polymorphisms. *Immungenetics* 56(3), 135–141 (2004).
- Relationship between IL-10 and HLA-G expression.
- 29 Svendsen SG, Hantash BM, Zhao L et al. The expression and functional activity of membrane-bound human leukocyte antigen-G1 are influenced by the 3'-untranslated region. *Hum. Immunol.* 74(7), 818–827 (2013).
- 30 Yie SM, Li LH, Xiao R, Librach CL. A single base-pair mutation in the 3'-untranslated region of *HLA-G* mRNA is associated with pre-eclampsia. *Mol. Hum. Reprod.* 14(11), 649–653 (2008).
- 31 Tan Z, Randall G, Fan J *et al.* Allele-specific targeting of microRNAs to *HLA-G* and risk of asthma. *Am. J. Hum. Genet.* 81(4), 829–834 (2007).
- 32 Manaster I, Goldman-Wohl D, Greenfield C et al. MiRNA-mediated control of HLA-G expression and function. PLoS ONE 7(3), e33395 (2012).
- 33 Castelli EC, Moreau P, Oya e Chiromatzo A et al. In silico analysis of microRNAS targeting the HLA-G 3' untranslated region alleles and haplotypes. *Hum. Immunol.* 70(12), 1020–1025 (2009).
- 34 Chang CC, Murphy SP, Ferrone S. Differential *in vivo* and *in vitro HLA-G* expression in melanoma cells: potential mechanisms. *Hum. Immunol.* 64(11), 1057–1063 (2003).
- 35 Mouillot G, Marcou C, Zidi I *et al.* Hypoxia modulates *HLA-G* gene expression in tumor cells. *Hum. Immunol.* 68(4), 277–285 (2007).
- 36 López AS, Alegre E, LeMaoult J, Carosella E, González A. Regulatory role of tryptophan degradation pathway in *HLA-G* expression by human monocyte-derived dendritic cells. *Mol. Immunol.* 43(14), 2151–2160 (2006).
- Rizzo R, Hviid TV, Stignani M et al. The HLA-G genotype is associated with IL-10 levels in activated PBMCs. Immunogenetics 57(3–4), 172–181 (2005).
- 38 Bamberger AM, Jenatschke S, Schulte HM, Löning T, Bamberger MC. Leukemia inhibitory factor (LIF) stimulates the human *HLA-G* promoter in JEG3 choriocarcinoma cells. *J. Clin. Endocrinol. Metab.* 85(10), 3932–3936 (2000).

- 39 Rizzo R, Rubini M, Govoni M et al. HLA-G 14-bp polymorphism regulates the methotrexate response in rheumatoid arthritis. *Pharmacogenet. Genomics* 16(9), 615–623 (2006).
- Role of *HLA-G* in methotrexate response in RA.
- 40 Ugurel S, Rebmann V, Ferrone S, Tilgen W, Grosse-Wilde H, Reinhold U. Soluble human leukocyte antigen-G serum level is elevated in melanoma patients and is further increased by interferon-alpha immunotherapy. *Cancer* 92(2), 369–376 (2001).
- Lefebvre S, Moreau P, Guiard V et al. Molecular mechanisms controlling constitutive and IFN-gamma-inducible HLA-G expression in various cell types. J. Reprod. Immunol. 43(2), 213–224 (1999).
- 42 LeMaoult J, Caumartin J, Daouya M et al. Immune regulation by pretenders: cell-to-cell transfers of HLA-G make effector T cells act as regulatory cells. *Blood* 109(5), 2040–2048 (2007).
- 43 Caumartin J, Favier B, Daouya M, Guillard C, Moreau P, Carosella ED, LeMaoult J. Trogocytosis-based generation of suppressive NK cells. *EMBO J.* 26(5), 1423–1433 (2007).
- 44 Kovats S, Main EK, Librach C, Stubblebine M, Fisher SJ, DeMars R. A class I antigen, *HLA-G*, expressed in human trophoblasts. *Science* 248(4952), 220–223 (1990).
- 45 Hara N, Fujii T, Yamashita T, Kozuma S, Okai T, Taketani Y. Altered expression of human leukocyte antigen G (HLA-G) on extravillous trophoblasts in preeclampsia: immunohistological demonstration with anti-HLA-G specific antibody '87G' and anti-cytokeratin antibody 'CAM5.2'. *Am. J. Reprod. Immunol.* 36(6), 349–358 (1996).
- 46 Yie SM, Li LH, Li YM, Librach C. HLA-G protein concentrations in maternal serum and placental tissue are decreased in preeclampsia. *Am. J. Obstet. Gynecol.* 191(2), 525–529 (2004).
- 47 Peng B, Zhang L, Xing AY, Hu M, Liu SY. The expression of human leukocyte antigen G and E on human first trimester placenta and its relationship with recurrent spontaneous abortion. *Sichuan Da Xue Xue Bao Yi Xue Ban* 39(6), 976–979 (2008).
- 48 Le Discorde M, Moreau P, Sabatier P, Legeais JM, Carosella ED. Expression of HLA-G in human cornea, an immune-privileged tissue. *Hum. Immunol.* 64(11), 1039–1044 (2003).
- 49 Mallet V, Blaschitz A, Crisa L et al. 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(6), 889–898 (1999).

- 50 Blaschitz A, Lenfant F, Mallet V et al. Endothelial cells in chorionic fetal vessels of first trimester placenta express HLA-G. Eur. J. Immunol. 27(12), 3380–3388 (1997).
- 51 Rizzo R, Andersen AS, Lassen MR *et al.* Soluble human leukocyte antigen-G isoforms in maternal plasma in early and late pregnancy. *Am. J. Reprod. Immunol.* 62(5), 320–338 (2009).
- 52 Rebmann V, Busemann A, Lindemann M, Grosse-Wilde H. Detection of HLA-G5 secreting cells. *Hum Immunol.* 64(11), 1017–1024 (2003).
- 53 Derrien M, Pizzato N, Dolcini G et al. Human immunodeficiency virus 1 downregulates cell surface expression of the non-classical major histocompatibility class I molecule HLA-G1. J. Gen. Virol. 85(Pt 7), 1945–1954 (2004).
- 54 Matte C, Lajoie J, Lacaille J, Zijenah LS, Ward BJ, Roger M. Functionally active *HLA-G* polymorphisms are associated with the risk of heterosexual HIV-1 infection in African women. *AIDS* 18(3), 427–431 (2004).
- 55 Onno M, Pangault C, Le Friec G, Guilloux V, André P, Fauchet R. Modulation of HLA-G antigens expression by human cytomegalovirus: specific induction in activated macrophages harboring human cytomegalovirus infection. J. Immunol. 164(12), 6426–6434 (2000).
- 56 Donaghy L, Gros F, Amiot L et al. 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(2), 236–240 (2007).
- 57 Rouas-Freiss N, Moreau P, Menier C, LeMaoult J, Carosella ED. Expression of tolerogenic HLA-G molecules in cancer prevents antitumor responses. *Cancer Biol.* 17(6), 413–321 (2007).
- 58 Pistoia V, Morandi F, Wang X, Ferrone S. Soluble HLA-G: are they clinically relevant? *Cancer Biol.* 17(6), 469–479 (2007).
- 59 Crispim, JC, Duarte, RA, Soares CP et al. Human leukocyte antigen-G expression after kidney transplantation is associated with a reduced incidence of rejection. *Transpl. Immunol.* 18(4), 361–367 (2008).
- 60 Sebti Y, Le Maux A, Gros F *et al.* Expression of functional soluble human leucocyte antigen-G molecules in lymphoproliferative disorders. *Br. J. Haematol.* 138(2), 202–212 (2007).
- 61 Lila N, Carpentier A, Amrein C, Khalil-Daher I, Dausset J, Carosella ED. Implication of HLA-G molecule in heart-graft acceptance. *Lancet* 355(9221), 2138 (2000).

- 62 Lila N, Amrein C, Guillemain R *et al.* Human leukocyte antigen-G expression after heart transplantation is associated with a reduced incidence of rejection. *Circulation* 105(16), 1949–1954 (2002).
- 63 Qiu J, Terasaki PI, Miller J, Mizutani K, Cai J, Carosella ED. Soluble *HLA-G* expression and renal graft acceptance. *Am. J. Transplant.* 6(9), 2152–2156 (2006).
- 64 Fainardi E, Rizzo R, Melchiorri L *et al.* Soluble HLA-G molecules are released as HLA-G5 and not as soluble HLA-G1 isoforms in CSF of patients with relapsingremitting multiple sclerosis. *J. Neuroimmunol.* 192(1–2), 219–225 (2007).
- 65 Rizzo R, Hviid TVF, Govoni M et al. 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(6), 520–529 (2008).
- 66 Lee HM, Sugino H, Aoki C et al. Abnormal networks of immune response-related molecules in bone marrow cells from patients with rheumatoid arthritis as revealed by DNA microarray analysis. Arthritis Res. Ther. 13(3), R89 (2011).
- Genetic association of *HLA-G* with RA.
- 67 Bakir-Gungor B, Sezerman OU. A new methodology to associate SNPs with human diseases according to their pathway related context. *PLoS ONE* 6(10), e26277 (2011).
- Genetic association of *HLA-G* with RA.
- 68 Veit TD, Vianna P, Scheibel I et al. Association of the HLA-G 14-bp insertion/ deletion polymorphism with juvenile idiopathic arthritis and rheumatoid arthritis. *Tissue Antigens* 71(5), 440–446 (2008).
- Analysis of *HLA-G* polymorphisms in RA.
- 69 Kim SK, Chung JH,, Kim DH, Yun DH, Hong SJ, Lee KH. Lack of association between promoter polymorphisms of *HLA-G* gene and rheumatoid arthritis in Korean population. *Rheumatol. Int.* 32(2), 509–512 (2012).
- 70 Verbruggen LA, Rebmann V, Demanet C, De Cock S, Grosse-Wilde H. Soluble HLA-G in rheumatoid arthritis. *Hum. Immunol.* 67(8), 561–567 (2006).
- Modulation of HLA-G molecules in RA.
- 71 Prigione I, Penco F, Martini A, Gattorno M, Pistoia V, Morandi, F. HLA-G and HLA-E in patients with juvenile idiopathic arthritis. *Rheumatology* 50(5), 966–972 (2011).
- 72 LeMaoult J, Le Discorde M, Rouas-Freiss N et al. Biology and functions of human leukocyte antigen-G in health and sickness. *Tissue Antigens* 62(4), 273–284 (2003).
- 73 Ongaro A, Stignani, M, Pellati A *et al.* Human leukocyte antigen-G molecules are

constitutively expressed by synovial fibroblasts and upmodulated in osteoarthritis. *Hum. Immunol.* 71(4), 342–350 (2010).

- 74 Kuroki K, Hirose K, Okabe Y *et al.* Maenaka K. The long-term immunosuppressive effects of disulfide-linked HLA-G dimer in mice with collagen-induced arthritis. *Hum. Immunol.* 74(4), 433–438 (2013).
- Therapeutic effect of the HLA-G dimer in mice with collagen-induced arthritis.
- 75 Stamp LK, O'Donnell JL, Chapman PT, Barclay, M.Let al. Lack of association between *HLA-G* 14 bp insertion/deletion polymorphism and response to long-term therapy with methotrexate response in rheumatoid arthritis. *Ann. Rheum. Dis.* 68(1), 154–155 (2009).
- 76 Kooloos WM, Wessels JA, van der Straaten T, Allaart CF, Huizinga TW, Guchelaar HJ. Functional polymorphisms and methotrexate treatment outcome in recent-onset rheumatoid arthritis. *Pharmacogenomics* 11(2), 163–175 (2010).
- 77 Rizzo R, Farina I, Bortolotti D *et al. HLA-G* may predict the disease course in patients with early rheumatoid arthritis. *Hum. Immunol.* 74(4), 425–432 (2013).
- == HLA-G as a biomarker in RA.
- 78 Needleman BW. Immunologic aspects of scleroderma. *Curr. Opin. Rheumatol.* 4(6), 862–868 (1992).
- 79 Wastowski IJ, Sampaio-Barros PD, Amstalden EM *et al. HLA-G* expression in the skin of patients with systemic sclerosis. *J. Rheumatol.* 36(6), 1230–1234 (2009).
- 80 Rosado S, Perez-Chacon G, Mellor-Pita S et al. Expression of human leukocyte antigen-G in systemic lupus erythematosus. Hum. Immunol. 69(1), 9–15 (2008).
- 81 Chen J, Shen B, Jiang Y *et al.* Analysis of immunoglobulin-like transcripts (ILTs) in lymphocytes with sHLA-G and IL10 from SLE patients. *Clin. Exp. Med.* 13(2), 135–142 (2013).
- 82 Rizzo R, Hviid TV, Govoni M et al. 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(6), 520–529 (2008).
- 83 Rudstein-Svetlicky N, Loewenthal R, Horejsi V, Gazit E. HLA-G levels in serum and plasma. *Tissue Antigens*. 69(2), 140–142 (2007).
- 84 Monsiváis-Urenda AE, Baranda L, Alvarez-Quiroga C, Abud-Mendoza C, González-Amaro R. Expression and functional role of HLA-G in immune cells from patients with systemic lupus erythematosus. J. Clin. Immunol. 31(3), 369–378 (2011).

- 85 Fernando MM, Freudenberg J, Lee A et al. Transancestral mapping of the MHC region in systemic lupus erythematosus identifies new independent and interacting loci at MSH5, HLA-DPBI and HLA-G. Ann. Rheum. Dis. 71(5), 777–784 (2012).
- 86 Veit TD, Cordero EA, Mucenic T *et al.* Association of the *HLA-G* 14 bp polymorphism with systemic lupus erythematosus. *Lupus* 18(5), 424–430 (2009).
- 87 Pedroza LS, Sauma MF, Vasconcelos JM et al. Systemic lupus erythematosus: association with KIR and SLC11A1 polymorphisms, ethnic predisposition and influence in clinical manifestations at onset revealed by ancestry genetic markers in an urban Brazilian population. Lupus 20(3), 265–273 (2011).
- 88 Consiglio CR, Veit TD, Monticielo OA et al. Association of the HLA-G gene +3142C>G polymorphism with systemic lupus erythematosus. *Tissue Antigens* 77(6), 540–545 (2011).
- 89 Park KS, Nam J, Lee ES, Choi JS, Bang D, Lee S. Increased risk of human leukocyte antigen-G gene variants in Behçet's disease. *Clin. Exp. Rheumatol.* 24(5 Suppl. 42), S126–S127 (2006).
- 90 Park KS, Park JS, Nam JH, Bang D, Sohn S, Lee ES. *HLA-E*0101* and *HLA-G*010101* reduce the risk of Behcet's disease. *Tissue Antigens* 69(2), 139–144 (2007).
- 91 Kim JJ, Hong SJ, Hong YM et al. Genetic variants in the HLA-G region are associated with Kawasaki disease. Hum. Immunol. 69(12), 867–871 (2008).

- 92 Rossman MD, Thompson B, Frederick M et al. HLA-DRB1*1101: a significant risk factor for sarcoidosis in blacks and whites. Am. J. Hum. Gen. 73(4), 720–735 (2003).
- 93 Hviid TV, Milman N, Hylenius S, Jakobsen K, Jensen MS, Larsen LG. HLA-G polymorphisms and HLA-G expression in sarcoidosis. Sarcoidosis Vasc. Diffuse Lung Dis. 23(1), 30–37 (2006).
- 94 Klareskog L, Catrina AI, Paget S. Rheumatoid arthritis. *Lancet* 373, 659–672 (2009).
- 95 Naredo E, Bonilla G, Gamero F, Uson J, Carmona L, Laffon A. Assessment of inflammatory activity in rheumatoid arthritis: a comparative study of clinical evaluation with grey scale and power Doppler ultrasonography. *Ann. Rheum. Dis.* 64, 375–381 (2005).
- 96 Brenol CV, Veit TD, Chies JA, Xavier RM. The role of the *HLA-G* gene and molecule on the clinical expression of rheumatologic diseases. *Rev. Bras. Reumatol.* 52(1), 75–91 (2012).
- 97 Kawashiri SY, Kawakami A, Iwamoto N et al. The power Doppler ultrasonography score from 24 synovial sites or 6 simplified synovial sites, including the metacarpophalangeal joints, reflects the clinical disease activity and level of serum biomarkers in patients with rheumatoid arthritis. *Rheumatology (Oxford)* 50, 962–965 (2011).
- 98 Wu CC, Wu TC, Liu FL, Sytwu HK, Chang DM. TNF-α inhibitor reverse the effects of human umbilical cord-derived stem cells on

experimental arthritis by increasing immunosuppression. *Cell. Immunol.* 273(1), 30–40 (2012).

- 99 Wang L, Wang L, Cong X, Liu G, Zhou J. Human umbilical cord mesenchymal stem cell therapy for patients with active rheumatoid arthritis: safety and efficacy. *Stem Cells Dev.* doi:10.1089/scd.0023 (2013) (Epub ahead of print).
- Boyson JE, Erskine R, Whitman MC *et al.* Disulfide bond-mediated dimerization of
 HLA-G on the cell surface. *Proc. Natl Acad. Sci. USA* 99(25), 16180–16185 (2002).
- 101 Morales PJ, Pace JL, Platt JS, Langat DK, Hunt JS. Synthesis of beta(2)-microglobulinfree, disulphide-linked HLA-G5 homodimers in human placental villous cytotrophoblast cells. *Immunology* 122(2), 179–188 (2007).
- 102 Morales PJ, Pace JL, Platt JS *et al.* Placental cell expression of HLA-G2 isoforms is limited to the invasive trophoblast phenotype. *J. Immunol.* 171(11), 6215–62124 (2003).
- 103 Zilberman S, Schenowitz C, Agaugué S et al. HLA-G1 and HLA-G5 active dimers are present in malignant cells and effusions: the influence of the tumor microenvironment. *Eur. J. Immunol.* 42(6), 1599–1608 (2012).

Websites

- 201 Online Mendelian Inheritance in Man. www.omim.org
- 202 IMGT/HLA. www.ebi.ac.uk/ipd/imgt/hla