The role of the HLA-G gene and molecule on the clinical expression of rheumatologic diseases

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ABSTRACT

Human leukocyte antigen G (HLA-G) is a non-classic class I major histocompatibility complex (MHC) molecule characterized by low polymorphism in its coding region, a limited tissue distribution pattern in physiologic conditions, and expression through soluble isoforms and isoforms bound to surface membranes through alternative splicing. HLA-G is fairly known since it is involved in induction and maintenance of tolerance between the maternal immunologic system and the semi-allogeneic fetus at the level of the fetal-placental interface. Besides, several studies have indicated a wider immunoregulatory role of this molecule. In this context, the expression of HLA-G in inflammatory and rheumatologic diseases is a relatively recent research area. The first studies described the expression of HLA-G in several inflammatory myopathies, atopic dermatitis, and cutaneous psoriasis. Based on the findings that HLA-G could divert T helper responses to the Th2 type, it was hypothesized that HLA-G would be a protective molecule in inflammatory responses. In this article, we review the potential roles of the HLA-G molecule in the immune system and in several rheumatologic diseases, such as systemic lupus erythematosus, rheumatoid arthritis, systemic sclerosis, and others.

Keywords: HLA antigens, rheumatologic diseases, genetic polymorphism.

INTRODUCTION

The study of the function of the immune system-related genes in rheumatologic disorders has been the focus of increasing interest. For a long time, class I and II major histocompatibility complex (MHC) genes, historically known as highly polymorphic and as the mainly responsible for the rejection of allogeneic transplantations, have generated a large volume of publications on their role in the autoimmune processes that characterize those disorders. In the decade of 1980, the discovery of MHC genes that did not share that high degree of polymorphism raised questions on their physiologic usefulness.1 However, from 1990 on, with the identification of one of those molecules, HLA-G, on fetal-placental interface, expressed on the surface of the trophoblast, those issues began to be answered. In addition to being responsible for fetal protection against maternal immune response, HLA-G was related to important immunoregulatory processes in transplantation, tumors, viral infections, and inflammatory disorders. This review aimed at presenting the HLA-G molecule from an immunologic point of view, addressing its effects on the immune system and clinical research, in addition to providing an update of the most relevant results in the field of rheumatologic diseases.

HLA-G GENE AND MOLECULE

HLA-G is a class Ib molecule, whose structure resembles other classic class I HLA molecules, with an alpha chain composed by up to three domains, noncovalently bonded to...
a β2-microglobulin chain. The HLA-G gene has low polymorphism in its coding region, a limited expression pattern in healthy conditions, and the unique characteristic among HLA molecules of forming multimers. In addition, seven different isoforms can be formed through alternative splicing. All those characteristics contribute for the increasing scientific interest on that molecule, some of which have vital importance in the biologic functions of HLA-G, characterized, mainly, by the induction of immune tolerance.2

So far, 47 alleles of the HLA-G gene, which encode 15 different proteins, have been described as compared with 1729, 2329, and 1291 alleles of the HLA-A, B, and C, respectively, according to the IMGT/HLA database in December 2011 (http://www.ebi.ac.uk/imgt/hla/stats.html). This low polymorphism is distributed along the three domains of the alpha chain, while, in classic HLA molecules, almost all polymorphism is concentrated around the peptide binding site. In the HLA-G molecule, the peptide is located more deeply into the cleft between domains 1 and 2 as compared with classic HLA molecules.4 These special characteristics of HLA-G make it unlikely that this molecule plays an important role in antigen presentation.

HLA-G gene also has polymorphisms in introns, promoter region, and 3’ untranslated region (3’UTR). The last consists in the deletion/insertion of 14 base pairs (bp), and their insertion is associated with reduced levels of messenger RNA. The polymorphism of 14-bp insertion/deletion located in position +2960 at exon 8 (rs1704) has attracted attention due to its potential role of alternative splicing and RNA stability. The transcriptions with the 14-bp (ins) sequence have been shown to undergo an additional splicing step that removes 92 bp from the region in which the sequence is located.2 Although the 14-bp polymorphism has been the most investigated, there is no strong evidence that it has any effect on protein production.

HLA-G proteins can be found in different isoforms (four membrane-bound, G1-G4, and three soluble, G5-G7), being generated by alternative splicing.6 Different isoforms of HLA-G are produced, depending on the type of cell and physiologic condition.7 All isoforms contain at least one alpha-1 domain, and the HLA-G1 isoform is complete. In isoforms G5-G7, transmembrane and cytoplasmic domains are not translated, resulting in soluble isoforms.6 Due to a mutation, HLA-G has a cytoplasmic tail shorter than that in HLA-A, B, and C.5 This characteristic has important implications for the expression of HLA-G, because it provides more prolonged expression of HLA-G on the cell surface when compared to classic HLA molecules.10

The expression of HLA-G is highly restricted to specific tissues – in addition to being expressed in fetal tissues, such as the trophoblast cells, it is constitutively expressed in adult thymus, cornea, pancreatic islets, and precursors of endothelial and erythroid cells. However, HLA-G expression can be induced in situations such as transplantations, inflammatory diseases, tumor cells, multiple sclerosis, and viral infections.5

HLA-G AND IMMUNE TOLERANCE
Immunoregulatory effects of HLA-G

The expression of HLA-G was first described in the cytotrophoblast and, therefore, the first studies on this molecule evaluated its role in pregnancy. During pregnancy, the maternal immune system is in close contact with the cells and tissues of the semi-allogeneic fetus. This suggests that specific mechanisms should modulate the maternal immune system to avoid fetal rejection, i.e., to promote fetal tolerance. In fact, some complications during pregnancy, such as preeclampsia (PE), have been associated with Th1 immune response.11 To protect the fetus from the maternal immune system, preventing cytotoxic T cell-mediated cytolysis (CTL), cytotrophoblast cells do not express HLA-A or B and express little HLA-C. Additionally, the expression of HLA-G by these cells inhibit activation of maternal T cells, as well as cytolysis by natural killer cells (NK) – and CTL by specific receptors.12,13 Recently, the role of HLA-G has been suggested to be more related to the production of soluble mediators important for gestational success.14

Several mechanisms through which HLA-G exerts its regulatory functions have been identified (Figure 1). As mentioned earlier, HLA-G is able to inhibit the cytotoxic activity of NK and CTL cells.13 Likewise, it is able to protect class I HLA-negative cells or allogeneic tumors from anti-tumor NK cell-mediated immune response.15 It has also been shown that HLA-G can inhibit the response of alloproliferative CD4+ T cells,16 proliferation of T and NK cells,17 and the maturation and function of antigen presenting cells (APC).18 Additionally, HLA-G is capable of generating apoptosis in endothelial cells.18

There are convincing arguments that HLA-G has an important role in regulating the immune system: (1) HLA-G is capable of binding to several types of receptors, some of which are widely distributed among immune cells; (2) HLA-G can exert long-term tolerogenic effects by generating suppressor cells; and (3) even cells that do not transcribe HLA-G can become temporarily HLA-G-positive, acquiring an immunosuppressive profile through a phenomenon known as trogocytosis (intercellular capture of HLA-G by incorporating membrane fragments that express this molecule).3

HLA-G exerts its immunoregulatory effects by binding specific receptors in different types of immune cells.1 The
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Leukocyte receptor complex in chromosome 19 includes two families of polymorphic genes: leukocyte immunoglobulin-like receptors (LILR) and killer cell immunoglobulin-like receptors (KIR). Of the LILR molecules, LILR1 (ILT2, CD85j, LILRB1) and LILR2 (ILT4, CD85d, LILRB2) are inhibitory receptors that recognize all class I HLA molecules.20 LILR1 is expressed by B cells, some T and NK cells, and all monocytes, while LILR2 is specific of myeloid lineages. There is evidence that LILRB1 and LILRB2 bind HLA-G.21,22 Recent data have shown that the binding sites of ILT-2 and ILT-4 in HLA-G dimmers are more accessible than in monomers, resulting in 100-times greater binding affinity, corroborating the importance of dimmers for the physiologic activity of HLA-G.23

The KIR2DL4 receptor (CD158d) is a member of the KIR family that also binds HLA-G. Its gene is located in the center of the KIR gene complex and is present in all KIR haplotypes. Signaling by this receptor is different from that of LILR receptors: after its binding to the receptor, soluble HLA-G is internalized in endosomes. Interestingly, this signaling results in a proinflammatory and pro-angiogenic response, with important functions in sites that express the ligand, such as the maternal-fetal interface during the initial phases of pregnancy.14

**HLA-G and suppressor cells**

A growing body of evidence indicates that, in addition to its direct inhibitory effect, HLA-G can exert long-term tolerogenic effects by generating suppressor cells. Several HLA-G-related suppressor cells have been identified.24 HLA-G+ regulatory T cells are present in the peripheral blood in physiologic conditions. These cells can be CD4+ or CD8+ and constitutively express HLA-G1 on their surfaces. HLA-G1+ T cells are hyporesponsive and mediate their suppressor functions through soluble factors, which include sHLA-G, but neither IL-10 nor TGF-B. Its presence has also been identified at inflammation sites.25

HLA-G+ T cells can also be induced by allostimulation, producing soluble HLA-G5, and, on rare occasions, HLA-G1.26 Although their origin is obscure, these are suppressive cells and limit the alloproliferation of autologous CD4 T cells.

**Figure 1**

HLA-G-mediated immunoregulatory functions, target cells, and receptors.

ILT2 = immunoglobulin-like transcript 2; ILT4 = immunoglobulin-like transcript 4; NKG2A = natural killer, group 2, member A receptor; KIR2DL4 = killer cell immunoglobulin-like receptor 2DL4.

Source: Adapted from Veit, Vianna, Chies.3

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HLA-G-mediated immunoregulatory functions, target cells, and receptors.

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Source: Adapted from Veit, Vianna, Chies.3
Antigen presenting cells can also express HLA-G in pathologic conditions. These cells were identified in transplanted tissues, tumors, inflammatory disorders, and viral infections. They are capable of blocking T cell reactivity and inducing suppressor T cells, and seem to have a prognostic role in chronic lymphocytic leukemia. Bone marrow mesenchymal stem cells (MSC) are multipotent cells capable of differentiating in several lineages, with strong immunomodulatory properties. Recently, HLA-G has been shown to be a determining factor in the immunomodulatory functions of MSC.

To conclude, HLA-G-dependent regulatory cells have very different origins, resulting in different induction modes, phenotypes, and mechanisms of action. Thus, it is unlikely that all these cells have the same role in the same situation.

Trogocytosis

Trogocytosis is a type of contact between cells leading to the exchange of membrane parts and associated molecules. However, during trogocytosis, all molecules contained in a specific area of the membrane are transferred, including some that do not participate in the intercellular communication and that end up being non-specifically transferred. The majority of the studies on trogocytosis involved murine T cells; these studies have shown that CD4+ and CD8+ can acquire class II and class I MHC molecules of APCs, respectively, in an antigen-specific way. Recently, trogocytosis of APC HLA-DR, CD80, and HLA-G1 by T cells in humans has been described, and it follows the same rules of murine models. Thus, T cells that acquire HLA-DR and CD80 behave like APCs after receiving an antigen-specific stimulus, while the acquisition of HLA-G1 makes them unresponsive. This can constitute an efficient way of immune system modulation.

In fact, it has been shown that HLA-G1 can be acquired by tumor cells through from trogocytosis-activated NK cells; this can be an immune escape mechanism for originally HLA-G-negative tumor cells. Almost all activated NK cells can acquire detectable levels of HLA-G1 in a few minutes through some mechanism depending on non-specifically transferred. Unlike cells that express HLA-G1, the expression of surface acquired HLA-G1 in NK cells is temporary, since they do not transcribe HLA-G. Functionally, NK cells that acquire HLA-G1 stop proliferating, are no longer cytotoxic, and behave like suppressor cells capable of inhibiting other NK-cell functions. All these functional properties are due to the acquired HLA-G1, and could be nullified by blocking the HLA-G1 or its LILR1 receptor on the surface of NK cells.

HLA-G – CLINICAL IMPLICATIONS

The new aspects of the HLA-G biology, such as the highly inhibitory functions of its multimers, regulatory cells, and trogocytosis, are critical for understanding the relevance of this molecule in pathologic conditions. In addition, they should help project HLA-G-mediated diagnostic and therapeutic strategies in different medical areas, such as obstetrics, oncology, organ transplantation, and inflammatory disorders.

The importance of HLA-G was primarily indicated in studies on the implantation of human embryos, a complex process requiring an adequate endometrial receptivity and the capacity to implant in the uterus. Evidence indicates that during this process, several adaptations in the maternal immune system are necessary to allow the development of a viable pregnancy. The relationship between HLA-G and implantation of the human embryo is the fundamental starting point in maternal immune tolerance of the semi-allogeneic fetus. In physiologic conditions, the strong expression of HLA-G by the invasive trophoblast can partly explain the maintenance of the semi-allogeneic fetus during pregnancy. Several studies have suggested the importance of maternal HLA-G expression during cleavage and embryonic and fetal development. In addition, the role of genetic polymorphisms, such as deletion/insertion of 14 bp (14 bp-rs1704), located in the 3’UTR region of the gene, was implicated in pregnancy complications, such as PE and repeated abortions.

Other situations in which HLA-G molecules are involved with clinical outcomes include organ transplantations and oncology. Since the first description of HLA-G expression by tumor cell in 1998, a considerable number of studies indicating the transcription of the gene and expression of the HLA-G protein in neoplastic lesions have suggested that this would represent a protection of malignant cells from cytolyis by NK cells. Thus, HLA-G expression would favor tumor development by changing cytotoxic immunity. Finally, this molecule could constitute a tumor marker and potential therapeutic target that could be blocked or eliminated.

In the context of transplantations, HLA-G expression can be beneficial and promote graft tolerance. The HLA-G expression has been widely studied in patients after heart, kidney, liver, and liver-kidney transplantations. In these populations, those expressing HLA-G in the graft or plasma showed significantly better graft acceptance. Lines of research indicate that HLA-G could be used as a tolerogenic therapeutic agent if administered as alternative and/or complementary therapy.
Rheumatologic diseases and HLA-G

HLA-G expression in inflammatory and autoimmune diseases is a relatively new area of investigation. In the first studies, HLA-G expression was evaluated in muscular fibers in different inflammatory myopathies, atopic dermatitis, and psoriasis. Based on the findings that HLA-G could divert helper T responses to Th2, it was hypothesized that HLA-G would be a protective molecule in inflammatory responses. Since then, several studies in rheumatology have been performed, and their results are summarized in Table 1.

Currently, studies of genomic scan have been gaining attention in the investigation and discovery of alleles and regions related to genetic susceptibility to autoimmune diseases. In a study of genomic scan that analyzed 2360 SNPs within the MHC region in 92 patients with Kawasaki disease (KD), a systemic vasculitis of childhood of unknown cause, HLA-G was identified as the only candidate locus for a significant association with that vasculitis.

The 14-bp polymorphism was investigated in other situations and has been associated with several diseases – the insertion allele has been observed with higher frequency in patients with sarcoidosis and Behçet’s disease (BD), while the deletion allele has been reported as a risk factor for idiopathic dilated cardiomyopathy and pemphigus vulgaris (PV). HLA-G expression in the skin of patients with PV has been recently reported. However, such results should be replicated in future studies.

In addition to KD and BD, other rheumatologic disorders have been evaluated for the presence of HLA-G, such as systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA). In SLE, studies have demonstrated diverging results:

Table 1

<table>
<thead>
<tr>
<th>Disease</th>
<th>Type of study</th>
<th>n</th>
<th>Materials</th>
<th>HLA-G expression</th>
<th>Outcome investigated</th>
<th>Polymorphism</th>
<th>Associated allele/genotype</th>
<th>Ref.</th>
</tr>
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<tbody>
<tr>
<td>Behçet’s disease</td>
<td>Genetic</td>
<td>312</td>
<td>—</td>
<td>—</td>
<td>Susceptibility</td>
<td>Several</td>
<td>C*010101 – protection</td>
<td>54</td>
</tr>
<tr>
<td>Inflammatory myopathy</td>
<td>Expression</td>
<td>20</td>
<td>Muscle</td>
<td>Present</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>47</td>
</tr>
<tr>
<td>JIA</td>
<td>Genetic</td>
<td>106</td>
<td>—</td>
<td>—</td>
<td>Susceptibility</td>
<td>14 bp (rs1704) del (girls)</td>
<td>64</td>
<td></td>
</tr>
<tr>
<td>Kawasaki disease</td>
<td>Genetic</td>
<td>92</td>
<td>—</td>
<td>—</td>
<td>Susceptibility</td>
<td>GWS</td>
<td>HLA-G locus</td>
<td>52</td>
</tr>
<tr>
<td>Rheumatoid arthritis</td>
<td>Expression</td>
<td>106</td>
<td>Serum</td>
<td>—</td>
<td>Reduced</td>
<td>14 bp (rs1704)</td>
<td>—</td>
<td>62</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30</td>
<td>PBMC</td>
<td>Increased after MTX use</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>63</td>
</tr>
<tr>
<td>Genetic</td>
<td>156</td>
<td>—</td>
<td>—</td>
<td>Susceptibility</td>
<td>14 bp (rs1704) del/del</td>
<td>63</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Genetic</td>
<td>130</td>
<td>—</td>
<td>—</td>
<td>Response to MTX</td>
<td>14 bp (rs1704)</td>
<td>NA</td>
<td>71</td>
<td></td>
</tr>
<tr>
<td>Genetic</td>
<td>265</td>
<td>—</td>
<td>—</td>
<td>Susceptibility</td>
<td>14 bp (rs1704)</td>
<td>NA</td>
<td>64</td>
<td></td>
</tr>
<tr>
<td>Genetic</td>
<td>186</td>
<td>—</td>
<td>—</td>
<td>Response to MTX</td>
<td>14 bp (rs1704)</td>
<td>NA</td>
<td>73</td>
<td></td>
</tr>
<tr>
<td>Sarcoïdosis</td>
<td>Genetic</td>
<td>47</td>
<td>—</td>
<td>—</td>
<td>Susceptibility</td>
<td>Several 14 bp (rs1704) alleles containing 14 bp (NS)</td>
<td>53</td>
<td></td>
</tr>
<tr>
<td>Expression</td>
<td>ND</td>
<td>Granulomas</td>
<td>Rare and weak</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td></td>
<td></td>
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<tr>
<td>SLE</td>
<td>Expression</td>
<td>50</td>
<td>Serum</td>
<td>Greater</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>58</td>
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<tr>
<td></td>
<td></td>
<td>130</td>
<td>Plasma</td>
<td>Smaller</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>59</td>
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<tr>
<td>Genetic</td>
<td>200</td>
<td>—</td>
<td>—</td>
<td>Susceptibility</td>
<td>14 bp (rs1704) ins</td>
<td>59</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Genetic</td>
<td>293</td>
<td>—</td>
<td>—</td>
<td>Susceptibility</td>
<td>14 bp (rs1704) ins/del</td>
<td>60</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systemic sclerosis</td>
<td>Expression</td>
<td>21</td>
<td>Skin</td>
<td>Present in 57% of patients associated with better prognosis</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>61</td>
</tr>
</tbody>
</table>

GWS = genomic-wide selection; JIA = juvenile idiopathic arthritis; LPS = lipopolysaccharide; MTX = methotrexate; NA = no association; ND = non-determined; NS = non-significant; PBMC = peripheral blood mononuclear cells; SLE = systemic lupus erythematosus.

Source: Adapted from Vet, Varma, Chies.
one has reported higher plasma levels of HLA-G, while another has reported lower levels. The latter has also reported a genetic association with the 14-bp polymorphism in which the ins/ins genotype was a risk factor for the development of SLE. However, this result has not been replicated in a research carried out at the Hospital de Clínicas de Porto Alegre, where an increased frequency of the heterozygous genotype was observed.

In systemic sclerosis, HLA-G expression has been reported in skin biopsies of Brazilian patients, and this expression has been associated with a lower frequency of cutaneous vascular ulcers, telangiectasia, and polyarthritis, and with better survival.

The number of studies on the prevalence and expression of HLA-G polymorphisms in RA is also limited. In a recent publication, Verbruggen et al. identified lower plasma levels of HLA-G in patients with RA when compared with those in healthy individuals. Thus, at first, one would conclude that the low levels of sHLA-G observed in those patients would translate into an inability to suppress the development of self-reactive cells, facilitating the development of an autoimmune disease. It is worth noting that, in the group of patients with HLA complex shared epitopes associated with the disease, especially HLA-DRB1*01, 04, and 10, higher levels of sHLA-G have been detected. Such levels showed a positive correlation with disease activity parameters, such as C-reactive protein and the number of swollen joints. Those authors have postulated that low sHLA-G levels could contribute to susceptibility to RA, while increased levels in the group of patients with bad prognostic factors would represent secondary defense mechanisms emerging after the onset and perpetuation of inflammation.

So far, all attempts to find a genetic association between HLA-G polymorphisms and RA susceptibility have been unsuccessful. In 2008, our group published a study of cases and controls that included 256 patients with RA and 356 healthy controls genotyped for the HLA-G 14-bp polymorphism. No differences in allelic and genotypic frequencies were observed between patients and controls. In a cross-sectional analysis, no correlation between disease characteristics (extra-articular manifestations, atlantoaxial subluxation, clinical activity and functional scores, and disease duration) and the genotype was observed. In that study, 106 patients with juvenile idiopathic arthritis (JIA) were also included. Interestingly, a significant association was observed between the deletion allele and JIA susceptibility in girls when compared with controls of the same gender (0.743 and 0.576, respectively, P < 0.001). These results suggest that those diseases have different physiopathogenic elements.

Pharmacogenetics is another field of investigation related to HLA-G 14-bp polymorphisms with potential applicability in the therapy of patients with RA. Methotrexate (MTX), the major disease-modifying anti-rheumatic drug (DMARD), is implicated in the increased production of IL-10 in patients with RA, which correlates with better therapeutic response. IL-10 and HLA-G might act together in the immunosuppression of in vivo inflammatory processes and might be important factors in the susceptibility to inflammatory disorders and their course. IL-10 induces HLA-G expression on the surface of monocytes, and sHLA-G seems to stimulate the expression of IL-10 in peripheral blood mononuclear cells (PBMC).

The close relationship between the HLA-G and IL-10 molecules could also be illustrated by the study by Rizzo et al. In that study, performed in cultures of PBMC activated by lipopolysaccharides (LPS), the highest levels of IL-10 were observed in the +14/+14 genotype, while the lowest levels were observed in the -14/-14 genotype. Secretion of HLA-G5/sHLA-G1 was preceded by the IL-10 expression, which can indicate an autocrine feedback. Considering that the +14/+14 genotype associates with less stable mRNA transcripts and lower production of sHLA-G, higher IL-10 levels would be needed for the secretion of those molecules, as compared with those in the -14/-14 genotype. Thus, the authors have demonstrated, for the first time, functional differences related to the HLA-G polymorphism at cellular level.

In view of those findings, the same authors have evaluated sHLA-G and IL10 production and their relationship with the HLA-G 14-bp polymorphism in 156 patients with RA (treated with MTX or not). Peripheral blood mononuclear cells of healthy individuals and of patients with AR not previously treated with MTX were exposed to different concentrations of MTX, and IL-10 and sHLA-G productions were determined by immunoenzymatic assays. Methotrexate clearly induced the production of sHLA-G, and a statistically significant correlation between higher concentrations of sHLA-G and the +14/-14 genotype was observed. Patients responsive to MTX (reduction in disease activity score (DAS) > 0.6–1.2; measured before and after six months of treatment with MTX) showed the -14/-14 bp genotype more frequently than non-responders. Despite the consistent result, the retrospective character of the study and the low doses of MTX used (mean dose, 10.5 mg/week) could have been relevant limitations. It is widely accepted that oral MTX should be initiated at 10–15 mg/week doses, with 5-mg increases every 2–4 weeks until the 20–30 mg/week dose is achieved, depending on the clinical response and tolerability. Additionally, when verifying that the production of sHLA-G correlated with the therapeutic
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response, the authors proposed that that polymorphism could become a marker of therapeutic response in the initial phases of the disease. They emphasized the need to confirm those results in prospective studies in different populations.63

In contrast to the previous result, there are two studies with negative results. A study with 130 patients with RA has shown statistically significant difference in neither allelic nor genotypic distribution of DAS28 (≤ 3.2 or > 3.2) in patients treated with MTX.71 Patients had long-standing RA (mean, 10 years) and were exposed to adequate doses of MTX (mean dose of 16.6 mg/week for non-responders). Unlike the study by Rizzo et al.,63 the limitation of that study was the cross-sectional design. In addition, the sample size had enough statistical power to detect only a very large difference in the response rates between genotype groups (30%). On the other hand, the lack of enough power to draw definitive conclusions is shared by the majority of pharmacogenetic studies in RA.72

In 2010, Kooloos et al.73 published a prospective study with 186 patients with RA, who had never been treated with MTX. One of the inclusion criteria was the duration of symptoms shorter than two years. Data were obtained from a subcohort of patients who participated in the multicenter study Behandelstrategieen voor Reumatoïd Artritis (BeSt). Responders were defined as those who were receiving MTX and had a DAS of up to 2.4 after six months of treatment (good clinical response); otherwise, individuals were considered non-responders. In addition to the insertion/deletion of 14 bp of the HLA-G gene, functional polymorphisms were tested in six other genes: DHFR (dihydrofolate reductase) 829C>T; ABCB1 (transmembrane ATP-binding cassette transporter B1) 3435C>T; ITPA (inosine triphosphate pyrophosphatase) IVS2 + 21A>C; IMPDH2 (inosine-5-monophosphate dehydrogenase 2) 787C>T; TGFB1 869T>C; and TLR4 896A>G. Finally, no significant association between these variants and MTX efficacy was observed.

Comparing with the single study with positive results, the opposite results in other studies may reflect interethnic differences in the frequencies of the investigated polymorphism,74 which can influence the power of association studies. Several factors (genetic and others), influential in different populations, can modify the expression of a gene and lead to different levels of association.75 In addition, outcomes in pharmacokinetic studies in RA have not been standardized, making them difficult to compare.

In summary, homozygosity for the HLA-G 14-bp deletion might be a potential marker of response to MTX. This hypothesis is reinforced by the functional consequences of the IL-10-mediated interaction of HLA-G 14-bp polymorphism and MTX, as well as by the existence, in the literature, of a clinical study with positive result. Since the real role of that polymorphism to predict the therapeutic response to MTX in RA has not been well established, positive findings should be replicated in independent cohorts. Future studies are required to identify genetic profiles capable of selecting patients more prone to properly respond to MTX.

CONCLUSION

It has become increasingly evident that the HLA-G molecule is capable of exerting direct and sustained action on the immune system by its immunoregulatory effects, induction of suppressor cells, and trogocytosis. Thus, it is a potential candidate to influence the clinical expression of several rheumatologic diseases. Although several studies have indicated the association of polymorphisms or even of plasma levels of HLA-G with several clinical situations, including modulation of the therapeutic response, it is prudent to emphasize that these results are still controversial and more scientific investigation is required to prove its potential properties.
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O papel do gene e da molécula HLA-G na expressão clínica das doenças reumatológicas


