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HLA-G MOLECULES
IN INFECTION AND AUTOIMMUNE DISEASES

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Abstract

Introduction: Human leukocyte antigen-G (HLA-G) is a non-classical HLA class I molecule that differs from classical HLA class I molecules for low allelic polymorphism and restricted tissue distribution. In physiological conditions HLA-G molecules are expressed on trophoblast at the fetal-maternal interface and other sites of immune privilege, such as thymus and cornea, where have an important role in the maintenance of a tolerogenic condition. In fact, HLA-G regulates the immune response through its immune-modulatory and anti-inflammatory function on both innate and adaptive immunity. HLA-G molecules are expressed as membrane bound and soluble isoforms (mHLA-G, sHLA-G) that bind and activate immune-inhibitory receptors (ILT2, ILT4, KIR2DL4) expressed on immune cells. Recently, HLA-G molecule has been shown to have an important implication in different pathological situations, such as infections and autoimmune diseases, where HLA-G shows aberrant expression and specific HLA-G gene polymorphisms correlate with disease severity and outcome. Moreover, HLA-G could be up-modulated and used as a immune-escape mechanism by virus and tumours. On the basis of this knowledge, HLA-G may exhibit two distinct effects in pathological conditions: it could be protective in Th1-based inflammatory and autoimmune diseases or it could be detrimental in tumors or infectious diseases. The aim of this thesis is to investigate the role of HLA-G mlecules in different pathological conditions: microbial infections and autoimmune diseases.

Material and methods: The data herein reported were achieved using different molecular, proteomic and cellular techniques. The most important are the following: soluble (s)HLA-G was analysed by ELISA assay and Western Blot analysis; HLA-G gene polymorphism and mRNA were analysed by PCR, RT-PCR and RealTime PCR; membrane-bound (m)HLA-G was investigate using flow cytometry and immunofluorescence; to perform in vitro studies both continuous and primary cell lines culture were used.

Results: The role of HLA-G was determined during microbial infections [(1) viral and (2) bacterial] and autoimmune diseases [3].

 1) *HLA-G in viral infections*. I focused my research on three viruses : HPV, HBV and hCMV.

Firstly, I focused on the role of HLA-G polymorphisms in HPV and HBV infection susceptibility.

We identify that two polymorphisms in the 3' untranslated region of the HLA-G gene (3' UTR) (14 bp ins/del, +3142C>G) are involved in susceptibility to HPV infection. Indeed, we reported that the 14 bp del allele is associated with a high risk of HPV infection, and the del/C haplotype facilitates

the development of invasive cervical cancer. By literature it is known that HLA-G is implicated in chronic hepatits B pathogenesis. Our study evidenced the importance of 14 bp ins/del polymorphism influence also concerning HBV infection, revealing an association between the 14 bp ins/del polymorphism and an enhanced HBV activity in presence of high HBV DNA levels.

Again, the analysis of the correlation between HLA-G and primary hCMV infection during pregnancy reported a strong correlation between sHLA-G amount found in maternal plasma and in the amniotic fluids and the symptomatology in the hCMV infected foetuses.

Last, the ability of inducing HLA-G was found also in HPV positive Sinonasal polyposis (SNP), a chronic inflammatory pathology that could develop after HPV infection. Our data showed that epithelial cells from nasal polyps obtained from HPV-11 positive SNP patients presented the expression of mHLA-G and IL-10R and secretion of sHLA-G and IL-10 in culture supernatants, while no HLA-G expression was observed in HPV negative polyps.

2) HLA-G in bacterial infections. I investigate the role of HLA-G during *P.aeruginosa* infection.

First of all, I analysed HLA-G modulation in patients affected by Cystic Fibrosis (CF) .

We found lower HLA-G levels in plasma of CF patients that, after treatment, became undistinguishable from those found in CTRLs. HLA-G was higher in the exhaled breath condensate (EBC) of CF patients and was normalized after antibiotic therapy only in CF patients that were free of *P. aeruginosa* infection. Thus, HLA-G levels correlate with the efficacy of antibiotic treatment and the higher HLA-G expression observed in the CF lung microenvironment is associated with *P. aeruginosa* infection, suggesting that this molecule could play a role in bacterial immune-escape mechanisms. Furthermore, we identify that a specific soluble molecules of *P.aeruginosa* Quorum Sensign (QS), 3-o-C12-HSL, is able to induce HLA-G expression particularly in monocyte U937 and Jurkat T cell lines through the CREB\p38 phosphorylation.

3) *HLA-G in autoimmune and inflammatory diseases*. I investigated HLA-G in different pathologies characterized by a dysregulation in host immune system in which HLA-G plays a central role: rheumatoid arthritis, Multiple sclerosis, Crohn's disease and psoriasis.

In all this conditions we identify a strong correlation between HLA-G 14bp INS/DEL and +3142C>G polymorphisms and soluble protein levels. In particular, early rheumatoid arthritis (ERA) treated patients with low sHLA-G and membrane HLA-G expression suffered a more severe disease while those with a significantly improved disease status were characterized by the presence of the DEL allele and higher HLA-G expression that correlates with lower DAS28 scores.

Concerning Multiple Sclerosis, serum and CSF sHLA-G levels were more elevated in high producers MS patients with C/C,DEL/DEL HLA-G genotype. Our group also reported higher frequency of HLA-G dimers in relapse-remitting (RRMS) patients that suggests their implication in reducing the inflammatory status in MS. The implications of HLA-G polymorphisms and protein expression in autoimmunity was also confirmed in psoriasis, where we found an increase in HLA-G plasmatic levels of systemic treated patients and a significant association between HLA-G14bpDEL allele and 14bpDEL/DEL genotype with acitretin clinical outcome, and in Crohn's Disease (CD), in which we reported that the 14-bp Del/Ins polymorphism of HLA-G gene and the presence of dimers are associated with the risk of CD and suggest a role for sHLA-G as prognostic marker for progressive disease.

Conclusion: The implication of HLA-G proteins in creating an impaired immune response during autoimmunity and microbial infections has been here confirmed. It appears even more evident that understanding the functions of HLA-G molecules in these disorders could help in the identification of new approaches to control HLA-G production. Moreover, HLA-G could fulfil the necessity of an easy and fast identification biomarker for disease diagnosis and prevention. HLA-G could represent a concrete help in disease outcome prediction and in supporting treatment decisions in different pathological conditions, such as infection and autoimmune diseases.