The discovery of HLA-G-bearing extracellular vesicles: new perspectives in HLA-G biology

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In contrast to classical human histocompatibility antigen (HLA) class I molecules, HLA-G has been discovered quite recently, in 1990. Since its discovery several efforts have been made to define HLA-G biology and its role in regulating immune responses. The main distinctive characteristics of HLA-G are the limited protein variability, alternative mRNA splicing that generates seven different isoforms(both soluble and membrane-bound) with the ability to form multiple structures via disulfide bounds, and restricted expression to certain tissues (1). Moreover, the discovery that HLA-G binds different inhibitory receptors (i.e., ILT2 and ILT4, and KIR2DL4) led to the classification of HLA-G as a tolerogenic molecule (2).

In the past decade much effort has been devoted to define the role of HLA-G in modulating immune responses in transplantation, inflammatory and autoimmune diseases, and cancer. HLA-G and its secreted forms are considered key players in the induction of short- and long-term tolerance. These tolerance-inducing activities render membrane-bound and soluble HLA-G attractive biomarkers for clinical approaches as prognostic factor to monitor disease stage and progression, or efficacy of treatments (3,4).

In different settings of transplantation it was demonstrated that the expression levels of membrane-bound and soluble HLA-G could be a predictive marker for graft stability, suggesting that increased HLA-G levels are associated with down-regulation of immune responses (5). Similarly, several studies performed in solid tumors highlighted the relationship between membrane-bound HLA-G expressed by tumor cells or soluble HLA-G in sera of patients with advanced disease stage, tumor load, or clinical outcome (5). Thus far, membrane-bound HLA-G expression has been proposed as diagnostic tool to stage breast cancer (6). In hematological malignancies, although several studies reported higher plasma levels of HLA-G in patients compared to healthy controls, the correlation of membrane-bound and soluble HLA-G expression with disease staging gave controversial results; therefore, it is still debated whether HLA-G can be used as biomarker of disease progression (5).

The complexity to apply HLA-G as a significant clinical biomarker has been limited by structural diversity of the molecule: HLA-G can be expressed as monomer and dimers in soluble form, and, more recently, it has been shown that it can be expressed associated with extracellular vesicles (EVs) (3). EVs are membrane-limited vesicles released in biological fluids by normal and malignant cells (7). Cells of the innate and adaptive immune system, including T and NK cells and antigen presenting cells, have been reported to release or to acquire informations via EVs. EVs indeed can contain proteins, lipids, and microRNA and, therefore, can provide molecules for immune modulation, modification in gene expression, and induction of apoptosis (8). Tumor cells can release EVs and can contribute to immune escape

by limiting tumor-specific effector T cells and promoting T regulatory cells expansion or expansion (9,10).

Tumor-derived EVs contain HLA-G, as demonstrated by Riteau *et al*. (11), who described for the first time the existence of HLA-G-bearing EVs in culture supernatants of a melanoma cell line expressing HLA-G. Subsequently, the presence of HLA-G-bearing EVs was observed *in vivo* in ascites and pleural exudates from cancer patients (12), and in exosomes released from first trimester and term placental explants (13). Recently, König *et al*. (14) isolated, for the first time, HLA-G-bearing EVs from plasma of breast cancer patients. Through the analysis of the levels of soluble HLA-G free and HLA-G-linked to EVs, König *et al*. demonstrated the prognostic relevance of HLA-G-bearing EVs, since HLA-G-linked to EVs was associated with disease progression, whereas the levels of soluble HLA-G free form were associated with response to treatment (14). This study highlighted the critical importance of defining the presence of HLA-G free or EVs-linked and how these two molecules may have opposite impact on disease progression, as they may promote immune escape or not. Based on this first demonstration, Rebmann *et al*. (15) discussed and proposed in their perspective the importance to discriminate the source of the different forms of soluble HLA-G, free or EV-linked, present in plasma or in serum of patients to better define their contribution in cancer, and, therefore, their use as biomarker of disease stage, progression, or response to therapy.

Rebmann *et al*. (15) also highlighted the consequences of the discovery of HLA-G-bearing EVs in the biology of HLA-G. In this contest, it has to be taken into account that EV-linked HLA-G can be expressed either as membrane-bound or as soluble form within the vesicles. Up to now, it has not been defined whether membrane-bound HLA-G expressed by EVs is structurally and biologically superimposable to the "classical" membrane-bound HLA-G. Thus, it cannot be excluded that EV-linked HLA-G may counteract the activity of "classical" soluble or membrane-bound HLA-G by competing for receptor occupancy. However, the evidence described by König *et al*. (14) suggested that HLA-G-linked to EVs is functional active, since higher levels of HLA-G-bearing EVs are associated with disease progression indicating its involvement in the down-regulation of immune responses. Future investigations are warranted to better define the mode of action of this new form of HLA-G (3). On the other side, the presence of soluble HLA-G within EVs suggests that HLA-G may be released within target cells independently from the expression of its receptors LILRBs and KIR2DL4 on the cell surface. It can indeed be possible that, upon interaction with target cells, EVs release soluble HLA-G directly into the cytoplasm, where it can interact with unknown receptors participating to yet undefined intracellular pathways. Moreover, since EVs have different composition based on the cell type from which they originate, it can also be speculated that the release of different bioactive effector molecules in conjunction with soluble HLA-G may have either positive or negative effects on HLA-G-mediated activity.

Several evidences indicated that HLA-G-expressing EVs mediate a number of immune-modulatory activities: HLA-G-expressing EVs isolated from renal cancer stem cells modulate monocyte-derived maturation and their ability to stimulate T cells *in vitro* (16). Recently, it was shown that mesenchymal stromal cells (MCS) isolated from patients with refractory graft versus host disease released EVs containing high levels of HLA-G, IL-10, and TGF-β (17). Taken together, these studies highlighted that HLA-G-expressing cells may exert tolerogenic functions not only via the expression of membrane-bound or the secretion of soluble HLA-G, but also through the release of HLA-G-expressing EVs. This can be the case also for HLA-G-expressing DC-10, a population of human tolerogenic dendritic cells, present in peripheral blood (18) and enriched in human decidua of pregnant women (19), that play an important role in promoting tolerance via T regulatory cells. Our group showed that the tolerogenic activity of DC-10 is associated with the expression of membrane-bound HLA-G: DC-10-expressing high levels of HLA-G are more potent inducers of T regulatory cells compared to DC-10-expressing low levels of HLA-G (20). DC-10, as other dendritic cells release EVs, and preliminary data indicated that DC-10-derived EVs contain IL-10 and HLA-G, suggesting that they may contribute to DC-10-mediated tolerance.

In conclusion, a more in depth study on the HLA-G-linked EVs will better define the role on HLA-G-mediated tolerance. Results will lead to improve the knowledge on the activity of the different forms of HLA-G and will shed light on the selection of the best-suited HLA-G form to be used as biomarker for disease stage, progression, and response to therapy.

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Footnote

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