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Chapter

Dynamic Interaction between Immune Escape Mechanism and HLA-Ib Regulation

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Abstract

HLA molecules scan the intracellular proteome and present self- or non-self-peptides to immune effector cells. HLA-Ia (HLA-A, HLA-B and HLA-C) are the most polymorphic genes, resulting in various numbers of allelic variants expressed on the surface of almost all nucleated cells. In contrast to HLA-Ia molecules that activate the immune system during pathogenic invasion, the marginal polymorphic HLA-Ib molecules (HLA-E, HLA-F and HLA-G) are upregulated during pathogenic episodes and mediate immune tolerance. A fine tuning between downregulation of HLA-Ia and upregulation of HLA-Ib can be observed through immunological episodes that require to remain unrecognized by immune effector cells. While HLA-Ia molecules collaborate by presenting a wide range of peptides, every HLA-Ib molecule is highly specialized in its protective immune function and seems to be restricted in the presentation of peptides. Additionally, Ia molecules are expressed ubiquitously while the expression of HLA-Ib molecules is strictly restricted to certain tissues and occurs instantly on demand of the cells/tissue that attempt to be hidden from the immune system. The more knowledge becomes available for the function of HLA-Ib molecules; the question emerges if the molecular typing of HLA-Ib molecules would be reasonable to take a decision post treatment for personalized cellular therapies.

Keywords: HLA-Ia, HLA-Ib, immune modulation, immune escape

1. Introduction

Human leukocyte antigens (HLA) are responsible for the regulation of the immune system. HLA molecules scan the proteome and present self- or non-self-peptides to immune effector cells [1]. T cells are highly specific and genetically restricted to recognizing HLA molecules. The capability of immune effector cells to recognize HLA-bound peptides is called MHC restriction and was first discovered by Zinkernagel and Doherty [2]. During pathogenic episodes, e.g. viral infection, the interaction between peptide and HLA-Ia (HLA-A, HLA-B and HLA-C) molecules with T cell receptors activates the immune system and enables the surveillance of the health statues of the cell. HLA molecules present an abundance of peptide antigens to T cells for a continuous immune surveillance through different

infectious/pathogenic stages. Peptides are bound by certain amino acids (AAs) located in the α1 and/or α3 domain of the HLA heavy chain (hc), those AAs form a cleft, the peptide-binding region (PBR). Alterations in the PBR are crucial to achieve maximum diversity of the selectable and presentable antigen repertoire [3, 4]. Consequently, HLA-Ia molecules are the most polymorphic genes in the human genome, resulting in various numbers of allelic variants expressed on the surface of almost all nucleated cells [1].

Furthermore, the human genome contains HLA-Ib genes like HLA-E, HLA-F and HLA-G. Contrary to HLA-Ia molecules, they exhibit only a few polymorphisms [5]. HLA-Ib molecules differ not only in their limited polymorphisms from HLA-Ia molecules but also in their restricted peptide repertoire and their distinct tissue distribution [6–8]. Typically, the function of classical HLA-Ia molecules is the presentation of peptide antigens to immune effector cells, yet HLA-Ib molecules exhibit a diverse range of functions in innate and adaptive immunity. In recent years, HLA-Ib molecules play a fundamental role in the understanding of virus-induced immunopathology, pathogen recognition, tumor immunosurveillance and regulation of autoimmunity [9–11]. In contrast to HLA-Ia molecules that activate the immune system during pathogenic invasion, the marginal polymorphic HLA-Ib molecules are upregulated during pathogenic episodes and mediate immune tolerance. A fine tuning between the downregulation of HLA-Ia molecules and the individual upregulation of highly specialized HLA-Ib molecules can be observed through immunological episodes that require to remain unrecognized by immune effector cells [12–14].

HLA-E features a specialized role between HLA and the immune system through engagement between innate and adaptive immunity; its interaction with an NK cell receptor or the T cell receptor could be distinguished [6, 15, 16]. During viral infections, viral immune evasion proteins disable through distinct actions: (i) the loading of peptides on HLA molecules, (ii) the trafficking of peptide-loaded HLA molecules to the cell surface or (iii) the retention of immature or mature peptide-HLA complexes in the endoplasmic reticulum. These entire virus-mediated actions result in infected cells that do not present peptide-HLA complexes on their surface and would be susceptible to NK cell-mediated lysis. HLA-E is upregulated in infected cells during infection and protects infected HLA-Ia empty cells from being recognized by NK cells [17, 18]. HLA-G is only expressed in restricted tissue, exclusively in immune-privileged sites. The protective potential of HLA-G is expressed through the capability of HLA-G to alter the phenotype of cytotoxic T cells towards non-reactive Tregs. HLA-G is the ligand for different NK cell receptors dependent on the tissue where it is expressed. Additionally, HLA-G can be found as membrane bound and soluble isoforms. In comparison to the other Ib molecules, HLA-G exhibits a unique molecular structure and diverse modulatory function in immune response [19, 20]. The presence of HLA-G is associated with immune tolerance. It is expressed in immune-privileged tissues, e.g. cornea and placenta [21]. It confers protection to the fetus from destruction by the maternal immune system during pregnancy and displays an immune checkpoint molecule in tumor immune evasion strategies [22, 23]. Among the Ib molecules, HLA-F is still an enigma. It is known that HLA-F molecule is a ligand for KIR3DS1 receptor on NK cells [24]. One remarkable immune function is its upregulation on the surface of HIV-infected CD4$^+$ T cells [25] where it enables NK cells to recognize the infection and destroy the infected host cells.

The aim of this chapter is to focus on the role of HLA-Ib during pathogenic episodes and their position in immunomodulatory mechanisms.

2. HLA-E

The *HLA-E* gene is located on the short arm of chromosome 6 and is composed of eight exons. In contrast to the other HLA-Ib molecules, HLA-E shows a broad tissue distribution. HLA-E is expressed in all nucleated cells [26]. Among the HLA molecules HLA-E is the least polymorphic; and proteins only 27 allelic variants enoding for 8 proteins are known [27]. However, of the described coding HLA-E variants, only two functional variants HLA-E*01:01 and HLA-E*01:03 are predominately distributed in the population. The frequency of both allelic variants in the population is approximately equal [28]. HLA-E*01:01 and HLA-E*01:03 differ only in one amino acid (AA) substitution at position 107 located in the α2 domain. An arginine for HLA-E*01:01 is substituted by a glycine for HLA-E*01:03. Interestingly, this polymorphism in contrast to the classical HLA molecules is not located in the PBR. A substitution at this position is unlikely influencing peptide presentation. We recently, however, demonstrated that this single polymorphism affects its immune function considerably [14]. Peptide studies utilizing soluble HLA technology revealed a shift in the peptide-binding repertoire between these two alleles [13, 29]. Despite the availability of the same proteomic content, HLA-E*01:03 selected and presented a smaller set of peptides than E*01:01; moreover, the C-terminal peptide-binding motif was altered towards a preference for lysine [13]. In comparison to HLA-E*01:01, the allelic variant HLA-E*01:03 shows higher thermal stability and higher level of surface expression [5]. Furthermore, clinical studies that analyzed the role of HLA-E allelic subtypes in hematopoietic stem cell transplantation (HSCT) showed the clinical relevance of HLA-E matching and recommended prospective HLA-E screening pre-HSCT. The analysis of the overall survival, non-relapse mortality and disease-free survival revealed that HLA-E incompatibility however significantly improves these factors [30].

Like classical HLA-Ia molecules, HLA-E forms a trimeric complex consisting of the heavy chain, β2-microglobuline (β2m) and a peptide (pHLA) that is presented on the cell surface [31]. The main protein source of HLA-E-restricted peptides is the leader sequence derived from other HLA molecules. Thus, the expression level of other HLA molecules determines the surface expression of HLA-E. Peptide binding studies with random peptide libraries show that HLA-E is also capable of binding peptides from stress signals or pathogens [32]. The peptide-binding features of HLA-E indicate its special immunomodulatory qualities. Due to the low polymorphism of HLA-E, the fine tuning of HLA-E immune response is exclusively dependent on the bound antigenic peptide.

2.1 Immunomodulatory qualities of HLA-E

HLA-E exhibits a dual role in the immune system. On the one hand, HLA-E regulates the innate immunity through interaction with the NK cell receptor, and on the other hand, it can activate the adaptive immunity through interaction with the T cell receptor. The interaction of HLA-E with the respective immune effector cell depends considerably on the peptide presented on HLA-E (**Figure 1**) [6, 15, 16].

HLA-E is a mediator of NK cell inhibition and activation. HLA-E constitutes a ligand for both the inhibitory CD94/NKG2A and the stimulatory CD94/NKG2C NK cell receptor on NK cells. The reason for the differential binding to these functionally diverse receptors could be explained by the presentation of a diverse set of peptides. We could demonstrate an unknown HLA-E peptide-driven NK cell reactivity [14]. Through surface plasmon resonance (SPR) binding studies where the same peptide sequence derived from leader peptides of HLA-Ia molecules have

been used to assemble pHLA-E complexes, it could be demonstrated that the HLA-E-binding affinity differs between these two NKG2 subunits. HLA-E binds CD94/NKG2A with a higher affinity than CD94/NKG2C [33]. The main peptide source of HLA-E is the leader sequence of HLA-Ia molecules; therefore, the role of HLA-E in innate immunity is to present HLA-E bound to these peptides to NK cells to inhibit NK-mediated lysis through pHLA-E/CD94/NKG2A engagement [7].

In adaptive immunity, certain HLA-E peptide complexes can be recognized by CD8^{+} T cells [34]. In addition to the presentation of self-peptides, HLA-E can also present a various number of pathogen-derived peptides, and these pHLA-E complexes elicit specific T cell responses. Although peptides presented by HLA-E are very restricted, it could be demonstrated that peptides derived from Epstein-Barr virus (EBV) [35], Cytomegalovirus (CMV) [36] or hepatitis C virus (HCV) [37] can be presented by HLA-E and recognized by virus-specific T cells. Furthermore, it has been reported that HLA-E also binds bacteria-derived peptides from Mycobacterium tuberculosis (Mtb) [38] and Salmonella enterica serovar Typhi [39]. These bacteria-derived peptides do not prevent NK-mediated lysis; instead, they elicit CD8^{+} T cell response [40, 41]. Like HLA-Ia-activated T cells, they combat intracellular bacteria through lysis of infected target cells [41]. However, interestingly, the HLA-E-specific CD8^{+} T cells uniquely produce Th2 (Il-4, Il-5, IL-13) cytokines instead of Th1 cytokines. Through B cell activation assays, it could be demonstrated that the T cells activate B cells that are able to induce cytokine production (**Figure 1**; [41]). These findings emphasize the important role of HLA-E in the adaptive immune response. It becomes obvious that also viruses and tumor cells use HLA-E for their own advantage to escape T cell recognition.

2.2 HLA-E in tumors

In order to evade CTL recognition due to tumor-peptide presentation, the downregulation of HLA molecules is a widespread mechanism of tumor cells.
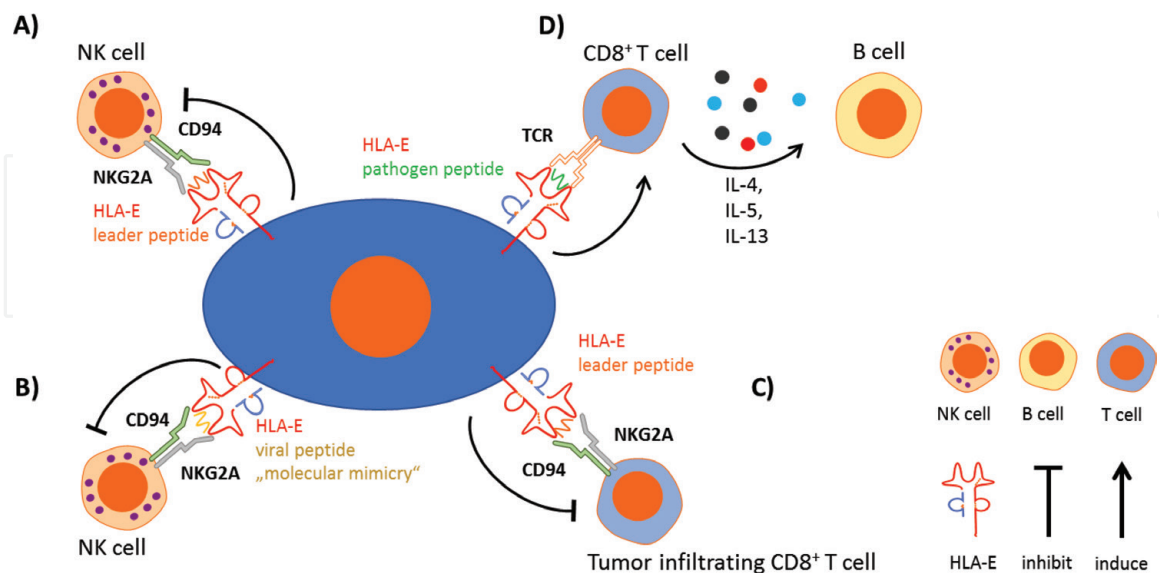

Figure 1.
The multiple roles of HLA-E in immune system. (A) HLA-E presents leader peptides to the unique inhibitory heterodimeric CD94/NKG2A receptor present on NK cells. (B) As part of immune evasion, HCMV glycoprotein UL40 provides a peptide mimicking the leader sequence of HLA-Ia molecules, thus inhibiting the NK cell by providing a ligand for CD94/NKG2A receptor. (C) As part of tumor immune evasion, the expression of the inhibitory NK cell receptor CD94/NKG2A is upregulated on tumor infiltrating CD8^{+} T cell leading to an inhibitory effect on these cells. (D) HLA-E can bind pathogenic peptides and elicit a CD8^{+} T cell response. Through HLA-E binding, CD8^{+} T cells release Th2 cytokines (IL-4, IL-5 and IL-13) and activate B cells.

Interestingly, the expression of HLA-E and HLA-G is upregulated in various types of cancers [42], indicating the use of these molecules as a special mechanism for immune evasion [9]. The cellular surface expression of pHLA complexes is required to avoid NK cell-mediated lysis. During tumor escape episodes, pHLA-Ia expression is downregulated; however, the expression of the HLA hc remains mostly unaffected, enabling the assembly of HLA-Ia hc and HLA-E. Since those pHLA-E complexes inhibit the NK cell reaction through its interaction with CD94/NKG2A, HLA-E expression provides an advantage for survival of the tumor cell. Furthermore, novel insights from cancer research suggest that the expression of HLA-E contributes to disease progression and is associated with a poor clinical prognosis [43, 44]. In patient with cervical and ovarian cancer, tumor infiltrating CD8$^+$ CTLs showed a higher expression of the CD94/NKG2A inhibitory receptor, and only a very low number of NK cells were found in tumor tissues (**Figure 1**) [45–47]. Regarding to the high expression of HLA-E in these tissues, those findings lead to the assumption that HLA-E plays a protective role and benefits the tumor through inhibition of CD94$^+$/NKG2A$^+$ CTLs and recognition of NK cells. Additionally, immunohistochemically stained breast cancer tissues showed that HLA-E expression is a prognostic marker for tumor progression [48]. Also in patients with non-small cell lung cancer (NSCLC), immunohistochemical staining demonstrated the association of HLA-E expression with a worse outcome for survival especially in the cells that are HLA-Ia negative and HLA-E positive [49]. In studies on colorectal cancer, an overexpression of HLA-E correlated with the malignancy stage. Remarkably, the release of soluble HLA-E could be detected in these cells [50]. In recent years, the focus of soluble HLA-Ib molecules as potential biomarker increased. In particular, soluble HLA-E and HLA-G are in the focus, because their expression is highly associated with the disease progression in tumor cells. In case of HLA-E, soluble molecules were significantly increased in neuroblastoma [51], melanoma [52] and chronic lymphocytic leukemia [53]. However, the overexpression of HLA-E is associated with the inhibition of tumor infiltrating NK cells and CD94$^+$/NKG2A$^+$ CTLs and contributes as one factor for the immune evasion strategies of tumor cells.

2.3 HLA-E in viral infections

Downregulation of HLA-Ia molecules is not only an escape mechanism of tumor cells but also a mechanism of pathogens like viruses. Human cytomegalovirus (HCMV) is one of the most intensive investigated pathogen related to HLA immune evasion. HCMV encodes several proteins that interfere with the antigen presentation and HLA expression. The glycoproteins US2 and US11 redirect the HLA hc from endoplasmic reticulum (ER) to the cytosol and induce the proteasomal degradation of the molecule. US3 inhibits the tapasin-dependent peptide loading leading to the retention of the HLA hc in the ER. Glycoprotein US6 inhibits the function of TAP. The loss of ligands for the inhibitory receptor increased the risk of NK cell recognition of the infected cells and for that reason HCMV developed mechanisms that inhibit NK cell responses [54]. One of these mechanisms involves the expression of HLA-E. HCMV encodes for the glycoprotein UL40. This protein has the same nonapeptide sequence (VMAPRTLIL) as the leader sequence of different HLA-C alleles that can bind to HLA-E [17] and thus inhibit NK cell-mediated lysis. TAP is a protein from the peptide loading complex (PLC) that is fundamental for the loading of peptides into the HLA PBR. TAP deficiency results in the presentation of empty HLA molecules on the cell surface. Interestingly, HLA-E expression analysis in TAP-deficient cells showed that HLA-E binds the HCMV

peptide TAP-independently [17, 55]. Thus, UL40 can inhibit the NK cell recognition via HLA-E, even when the other HCMV glycoproteins prevent the presentation of pHLA-Ia complexes. HCMV utilizes the protective effect of HLA-E towards NK cell lysis through molecular mimicry (**Figure 1**). Furthermore, it could be demonstrated that UL40 polymorphism in HCMV impacts the recognition of HLA-E by NK cells. SPR analysis and cytotoxicity assays with 14 UL40 polymorphisms were performed to confirm that the binding affinity of HLA-E and CD94/NKG2A or CD94/NKG2C and the mediated reaction depend on the presented peptide [56]. These studies show that the alteration of the peptide sequence influences the recognition by CD94/NKG2A or CD94/NKG2C significantly and consequently impacts NK cell-mediated cytotoxicity. The HCV protein YLLPRRGPRL also binds into the PBR of HLA-E. Thus, peptides of other viruses also stabilized the HLA-E expression on infected cells and reduced the NK cell-mediated toxicity [57]. These findings show that the peptide presentation on HLA-E impairs the interaction with NK cell receptors considerably.

3. HLA-G

Among the very oligomorphic family of HLA-Ib molecules, HLA-G is the most polymorphic representative with 58 different alleles compared to 27 and 30 alleles of HLA-E and HLA-F, respectively [27]. The *HLA-G* gene is located on chromosome 6p21.3 close to HLA-A locus and is composed of eight exons with an internal stop codon after Exon 6. Through intron variability, those 58 encode eventually for 17 proteins. HLA-G*01:01 resembles the most common variant worldwide; in Europe, it is followed by HLA-G*01:04 and HLA-G*01:03 [58]. Mediated by alternative splicing, four membrane-bound (HLA-G1-G4) and three (HLA-G5-G7) soluble isoforms of HLA-G exist [59, 60]. The full-length membrane bound molecule is resembled by the HLA-G1 isoform, and its soluble equivalents are either generated by a stop codon after Exon 4 (HLA-G5) or by cleaving the membrane bound HLA-G1 from cell surface (soluble HLA-G1). The cleaving process of HLA-G1 is mediated by IL-10-dependent matrix metalloproteinase-2 (MMP2) [61]. The cleaving process was verified through coincubation of MMP2 with HLA-G-expressing cells and IL-10. A sharp increase in soluble HLA-G1 could be detected, whereas HLA-G5 mRNA levels remain constant [61]. MMP2 is predominantly expressed in the placenta and the lung [6]. The other membrane-bound isoforms are generated through elimination of one or more α-domains and for the soluble equivalents a stop codon after Exon 4 or Exon 2 (HLA-G7) (**Figure 2**). So far, receptors are reported only for the isoforms HLA-G2 and HLA-G6 [62]. The most expressed isoforms appear to be HLA-G1 and HLA-G5 [63–65]. For the membrane-bound isoforms (HLA-G2–HLA-G4), it could be shown that they could be detected on cell surface of transfected cells [66], whereas for the soluble isoforms only HLA-G5 and HLA-G6 (only after transplantation [67]) could be detected in supernatant of transfected cells as well as in the blood [65]. Nevertheless, it could be demonstrated that the membrane-bound isoforms convey also a protective status as well as HLA-G1 despite their different α-domain composition [33]. In addition, a recent study could demonstrate that HLA-G2 and HLA-G6 could bind to ILT4 through their α3 domain [62] but not to ILT2 due to the fact that the ILT2 binding to HLA-G is β2m-dependent [68]. Those findings suggest that the biological function and implementation of HLA-G are crucially depending on its structure. Nevertheless, despite those findings, it is not known yet whether the isoforms of HLA-G1 and HLA-G5 are relevant *in vivo* under physiological conditions [62].

In terms of peptide presentation, HLA-G differs from other HLA-Ib molecules like HLA-E that presents a very restricted peptide repertoire derived from the signal sequence of other HLA molecules irrespectively of the HLA-E-expressing tissue [7]. In contrast, HLA-G is considered to be a classical peptide presenter like HLA-Ia molecules; however, its peptide repertoire is restricted to the tissue distribution and cell type [7, 69, 70]. Peptide identification of HLA subtypes is usually performed by affinity purification of the desired HLA molecule from a selected tissue/cell type followed by peptide isolation and mass spectrometric sequencing. A reason for the detection of a restricted HLA-G peptide repertoire might therefore be the selection of the HLA-G-expressing tissue/cell type or the unintended selection of HLA-G*01:01 exclusively. We recently demonstrated that the HLA-G-restricted peptide repertoire is distinctively determined by the HLA-G allelic subtype. Despite the fact that the allelic variants HLA-G*01:04, HLA-G*01:03 and HLA-G*01:01 differ from each other by a single AA in an outer loop position outside the PBR, the selected peptide repertoire and the peptide binding motif are fundamentally different [12].

While the HLA-Ib molecule HLA-E and HLA-Ia molecules are ubiquitously expressed on every nucleated cell, HLA-G expression is restricted under physiological conditions to immune privileged sites. HLA-G is expressed in placenta [71], thymus [72], cornea [21], nail matrix [73], pancreas [74] and erythroid and endothelial precursors [75]. Also, HLA-G is ectopically expressed in transplanted organs, tumors, monocytes, viral infections and autoimmune diseases [76–78].

3.1 HLA-G in pregnancy

Under healthy conditions, the main expressing site of HLA-G is the placenta [79]. In the placenta, extravillous cytotrophoblast (EVT) cells are the only cells expressing membrane-bound HLA-G (G1) and secreting soluble HLA-G (G5) [79, 80]. The fetus can be considered a semi-allograft; hence the immune system of the mother has to be regulated in tolerogenic direction to avoid rejection. Here, HLA-G
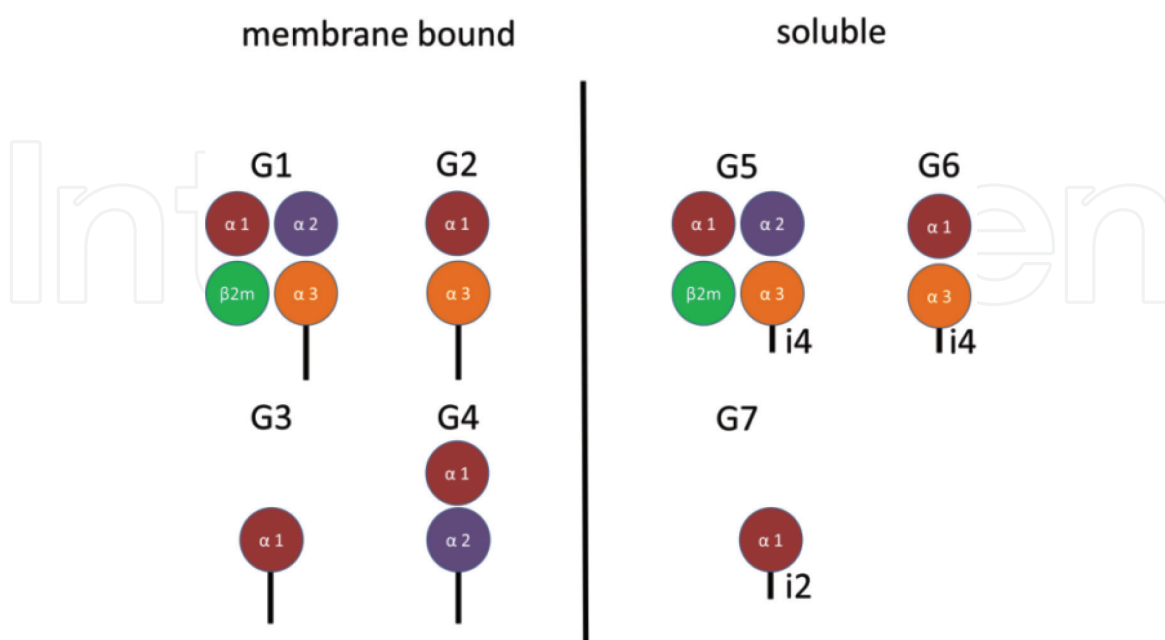

Figure 2.
Structural isoforms of HLA-G. Generation of HLA-G isoforms is done by alternative splicing of the HLA-G mRNA. HLA-G1 resembles the full-length membrane-bound HLA-G molecule. Membrane-bound isoforms are generated by abundance of certain α domains. Soluble isoforms are achieved by a stop codon after Exon 4 and Exon 2, respectively. Modified after Foroni et al. [7].

is the key element for maternal-fetal tolerance induction [80–82]. Interaction with HLA-G leads commonly to an inhibition of the interacting immune effector cells [83–86]. Those interactions are mediated through inhibiting receptors like KIR2DL4 and ILT2 on NK cells [87], ILT2 on T cells, ILT4 on macrophages and ILT4/CD160 on dendritic cells [88]. The binding site for those receptors is suggested to be the alpha-3 domain of HLA-G (G1 or G5) [89]. KIR2DL4 and ILT2 interaction leads to inhibition of NK-mediated lysis [87]. Additionally, it has been reported that at the maternal interface decidual NK cells (a unique immunosuppressive and proangiogenic subset of NK cells [90]; dNK) are up-taking and internalizing HLA-G from the EVT cellular surface via trogocytosis mediated by a yet not clearly identified receptor [91]. Internalization of HLA-G is necessary for maintaining a low cytotoxicity and immunosuppressive status of dNK cells. It could be shown that the disappearance of internal HLA-G leads to cytokine production and an overall higher cytotoxicity of dNK cells, the functional background of this mechanism is still unclear and further research has to be performed [91, 92]. Although, it is assumed that KIR2DL4, through its highly intracellular occurrence, is involved in an intracellular cascade leading to this immunosuppressive status [91]. Moreover, alloproliferative response of CD4$^+$ T cells is inhibited by ILT2 interaction with HLA-G [93], and the population is driven to a suppressive and passive phenotype [94]. Furthermore, it is known that HLA-G5 induces in ILT4$^+$ dendritic cells (DCs) the production of the immunosuppressive cytokine IL-10 and an arrest of maturation [95, 96] as well as the induction of Tregs (CD4$^+$ CD25^{high}FOXP3$^+$) [97] and Tr1 cells by IL-10-producing dendritic cells [96, 98]. Moreover, through interaction with the ILT2 receptor on B cells, HLA-G inhibits proliferation, differentiation and antibody secretion [85]. In addition, HLA-G5 induces apoptosis of CD8$^+$ T cells and
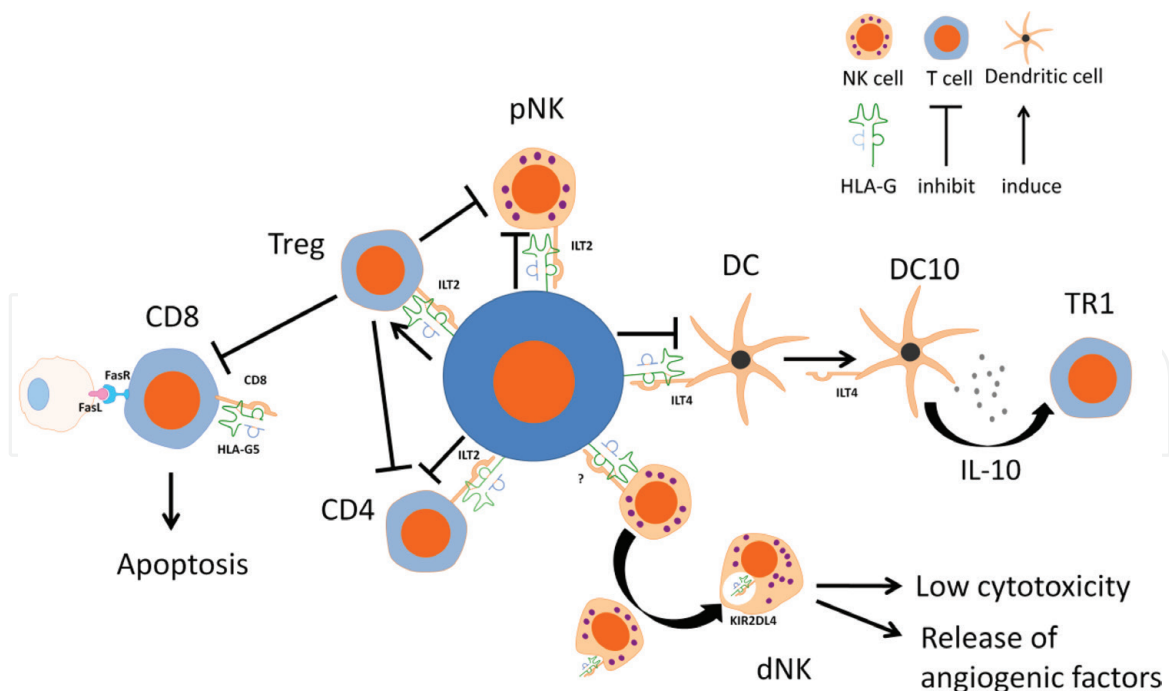

Figure 3.
HLA-G binding inhibiting immune effector cell activation. HLA-G regulates the immune system to an immunosuppressive and tolerogenic status by inducing the development of CD4$^+$ and CD8$^+$ to regulatory T cells and IL-10 production by DCs. Furthermore, HLA-G is able to inhibit CD4$^+$ and pNK cells directly through binding to ILT2. In addition, soluble HLA-G induces apoptosis in CD8$^+$ T cells by activation of the FasL/FasR pathway. Decidual NK cells are up-taking HLA-G from the extravillous cytotrophoblast cell surface by a yet unidentified receptor. Intracellular KIR2DL4 is assumed to be responsible for an intracellular cascade that leads to immunosuppressive status of decidual NK cells. Treg: T regulatory cell; pNK: peripheral natural killer cell; DC: dendritic cell; DC10: IL-10-producing dendritic cell; TR1: type 1 T regulatory cell; dNK: decidual natural killer cell; CD4: CD4$^+$ T cells; CD8: CD8$^+$ T cells. Modified after Rizzo et al. [78].

endothelial cells through interactions with CD8 receptor and activation of FasR/FasL pathway in CD8⁺ T cells and CD160 on endothelial cells (**Figure 3**) [69]. To conclude, HLA-G has a strong immunosuppressive impact and thus plays a critical role in maintaining an immunotolerant status in pregnancies.

3.2 Interactions of HLA-G

In recent years, the impact of the presented peptide on the interaction between HLA-G and its cognate receptors is controversially discussed. It has been reported that the interaction between HLA-G and its receptors is mediated exclusively by the HLA-G α3 domain and not by the α1 and/or α2 domain. Therefore, it was concluded that the presented peptide has no influence on the receptor-HLA-G interaction [68, 99], whereas we could demonstrate that the HLA-G alleles, HLA-G*01:01, G*01:03 and G*01:04, differing from each other by single AA polymorphism within the α2 region and a variability in peptide-binding features, convey a different degree in protection from NK cell-mediated lysis [12]. These results imply that the available membrane-bound HLA-G for a given NK cell receptor is influenced by the peptide-mediated alteration of the molecule [12]. Supporting our functional results, a recent study describes the x-ray structure of the D3D4 domain of the ILT2 receptor; this structural insight indicates that ILT2 would be theoretically able to interact with the α1 and α2 domain of an HLA-G molecule [100]. Taken all those findings into account, it seems apparent that the α3 domain is the main receptor-binding site of HLA-G; however, it should be considered that the α1 and α2 domain as well as the bound peptide directly or indirectly have a great impact on the HLA-G-NK-receptor interactions; therefore, it becomes obvious that the HLA-G allele has more functional impact than previously thought.

3.3 HLA-G in cancer

As a consequence of its immunosuppressive abilities, the ectopic expression of HLA-G1 is a part of immune evasion strategies of many tumors to escape immunosurveillance by T and NK lymphocytes [101]. In esophageal squamous cell carcinoma, heighten IL-10 and HLA-G levels could be detected and correlated with a poor outcome [102]. HLA-G was found to be an independent factor for overall survival in colorectal cancer [103]. Heighten soluble HLA-G levels were found in plasma levels of patients with chronic lymphatic leukemia, T-non-Hodgkin lymphoma (NHL), B-NHL [104], multiple myeloma [105] and breast cancer, whereas in the later one heighten soluble HLA-G levels are associated with a better outcome of neoadjuvant chemotherapy [19] In pancreatic cancer, HLA-G expression is common and positively correlated with metastasis and a worse overall survival [106]. In essence, HLA-G ectopic expression in tumors is common and correlated with tumor progression and patients' survival as recent studies could demonstrate [101].

3.4 HLA-G in transplantations

In past years, HLA-G has received more and more attention as a potential biomarker in transplantations due to its immunomodulatory abilities [107]. It could be shown that myocardial HLA-G expression is correlated with low risk of acute cellular rejection in heart transplant recipients [108]. In lung transplantation, acute rejections were observed in patients without HLA-G expression in the donor lung, whereas in stable patients, HLA-G expression was frequently detected. Also, in long-term follow-ups, HLA-G expression correlated significantly with a lower

occurrence of steroid-resistant acute rejection and bronchiolitis obliterans syndrome [109]. Furthermore, HLA-G expression in biliary epithelial cells is positively correlated with an overall better acceptance in liver-kidney transplantations [110]. Moreover, it could be shown that HLA-G expression in endomyocardial biopsies is negatively correlated with C4d staining, a marker for antibody-mediated rejection, implying that HLA-G expression protects the graft from antibody-mediated rejection [111]. Contrastingly, heighten levels of membrane-bound isoforms (G1 and G3) combined with lower amount of G5 corresponded with acute rejection in end-stage renal disease [107, 112]. Although HLA-G seems to play a critical role in terms of graft acceptance and long-term survival, it is however not comprehensively used as a biomarker, yet.

Given the fact that HLA-G expression is often correlated with a better graft acceptance, LeMaoult et al. investigated HLA-G as a potential therapeutic target to improve graft acceptance. By utilizing synthetic HLA-G molecules, they could demonstrate that synthetic HLA-G molecules are capable of improving skin graft survival and tolerance induction in mice [113]. These findings underline the pivotal role of HLA-G for transplantations in the future.

3.5 HLA-G in autoimmune diseases and inflammation

In the context of autoimmune and inflammatory diseases, several studies have investigated the role of HLA-G that controls the disease progression [101]. In return, HLA-G polymorphisms and expression levels have been linked to several autoimmune/inflammatory diseases such as ulcerative colitis (UC) [78], Crohn's disease [101], celiac disease [114], psoriasis [115], pemphigus vulgaris [116], rheumatoid arthritis [117] and multiple sclerosis [78, 101]. Ulcerative colitis and Crohn's disease are both inflammatory gastrointestinal diseases in which sHLA-G secretion by peripheral blood mononuclear cells (PBMCs) could serve as a marker to distinguish between them and further could be utilized for controlling treatment progression [118]. PBMCs from healthy patient and patients with UC do not secrete sHLA-G under physiological conditions, whereas PBMCs from patients with Crohn's disease secrete sHLA-G. This pathological pattern reverses under immunosuppressive treatment PBMCs from UC patients start secreting sHLA-G whereas secretion is reduced in Crohn's disease patients [78, 119]. In chronic skin inflammation such as pemphigus vulgaris, heighten HLA-G expression could be observed [120] together with a higher frequency of HLA-G 14 bp DEL allele [116]. These findings advocate the role of HLA-G expression as a pivotal determinant in the evolution of pemphigus vulgaris [101]. In multiple sclerosis (MS), several studies indicate that HLA-G downregulates the autoinflammatory reaction in the microenvironment of the brain, and it could be shown that HLA-G suppresses cytokine production and CD4^{+} T cell proliferation of MS patients *in vitro*. In addition, it has been found that sHLA-G serum levels are heighten *post-partum* in patients without clinical attacks [121], and a suppressive subset of CD4^{+}CD8^{+} HLA-G secreting and expressing regulatory T cells could be observed in MS patients [122–124]. Contrastingly, a recent correlation analysis could find no significant evidence for an impact of sHLA-G levels on any parameter of multiple sclerosis [125].

3.6 HLA-G in infection diseases

Infection diseases can be divided into three groups (bacterial, parasitic and viral infection) in which HLA-G is associated with different outcomes and different diseases progression [126].

3.7 HLA-G in bacterial infections

As consequence of a far advanced bacterial infection, a septic shock leads to a systemic inflammation and is defined by a high fatality rate [127]. In this scenario, a highly increased HLA-G5 expression is correlated with a higher chance for survival by supporting the anti-inflammatory feedback loop [127].

3.8 HLA-G in parasitic infections

Toxoplasma gondii infection leads often to adverse pregnancy outcomes such as miscarriage and still birth [128]. It has been shown that *Toxoplasma gondii infection* leads to a higher secretion of sHLA-G by extravillous cytotrophoblast. Notably, those heightened sHLA-G expression leads to apoptosis of dNK cells when they were co-cultured with *Toxoplasma gondii*-infected extravillous cytotrophoblast [101]. Contrastingly, in healthy pregnancies, sHLA-G is a necessary factor for dNK cells to maintain their immunosuppressive and cytokine production status in order to sustain a correct placentation [91]. In conclusion, this differing mechanism could be one key determinant for a poor outcome of pregnancies while *Toxoplasma gondii infection.*

A recent study has observed a correlation of high maternal HLA-G serum levels with low birth weight and a higher risk of *Plasmodium falciparum* infection in infancy. Suggesting sHLA-G levels could be utilized as biomarker for sensitivity of infants for malaria infection [129].

3.9 HLA-G in viral infections

Commonly, sHLA-G levels are uprising during viral infection such as infections with HIV, hCMV, HCV and HBV [126]. This is due to heightened levels of cytokine production especially due to interferon secretion that stimulates HLA-G shedding from cell surface mediated by metalloproteases during viral infection [126]. During viral infection, IL-10 serum levels rise to dampening the proinflammatory TH1 immune response [130]. As a consequence, the IL-10-dependent MMP2 is activated and cleaved HLA-G1 from cell surface; this leads to a rise in soluble HLA-G1 levels [61].

In hCMV, increased membrane bound and soluble HLA-G levels could be detected during infection. It could also be shown that macrophages derived from latency infected monocytes upregulating HLA-G expression when the hCMV infection reactivates in order to avoid recognition by the immune system [131]. Further, sHLA-G serum level correlates with IL-10 and IFN-γ concentration due to IL-10- and interferon-dependent MMP [126].

In HBV and HCV infections, HLA-G serum levels also correlate with IL-10 and IFN-γ concentration. Furthermore, sHLA-G levels were observed to be higher in the chronic HBV situation than in the acute infection. In addition, higher HLA-G expression has been correlated with fibrotic areas within the liver in patients with a chronic HCV infection [132–135].

In context of HLA-G and viral infections, HIV infection is currently the most widely researched [126]. In HIV infections, HLA-G is expressed on nearly all monocytes and on 34% of T-cells [136]. HLA-G serum levels increased during first phase of infection and lowered to normal again when infection progresses to a chronic stage [136, 137]. If HLA-G serum levels remain high during infection progression, it indicates a fast progressor [136]. On basis of this change in HLA-G serum levels, it is proposed that HLA-G can be utilized as a biomarker for disease progression [126]. In addition, under antiretroviral therapy, serum

HLA-G levels decreased significantly and are correlated with virus clearance and an increase of CD4^{+} T lymphocytes [126]. This is probably due to a decrease of an immune response and decreasing IL-10 and interferon levels. Furthermore, it is suggested that HLA-G-expressing monocytes serve as reservoir in HIV-infected patients, since they are protected from an immune response through their HLA-G surface expression [136]. In coherency to those findings, it is reported that HLA-G null allele, HLA-G*01:05 N allele, correlates with a lower risk of a HIV infection, whereas HLA-G*01:01:08 correlates with a higher risk of getting a HIV infection [138, 139]. Null alleles are characterized by a mutation that leads to a non-functional molecule [140]. Consequently, in patients with the allelic variant HLA-G*01:05 N, monocytes could not be served as HIV reservoir in an infection, because they are no longer protected from the immune system by a functional HLA-G expression [126].

4. HLA-F

The *HLA-F* gene is located on chromosome 6p21.3 telomeric to the HLA-A locus and is composed of seven exons. Exon 6 contains an internal stop codon leading to the exclusion of Exon 7 from the mature mRNA transcript. The association of HLA-F hc with β2m forms a 40–41 kDa protein with a truncated cytoplasmic tail [141, 142]. It has been proposed that this distinct cytoplasmic tail enables HLA-F to conquer the cell surface independent of the classical peptide-loading pathway in the ER [143].

Like HLA-E and HLA-G, HLA-F has limited allelic polymorphism when compared to the allelic variances of HLA-Ia molecules. Until now, 30 alleles encoding for five proteins (HLA-F*01:01, F*01:02, F*01:03, F*01:04, F*01:05) have been described [27].

AA residues within the PBR of HLA class I molecules determine their biophysical properties and thereby the feature of bound peptides. Ten of these amino acid residues are highly conserved in all HLA class I PBRs, except HLA-F that shows alterations in five of them, implying an unidentified immune function of HLA-F. Four of these alterations are located within the α1 domain where a methionine is substituted by a leucine at position 5, a tyrosine by a phenylalanine at position 22, a glycine by a glutamine at position 26 and a tyrosine by an arginine at position 84. The fifth alteration is located within the α1 domain with a substitution of phenylalanine for leucine at position 146 [141]. On this account, the electrostatic characteristics of the PBR are modified and HLA-F exclusive. It could be demonstrated that HLA-F-presented peptides are not restricted by length, because the A pocket of the PBR is blocked off due to the aforementioned substitutions. This allows binding of peptides of 7 to >30 AA in length that is more consistent with HLA class II molecules [144].

HLA-F expression is highly cell- and tissue-specific. Expression of HLA-F has been detected intracellularly in leukocytes, including monocytes, B cells, T cells and NK cells, as well as on the cell surface of activated lymphocytes excluding Treg cells [145]. As well as HLA-G and HLA-E, HLA-F has been detected on extravillous trophoblasts invading maternal decidua [146], even though its function during pregnancy is still unclear. It has been found that HLA-F underexpression due to single nucleotide polymorphisms (SNP) within the *HLA-F* gene correlates with reduced fecundity [147], suggesting a role in maternal-fetal tolerance.

In contrast to other HLA class I molecules, HLA-F has been described to be expressed as an open conformer (OC) without association with β2m and peptides; however, the usual trimeric hc/β2 m/peptide complexes could be detected as well.

These distinct HLA-F conformers are recognized by various binding partners of the NK cell receptor (NKR) family. While HLA-F OCs are mainly recognized by the killer cell immunoglobulin-like NKRs (KIR) KIR3DL2, KIR3DS4 [148] and KIR3DS1 [25], peptide-bound HLA-F complexes are ligands for the NKRs of the leukocyte immunoglobulin-like receptor (LIR) family, ILT2 and ILT4 [8, 144].

4.1 HLA-F in viral infections

HLA-F is the most enigmatic HLA-Ib molecule and little is known about its function. It has been found that through interaction with the KIR3DS1 receptor, HLA-F has a beneficial effect on HIV outcome.

Stimulation of KIR3DS1[+] and KIR3DS1[−] NK cell lines (NKCLs) with recombinant HLA-F monomers elicits downstream immune responses as production of antiviral cytokines (IFNγ, TNFα and MIP1β) measured by intracellular staining and NK cell degranulation measured by surface expression of the lysosome-associated marker CD107a in KIR3DS1[+] NKCLS, but not in KIR3DS1[−] NKCLs. Moreover, *in vitro* studies showed that KIR3DS1[+] NKCLs reduce the quantitative frequency of HIV-infected cells elucidating that KIR3DS1-HLA-F ligation inhibits HIV-1 replication (**Figure 4**). Interestingly, *HLA-F* transcription is upregulated in HIV-1-infected activated CD4^{+} T cells. However, recognition of HLA-F by KIR3DS1 is weakened in 'early' infected cells that are characterized by low HIV p24 expression, CD4 positivity and HLA-Ia and tetherin expression and particularly diminished in 'late' infected cells with high p24 expression, low CD4 positivity and low HLA-Ia and tetherin expression, implying the involvement of HLA-F in an immune evasion strategy of HIV-1 [25].

4.2 HLA-F in tumors

Tumor cells evolved strategies to evade the immune system. One of the most frequently used mechanisms is the alteration of HLA expression, including the downregulation of HLA-Ia molecules to escape from recognition by cytotoxic T cells. HLA-Ib molecules exhibit protective features that protect the cells from NK cell lysis, and based on this, tumor cells upregulate these molecules for their own advantage.

In contrast to HLA-E and HLA-G, little is known about the function of HLA-F in tumor. Until now, HLA-F has been detected immunohistochemically in various cancers, i.e. non-small cell lung cancer (NSCLC) [149], esophageal squamous cell carcinoma [150], gastric adenocarcinoma [151] and breast cancer [152].

It has been reported that HLA-F detection is associated with a poor outcome in NSCLC and gastric adenocarcinoma. In patients with gastric adenocarcinoma, HLA-F detection leads to a more invasive carcinoma type and further lymph knot involvement and infiltration into blood vessels [151]. HLA-F has been found to
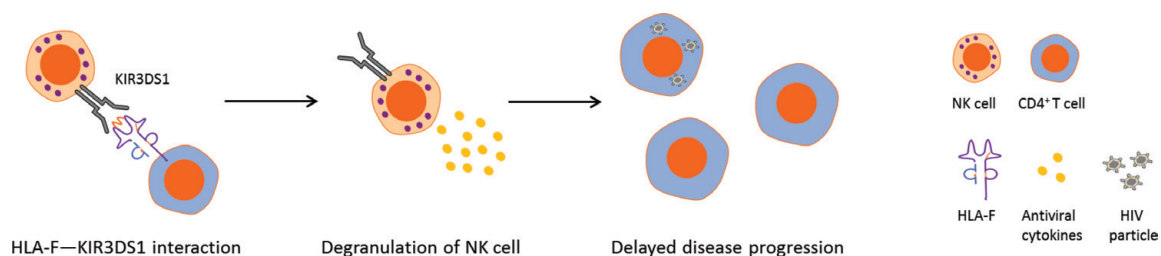

Figure 4.
Effect of HLA-F-KIR3DS1 interaction on AIDS disease progression.

interact with the inhibitory NKRs, ILT2 and ILT4 [8], indicating its expression on tumor cells as protection against anticancer responses by the immune system. Yet in NSCLC, positive HLA-F expression is not associated with disease progression and differentiation status of the tumor [149]. The occurrence of HLA-F in breast cancer correlates with the size of tumor, but there is no evidence for nodal involvement [152].

Taken together, these findings imply organ-specific effects of HLA-F-positive tumors. Further investigation is necessary to verify and decode the underlying mechanism of tumor evasion strategies by cancers.

The immunomodulatory potential of HLA-F subtypic variants is still unknown. For that reason, we developed a typing strategy for typing HLA-F in certain patients (Ho et al., manuscript in preparation). It seems that the function of HLA-F as well as that of the other HLA-Ib molecules is influenced by the AA composition of the heavy chain. A single AA mismatch seems to tip the immunological balance (Ho et al., manuscript in preparation).

Taken together, the previously assumed invariability of the HLA-Ib heavy chain has to be rethought, and HLA-Ib typing seems to support intelligent patient management protocols.

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