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Non-Classical MHC Class Ib Molecules and their Receptors-Role in Allogeneic Transplantation of Hematopoietic Stem Cells

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Abstract

HLA-E, HLA-G, and major histocompatibility complex class I-related chain A and B (MICA and MICB) proteins belong to human non-classical Major Histocompatibility Complex (MHC) class Ib molecules. These molecules are well known as powerful modulators of the immune response. The majority of the mentioned proteins interact exclusively with receptors from the CD94/NKG2 c-type lectin family. The peptide-presenting HLA-E molecule is recognized by the NKG2A or NKG2C receptor, triggering a signal cascade leading to inhibition or activation of immune cell activity, respectively. Another member of this family is the NKG2D receptor that interacts with the MICA/B proteins, transducing signals that result in activation of immune cells.

This article discusses the current knowledge in regard to the genetic properties and physiological functions of the molecules HLA-E and MICA/B as well as the CD94/NKG2 receptors in the context of the potential role of the signaling pathways they constitute in the outcome of hematopoietic stem cell transplantation. Furthermore, the possible role of HLA-G, also a member of the non-classical MHC class Ib family, is considered.

Keywords: HLA-E; HLA-G; MICA/B; CD94/NKG2; Hematopoietic stem cell transplantation

Introduction

The outcome of allogeneic Hematopoietic Stem Cell Transplantation (HSCT) has been documented to be affected by a number of risk factors, including polymorphisms of the genes located within the human Major Histocompatibility Complex (MHC) region, classical Human Leukocyte Antigen (HLA) class I and class II, but also other genes located in the region such as genes coding for Tumor Necrosis Factor (TNF)-α and TNF-β or Heat Shock Protein (HSP)-70 [1-7]. The role of non-classical molecules have been less elucidated although more recently a role of HLA-E polymorphism as a risk factor of microbial infections or Graft-Versus-Host Disease (GvHD) in the recipients of allogeneic stem cells has been reported.

This review focuses on the association between non-classical HLA-E and Major Histocompatibility Complex class I-related chain A and B (MICA and B) molecules and their receptors belonging to the CD94/NKG2 family in relation to the manifestation of post-transplant complications, acute and chronic GvHD, viral and microbial infections, and mortality. In addition, the possible role of HLA-G for the transplant outcome is described.

HLA-E-NKG2A interaction

Ubiquitously expressed Human Leukocyte Antigen-E belongs to the non-classical HLA class Ib family. HLA-E molecules are among the least polymorphic members of the HLA I family, with eight alleles encoding three different proteins described in human populations. However, only two functional alleles, differing at one amino acid position (non-synonymous mutation) in the α2 heavy chain domain, have been reported. In *HLA-E*01:01* it is arginine and glycine in *HLA-E*01:03*. These isoforms vary in peptide binding affinity, with *HLA-E*01:03* encoded protein having higher affinity. Proteins encoded by both alleles have the same intracellular protein expression levels, but *HLA-E*01:01* encoded molecules have lower cell surface expression in comparison to *HLA-E*01:03* encoded proteins [8].

The HLA-E protein binds peptides derived from the conserved region of the leader sequences (amino acid residues 3-11) of other MHC class I molecules [9]. The binding process requires proteasomal trimming and functional transporter associated with antigen processing (TAP) [10]. The loading of a peptide onto a HLA-E protein allows the latter to be expressed on the cell surface. The presence of HLA-E on the outside of a cell is indicative of a cell that has normal MHC class I expression and functional antigen processing machinery [11]. Although HLA-E appears to bind a narrow peptide repertoire, recent evidence revealed that several proteins other than MHC class I molecules encode peptides that can bind to HLA-E. It has been shown that HLA-E binds peptides derived from signal sequences of HLA-G, Heat Shock Protein (Hsp60) [12] and Multidrug Resistance-Associated Protein (MRP7) [13]. In addition, some xenogenic peptides can also make complexes with HLA-E, allowing its surface expression. Such ability was shown for peptides encoded by HCMV, HCV, HIV, EBV and influenza viruses, as well as peptides derived from *S.* vaccine and *Mycobacterium sp.*

The HLA-E molecule is a ligand for the CD94/NKG2 receptors [14,15] that are mainly expressed on natural killer (NK) cells, thus playing a significant role in regulation of activity of these immune cells (Figure 1). CD94/NKG2 is a member of the C-type lectin super family and consists of an invariant CD94 subunit disulfide-linked to a member of the NKG2 family. Genes encoding CD94/NKG2 receptor components are grouped into a cluster located on chromosome 12. The NKG2 family comprises inhibitory (A and B) and activating (C, E and H) isoforms. The inhibitory isoforms contain Immunoreceptor Tyrosine-Based Inhibition Motifs (ITIM) in their cytoplasmic domains. Their interaction with peptide-presenting HLA-E molecule induces phosphorylation of ITIM motifs leading to recruitment of

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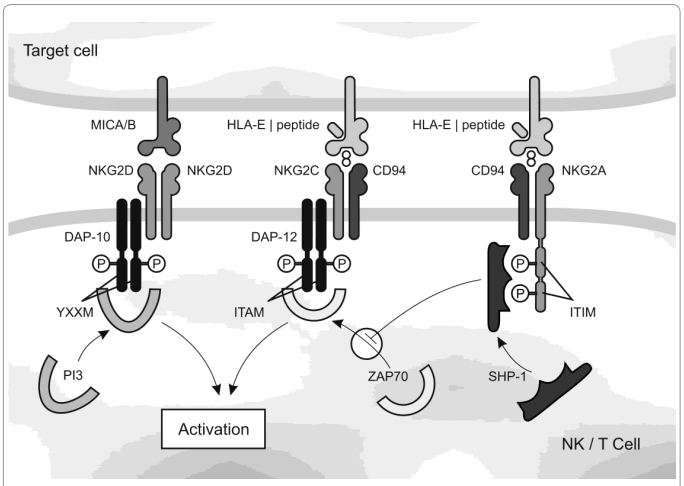

Figure 1: Schematic representation of the interactions between HLA-E and MIC molecules with their NKG2 receptors. Activating and inhibitory signal transduction and regulation in NK or CTL (cytotoxic T lymphocyte) cells. The CD94/NKG2C activating receptor's interaction with peptide-presenting HLA-E molecule leads to binding of adapter molecule DAP-12 containing immunoreceptor tyrosine-based activation motifs (ITAM), which in turn interact with ZAP70 protein, triggering the signal cascade resulting in NK or CTL cell activation. The CD94/NKG2A inhibitory receptor interaction with peptide-presenting HLA-E molecule results in phosphorylation of NKG2A immunoreceptor tyrosine-based inhibition motifs (ITIM), leading to recruitment of SHP-1 protein, which causes dephosphorylation of ZAP70 protein, preventing its ability to bind ITAM domains, thus restraining the activatory pathway. The NKG2D activating receptor's interaction with MICA/B molecule leads to binding of adapter molecule DAP-10 containing YXXM motifs, which in turn interact with PI3 kinase (PI3K), sending signals resulting in immune cell activation.

Src Homology Phosphatase (SHP)-1 protein, resulting in inhibition of NK (natural killer) cells' cytotoxicity. In contrast, the activating isoforms lack Immuno-Tyrosine Inhibitory Motifs (ITIM), but during interaction with the peptide-presenting HLA-E molecule their transmembrane regions binds an adaptor molecule, DAP-12, which contains Immuno-Tyrosine Activating Motifs (ITAM) responsible for triggering the cascade that leads to NK cell activation and cytotoxic function [16]. Despite the sequence similarity between the extracellular domains of NKG2A and NKG2C, the interaction of CD94/NKG2C with HLA-E is presenting standard HLA class I-derived peptide has a binding affinity approximately 8-fold lower than that of CD94/NKG2A [17]. The physiological roles of activating CD94/NKG2 receptors are unclear. It has been proposed that lysis is triggered when the reactive dominance of inhibitory receptors falls below a critical threshold. It may also be possible that CD94/NKG2C has higher affinity to HLA-E presenting non-standard or non-self peptides and such interaction triggers the activation signal [18].

The dominant interaction of HLA-E-expressing cells with NK cells is a reaction between HLA-E and NKG2A inhibitory receptor. This interaction acts as a crucial checkpoint in NK cell surveillance. After HLA-E recognition the NKG2A receptor triggers a signal cascade leading to inhibition of NK cell cytotoxicity [19]. Detection of HLA-E by CD94/NKG2A receptors is a simple yet powerful sensitive mechanism evolved to monitor the expression of a broad array of polymorphic MHC class I molecules. This way the single receptor is able to test the correctness of expression of such molecules and to prevent self-reactivity against normal cells. Events such as a viral infection or malignant transformation can affect the supply of leader sequence peptides derived from MHC class I proteins either directly through down-regulation of expression of these proteins or indirectly through inhibition of TAP function. In the absence of suitable peptides, HLA-E is degraded in the endoplasmic reticulum and doesn't make it to the cell surface. This way NK cells don't receive inhibitory signals from their CD94/NKG2A receptors and can proceed to lyse such anomalous cell.

In addition to the recognition of HLA-E by CD94/NKG2 receptors, CD8+ T cells have been shown to recognize target cells in an HLA-E restricted, T Cell Receptor (TCR) dependent manner [20]. These T cells are called natural killer-cytotoxic T lymphocytes (NK-CTL) because of their NK-like activity, i.e. the ability to lyse certain NK-susceptible target cells. NK-CTLs are a subset of CD8+CD28- CTLs composed of cells that express one single TCR Vβ expansion, display a memory phenotype and express HLA class I-specific inhibitory NK receptors (iNKR). NK-CTLs are capable to recognize HLA-E-bound viral peptides derived from CMV, HCV or EBV and induce a cytotoxic response against such target cells [21,22]. HLA-E can also bind bacterial peptides e.g. peptides derived from *Mycobacterium tuberculosis* [23] and *Salmonella enterica* and present them to CD8+ T cells triggering CD8+-mediated lysis and IFN-γ production. This mechanism might contribute to host defence against intracellular viral and bacterial infections.

HLA-E and *NKG2A* polymorphisms and HSCT outcome

The potential involvement of HLA-E in the immunological response following allogeneic Hematopoietic Stem Cell Transplantation has been suggested by the results of studies using transgenic mice (*HLA-E*01:03* and β*2*-microglobulin gene) [24,25] in which HLA-E exhibited alloantigenic properties that are indistinguishable from classical HLA class I molecules, and it was observed that HLA-E specific alloreactive T cells could proliferate in response to allogeneic stimulation and could lyse most donor cells. There have been a number of studies in humans showing the effect of the HLA-E polymorphism on the transplant outcome (Table 1).

It has been reported that homozygous state for the *HLA-E*01:01* allele is a risk factor for early severe bacterial infections in matched unrelated bone marrow transplantation [26]. Since the HLA-E proteins are involved in the presentation of pathogen-derived peptides to CD8+ T cells, a homozygous state of an inefficiently expressed *HLA-E*01: 01* allele may impair binding of bacterial peptides and thus diminish the possibility of T cell activation and destruction of infected cells [26]. The polymorphism of the HLA-E locus may also influence susceptibility to severe bacterial infections in sickle cell anemia [27]. Moreover, it has been observed that the presence of the *HLA-E*01:03* allele in recipients of allogeneic HSCT is associated with an increased survival rate expressed by decreased Transplant Related Mortality (TRM) and decreased risk of acute GvHD [28-32]. Furthermore, a mismatch between donor and recipient with respect to the HLA-E alleles is connected with higher incidence of acute GvHD [33,34]. Various mechanisms have been proposed to explain the influence of the HLA-E polymorphism on the outcome of HSCT.

In HLA mismatched transplants allogeneic HSCT donor T cells react directly with major histocompatibility antigens; however, in HLA matched transplants, when donor and recipient share the same HLA-A, -B and -C alleles, the T cells recognize minor Histocompatibility antigens (mHags). mHags are presented primarily by classical HLA molecules and such complexes may activate T cells. There is a possibility that HLA-E**01:03* is able to bind mHag molecules, but the presentation of these peptides to T cells is impaired and does not trigger their activation. It is hypothesized that either HLA-E outcompetes classical HLA proteins in mHag binding and disrupts the classical pathway, or

Effect	Allele / genotype (individuals studied)	References
Bacterial infections	*HLA-E*01:01, 01:01* in donor (77 unrelated donor-recipient pairs identically matched for HLA class I and II alleles)	[26]
Transplant related mortality at day 180	*HLA-E*01:01, 01:01* in donor (77 unrelated donor-recipient pairs identically matched for HLA class I and II alleles)	[26]
Protection from aGvHD	*HLA-E*01:03, 01:03* in donor and in recipient (187 HLA-identical sibling pairs)	[28]
Protection from TRM at day 180	*HLA-E*01:03, 01:03* in donor and in recipient (187 HLA-identical sibling pairs)	[28]
Higher probability of overall survival	*HLA-E*01:03, 01:03* in patients (83 HLA-matched donor-recipient pairs)	[29]
Disease free survival	*HLA-E*01:03, 01:03* in patients (83 HLA-matched donor-recipient pairs)	[29]
Decreased incidence of transplant related mortality	*HLA-E*01:03, 01:03* in patients (83 HLA-matched donor-recipient pairs)	[29]
Higher risk of GvHD	*HLA-E*01:01 and 01:03* unmatched in donor and recipient (100 allogeneic donor-recipient pairs)	[30]
Higher survival rate	*HLA*01:03, 01:03* (100 allogeneic donor-recipient pairs)	[30]
Decreased risk of aGvHD (II-IV)	*HLA-E*01:03, 01:03* in donors (121 unrelated donor-recipient pairs)	[31]
Decreased risk of overall aGvHD (cumulative incidence at 3 years)	*HLA-E*01:03, 01:03* in donors (102 unrelated donor-recipient pairs)	[31]
Higher risk of relapse (cumulative incidence at 3 years)	*HLA-E*01:03* in donors (124 unrelated donor-recipient pairs)	[31]
TRM (cumulative incidence at 189 days)	*HLA-E*01:03, 01:03* in donors was a risk factor, whereas the presence of *HLA-E*01:01* alleles was protective (124 unrelated donor-recipient pairs)	[31]
Lower risk of aGvHD and cGvHD	*HLA-E*01:03, 01:03* in patients (56 HLA-E matched donor-recipient pairs)	[32]
Improved overall survival	*HLA-E*01:03, 01:03* in patients (56 HLA-E matched donor-recipient pairs)	[32]
No effect	*HLA-E*01:03, 01:03* in patients and *HLA-E*01:01* in donors (116 HLA-matched unrelated donors)	[73]
Risk of cGvHD development	*MICA-129 val/val* in recipients (211 HLA-identical HSCT sibling pairs)	[65]
Higher incidence of disease relapse	*MICA-129 met/met* in recipients (211 HLA-identical HSCT sibling pairs)	[65]
Higher risk of aGvHD	*MICA* donor-recipient mismatches (236 unrelated HSCT donor-recipient pairs)	[66]
Improved overall survival	*MICA* and *MICB* donor-recipient matches (44 unrelated HSCT donor-recipient pairs)	[67]
Higher risk of aGvHD	*14 bp del/del HLA-G* genotype (position+2961 of 3' UTR) in recipients (53 patients of allo-BMT)	[81]
Higher risk of aGvHD	*14 bp ins/ins HLA-G* genotype (position+2961 of 3' UTR) in donors (unrelated HSCT donor-recipient pairs)	[79]
Decrease in the overall and disease-free survival	*14 bp ins/ins HLA-G* genotype in recipients (47 recipients for allo-HSCT receiving MTX therapy and their donors)	[80]

Table 1: Effect of non-classical HLA polymorphisms on allogeneic HSCT outcome.

it binds non-standard mHag peptides that could specifically mediate Graft-versus-Leukemia (GvL) rather than GvHD [17].

NK cells generated following transplantation are characterized by specific phenotypic features including elevated expression level of the CD94/NKG2A receptors and increased secretion of cytokines, mainly IFN-γ [22]. These cytokines induce upregulation of expression of the HLA-E proteins, which in turn transduce inhibitory signals through the overexpressed CD94/NKG2A receptors back to the immune cells. Such a feedback loop constitutes a defense mechanism against autoreactive incidents involving immature NK cells. It can be hypothesized that cells expressing the *HLA-E*01:03* allele in a highly inflammatory environment such as the post-transplant period may be protected from NK-mediated tissue damage. At the same time, those cells with a low expression of HLA-E molecules may become the target of NK-mediated destruction [28].

On the other hand, the presence of the *HLA-E*01:03* allele in the recipient's cells may negatively influence the GvL reaction. Nguyen et al. reported that the impaired cytotoxicity of NK cells with high expression of the NKG2A receptors was associated with both relapse and patient death in T-Cell Depleted (TCD) haploidentical HSCT [35]. Immature NK cells generated after haplo-mismatched stem cell transplantation were ineffective in destroying Acute Myeloid Leukemia (AML) blasts. These immature immune cells are indirectly responsible for their inefficiency, because the IFN-γ they produce upregulates cell surface expression of HLA-E on AML blasts and this upregulation protects leukemic cells from NK-mediated cell lysis through the mediation of CD94/NKG2A [36]. This suggests that the HLA-E-NKG2A pathway might represent a mechanism of tumor escape from the immune response. Thus, all the facts discussed above show that HLA-E-NKG2A interaction is involved in preventing both autoreactivity and the GvL effect, the former beneficial and the latter deleterious for a patient. Besides Nguyen's group, other groups have also observed that CD94/NKG2A was the predominant NK cell

receptor in the early post-transplantation period [37-40]. Furthermore, Zhao et al. reported that high levels of CD94 expression in donors or in recipients were associated with increased TRM and poorer Leukemia-Free Survival (LFS) [37].

Kawamura et al. [41] using the murine acute GvHD model observed that the CD94/NKG2A receptor is expressed on donor T cells during the course of the disease. Similar expression of CD94/NKG2A on donor T cells was reported for human peripheral blood T cells after allogeneic HSCT. This may suggest that the role of these receptors in this context is to restrain excessive T cell activation, limiting the acute GvHD pathology. Interestingly, administration of anti-NKG2A monoclonal antibodies markedly inhibited the expansion of donor T cells and ameliorated the acute GvHD pathologies. So the exact functional role of CD94/NKG2A in the pathogenesis of GvHD remains unclear [41].

Moreover, Robertson et al. explored the function of CD94/NKG2 in regulation of CD8+ T cell immunity to minor histocompatibility antigens in a murine model using a skin graft as a surrogate GvH target [42]. They observed dramatic differences in the graft rejection rates between mice with viable and inoperative NKG2 inhibitory pathways. These data confirm that the CD94/NKG2 receptors could modulate CD8+ T cell activity and could possibly play a role in limiting disease.

Our preliminary study also suggests that *NKG2A* gene polymorphism may be of prognostic value for the outcome of allogeneic HSCT. Patients carrying the *NKG2A* (-4258 C>G) *C* allele more frequently suffered from herpes virus reactivations than *GG* homozygotes. Moreover, patients transplanted with donors carrying the *NKG2A* heterozygous genotype were characterized by the worst overall survival in comparison to patients grafted from *CC* homozygous donors [43].

MICA/MICB and NKG2D pathway in HSCT

NKG2D is also a member of the NKG2 C-type lectin surface receptor family, but is only distantly related to the other members of the family. It does not dimerize with CD94 and is expressed as a homodimer (Figure 1). NKG2D associates with the signaling adaptor peptide DAP10 (an alternative splice variant can also associate with DAP12 in mice) and, following receptor engagement, induces cytotoxicity, cytokine production, and/or proliferation. The intracellular domain of NKG2D does not have any signaling motif, and therefore signaling is exclusively through its association with DAP10, which does not contain a cytoplasmic ITAM but recruits phosphatidylinositol (PI)-3 kinase. NKG2D is expressed on NK cells, CD8+αβ+T cells, and γδ+ T cells. It binds to the stress-inducible proteins MHC class I chain-related molecule (MIC)-A and -B that are generally not expressed on normal cells but can be induced under pathological conditions. Ligand engagement of NKG2D activates NK cells and potently costimulates effector T cells. NKG2D provides an efficient mechanism for the rapid activation of NK cells which does not require the down-regulation of MHC class I.

To date there has been published just one study concerning the impact of NKG2D polymorphism on the outcome of bone marrow transplantation. Espinoza et al. found an association between the NKG2D-HNK1 haplotype in unrelated donors of HLA-matched bone marrow transplants and a significantly reduced transplant-related mortality and better overall survival. Since the HNK1 haplotype is associated with greater activity of NK cells, one might hypothesize that this beneficial effect of the HNK1 haplotype may be a consequence of increased resistance to infections in the recipients [44].

Regarding the possible role of the NKG2D receptor in the Graft-versus-Tumor (GvT) response in allogeneic HSCT, Boyiadzis et al. reported increased expression of this receptor on NK cells during the early post-transplant period that may enhance the NK-mediated attack on residual tumor cells [45]. Indeed such upregulation was associated with an increase in anti-tumor lytic activity. These data suggest that NKG2D and its ligands may play important roles in the GvT response.

It has also been shown that NKG2D is a key component responsible for bone marrow graft rejection in mice. The rejection of transplanted hematopoietic cells by the recipient's NK cells is regulated by a dynamic balance between positive signals from NKG2D and negative signals from inhibitory receptors for MHC class I. If the graft's cells do not provide adequate ligands for these inhibitory receptors, the signaling from NKG2D receptors suffices to activate the NK cells. Consequently, neutralization of the NKG2D receptors by antibodies prevents rejection [46-47].

Rivas et al. [48] observed in a mouse model involvement of NK cells in the control of proliferation of CD4+ T cells under persistent antigenic stimulation. It has been confirmed that the activating NKG2D receptor plays a major role in this process. The studied T cells, which are responsible for chronic GvHD, showed upregulated expression of the NKG2D ligands. Moreover, blocking in vivo the interaction between NKG2D and its ligands results in the NK cells' failure to control the T cells and in consequence to proliferation of the latter cells and induction of chronic GvHD. These data highlight the potential role played by NK cells via the NKG2D receptor in controlling the Ag-specific CD4+ T cells responsible for chronic GvHD [48].

Among a number of ligands activating this receptor, the most intriguing ones are a pair of closely related proteins called MICA and MICB (major histocompatibility complex class I-related chain A and B). These peptides are encoded by genes located on the short arm of chromosome 6, near the classical (HLA-A, -B and -C) and non-classical (HLA-E, -F and -G) human MHC class I genes [49]. The *MIC* family comprises 2 functional genes (*MICA* and *MICB*) and several pseudogenes (*MICC* to *MICF*) [50]. The genes possess elements of heat shock promoters and are expressed mostly during cell stress. The overall homology between *MICA* and *MICB* reaches 83%, but their homology with the classical MHC class I genes are quite low, being between 15 and 35% [51]. It is also of importance that despite high gene and protein homology, *MICA* and *MICB* seem to be expressed independently and in response to distinct signals, due to the different promoter regulation of both genes [52]. *MIC* family genes are highly polymorphic. This fact may contribute to a considerable variability in immune responses, as was observed in various clinical settings, including organ transplantation.

Polypeptides encoded by both *MICA* and *MICB* genes are, typically, 383 amino acids long and their organization is similar to that of the MHC class I molecules' α chain. Upon alternative splicing MICA can be derived into 2 isoforms and MICB into 3 isoforms. Both MICA and MICB molecules do not associate with β2-microglobulin, lack a CD8 binding site, have a few potential N-glycosylation sites, which are located along the 3 extracellular domains [53], and do not present any antigens [54] (Figure 1). These proteins can be cleaved by matrix metalloproteinases and ADAM proteinase, and released into the blood stream or tissue culture medium as soluble molecules (sMICA and sMICB) [55]. The soluble isoform of MICA has been implicated in the pathogenesis of a variety of cancers as well as autoimmune and other organ transplant-related disorders through NKG2D receptor down-regulation [55-57].

MICA and MICB are non-classical human leukocyte antigens poorly expressed on some healthy cell lines, e.g. on leukocytes, epithelial and endothelial cells, kidney, lung and prostate, but seem to be over-expressed on cancer cells, for example on epithelial tumor cells, some melanomas and leukemic cell lines [49]. This MIC protein expression seems to be the cause of NK cell-mediated death of those malignant cells; for example, there is evidence that over-expression of MICA/B ligands, combined with other factors, is associated with the survival and prognosis of cervical cancer patients [58]. Thus, regulation of the pathway via these proteins presents new clinical possibilities of cancer treatment [58,59].

The molecular mechanisms involved in the expression of MICA and MICB are still poorly understood. However, some experimental data have shown that their over-expression is the result of a DNA damage response that involves the protein kinases ATM (ataxia telangiectasia mutated) and ATR (ATM- and Rad3-related) [60,61]. Interestingly, the ATM/ATR axis is crucial for nuclear factor NF-κB regulation during stress induction [62], and NF-κB was demonstrated to play a role in MICA expression in T cells [63].

The association of the microsatellite loci within the MHC with survival, acute GvHD and leukemia relapse after unrelated HSCT was investigated by Li et al. [64]. The results showed that microsatellite loci localized within 100 kb of the classical HLA loci (5 genes investigated) have a high matching ratio (e.g. MICA – 93%), whereas loci located further away from the HLA classical loci (8 investigated genes) have much lower percentages of allele matching (e.g. TNFα-73%). However, no significant correlation with graft survival, acute or chronic GvHD or leukemia relapse was found.

Boukouaci et al. [65] investigated an influence of the *MICA* polymorphism (*MICA-129*) on the outcome of the HSCT. This *MICA-129* polymorphism categorizes MICA molecules as either strong or weak binders to NKG2D, depending respectively on the presence of methionine or valine residue in position 129 of the α2-heavy chain domain of the molecule. An association has been observed between the *MICA-129 val/val* genotype and increased risk of chronic GvHD. It is proposed that the MICA-129 val protein due to its low affinity to the NKG2D receptor may induce overexpression of this receptor which in turn may favor binding of its alternative ligands, e.g. UL16 peptide. Such non-typical interactions in an environment rich in IL-15 may lead to over-stimulation of NK and T cells and finally to destabilization of the immune control system manifesting in autoreactive incidents. Boukouaci's group has also found that the homozygous state for the strong NKG2D binder *MICA-129 met* allele was associated with high incidence of relapse. The results suggest that the *MICA-129 met/met* genotype, resulting in a protein variant with higher ability to activate NK cells, contributes to inhibition of both GvH and GvL effects. These data are in agreement with the observed phenomenon that an effort to diminish the deleterious GvH effect by silencing autoreactive incidents is accompanied by a similar decrease in the beneficial GvL effect. An alternative hypothesis explaining the results mentioned above are based on studies on the influence of alloreactive NK cells on the HSCT outcome. It has been observed that such NK cells protected patients against rejection and GvHD after transplantation, as well as contributing to a beneficial GvL effect. In this regard Boukouaci's group showed that the activatory MICA-129 met/met protein variant protects against GvHD, although the presence of this allele did not enhance the GvL effect, but correlated with higher relapse risk instead.

A number of *MICA* polymorphisms located in exons 2, 3, 4, and 5 were also studied to establish a possible relationship between donor-recipient *MICA* mismatches and the risk of acute GvHD manifestation [66]. The presence of *MICA* mismatches was found to be associated with more frequent development of gastrointestinal acute GvHD. An unfavorable effect of *MICA* mismatches was also observed by Kitcharoen et al. [67] in relation to patient survival (Table 1).

Role of HLA-G in HSCT

Human leukocyte antigen-G (HLA-G) belongs to the group of non-classical HLA class Ib major histocompatibility complex antigens. Due to alternative splicing of the primary transcript this gene can be expressed as seven isoforms, including four membrane-bound (HLA-G1 to -G4) and three soluble (HLA-G5 to -G7) forms [68]. The HLA-G molecule functions as an immune suppressive molecule. It inhibits the cytotoxicity of CD8+ T cells and NK cells, the alloproliferative response of CD4+ T cells and the maturation of dendritic cells, as well as inducing tolerogenic regulatory T cells [69,70]. Due to these properties, HLA-G contributes to both decrease in reactivity and increase in tolerance of the immune system. The inhibitory effects of this antigen are mediated through direct binding to the inhibitory receptors Immunoglobulin-like Transcript-2 and -4 (ILT2, ILT4), and Killer Immunoglobulin-like Receptor (KIR) 2DL4, which are differentially expressed by immune cells [71]. ILT2 is expressed by monocytes, dendritic cells (DC), NK, T and B cells, ILT4 primarily by monocytes, macrophages and dendritic cells 005 [72], and KIR2DL4 is present on NK and T cells [73]. The expression of HLA-G is highly restricted to specific tissues, with high expression levels on fetal extravillous trophoblasts, where these antigens maintain a tolerogenic status between the mother and the semi-allogeneic fetus [74]. In non-pathological conditions HLA-G may also be constitutively expressed in adult thymus, cornea, pancreatic islets and the precursors of endothelial and erythroid cells [75]. In addition, the HLA-G proteins can be found after organ transplantations, malignant transformations, viral infections and inflammatory/autoimmune diseases [75]. In such pathological conditions this antigen might be expressed by tumor, virus-infected or grafted cells and also by infiltrating immune cells. The tolerogenic properties of the HLA-G molecule is beneficially applied during the promotion of uterine implantation of the embryo or acceptance of solid allografts, but on the other hand these properties allow the evasion of tumours or viruses from the immune response. In the context of transplantation, HLA-G is involved in the protection of the transplanted tissues via the inhibition of immune effectors that mediate graft rejection.

The 3' UTR 14-bp polymorphism, a 14 base pair deletion/insertion at position +2961 of the gene, is the most frequently studied *HLA-G* polymorphism. The presence of the insertion variant is known to generate an additional splice site resulting in the removal of 92 bases from the mRNA coding exon 8. It was shown that this insertion improves the mRNA stability [76], but subsequently it proved to be also associated with low levels of the HLA-G mRNA [77] and serum sHLA-G isoforms [78]. Therefore this polymorphism plays an important role in the regulation of HLA-G molecule expression with likely consequences on functional properties of this antigen. An inconsistency between better stability of the RNA and lower synthesized protein output, termed "the 14-bp polymorphism paradox", has not been ultimately explained to date. Probably the interaction of several independent factors is relevant in this case.

The results of deletion typing in the *HLA-G* gene published to date in the context of HSCT is ambiguous. Boukouaci et al [79] observed that the *14-bp ins* allele is associated with higher GvHD risk in patients undergoing HSCT. Taking into account that the HLA-G protein enhances the tolerance of the immune system, the down-regulated

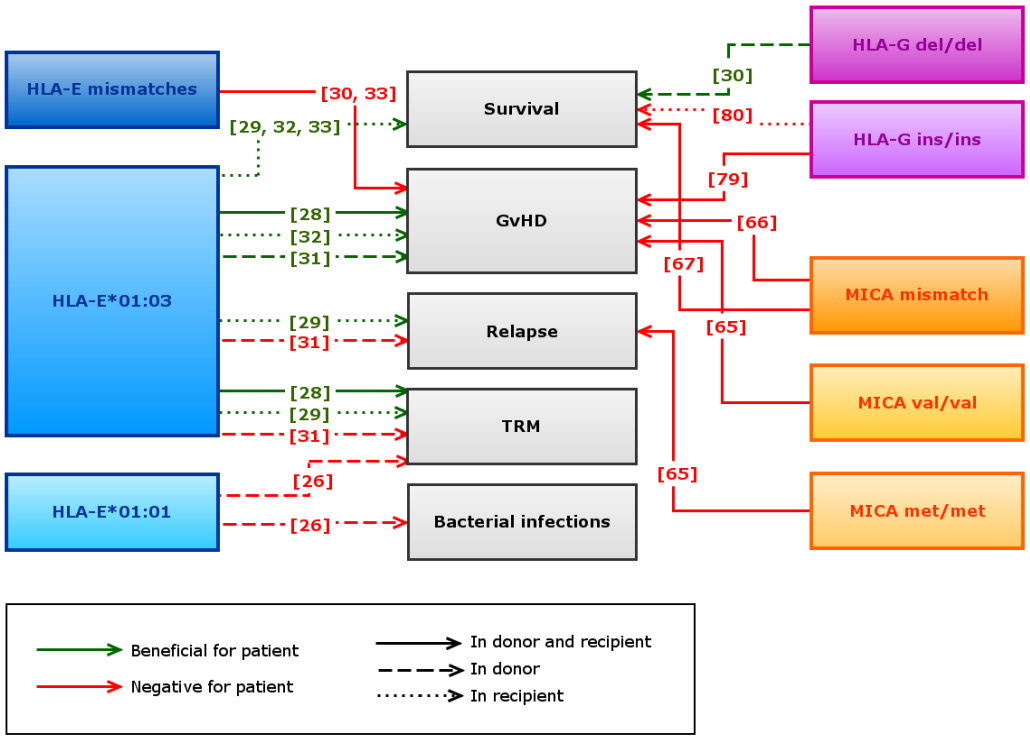

Figure 2: Impact of donor and recipient *HLA-E*, *HLA-G* and *MICA* genotypes on the outcome of HSCT.

surface expression of this molecule, associated with the *HLA-G 14-bp ins/ins* genotype, and may result in increased immunoreactivity with consequent acute GvHD (aGvHD) development. This hypothesis is in line with conclusions from studies on the influence of surface expression of the HLA-G protein on outcome of solid organ transplantations evidencing the beneficial effect of the HLA-G molecules against rejection/chronic dysfunction of the transplanted organ. In regard to HSCT, the results obtained by Chiusolo et al. [80] also suggest a negative effect of the *HLA-G 14-bp ins* allele on the outcome of transplantation. This allele was associated with a lower survival rate and increased incidence of relapse of the disease. Similarly, our group observed a beneficial effect of the *HLA-G 14-bp del/del* genotype on the result of HSCT [30]. On the other hand, the data published by La Nasa et al. oppose the presented paradigm [81]. They found that the homozygous genotype *HLA-G 14-bp del/del* correlates with higher risk of GvHD in bone marrow transplanted patients. It should be noted, however, that in this case a relatively small group of beta-thalassemia patients was studied, while the previously mentioned studies concerned patients who had undergone transplantations in consequence of hematological malignancies. Moreover, the mechanism of the regulation of HLA-G expression is not influenced exclusively by the mentioned alleles, but other factors are certainly involved. Recent studies implicate the potential role of microRNAs interacting with the HLA-G 3' mRNA region, which may regulate the expression of this protein. One of the single nucleotide polymorphisms (SNPs) from this region (rs1063320) promotes the interaction, resulting in lower expression of the protein. Another *HLA-G* SNP (rs9380142), associated with decreased stability of the produced mRNA, has a similar effect. The observed effect of the allele on GvHD could be either due to the combined effect of the above presented factors or due to another yet to be identified linked functional variant [79].

Obviously, the role of HLA-G molecules in hematopoietic stem cell transplantation needs further investigation. It is of note that a number of works described a positive effect of increased expression of this molecule on the risk of graft rejection after solid organ transplantation. Expression of HLA-G molecules by transplanted organs and the levels of soluble HLA-G in the patients' sera significantly correlated with a reduced transplant rejection rate, confirming the immunosuppressive role of these molecules.

Summary and Conclusion

The results of the studies presented and discussed in this review provide additional data concerning the relationship between the polymorphic features within the human MHC region and transplantation outcome, suggesting a role for the *HLA-E, G*, and *MICA* alleles as other (non-conventional HLA class I and class II) genotypic risk factors associated with susceptibility to development of GvHD, infections, and patient survival (Figure 2). These data imply that donor-recipient genotyping; extended to non-classical HLA loci, as well as the CD94/NKG2 family, may be of prognostic value for transplantation outcome.

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