Chapter 2

HLA Class I Polymorphism and Tapasin Dependency

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Additional information is available at the end of the chapter

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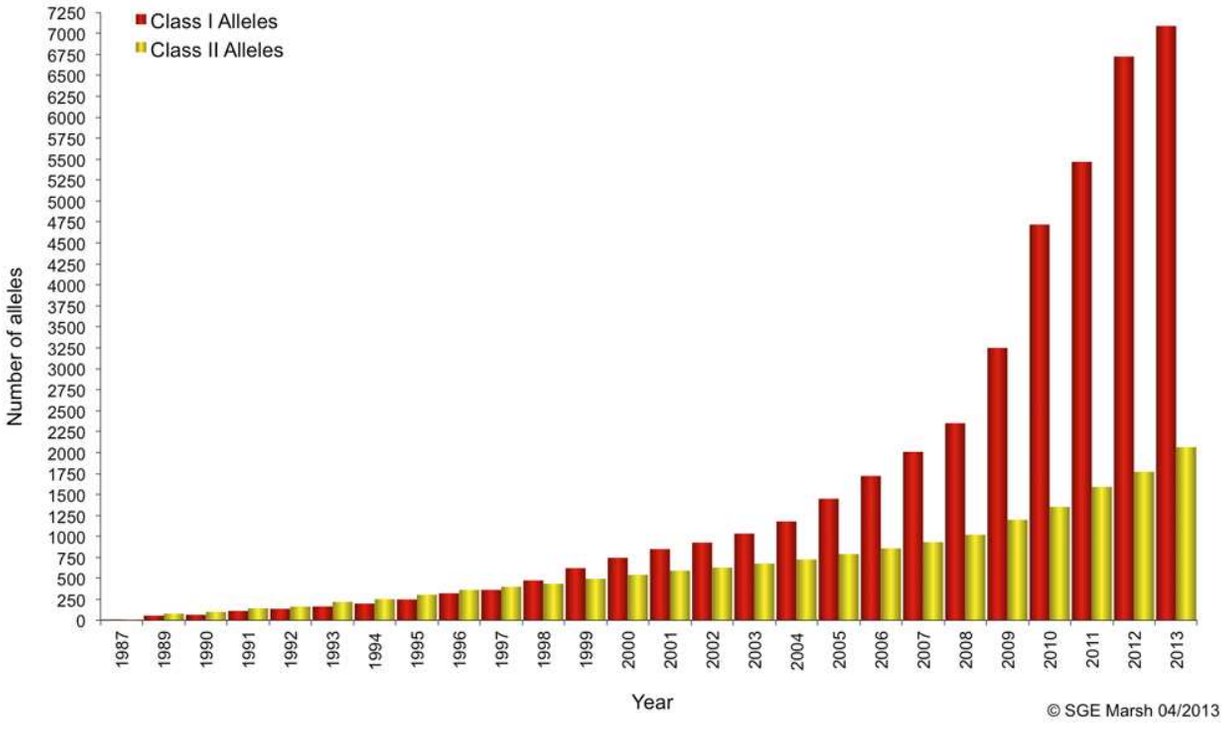
1. Introduction

Human leucocyte antigens (HLA) are highly specialized proteins, expressed on all nucleated cells and platelets, that form stable complexes with peptides of self or pathogenic proteins generated by proteasomal degradation. These peptide-HLA (pHLA) complexes are presented at the cell surface where they are subsequently recognized by T cells. Thus, T cells with their specific antigenic receptor (TCR) continuously scan an array of pHLA complexes which are presented on the cell surface [1]. One of the distinct properties of TCRs is that they can recognize an antigen only when it is associated with a host or "self"-encoded HLA molecule. This property of T cells was discovered by Zinkernagel and Doherty in 1974 and is called ´MHC-restriction´ [2]. The recognition of pHLA complexes by TCRs regulates many immunological responses such as antiviral cytolysis, graft or tumor rejection and regulation of B cell function. The genes encoding for HLA molecules are known to be the most polymorphic genes present in the whole genome. To date more than 9,000 HLA alleles have been identified of which there are ~7,300 HLA class I and ~2,200 class II polymorphic alleles (Figure 1) [3].

These polymorphisms do not occur throughout the HLA molecule, but are confined to specific AA positions in the peptide-binding region (PBR) [4, 5, 6]. They can cause alterations in the conformation of the PBR, thus changing the peptide binding specificities of these molecules [7]. The frequency of HLA alleles varies greatly among different ethnic groups. It has been postulated that evolutionary pressures such as those exerted by epidemics of infectious diseases might lead to the evolution of new HLA alleles having distinct peptide binding properties.

Following hematopoietic stem cell transplantation (HSCT), polymorphic differences between donor and recipient HLA class I molecules can lead to transplant rejection or graft-versus-host disease (GvHD). Extensive clinical data have demonstrated that the risk of GvHD strongly



There are currently 9,719 HLA and related alleles described by the HLA nomenclature and included in the IMGT/HLA Database. Red bars represent class I alleles and yellow bards represent class II alleles. As of 2013, there are around 7,353 class I alleles and 2,202 class II alleles that have been identified [3].

Figure 1. Number of HLA alleles that have been identified from the year 1987 until 2013

correlates with the number and kind of HLA mismatches and that both the type of amino acid (AA) substitution and its location within the HLA molecule can directly influence the transplantation outcome. Polymorphisms occurring within the PBR of HLA class I molecules determine which allogenic peptides are selectively bound and subsequently recognized as self or non-self by the effector T-lymphocytes that survey pHLA complexes on antigen presenting cells. Assembly of MHC class I heavy chain (hc) and β2 microglobulin (β2m) subunits with peptides is assisted by the peptide loading complex (PLC). Initially, proteasomally digested peptides are transported into the endoplasmic reticulum (ER) via the transporter associated with antigen processing (TAP) and are then loaded onto HLA class I molecules with the assistance of the PLC. The transmembrane glycoprotein tapasin (TPN) functions within this multimeric PLC as a disulfide-linked heterodimer along with the thiol oxidoreductase ERp57 to stabilize the empty class I molecule and promotes the selection of high affinity peptides. In addition to bridging HLA class I molecules with TAP, TPN was found to stabilize the peptide-receptive state of HLA class I molecules and increased the steady state levels of TAP heterodimers. Certain HLA class I polymorphisms within the PBR appear not only to influence the repertoire of bound peptides, but also determine the requirement for PLC mediated acquisition and optimal loading of peptides for the given HLA class I molecule. Whereas most class I allotypes associate strongly with the PLC and are highly dependent upon TPN for effective presentation of high affinity peptides and cell surface expression, others can acquire peptides

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even without assistance from the PLC but are then sub-optimally loaded. Due to the crucial role of TPN in selecting peptides, its indirect role in the immunorecognition of pathogens becomes obvious. This makes TPN an ideal target for viruses to interfere with the presentation of viral peptides to CTLs. For instance, US3 protein of HCMV binds to TPN and acts as a TPN inhibitor. This affects the antigen presentation by TPN-dependent HLA class I molecules. However, TPN-independent molecules can selectively escape the US3 mediated class I retention and continue to present the viral peptides.

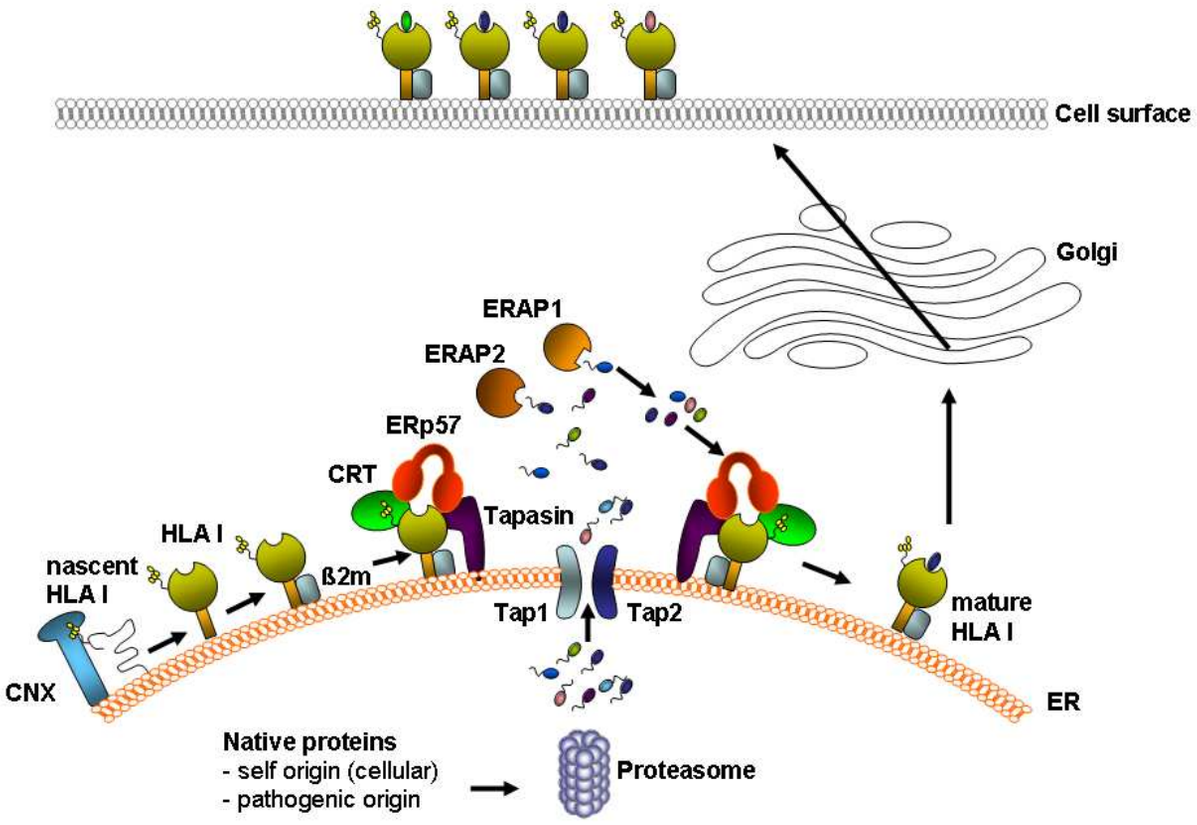
Given HLA class I polymorphisms affect the generic antigen processing pathway and their dependency on TPN for antigen presentation. TPN-independency is occurring very rarely and might have evolved as an evolutionary trade-off to combat viral infections. However, presentation of unusual ligands by certain HLA class I alleles could be a risk factor during stem cell transplantation and needs to be considered during donor selection process. The future of HSCT relies on our understanding of how successful clinical outcomes can be achieved despite patient-donor allelic mismatches.

2. HLA polymorphisms and transplantation

The HLA system is one of the major barriers in hematopoietic stem cell transplantation (HSCT) and the degree of HLA matching is found to reflect on the outcome following transplantation. The best result following HSCT is achieved when an identical twin or a genotypically HLA identical sibling is used as donor. However, only 30 % of the donors for HSCT have a HLA identical sibling donor available [8]. Therefore, in most of the situations, HLA haplo-identical related, matched unrelated or partially matched related or unrelated donors are considered for transplantation. However, these transplants are associated with high risks of post-transplant complications such as graft failure/rejection or graft-versus-host disease (GvHD), mainly because of undefined or HLA incompatibilities. Many studies have demonstrated the negative impacts of HLA mismatches on the outcome following HSCT [9-11]. To understand the magnitude of distinct mismatches between HLA variants, several studies analyzed allele specific peptide motifs towards a rating of incompatibility [12-21] [22] [23, 24]. The knowledge of the peptide binding motifs of individual alleles and their comparison within allelic groups is the basis for understanding the impact of a given mismatch and is fundamental in predicting the relevance of allelic differences. Since allelic mismatches occurring at critical residues within the class I heavy chain may cause allorecognition, high resolution matching of patients and unrelated donors have been found to significantly improve post-transplant survival [25], lower the incidence and severity of GvHD [26, 27] and improve engraftment [28, 29]. The question whether a mismatch is permissive or not is critical in deciding which individual is the best matched donor. This could be achieved by conducting a systematic study to determine the effect of AA sequence polymorphisms on the function of a particular HLA molecule and on the immune responses post-transplantation.

3. HLA class I molecules and the peptide loading complex

HLA class I molecules loaded with high affinity peptides are essential for efficient immune surveillance and elimination of virally infected cells by CTLs. Newly synthesized class I hc and ß2m are translocated into the ER by their amino terminal signal sequences. Following translocation, HLA class I hcs are glycosylated and folded by the formation of two intra-chain disulphide bonds. Calnexin (CNX) facilitates the stabilization of class I hc and its association with ß2m. Following the formation of class I hc - ß2m heterodimer, CNX is replaced by calreticulin (CRT). Peptides are loaded onto the class I heterodimer by a complex machinery consisting of many chaperons, known as the peptide loading complex (PLC). The PLC consists of the transporter associated with antigen processing (TAP) heterodimers, transmembrane glycoprotein – TPN, lectin like chaperon – CRT, thiol oxidoreductase – Erp57 which is non-covalently associated with CRT and disulphide linked to TPN (Figure 2).



Peptides are loaded onto MHC class I molecules with the assistance of the Peptide Loading Complex (PLC). Processed peptides are transported into the ER via TAP. N-terminal trimming of peptides via the ER aminopeptidases (ERAP) 1 and 2. TPN functions within the multimeric PLC as a disulfide-linked, stable heterodimer with the thiol oxidoreductase ERp57.

Figure 2. Peptide loading complex

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Peptides presented by HLA class I molecules originate mostly from cytosolic or nuclear proteins and are processed by the proteasome, a multicatalytic protease complex. TAP helps in the translocation of peptides from cytosol into the ER lumen. TPN bridges class I - ß2m heterodimer to TAP and acts as a peptide editor, facilitating the loading of high affinity peptides onto HLA class I molecules. Stable HLA class I molecules dissociate from TAP heterodimers and are transported through the golgi complex to the cell surface where they present peptides to CD8+ T cells.

4. Tapasin

TPN is a type 1 transmembrane glycoprotein, 428 amino acid long and consisting of three parts: an N-terminal ER luminal region consisting of two domains, a transmembrane domain and a short cytoplasmic tail. TPN has multitudinous roles within the PLC, all of which are directed towards peptide presentation by the class I molecules. The transmembrane domain of TPN interacts with TAP and bridges TAP to class I molecules. TPN facilitates the stabilization of TAP and promotes the binding and translocation of peptides by TAP. Absence of TPN abrogates the binding of class I molecules to TAP. It was also shown that certain class I molecules presented at the cell surface in the absence/independent of TPN were very unstable and dissociated rapidly. The double lysine motif at the C-terminus of the TPN molecule mediates its interaction with coat protein type I (COP I) vesicle and facilitates the recycling of class I molecules which have not been not loaded with optimal peptides. Mutational analysis identified a conserved region on the ER-luminal domain of TPN that interacts with HLA class I molecules and was found to be critical for peptide loading and its editing function. Polymorphisms occurring in HLA class I molecules are found to affect the dependency of these molecules on TPN for antigen presentation and cell surface expression.

5. Inhibitors of TPN

TPN is a critical component of the PLC which plays an important role in optimization and selection of peptides subsequently presented on the cell surface by HLA class I molecules [30, 31]. Transmembrane glycoprotein US3 expressed during the immediate early phase of HCMV infection binds to TPN and inhibits its ability to load kinetically stable peptides onto HLA class I molecules, thus retaining class I molecules in the ER [32]. However, not all HLA class I molecules are equally affected by US3, thus highlighting that not all HLA class I molecules are equally dependent upon TPN for maturation in the ER [33]. US3 and TPN are associated by their ER luminal domains, but the transmembrane domains are also required for the inhibition of TPN [32]. Another transmembrane glycoprotein E3-19K from the adenovirus also inhibits crucial functioning of TPN by blocking its ability to bridge TAP to HLA class I molecules. E3-19K associates with TAP and impairs the formation of TAP-TPN complex and inclusion of TAP in the PLC [34]. This competitive inhibition by E3-19K delays the maturation and assembly of TPN-dependent HLA class I loading complex [34].

Certain other viral proteins prevent surface expression of HLA class I molecules by retention or degradation of HLA molecules in the ER. For example, cowpox virus protein 203 (CPXV 203] causes retention of HLA class I molecules in the ER [35]. US2 and US11 proteins from HCMV and mK3 protein from mouse herpes virus directs HLA class I molecules towards proteasomal degradation [36] [37] [38] [39]. Sorters such as HIV-1 protein Nef and murine CMV protein gp48 averts the trafficking of HLA class I molecules from golgi to lysosomal compartment where they are subsequently degraded [40] [41] [42].

6. Interactions between HLA class I molecules and TPN

In addition to bridging HLA class I molecules to TAP, TPN was found to stabilize the peptide-receptive state of class I molecules [43] and increase the steady state levels of TAP heterodimers [44]. TPN also facilitated the retention of empty class I molecules in the ER of insect cells [30]. Barnden *et al* demonstrated that TPN prevented premature exit of HLA class I molecules from the ER of mammalian cells, thus suggesting a potential role for TPN in the retention of sub-optimally loaded class I molecules in the ER [45]. HLA class I molecules expressed in TPN-deficient cells were found to be unstable and were loaded with a significant proportion of suboptimal ligands [33, 46, 47]. Yu *et al* demonstrated that mutations occurring at residues 128-136 in the α2 helix of class I heavy chain affected the interaction of HLA class I molecules with TPN. These residues are located in the loop region connecting β-pleated sheets below the peptide binding groove with an α-helix reaching above the groove. This region of the heavy which forms a potential interacting site with TPN was found to be sensitive to peptide binding and underwent conformational changes, thus implying the ability of TPN to distinguish between empty and peptide-bound HLA class I molecules [48, 49]. Some groups have speculated about the interaction of TPN with the α3 domain of HLA class I heavy chain [50]. However, given that residues 128–136 in the α2 domain and residues 227–229 in the α3 domain are located on the same plane along the side of class I heavy chain, TPN might be able to interact with both these determinants. Mutations occurring in the HLA class I heavy chain were found to affect the interaction of these molecules with the loading complex components [49, 51-53]. However, precise interacting surfaces/interfaces between the class I molecules and TPN are yet to be defined. To determine the potential interacting surfaces of TPN with HLA class I molecules, Dong *et al* initially compared the sequence of TPN across different species and identified a region in the N terminal domain of TPN that was highly conserved amongst these species [54]. Residues occurring in this region and in other parts of the TPN molecule were mutated and the effect of these mutations on PLC function was tested. Eight different TPN mutants conjugated with ERp57 were incubated with extracts from LCL 721.220/B*08:01 cells that are enriched with empty HLA class I molecules. Co-immunoprecipitation experiments were performed to determine the interaction of HLA class I molecules with these different TPN mutants. HLA class I molecules associated at normal levels with the wild type TPN; with two other TPN mutants in which amino terminal residues (TN1 and TN2) located farthest from the conserved patches were mutated and TPN mutant with a single polymorphism located in the carboxy terminal region (TC1). Mutating the residues in the central, conserved patch of

TPN molecule (TN6 - Glu185Lys, Arg187Glu, Gln189Ser and Gln261Ser) completely abolished its binding with the class I heavy chain. Also, only small amounts of the heavy chain were found to interact with the mutants located in or around the conserved patch. The ability of HLA class I binding to wild type/mutant TPN molecules reflected their relative capacities in mediating peptide loading. TN6 mutant mediated only 8 % of peptide loading activity in. 220.B*08:01 molecules as compared to the activity of wild type TPN. It was also observed that the transduction of TN6 mutant into TPN independent 220.B*44:02 cells did not favour the surface expression of these molecules on the cell surface. The conserved, functionally important central region of TPN was suggested to be responsible for the stabilization of the α2-helix of the PBR. This stabilization was found to maintain the peptide binding groove in an open peptide-receptive conformation until an optimal peptide binds to it [54]. The findings from this study are in agreement with previous studies conducted where Thr134Lys mutation in HLA-A*02:01 was found to disrupt its interaction with TPN [47]. Co-immunoprecipitation experiments performed by Lehner *et al* demonstrated that deletion of the transmembrane region of TPN did not have any effect on the interaction of HLA class I molecules with TPN. However, HLA class I molecules failed to co-precipitate with TPN molecules truncated at the N terminus, suggesting that the residues in this region were important for the interaction of HLA class I molecules with TPN [43].

7. Peptide editing function of TPN

Many studies have highlighted the role of TPN in stabilizing HLA class I molecules [30, 31, 45, 55] and maintaining them in a peptide-receptive conformation [56]. It has also been shown that TPN facilitates peptide optimization, a process in which bound peptides of low affinity are exchanged for the high affinity ones [45, 57-61]. These functions were attributed to TPN based on the findings that the class I-peptide complexes expressed on the surface of cells lacking TPN were less stable than those complexes expressed on normal cells containing TPN. However, analysis of peptides eluted from HLA class I complexes expressed on the cell surface in the presence and absence of TPN demonstrated no co-relation between the decreased stability of HLA class I-peptide complexes and binding of low-affinity peptides in TPN deficient cells [62]. The authors suggested that the plausible ability of TPN to stabilize immature HLA class I molecules in the ER instead broadens the bound peptide repertoire both in terms of complexity of bound peptides and their binding affinities [62]. A more recent study conducted by Howarth *et al* demonstrated key functions of TPN in shaping the peptide repertoire presented to the cell surface based on their intrinsic half-lives [63]. They investigated the effect of TPN on the presentation of a hierarchy of peptides generated based on the H2-K^b-binding peptide SIINFEKL by varying the anchor residues in order to produce peptides having a wide array of binding affinities. These peptides were expressed stably as mini-genes in the cytosol of TPN deficient cell line.220K^b and in.220K^b TPN transfected cell line. Results indicated all the peptides to be presented at high levels in the presence of TPN and their relative expression levels were found to be in accordance to their peptide-half lives. However, in the absence of TPN, this hierarchy was disrupted and a peptide with intermediate half-life was

found to be presented more dominantly than the rest of the peptides. Since all the peptides utilized in this study had similar affinities to H2-K^b-binding peptide SIINFEKL, editing function by TPN was suggested to be influenced primarily by the peptide-off rate rather than peptide-affinity per se [63].

Many groups have established *in vitro* assays to provide a molecular understanding of the mechanisms of peptide editing by TPN. However, weak intrinsic interactions between TPN and HLA class I molecules make it difficult to perform *in vitro* experiments using recombinant TPN to assess its functions. In order to overcome this problem, Chen *et al* used leucine zippers to tether soluble TPN together with HLA class I molecules [56]. For this study, they selected HLA-B*08:01 as this allele has earlier shown to be TPN dependent [33] for normal levels of cell surface expression. The results of this study indicated that TPN acts as a chaperon by accelerating the ratio of active to inactive-peptide deficient HLA class I molecules. In addition to stabilizing HLA class I molecules, TPN was also found to increase the association-dissociation rates of peptides with class I molecules owing to its ability to widen the peptide binding groove, thereby enabling a diversified set of peptides to initially bind into the groove. This TPN-assisted mechanism of peptide selection was suggested to be mediated by disruption of the conserved hydrogen bonds at the C terminus of the binding groove [56]. In yet another approach to determine the peptide-editing mechanism of TPN, Wearsch *et al* reconstituted the PLC subcomplex *in vitro* by co-incubating recombinant soluble TPN-ERp57 conjugate with additional cell extracts containing CRT and peptide receptive heavy chain-β2m complex [59]. The results from their study demonstrated that the TPN-ERp57 conjugate promoted rapid exchange of sub-optimal low and intermediate peptides with high affinity ones [59]. Praveen *et al* demonstrated an alternative approach to explore the TPN-mediated peptide editing function in the lumen of ER microsome [60], wherein components of the loading complex can interact with each other with their native affinities. For their experiments, they used the allomorphs K^b wild type (WT) and K^b mutant (T134K) in which the replacement of Thr134Lys abolished the interaction of these molecules with TPN. They found that when these allomorphs were incubated with a mixture of high affinity peptides and a 100-fold excess of a low affinity peptide or alternatively with the low affinity peptides and a 100-fold reduced concentration of a high affinity peptide, the high affinity peptide was predominantly bound by wild type K^b while K^b mutant (T134K) mostly bound the low affinity ones [60].

8. TPN dependence/ independence of HLA class I molecules

Polymorphisms occurring at specific AA positions within the HLA class I hc are found to influence the dependency of these molecules on TPN for efficient cell surface expression and peptide presentation. It has been hypothesized that the nature of AAs occurring at the bottom of the F pocket influences the conformational flexibility of empty class I molecules [55, 64], which could in turn determine the ability of a particular allotype to bind peptides in the presence or absence of TPN [65]. It has been shown that in the TPN dependent alloforms, the region around the F pocket of the peptide binding groove is in a disordered conformation due

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to a partially open disulphide bond in the α2 domain [66] and that TPN facilitates the conversion of this disordered conformation into a stable, peptide-receptive conformation [55].

Studies performed using TPN deficient cell lines (LCL 721.220] transfected with various HLA-A and B allotypes demonstrated an altered dependency of these class I variants on TPN for their cell surface expression [33, 67, 68] [18]. HLA-B*27:05 molecules showed high levels of surface expression and were able to present specific viral peptides even in the absence of TPN. On contrary, HLA-B*44:02 molecules were found to be highly dependent upon TPN for these functions and HLA-B8 molecules showed intermediate dependency on TPN [33]. It has also been observed that while HLA-A1 molecules fail to present antigens in the absence of TPN [31], HLA-A2 molecules present peptides very efficiently on the surface of these cells [69].

Many studies have highlighted the importance of AAs occurring at position 114 of class I hc in determining their dependency on TPN for efficient antigen processing and presentation [33, 55, 64, 70, 71]. Park *et al* demonstrated that the class I molecules having an acidic AA at position 114 such as HLA-B*44:02^{114Asp} or HLA-A*30:01^{114Glu} are highly dependent upon TPN for their cell surface expression, alleles with neutral AAs such as HLA-B*08:01^{114Asn} or HLA-B*54:01^{114Asn} are weakly dependent while alleles with basic AAs such as HLA-B*27:02^{114His}, HLA-B*27:05^{114His}, HLA-A*02:10^{114His} or HLA-A*24:01^{114His} are independent of TPN for their surface expression [71]. However, both HLA-B*44:02 and HLA-B*44:05 are found to have an acidic AA at position 114 and yet show opposite ends of the TPN dependency spectrum. In the absence of TPN, HLA-B*44:02 fails to bind high affinity peptides and is prone to intracellular degradation. In contrast, HLA-B*44:05 shows efficient cell surface expression both in the presence and absence of TPN [58]. These two allotypes differ exclusively at AA position 116 which is located in the F pocket of the peptide binding groove and contacts C terminus of the bound peptide. HLA-B*44:02 has an Asp while B*44:05 has a Tyr at position 116. While HLA-B*44:02 binds very efficiently to TAP and undergoes significant optimization of its peptide cargo, B*44:05 molecules are not incorporated in the PLC and undergo only partial optimization of their peptide cargo in the presence of TPN [58]. Asp116His mutation in HLA-B*44:02 resulted in a TPN-independent molecule [55]. Sieker *et al* hypothesized that the presence of two acidic residues at positions 114 and 116 in HLA-B*44:02 hc resulted in the disruption of the F-pocket conformation due to excessive hydration [65] and that the ability of HLA-B*44:05 to acquire limited peptides without being incorporated into the PLC was due to the aspartic acid to tyrosine exchange at residue 116 which decreased the electronegativity and increased the hydrophobicity around the F pocket [64]. Experiments performed by Neisig *et al* demonstrated that among the HLA-B allotypes investigated, those containing an aromatic AA at position 116 bound efficiently to TAP while the others did not [70]. HLA-B*35:02 and B*35:03 having aromatic AAs Tyr and Phe respectively at position 116 demonstrated strong associations with TAP while B*35:01 and B*35:08 both containing Ser at position 116 showed no significant association with TAP [70]. Similarly, among the HLA-B*15 allotypes, B*15:10 having a Tyr at position 116 showed stronger association not only to TAP but also to TPN and CRT when compared to B*15:18 or B*15:01 having Ser at this position [72]. It was also observed that HLA-B*68:07^{116His} associated much stronger with TAP than B*68:03^{116Asp} [73]. The authors pointed out that residue 116 pointing upwards from the F pocket into the binding groove might

be involved in the association of TPN with the class I heavy chain, which in turn regulates the differential binding of position 116 variants with TAP. However, it is seen that HLA-B*44:02 and B*44:03 both have an Asp at position 116 and yet associate differentially with TAP. These two alleles differ from each other by a single AA at position 156. While HLA-B*44:02 binds efficiently to TAP, B*44:03 is found to be a weak TAP binder. The authors pointed out that the AA at position 156 located at the centre of the α2 helix of class I heavy chains might determine the strong and the weak binding of HLA-B*44:02 and B*44:03 respectively to TAP [70].

Some of the more recent studies have shed light on the functional consequences of HLA class I polymorphisms in modulating the presented peptide repertoire. It could be demonstrated that the TPN dependent B*44:02^{116Asp} and TPN-independent variant B*44:05^{116Tyr} differed in their preference at the PΩ anchor residue [64]. Binding preference of HLA-B*44:05 at P9 was restricted to Phe while B*44:02 showed preference for both Phe and Tyr at this position, largely due to the ability of Asp116 in B*44:02 to make hydrogen bond with Tyr at P9 [64]. In yet another study, it was demonstrated that although the surface expression of HLA-B*27:05 was similar both in the presence and absence of TPN, there was a difference in the cytotoxic lysis of B*27:05 targets upon infection with recombinant vaccina viruses under these two circumstances. Measurement of cytotoxicity at four hours post infection demonstrated that the lysis of B*27:05/TPN negative targets was only half the cytotoxicity level observed for B*27:05/TPN positive target cells. At 12 hours post infection, the cytotoxic lysis of B*27:05/TPN negative targets was similar to B*27:05/TPN positive target cells. However, this study pointed out an impairment in the presentation of specific viral peptides by B*27:05 in the absence of TPN. Although there was some overlap in the peptides presented in the presence and absence of TPN, unique set of peptides were selected and presented by B*27:05 under these two conditions. B*27:05 molecules on the surface of cells lacking TPN are more unstable probably owing to the nature of peptides selected and bound by them in the absence of TPN [33].

Studies examining single AA exchanges within the hc of naturally occurring HLA class I alleles have identified some of the residues in the α2 domain which are of critical importance for the interaction of class I molecules with the PLC components. Elliott *et al* demonstrated that the replacement of Thr with Lys at residue 134 (T134K) in HLA-A2 resulted in disruption of the interaction between class I and the PLC components [47, 52]. In contrast, replacement of Ser with Cys at residue 132 (S132C) in these molecules resulted in prolonged association of class I molecule with the PLC components, slower maturation of the complex and binding of optimal high-affinity ligands [52].Yu *et al* have shown that residues 128-136 occurring in the α2 domain play an important role in peptide loading and formation of the class I loading complex [49]. These studies have led to the identification of a putative PLC binding surface of the class I heterodimer that is located on the α2 domain of the molecule. The surface on the class I molecule that these regions contribute to defines a pronounced groove which might form a docking structure for one or more components of the PLC. Also, the conserved disulphide bond between AAs Cys101 and Cys164 are located within this region of the α2 domain. This disulphide bond is responsible for linking the α2 helix to the peptide binding floor and isomerisation of this bond has been implicated during peptide binding [74].

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Previous studies have demonstrated that the nature of AAs occurring at residues 114 or 116 determine the interaction of these different class I allotypes with the PLC components [64]. These two residues are located in the F pocket of the PBR, that interacts with the C-terminal peptide residue and thus determine the nature of a bound peptide. It is found that certain AA polymorphisms occurring at these positions result in loading and presentation of peptides independent of the loading complex (TAP/ TPN) [64, 71] via a non-classical pathway resulting in the presentation of pHLA complexes, that might be poorly tolerated by the self immune system.

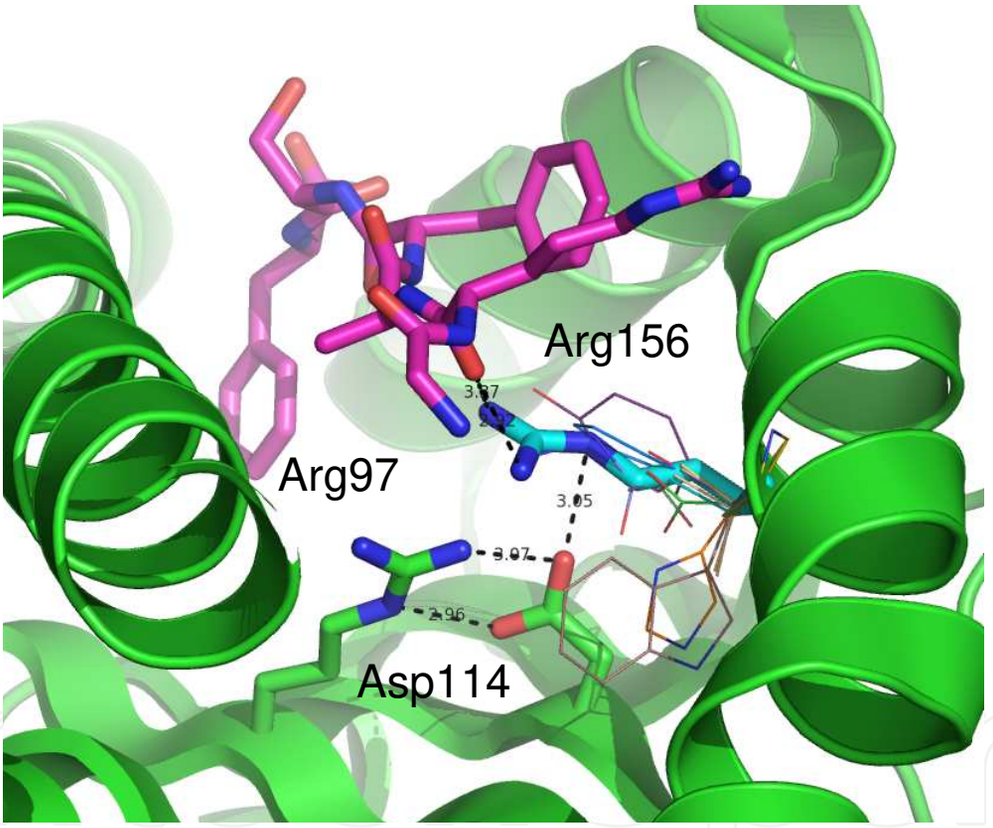
9. Impact of mismatch at residue 156 in B*44 allotypes

Recently the association of TPN with HLA subtypes featuring micro-polymorphisms at AA position 156 was discovered [18]. This position is part of the pockets D and E within the peptide binding region and contacts peptide of canonical length at positions p3 and p7 [75], explaining its distinct structural role in influencing the conformation of the pHLA complex [76] [77]. Polymorphisms at residue 156 represent one of the most non-permissive transplantation scenarios and are associated with acute GvHD for HLA-A, -B and -C alleles [58] [78] [76] [77] [79] [80].

The HLA-B*44 allelic group occurring in approximately 25 % of the Caucasian population has four naturally occurring variants (B*44:02^{156Asp}, 44:03^{156Leu}, 44:28^{156Arg}, 44:35^{156Glu}), which exclusively differ by just one single AA at residue 156. The mismatch B*44:02^{156Asp} / B*44:03^{156Leu} e.g. is described to represent a non-permissive transplantation scenario. The association with strong alloreactive T-cell responses due to distinct structural differences between B*44:02 and B*44:03 pHLA complexes leads to acute GvHD [79]. It has also been demonstrated that the resulting mismatch leads to a disparity in the derived peptide repertoire, which explains the cytotoxic T-lymphocyte recognition of different pHLA landscapes between B*44:02 and B*44:03. Structural involvement of position 156 in influencing the conformation of PBR was demonstrated by comparing the crystal structures of HLA-B*44:02 and HLA-B*44:03 complexed with the same, natural high affinity ligand [18]. In order to determine if position 156 is also involved in the PLC/HLA association and if polymorphism at this position affect TPN dependency through alteration of the structure and property of the PBR and subsequently the peptide repertoire, we investigated the mode of peptide loading for the B*44/156 mismatched variants.

Our data demonstrates that exclusively HLA-B*44:28^{156Arg} variant can acquire peptides independently of TPN and that AA position 156 is unambiguously responsible for the HLA/TPN interaction within B*44 subtypes. Based on its position and orientation, residue 156 is unlikely to contact TPN directly. Similarly, TPN-dependent B*44:02 and TPN-independent B*44:05 alleles with a micropolymorphic difference at residue 116 also appear unlikely to contact TPN directly. Although AA residue 156 is not a part of the first segment of α2-helix, it is likely that it influences the strand/loop region that TPN interacts with and in a similar manner to residue 116 affects the stability/dynamics of the unloaded HLA molecule.

By systematically analysing the influence of residue 156 in B*44 variants and their interaction with TPN could clearly be demonstrated. Using mass spectrometry we sequenced those peptides derived from B*44:02 aquired with the assistance of TPN and hence through the optimization machinery of the PLC and compared those with peptides bound to B*44:28 aquired in a TPN-independent manner. Significant differences between these sets of peptides could be observed, both in their attributed binding affinity and in the length of the derived peptides. The peptide repertoires of sHLA-B*44:02 and sHLA-B*44:28 display subtle differences, suggesting an alternate antigen presentation pathway, the core binding motifs are strongly retained [18]. The results from the structural insight through computational analysis indicated a role for 156Arg in increasing the stability of the pHLA complex through contacts to both Asp114 and to peptide backbone at P5 (Figure 3).



Based on the B*44:02 structure (1M6O) [76] all 20 AAs were modelled at position 156 fitting the best side chain rotamer. Arg156 shows increased hydrogen bonding both to residue Asp114 and to peptide backbone. This is likely to increase stability of the HLA-peptide complex.

Figure 3. B*44/156 substitution model

Our results indicate that the HLA-B*44:28^{156Arg} variant stabilises the binding groove in its empty state, thus negating the contribution of the PLC and allowing independent loading of high affinity peptides. The interaction between Arg156 and Asp114 on the floor of the peptide binding groove seems to be able to generate a stable peptide receptive state.

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10. Conclusion

TPN independency offers flexibility on one hand, because it provides an effective pathogen evasion, however peptides are loaded suboptimally and that might influence the immunogenicity and half-life time of pHLA complexes and this might result in autoimmunity.

The question whether TPN-dependency or TPN-independency is advantageous or not is likely to depend on the combination of HLA-A, -B, and C- alleles of an individual. An appreciation of the interaction between TPN, HLA class I molecules and peptide loading may therefore be important not only during viral infections, but also while considering transplantation scenarios.

Nomenclature

- Human leucocyte antigens (HLA)
- Major Histocompatibility Complex (MHC)
- T cell receptor (TCR)
- peptide-HLA complexes (pHLA)
- peptide-binding region (PBR)
- hematopoietic stem cell transplantation (HSCT)
- graft-versus-host disease (GvHD)
- amino acid (AA)
- heavy chain (hc)
- β2 microglobulin (β2m)
- peptide loading complex (PLC)
- endoplasmic reticulum (ER)
- transporter associated with antigen processing (TAP)
- tapasin (TPN)
- cytotoxic T lymphocyte (CTL)
- human cytomegalie virus (HCMV)
- Calnexin (CNX)
- Calreticulin (CRT)

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References

[1] Germain RN, Margulies DH. The biochemistry and cell biology of antigen processing and presentation. Annu Rev Immunol. 1993;11:403-50.

[2] Zinkernagel RM, Doherty PC. Restriction of in vitro T cell-mediated cytotoxicity in lymphocytic choriomeningitis within a syngeneic or semiallogeneic system. Nature. 1974 Apr 19;248(450):701-2.

[3] Marsh SG, Albert ED, Bodmer WF, Bontrop RE, Dupont B, Erlich HA, et al. An update to HLA nomenclature, 2010. Bone Marrow Transplant. May;45(5):846-8.

[4] Bjorkman PJ, Saper MA, Samraoui B, Bennett WS, Strominger JL, Wiley DC. Structure of the human class I histocompatibility antigen, HLA-A2. Nature. [Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, P.H.S.]. 1987 Oct 8-14;329(6139):506-12.

[5] Bjorkman PJ, Parham P. Structure, function, and diversity of class I major histocompatibility complex molecules. Annu Rev Biochem. 1990;59:253-88.

[6] Klein J, Sato A. The HLA system. First of two parts. N Engl J Med. 2000 Sep 7;343(10): 702-9.

[7] Falk K, Rotzschke O, Stevanovic S, Jung G, Rammensee HG. Allele-specific motifs revealed by sequencing of self-peptides eluted from MHC molecules. Nature. 1991 May 23;351(6324):290-6.

[8] Zuckerman T, Rowe JM. Alternative donor transplantation in acute myeloid leukemia: which source and when? Curr Opin Hematol. 2007 Mar;14(2):152-61.

[9] Mickelson EM, Petersdorf E, Anasetti C, Martin P, Woolfrey A, Hansen JA. HLA matching in hematopoietic cell transplantation. Hum Immunol. 2000 Feb;61(2): 92-100.

[10] Ottinger HD, Ferencik S, Beelen DW, Lindemann M, Peceny R, Elmaagacli AH, et al. Hematopoietic stem cell transplantation: contrasting the outcome of transplantations from HLA-identical siblings, partially HLA-mismatched related donors, and HLA-matched unrelated donors. Blood. 2003 Aug 1;102(3):1131-7.

[11] Schaffer M, Aldener-Cannava A, Remberger M, Ringden O, Olerup O. Roles of HLA-B, HLA-C and HLA-DPA1 incompatibilities in the outcome of unrelated stem-cell transplantation. Tissue Antigens. 2003 Sep;62(3):243-50.

[12] Bade-Doeding C, Cano P, Huyton T, Badrinath S, Eiz-Vesper B, Hiller O, et al. Mismatches outside exons 2 and 3 do not alter the peptide motif of the allele group B*44:02P. Hum Immunol. Nov;72(11):1039-44.

[13] Bade-Doeding C, DeLuca DS, Seltsam A, Blasczyk R, Eiz-Vesper B. Amino acid 95 causes strong alteration of peptide position Pomega in HLA-B*41 variants. Immunogenetics. 2007 Apr;59(4):253-9.

[14] Bade-Doeding C, Eiz-Vesper B, Figueiredo C, Seltsam A, Elsner HA, Blasczyk R. Peptide-binding motif of HLA-A*6603. Immunogenetics. 2005 Jan;56(10):769-72.

[15] Bade-Doeding C, Elsner HA, Eiz-Vesper B, Seltsam A, Holtkamp U, Blasczyk R. A single amino-acid polymorphism in pocket A of HLA-A*6602 alters the auxiliary anchors compared with HLA-A*6601 ligands. Immunogenetics. 2004 May;56(2):83-8.

[16] Bade-Doeding C, Huyton T, Eiz-Vesper B, Blasczyk R. The composition of the F pocket in HLA-A*74 generates C-terminal promiscuity among its bound peptides. Tissue Antigens. Nov;78(5):378-81.

[17] Badrinath S, Huyton T, Schumacher H, Blasczyk R, Bade-Doeding C. Position 45 influences the peptide binding motif of HLA-B*44:08. Immunogenetics. Mar;64(3): 245-9.

[18] Badrinath S, Saunders P, Huyton T, Aufderbeck S, Hiller O, Blasczyk R, et al. Position 156 influences the peptide repertoire and tapasin dependency of human leukocyte antigen B*44 allotypes. Haematologica. Jan;97(1):98-106.

[19] Elamin NE, Bade-Doeding C, Blasczyk R, Eiz-Vesper B. Polymorphism between HLA-A*0301 and A*0302 located outside the pocket F alters the POmega peptide motif. Tissue Antigens. Dec;76(6):487-90.

[20] Huyton T, Ladas N, Schumacher H, Blasczyk R, Bade-Doeding C. Pocketcheck: updating the HLA class I peptide specificity roadmap. Tissue Antigens. Sep;80(3): 239-48.

[21] Huyton T, Schumacher H, Blasczyk R, Bade-Doeding C. Residue 81 confers a restricted C-terminal peptide binding motif in HLA-B*44:09. Immunogenetics. Sep;64(9): 663-8.

[22] Krausa P, Munz C, Keilholz W, Stevanovic S, Jones EY, Browning M, et al. Definition of peptide binding motifs amongst the HLA-A*30 allelic group. Tissue Antigens. 2000 Jul;56(1):10-8.

[23] Prilliman K, Lindsey M, Zuo Y, Jackson KW, Zhang Y, Hildebrand W. Large-scale production of class I bound peptides: assigning a signature to HLA-B*1501. Immunogenetics. 1997;45(6):379-85.

[24] Prilliman KR, Jackson KW, Lindsey M, Wang J, Crawford D, Hildebrand WH. HLA-B15 peptide ligands are preferentially anchored at their C termini. J Immunol. 1999 Jun 15;162(12):7277-84.

[25] Bray RA, Hurley CK, Kamani NR, Woolfrey A, Muller C, Spellman S, et al. National marrow donor program HLA matching guidelines for unrelated adult donor hematopoietic cell transplants. Biol Blood Marrow Transplant. 2008 Sep;14(9 Suppl):45-53.

[26] Morishima Y, Kawase T, Malkki M, Petersdorf EW. Effect of HLA-A2 allele disparity on clinical outcome in hematopoietic cell transplantation from unrelated donors. Tissue Antigens. 2007 Apr;69 Suppl 1:31-5.

[27] Morishima Y, Sasazuki T, Inoko H, Juji T, Akaza T, Yamamoto K, et al. The clinical significance of human leukocyte antigen (HLA) allele compatibility in patients receiving a marrow transplant from serologically HLA-A, HLA-B, and HLA-DR matched unrelated donors. Blood. 2002 Jun 1;99(11):4200-6.

[28] Petersdorf EW. Optimal HLA matching in hematopoietic cell transplantation. Curr Opin Immunol. 2008 Oct;20(5):588-93.

[29] Petersdorf EW, Hansen JA, Martin PJ, Woolfrey A, Malkki M, Gooley T, et al. Major-histocompatibility-complex class I alleles and antigens in hematopoietic-cell transplantation. N Engl J Med. 2001 Dec 20;345(25):1794-800.

[30] Schoenhals GJ, Krishna RM, Grandea AG, 3rd, Spies T, Peterson PA, Yang Y, et al. Retention of empty MHC class I molecules by tapasin is essential to reconstitute antigen presentation in invertebrate cells. Embo J. 1999 Feb 1;18(3):743-53.

[31] Ortmann B, Copeman J, Lehner PJ, Sadasivan B, Herberg JA, Grandea AG, et al. A critical role for tapasin in the assembly and function of multimeric MHC class I-TAP complexes. Science. 1997 Aug 29;277(5330):1306-9.

[32] Lee S, Yoon J, Park B, Jun Y, Jin M, Sung HC, et al. Structural and functional dissection of human cytomegalovirus US3 in binding major histocompatibility complex class I molecules. J Virol. 2000 Dec;74(23):11262-9.

[33] Peh CA, Burrows SR, Barnden M, Khanna R, Cresswell P, Moss DJ, et al. HLA-B27-restricted antigen presentation in the absence of tapasin reveals polymorphism in mechanisms of HLA class I peptide loading. Immunity. 1998 May;8(5):531-42.

[34] Bennett EM, Bennink JR, Yewdell JW, Brodsky FM. Cutting edge: adenovirus E19 has two mechanisms for affecting class I MHC expression. J Immunol. 1999 May 1;162(9): 5049-52.

[35] Byun MW, Kim JH, Kim DH, Kim HJ, Jo C. Effects of irradiation and sodium hypochlorite on the micro-organisms attached to a commercial food container. Food Microbiol. 2007 Aug;24(5):544-8.

[36] Lilley BN, Ploegh HL. Viral modulation of antigen presentation: manipulation of cellular targets in the ER and beyond. Immunol Rev. 2005 Oct;207:126-44.

[37] Lybarger L, Wang X, Harris M, Hansen TH. Viral immune evasion molecules attack the ER peptide-loading complex and exploit ER-associated degradation pathways. Curr Opin Immunol. 2005 Feb;17(1):71-8.

[38] Wiertz EJ, Jones TR, Sun L, Bogyo M, Geuze HJ, Ploegh HL. The human cytomegalovirus US11 gene product dislocates MHC class I heavy chains from the endoplasmic reticulum to the cytosol. Cell. 1996 Mar 8;84(5):769-79.

[39] Wiertz EJ, Tortorella D, Bogyo M, Yu J, Mothes W, Jones TR, et al. Sec61-mediated transfer of a membrane protein from the endoplasmic reticulum to the proteasome for destruction. Nature. 1996 Dec 5;384(6608):432-8.

[40] Schwartz O, Marechal V, Le Gall S, Lemonnier F, Heard JM. Endocytosis of major histocompatibility complex class I molecules is induced by the HIV-1 Nef protein. Nat Med. 1996 Mar;2(3):338-42.

[41] Collins KL, Chen BK, Kalams SA, Walker BD, Baltimore D. HIV-1 Nef protein protects infected primary cells against killing by cytotoxic T lymphocytes. Nature. 1998 Jan 22;391(6665):397-401.

[42] Reusch U, Muranyi W, Lucin P, Burgert HG, Hengel H, Koszinowski UH. A cytomegalovirus glycoprotein re-routes MHC class I complexes to lysosomes for degradation. Embo J. 1999 Feb 15;18(4):1081-91.

[43] Lehner PJ, Surman MJ, Cresswell P. Soluble tapasin restores MHC class I expression and function in the tapasin-negative cell line.220. Immunity. 1998 Feb;8(2):221-31.

[44] Li S, Paulsson KM, Chen S, Sjogren HO, Wang P. Tapasin is required for efficient peptide binding to transporter associated with antigen processing. J Biol Chem. 2000 Jan 21;275(3):1581-6.

[45] Barnden MJ, Purcell AW, Gorman JJ, McCluskey J. Tapasin-mediated retention and optimization of peptide ligands during the assembly of class I molecules. J Immunol. 2000 Jul 1;165(1):322-30.

[46] Purcell AW, Kelly AJ, Peh CA, Dudek NL, McCluskey J. Endogenous and exogenous factors contributing to the surface expression of HLA B27 on mutant APC. Hum Immunol. 2000 Feb;61(2):120-30.

[47] Lewis JW, Elliott T. Evidence for successive peptide binding and quality control stages during MHC class I assembly. Curr Biol. 1998 Jun 4;8(12):717-20.

[48] Yu YY, Myers NB, Hilbert CM, Harris MR, Balendiran GK, Hansen TH. Definition and transfer of a serological epitope specific for peptide-empty forms of MHC class I. Int Immunol. 1999 Dec;11(12):1897-906.

[49] Yu YY, Turnquist HR, Myers NB, Balendiran GK, Hansen TH, Solheim JC. An extensive region of an MHC class I alpha 2 domain loop influences interaction with the assembly complex. J Immunol. 1999 Oct 15;163(8):4427-33.

[50] Suh WK, Derby MA, Cohen-Doyle MF, Schoenhals GJ, Fruh K, Berzofsky JA, et al. Interaction of murine MHC class I molecules with tapasin and TAP enhances peptide loading and involves the heavy chain alpha3 domain. J Immunol. 1999 Feb 1;162(3): 1530-40.

[51] Carreno BM, Schreiber KL, McKean DJ, Stroynowski I, Hansen TH. Aglycosylated and phosphatidylinositol-anchored MHC class I molecules are associated with calnexin. Evidence implicating the class I-connecting peptide segment in calnexin association. J Immunol. 1995 May 15;154(10):5173-80.

[52] Lewis JW, Neisig A, Neefjes J, Elliott T. Point mutations in the alpha 2 domain of HLA-A2.1 define a functionally relevant interaction with TAP. Curr Biol. 1996 Jul 1;6(7):873-83.

[53] Peace-Brewer AL, Tussey LG, Matsui M, Li G, Quinn DG, Frelinger JA. A point mutation in HLA-A*0201 results in failure to bind the TAP complex and to present virus-derived peptides to CTL. Immunity. 1996 May;4(5):505-14.

[54] Dong G, Wearsch PA, Peaper DR, Cresswell P, Reinisch KM. Insights into MHC class I peptide loading from the structure of the tapasin-ERp57 thiol oxidoreductase heterodimer. Immunity. 2009 Jan 16;30(1):21-32.

[55] Garstka MA, Fritzsche S, Lenart I, Hein Z, Jankevicius G, Boyle LH, et al. Tapasin dependence of major histocompatibility complex class I molecules correlates with their conformational flexibility. Faseb J. Nov;25(11):3989-98.

[56] Chen M, Bouvier M. Analysis of interactions in a tapasin/class I complex provides a mechanism for peptide selection. EMBO J. [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. 2007 Mar 21;26(6):1681-90.

[57] Garbi N, Tan P, Diehl AD, Chambers BJ, Ljunggren HG, Momburg F, et al. Impaired immune responses and altered peptide repertoire in tapasin-deficient mice. Nat Immunol. 2000 Sep;1(3):234-8.

[58] Williams AP, Peh CA, Purcell AW, McCluskey J, Elliott T. Optimization of the MHC class I peptide cargo is dependent on tapasin. Immunity. 2002 Apr;16(4):509-20.

[59] Wearsch PA, Cresswell P. Selective loading of high-affinity peptides onto major histocompatibility complex class I molecules by the tapasin-ERp57 heterodimer. Nat Immunol. [Research Support, Non-U.S. Gov't]. 2007 Aug;8(8):873-81.

[60] Praveen PV, Yaneva R, Kalbacher H, Springer S. Tapasin edits peptides on MHC class I molecules by accelerating peptide exchange. Eur J Immunol. Jan;40(1):214-24.

[61] Barber LD, Howarth M, Bowness P, Elliott T. The quantity of naturally processed peptides stably bound by HLA-A*0201 is significantly reduced in the absence of tapasin. Tissue Antigens. 2001 Dec;58(6):363-8.

[62] Zarling AL, Luckey CJ, Marto JA, White FM, Brame CJ, Evans AM, et al. Tapasin is a facilitator, not an editor, of class I MHC peptide binding. J Immunol. 2003 Nov 15;171(10):5287-95.

[63] Howarth M, Williams A, Tolstrup AB, Elliott T. Tapasin enhances MHC class I peptide presentation according to peptide half-life. Proc Natl Acad Sci U S A. 2004 Aug 10;101(32):11737-42.

[64] Zernich D, Purcell AW, Macdonald WA, Kjer-Nielsen L, Ely LK, Laham N, et al. Natural HLA class I polymorphism controls the pathway of antigen presentation and susceptibility to viral evasion. J Exp Med. 2004 Jul 5;200(1):13-24.

[65] Sieker F, Straatsma TP, Springer S, Zacharias M. Differential tapasin dependence of MHC class I molecules correlates with conformational changes upon peptide dissociation: a molecular dynamics simulation study. Mol Immunol. 2008 Aug;45(14): 3714-22.

[66] Fussell HE, Kunkel LE, Lewy CS, McFarland BH, McCarty D. Using a standardized patient walk-through to improve implementation of clinical trials. J Subst Abuse Treat. 2008 Dec;35(4):470-5.

[67] Greenwood R, Shimizu Y, Sekhon GS, DeMars R. Novel allele-specific, post-translational reduction in HLA class I surface expression in a mutant human B cell line. J Immunol. 1994 Dec 15;153(12):5525-36.

[68] Grandea AG, 3rd, Androlewicz MJ, Athwal RS, Geraghty DE, Spies T. Dependence of peptide binding by MHC class I molecules on their interaction with TAP. Science. 1995 Oct 6;270(5233):105-8.

[69] Lewis JW, Sewell A, Price D, Elliott T. HLA-A*0201 presents TAP-dependent peptide epitopes to cytotoxic T lymphocytes in the absence of tapasin. Eur J Immunol. 1998 Oct;28(10):3214-20.

[70] Neisig A, Wubbolts R, Zang X, Melief C, Neefjes J. Allele-specific differences in the interaction of MHC class I molecules with transporters associated with antigen processing. J Immunol. 1996 May 1;156(9):3196-206.

[71] Park B, Lee S, Kim E, Ahn K. A single polymorphic residue within the peptide-binding cleft of MHC class I molecules determines spectrum of tapasin dependence. J Immunol. 2003 Jan 15;170(2):961-8.

[72] Turnquist HR, Thomas HJ, Prilliman KR, Lutz CT, Hildebrand WH, Solheim JC. HLA-B polymorphism affects interactions with multiple endoplasmic reticulum proteins. Eur J Immunol. 2000 Oct;30(10):3021-8.

[73] Turnquist HR, Schenk EL, McIlhaney MM, Hickman HD, Hildebrand WH, Solheim JC. Disparate binding of chaperone proteins by HLA-A subtypes. Immunogenetics. 2002 Feb;53(10-11):830-4.

[74] Cresswell P, Bangia N, Dick T, Diedrich G. The nature of the MHC class I peptide loading complex. Immunol Rev. 1999 Dec;172:21-8.

[75] Chelvanayagam G. A roadmap for HLA-A, HLA-B, and HLA-C peptide binding specificities. Immunogenetics. 1996;45(1):15-26.

[76] Macdonald WA, Purcell AW, Mifsud NA, Ely LK, Williams DS, Chang L, et al. A naturally selected dimorphism within the HLA-B44 supertype alters class I structure, peptide repertoire, and T cell recognition. J Exp Med. 2003 Sep 1;198(5):679-91.

[77] Archbold JK, Macdonald WA, Gras S, Ely LK, Miles JJ, Bell MJ, et al. Natural micropolymorphism in human leukocyte antigens provides a basis for genetic control of antigen recognition. J Exp Med. 2009 Jan 16;206(1):209-19.

[78] Park B, Kim Y, Shin J, Lee S, Cho K, Fruh K, et al. Human cytomegalovirus inhibits tapasin-dependent peptide loading and optimization of the MHC class I peptide cargo for immune evasion. Immunity. 2004 Jan;20(1):71-85.

[79] Fleischhauer K, Kernan NA, O'Reilly RJ, Dupont B, Yang SY. Bone marrow-allograft rejection by T lymphocytes recognizing a single amino acid difference in HLA-B44. N Engl J Med. 1990 Dec 27;323(26):1818-22.

[80] Fleischhauer K, Avila D, Vilbois F, Traversari C, Bordignon C, Wallny HJ. Characterization of natural peptide ligands for HLA-B*4402 and -B*4403: implications for peptide involvement in allorecognition of a single amino acid change in the HLA-B44 heavy chain. Tissue Antigens. 1994 Nov;44(5):311-7.