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Polymorphisms of HLA-B: influences on assembly and immunity

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The major histocompatibility class I (MHC-I) complex functions in innate and adaptive immunity, mediating surveillance of the subcellular environment. In humans, MHC-I heavy chains are encoded by three genes: the human leukocyte antigen (*HLA*)-*A*, *HLA-B,* and *HLA-C*. These genes are highly polymorphic, which results in the expression, typically, of six different HLA class I (HLA-I) proteins on the cell surface, and the presentation of diverse peptide antigens to $CD8^+$ T cells for broad surveillance against many pathogenic conditions. Recent studies of HLA-B allotypes show that the polymorphisms, not surprisingly, also significantly impact protein folding and assembly pathways. The use of non-canonical assembly routes and the generation of non-canonical HLA-B conformers has consequences for immune receptor interactions and disease therapies.

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Introduction

The MHC-I complex provides a means for the immune system to identify cells with non-self (foreign) or altered-self (cancer or cell stress-induced) proteins. In humans, the high degree of polymorphism of HLA-I genes results in significant diversity of peptide antigen presentation to T cells (Figure 1), but also in differences in the intracellular folding and assembly characteristics of the proteins (Figures 2 and 3) and in cell-surface interactions with immune receptors (Figure 4). Ultimately, at the organismal level, the polymorphisms link to protection from or association with disease. This review will summarize the recent literature which examines all these aspects of the biology of HLA-B, the most polymorphic of the HLA-I genes.

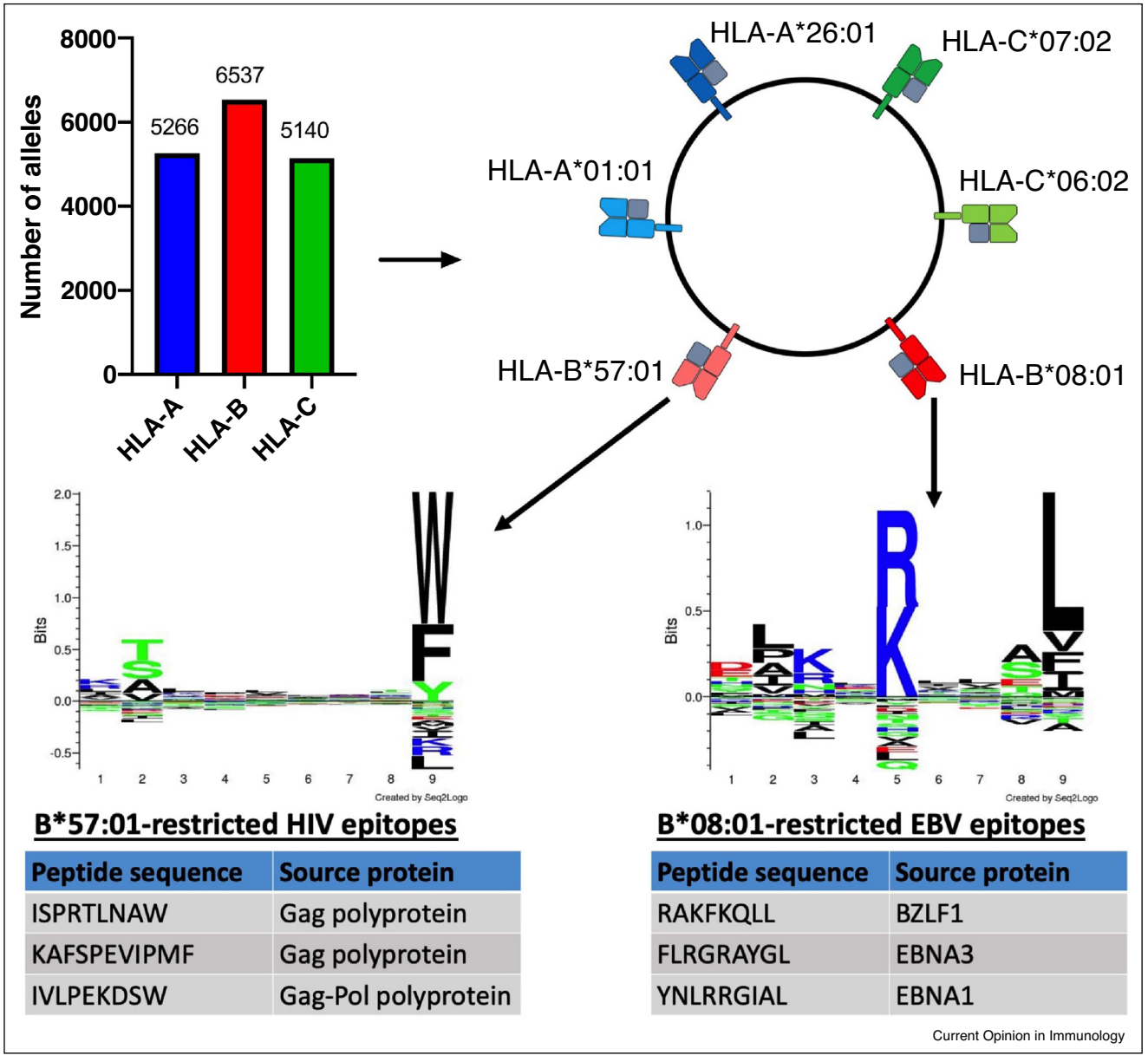
Canonical MHC-I assembly in the ER

MHC-I assembly is highly regulated and involves the coordinated action of a number of proteins [1]. Canonical peptide loading occurs in the ER, beginning with MHC-I heavy chain dimerization with the invariant β_2-microglobulin (β_2m) light chain. The heavy chain-β_2m dimer is generally (but not always) unstable in the absence of peptides [2,3•], and associates with the peptide loading complex (PLC) which comprises the chaperone proteins calreticulin and ERp57, tapasin, and the transporter associated with antigen processing (TAP). Peptides that are processed by the cytoplasmic proteasome are bound by and transported into the ER lumen by heterodimeric TAP1/TAP2 complexes, the two subunits of TAP. Peptides of appropriate sequence specificity bind to the MHC-I heterodimers with the help of the PLC [1]. Long peptides may be further trimmed by ER-aminopeptidases (ERAP1 and ERAP2) [4]. The fully assembled heavy chain-β_2m-peptide complex dissociates from the PLC and traffics to the cell surface via the Golgi apparatus.

Recently, the electron microscopy (EM) structure of a PLC was solved [5•], demonstrating the full network of chaperone-MHC-I interactions within the PLC. These structures revealed a stoichiometric ratio of two sets of chaperone-MHC-I complexes per TAP1/TAP2 dimer. Tapasin, a key component of the PLC, is important for generation of stable MHC-I complexes with long half-lives [6]. Unloaded or suboptimally loaded MHC-I are preferentially bound by tapasin and tapasin-ERp57 complexes. Additionally, unloaded or suboptimally loaded MHC-I are glucosylated by UDP-glycoprotein glucosyltransferase (UGGT), leading to a glycan-dependent interaction between MHC-I and calreticulin. This interaction is important for stable and productive PLC binding and quality control [7]. The entire network of chaperone-MHC-I interactions [5•] is generally important for full stability of the PLC, for stable surface peptide-MHC-I complexes, and efficient antigen presentation to CTLs.

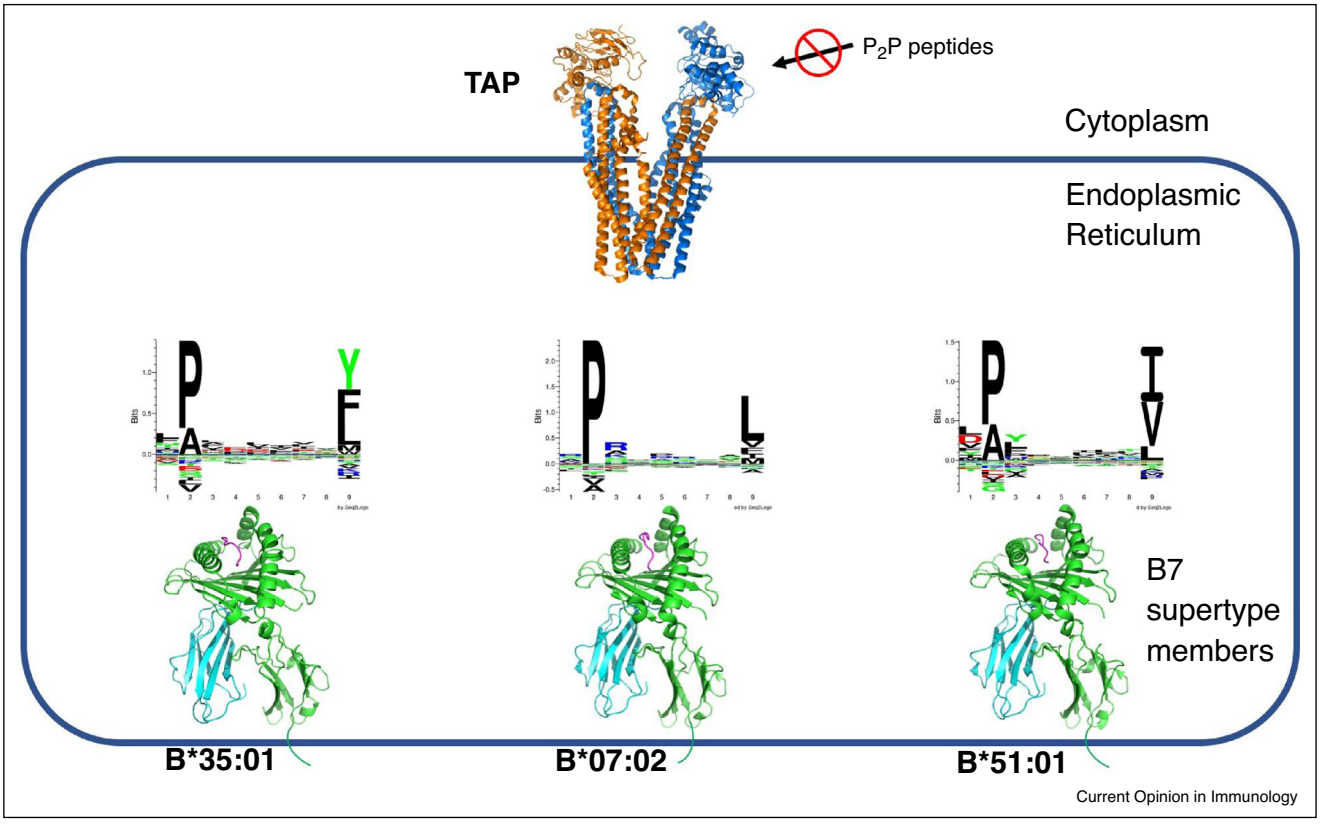
Much work has been done recently to elucidate the role of TAPBPR, which modulates HLA-I assembly downstream of the PLC in a manner that is mutually exclusive with tapasin [8]. Crystal structures have been solved of MHC-I complexes with TAPBPR; TAPBPR can be seen looping into the MHC-I binding groove to provide

Figure 1



As in all domains of science, diversity of representation is critical for effective CD8$^+$ T cell immunity. The HLA class I genes are highly polymorphic, with the HLA-B gene being the most diverse at 6537 alleles (as of 2019: https://www.ebi.ac.uk/ipd/imgt/hla/stats.html). The high degree of polymorphism at each locus typically results in expression of six different HLA-I proteins on the cell surface: two HLA-A, two HLA-B, and two HLA-C. Two HLA-I haplotypes (co-inherited allele combinations present on a single chromosome) with high frequency worldwide (A*01:01-B*57:01-C*06:02 and A*26:01-B*08:01-C*07:02 [56]) are shown on the cell. While each HLA-I allotype can bind a large number of peptides, the polymorphic residues located within the HLA-I peptide-binding site affect the peptide repertoire. Mass spectrometry-derived peptide motifs [15,57] for the two HLA-B allotypes are shown as Seq2Logo diagrams [58]. Peptides bound to each allotype share specific amino acid features at 'anchor' positions where the peptides interact with the HLA-I peptide-binding site. The surface expression of six distinct allotypes ensures a diversity of peptide antigen presentation to CTLs, such that in a given pathogenic condition, at least one of six HLA-I of the cell can present peptides that trigger CD8$^+$ T responses. Representative known immunodominant viral epitopes presented by the two HLA-B allotypes in HIV and EBV infection are shown. HLA-I heterozygosity at each locus is thought to confer immune response advantages.

Figure 2



A mismatch between the peptide-binding preferences of TAP and HLA-B7 supertype members. The HLA-B7 supertype comprises several allotypes with a preference for prolines at position 2 (P_2P peptides). The crystal structures of three common B7 members, B*35:01, B*07:02, and B*51:01, are shown with the heavy chains (green) in complex with a peptide (purple) and β_2m (cyan) (PDB 1a1n, 5eo0, and 1e28, respectively). All three allotypes have strong P_2P preferences, as shown in the Seq2Logo plots based on previously published peptidome data [15,57]. Notably, the peptide transporter TAP (PDB code 5u1d), comprising the TAP1 (blue) and TAP2 (orange) subunits, has a cytosolic peptide-binding site that sequesters 9-15 mer peptides before their ATP-dependent transport. TAP's peptide-binding strongly disfavors P_2P peptides for binding and transport [28], creating a discrepancy between the availability of high-affinity peptides from TAP and the binding preference of HLA-B7 members in the ER. Thus, HLA-I allotypes with a P_2P specificity are predicted to at least partially rely on TAP-independent peptide sources and/or ERAP-dependent trimming of long peptide sequences transported by TAP.

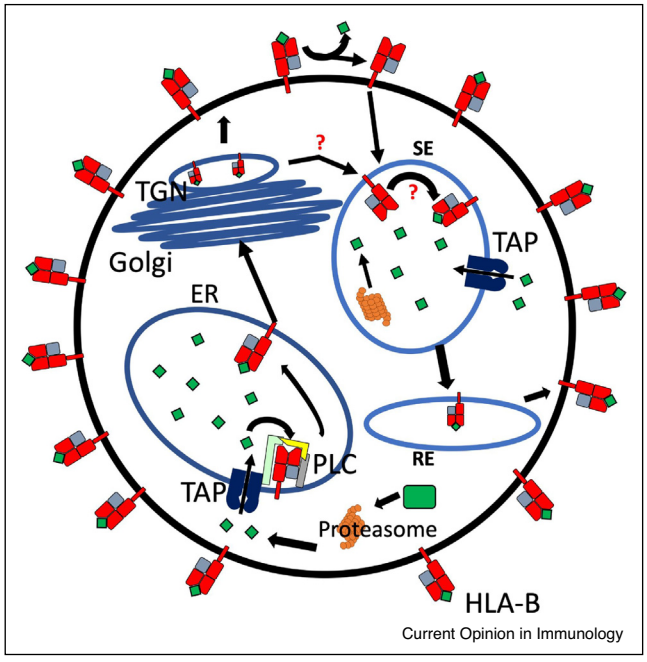
stabilization in the absence of peptide [9•,10•]. Because of the structural relatedness of tapasin and TAPBPR, these structures also provide high resolution models for better understanding of tapasin-MHC-I interactions.

Varying assembly and expression characteristics of HLA-B allotypes

Assembly of MHC-I complexes in the ER via the PLC is generally thought to be optimized in primary human cells so that stable complexes are always generated. However, the high degree of polymorphism of the HLA-I locus also results in a high degree of variability in peptide loading pathway requirements. HLA-B allotypes have diverse requirements for tapasin, and several allotypes are able to efficiently assemble in tapasin-deficient cells [11–13]. Tapasin-independent allotypes generally have high intrinsic conformational stabilities of their peptide-free (empty) forms [3•,6,13]. Paradoxically, in HIV infections, the presence of tapasin-independent HLA-B conferred more rapid progression to death [13], although more detailed analyses examining the functional effects of tapasin-independence of HLA-A, B and C allotypes are required for full understanding of the immune consequence of tapasin-independence.

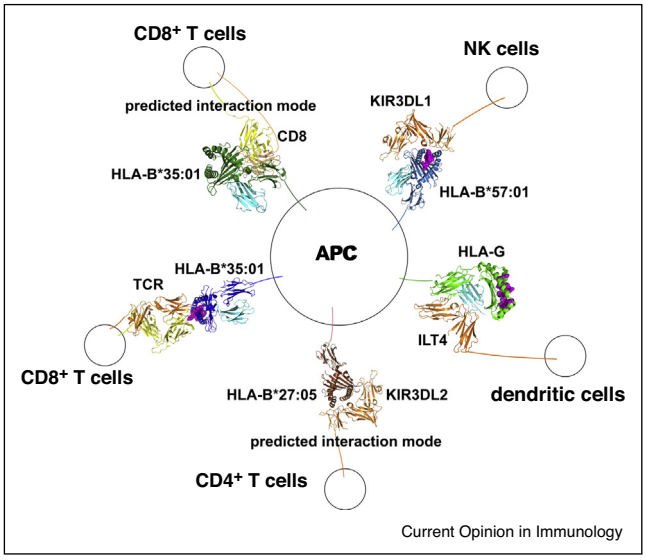
Each HLA-I allotype has its own preference for peptides (Figure 1) [14,15]. Residues at the P_2 position are often highly conserved among related allotypes, which are grouped into supertypes [16]. For example, B7 supertype members prefer proline at P_2 (P_2P), whereas B44 supertype members prefer P_2E peptides [16]. TAP has its own binding preference, in particular disfavoring P_2P peptides [17]. Thus, B7 supertype members must either encounter a suboptimal loading environment in the ER or bind to

Figure 3



Canonical and non-canonical assembly and surface expression of peptide-filled and empty HLA-I. MHC-I complexes are canonically assembled in the endoplasmic reticulum (ER) by the peptide loading complex (PLC). Peptide-loaded MHC-I molecules are exported to the cell surface via the secretory pathway. While on the surface, peptide may dissociate from the peptide-binding groove due to stochastic processes, leading to a loss of complex stability and endocytosis into the sorting endosome (SE). Additionally, an MHC-I trafficking route to the endosomes from the trans-Golgi network (TGN) has been proposed involving the clathrin adaptor protein AP-1 [18••,21]. Whereas optimal peptide binding results in high cell-surface stability (half-life), the cell-surface half-lives of HLA-B allotypes vary in both allotype and cell-type dependent manners, indicating that optimized peptide-loading is not always achieved in primary human leukocytes [18••]. Additionally, peptide-deficient forms of HLA-I are detected on the cell-surface for many allotypes under PLC-deficiency conditions, and function in augmenting antigen-specific T cell responses, although their precise cell-surface trafficking pathway remains to be defined [3•,18••,19•]. Recent studies have demonstrated the presence of two potential peptide sources in the SE: the immuno-proteasome and the peptide transporter TAP [27•,29•]. These factors, in addition to endo-lysosomal proteases, could provide peptides to endosomal MHC-I complexes, which are loaded and transported/recycled to the surface via the recycling endosome [24]. Of note, the endosomal locations of the proteasome and TAP has only been shown in certain professional antigen-presenting cells, such as monocytes and dendritic cells.

Figure 4



Interactions mediated by canonical and non-canonical cell-surface HLA-I conformers. HLA-I molecules modulate immune responses in a peptide specificity-dependent or peptide-independent manners. **CD8$^+$ T cells**: HLA-I molecules (blue, cyan, purple) present peptides (purple) to cognate T cell receptors (TCR) (yellow, orange). TCRs display exquisite allele and peptide specificity for HLA-I complexes. Shown here is the structure of extracellular domains of HLA-B*35:01-HPVGEADYFEY-TCR complexes (PDB 3mv7). In the second CD8$^+$ T cell panel, HLA-B*35:01 (green, cyan) in its empty (peptide-depleted) version, is shown as binding the T cell co-receptor CD8 (yellow, orange). This interaction was shown to have stronger affinity compared with CD8 binding to peptide-filled HLA-B*35:01 and also enhance CD8$^+$ T cell adhesion to target cells in peptide antigen-dependent CD8$^+$ T cell responses [3•]. The extracellular domain of HLA-B*35:01 derived from HLA-B*35:01-VPLRPMTY (PDB 1a1n) was superimposed onto H-2D^d-RGPGRAFVTI-CD8$\alpha\beta$ complex (PDB 3dmm) by aligning Cαs of HLA-B•35:01 and H2-D^d, followed by deletion of peptides and H2-D^d. **NK Cells**: KIR receptors (orange) bind select HLA-I allotypes (blue, cyan, purple). For inhibitory KIR, the binding is not very peptide-specific, whereas the structurally related activating KIR bind HLA-I in significantly peptide-dependent manners [40,41]. Shown here is the structure of extracellular domains of HLA-B*57:01-LSSPVTKSF-KIR3DL1 complex (PDB 3vh8). **Dendritic Cells**: ILT2/4 (orange) recognize HLA-I molecules (green, cyan, purple) in a peptide-sequence independent manner. ILT4 contacts HLA-I at α3 domain, away from the peptide binding pocket. Shown here is the structure of extracellular domains of HLA-G-RIIPRHLQL-ILT4 complex (PDB 2dyp). **CD4$^+$ T cells:** Heavy chains of HLA-B*27:05 (brown) are proposed to be ligands for KIR3DL2 (orange), and to modulate CD4$^+$ T cell responses [49]. Structure of HLA-B*27:05 heavy chain (brown) is derived from HLA-B*27:05-GRFAAAIAK by deleting peptide and β_2M (PDB 1jge); a structure of KIR3DL2 is not available, thus the D0D1D2 domain of KIR3DL1 derived from HLA-B*57:01-LSSPVTKSF-KIR3DL1 complex (PDB 3vh8) is used here to depict the predicted mode of binding. The HLA-B*27:05 structure was superimposed onto the KIR3DL1-LSSPVTKSF-HLA-B*57:01 by aligning Cαs of HLA-B*27:05 and HLA-B*57:01, and HLA-B*57:01 was then deleted.

amino-terminally extended peptides that are then trimmed to size by ERAP (Figure 2). To explore the consequences of the mismatch in peptide-binding preferences between TAP and the B7 supertype, we recently measured the expression levels and half-lives of select HLA-B allotypes in primary human lymphocytes. Individual HLA-I expression/half-life measurements on primary cells requires a careful choice of antibodies, understanding of antibody binding specificities for the closely related polymorphic HLA-I variants, as well as a knowledge of full HLA-I genotypes of donors [18••]. We found that, indeed, B7 supertype members were expressed at lower levels than non-P_2P-restricted

allotypes in T cells, which correlated in some cases with shorter global half-lives [18••]. As further evidence for suboptimal assembly, we found that some B7 allotypes have high peptide-receptivity on the surface. These results indicate that in lymphocytes, limitations in the ER peptide pool can lead to lower cell-surface expression and stability, as well as the surface expression of peptide-receptive forms. In a parallel study, we found that B7 allotypes such as B*35:01 and B*51:01 are expressed at relatively high levels in TAP-deficient cell lines, confirming the abilities of these allotype to assemble via alternate TAP-independent pathways [19•]. This study also suggested that there is a potential alternative export pathway for suboptimally loaded conformers involving Golgi-independent trafficking, but the nature of this pathway remains to be elucidated.

Surprisingly, the surface density and peptide-receptivity trends in T cells were reversed in monocytes, which express B7 allotypes at higher levels compared with lymphocytes. Monocytes contain larger intracellular pools of HLA-I than lymphocytes. On imaging cytometry studies, we found that the intracellular HLA-B co-localizes with an AP-1$^+$ compartment. AP-1 is an adaptor protein that facilitates trafficking between the trans-Golgi network and recycling endosomes [20] (Figure 3). Interestingly, AP-1 has been shown to functionally interact with and redirect HLA-I to an intracellular (endocytic) compartment in antigen presenting cells (APCs), but not T cells [21]. These findings together suggest that monocytes possess trafficking pathways which supplement canonical assembly for certain HLA-B allotypes. Overall, it appears that there are cell type-specific mechanisms for optimizing HLA-B assembly, and indeed that assembly optimization, assessed by global cell-surface half-life, is both HLA-B allotype and cell type-dependent. Furthermore, there are cell and allele-dependent differences in the cell-surface levels of HLA-B variants [18••,22] that do not arise from differences in mRNA levels of HLA-B [18••,23]. These differences are predicted to have important consequences for HLA-B-mediated immunity, but in a cell-context dependent manner.

Post-ER HLA-I assembly pathways

It is well known that surface HLA-I is internalized and recycled through endocytic compartments. Additionally, professional APCs have specialized vacuolar pathways that facilitate the presentation of exogenous antigen (cross-presentation) [24]. However, it is possible that these compartments are also important for endogenous antigen presentation, consistent with several studies which have shown the requirement of autophagy pathways for presentation of epitopes derived from endogenously expressed proteins (for example, [25]). Degradation of autophagosome cargo typically requires fusion with lysosomes [26], spatially linking the antigen source to recycling HLA-I in endo-lysosomal compartments.

Recently, a study found that TAP localizes to early endosomes in monocytes and re-distributes to the ER and lysosomes upon differentiation to dendritic cells (DCs) [27•]. While TAP has been shown to be recruited to endosomes or phagosomes of DCs during cross-presentation, this recruitment usually requires TLR4 stimulation [28]. A recent study has also found evidence for the presence of the cytosolic proteasome within phagosomes and endosomes, providing an additional mechanism of antigen processing in these compartments [29•]. The convergence of these assembly factors in a compartment where HLA-I is known to cycle through merits further exploration (Figure 3).

HLA-I and disease

Population studies have shown that particular genotypes of HLA-I, including HLA-B, are linked to differential outcomes in many human diseases, including autoimmune diseases [30], infectious diseases [30–32], and cancers [33]. Despite strong evidence of HLA-disease associations, the underlying mechanisms, in many cases, are not understood. The antigen presentation hypothesis postulates the involvement of immune responses to specific foreign or self-peptides, which in some cases are identified (for example, [34]). As discussed below, differential interactions with innate immune receptors and assembly properties of HLA-I molecules provide additional perspectives to understand HLA-disease associations.

Allele and peptide-dependent influences of HLA-B on immunity to HIV

HLA-B alleles are known to have strong influences on acquired immunodeficiency syndrome (AIDS) progression. Several HLA-B alleles, such as HLA-B*57 and HLA-B*27, are protective in HIV infections by prolonging progression to AIDS, while other alleles, such as HLA-B*35, are linked to rapid progression to AIDS [31,32]. Supporting the antigen presentation hypothesis, a recent study shows that differential HLA-B-mediated protection in HIV infection is at least partially attributable to different preferences of HLA-I allotypes for conserved and topologically important epitopes within viral proteins [35•].

In addition to triggering CTL responses, HLA-I molecules regulate natural killer (NK) cell responses (Figure 4). NK cells express receptors for HLA-I, (the killer-cell immunoglobulin-like receptors, KIRs) that have either inhibitory or activating functions [36]. The inhibitory receptor interactions are broadly HLA-I allotype-dependent, but have low peptide specificity compared with CTL responses. For example, several HLA-B allotypes carrying the Bw4 motifs, specified by residues 77–83 of the heavy chain, are ligands for inhibitory NK cell receptor, KIR3DL1 [37]. In HIV infections, NK cells in individuals carrying highly expressed, highly inhibitory

KIR3DL1 and HLA-Bw4 allotypes were more effective at inhibiting viral load and AIDS progression [38]. These effects were suggested to arise due to the stronger intrinsic activities of the corresponding KIR3DL1$^+$ NK cells, as NK cell activities are thought to be dependent on the expression levels of the inhibitory KIR receptors, their HLA-I ligands and the HLA-I-KIR binding affinities [39]. Recent studies also show HLA-I-allele and peptide-specific responses of some activating NK receptors [40,41], resembling CTL responses. It is thus likely that the peptide-binding preferences of HLA-I influence disease outcomes via activating NK cell receptors, in addition to CTL. Another set of functionally important innate interactions mediated by HLA-I is with the inhibitory immunoglobulin-like transcript 4 (ILT4) receptor. HLA-B*35:03, which is strongly associated with rapid AIDS progression, binds with relatively high affinity to ILT4, which is suggested to inhibit the function of dendritic cells in priming CTL [42]. These findings illustrate the multiple levels of HLA-B allele-dependent influences on immunity, with consequences for disease outcomes.

HLA-I expression variations and disease outcomes

A single nucleotide polymorphism (SNP) upstream of the HLA-C locus was shown to cause higher expression of specific HLA-C alleles, enhancing HIV control but having a negative effect in Crohn's disease [43,44]. More recent studies also show that epigenetic regulation influences allelic expression at the HLA-A locus [45]. Surprisingly, however, elevated HLA-A expression levels associated with poor HIV control [46•]. Elevated HLA-A expression was suggested to enhance activity of the inhibitory NK cell receptor NKG2A by providing peptides for assembly of the non-classical HLA-I molecule, HLA-E, the ligand for NKG2A receptors [46•]. Overall, these findings indicate that the effects of HLA expression levels on disease outcomes are complex and context-dependent.

As noted above, there are not significant allele-dependent variations in the levels of HLA-B transcripts [18••,23]. However, allotypes such as HLA-B*35:01 and HLA-B*07:02, which are linked to poor HIV control [32], display low cell surface expression and stability in CD4$^+$ T cells [18••], and thus are primary targets of HIV-1 due to variations at the level of protein assembly. HLA-B*35:01 and HLA-B*07:02 are not known to engage NK receptors. Whether the lower expression/stability of these allotypes on CD4$^+$ T cells differentially influences CTL responses against HIV infected cells remains to be investigated.

Varying cell-surface conformers of HLA-B, and their influences on immunity

Among the best-known example of HLA-disease associations is ankylosing spondylitis (AS), which is associated with specific HLA-B*27 variants. HLA-B*27 allele carriers account for a large percentage of AS patients among Caucasians. Among HLA-B*27 subtypes, B*27:02, B*27:04, and B*27:05 are associated with AS, while other closely related alleles such as B*27:06 and B*27:09 have no association with AS [47,48]. The mechanism underlying the association between HLA-B*27 and AS is unknown. In line with the antigen presentation hypothesis, the arthritogenic peptide theory proposes that selected HLA-B*27 allotypes can present certain foreign or altered-self peptides which induce cross-reactive CTL responses, although no such epitope has been identified to date. In contrast, the 'aberrant assembly theory' focuses on the unique assembly properties of HLA-B*27 molecules. HLA-B*27 allotypes have a free thiol in their peptide-binding site, and an increased propensity to be expressed as β_2M-free heavy chains, disulfide bond-linked homodimers, or even oligomers [48]. These abnormal species are shown to engage KIR3DL2 on CD4$^+$ T cells to drive an inflammatory IL-17 response [49] (Figure 4). Previous studies also showed that accumulation of misfolded HLA-B*27 induces ER stress and activates the unfolded protein response [50], which is linked to enhanced inflammation [51]. It remains unclear whether many of these HLA-B*27-linked features are specific to AS-associated HLA-B*27 allotypes, particularly in pathologically relevant cells *in vivo*. Genome wide association studies have shown that specific polymorphic variants of ERAP1 are linked to AS [47]. AS-linked ERAP1 variants have higher enzymatic activities, although whether such variants induce the *in vivo* cell surface expression of misfolded HLA-B*27 requires further clarity [48].

Our recent studies show that cell surface peptide-free (empty) conformers of HLA-B*35:01 are detectable on activated T cells and TAP-deficient cells. Empty HLA-B*35:01 binds to the T cell co-receptor CD8 with enhanced affinity compared to the peptide-filled versions (Figure 4), an interaction that enhances antigen-specific CTL responses via increased CTL-target cell adhesion [3•]. Since suboptimal MHC-I peptide-loading is induced by many infections and cancers, the expression of empty HLA-I could help counter pathogenic immune evasion strategies that target the HLA-I pathway. Allele-dependent variations in empty HLA-I cell surface expression are expected, based upon their allele-dependent stability differences [3•,13], as well as incompatibility of the binding preference of some allotypes with the specificity of TAP [18••], as discussed above.

Exploitation of non-canonical HLA-I assembly pathways in cancer therapy

Many tumor cells have somatic mutations within the HLA locus, including mutations of HLA-I alleles or those that disrupt the expression of the components of the PLC, including β_2M, tapasin, and TAP [52]. There is accumulating evidence that the presentation of TAP-independent

self-peptides is induced in TAP-deficient cells, eliciting CTL responses, which can be used as a new strategy to eliminate tumor cells [53,54•,55]. Because of different TAP dependencies and TAP-independent assembly pathways of HLA-B as discussed above [13,19•], HLA-I allotypes are expected to present TAP-independent self-peptides with different efficiencies, leading to the expectation of allele-dependent differences in the protective effects of specific allotypes under TAP-deficiency conditions.

Concluding remarks

While it is clear that HLA-B polymorphisms have significant impact on disease outcomes, the cellular and molecular mechanisms for these differences are often not well understood. The assembly characteristics discussed above likely play a role, but few studies have examined effects of allotype-dependent differences in HLA-B assembly in the context of infectious or autoimmune diseases. More work is necessary to link these branches of the field and provide a cohesive understanding of the role of HLA-B polymorphisms in disease and health.

Conflict of interest statement

Nothing declared.

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- •• of outstanding interest

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