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Conformational sensing of major histocompatibility complex (MHC) class I molecules by immune receptors and intracellular assembly factors

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Abstract

Major histocompatibility complex class I (MHC-I) molecules play a critical role in both innate and adaptive immune responses. The heterodimeric complex of a polymorphic MHC-I heavy chain and a conserved light chain binds to a diverse set of peptides which are presented at the cell surface. Peptide-free (empty) versions of MHC-I molecules are typically retained intracellularly due to their low stability and bound by endoplasmic reticulum chaperones and assembly factors. However, emerging evidence suggests that at least some MHC-I allotypes are relatively stable and detectable at the cell-surface as peptide-deficient conformers, under some conditions. Such MHC-I conformers interact with multiple immune receptors to mediate various immunological functions. Furthermore, conformational sensing of MHC-I molecules by intracellular assembly factors and endoplasmic reticulum chaperones influences the peptide repertoire, with profound consequences for immunity. In this review, we discuss recent advances relating to MHC-I conformational variations and their pathophysiological implications.

Keywords

Major histocompatibility complex class I (MHC-I); Peptide-free MHC-I; Empty MHC-I; Endoplasmic reticulum (ER); Peptide repertoire

Introduction

Cell surface major histocompatibility complex class I (MHC-I) molecules comprise a highly polymorphic heavy chain (HC), a conserved light chain β_2-microglobulin (β_2m), and a diverse set of short peptides with sequences complementary to residues within the peptide-binding site in the HC. Peptides that bind MHC-I molecules are frequently derived from the cytosol of cells, where they undergo cleavage by the proteasomes. The resulting peptides are translocated to the endoplasmic reticulum (ER) lumen by the transporter associated with

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antigen processing (TAP). Before peptide binding, MHC-I molecules are localized in the ER as heterodimers of HC and β_2m. Such peptide-free (empty) heterodimers are generally unstable and in complex with several ER chaperones and other factors that comprise what is called the peptide-loading complex (PLC). The PLC includes TAP, tapasin, calreticulin and ERp57 [1,2]. In the ER lumen, peptides are assembled with MHC-I molecules with the assistance of the PLC components, which facilitate the selection of high affinity peptides. After such peptide loading, MHC-I molecules are released from the PLC and transported to the cell surface via the *Golgi* [1]. Polymorphisms of MHC-I molecules influence their assembly pathways. Many MHC-I allotypes can be successfully assembled in the absence of specific PLC components [3,4].

The α1 and α2 domains of MHC-I molecules, which include the peptide-binding site, constitute the recognition site for T cell receptors (TCRs) (Figure 1). This site partly overlaps with binding sites for other immune receptors for MHC-I, such as specific killer cell immunoglobulin-like receptors (KIRs) (Figure 1), expressed on natural killer (NK) cells and other immune cells [5]. In addition, the membrane-proximal α3 domain of MHC-I modulates immune responses. The major binding site for CD8, a co-receptor for TCRs [6] and some KIRs [7], is contained within the α3 domain (Figure 1). The α3 domain is also the binding site for leukocyte immunoglobulin-like receptors (LIR) 1 and LIR2 [8] (Figure 1), which carry immunoreceptor tyrosine-based inhibitory motifs in their cytosolic domains, and deliver inhibitory signals [9]. Although the membrane-proximal and membrane-distal receptor recognition sites correspond to distinct MHC-I surfaces, accumulating evidence suggests the domains are dynamically coupled [10-13]. Recent studies have revealed important functions of peptide-free MHC-I conformers. Here we review recent advances relating to MHC-I-specific immune receptors and assembly factors, which modulate immune responses through sensing MHC-I conformations.

Polymorphisms of Human MHC-I Molecules and Expression of Sub-Optimally Loaded and Empty Conformers

The human MHC-I HC genes (human leukocyte antigens (HLAs); *HLA-A, HLA-B and HLA-C)* are the most polymorphic genes in humans with thousands of variants in the population [14]. The structures of the variants are broadly conserved. The vast majority of the polymorphic residues are located within the peptide-binding cleft (Figure 1) conferring variants with different peptide-binding specificities. TCRs have fine specificity for individual cell-surface peptide-MHC-I (pMHC-I) complexes, whereas KIR receptor recognition has broad specificity [5,15], although some peptides are known to interfere with KIR binding [16].

MHC-I molecules typically exist as peptide-free forms in the ER during their folding and assembly and in peptide-loaded forms on the cell surface. However, peptide-free versions or free HCs are also observed on the cell surface under certain conditions, such as upon lymphocyte activation [17,18], viral infection [18], or TAP deficiency [19-21]. The exact mechanism of their expression is not elucidated under some of these conditions. One factor that can influence the cell surface expression of empty conformers is the peptide loading

efficiency. When optimal peptide loading is compromised, MHC-I allotypes become loaded with suboptimal peptides, which are more likely to be released after trafficking to the cell surface, thus generating empty conformers of MHC-I [22]. The intrinsic peptide loading efficiency is dictated by the conformational stability of the empty form [23], and for many allotypes, stabilization by the PLC is critical for effective peptide loading and cell surface expression. MHC-I molecules on the surface of cells deficient in TAP [24,25], tapasin [24,26], calreticulin [27], or ERp57 [28], have been shown to be loaded with suboptimal peptides and are thus more peptide-receptive at the cell surface. Given the importance of peptide loading in the antigen presentation process, the PLC is widely targeted by tumor cells and viruses to escape MHC-I mediated immune surveillance. Therefore, under these pathological conditions, suboptimally loaded or empty conformers of MHC-I are more easily produced. Due to varying conformational stabilities of suboptimally loaded forms of different MHC-I allotypes, varying cell surface expression levels are measured in the presence of viral inhibitors of the PLC [21,29,30].

Although the interaction between the highly polymorphic MHC-I molecules and PLC is largely conserved, the functions of the PLC are not fully optimized for the assembly of all MHC-I allotypes [3]. For example, TAP is the major known source of peptides for MHC-I assembly, but in fact, some allotypes are mismatched with TAP in their peptide-binding specificity [31]. Human MHC-I (HLA-I) molecules from the HLA-B7 supertype [32], including the frequent HLA*B35:01 and HLA-B*07:02 allotypes, have specificities for peptides with a proline at the second position, while these peptides are highly disfavored by TAP [33]. For such allotypes, peptide precursors could be transported and further trimmed to optimal sequence by the ER aminopeptidase ERAP, that functions to optimize MHC-I assembly [34]. Nonetheless, the mismatch with TAP specificity results in lower expression and stability of members of the HLA-B7 supertype on the surface of lymphocytes [31]. These studies suggest that even under normal conditions, certain MHC-I allotypes have tendencies to be expressed as suboptimally-loaded or empty conformers.

Suboptimally loaded or empty MHC-I molecules are generally quickly internalized and degraded, and their presence on the cell surface depends on their stabilities [35]. Increasing the stability of MHC-I by lowering the cell culture temperature is known to enhance the cell surface expression level of peptide-receptive conformers of MHC-I in TAP-deficient cells [19]. Cell surface expression of empty or suboptimally loaded MHC-I in TAP-deficient cells is also elevated by introducing a disulfide bond at the C-terminal end of the peptide binding pocket, which increases the stability of MHC-I molecules but does not influence the peptide binding specificity [36]. The polymorphisms of HLA-I molecules influence not only the peptide binding specificities, but also the intrinsic stabilities in the absence of peptides. MD simulations showed that empty conformers of MHC-I variants have different conformational dynamics, particularly near the peptide binding region [11]. In a recent study, we compared the thermostabilities of purified empty conformers of several HLA-B allotypes (HC-β_2m heterodimer) and found a strong hierarchy, with HLA-B*35:01 and HLA-B*18:01 displaying high stability compared with HLA-B*44:02 and HLA-B*51:01 [12]. Correspondingly, HLA-B*35:01 and HLA-B*18:01 are expressed at higher levels than HLA-B*44:02 and HLA-B*51:01 in TAP-deficient cells, in peptide-receptive forms [21]. TAP is widely targeted by tumor cells or virus to escape MHC-I mediated immune

surveillance. Higher expression levels of certain HLA-I allotypes in TAP-deficient cells suggest such allotypes could be more efficient at antigen presentation under these conditions [21].

The most famous MHC-I molecules known to be expressed in multiple conformations are HLA-B*27 allotypes, some of which have strong genetic associations with ankylosing spondylitis (AS) , an inflammatory disease [37]. HLA-B*27 is known to be expressed on the cell surface as either free HCs or disulfide bond-linked homodimers, following recycling of endocytosed cell surface pMHC-I from endosomes, where some molecules lose their peptides [38]. HLA-B*27 allotypes have a unique free cysteine at position 67, which impairs the folding, and confers the ability to form homodimers [39]. However, the free thiol *per se* is not enough to explain the cell surface expression of HLA-B*27 as aberrant conformers. Since peptides are more easily released from suboptimally loaded MHC-I molecules, cell surface expression of suboptimally loaded HLA*B27 might also be a prerequisite for expression of aberrant conformers. In fact, expression of aberrant HLA-B*27 conformers is correlated with abnormal ERAP1 activity, via the presence of specific ERAP1 polymorphic variants, which reduce the level of optimal peptides in the ER [40,41]. Indeed, ERAP1 polymorphisms are linked to AS, based on genetic studies [42]. These findings are consistent with the view that compromised peptide loading efficiency is important for cell surface expression of aberrant conformers. Aberrant conformers of HLA-B*27 are shown to be ligands for multiple immune receptors [37], but the causal association of such interactions with AS remains to be established.

Tapasin and TAP-Binding Protein Related (TAPBPR) as Intracellular Sensors of Empty and Peptide-Loaded MHC-I Conformations

Tapasin, an ER chaperone specialized for MHC-I peptide loading, binds and stabilizes HC-β_2m heterodimers within the PLC [1]. Tapasin preferentially binds peptide-free class I [43]. HLA-I variants are known to vary widely in their requirements for tapasin [23,44], which is correlated with the intrinsic stabilities of empty HLA-I [23] and their conformational plasticity during their folding in the ER [11,45,46] (reviewed in [13]). As a component of its functions, tapasin edits the MHC-I peptide repertoire, facilitating loading of HLA-I with high affinity peptide, replacing low affinity peptides [47-49]. An open question in the field relates to whether HLA-I allotypes that assemble in a tapasin-independent manner acquire a broader or more suboptimal peptide repertoire compared to tapasin-dependent allotypes that strictly assemble within the PLC and may thus be subject to more stringent quality control. In a recent study [44], tapasin dependencies of 97 HLA-I were determined by analyzing their expression levels on the surface of tapasin-deficient cells in comparison with the corresponding tapasin-sufficient cells. The tapasin dependency scores were negatively correlated with the breadth of HIV peptides that elicited responses from $CD8^+$ T cells of HIV-infected individuals, and higher tapasin dependency was linked to higher viral load and more rapid progression to AIDS. An implication of these associations is that tapasin-independent allotypes in general display greater peptide repertoire diversities. Based on these studies, further assessments of these key differences between tapasin-dependent and the relatively tapasin-independent allotypes are important.

TAPBPR is a recently identified protein homologous to tapasin. Different from tapasin, TAPBPR is not a component of PLC [50]. It is localized in multiple organelles along the secretory pathway [50]. TAPBPR and tapasin share similar overall structures and interaction mode with HLA-I [2,51,52]. High resolution crystal structures (Figure 2A) and NMR studies of TAPBPR in complex with MHC-I have uncovered mechanisms underlying the modulation of MHC-I peptide loading and selection by TAPBPR and tapasin. TAPBPR and tapasin bind MHC-I at two major regions. While the N-terminal domain of TAPBPR interacts with the α2-1 helix and the bottom side of the β-sheet in the peptide-binding groove, the C-terminal domain interacts with both α3 domain and β_2m [2,51,52]. Peptide binding stabilizes a relatively closed conformation of MHC-I molecules, and whereas the TAPBPR-bound conformation is more open (Figure 2B). In addition, peptide and TAPBPR binding induce different conformations of the α3 domain, including the CD8 binding loop region (Figure 2C). There is negative allosteric coupling between the peptide binding site and the TAPBPR binding site [53]. Only high affinity peptides can effectively stabilize the closed conformation, and thus induce MHC-I release from TAPBPR. Thus, like tapasin, TAPBPR preferentially binds MHC-I molecules lacking peptide or those bound to specific peptides, constricting the peptide repertoire, and the effects of TAPBPR are allele-specific [54-56]. TAPBPR is suggested to recognize discrete conformational states of peptide-bound MHC-I allotypes to achieve its peptide editing function [56]. Overall, interactions with both tapasin and TAPBPR alter the MHC-I peptidome. As noted above, recent studies indicate that enhanced peptide repertoire diversity is a functionally significant feature of tapasin-independent allotypes [44].

CD8 Stabilizes Diverse MHC-I-Receptor Interactions and Senses the Conformation of Cell Surface MHC-I

CD8 is a well-known co-receptor for MHC-I molecules. It plays a crucial role in $CD8^+$ T cell development and activation. The interaction between a TCR and pMHC-I complex determines the specificity of each $CD8^+$ T cell. The binding affinity between TCR and MHC-I is generally weak, typically around 1~100 μM. The interactions are characterized by slow association rates and fast dissociation rates [57]. Such low binding affinity could reduce the likelihood of autoimmune responses. Fast dissociation rates are also good for broad immune surveillance. However, the disadvantages of fast dissociation rates are also obvious. $CD8^+$ T cells can get readily released from antigen presenting cells. A major function of CD8 is to enhance the dwelling time of TCR-pMHC-I complexes, and thus allowing a given pMHC-I sufficient interaction time to activate $CD8^+$ T cells [58]. Another function of CD8 is to synergize TCR/CD3 phosphorylation by recruiting Lck [59,60]. The combination of interactions mediated by TCR and CD8 allow $CD8^+$ T cells to precisely and quickly identify target cells. Previous studies have shown that $CD8^+$ T cells can lyse cells carrying extremely low numbers of HLA-I loaded with cognate peptides [61]. The exact mechanism underlying the synergistic effect of CD8 is still under debate.

The diversity of TCRs is generated by somatic recombination during T cell development. While TCRs recognize the highly polymorphic α1 and α2 domains, CD8 binds MHC-I molecules at the highly conserved α3 domain, with loop residues 222-232 constituting

the major binding site [6] (Figure 3A). CD8 has two isoforms, the CD8αβ heterodimer and the CD8αα homodimer. Cytotoxic T lymphocytes (CTLs) express both CD8αβ and CD8αα. However, it was proposed that only CD8αβ synergizes CTL activation. CD8αα, on the other hand, is thought to be inhibitory in CTL activation [62]. While the CD8αβ heterodimer is exclusively expressed in CTLs, the CD8αα homodimer is widely expressed by subsets of other immune cells, such as NK cells, γδ T cells and NKT cells. The expression levels of CD8 in these non-CTL immune cells are significantly lower than in CTL. The function of CD8 in such cells is less clear. Earlier studies have shown that $CD8^+$ dendritic cells are more efficient at cross-presentation, and $CD8^+$ NK cells are more cytotoxic, findings which imply that CD8 might also play a role in the function of these cells. $CD8\alpha\alpha^+$ γδ T cells were recently shown to be activated by Qa-1^b, a strong CD8 binder, via CD8αα binding [63]. In a recent study, we investigated the function of CD8 upon NK cell activation and education. We found that CD8αα functions as a co-receptor for the NK cell inhibitory receptor KIR3DL1 (Figure 3B), facilitating binding of its HLA-B ligands (those which are the Bw4 serotype), and enhancing the inhibitory signal delivered by HLA-Bw4 via KIR3DL1 [7]. Inhibitory signaling is known to be important for NK cell education. Consistent with the co-receptor function of CD8αα, in NK cells, our studies showed that $CD8^+$ NK cells were more cytotoxic than $CD8^-$ NK cells only in $HLA\text{-}Bw4^+$ donors [7]. These results suggest that CD8 might be a universal co-receptor for MHC-I, and that its function depends on the property of the receptor with which it cooperates.

MHC-I molecules bound to specific pathogen-derived or mutant peptides are typically recognized by a small subset of peripheral $CD8^+$ T cells in a TCR dependent manner. Different from such interactions, our recent study also found that empty conformers of HLA-B*35:01 stain bulk $CD8^+$ T cells in a TCR-independent but CD8-dependent manner [12]. *In vitro* binding assays indicated that empty conformers of HLA-B*35:01 bind CD8 with higher affinity than HLA-B*35:01 bound to specific peptides (Figure 3C). Further studies are needed to determine whether CD8 binding preferences for empty conformers are conserved across all HLA-I allotypes, and whether CD8 binding is also influenced by the sequence of the HLA-I-bound peptides. Although the CD8-binding site is distal from the peptide binding site, the two binding sites have correlated motions. MD simulations [11] and the structures of TAPBPR-MHC-I complexes [51,52] showed that opening of the F pocket induces an allosteric movement of the α3 domain. A comparison of the peptide-filled and TAPBPR-associated empty MHC-I also indicates a significant conformational change of the CD8-binding loop [51,52] (Figure 2C). Similar conformational changes between empty HLA-B*35:01 and specific peptide-filled versions could explain why empty HLA-B*35:01 was preferentially bound by CD8 [12]. Importantly, enhanced binding between empty HLA-I and CD8 did not bypass the TCR to activate $CD8^+$ T cells directly. Rather, empty conformers of HLA-B*35:01 augmented pHLA-I-mediated $CD8^+$ T cell activation. Empty conformers of MHC-I were found to be enriched within the immunological synapse, which enhanced the adhesion between target cells and CTLs. Enrichment of CD8 by empty conformers of HLA-B*35:01 might further activate CD3 through recruiting more Lck, although this was not examined in the study [12]. As described above, empty conformers of HLA-I are expressed under certain pathological conditions. The enhanced interaction between empty conformers of HLA-I and CD8 might be a mechanism to augment the ability

of $CD8^+$ T cells to eliminate target cells under pathogenic conditions that target HLA-I expression or assembly, which could result in low levels of HLA-I loaded with antigenic peptides.

Other Immune Receptors Recognize Empty MHC-I Conformers

LIR1 and LIR2 are two inhibitory innate immune receptors for HLA-I molecules. They share similar HLA-I binding sites as CD8 and compete with CD8 for HLA-I interactions [8]. LIR1 prefers peptide-filled HLA-I molecules, while LIR2 binds both conformations [64]. Thus, as seen with CD8-HLA-I interactions [12], peptide binding is linked to α3 domain conformational changes to regulate the functional interactions of HLA-I with other immune receptors. Engagement of LIR1 and LIR2 by MHC-I regulates the differentiation [65] and antigen-presenting properties of DCs [66] and phagocytosis by macrophages [67], correlating with HIV infection outcomes [66] and inhibition of tumor elimination by macrophages [67]. As noted previously, empty conformers of HLA-I were observed on the surface of certain viral infected cells and tumor cells. How these empty conformers affect the functions of DCs and phagocytosis by macrophages through LIR-1 and LIR-2 needs further investigation.

Concluding Remarks

It is becoming clear that MHC-I molecules exist in different conformations and that conformational variants can also represent functional variants. Specific conformers are recognized by intracellular chaperones to modulate the peptide repertoires and by cell surface immune receptors to modulate the immune cell activation or inhibition. MHC-I allotypes have different tendencies to be expressed as suboptimally loaded or empty conformers, or with variable peptide repertoire diversities because of their peptide specificities, assembly pathways and due to differential influences of pathophysiological conditions. These types of variations have key functional consequences for the immune response.

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Highlights

- MHC-I allotypes have different tendencies to be expressed as empty or sub-optimally loaded conformers.
- Tapasin and TAPBPR sense intracellular MHC-I conformations and modulate the peptide repertoire.
- CD8 preferentially recognizes specific MHC-I conformations.

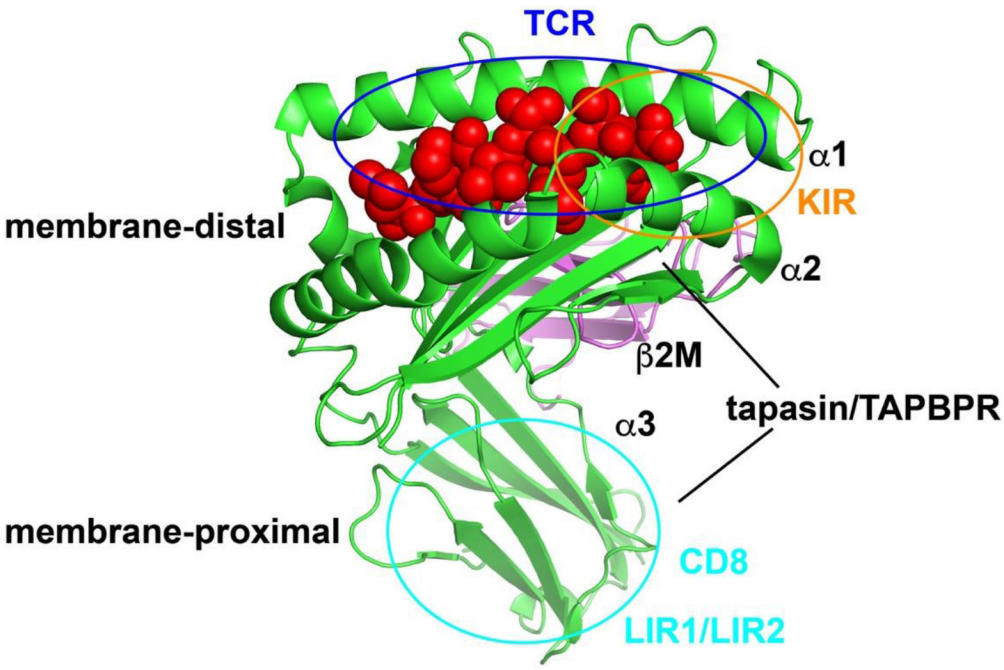

Figure 1.

Structure and interaction sites of MHC-I. MHC-I is composed of a heavy chain (green) that has three domains (α1, α2 and α3), a light chain β_2m (violet), and a peptide (red). The recognition sites of TCR, KIR, CD8, LIR1/LIR2 and tapasin/TAPBPR are indicated. There are two major tapasin/TAPBPR binding regions on MHC-I. The crystal structure of HLA-B*5701 in complex with peptide LSSPVTKSF (pdb: 2rfx) is used and the figure was generated with PyMOL.

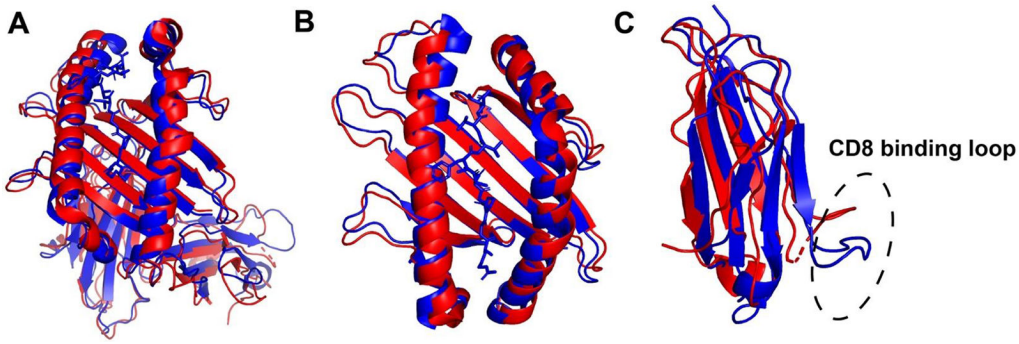

Figure 2.

Structural alignments of TAPBPR-associated (red) and peptide-filled (blue) MHC-I molecules show significant movements of both the α1-α2 and α3 domains. (A) The structure of H2-D^d-TAPBPR complex (5wer) was superimposed onto H2-D^d with a 10mer peptide (5weu). TAPBPR was omitted to compare the conformations of H2-D^d in peptide and TAPBPR-bound forms. (B) α1 and α2 domain of the structures shown in A. (C) α3 domain of the structures shown in A, with the CD8 binding loop indicated. The figure was generated with PyMOL.

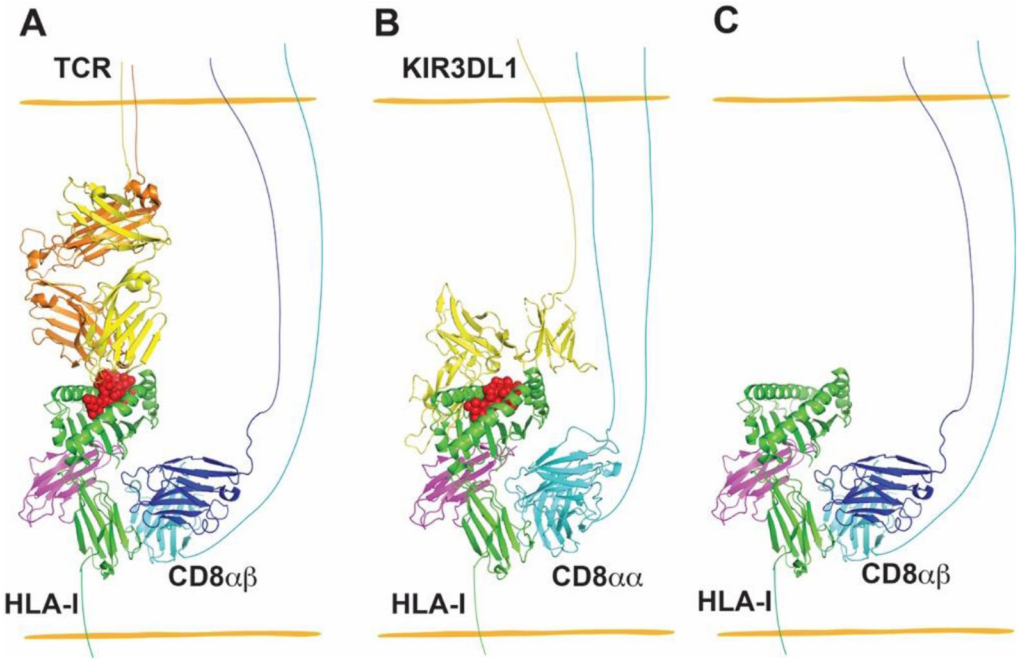

Figure 3.

Interactions between MHC-I and CD8 provide co-receptor-based synergy for receptors of $CD8^+$ T cells and NK cells (A and B), and enhanced binding between CD8 and peptide-free versions of some MHC-I allotypes contributes to increased adhesion between CD8-expressing cells and MHC-I-expressing target cells (C). (A) CD8αβ heterodimers are co-receptors for TCR recognizing cognate pHLA-I. The structure of TCR–HLA-A*02:01 complex (PDB: 5c0a) was superimposed with H2-D^d- CD8αβ (PDB: 3dmm) by aligning HLA-A*02:01 and H2-D^d, and H2-D^d was then deleted to generate a model for the TCR–HLA-A*02:01-CD8αβ complex. (B) A model for the complex between CD8αα and KIR3DL1 of NK cells with HLA-I of target cells, wherein CD8αα has co-receptor function in NK cells, similar to that for CD8αβ in T cells. The structure of KIR3DL1–HLA-B*57:01 complex (PDB: 3vh8) was superimposed onto HLA-A*02:01-CD8αα (PDB: 1akj) by aligning HLA-B*57:01 and HLA-A*02:01, followed by deletion of HLA-A*02:01 to generate a model for the KIR3DL1-HLA-B*57:01-CD8αα complex. (C) CD8 has been shown to preferentially bind to the empty version of HLA-B*35:01 relative to specific peptide-filled versions. To depict this type of interaction, peptide was deleted from the structure of H2-D^d-CD8αβ (PDB: 3dmm) to model a peptide-free MHC-I allotype binding to CD8αβ of $CD8^+$ T cells. PyMOL was used to visualize the structures of HLA-I and its receptors.