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The Mechanism of HLA-DM Induced Peptide Exchange in the MHC class II Antigen Presentation Pathway

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Abstract

HLA-DM serves a critical function in the loading and editing of peptides on MHC class II molecules. Recent data showed that the interaction cycle between MHC class II molecules and HLA-DM is dependent on the occupancy state of the peptide binding groove. Empty MHC class II molecules form stable complexes with HLA-DM, which are disrupted by binding of high-affinity peptide. Interestingly, MHC class II molecules with fully engaged peptides cannot interact with HLA-DM, and prior dissociation of the peptide N-terminus from the groove is required for HLA-DM binding. There are significant similarities to the peptide loading process for MHC class I molecules, even though it is executed by a distinct set of proteins in a different cellular compartment.

Introduction

HLA-DM and its mouse homolog H-2M (referred to as 'DM') play a central role in the MHC class II antigen presentation pathway [1]. The human DM genes are located in the class II region of the MHC locus and apparently arose through duplication of ancestral MHC class II genes [2]. Despite similarities in primary sequence and overall structure with conventional MHC class II molecules, DM lacks the ability to bind and present peptides [3,4]. Rather, it plays a crucial role in the loading of peptides into the groove of MHC class II (MHCII) molecules.

CLIP (class II-associated invariant chain peptide) is a segment of invariant chain that remains bound in the MHCII groove after invariant chain cleavage by endosomal proteases [5]. It is frequently stated that DM is required to induce dissociation of CLIP from MHC class II molecules so that peptides from exogenous antigens can enter the binding groove. However, CLIP binds with a wide range of affinities to MHC class II molecules, due to the highly polymorphic nature of the binding groove. For MHCII molecules that bind CLIP with

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high affinity (such as HLA-DR1 or I-A^b), DM is essential for the displacement of CLIP. Other MHCII molecules have a much lower affinity for CLIP (certain HLA-DR4 alleles or I-A^{g7}) and CLIP spontaneously dissociates following invariant chain cleavage [6–8]. A subset of MHC class II molecules thus becomes dysfunctional in the absence of DM.

DM actually plays a more general role in the MHCII pathway. It induces dissociation of any peptide from MHCII molecules and thereby performs a critical editing function that favors display of high affinity peptides on the surface of antigen presenting cells (APC) [9–12]. This editing function substantially changes the peptide repertoire presented to T cells [12–19]. Recent work has shown that almost all T cells with a given peptide specificity come in contact with the relevant pMHC complex following immunization [20]. Recruitment of these rare naïve T cells requires a substantial amount of time, making long-lived display of pathogen-derived peptides essential.

Another crucial function of DM is the stabilization of empty MHCII molecules [21–23]. Peptides are deeply buried in the MHCII binding site, and MHCII molecules are unstable in the absence of bound peptide [24,25]. Empty molecules quickly lose their ability to bind peptides with rapid kinetics; rebinding of new peptide occurs very slowly and a substantial fraction of molecules aggregate [24,26]. DM stabilizes empty MHCII and keeps them in a peptide-receptive state that enables rapid binding of incoming peptides [21–23]. In the endosomal/lysosomal compartment, rapid binding of peptides to MHCII molecules is essential to prevent proteolytic destruction of epitopes.

The interaction of DM and MHCII is determined by peptide

A recent study showed that peptides play a key role in the DM-MHCII interaction cycle [23]. Direct binding of DR-CLIP complexes to DM was examined in real time using surface plasmon resonance (SPR, Biacore) because this technique permits independent assessment of association and dissociation stages [27]. DR-CLIP complexes were run over chips with immobilized DM, and dose-dependent binding was observed. Surprisingly, dissociation of DR from DM occurred very slowly. This DM-DR complex was devoid of peptide, and peptide injection resulted in rapid dissociation of DM and DR. This means that DM forms long-lived, stable complexes with empty DR that are disrupted by binding of peptides to the groove. DM-DR complexes had previously been isolated from cells and mass spectrometry analysis had shown that they were devoid of peptide [21,28,29].

Peptide-induced dissociation of the DM-DR complex was dependent on the affinity of the peptide for the respective DR molecule. Furthermore, DM bound only very slowly to high-affinity DR/peptide complexes. High-affinity DR/peptide complexes are thus protected from the action of DM by two mechanisms: binding of such peptides to the DR groove induces rapid DM dissociation and rebinding of such complexes to DM is very slow. In contrast, low affinity peptides induce substantially slower dissociation of DM-DR complexes and are more likely to be removed through the action of DM. These results explain how editing by DM favors presentation of high affinity peptides [12–19].

Dissociation of the peptide N-terminus precedes DM binding

DM did not bind to DR molecules that carried peptides covalently attached through a flexible linker to the N-terminus of the DR β chain [23]. This result was not due to steric hindrance, because covalent linkage through a disulfide bond in one of the DR pockets gave the same result. These results raised an interesting question: what changes would need to be made to such DR/peptide complexes to enable DM binding? Deletion of the first three N-terminal residues (P-2, P-1 and P1) of such a covalently linked peptide enabled strong DM binding, while deletion of the first two residues was not sufficient [23]. These residues form

conserved hydrogen bonds to the DR α and DR β helices (DR α F51 and S53, as well as DR β H81); the side chain of the third peptide residue occupies the critical P1 pocket of the groove [25] (Figures 1 & 2). DM thus binds to a short-lived transition state in which the N-terminal peptide segment has transiently disengaged from key interactions with the groove due to spontaneous peptide motion. This mechanism of action is consistent with a large body of mutagenesis data which showed that DM binds to DR molecules in the vicinity of the peptide N-terminus (Figures 1 & 2) [23,30]. This conclusion is also supported by the finding that loss of conserved hydrogen bonds between the peptide N-terminus and DR α (F51 and S53) resulted in greater susceptibility to HLA-DM (six to nine-fold) [31].

Model of DM action

These results provide a unifying model of DM action (Figure 3). DM fails to interact with DR molecules whose peptides are fully engaged in the groove (Figure 3, step 1), and it can only bind when the N-terminal part of the peptide dissociates through constant motion within the DR/peptide complex (steps 2 & 3). DM captures this short-lived transition state and shifts the equilibrium to the empty state (step 4), due to its higher affinity for empty DR molecules [23]. The empty DM-DR complex retains the ability to quickly bind a new peptide over extended periods of time [22,23,28]. Newly generated peptides can thereby be rapidly captured in the processing compartment, rescuing them from proteolytic degradation. If an interacting peptide has a low affinity (step 5), DM may catalyze its removal (editing) while binding of a high-affinity peptide (step 6) is more likely to induce dissociation of the DM-DR complex. The resulting high-affinity DR/peptide complex has a low likelihood of rebinding DM and can reach the cell surface (step 7). This model is consistent with a large body of prior work in the field, including the identification of empty DR-DM complexes in cells [21,28] and the demonstration of an editing function of DM that drives selection of high-affinity peptides [12–19].

Functional similarities between the MHC class I and II peptide loading mechanisms

There are striking similarities in the peptide loading processes for MHC class I and class II molecules (Figure 4), even though peptide acquisition is facilitated by entirely different sets of proteins in distinct cellular compartments [32,33]. Peptides are buried deeply in the binding grooves of MHCI and MHCII, and both sets of molecules are highly unstable in the absence of peptide [24,33]. In the ER, the MHC class I heavy chain first associates with β_2m to generate a peptide-receptive heterodimer which is then incorporated into the multi-subunit peptide loading complex (PLC) [33]. A key component of the PLC is tapasin, a protein that provides a physical link between the MHC class I heavy chain and the TAP peptide transporter [34]. Tapasin forms a disulfide-linked dimer with ERp57, and this dimer serves a crucial function in peptide loading analogous to the role of DM in the MHCII pathway [35,36]. The tapasin-ERp57 dimer stabilizes empty MHC class I molecules in a peptide-receptive conformation and greatly enhances peptide binding. It also promotes peptide editing and thereby favors binding of peptides with high affinity for display on the cell surface. Binding of high affinity peptide induces dissociation of class I molecules from the PLC [35]. The tapasin-ERp57 dimer has a higher affinity for empty MHC class I molecules than tapasin alone because it possesses two binding sites: tapasin binds directly to MHC class I molecules while ERp57 interacts with calreticulin bound to the mono-glucosylated N-linked glycan of recruited MHC class I molecules [33,35]. When tapasin is linked to MHC class I molecules through artificial leucine zippers, it can promote peptide exchange in the absence of ERp57 or other PLC components [37]. Thus, key principles of the peptide loading process are remarkably similar between MHC class I and II molecules: 1. dedicated chaperones stabilize empty molecules and thereby greatly accelerate peptide binding; 2. an

editing process favors acquisition of high affinity peptides; and 3. the binding of such peptides induces dissociation of the peptide loading complex.

Connection to autoimmune diseases

Particular alleles of MHC class II genes are strongly associated with autoimmune diseases [38]. For example, HLA-DQ2 (DQ2) is associated with type 1 diabetes and celiac disease. The association with celiac disease is particularly strong as ~90–95% of patients express this MHCII molecule [39]. DQ2 is resistant to the action of DM, due to a deletion at position DQ α 53 which is located close to the putative DM interaction site. This deletion is not seen in DQ1 (DQA1*0101) and DQ8 (DQA1*0301), molecules that are sensitive to the action of DM. Insertion of a glycine residue at this position (as in DQ1) rendered DQ2-peptide complexes sensitive to editing by DM [40]. Celiac disease is initiated by CD4 T cells specific for peptides from gluten, a component of wheat, barley and rye [39]. The DQ α 53 mutant showed substantially reduced presentation of an immunodominant gluten peptide [40]. The documented DM resistance of DQ2 may thus be involved in the chronic inflammatory process by two related mechanisms. First, it prevents editing of DQ2-bound peptides, potentially including pathogenic epitopes. Second, the predominance of CLIP peptide on the cell surface reduces the diversity of peptide species available for negative selection of self-reactive T cells in the thymus.

Inhibition of DM by DO

HLA-DO (DO) is another non-classical class II molecule that modulates the presentation of antigens in the endocytic pathway. Biochemical studies have shown that DO forms stable complexes with DM and blocks its catalytic function [41,42]. DM-DO complexes are formed in the ER and efficient exit of DO from the ER requires association with DM [43]. In B cells, DO favors presentation of antigens internalized through the B cell receptor [44]. DO is expressed by naïve B cells, thymic epithelial cells, and subsets of immature dendritic cells and its expression is down-regulated with activation. Down-regulation of DO by germinal center B cells and the resulting increase in antigen presentation capability enhances the interaction of these B cells with follicular helper T cells [45,46]. Another recent study showed that over-expression of DO in dendritic cells prevents development of type 1 diabetes in NOD mice [47]. DO may thus dampen self-antigen presentation by naïve B cells and immature dendritic cells and thereby reduce the risk of autoimmunity.

Bidirectional binding of CLIP peptide to HLA-DR1

A substantial number of crystal structures of pMHCII complexes identified a common orientation of peptides in the binding groove, with the peptide N-terminus being located in the proximity of the P1 pocket [25]. Interestingly, a recent study reported that a CLIP peptide can bind to DR1 also in an inverted orientation. This CLIP peptide was shortened at the N-terminus and therefore not optimally bound in the conventional orientation (lacking three hydrogen bonds to DR α Phe51 and Ser53); in the flipped orientation hydrogen bonds to these DR residues were made [48]. Surprisingly, the backbone of the inverted CLIP peptide formed hydrogen bonds with the same set of conserved DR residues as peptides bound in the conventional orientation. Inversion of the CLIP peptide was favored by its pseudo-symmetry: it has methionine residues at the P1 and P9 positions and small residues (alanine and proline) at P4 and P6.

DM was able to catalyze peptide exchange on complexes containing CLIP in either orientation [48]. This is explained by the fact that DM only binds to DR molecules following disengagement of the peptide N-terminus, as explained above [23]. DM also substantially accelerated exchange of CLIP between the two orientations, suggesting that the

flipped orientation may be presented on the cell surface by some DR molecules [48]. Are some T cell epitopes from microbial antigens actually recognized in such a non-canonical orientation? Also, is this mechanism involved in some instances of autoimmunity? Differences between thymic and peripheral APC (such as DM expression levels) may enable peripheral presentation of self-peptides in an orientation to which there is insufficient central tolerance.

A cell-free system for determination of T cell epitopes

Epitope prediction is more challenging for MHCII than MHCI restricted T cell responses because MHCII peptide binding motifs are more degenerate. An *in vitro* system for epitope discovery was developed using the key proteins in the peptide loading compartment, DR1, DM and proteases, along with a folded antigen of interest [49]. DM is a critical component of this system because it enables rapid binding of peptides to MHCII before they are degraded by proteases. Three endosomal proteases were shown to be sufficient: cathepsin S (an endoprotease), cathepsin H (an aminopeptidase) and cathepsin B (a carboxypeptidase); cathepsin B and H also have endoprotease activity. DR1 bound peptides were sequenced by mass spectrometry analysis of immunoprecipitated DR/peptide complexes. Novel epitopes were identified from two antigens, hemagglutinin from influenza strain H5N1 and a liver-stage specific protein (LSA-1) of *plasmodium falciparum* [49]. This approach enables simultaneous identification of T cell epitopes as well as post-translational modifications that can be important for recognition of self-antigens.

Concluding remarks

Significant advances have thus been made in our understanding of DM function in the MHCII antigen presentation pathway. We propose that the ability of DM to stabilize empty MHCII molecules is closely related to its function in peptide editing. The DM-stabilized conformer is highly peptide-receptive and peptides can diffuse in and out until a peptide forms strong interactions with the groove. Tight binding of peptide then induces dissociation of DM. Similar processes may control the release of peptide-filled MHC class I molecules from the peptide loading complex in the ER.

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Highlights

- DM and MHCII molecules form stable complexes in the absence of peptide
- Peptide binding induces dissociation of DM from MHCII
- MHCII molecules with fully bound peptides do not interact with DM
- Dissociation of the peptide N-terminus from the MHC groove is required for DM binding
- Key principles of peptide loading are similar between MHC class I and II molecules

Figure 1. Lateral interaction surfaces of HLA-DM and HLA-DR molecules

Contact residues are colored red on both proteins, based on mutants that substantially reduced susceptibility of DR/peptide complexes to DM [30,23] or the activity of DM [50]. Mutants that only showed small effects or introduced a glycosylation site (and thereby steric hindrance) were omitted. A functionally important cluster is located in the DR α 1 domain close to the peptide N-terminus; a second cluster is present in the membrane proximal DR β 2 domain. DM also shows two clusters of contact residues, located in the membrane-distal α 1/ β 1 domains and the membrane proximal α 2/ β 2 domains. DM chains are colored yellow (DM α) and orange (DM β), DR chains light blue (DR α) and turquoise (DR β). Models are based on crystals structures of HLA-DM (PDB 1HDM and 2BC4) and HLA-DR3/CLIP (PDB 1A6A).

Figure 2. The peptide N-terminus is located in close vicinity to critical DM-interacting residues
A. Top view of the peptide binding groove. Three of four DR residues shown to be critical for the interaction with DM are located in close proximity to the peptide N-terminus: DRα F51, S53 and W43. DR chains are colored light blue (DRα) and turquoise (DRβ); the bound peptide is shown as a stick model. Three N-terminal peptide residues (P-2, P-1, P1) that need to dissociate prior to DM binding are indicated. **B.** Side view of the peptide, following removal of the DRβ chain. DRα W43 (a key DM interacting residue) forms part of the lateral wall of the P1 pocket of the groove and is accessible on the outer surface of the DR molecule. Models are based on the crystal structure of DR1/HA₃₀₆₋₃₁₈ (PDB 1DLH).

Figure 3. Model of DM action

DM cannot bind to DR molecules when the peptide is fully bound in the groove (1). Spontaneous dissociation of the peptide N-terminus due to continuous peptide motion (2) creates the DM binding site. DM induces dissociation of the remainder of the peptide (3), and the resulting DM – empty DR complex (4) is stable and can bind new peptides with very rapid kinetics. Binding of low affinity peptides (5) leads to cycles of peptide editing by DM, while binding of high affinity peptides results in dissociation of DM from DR molecules (6). These stable DR/peptide complexes display their peptides for many days on the cell surface (7). DR molecules are colored in shades of blue and DM molecules shades of yellow/orange. The ribbon diagrams in the top left corner show the hydrogen bond network between the DR helices (light and dark blue) and the peptide (red), with the peptide either fully bound (left) or with the N-terminus released from the groove (right).

Figure 4. Similarities between the peptide loading mechanisms utilized by MHC class I and class II molecules

Empty MHCI and MHCII molecules are highly unstable in the absence of peptide, and peptide loading requires chaperones that stabilize the empty state in a functional form. Empty MHCI molecules become part of a peptide loading complex involving tapasin, ERp57 and calreticulin; tapasin links the peptide loading complex to the peptide transporter TAP (not shown). Tapasin is covalently linked to ERp57 and this heterodimer performs a peptide editing function. Peptide loading occurs in different compartments for MHCI (ER) and MHCII (endosomes-lysosomes), but key features of the peptide loading/editing process are similar, as illustrated here. In both cases, binding of high affinity peptides results in release from the respective chaperones.