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Review

# Structural insights into HLA-DM mediated MHC II peptide exchange

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## ABSTRACT

Antigen presentation by class II MHC proteins (MHC-II) is a critical component of the adaptive immune response to foreign pathogens. Our understanding of how antigens are presented has been greatly enhanced by crystallographic studies of MHC-II-peptide complexes, which have shown a canonical extended conformation of peptide antigens within the peptide-binding domain of MHC-II. However, a detailed understanding of the peptide loading process, which is mediated by the accessory molecule HLA-DM (DM), remains unresolved. MHC-II proteins appear to undergo conformational changes during the peptide loading/ exchange process that have not been clearly described in a structural context. In the absence of a crystal structure for the DM-MHC-II complex, mutational studies have provided a low resolution understanding as to how these molecules interact. This review will focus on structural and biochemical studies of the MHC-II-peptide interaction, and on studies of the DM-MHC-II interaction, with an emphasis on identifying structural features important for the mechanism of DM mediated peptide catalysis.

**KEYWORDS:** MHC protein, antigen presentation, immune response, HLA-DR, HLA-DP, HLA-DQ, H2-M, I-A, I-E

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## ABBREVIATIONS

CD, circular dichroism; CLIP, class II invariant chain peptide; HLA, Human Leukocyte Antigen; kDa, kilodalton; MHC, Major Histocompatibility Complex; SDS, sodium dodecyl sulfate; SPR, surface plasmon resonance

## INTRODUCTION

MHC-II proteins are ~50 kDa heterodimeric transmembrane glycoproteins that play an important role in immune recognition [1]. The nascent complex as expressed in the endoplasmic reticulum is comprised of three alpha-beta MHC-II proteins in complex with the invariant chain (Ii) trimer [2, 3]. The invariant chain has been proposed to carry out a dual function to both direct the MHC-II to endosomal compartments as well as to block the peptide binding groove of the MHC-II [4, 5]. In endosomal compartments, regions of the Ii that are not protected by the MHC-II are proteolyzed by cysteine proteases [6]. This results in an MHC-II protected peptide fragment, CLass II Invariant chain Peptide (CLIP), which remains bound to the MHC-II in the antigen binding site [7]. In order for antigen presentation to occur, CLIP must be exchanged with endosomal peptides, a process which is catalyzed by the MHC-II homologue HLA-DM [8]. There is evidence that HLA-DM has a dual function to both stabilize an empty conformation of MHC-II [9], as well as to catalyze peptide exchange [10], as discussed below. Although the involvement of HLA-DM in promoting peptide exchange has been definitively

described, the mechanism of this process is poorly understood [11-18].

**MHC-II structure**

Prior to the first solved MHC-II crystal structure, a hypothetical model of the MHC-II was developed based on the MHC I structure [19]. Genetic analysis had revealed that class I and II molecules shared similar domain and genomic organization [20]. It was therefore predicted that the MHC-II structure would reveal one membrane distal peptide binding site that would be composed of regions with high amino acid polymorphism [20]. In 1993, a 3.3Å structure of the extracellular portion of HLA-DR1 in complex with a mixture of peptides isolated from B-cells provided a detailed molecular model describing the fold of the MHC-II as well as the architecture of the peptide binding groove [21]. The crystal structure revealed a peptide-binding groove that is formed by the α1 and β1 domains (Fig. 1). The binding groove has a beta sheet floor flanked by two alpha helices formed by both the alpha and beta chains. Ig domains comprise the membrane proximal portion from both the a2 and β2 regions (Fig. 1). Although the structures were obtained from soluble ectodomains of the protein, the full-length protein also includes alpha and beta chain transmembrane domains as well as short cytoplasmic tails that have been implicated in signal transduction [22]. This first structure of an MHC-II peptide complex revealed that peptides bind in an extended conformation. In contrast to the MHC I peptide structures, the MHC-II structure showed that the peptide binding groove is open-ended at both the N and C-terminus of the peptide suggesting that peptides of variable length could bind into the groove [21]. Subsequently, it has been demonstrated that MHC-II proteins can bind peptides with lengths ranging from 2-4 residues [23] all the way to unfolded intact proteins [24].

In 1994, the crystal structure of HLA-DR1 with a single defined peptide, an immunodominant fragment from influenza A haemagglutinin (HA), was solved and the molecular details of the peptide MHC-II interaction were described [25]. The peptide bound in an extended conformation corresponding to a type II polyproline twist, in which 35% was solvent exposed and therefore theoretically able to interact with CD4+ T-cells. This structure showed pockets in the overall peptide-binding groove that accommodated five of the thirteen side chains from the peptide. In addition, a hydrogen-bonding network was clearly defined in this structure, with twelve hydrogen bonds involving the main chain of the peptide and conserved residues in the MHC-II (Fig. 2). Further evidence that the hydrogen bond network was a common motif in MHC-II–peptide interactions was revealed by another MHC-II structure bound to a mixture of endogenous peptides determined at higher resolution by co-crystallization with a bacterial superantigen, which showed the peptide conformation to be constrained by the pattern of side-chain binding pockets as well as by the hydrogen bonds that extended from the backbone of the peptide to conserved asparagine and glutamine residues on the MHC-II [26].

Through these structural studies, it became evident that there are two different aspects of the peptide-MHC-II interaction. One is mediated through a hydrogen bond network that is formed by the peptide backbone and both main chain and side chain atoms of the MHC-II, and the other mediated by peptide side chain interactions with binding groove pockets (Figs. 2, 3).

**MHC-II variation and peptide binding**

There are four main binding pockets present in all MHC-II structures determined to date, P1, P4, P6 and P9, which accommodate peptide side chains. These pockets are highly polymorphic and therefore diverse in the shape and charge specificity between different MHC-II proteins (Fig. 3). HLA-DR1 has been extensively studied and numerous structures show a large P1 hydrophobic binding pocket (Fig. 4) [25, 26]. This pocket is the most important determinant of binding specificity for HLA-DR1 [23, 27]. However, there is sequence variation for residues lining the P1 pocket of the MHC-II proteins, which affects the charge and shape of the pocket, ultimately leading to differences in which peptide side chains can be accommodated at this position (Fig. 4). Indeed, the sequence variations within the peptide-binding domain of the MHC-II

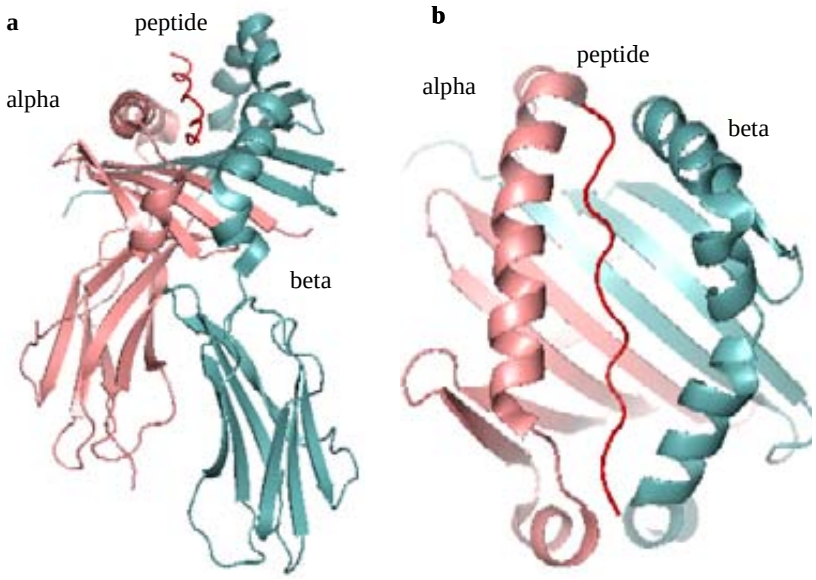


**Fig. 1. Crystal structure of MHC II.** (a) Cartoon representation of an HLA-DR1 peptide complex (1DHL). The alpha chain is shown in salmon, the beta chain in teal and the peptide in red. (b) 90° rotation showing the top of the peptide binding groove.

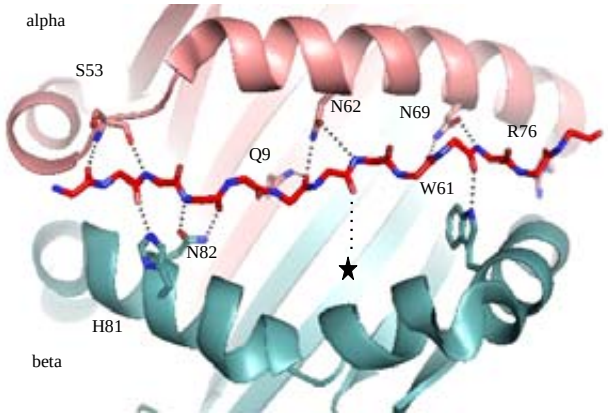


**Fig. 2. Conserved hydrogen bond network of MHC II.** Cartoon representation of peptide binding groove, alpha chain in salmon, beta chain in teal, peptide in red stick with amine groups colored blue, and carboxyl groups colored pink. Conserved residues in MHC II are shown in stick with dashed lines representing hydrogen bonds to the peptide backbone. Note that bonds are formed between conserved side chains in the MHC II except for the S53, where the bonds are mediated by the main chain atoms in the MHC II. The beta 71 position, represented by a star, is polymorphic. In most HLA-DR alleles, there is an H-bond formed between this reside and the carbonyl of the peptide backbone, shown as a dashed line, however, in the HLA-DQ, HLA-DP, IA alleles, there is no H-bond formed at this position.

proteins cause differences in specificity for the anchor residues all along the peptide binding groove (see below) (Fig. 3).

After the first HLA-DR1 structures were solved, the residues forming the anchor pockets were defined. Subsequent structures of different MHC-II variants allowed a detailed look into the effect of polymorphisms and sequence variations on the size, shape and specificities of the different pockets. Numerous crystal structures for MHC-II alleles of HLA-DR, HLA-DP and HLA-DQ have helped to refine our understanding of the peptide MHC-II interaction (Table 1). In addition, crystal structures for I-E the mouse MHC-II homologue of the human HLA-DR, as well as I-A, the homologue for the human HLA-DQ, have been solved (Table 1). To date, there are 81 MHC-II structures deposited in the PDB, encompassing over 15 different allelic variants of the five human and murine class II MHC proteins (Table 1). Although this is a narrow view into the thousands of documented MHC-II alleles [28], these structures are strikingly similar in overall fold with polymorphic differences concentrated in the binding groove.

An example of how MHC-II sequence variation can alter the size, shape and specificity of the key

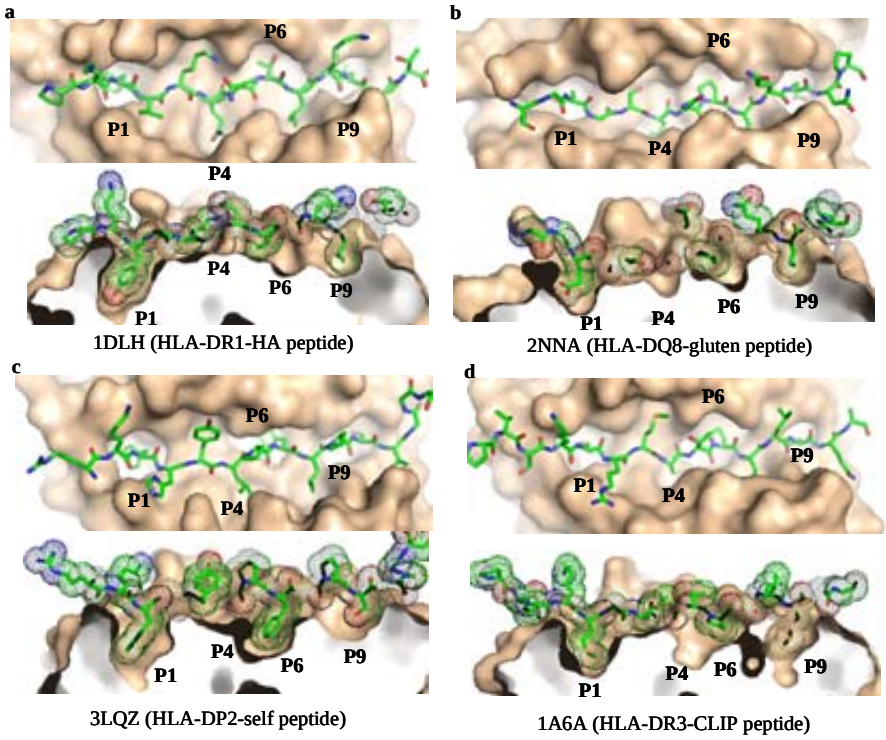


**Fig. 3. Peptide binding grooves of multiple MHC II alleles show differences in peptide side chain specificity pockets**. (a top) Surface representation of the top of the peptide binding groove for HLA-DP2 (PDB ID: 3LQZ), with peptide shown in stick and dots. Main anchoring specificity pockets P1, P4, P6 and P9 are designated below. (bottom) 90° rotation showing side of peptide binding groove with peptide side chains lodged into specificity pockets. (b) HLA-DR1 (PDB ID: 1DLH) shown as in same orientation as (a). (c) HLA-DQ8 (PDB ID: 2NNA) shown as in same orientation as (a). (d) HLA-DR3 (PDB ID: 1A6A) shown as in same orientation as (a).

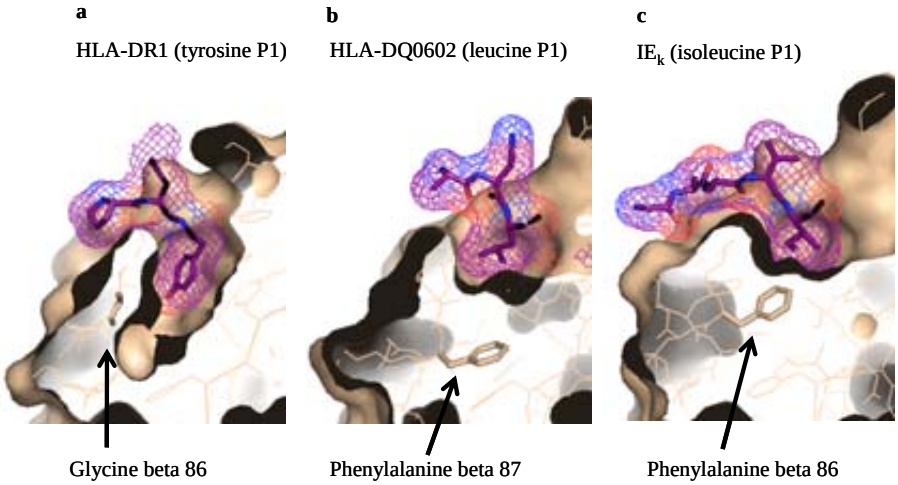


**Fig. 4. Diversity of size and shape of the P1 pocket.** Changes in beta 86/87 contribute to alterations in the size and shape of the P1 pocket. Each of the structures are represented in both surface and lines with the beta 86 or 87 residue shown in stick. The peptide is in purple stick and mesh with the P1 residue indicated above. (a) HLA-DR1 (PDB ID# 1DLH) has a glycine at beta 86, which allows a large hydrophobic pocket at the P1. (b) the HLA-DQ0602 structure (PDB ID# 1UV6) has a phenylalanine at the beta 87 residue. (c) the IEk (PDB ID# 1IEA) has a phenylalanine at the beta 86 position.

**Table 1.** MHC II structures.

| PBD ID | Allele | Peptide | Res. | Citation |
|---|---|---|---|---|
| 3LQZ | HLA-DP2 (A1*0103/B1*0201) | self DRa | 3.25 | (2010) Proc. Natl. Acad. Sci. |
| 1UVQ | HLA-DQ6 (A1*0102/B1*0602) | hypocretin | 1.80 | (2004) Proc. Natl. Acad. Sci. |
| 3PL6 | HLA-DQ1 (A1*0102/B1*0502) | MBP | 2.55 | (2011) J. Exp. Med. |
| 1S9V | HLA-DQ2 (A1*0501/B1*0201) | Deam. Gliadin | 2.20 | (2004) Proc. Natl. Acad. Sci. |
| 2NNA | HLA-DQ8 (A1*0301/B1*0302) | Deam. Gluten | 2.10 | (2007) Immunity |
| 1JK8 | HLA-DQ8 (A1*0301/B1*0302) | human Insulin | 2.40 | (2001) Nat. Immunol. |
| 1KLU | HLA-DR1 (B1*0101) | TPI | 1.93 | (2002) J. Mol. Biol. |
| 3PDO | HLA-DR1 (B1*0101) | CLIP (102-120) | 1.95 | (2010) Proc. Natl. Acad. Sci. |
| 2G9H | HLA-DR1 (B1*0101) | HA | 2.00 | (2006) J. Biol. Chem. |
| 1PYW | HLA-DR1 (B1*0101) | HA variant | 2.10 | (2003) J. Biol. Chem. |
| 3L6F | HLA-DR1 (B1*0101) | MART-1 | 2.10 | (2010) J. Mol. Biol. |
| 1SJH | HLA-DR1 (B1*0101) | HIV 13mer | 2.25 | (2004) Proc. Natl. Acad. Sci. USA |
| 1JWU | HLA-DR1 (B1*0101) | HA | 2.30 | (2003) Structure |
| 2IPK | HLA-DR1 (B1*0101) | HA variant Fluor | 2.30 | (2007) Nat. Chem. Biol. |
| 1KLG | HLA-DR1 (B1*0101) | TPI | 2.40 | (2002) J. Mol. Biol. |
| 1T5W | HLA-DR1 (B1*0101) | synthetic peptide | 2.40 | (2004) Chem. Biol. |
| 2ICW | HLA-DR1 (B1*0101) | HA | 2.41 | (2007) Nat. Struct. Mol. Biol. |
| 1AQD | HLA-DR1 (B1*0101) | endogenous | 2.45 | (1997) Structure |
| 1SJE | HLA-DR1 (B1*0101) | HIV 16mer | 2.45 | (2004) Proc. Natl. Acad. Sci. |
| 1TX5 | HLA-DR1 (B1*0101) | synthetic peptide | 2.50 | (2004) Chem. Biol. |
| 1FYT | HLA-DR1 (B1*0101) | HA | 2.60 | (2000) EMBO J. |
| 1HXY | HLA-DR1 (B1*0101) | HA | 2.60 | (2001) EMBO J. |
| 1JWS | HLA-DR1 (B1*0101) | HA | 2.60 | (2003) Structure |
| 1R5I | HLA-DR1 (B1*0101) | MAM | 2.60 | (2004) Structure |
| 1KGO | HLA-DR1 (B1*0101) | EBV | 2.65 | (2002) Mol. Cell |
| 3PGC | HLA-DR1 (B1*0101) | CLIP (Flipped) | 2.66 | (2010) Proc. Natl. Acad. Sci. |
| 1JWM | HLA-DR1 (B1*0101) | HA | 2.70 | (2003) Structure |
| 1SEB | HLA-DR1 (B1*0101) | endogenous | 2.70 | (1994) Nature |
| 3PGD | HLA-DR1 (B1*0101) | CLIP (102-120) | 2.72 | (2010) Proc. Natl. Acad. Sci. |
| 1DLH | HLA-DR1 (B1*0101) | HA | 2.80 | (1994) Nature, 368 |
| 2IAM | HLA-DR1 (B1*0101) | TPI | 2.80 | (2007) Nat. Immunol. |
| 2IAN | HLA-DR1 (B1*0101) | TPI | 2.80 | (2007) Nat. Immunol. |
| 2OJE | HLA-DR1 (B1*0101) | HA | 3.00 | (2007) J. Biol. Chem. |
| 2WBJ | HLA-DR1 (B1*0101) | ENGA | 3.00 | To be published |
| 2FSE | HLA-DR1 (B1*0101) | Collagen II | 3.10 | (2006) J. Immunol. |
| 1LO5 | HLA-DR1 (B1*0101) | HA | 3.20 | (2002) Structure |
| 1FV1 | HLA-DR2a (B5*0101) | MBP | 1.90 | (2000) J. Mol. Biol. |

Table 1 continued..

| | | | | |
|---|---|---|---|---|
| 1H15 | HLA-DR2a (B5*0101) | EBV | 3.10 | (2002) Nat. Immunol. |
| 1ZGL | HLA-DR2a (B5*0101) | MBP | 2.80 | (2005) EMBO J. |
| 1HQR | HLA-DR2a (B5*0101) | MBP | 3.20 | (2001) Immunity |
| 1BX2 | HLA-DR2b (B1*1501) | MBP | 2.60 | (1998) J. Exp. Med. |
| 1YMM | HLA-DR2b (B1*1501) | MBP | 3.50 | (2005) Nat. Immunol. |
| 1A6A | HLA-DR3 (B1*0301) | CLIP | 2.75 | (1995) Nature |
| 1D5M | HLA-DR4 (B1*0401) | pep mimetic | 2.00 | (2000) J. Med. Chem. |
| 1D5Z | HLA-DR4 (B1*0401) | pep mimetic | 2.00 | (2000) J. Med. Chem. |
| 2XN9 | HLA-DR4 (B1*0401) | HA | 2.30 | (2010) Nat. Commun. |
| 1J8H | HLA-DR4 (B1*0401) | HA | 2.40 | (2002) J. Exp. Med. |
| 1D5X | HLA-DR4 (B1*0401) | pep mimetic | 2.45 | (2000) J. Med. Chem. |
| 1D6E | HLA-DR4 (B1*0401) | pep mimetic | 2.45 | (2000) J. Med. Chem. |
| 2SEB | HLA-DR4 (B1*0401) | Collagen II | 2.50 | (1997) Immunity |
| 3O6F | HLA-DR4 (B1*0401) | MBP | 2.80 | (2011) EMBO J. |
| 2Q6W | HLA-DR52a (B3*0101) | platelet integrin | 2.25 | (2007) J. Mol. Biol. |
| 3C5J | HLA-DR52c (B3*0301) | UNP | 1.80 | (2008) P.N.A.S. |
| 1ES0 | I-Ag7 | Gad65 | 2.6 | (2000) Science |
| 3MBE | I-Ag7 | HEL | 2.89 | (2010) J. Clin. Invest. |
| 3CUP | I-Ag7 | GAD | 3.09 | To be published |
| 1F3J | I-Ag7 | HEL | 3.10 | (2000) Immunity |
| 1MUJ | $I\text{-}A^{b}$ | CLIP | 2.15 | (2003) J. Mol. Biol. |
| 1LNU | $I\text{-}A^{b}$ | EALPHA3K | 2.50 | (2002) P.N.A.S. |
| 3C5Z | $I\text{-}A^{b}$ | 3K | 2.55 | (2008) Immunity |
| 3C60 | $I\text{-}A^{b}$ | 3K | 3.05 | (2008) Immunity |
| 3C6L | $I\text{-}A^{b}$ | 3K | 3.40 | (2008) Immunity |
| 1IAO | $I\text{-}A^{d}$ | Ova | 1.60 | (1998) Immunity |
| 2IAD | $I\text{-}A^{d}$ | HA | 2.40 | (1998) Immunity |
| 1IAK | $I\text{-}A^{k}$ | HEL | 1.90 | (1998) Immunity |
| 1D9K | $I\text{-}A^{k}$ | Conalbumin | 3.20 | (1999) Science |
| 1JL4 | $I\text{-}A^{k}$ | Ovotransferrin | 4.30 | (2001) P.N.A.S. |
| 2P24 | $I\text{-}A^{u}$ | MBP | 2.15 | (2008) J. Mol. Biol. |
| 1K2D | $I\text{-}A^{u}$ | MBP | 2.20 | (2002) Immunity |
| 2PXY | $I\text{-}A^{u}$ | MBP | 2.23 | (2007) Nat. Immunol. |
| 1U3H | $I\text{-}A^{u}$ | MBP | 2.42 | (2005) Immunity |
| 2Z31 | $I\text{-}A^{u}$ | MBP | 2.70 | (2007) Nat. Immunol. |
| 1FNE | $I\text{-}E^{K}$ | HB | 1.90 | (2001) J. Immunol. |
| 1FNG | $I\text{-}E^{K}$ | HB | 1.90 | (2001) J. Immunol. |
| 1IEA | $I\text{-}E^{K}$ | HB | 2.30 | (1996) Science |
| 1I3R | $I\text{-}E^{K}$ | Hemoglobin β2 | 2.40 | (2001) Immunity |
| 1KTD | $I\text{-}E^{K}$ | Pigeon Cyto C | 2.40 | (2002) J. Exp. Med. |
| 1R5V | $I\text{-}E^{K}$ | artificial pep. | 2.50 | (2003) Mol. Cell |
| 1IEB | $I\text{-}E^{K}$ | HSP70 | 2.70 | (1996) Science |
| 1KT2 | $I\text{-}E^{K}$ | Moth Cyto C | 2.80 | (2002) J. Exp. Med. |
| 1R5W | $I\text{-}E^{K}$ | artificial peptide | 2.90 | (2003) Mol. Cell |

anchoring pockets was provided by Fremont *et al.* when the crystal structure of the mouse HLA-DR homologue, I-$E^k$, was solved (PDB ID: 1IEA) [29]. This structure shows that polymorphisms in the beta chain give rise to differences along the binding pockets. The P1 pocket is smaller in I-$E^k$ due to a phenlyalanine at the beta86 position, which in DR1 is a glycine (Fig. 4). The beta 86 position forms the side of the P1 pocket, so larger residues at this position decrease the volume of the pocket, which restricts binding to smaller residues. In I-$E^k$, the largest pocket is the P4. Unlike HLA-DR1, this allows a large hydrophobic residue to be engaged in the middle of peptide [29].

The beta 86 position also contributes to alterations in the shape of HLA-DR alleles. For instance, in some HLA-DR alleles, there is a valine at this position instead of the glycine found in HLA-DR1. This dimorphism allows discrimination between allelic variants of HLA-DR based on the amino acid sequence at a single position within a peptide [30]. Other polymorphisms within HLA-DR variants alter structural properties of the P4 and P6 pockets pocket. For example, differences in charge for residues lining the P4 pocket in HLA-DR alleles play a role in susceptibility to rheumatoid arthritis (RA) [31], such that a positive residue at beta71 in HLA-DRB1*0401 and HLA-DRB1*0101 is linked to RA, but a negative residue as found in HLA-DRB1*0402 confers a non-RA associated phenotype. In addition, polymorphic differences extend to the P6 position of HLA-DRB1*0101 which prefers small, polar or neutral residues, whereas the DRB1*0401 can accommodate Thr, Asn, and Ser [32].

Structures of other MHC-II variants have highlighted the diversity in size, shape, specificities, and general architecture of the peptide binding groove. The mouse HLA-DQ homologue is I-A. One major structural difference between the I-A/HLA-DQ and the I-E /HLA-DR proteins is due to an alpha 9 glycine insertion which contributes to a betabulge in the floor of the peptide-binding groove (Fig. 1.5 b) [33-35]. Intriguingly, this bulge precludes large anchor residues from engaging anchor pockets within the core of the peptide binding groove, while at the same time, it draws the peptide deeper into the groove because of additional hydrogen bonds that form between the peptide and the main chain atoms of the beta bulge residues [33]. Ultimately, these effects allow tight binding to peptides that are not anchored by side chain specificity pockets within the core of the peptide binding domain [33].

HLA-DP2 is the only HLA-DP allele for which a structure has been solved. It has a similar charge and shape as HLA-DR1 for the P1 pocket, which can accommodate large hydrophobic anchor side chains [36] (Fig. 3). However, unlike other HLA-DR alleles, this structure shows that the P6 pocket is equal in size to the P1 and can also act as a major anchoring site [36]. One of the interesting features from this structure is that there is a solvent exposed acidic pocket between the peptide backbone and the β-chain α-helix that is believed to be responsible for the linkage of this allele with Chronic beryllium disease [37], a T-cell mediated hypersensitivity to beryllium metal, oxides, and alloys [38].

Structures of other MHC-II variants show yet even more diversity in the size and shape of the binding groove pockets. The HLA-DQ8 (A1*0301/B1*0302) structure (PDB ID: 2NNA), has a P1 pocket that is deep and highly charged (Fig. 3b). However, unlike HLA-DR1, there are deep pockets for the P4 position, which can accommodate large hydrophobic residues, and the P9 position, which is deep and charged. The P6 pocket is shallow and accommodates only minor anchor residues [39, 40].

The crystal structure of HLA-DQ06 (A1*0102/B1*0602), an allele which is linked to protection from type I diabetes [41], shows significant main chain and side chain conformational differences for the alpha 46 to 55 region as well as the beta 85 to 91 region (Fig. 5d). The P1 pocket is shallow as compared to HLA-DR1, mostly due to a phenylalanine at the beta 87 position, which “fills” the P1, much like other alleles which have polymorphisms at the beta 86 position that alter the P1 size and shape (Fig. 4). This structure reveals fairly equivalent sizes and shape for the P4, P6 and P9 pockets [42].

The structure of HLA-DQ2 (A1*0501/B1*0201, PDB ID:1SV9) also deviates from the canonical

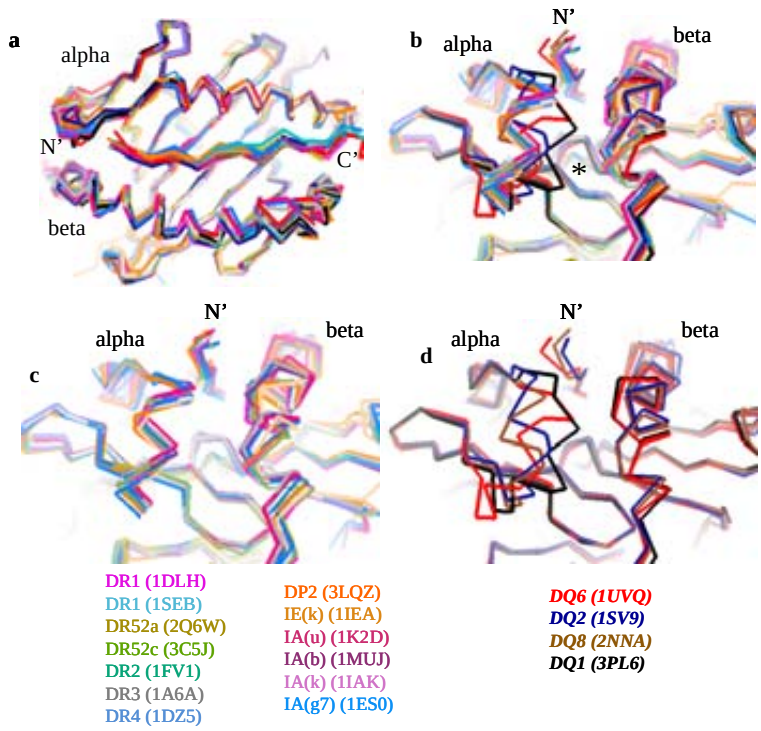
a
alpha
N'
C'
beta
b
N'
alpha
beta
*
c
N'
alpha
beta
d
N'
alpha
beta
DR1 (1DLH)
DR1 (1SEB)
DR52a (2Q6W)
DR52c (3C5J)
DR2 (1FV1)
DR3 (1A6A)
DR4 (1DZ5)
DP2 (3LQZ)
IE(k) (1IEA)
IA(u) (1K2D)
IA(b) (1MUJ)
IA(k) (1IAK)
IA(g7) (1ES0)
DQ6 (1UVQ)
DQ2 (1SV9)
DQ8 (2NNA)
DQ1 (3PL6)

**Fig. 5**

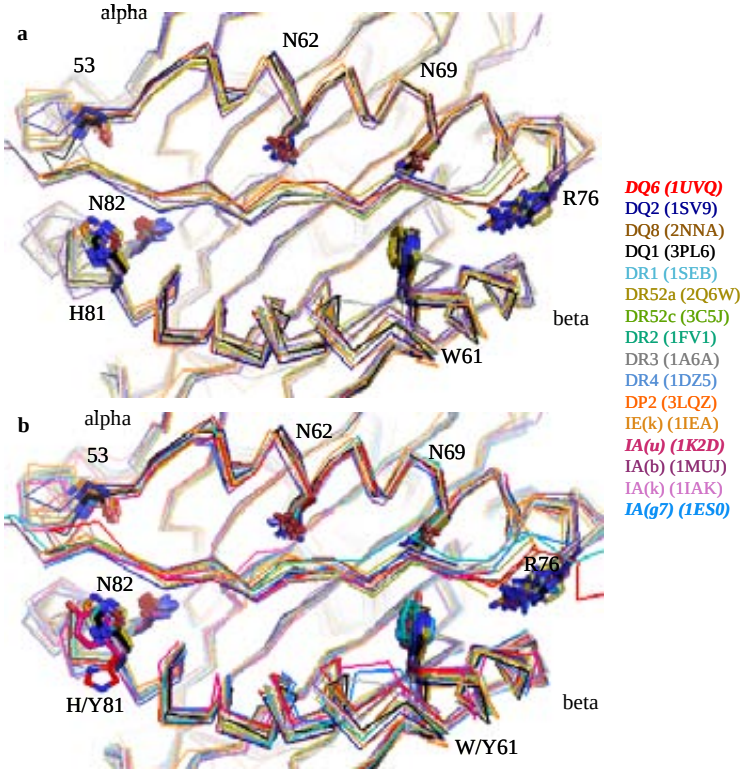
a
alpha
53
N62
N69
N82
R76
H81
beta
W61
b
alpha
53
N62
N69
N82
R76
H/Y81
beta
W/Y61
DQ6 (1UVQ)
DQ2 (1SV9)
DQ8 (2NNA)
DQ1 (3PL6)
DR1 (1SEB)
DR52a (2Q6W)
DR52c (3C5J)
DR2 (1FV1)
DR3 (1A6A)
DR4 (1DZ5)
DP2 (3LQZ)
IE(k) (1IEA)
IA(u) (1K2D)
IA(b) (1MUJ)
IA(k) (1IAK)
IA(g7) (1ES0)

**Fig. 6**

MHC-II structure with deviations in the tilt of the alpha 45-51 helical region toward the long alpha helical stretch of the alpha chain (Fig. 5d). In addition, the beta 85-91 region is tilted in toward the peptide binding domain relative to other MHC-II structures. HLA-DQ2 (1SV9) has a region of positive electrostatic charge between the P4 and P6 pockets unlike the HLA-DQ8 (2NNA) structure, which has a neutral electrostatic potential in this region. The P9 can accommodate bulky hydrophobic residues [43].

There is tremendous diversity within the peptide-binding groove of MHC-II proteins, which in turn allows peptide antigens with variable sequences to bind these proteins for presentation to CD4+ T cells. With the exception of HLA-DR alleles [44], the role that these pocket differences confer on the ability of DM to mediate peptide exchange has not been thoroughly explored (see below).

## Hydrogen bonding interactions between the peptide and MHC-II

Even though there is great diversity of the residues lining the peptide binding groove, highly conserved residues across all MHC-II alleles give rise to a canonical hydrogen bonding network which provides a peptide sequence independent type of interaction (Figs. 2, 6). There are however a few deviations from the canonical network. Notably, HLA-DQ06 (1UVQ) has a rotamer change for βH81 such that the imidazole group is positioned away from the peptide backbone and therefore unable to form a hydrogen bond at this position. Thus, a conserved residue can adopt different conformations depending on the structural context, which can alter its ability to participate in the canonical hydrogen-bonding network. The hypocretin peptide in this complex has a proline in the P2 position which may induce structural changes that propogate through the protein's secondary structure (discussed below). The βH81 position has been implicated in peptide binding stability and has been posited as a key residue involved in the function of DM mediated peptide exchange [14, 45]. However, the βH81 is not invariant, for example it is a tyrosine in IA(u) (Fig. 6). Because MHC-II βH81 hydrogen currently is understood to be a major component of the MHC-II peptide interaction, alleles that can bind peptides without forming this hydrogen bond should be more completely characterized.

## Conformational changes in MHC-II

Conformational changes have been reported for MHC-II proteins [46-54]. Although direct structural analysis by X-ray crystallography of MHC-II peptide complexes suggests a highly stereotypic structure for these proteins, biochemical data does exist for alternate conformers under conditions that differ primarily in peptide occupancy and pH. Evidence for a peptide loading-induced conformational change suggests that prior to peptide loading, empty MHC-II exist in a "floppy" conformation [46, 48]. Peptide-free "empty" MHC-II are less stable in sodium dodecyl sulfate (SDS) such that the subunits of the empty MHC-II dissociate and migrate as individual chains, whereas MHC-II in complex with stably bound peptide migrates as an intact complex [55].

**Legend to Fig. 5. Structural alignment of MHC II alleles.** Representative crystal structures for each of the MHC II alleles that have solved crystal structures are shown aligned by residues alpha 4-80 and beta 4-90, which comprise the peptide binding groove. The alpha and beta chains are labeled for each panel, and the peptide is labeled N' for the N-terminus and C' for the C terminus. (a) Ribbon diagram of the top of the binding groove for each of the alleles (listed to right by color). (b) Ribbon diagram of the N-terminal peptide binding region, asterisk designates beta bulge at alpha Gly9 in IA and DQ structures. (c) Same view as in (b) with the DQ alleles omitted. (d) same view as in (b) with only DQ alleles.

**Legend to Fig. 6. Conserved hydrogen bond network across multiple MHC II proteins.** Representative structures from each of the MHC II proteins/alleles that have crystal structures were aligned by the alpha1/beat1 peptide binding domain. (a) Proteins invariant at positions considered to be part of the "canonical" hydrogen bonding network. (b) Proteins that have deviations in the hydrogen bond network are overlaid with the invariant alleles, shown as italicized and bold to the right. HLA-DQ6 (1UVQ) has a rotamer shift of the betaH81 away from the peptide backbone. The IA(u) has tyrosines at the b81 and the b61 and the and IA(g7) has a tyrosine at the b61 position.

Peptide loading to MHC-II proteins has been shown to alter the apparent molecular weight as assessed by gel filtration, which changes from 50kDa, for MHC-II in complex with stable peptide to ~60kDa for empty MHC-II [49]. Since the actual molecular weight of the peptide complex is larger than the empty protein, the larger apparent molecular weight for the empty protein implies that there is a difference in shape. In addition, a change in hydrodynamic radius from 37.4 Å for empty MHC-II, to 31.8 Å for MHC-II-peptide complex has been observed [49]. Studies using far-UV circular dichroism (CD) spectroscopy suggest that peptide-empty MHC-II exists in a conformation that has slightly less helical content [49, 56].

In addition to peptide-induced variations in MHC-II structure, there is evidence that pH may play a role in MHC-II conformational changes. The effect of pH on MHC-II conformation has been partially attributed to increased peptide binding at low pH [57, 58]. In endosomal compartments (pH ~5), exposure to peptides that can form stable MHC-II peptide complexes decreased the floppy conformation, noted above, and promoted an SDS-stable conformation of the complex [58]. In addition, there are changes in the CD spectrum for MHC-II under acidic conditions as compared to spectra at neutral pH, suggesting that there is loss of helicity for MHC-II under acidic conditions [54, 59]. A histidine at position alpha 33 also has been implicated as a pH “trigger” for MHC-II conformational changes [60]. When the alpha His 33 is mutated to a tyrosine, a relatively isosteric analogue that is unable to undergo protonation at endosomal pH, the stability of the peptide-MHC-II complex is unaffected by changes in pH [60].

Although the aforementioned studies suggest that MHC-II can undergo alterations in conformation as a result of peptide binding and/or changes in pH, they do not reveal the regions of the MHC-II that are involved. Studies designed to better understand which regions of the MHC-II participate in peptide-inducedchanges have subsequently been carried out, and include epitope mapping of antibodies specific for empty MHC [51], differential chemical modification due to peptide occupancy [47], as well as molecular dynamics simulations of the peptide free form of MHC-II [61]. These studies will be described below.

Using a panel of monoclonal antibodies raised against the denatured beta chain of HLA-DR1 and screened for preferential reactivity against peptide-empty vs. peptide-loaded HLA-DR1, Carven *et al.* were able to map regions along the beta chain of HLA-DR1 that had different activity when the MHC-II complex was empty or loaded with peptide [51]. Antibodies that reacted differentially to empty and peptide loaded HLA-DR1 were mapped to two distinct regions along the beta chain. One set of antibodies mapped to the alpha helical region in the beta 1 domain from the 53-73 positions; notably, this region is kinked, with the Leu 53 pointing down toward the peptide binding groove which is seen in structures of HLA-DR peptide complexes. In order for there to be a recognizable epitope at the Leu 53, presumably there would have to be an alteration of this region that exposed that residue. The other epitope recognized on the empty but not peptide-loaded HLA-DR1 mapped to the membrane-proximal C-terminal region of the beta chain and encompassed residues 186-189. The implication of having this region differentially recognized is that the conformation of the empty protein must have differences that propagate to regions far away from the peptide binding domain [51].

Carven *et al.* continued to define differences in conformation between empty and peptide loaded MHC-II by probing for differential reactivity towards selective chemical modifications between the bound and unbound complexes [47]. Here, they determined differential reactivity for a residue in the alpha chain proximal to the P1 pocket, αArg50, as well as two other residues, βArg198 and βArg98. These three residues map to the same lateral face of HLA-DR1 that is predicted to be at the binding interface between MHC-II and HLA-DM [47]. An additional residue in the alpha chain, αLys67, was also observed to be differentially reactive between the empty and peptide loaded structures [47].

In addition to biochemical studies identifying regions involved in the peptide-induced conformational change, molecular dynamics simulations and normal mode analysis of peptide free MHC-II have been performed [61-63]. These

studies have implicated beta 57-67, described as a region differentially recognized in empty MHC-II by monoclonal antibodies above, as well as the short alpha chain $3_{10}$ helix and adjacent strand proximal to the P1 pocket, as undergoing conformational changes during peptide loading.

The conformational changes described in the aforementioned studies are based on HLA-DR alleles. However, an overlay of the structures for different MHC-II alleles reveal that even in the peptide-bound form there are regions that can adopt alternate conformations, some of which are beyond the side chain specificity pockets (Fig. 5). The beta-chain helical region has a distinct kink, which has been implicated as a region that undergoes conformational changes based on peptide occupancy, as described above. However, even in the peptide bound form, the position of this kink can move toward or away from the peptide binding groove (Fig. 5). In addition to this variable conformation on the beta chain, the alpha $3_{10}$ helical region can adopt alternate conformations in the HLA-DQ alleles (Fig. 5). Intriguingly, the HLA-DQ0602 structure (1UVQ; A1*0102/B1*0602) and the HLA-DQ1 (3PL6; A1*0102/B1*0502) have the same alpha chain, yet have alterations in the conformation of the alpha $3_{10}$ helix (Fig. 7). The HLA-DQ2 (1SVP; A1*0501/B1*0201) also deviates from the majority of MHC-II structures in the $3_{10}$ helical region (Fig. 5).

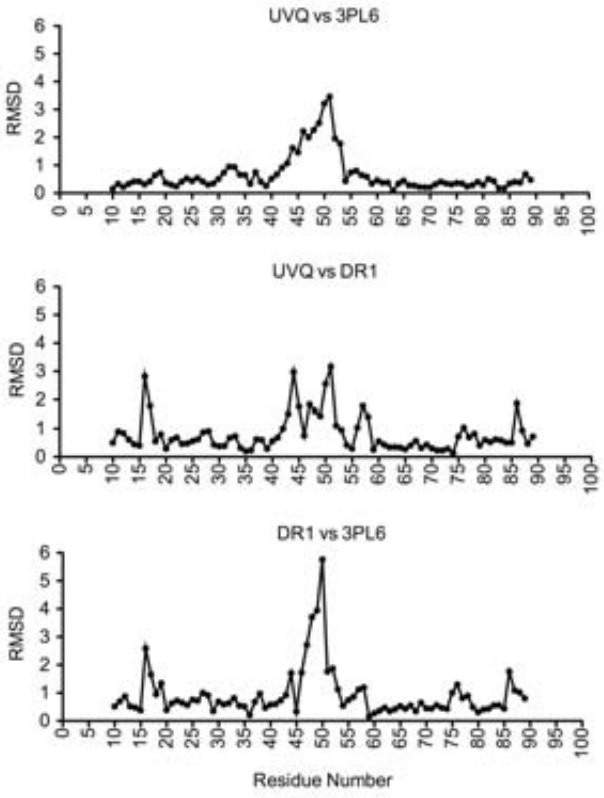


**Fig. 7. The alpha $3_{10}$ helical region can adopt alternated conformations even in HLA-DQ alleles that contain the same alpha chain**. (top) LSQ alignment, between the HLA-DQ structures, 1UVQ and 3PL6 (both contain the same alpha chain), of the C-alpha atoms for residues in the $3_{10}$ alpha helix. (middle) The same alignment was performed for the HLA-DQ structure 1UVQ vs. the HLA-DR1 structure, 1DLH. (bottom) The same analysis is shown for the HLA-DR1 structure, 1DLH and the HLA-DQ structure 3PL6. In all cases, there is a deviation in RMSD in the $3_{10}$ helical region.

## HLA-DM

HLA-DM (DM), like its murine ortholog H-2M, is an MHC-II-like alpha beta heterodimer that is structurally homologus to classical MHC-II proteins (Fig. 8) [64]. The structure of DM revealed that it is composed of two membrane proximal immunoglobulin-like domains, α2 and β2, and two membrane distal domains that contain two anti-parallel alpha helices that flank a beta sheet platform [64, 65]. Although DM shares only ~28% sequence identity with the MHC-II alleles, the structural similarities between the two molecules are striking. The crystal structure of DM has the same overall fold as DR1 with one obvious deviation: the region which constitutes the peptide binding domain of MHC-II is partially collapsed, leaving only a vestigial P4 pocket, and DM is devoid of bound peptide (Fig. 8) [64, 65].

HLA-DM is an endosomal resident that is necessary for proper antigen presentation by MHC-II proteins [66]. As noted above, in the absence of DM, most MHC-II molecules remain occupied with CLIP indicating that the repertoire of peptides displayed at the cell surface requires the catalytic action of DM [66, 67]. The individual crystal structures of DM and MHC-II reveal no obvious docking site. Several subsequent studies have aimed at identifying the DM-MHC-II interface, as described below.

The mechanism through which DM mediates peptide catalysis remains to be elucidated. However, various functions of DM have been described.

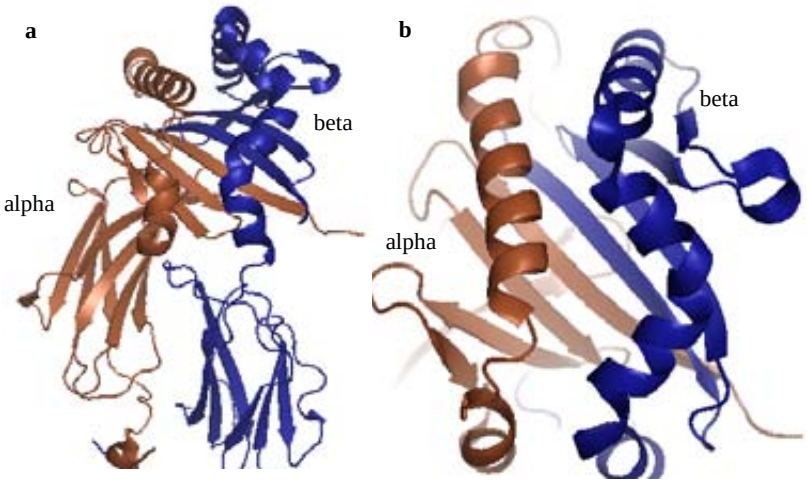
a
beta
alpha
b
beta
alpha

**Fig. 8**

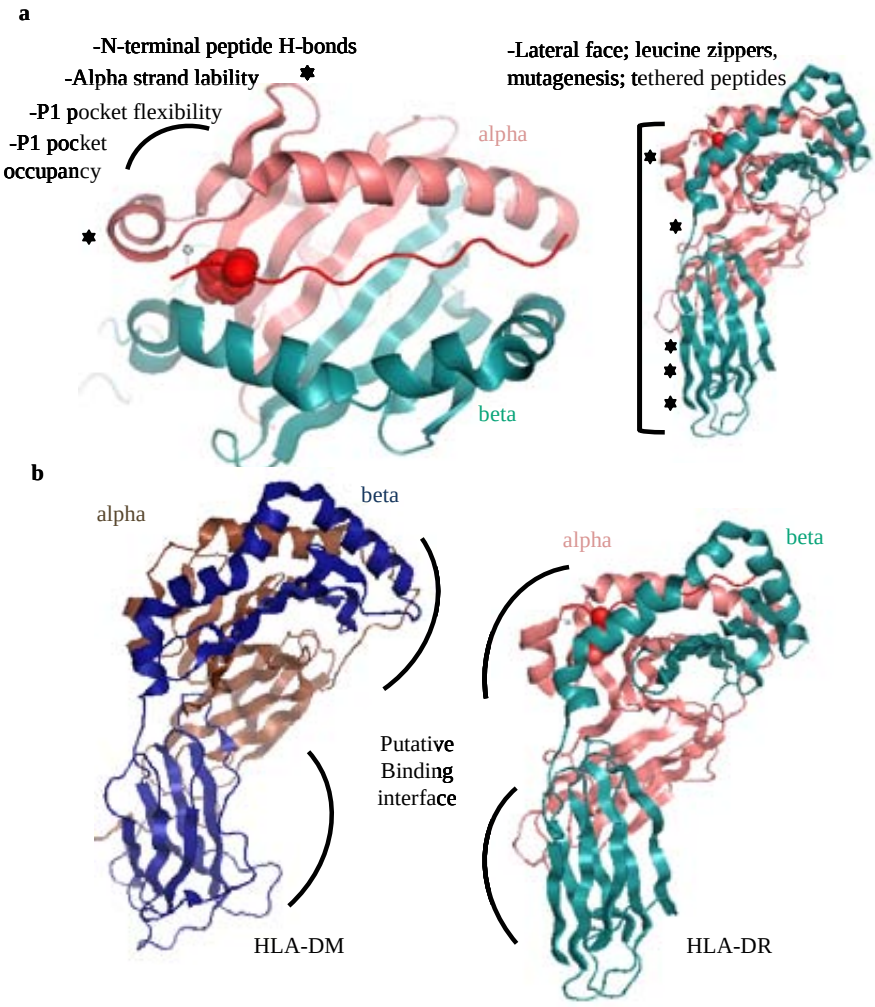
a
-N-terminal peptide H-bonds
-Alpha strand lability
-P1 pocket flexibility
-P1 pocket occupancy
alpha
beta
-Lateral face; leucine zippers, mutagenesis; tethered peptides
b
alpha
beta
Putative Binding interface
alpha
beta
HLA-DM
HLA-DR

**Fig. 9**

DM acts as a chaperone to protect peptide-free MHC-II from forming inactive aggregates [55, 68, 69]. In addition, DM promotes peptide exchange from MHC-II [11, 66, 70], and increases peptide binding to empty MHC-II [71]. Given the structural differences between empty and loaded MHC-II, and the roles for DM in promoting peptide exchange and stabilizing empty protein, one might expect that DM would interact specifically with particular conformations of MHC-II.

**Identification of the DM-MHC-II interface**

Given that there is no co-crystal of the DM-MHC-II complex, nor structural information regarding the dynamic nature of this interaction, studies have focused identification of key residues and regions involved in the DM-MHC-II interaction in order to gain insight into the mechanism of peptide catalysis. Examination of the DM structure revealed a tryptophan-rich hydrophobic cluster on one lateral face of DM that was proposed to be the binding site for either MHC-II and/or HLA-DO, an inhibitor of DM catalyzed peptide exchange [64]. Early experimental work aimed at identifying interaction sites for DM on MHC-II was presented by Doeble *et al.* [72], in which random mutagenesis of both chains the MHC-II allele, HLA-DR3, was performed in order to test which residues were important for the DM-MHC-II interaction. Using a screening method for cell-surface HLA-DR3-bound CLIP, in a DM deficient cell line, this group identified HLA-DR3 mutations that led to elevated cell surface expression of CLIP because of reduced DM-induced peptide exchange. The results implicated one lateral face of HLA-DR3 in the interaction with DM [72] (Fig. 9).

Looking at other regions of both MHC-II and DM proteins, Weber *et al.* investigated the role of the transmembrane and cytoplasmic domains of DM and MHC-II [73]. These were determined to be of importance only in colocalization of the two proteins within the same membrane, and not involved in the mechanism *per se* [73].

Due to the low binding affinity of soluble DM and MHC-II [74], and the recent evidence that tethering to the membrane by both molecules was necessary for optimal DM mediated peptide catalysis, Busch *et al.* [75] devised a system whereby DM and HLA-DR could be tethered in parallel orientations that would differentially bring opposing lateral faces of the molecules in close proximity [75]. This was accomplished by tethering leucine zippers to alpha and beta chains of each protein with variable linker distances. This work further established that the lateral face implicated in earlier mutagenesis work was at the interface between DM and MHC-II. In addition, the orientation which led to optimal DM mediated peptide catalysis suggested that the HLA-DR and DM beta chains were in closer proximity than the alpha chains (Fig. 1.9).

Further details regarding the orientation of DM and MHC-II emerged from work carried out by Stratikos *et al.* [76]. This group tethered either the N- or C-termini of antigenic peptides to a free cyteine β46 on DM, and tested the ability of DM to “exchange” the tethered peptide on HLA-DR1. When the N-terminal peptide/DM complex was used, exchange rates were several orders of magnitude greater then when the C-terminal tethered peptide, or untethered peptide was used. This finding established the N-terminal peptide binding region of the MHC-II to be involved at

**Legend to Fig. 8. Crystal structure of HLA-DM.** (a) Cartoon representation of an HLA-DM peptide complex (PDB ID# 2BC4). The alpha chain is shown in brown, the beta chain in blue. (b) 90° rotation showing the top of the closed groove that corresponds to the peptide binding groove in other MHC II proteins.

**Legend to Fig. 9. DM-MHC II interactions.** (a left) Ribbon diagram of the top of the peptide binding domain of HLA-DR1 with the alpha chain in salmon, the beta chain in teal and the peptide in red. The P1 tyrosine of the peptide peptide in shown in spheres. Curve represents regions implicated in the DM-MHC II interaction, with brief descriptions noted. Stars represent residues found to be important in the DM-HLA-DR3 interaction. (a right) Side view of the HLA-DR1 peptide complex depicted on the left with residues important in the DM-HLA-DR3 interaction indicated as stars, bracket indicates the lateral face of HLA-DR thought to be at the interface with DM in the DM-MHC II complex. (b) Ribbon diagram depicting the putative DM HLA-DR binding interface

the interface between these molecules, thus supporting work implicating this lateral face (Fig. 1.9).

Providing further information on the orientation of the DM-MHC-II complex, another study used mutagenesis of DM residues to reveal that there was a lateral face on DM that contained residues important to the DM-MHC-II interaction (Fig. 9) [77]. This surface was distinct from the tryptophan-rich face proposed earlier [64]. This work helped to unequivocally define the faces of DM and MHC-II that were involved at the interface during peptide catalysis. However, taken together, these studies establish only a low resolution structural model for the DM/ MHC-II complex.

The P1 pocket MHC-II has been implicated also as important in the DM-MHC-II interaction. The flexibility of the P1 pocket has been postulated by Chou *et al.* as a key structural determinant of DM susceptibility [78]. They proposed that when the P1 pocket is empty and/or flexible, DM can recognize MHC-II and induce a peptide receptive state. From this work, the flexibility rather then the stability of the complex was introduced as a potential determinant for DM action [78] (Fig. 9).

Using a panel of peptides that were unable to form various hydrogen bonds along the peptide backbone, Stratikos *et al.* [13] determined that hydrogen bonds that formed between the N-terminal region of the peptide and conserved residues of the MHC-II, proximal to the P1 pocket, were critical determinants of DM action. When these bonds were broken, DM catalysis of peptide release was augmented up to an order of magnitude [13] (Fig. 9).

Recently, a new model has been proposed that suggests DM recognition of MHC-II peptide complexes only occurs for MHC-II that have a vacant P1 pocket as well as disrupted N-terminal hydrogen bonds [79]. In this model, spontaneous protein motions in the N-terminal region of the peptide would eject the peptide from the P1 pocket, providing a novel MHC-peptide complex structure that DM could recognize. Although this work provides a model for the MHCII conformation recognized by DM, a mechanistic understanding of the catalytic role of DM remains unresolved.

Recent work by our group on an MHC-II mutant, αF54C HLA-DR1, describes dramatic increases in DM susceptibility and affinity as well as a conformational change in the same alpha $3_{10}$ helical regions as those seen in some of the HLA-DQ alleles (Fig 5d). Structural analysis, peptide dissociation studies and mass spectrometry identified the alpha F54C mutation as a key determinant for increasing the DM mediated rate of peptide exchange as well as being a locus for stuctural lability within the MHC-II $3_{10}$ alpha helix and adjacent strand region. Therefore this region is a key structural determinant for DM mediated peptide exchange (manuscript submitted for publication).

Importantly, it should be noted that, the functional studies described above were limited to HLA-DR alleles. However, as described above there are conformational differences in regions of HLA-DQ alleles that lie at the presumptive DM-MHC-II interface (Figs. 5, 9). It has been established that DM has different susceptibility for allelic variants of HLA-DR [44], and there are hints that HLA-DQ structural differences may play a role in DM susceptibility [80]. To date, a thorough examination of HLA-DM susceptibility for non-HLA-DR MHC-II proteins has not been presented. This would appear to be necessary for a comprehensive understanding into the nature of DM mediated peptide catalyses.

## SUMMARY

Structural analyses of MHC-II proteins have revealed the key interactionsnecessary for formation of a stable MHC-peptide complex. Mutagenesis studies and biochemical characterizations have provided a structural modelof the bi-molecular DM-MHC-II complex. However, details that would enable a full understanding of the DM-mediated peptide exchange process remain elusive.

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