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HLA-DM and HLA-DO, key regulators of MHC-II processing and presentation

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Peptide loading of class II MHC molecules in endosomal compartments is regulated by HLA-DM. HLA-DO modulates HLA-DM function, with consequences for the spectrum of MHC-bound epitopes presented at the cell surface for interaction with T cells. Here, we summarize and discuss recent progress in investigating the molecular mechanisms of action of HLA-DM and HLA-DO and in understanding their roles in immune responses. Key findings are the long-awaited structures of HLA-DM in complex with its class II substrate and with HLA-DO, and observation of a novel phenotype — autoimmunity combined with immunodeficiency — in mice lacking HLA-DO. We also highlight several areas where gaps persist in our knowledge about this pair of proteins and their molecular biology and immunobiology.

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Introduction

HLA-DM and HLA-DO and their murine counterparts H-2M and H-2O (generically referred to here as DM and DO) are non-peptide binding class II major histocompatibility (MHC-II) homologs. Unlike the large family of class I MHC homologs, which have varied roles in many cell types as endocytic receptors, NK ligands, T cell decoys, and presenters of peptides, lipids, and vitamin derivatives [1], for the non-classical MHC-II proteins DM and DO known roles are only in antigen-presenting cells, where they regulate loading of peptides derived from self and foreign antigens. DM functions as a peptide exchange factor required for efficient loading of endosomal peptides onto MHC-II molecules. DO functions as a modulator of DM. The molecular mechanism by which DM promotes peptide exchange and the roles of DM and DO in the overall immune response are outstanding fundamental questions in MHC biology. In the period covered by this review, significant progress has been made towards understanding the structural basis for DM interaction with MHC-II, and new work strengthens the conclusion that DM plays a key role in immunodominance. However, important mechanistic questions about DM action still remain unanswered, and this constrains our ability to integrate these advances into deeper understanding of how DM functions in development, maintenance, and activation of the $CD4^+$ T cell response. For DO, the mechanism of action has been established: DO acts as substrate mimic to competitively inhibit HLA-DM-mediated catalysis of MHC-II peptide exchange. A key role for DO in regulating autoimmunity has been revealed through studies of H-2O knockout mice. However, the relationship of the molecular mechanism of DO action to its biological role still is not clear.

Insight into DM function from crystal structures of DM-DO and DM-DR

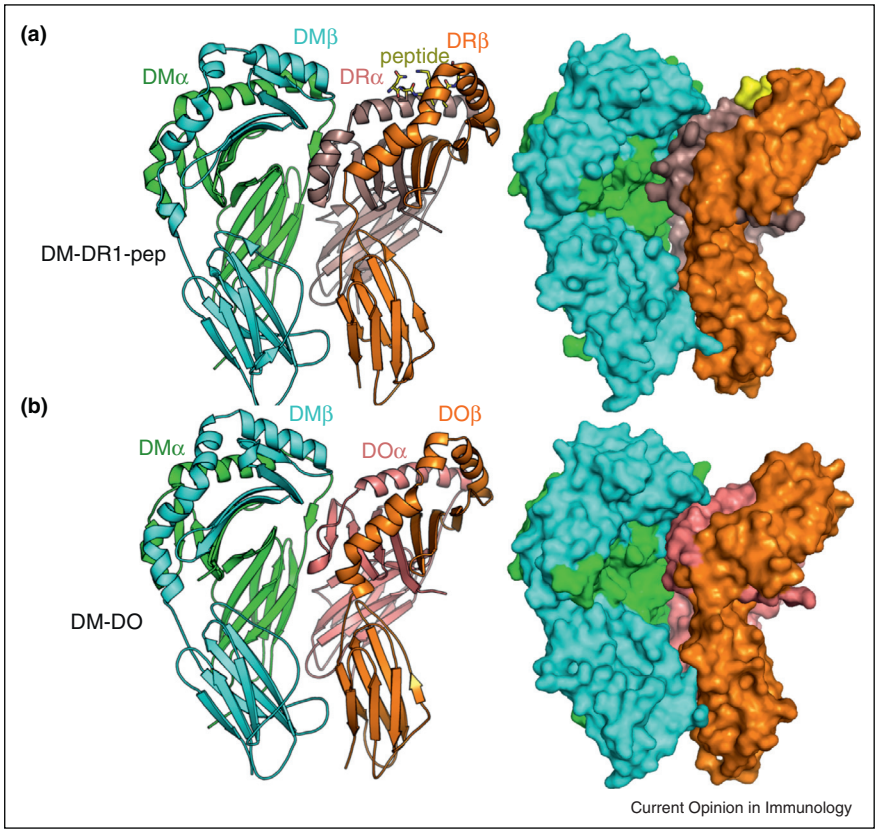
Two crystal structures of trapped DM-MHC complexes provided long-awaited insight into how DM engages MHC-II to promote peptide exchange [2••,3••]. DM acts as an enzyme to catalyze peptide exchange [4,5], and like other enzymes it binds only transiently to its substrate(s) before inducing conversion and releasing product(s). Thus, DM does not bind stably to MHC-peptide complexes [6•,7•]. DM does not appear to bind to recombinant peptide-free empty MHC molecules [7•], although DM binding to apparently empty MHC molecules produced in their normal cellular context has been reported [8,9]. The discrepancy may be due to differences between metastable 'peptide-receptive' species generated during peptide dissociation [9–11] and stable 'peptide-averse' species produced in the absence of peptide [11,12]. Previously, a few mutated HLA-DR-peptide complexes with weakened MHC-peptide interaction have been shown to bind to DM sufficiently tightly to be observed biochemically [6•,7•,13], but until recently all of these have resisted crystallization and detailed structural analysis. In one of the recent structure reports, Pos *et al.* crystallized a DM-MHCII complex after covalent attachment of DM to

HLA-DR1 via sortase-A mediated coupling of the DM beta-subunit C-terminus to the HLA-DR1 beta-subunit C-terminus, with the HLA-DR1 carrying a truncated peptide attached via a disulfide bond engineered into the P6 pocket [2••]. The peptide was designed to bind only to the C-terminal side of the binding site, leaving the N-terminal side empty; usually such peptides bind weakly if at all, but here the interaction was stabilized through covalent bonding to the MHC. Crucially, leaving the N-terminal side of the site open allows MHC conformational alteration and stable interaction with DM. In the second of the crystal structure reports, Guce *et al.* crystallized DM with HLA-DO [3••]. In the complex, DO adopts an overall conformaton highly similar to classical MHCII proteins with an open groove, but with conformational alterations at the N-terminal side. The DO structure provides insight into the nature of αβ chain association in the MHCII family and constrains possible functional roles for DO in antigen presentation. DO was shown through enzymatic and mutagenesis studies to act as a substrate mimic, binding tightly to DM and competitively inhibiting the interaction with MHC-peptide. In the crystal structure DO was observed to bind to the same lateral face of DM as does DR, with essentially all interface residues conserved.

The DM-MHC interaction in the two structures is nearly identical (Figure 1), alleviating concerns that the protein engineering necessary to trap DM with HLA-DR might have induced a non-physiologically relevant conformation, or that DO's mimicry of an MHC-peptide complex might not extend to structural details of its interaction with DM. A FRET study revealed a similar side-by-side arrangement for DM bound to DO in solution [14•], further supporting the physiological relevance of the complex visually by X-ray crystallography.

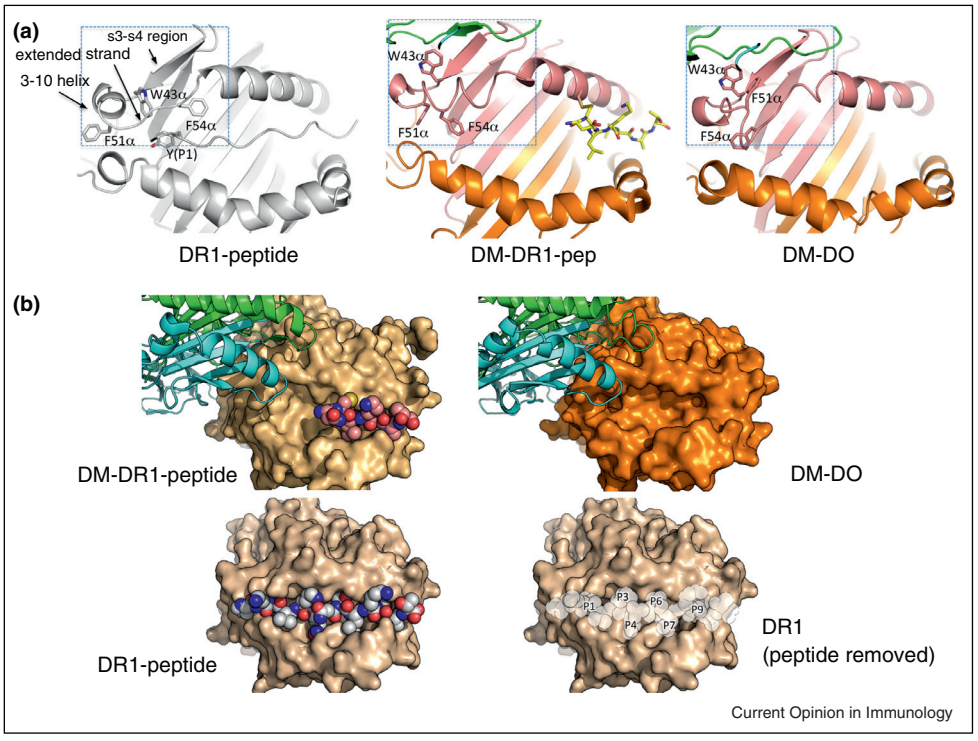
The crystal structures reveal an extensive interface between DM and MHC-II involving both membrane-proximal immunoglobulin-like domains and membrane-distal helices-atop-sheet domains, generally corresponding to the lateral surfaces previously identified by mutagenesis [15,16]. The interaction is surprisingly asymmetric, given the structural homology between DM and both HLA-DR and HLA-DO, and involves surfaces not previously observed to be involved in interactions of MHC proteins with other binding partners. Compared to crystal structures of DM alone [17–19], DM does not undergo any appreciable conformational alteration upon DR/DO binding, but HLA-DR undergoes a dramatic change in residues 35–57

Figure 1



DM engages DR1 and DO similarly. **(a)** Structure of DM bound to HLA-DR1 carrying a covalently attached peptide fragment to mimic a reaction intermediate, from PDB:4FZQ described in Pos *et al.* [2••]. **(b)** DM bound to DO, a tight-binding competitive inhibitor that acts as a substrate mimic, from PDB:4IOP described in Guce *et al.* [3••].

Figure 2



Conformational alteration in DM–DR1-peptide and DM–DO occludes the P1 regions of the MHCII peptide-binding site. **(a)** Major structural alterations are restricted to residues 35–57 in the MHCII alpha subunit (boxed), involving strands 3 and 4 of the beta sheet platform (s3–s4 region), the MHCII 3–10 helix adjacent to the peptide binding site, and the extended strand region alongside the P1 pocket. **(b)** These alterations sterically occlude the N-terminal side of the peptide-binding site. Locations of the peptide side-chain binding pockets are indicated after notional removal of the peptide from the structure of DR1 bound to an influenza-derived peptide.

of the alpha subunit (boxed in Figure 2a). This region comprises the last two strands (s3–s4) of the beta-sheet 'floor' of the peptide-binding site, the short 3–10 helix at the N-terminal end of the site, and the extended strand region in the vicinity of the P1 pocket. The structure of free HLA-DO has not been determined, so whether or not it undergoes conformational change upon binding DM is not known, but HLA-DO in complex with DM assumes a conformation that is similar to the altered MHC-II-peptide structure in the DM/DR complex (Figure 2a). The conformation includes a flipped-out orientation of Trpα43, previously identified as a crucial for the DM/DR interaction [6•], and occlusion of the N-terminal side of the peptide-binding site (Figure 2b). The extended strand rearrangement places a phenylalanine in the P1 pocket (Pheα51 for HLA-DR, Pheα54 for HLA-DO) and disrupts hydrogen-bonding interactions between the extended strand and the peptide main-chain atoms. Both the P1 pocket and the main-chain hydrogen bonds in this region are known to be important for stabilizing MHC-peptide interaction. Thus the structures provide a straightforward molecular mechanism for DM-catalyzed peptide exchange, in which DM binding destabilizes the MHC-peptide complex by interfering with key peptide main chain and side chain binding interactions.

Determinants for DM action

Much recent effort in the field has been devoted to identifying features of the MHCII-peptide complex associated with susceptibility to editing by DM. Resistance to DM editing is thought to be a key aspect of $CD4^+$ T cell epitope selection. Yin *et al.* validated this idea in the context of the long-term memory response to smallpox vaccination, adding to the existing literature of studies in mice [20]. Various features of the MHC-peptide interaction were measured for HLA-DR1 and a series of peptides from the vaccinia A10L major core protein, and the dissociation lifetime in the presence of DM was found to be the best predictor of immunogenicity. Chaves *et al.* reviewed currently available algorithms for predicting $CD4^+$ T cell epitopes and concluded that, while these algorithms are useful for many aspects of epitope discovery, their prediction efficiency remains poor [21]. It is likely that approaches that take into account the action of DM would have greatly improved prediction efficiency. However, the peptide sequence determinants for DM editing are not yet clear.

One line of evidence implicates P1 pocket occupancy as a key feature determining DM susceptibility. For HLA-DR (and its murine counterpart I-E), the P1 pocket is the largest and most important energetically of the MHCII peptide side-chain binding pockets. The P1 pocket becomes sterically blocked in the DM-MHCII peptide complexes as a result of the DM-induced conformational change (Figure 2). Anders *et al.* found that HLA-DR molecules carrying truncated peptides that left the P1 pocket vacant bound tightly to DM [6•]. As the P1 pocket is the major determinant of peptide binding affinity, the truncated peptides bound only very weakly to DR, but could be trapped in the site by engineered disulfide bonds. In a subsequent study, Schulze *et al.* found that that truncated peptides that retained P1 pocket occupancy were resistant to DM [22]. These considerations led Pos *et al.* to a model whereby the ability of a peptide to make strong interactions in the HLA-DR P1 pocket is the crucial factor for resistance to DM editing [23]. For HLA-DQ (and I-A) proteins, there is not as much information on binding specificity and DM susceptibility, but the P1 pocket is smaller and less consequential energetically [24], and so factors in addition to P1 interactions might play a greater role for these proteins.

A different line of evidence implicates MHC conformational change as a key feature determining DM susceptibility. Painter *et al.* found that substitution of DRα F54 resulted in MHC-peptide complexes highly sensitive to DM editing and able to form relatively stable DM complexes even with a large anchor residue in the P1 pocket [7•]. Structural analysis of the mutant protein revealed conformational changes in the alpha subunit 3–10 helix and extended strand region, smaller but in the same regions as the MHCII conformational changes observed in the DM-MHCII crystal structures. Hou *et al.* investigated HLA-DQ2, an allele that is relatively resistant to DM editing, and found that insertion of a glycine residue at position DQα 53 in the extended strand recovered DM susceptibility [25]. HLA-DQ2 has a deletion at this position relative to most other MHCII proteins, but the deletion does not dramatically change the MHC conformation in this region [26]. How insertion of glycine at DQα 53 increases DM susceptibility is not known, but increasing conformational flexibility would appear to be a likely explanation. Using a gel mobility assay and a series of DR1-binding peptides, Ferrante *et al.* observed two different MHC-peptide conformers populated according to peptide binding affinity, only one of which was a substrate for DM editing [27•]. All the peptides shared a tyrosine at the P1 position, but differed at other, non-anchor positions. These considerations lead to a model whereby the ability of a MHC-peptide complex to adopt a new conformation is the key feature determining DM susceptibility.

Most naturally occurring MHC-peptide complexes will occupy the P1 pocket, and indeed usually the entire P-2 to P10 region. Whether the relative DM susceptibility of these complexes is related to how frequently they vacate the P1 pocket, or to how efficiently they populate a DM conformer susceptible to editing, and whether these properties are related in any straightforward way to peptide sequence, remain topics for further investigation. To date conformational properties have been investigated only for a very few HLA-DR complexes, and DM susceptibilities investigated for a somewhat larger set of peptides but almost exclusively for a very few HLA-DR alleles (primarily DRB1*0101). Thus at present there are not enough empirical data available to resolve these important questions about the sequence and structural determinants for DM susceptibility.

Outstanding questions about the mechanism of DM action

Several important questions remain about the molecular mechanism of DM action. Despite the overall congruence of DM-DR and DM-DO structures, the actual conformation of the crucial region MHC-II α35–57 region is quite different in two structures, with either αF51 or αF54 occupying the P1 pocket (Figure 2a). These phenylalanine residues are conserved, but not invariant, among MHC-II sequences, and understanding their relative roles might bear on the relative DM susceptibilities of MHC alleles with differences at these positions. The relationship of these trapped complexes to an actual reaction intermediate is not clear, and the very basic mechanistic question of whether the DM-bound MHC-II intermediate contains one (destabilized) peptide [2••,6•], two (partially bound) peptides [28,29], or no peptide at all [3••,7•], remains to be established. Besides its mechanistic importance, this issue relates to question of peptide competition for MHC-II binding, important both for understanding the factors that regulate the spectrum of peptides displayed normally by antigen presenting cells and also for guiding efforts to alter this spectrum therapeutically. Finally, none of the mechanistic studies to date have addressed the relationship between DM action and those of small molecule [30–32] and short peptide [33,34] modulators of MHC-II peptide dissociation, which have been described as having a DM-like mechanism of action. A possible mechanism that could reconcile all these ways to promote MHC-peptide dissociation would include an aspect of facilitated dissociation by subsite occupancy [35], as recently proposed for IgE-Fcε receptor inhibitors [36]. In this mechanism, dissociation of a ligand with multiple attachment points is facilitated by blocking only one of the sites of interaction. It is possible that small molecules that occupy a side-chain binding pocket [30–32], short peptides that occupy only part of the peptide-binding site [33,34], and DM-induced conformational changes that block P1 pocket and/or hydrogen-bonding interactions

[2••,3••,6•,7•], all induce peptide dissociation by this mechanism.

Progress in elucidating the elusive role of DO in immunity

In an important paper, Gu *et al.* report immunodeficiency and autoimmunity in H2-O knockout mice [37••]. Previous biochemical and cellular work on H2-O had identified potential roles for DO in regulating antigen presentation [38–42] and in shaping the spectrum of MHC-II-bound peptides [41,42], but only very limited immune changes were identified in knockout mice [38,39]. Gu *et al.* show that H2-O$^{-/-}$ mice spontaneously develop high titers of anti-nuclear antibodies. Despite the production of autoantibodies, T-dependent IgG antibody responses to model antigens are delayed, perhaps due to the reduced frequency of marginal zone B cells in these mice. Thus DO decreases immunity to self-antigens while increasing immunity to (at least some) foreign antigens. CD4^{+} T cells that developed in H2-O$^{-/-}$ mice were required for autoantibody production and higher ANA titers were observed when B cells also lacked H2-O. T cell receptor repertoire was altered, as reflected by greater homeostatic proliferation of H2-O$^{-/-}$ T cells when they were transferred into H2-O$^{-/-}$ (compared to H2-O$^{+/+}$) hosts. The observations from these mice argue that DO's regulation of self-peptide presentation is important in restraining peripheral activation of autoreactive CD4+ T cells, consistent with Yi *et al.* [43]. In a separate avenue of research, Kremer *et al.* showed that DO is required for efficient MHC-II presentation of particular human self-antigens [44]. Similarly, DO expression generally is required for high levels of class II invariant chain peptide (CLIP) presentation at the cell surface; new work from the Pezeshki *et al.* shows that this increases binding of particular superantigens, SEA and TSST-1 [45]. The finding that HLA-DO is a competitive inhibitor of DM action would appear to indicate that DO acts simply by attenuating DM, and not, for example, by modulating DM specificity [3••], although Poluektov *et al.* have proposed an additional role for HLA-DO in direct interaction with MHC-II [46].

Gu *et al.* suggest that if DO is acting by inhibiting DM that inhibition should be spatially or temporally regulated to result in a qualitatively different spectrum of peptides presented on MHC-II [37••], as has been reported in class II peptide elution studies of B cells with and without DO [40]. DO is relatively unstable in the absence of DM and free DO does not traffic beyond the ER [47], so most or all DO in an antigen-presenting cell is associated with DM. However, there appear to be mechanisms that result in differential steady state distribution of DM/DO complexes and free DM within the endosomal pathway in B cells, with DM/DO enriched in early endocytic compartments and free DM enriched in late endosomal/lysosomal compartments [48]. Within the latter, evidence suggests that there is differential distribution of DM and DM/DO [49]. Consistent with this prior finding, Xiu *et al.* used B cell and HeLa cell transfectants to show that DM redistributes to the limiting membrane from the internal vesicles of lysosomal multivesicular bodies though its interaction with DO, in a process mediated by the sorting motif present in the HLA-DO beta subunit cytoplasmic tail [50]. Jahnke *et al.* report evidence supporting ubiquitination as a direct regulator of DO intracellular localization, with additional indirect effects on endocytic machinery [51]. Thus, intracellular sorting and localization might provide the spatial regulation hypothesized by Gu *et al.* to generate a different spectrum of self and foreign peptide antigens bound to MHC-II in the presence or absence of DO.

Role of DM in autoimmunity

Several papers have reported a requirement for DM activity in development of type 1 diabetes (TID). Hyperexpression of DO, with consequent reduced DM function, inhibited T1D in NOD mice [43]. Direct ablation of DM function in NOD mice, achieved by gene targeting of NOD ES cells, also blocked diabetes [52•]. In the second model, reduced numbers of pathogenic CD4 T cells were observed. The expression on antigen-presenting cells of the NOD class II molecule I-Ag7 is lower in both DO-overexpressing and DM-deficient strains as compared to wild type NOD, reflecting dependence of I-Ag7 on DM chaperoning. In these models, the role of DM in autoimmunity is hypothesized to be related to peripheral presentation of DM-dependent disease-initiating peptide(s), with reduced DM activity resulting in reduced presentation and reduced disease pathology. In other studies of the role of DM in the NOD model of type 1 diabetes, evidence has been presented supporting a critical role in disease pathology for CD4+ T cells that recognize DM-susceptible complexes of I-A/insulin peptide, generated by capture of extracellular peptide at the cell surface or in re-cycling vesicles, locations where DM activity is low. DM is hypothesized to edit out disease-related peptide(s) during tolerance development, with reduced DM activity resulting in increased tolerance to DM-susceptible self-antigens [53]. Further, evidence has been presented supporting a critical role in disease pathology for insulin-reactive CD4+ T cells recognizing a DM-independent/susceptible epitope [54]. An integrated picture would suggest multiple roles for DM in T1D pathogenesis. Notably, the DM-resistant DQ2 allele is a well-established high risk allele for human TID, and a recent bioinformatic analysis based on GWAS data indicates a DMB polymorphism with consequences for risk for several autoimmune diseases, including T1D [55].

Concluding remarks

The work described here provided long-sought answers to questions in the class II antigen presentation field: What is the conformation of a class II transition state stabilized by DM? What is the mechanism of DO inhibition of DM? And what are (at least some of) the immunological consequences of DO deficiency? However, key issues remain. The molecular basis for the difference in stability of transient DM/DR complexes as compared to tight and essentially irreversible binding of DM/DO complexes is not clear from current structural and mutational analysis. The peptide sequence determinants of DM susceptibility are not known, and the DM susceptibility of HLA-DQ/peptide and HLA-DP/peptide complexes has been investigated in few if any cases. The contribution of DO in central tolerance has not been directly addressed. The compartments and subdomains where DM and DO localize in primary antigen presenting cells are not known, nor is the function (if any) of DM expressed at the cell surface. How do DO deficient mice handle infection? These and other questions will shape the next efforts in the field.

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- •• of outstanding interest

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