[Frontiers in Bioscience S4, 1325-1332, June 1, 2012]

How HLA-DM molecules work: recognition of MHC II conformational heterogeneity

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1. ABSTRACT

Helper T cells respond to peptide antigens derived from exogenous sources presented by MHC II on antigen presenting cells. Antigens from pathogens are internalized by professional antigen presenting cells (APC) and processed for presentation. Certain epitopes are selected during processing as the final peptides for stimulation of T cells and are termed "immunodominant". Understanding how selection of immunodominant epitopes takes place has been a difficult task because of the complexity of the mechanisms governing both antigen processing and T cell recognition. In this review, we discuss our current understanding of HLA-DM function in peptide exchange and selection and its relevance to epitope immunodominance.

2. INTRODUCTION

T cell recognition of pathogens occurs through a short pathogen-derived peptide that sits within a structural groove found in major histocompatibility complex (MHC) molecules designated for peptide binding (the "peptide-binding groove"); T cell receptors specifically recognize and respond to those stretches of peptides together with their MHC restricting proteins to which they are bound. This is very different from antigen recognition by B cells, whose immunoglobulin receptors recognize their specific epitope within the context of a fully folded, and intact, protein antigen. The processing of antigens for MHC presentation requires a complex array of cellular machinery distributed among subcellular compartments specialized just for this purpose. The universe of antigens is divided

into two general categories: exogenous and endogenous. Each has distinct processing compartments, machinery, and MHC molecule subtypes dedicated to them, and each is presented to distinct T cell subtypes. The MHC class I processing pathway is optimized for presenting endogenous antigens, such as viruses, to $CD8^+$ cytotoxic T cells. The MHC class II antigen processing pathway is optimized for presenting exogenous antigens taken up by antigen presenting cells (APC) to $CD4^+$ helper T cells. We will focus on MHC class II antigen processing and presentation in this review, making an effort to describe the critical role that the chaperone-like molecule, HLA-DM (1) (H-2DM in mice, to be called DM from now on) plays in peptide exchange and in the selection of peptides designated for presentation to $CD4^+$ T cells.

3. MHC CLASS II SYNTHESIS AND ASSEMBLY WITH CHAPERONES

An important early step in MHC class II antigen processing is the assembly of newly synthesized MHC class II alpha beta heterodimers with its dedicated chaperone, the Invariant Chain (Ii). Ii ensures both the proper folding of the nascent MHC II heterodimer in the endoplasmic reticulum (ER) and its proper sorting through the Trans-Golgi Network to the low-pH endosomal compartments in the APC (2). As MHC II molecules move toward vesicular compartments enriched for exogenous antigens, Ii is gradually cleaved away by endosomal proteases leaving behind a portion called the Class II-associated Invariant peptide (CLIP), which remains bound to the MHC class II molecule's peptide-binding groove. The CLIP portion of Ii fulfills two functions. First, it prevents premature binding of self-peptides to the groove of MHC II molecules (3). Second, because the peptide-binding groove of MHC class II is structurally unstable in the absence of peptide, CLIP acts as a "place-keeper" for maintaining the groove's conformation. Removal of CLIP during peptide exchange generates a transient "peptide-receptive" conformation of MHC II that binds peptides extremely rapidly and stably (4-6). In the endosomal compartments specialized for the capture of exogenous antigenic peptides, another chaperon like molecule, DM, plays a vital role in exchanging CLIP for an exogenous antigen derived peptide (7-15).

4. BIOCHEMICAL AND STRUCTURAL CHARACTERIZATION OF DM

The role of DM in antigen processing has been explored for years and is still actively researched. In solution as well as in cells, DM is now known to be active at moderately acidic pH (8) and capable of accelerating dissociation of CLIP and other peptides from MHC II molecules (8, 16). In addition, DM can accelerate the rate of association of peptides to MHC II molecules (16-18), validating its role as a mediator of peptide exchange, since peptide association to MHC II molecules in its absence is slow and requires substantial conformational changes in the structure of MHC II (5, 17). However, characterizing DM as a typical catalyst was confounded by biochemical experiments showing that DM does not bind peptide and co-precipitates with DR only under very mild conditions. The reason for DM's inability to bind peptide became apparent when the crystal structures of DM and its mouse homolog H-2M were solved (19, 20). While DM is structurally analogous to MHC II molecules, the two helices lining the putative "peptide-binding groove" are too close to each other, preventing binding of peptides to DM. The other feature emerging from the crystal structure was the lack of an obvious catalytic domain on the molecule. In the absence of DM-MHC II co-crystals, mutational studies suggested that the acidic face of DM is important in mediating peptide exchange (21). Mutagenesis studies suggested a lateral interaction between DM and the MHC II molecule, HLA-DR3, involving the groove accommodating the N-terminus of the bound peptide. While the precise residues constituting the "active site" that mediates peptide exchange remains unknown, some short peptides (six to eight amino acid in length) with sequence similarities to the N-terminal sequence of the long form of the CLIP peptide have been shown to facilitate peptide exchange (22, 23). More recently, one such set of peptides, called helper peptides, was shown to closely mimic different functions of DM *in vitro* and to enhance T cell activation when administered *in vivo* (24).

5. THE MOLECULAR MECHANISM OF DM FUNCTION

There still exists much ambiguity regarding the actual effect of DM on MHC II molecules. The initial characterization of DM included evidence that the efficacy of DM in dissociating peptides was linked to the intrinsic rate of dissociation of the peptide from MHC II (14). Sant *et al.* showed that a peptide's susceptibility to DM was inversely proportional to its intrinsic kinetic stability (25). Whether the effect of DM is dependent or independent of peptide sequence is important as the two possibilities implicate two very different energy barriers for DM to bypass in order to dissociate peptides. These are (a) a sequence-independent array of 12-15 Hydrogen bonds of varying strengths that are formed between residues in the MHC II groove and the main chain of the bound peptide, and (b) a sequence-dependent set of interactions that are determined by the fit between the side chains of the peptides bound and the pockets of the groove that accommodates them (26).

5.1. Contribution of H-bonds to DM susceptibility of MHC class II-peptide complex

H-bonds have been shown to be critical for the stability of bound peptides. Introducing perturbations that reduce H-bond formation, such as targeted mutation of key amino acid residues in the peptide binding groove or derivatization of the peptide, have been shown to destabilize peptide binding (27-30). Small molecules such as alcohols (H-bond donors) that weaken H-bonds also increase peptide dissociation at high doses, although in either case, conformational perturbations of the MHC II molecule cannot be ruled out (31, 32). The involvement of H-bonds in DM-mediated peptide dissociation was first postulated by Jensen and coworkers (14). Later, Sant and colleagues (33) observed that a mutation in β81His to Asn

in MHC class II I-A^d generated molecules that bound peptides poorly. Working with soluble DM and the MHC II molecule HLA-DR1 (DR1 from now on), we have recently shown that the conformation of DR1 with disrupted H-bonds between the highly conserved residue β81His and peptide main chain may represent a post-DM-effected conformation (34). We found that disrupting a single conserved H-bond through mutation caused the bound peptide to rapidly dissociate with kinetics resembling "DM mediated" dissociation of peptide from wild-type DR1. This mutant molecule was also resistant to DM-mediated peptide dissociation, with the crucial difference that DM could still recognize and interact with it if it was bound with a peptide that did not fill the P1 pocket, just as observed with wild-type DR1. Importantly, the mutant DR1betaH81N molecule appeared functional in all respects tested, e.g. the complexes it forms with peptides $HA_{306-318}$ and CLIP were SDS-stable and migrated in a manner similar to wild-type DR1 complexes on a gel; also, DR1betaH81N bound peptides with association kinetics patterns similar to wild-type DR1. However, these complexes dissociated very rapidly and independently of peptide sequence. The rates of dissociation of these complexes were similar to the accelerated dissociation rates seen with wild-type DR1/peptide complexes in the presence of DM. Moreover, when we generated a compensatory mutant, DR1betaH81N/betaV85H, that potentially re-introduced an appropriate His-mediated H-bond with peptide, we observed that the DR1 mutant regained its susceptibility to DM-mediated peptide dissociation (34). We interpreted this data as evidence that the DR1betaH81N mutant, with its perturbed H-bond-forming capacity, represented a "post-DM effected" MHC II molecule. We could not rule out the role of other H-bonds, and we suspect that our results reflect the unusual strength of the His81 H-bond and the cooperative nature of the H-bond array (34).

A seemingly contradictory model has been proposed by Zhou *et al*. (30) and Ferrante *et al*. (29): Using a series of Ala-substituted DR1 molecules at every conserved residue forming H-bonds with the peptide backbone, Zhou *et al* concluded that while each H-bond might contribute to the overall DM interaction with DR1, DR1betaN82A mutant spontaneously released CLIP, a phenotype similar to DR1βH81N (35). Interestingly, Ferrante *et al*. examined the cooperativity of peptide binding to the DR1βH81N mutant and concluded that while the loss of the H-bond between His 81 and the peptide backbone did not alter cooperativity of binding, it did alter the overall energy of the complex. The authors proposed that, for the DR1βH81N mutant molecule, the exchange peptide requires only one less interaction with the MHC II (29). Ferrante *et al*. (29, 36) find cooperative association and dissociation of peptide from DR molecules consistent with the conformational changes induced upon peptide binding (37-40). Despite the apparent disagreement among these groups as to the contribution of individual H-bonds to the stability and DM susceptibility of DR/peptide complexes, the mechanisms of DM function that they propose are remarkably similar in that they all attribute DM function to its ability to change the overall conformation of the DR peptide-binding groove in such ways that the peptide can no longer form H-bonds with the DR molecule in order to become stably bound.

5.2. DM recognition of MHC class II conformation

The conformation of the MHC II molecule has long been known to be an important factor in determining its ability to participate in peptide association or dissociation (37, 38, 41-47). Many groups have shown that the binding of peptide to MHC II involves transitions through several distinct conformational stages; amongst these is a short-lived "peptide-receptive" conformation (4, 5, 18) to which a peptide can bind with rapid and monophasic kinetics. In the absence of DM, however, the conversion of MHC II from a stable "non-receptive" (closed) to "receptive" (open) conformation is the rate-limiting step during peptide association. Early studies revealed that peptides that fit suboptimally in the groove are susceptible to DM (17) and that variants of the CLIP peptide with different ligand binding motifs showed variable DM susceptibility (14). Using variants of the $HA_{308-316}$ peptide, it has previously been shown for DR1 molecules that the filling of its P1 pocket with large hydrophobic residues (e.g., Phe, Trp, Tyr) imparts resistance to DM-mediated dissociation while less bulky residues such as Ala, Leu, or Met make the complex susceptible to DM (17). Furthermore, a DR1 molecule whose P1 pocket is partially filled through the DR1beta G86Y mutation also proved to be resistant to DM recognition (6, 17). We observed that a mutation that partially filled the P1 pocket caused DR1 molecule to stay in an open conformation to which peptides could rapidly bind and unbind (35). Importantly, DM no longer interacted with this mutant molecule and hence did not accelerate peptide association or dissociation (17). We thus hypothesize that the primary function of DM is to induce DR molecules to assume a receptive conformation, thus mediating quick peptide association.

All studies up until this point had failed to demonstrate a stable binding interaction between DM and MHC class II; only transient interactions have been documented (17, 34). Chou *et al*., using intrinsic tryptophan fluorescence demonstrated that DM could only interact with empty DR1 molecules or those in complex with peptides with an Ala substitution at the P1 position and hence having rapid dissociation rates (17). When the P1 pocket of DR1 was filled with a bulky hydrophobic side chain from peptide or DR1 mutation as in DR1betaG86Y, no interactions between DR1 and DM were detected.

Recently, Wucherfpennig and colleagues, using Surface Plasmon Resonance, demonstrated a stable interaction between DM and DR1 (48). They showed that non-DM binding DR1–peptide complexes were converted into efficient HLA-DM binders when an N-terminal peptide segment was truncation leaving the P1 pocket empty and disrupted conserved hydrogen bonds to DR1. Peptides that filled the P1 pocket were protected from removal by HLA-DM through two mechanisms: a) peptide binding induced the dissociation of a long-lived complex of empty HLA-DR and HLA-DM, and b) tightly bound DR1–peptide complexes interacted with HLA-DM only very

slowly. They concluded that HLA-DM binds only to DR1 conformers in which a critical part of the binding site is already vacant because of spontaneous peptide motion. In all, it appears that the majority of the studies addressing the interaction of DM with DR molecules are in agreement with a model that DM targets the N-terminus of the bound peptide in the groove of DR molecules (6, 17, 21, 27, 28, 34, 48-53).

6. A UNIFYING MODEL TO EXPLAIN MECHANISM OF ACTION OF DM: A "*HIT-AND-HUG, THEN RUN*" MECHANISM MIGHT EXPLAIN HOW DM WORKS!

We suggest that these two mutants, DR1betaH81N and DR1betaG86Y, reveal the basis of the recognition and the effector functions of DM, and that this two-step functionality of DM may explain some of the earlier data. Depending on sequence, various peptide side chains will fit differently into the peptide-binding groove of MHC II molecules, resulting in slightly different peptide-MHC II conformations. While this may result in variable kinetic stabilities of the complexes, we propose that the actual criterion and true predictor for DM recognition is the conformation of the complex and that recognition by DM is independent of its intrinsic stability. If DM recognizes and interacts with the peptide-MHC complex, it mediates its effector function, which is to generate an open peptide-receptive conformation. This opening up of the groove is mediated by weakening the H-bonds between MHC II and peptide, allowing for rapid dissociation. If there is a peptide in the milieu, it can now bind rapidly; otherwise, the groove of MHC II will close, as the lifetime of this intermediate conformation is quite short (5, 18). The molecule stays in this closed conformation until another transient interaction with DM occurs. We suspect that empty MHC II is conformationally similar to a "DM susceptible" complex (44). This may explain why DM can also interact with empty MHC II molecules and convert them into a receptive conformation, allowing for rapid peptide binding (48). Once a MHC II molecule binds a peptide that converts it into a compact DM-insensitive conformation, DM no longer recognizes and interacts with the molecule, and is thus ineffective in mediating peptide dissociation. Putting everything together, a class II molecule (e.g., DR1), occupied by a peptide that does not fill P1 of the peptide binding groove is in a 'floppy,' or 'open,' DM-sensitive conformation. DM interacts transiently with the peptide/MHC II complex by using the proposed 'hit and run' mechanism and induces local conformational changes that lead to disruption of H-bonds between the peptide and DR1, resulting in peptide release. This generates an empty and peptide-receptive conformation of DR1, where DM can now "hug" the groove. At this point, subsequent events will vary depending on the type of peptides that bind to DR1. If DR1 binds another peptide that does not fill P1, the DR1/peptide complex will go through another round of DM-mediated dissociation. Alternatively, if DR1 remains unbound by peptide, it might close and become inactive over time under physiological conditions. This empty DR1 would then be rescued by a DM 'hit' and be reverted to its peptide-receptive form. Finally, if DR1 binds a peptide that fills P1, the molecule then changes to a tight, DM-insensitive conformation. DM can no longer interact productively with this complex, and the DR1 bound to peptide is exported to the cell surface (Figure 1).

7. A ROLE FOR DM IN EPITOPE SELECTION AND IMMUNODOMINANCE

DM stabilizes peptide-deficient MHC molecules; the stabilizing effect ensures that the peptide binding groove is maintained into a conformation receptive to capturing candidate peptides. DM may act by widening the peptide binding groove of MHC molecules, allowing for faster association of all peptides and faster dissociation of some peptides. Once class II molecules have captured optimal peptides, MHC class II undergoes conformational changes that render it insensitive to productive interactions with DM. As we see it, an MHC II molecule can bind many peptides of a given protein antigen but only a small subset of these peptides will impart conformational changes in the MHC molecule that render them insensitive to DM. Such screening for those selected peptides among those generated from a given protein antigen might well explain the concept of "immunodominance", where the selection of immunodominant epitopes from exogenous and endogenous antigens must be a necessary and stringent process to avoid having either too many T cell specificities per antigen, for which a memory T cells should be developed and preserved over long periods (54). The editing mechanisms imposed by DM on MHC II molecules may thus be nature's elegant solution to narrow the repertoire of peptides presented and ensure the generation of a robust, non-crossreactive and highly specific immune response.

In accordance with the scenario described above, a reductionist system for MHC class II molecules has been developed that incorporates a minimal number of ingredients, and predicts immunodominant epitopes from any protein antigen with surprising accuracy (55). The system includes only five components of antigen processing machinery besides the protein antigens, which are: soluble purified DR1, two exoproteases (cathepsin D and cathepsin H), an endoprotease (cathepsin S), and, importantly, DM. Protein antigen is subjected to processing, binding and editing, and the peptides that remain stably bound to DR1 are isolated and identified by mass spectrometry. It was demonstrated that this system could identify physiologically relevant epitopes from well-characterized test antigens as well as from antigens whose immunodominant epitopes had not previously been identified. Verification of the epitopes was done in DR1 transgenic mice and in DR1-positive humans (55). It is striking that this system with so few components closely mimics antigen processing *in vivo*. Furthermore, in accordance with the above discussion on how DM works, the system gives a central role to DM in the selection of immunodominant epitopes, a role that may not be downplayed. In this reductionist system, DM might select the immunodominant epitope just by leaving DM-insensitive epitope/MHC II complexes alone while continuing to dislodge those epitopes that form DM-

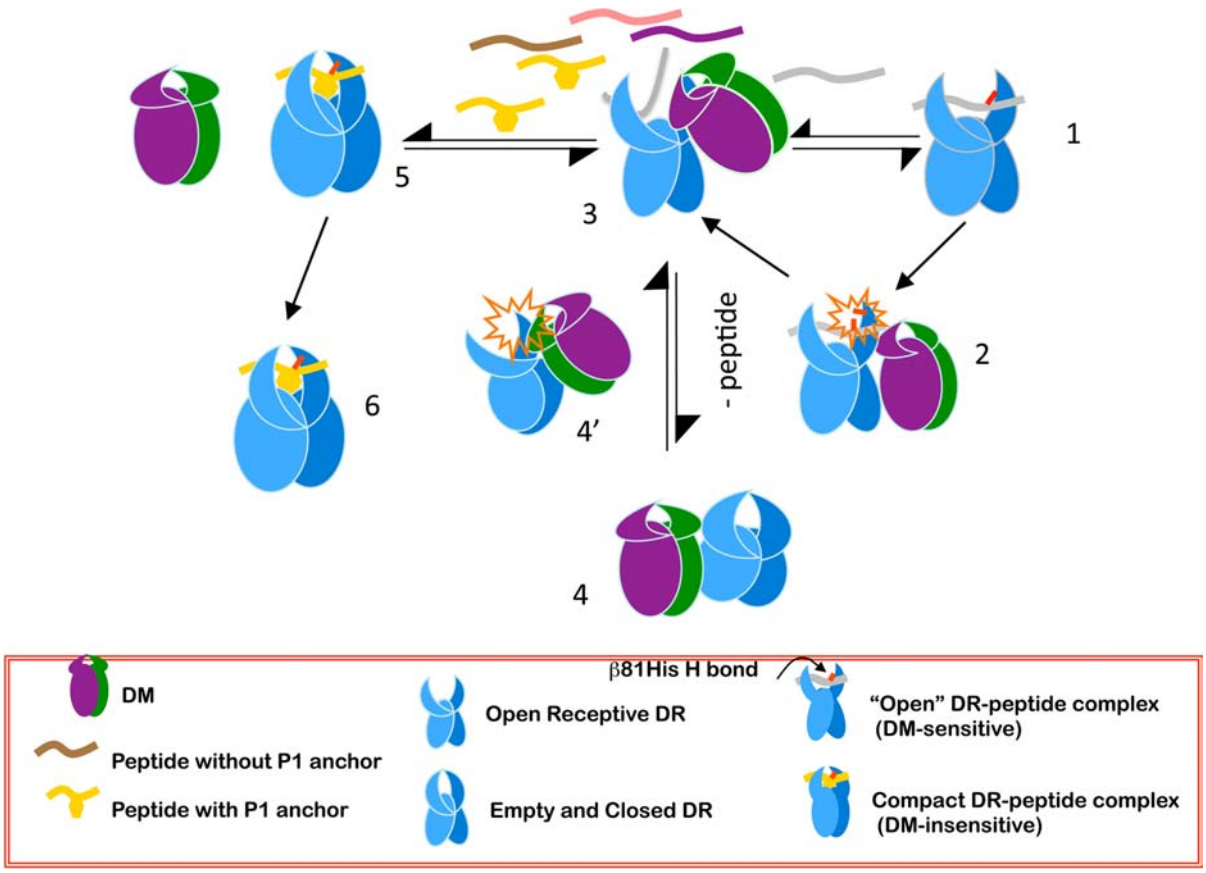

Figure 1. A "*Hit-and-Hug, then Run"* mechanism might explain how DM works! A newly synthesized MHC class II, or DR1, molecule, occupied here by a peptide that does not fill Pocket 1, is in a 'floppy' or 'open,' DM-sensitive conformation (1). DM can interact transiently with the molecule by using the proposed 'hit and run' mechanism and can induce local conformational changes that lead to break in hydrogen bonds or inhibition of formation (2) between the peptide and DR1, resulting in the release of peptide. This generates a peptide-receptive conformation with peptide being displaced gradually leaving the P1 empty, where DM can now "hug" the groove (3). Now several events might follow; the molecule can bind another peptide that is similar to the one described above and can then go through another round of DM mediated dissociation (steps 1 and 2). Alternatively, in the absence of peptide, the DR1 molecule might close and become inactive over time under physiological conditions (4). This empty DR1 might now be rescued by a DM 'hit' to generate the peptide-receptive form again (4'). Finally, if DR1 binds a peptide that fills P1, the molecule then changes to a tight, DM-insensitive conformation (5). DM cannot interact productively with this complex, and the DR1 bound to peptide is exported to the cell surface (6).

sensitive complexes with MHC II. As a result, those epitopes that form DM-resistant complexes with MHC II gain abundance over the others, resulting in immunodominance.

8. ACKNOWLEDGEMENTS

Authors wish to thank Yuri Poluektov for critical reading of the manuscript. This work was supported by R01 grants AI063764, and GM053549, a grant from Johns Hopkins Malaria Research Institute to SS-N, an NSF predoctoral award to IZH, JHU Rheumatology T32, and Immunology T32 postdoctoral Fellowships to AK.

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Key Words: HLA-DM, Immunodominance, Epitope selection, MHC class II, HLA-DR, Antigen processing, Antigen presentation, Molecular recognition, Conformational heterogeneity, Review

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