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HLA-DM: An In Vivo Facilitator of MHC Class II Peptide Loading

Minireview

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Initiation of cell-mediated immune responses requires processing of intracellular pathogens into peptide fragments and presentation of these fragments to effector T cells. In the past few years, considerable progress has been made in understanding the molecular mechanisms underlying antigen processing and presentation. In particular, recent work has led to an understanding of the function of HLA-DM (DM), a key player in antigen presentation, whose importance was first appreciated only 1 year ago.

Antigen Processing in Prelysosomal Compartments: The Basics

Major histocompatibility complex (MHC) proteins are responsible for binding and presenting antigen-derived peptides to T cells (Germain, 1994). Unlike class I MHC molecules, which bind peptides derived from cytosolic antigens and present them to CD8-restricted T cells, class II MHC molecules generally bind peptides generated by proteolysis of exogenous antigens in endocytic compartments and present these peptides to CD4-restricted antigen-specific T cells. Since class I and class II molecules coexist in the endoplasmic reticulum (ER) and are each capable of binding antigenic peptides, it was proposed that a mechanism must exist to "protect" the peptide-binding groove of class II molecules in the ER from occupancy by cytosolically produced endogenous peptides. In 1990, it was demonstrated that the class II–associated invariant chain (Ii) performed this function (Roche and Cresswell, 1990). Ii is a type II transmembrane glycoprotein that associates with newly synthesized class II α and β chains in the ER. Association with Ii prevents the binding of peptides to class II molecules (and vice versa) and newly synthesized αβIi complexes are devoid of antigenic peptides; thus, Ii prevents newly synthesized class II molecules from sampling ER peptides.

In addition to its role in blocking premature peptide binding to class II molecules, Ii contains an extremely efficient endosomal/lysosomal targeting motif in its amino-terminal cytosolic domain (Bakke and Dobberstein, 1990), and this signal is responsible for delivery of newly synthesized class II αβIi complexes to intracellular antigen-processing compartments. Once in these compartments, class II–associated Ii is degraded by proteinases such as cathepsin B and cathepsin D. Stepwise proteolysis of Ii leads to formation of distinct class II–associated Ii fragments that have been well characterized biochemically (Blum and Cresswell, 1988). Following dissociation of Ii fragments from class II molecules, antigenic peptides are able to bind to newly liberated class II αβ heterodimers. The resulting class II αβ–peptide complex is then transported to the plasma membrane for recognition by CD4-restricted T cells, thereby completing the antigen processing and presentation cycle.

APC Mutants Point to Additional Players in MHC Class II Peptide Loading

The rate of stable peptide association with purified class II αβ heterodimers in vitro is extremely slow as compared with the rapid acquisition of peptide by class II molecules in vivo, suggesting that a mechanism exists in vivo to load peptides onto class II molecules efficiently. Characterization of several mutant antigen-presenting cell (APC) lines confirmed this hypothesis (Mellins et al., 1990). Although the class II structural genes in these mutant cells were not themselves altered, certain class II conformation-specific monoclonal antibody epitopes were absent. In addition, class II molecules from these mutant APCs were unstable and dissociated into free α and β chains in the presence of SDS, whereas most class II molecules from their wild-type progenitors did not. Most importantly, these mutants were incapable of processing and presenting exogenously added foreign protein antigens to T cells, although they were perfectly capable of binding and presenting the preprocessed peptide fragments of these antigens.

Since SDS stability of class II molecules has been suggested to represent stable acquisition of peptides by αβ heterodimers (Sadegh-Nasseri and Germain, 1991), it seemed reasonable to assume that the antigen presentation defect in mutant APCs was a consequence of the inability of their class II molecules to bind peptides. It was subsequently determined that most class II molecules from these mutant cells did in fact contain peptides, but, unlike the diverse array of peptides found associated with wild-type class II molecules, essentially all of the peptides were derived from proteolytic fragments of Ii (Riberdy et al., 1992; Sette et al., 1992). These peptides were dubbed CLIP, for class II–associated invariant chain peptides, and represent a nested set of peptides corresponding to Ii amino acid residues 81–104. Because normal APCs also contain class II αβ–CLIP complexes, but in much smaller amounts, it was appreciated that CLIP represented a normal biosynthetic intermediate in the degradation of Ii from αβIi complexes. Consequently, it was proposed that the defect in mutant APCs involved their inability to generate antigenic peptides, their inability to exchange CLIP for antigenic peptides, or their inability to direct αβIi complexes (or antigenic peptides) into the proper peptide-loading compartments.

Elegant genetic studies established that mutant APCs in fact contained lesions in DM, originally identified as a novel gene of unknown function that mapped to the class II region of the MHC between HLA-DP and HLA-DQ (Morris et al., 1994; Fling et al., 1994). Like conventional class II molecules, DM is a transmembrane glycoprotein composed of an α chain and a β chain. The homology of DM with conventional class II proteins is only marginal, however, as DM appears to be almost as closely related to

class I molecules as to class II molecules. When mutant APCs containing lesions in DMα or DMβ genes were transfected with the corresponding wild-type cDNAs, the phenotype reverted to that of the wild-type progenitor cells and they were now able to present foreign antigens to T cells. However, the mechanism by which DM allowed class II molecules to adopt a proper conformation and to bind and present processed antigen to T cells remained elusive.

DM Induces CLIP Dissociation and Peptide Binding to MHC Class II Molecules

Within the last month, three different papers have demonstrated that DM catalyzes the dissociation of CLIP from class II αβ–CLIP complexes (Sloan et al., 1995; Denzin and Cresswell, 1995; Sherman et al., 1995). Following CLIP removal, class II αβ dimers are capable of effectively binding antigenic peptides, rapidly leading to generation of SDS-stable class II molecules. Thus, the phenotype of the APC mutants appears to be due to an inability to remove CLIP from class II molecules. This effect was observed using DM isolated from several different sources and similar results were obtained with both murine and human class II molecules. In agreement with earlier studies of peptide binding to purified class II molecules, the enhancement in the rate of class II peptide binding induced by DM is likely to be a consequence of CLIP removal from the class II peptide-binding groove. However, evidence suggesting that DM facilitates peptide binding to empty class II molecules has also been obtained (Denzin and Cresswell, 1995), although it is premature to conclude from such experiments that DM performs a peptide-binding enhancement function independent of its CLIP-dissociation function.

The steady-state amount of DM in professional APCs is thought to be quite low and DM has a remarkably long half-life in vivo, suggesting that DM may functional catalytically (Denzin et al., 1994). Experiments demonstrating that substoichiometric levels of DM were sufficient to enhance SDS stability of class II molecules support this hypothesis (Karlsson et al., 1994; Denzin and Cresswell, 1995). An alternative theory postulated for the function of DM, namely that DM acted as a CLIP "sink," capable of binding CLIP that has dissociated from αβ–CLIP complexes, is not consistent with these data. It appears likely, therefore, that one molecule of DM can lead to release of many CLIPs from αβ–CLIP complexes.

Does DM Enhance Release of Non-CLIP Peptides from MHC Class II Molecules?

In addition to the ability to remove CLIP from αβ–CLIP complexes, DM can also catalyze the removal of non-CLIP peptides from class II molecules and can facilitate peptide binding to class II molecules synthesized in Ii-negative cells (Sloan et al., 1995). Similarly, transfection with DM increases the amount of SDS-stable I-A^k molecules generated, even in cells not expressing Ii in vivo (Karlsson et al, 1994). These results strongly suggest that αβ–CLIP complexes are not the only substrates for DM. By contrast, DM had very little effect on SDS stability of DR αβ dimers isolated from wild-type B-LCL (Denzin and Cresswell, 1995). In this study, however, the class II molecules were mature, and any unstable class II–peptide complexes may have become stabilized by endogenous DM long before their isolation. A reasonable hypothesis that can reconcile both types of data is that DM catalyzes the release of prebound peptides from class II molecules, allowing DM to act as a peptide "editor," ensuring that only long-lived class II–peptide complexes escape to the cell surface (Sloan et al., 1995). This hypothesis is also consistent with the idea that DM catalyzes the release of peptides that do not produce SDS-stable αβ dimers, although this requires further substantiation.

DM Functions Intracellularly in Antigen-Processing Compartments

DM is maximally effective at pH ranges of 4.5–6, conditions that approximate those found in antigen-processing compartments. When the cellular localization of DM in B lymphoblastoid cell lines was investigated by immunoelectron microscopy, it was found to colocalize with the majority of conventional class II molecules in a prelysosomal compartment, termed the MHC class II compartment, or MIIC (Peters et al., 1991; Sanderson et al., 1994; Karlsson et al., 1994; Nijman et al., 1995). Similar, if not identical, prelysosomal compartments have been observed in B cell lines, melanoma cell lines, activated macrophages, and dendritic cells. The exact relationship between the MIIC originally defined in B lymphoblastoid cell lines and these other prelysosomal antigen processing compartments still needs to be resolved; however, for simplicity, all class II molecule-enriched prelysosomal antigen-processing compartments will be referred to as MIIC. These compartments are located late in the endocytic pathway, and most class II αβIi complexes appear to be targeted to these compartments directly from the *trans*-Golgi network. Several studies published within the last year strongly implicate these compartments as the predominant sites of antigenic peptide loading to newly synthesized class II molecules, although peptide loading in earlier endocytic compartments is also possible.

Unlike conventional class II molecules, DM does not significantly associate with Ii and transport of DM to MIICs is independent of Ii expression (Denzin et al., 1994; Karlsson et al., 1994). The recent finding that MIIC targeting information resides in a tyrosine-based motif in the DM β chain cytosolic domain is in excellent agreement with these findings (Marks et al., 1995). Almost no DM is found on the plasma membrane, consistent with its intracellular role in antigen presentation.

Model of DM Function in Antigen-Processing Compartments

A cartoon illustrating the role of DM in facilitating peptide binding and dissociation of CLIP from αβ–CLIP complexes in MIIC is shown in Figure 1. Class II αβIi complexes are transported from the *trans*-Golgi network to an early MIIC. Since few Ii epitopes are present in conventional MIIC, it is likely that αβIi complexes are transported to this compartment before eventual transport to MIIC. In this model,

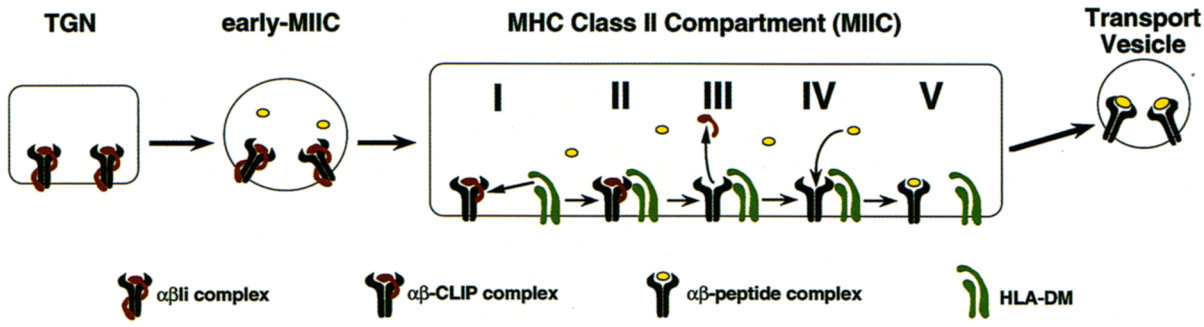

Figure 1. Model of HLA-DM Function in Antigen-Processing Compartments.

Following the delivery of class II αβIi complexes from the *trans*-Golgi network to an as yet uncharacterized early MIIC (where Ii proteolysis is initiated), αβ–CLIP complexes are targeted to MIIC compartments. Once in MIIC, CLIP is removed from the class II αβ–CLIP complex by a DM-dependent process involving the following steps. The affinity of DM for αβ–CLIP (I) results in the formation of an αβ–CLIP/DM complex (II). The binding of DM induces a conformational change in αβ–CLIP and leads to dissociation of CLIP from the complex (III). Following CLIP dissociation, antigenic peptides bind to empty class II αβ dimers that are stabilized by association with DM (IV). Antigenic peptide binding results in a conformational change in the αβ dimer/DM complex and leads to dissociation of DM from the complex (V). Although DM is retained in the MIIC to catalyze the release of CLIP from additional αβ–CLIP complexes, class II αβ–peptide complexes leave the MIIC in transport vesicles bound for the cell surface.

Ii proteolysis begins in the early MIIC, leaving only αβ–CLIP complexes to be transported to MIIC.

Once in MIIC, interaction of DM with the αβ–CLIP complex facilitates release of CLIP and allows antigenic peptide binding to the class II αβ dimer. One can imagine that the facilitation of peptide loading by DM occurs in the following sequence. DM binds to class II αβ–CLIP complexes, inducing a conformational change that results in rapid release of CLIP from the class II peptide-binding groove. DM remains associated with the empty class II molecule until a new peptide binds, preventing aggregation of empty αβ dimers. Antigenic peptides present in MIIC then bind to the recently emptied αβ dimer and, under the low pH conditions of MIIC, "lock" the class II αβ dimer into a conformation that is unfavorable for DM association. Since DM appears to function catalytically, released DM molecules are available for reuse in MIIC. Finally, the class II αβ–peptide complex receives signals (perhaps a consequence of peptide-induced conformational changes in the αβ dimer itself) to exit the MIIC and enter a transport vesicle, which eventually fuses with the plasma membrane to present the class II–bound antigenic peptide to T cells.

Is DM Required for Antigen Presentation Function?

Since the function of DM is to catalyze the removal of CLIP from αβ–CLIP complexes, one might expect that DM would not be necessary in situations in which CLIP is only weakly associated with class II molecules. In agreement with this hypothesis are recent results demonstrating that the ability of DM to enhance antigen presentation and SDS-stable αβ dimer formation is allele dependent (Brooks et al., 1994; Stebbins et al., 1995). In addition, there was good correlation between the requirement for DM to enhance antigen presentation and the stability of particular class II αβ–CLIP complexes; alleles in which CLIP was very tightly bound to class II molecules required DM for efficient antigen presentation, whereas alleles in which CLIP was not tightly bound did not. Thus, the ease with which CLIP can dissociate from αβ–CLIP complexes can be inversely correlated with the requirement for DM to facilitate peptide binding, SDS-stable class II αβ dimer formation, and antigen presentation.

What Is the Mechanism Behind the Function of DM?

Given the ability of DM to catalyze the release of CLIP from αβ–CLIP complexes, it seems reasonable to assume that DM binds to either CLIP or the class II αβ dimer. However, no biochemical association between DM and CLIP or class II molecules has yet been demonstrated. Nevertheless, the isolation and characterization of an HLA-DR (DR) α chain mutant cell line that has an identical phenotype to the DM mutant APCs further supports the speculation that DM binds directly to class II molecules (Mellins et al., 1994). The DR α chain mutant possesses an additional glycosylation site that has been postulated to interfere with normal class II αβ dimer–DM interactions, thereby leading to a DM mutant phenotype. Consistent with this hypothesis are experiments demonstrating that the in vitro effects of DM are not observed when class II molecules isolated from the DR α chain mutant APC were used as substrates (Sloan et al., 1995). In addition, an antibody that recognizes the amino-terminal region of CLIP can prevent the DM-mediated peptide loading of αβ–CLIP complexes (Denzin and Cresswell, 1995), lending additional evidence in favor of a direct αβ–CLIP/DM interaction.

One particularly puzzling feature of DM is that it appears to be able to discriminate between class II αβ–CLIP complexes and other αβ–peptide complexes. Crystallographic evidence suggests that CLIP is bound to the peptide-binding groove of αβ dimers like conventional antigenic peptides. The finding that αβ–CLIP complexes are unstable in SDS, whereas most other αβ–peptide complexes are not, argues that SDS stability may actually be the best indicator of whether a given αβ–peptide complex can serve as a substrate for DM or not. Since CLIP binds with high affinity to many class II alleles, it seems likely that SDS stability does not correlate with peptide binding affinity. Clearly, more work is required to understand the relationship between the SDS-unstable phenotype of αβ–

CLIP complexes and their ability to serve as a substrate for DM.

It is interesting to speculate that DM is acting in the capacity of a true molecular chaperone, binding to class II–peptide complexes that are relatively unstable and retaining them in antigen-processing compartments until peptides capable of inducing SDS-stable αβ–peptide conformations are bound. Since class II molecules aggregate in the absence of peptides (Stern and Wiley, 1992; Germain and Rinker, 1993), perhaps DM protects empty αβ dimers from aggregation while they are waiting for antigenic peptides to bind. In this model, DM would be acting in a similar manner to the MHC class I chaperone calnexin that retains class I molecules in the ER until peptide binding occurs.

Conclusion

The mechanisms involved in antigen processing and presentation have begun to be unravelled in the past few years. Although the identification of Ii groove-blocking ability helped explain the finding that cytosolic peptide antigens were not efficiently presented by class II molecules, it led to the question of how to get rid of Ii once this task was completed. Now that DM has been shown to be responsible for Ii CLIP dissociation (and perhaps the dissociation of other unstable peptides from class II molecules), we have a new problem to address; namely, how does a protein like DM distinguish "bad" class II–peptide complexes (such as αβ–CLIP complexes) from "good" stable class II–peptide complexes? If the progress of the last few years is any indication, we should have our answer shortly.

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