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Review

Peptide Loading of Nascent MHC Class I Molecules

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Abstract. A critical molecular interaction during assembly of the major histocompatibility complex (MHC) class I molecules takes place between the heavy chain and the transporter-associated with antigen-processing (TAP) complex. The recent mapping of regions of the heavy chain involved in the binding to TAP suggests a complex molecular interaction essential for the cell surface expression of the MHC class I. The advances made in understanding the TAP-MHC class I interaction are reviewed and discussed here.

Key words: MHC class I; antigen processing; TAP; $CD8^+$ cells; cytotoxic T lymphocytes.

Introduction

MHC class I molecules play several important roles in the immune system. They present antigenic peptides derived from the cytoplasmic proteolytic activity and, thereby, alert cytotoxic $CD8^+$ T cells of intracellular parasites or of other changes in the intracellular milieu that may be indicative of oncogenic transformation[44]. MHC class I molecules are also actively involved in thymic education, through which $CD8^+$ T cells acquire the ability to recognize peptides presented by particular alleles of MHC class I[22]. The "spontaneous" lytic activity of NK cells is prevented by MHC class I molecules and, in a manner analogous to the education of $CD8^+$ T cells for MHC restriction, NK cells appear educated to be both functional[31] and functionally inhibited by particular MHC class I alleles[7]. Recently, a new role of MHC class I molecules in maintaining naive $CD8^+$ cells in the periphery has been described[38, 63].

Peptides presented by the MHC class I are important in the activity of both NK and $CD8^+$ T cells, and microorganisms have found ways to interfere with peptide presentation[46]. Therefore, studying the manner in which peptides are associated with MHC class I is important not only for understanding the processes of $CD8^+$ and NK cell education and function but also for preventing interference with normal antigen processing. We review here recent advances made towards understanding the interactions of MHC class I heavy chain with TAP molecules that are crucial for the delivery of cytosolic peptides to the nascent MHC class I molecules.

MHC Class I Molecules: Structure and Function

The MHC class I molecule is a heterotrimeric complex comprised of a 44kDa heavy chain (HC),

Abbreviations used: β2m – β2-microglobulin, ER – endoplasmic reticulum, HC – heavy chain, TAP – transporter-associated with antigen-processing.

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a β2-microglobulin (β2m) light chain and a peptide of 8–10 residues[12, 52, 65, 67]. The heavy chain consists of three extracellular domains (α1, α2, α3) which are non-covalently associated with β2m. Each component of the MHC class I complex is essential for its stability. Consequently, any interference with the proper subunit addition (HC, β2m or peptide supply) results in intracellular retention and degradation of defective MHC class I molecules, reduction of the cell surface MHC class I expression and incapacitated MHC class I antigen processing[4, 26, 29, 45, 59, 71, 72].

In order to be presented on the cell surface, cellular proteins require processing. The principal pathway of antigen processing that is associated with MHC class I involves three main steps: cytosolic peptide generation, peptide transport into the endoplasmic reticulum (ER) and peptide assembly with class I molecules.

Cytosolic peptide generation

Degradation of cytosolic antigens is an essential step in the generation of most MHC class I-presented epitopes. The bulk of the peptides which are presented in peptide-MHC complexes are derived from the degradation of protein through the ubiquitin-proteasome pathway[48]. The essential role of proteasomes in antigen processing was established by the use of peptide aldehyde inhibitors, which inhibit proteasome-mediated protein degradation and cell surface expression of MHC class I molecules[49]. Further, single amino acid substitutions that prevent proteasome-mediated peptide cleavage also prevent antigen presentation[43]. However, proteins targeted for the 26S proteasome complex-mediated degradation are first "ubiquitinated", producing a protein-ubiquitin complex. The linkage of the 76-amino-acid-residue monomeric ubiquitin to the target protein is performed in three steps, details of which have recently been reviewed elsewhere[20]. The role of ubiquitination in antigen presentation was suggested by the inhibition of the processing and presentation of injected ovalbumin at non-permissive temperatures in mutant cell line with temperature-sensitive ubiquitin conjugating enzyme E1[33]. Further, protein (β-gal) modifications that alter the speed of ubiquitination also influence antigen presentation in osmotically loaded cells[17].

Several experimental approaches indicate the existance of alternative pathway(s) of protein degradation. First, presentation of other endogenous or exogenous antigens was not affected by inhibition of the ubiquitin pathway in temperature-sensitive mutants[9]. Second, denatured or chemically modified ovalbumin is presented in spite of E1 inactivation[34]. Third, proteasome inhibitors can significantly decrease the presentation of several, but not all peptides, suggesting that a fraction of the MHC class I-presented peptides may be processed by non-proteasomal proteases[60, 70]. Proteases in the secretory pathway have been implicated in the processing of peptides from antigens that can gain access to the ER[11, 13, 57]. However, for most antigens, processing has to take place in the cytosol. Recent evidence suggests the presence of a second cytosolic protease complex that can compensate for the loss in proteasome function and restore normal peptide production[14]. The relative contribution of this alternative pathway to the pool of peptides generated in normal cells, however, remains to be established.

Peptide transport into the ER

The peptides generated in the cytosol are translocated into the ER by the transporter-associated with antigen-processing (TAP)[36, 55], a member of the ATP-binding cassette (ABC)-family transporters[24, 44, 73], consisting of TAP1 and TAP2 subunits. Each subunit has an N-terminal hydrophobic region with multiple predicted transmembrane domains, and a cytosolic C-terminal ATP-binding domain. Cytosolic regions of both TAP1 and TAP2 are required for peptide binding, suggesting that a peptide binding site is combinatorial[3, 39]. TAP preferentially binds 8-13-amino-acid-long peptides[2, 53] in a relatively promiscuous manner with regards to the peptide sequence[53, 66]. Peptides bind to TAP in an ATP-independent manner, but their translocation requires ATP[1, 36]. Once localized to the ER lumen, peptides can bind to and, thereby, stabilize nascent class I molecules.

MHC class I assembly

Formation of the heterotrimeric MHC class I complex in the ER is facilitated by chaperons in an ordered sequence of molecular interactions that regulate the assembly and release of newly synthesized MHC class I molecules[44]. Among the chaperones that are involved in these interactions are calnexin[50, 54], the soluble calnexin homolog calreticulin[51, 68], the recently identified thiol-reductase ERp57[21, 32], the glycoprotein tapasin[15, 30, 42, 51] and the TAP1/TAP2 heterodimer[35]. These molecules have a role in the folding, quality control and retention of misassembled complexes.

Newly synthesized class I HC associate initially with calnexin[50, 54]. Although non-essential, calnexin appears to facilitate the folding and interchain disulfide bridge formation of the HC and the promotion of β2m

binding[64, 69]. MHC class I dimers (HC and β2m) then associate with a complex of proteins consisting of calreticulin, thiol reductase ERp57, TAP transporters and tapasin. The final step in class I assembly is the binding of high affinity peptides to the TAP-associated HC-β2m heterodimer. This appears to be a critical event, leading to the dissociation of these large complexes and the subsequent export of fully assembled class I proteins out of ER[18, 41, 62] via the constitutive, exocytic pathway.

Optimal binding of peptides to class I molecules is facilitated by the physical association between MHC class I and TAP heterodimers. This association is mediated largely by the glycoprotein tapasin[42]. Tapasin is a transmembrane glycoprotein encoded by an MHC-linked gene[16, 19, 30, 42]. In TAP-deficient cell lines, tapasin coprecipitates with the MHC class I, whereas in the β2m-deficient cell lines, tapasin coprecipitates with TAP[51]. Thus, tapasin can be described as a molecular bridge between MHC class I and TAP molecules that mediates their association. The C-terminal region is required for binding to TAP heterodimer[28], whereas the 50 N-terminal aminoacids appear crucial for binding to the HC as well as for maintaining calreticulin and ERp57 in the complex[5]. Tapasin increases the level of TAP and, thus, allows more peptide to be translocated to the ER[28]. Furthermore, when expressed in a tapasin negative cell line, recombinant soluble tapasin retains its association with class I and restores class I cell surface expression and function, even though it no longer binds TAP or increases TAP levels[28]. Thus, tapasin may have an important role in peptide loading and class I assembly that is unrelated to TAP association and is most probably based on its chaperone effect.

Sites of MHC Class I/TAP Interaction

Both the extracellular[62] and the transmembrane/cytoplasmic regions of the HC[27] have been implicated in the interaction with TAP complex (TAP heterodimer plus tapasin). Point mutations introduced in the α3 domains of both H-2L^d (position 227) and H-2D^d (position 222) resulted in the loss of TAP coprecipitation with the MHC class I HC[6, 62]. The latter mutant also failed to coprecipitate with tapasin[61]. However, these same point mutations only slightly affected the ability of these molecules to be expressed at the cell surface[8, 23, 47] and to present endogenous peptides[23]. In contrast, mutations in either TAP or β2m drastically affect both cell surface expression and antigen presentation of MHC class I[4, 59, 71, 72]. Recently, we have demonstrated that the physical association with the TAP complex, TAP-dependent peptide loading and cell surface expression of MHC class I was completely abolished by a 15-amino-acid substitution made in the H-2D^b α3 domain[26]. The mutant molecule was expressed at the mRNA and protein levels, but immunofluorescence staining for H-2D^b resulted in no detectable surface expression[26]. In addition, this mutant was unable to accumulate target structures for cytotoxic T lymphocyte (CTL) recognition unless peptides were introduced into the ER in a TAP-independent manner via the N-terminal ER retention signal sequence[26]. Further, more mutant molecules relative to the endogenous heavy chains appeared associated with calnexin indicating that this molecule failed to traffic efficiently to the cell surface and, hence, accumulated in the ER[26]. Thus, the α3 domain contains important sites that interact either directly or indirectly with the TAP complex and are critical for proper peptide loading and subsequent surface expression of MHC class I molecules[26].

Why is it that some mutant molecules reach the cell surface and can present antigens[6, 8, 23, 47; 62], while others are retained intracellularly[26], when both types can no longer be coprecipitated with TAP? It is known that the association between TAP and MHC class I is very labile in most detergents other than digitonin[41]. Perhaps the change of even one critical residue involved in TAP association can render this interaction subject to disruption by digitonin, even though the interaction still functionally exists. In other words, when two molecules coprecipitate one can conclude that they are associated, but when they do not coprecipitate one can only say that their interaction is not sufficiently strong to withstand the repulsive charges brought about by the use of detergent. This interpretation is also supported by the findings of allelic variations in the ability of human class I HC to associate with TAP, as HLA-B35 alleles do not coprecipitate with TAP[37] and yet are expressed at the cell surface and efficiently present antigenic peptides[25, 40]. Thus, cell surface expression and antigen presentation are the key tests for the association of MHC class I with TAP. Coprecipitation assay, on the other hand, can provide the very useful information that mutation has affected the TAP-complex binding site, but one has to be careful in interpreting absence of coprecipitation, as it does not necessarily result from a complete absence of interaction.

In addition to the HC α3 domain, the α2 domain also appears to bind to the TAP complex. A point mutation in the α2 domain of the human MHC class I molecule HLA-A*0201 (T to K substitution at position 134) resulted in a ~80% reduction of surface expression and a diminished ability to present endogenous

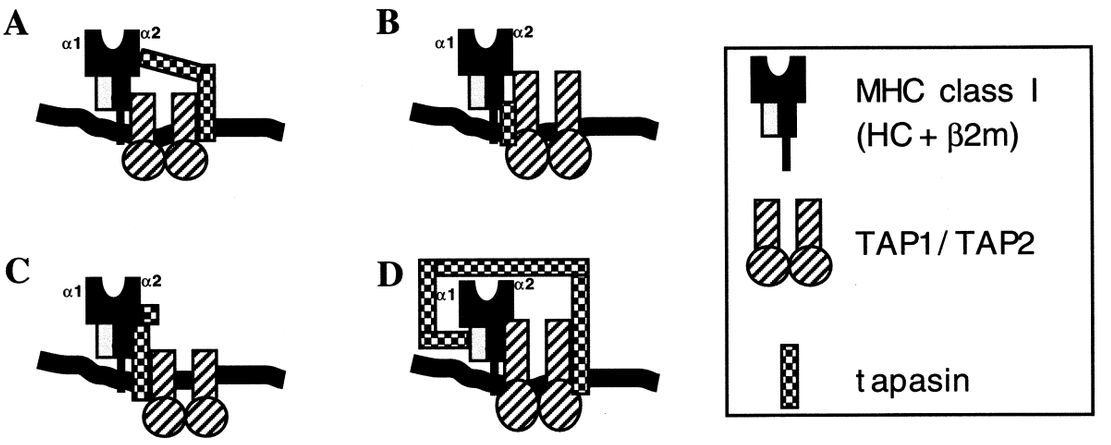

Fig. 1. Some possible modes of interaction between class I heterodimer, tapasin and TAP heterodimer. **A** – α2 interacts with tapasin, while α3 interacts with TAP. **B** – α2 interacts with TAP while α3 interacts with tapasin. **C** – α2 and α3 both interact with tapasin. **D** – α2 and α3 both interact with TAP, while tapasin interacts with β2m. Note that tapasin and TAP are presented in various sizes in **A**–**D** because of the constraints of two dimensional schematic representation of an actually three dimensional occurrance, and this does not indicate different sizes of these molecules in different models

antigens[29, 45]. However, in contrast to any α3-domain--mutant phenotype (reaching the cell surface with peptides or completely retained intracellularly), HLA--A*0201T134K mutant molecule was rapidly transported to the cell surface without bound peptide[29, 45]. Apparently, this molecule escaped the degradation that normally happens to the majority of partially assembled MHC class I molecules. Based on the phenotype of position 134-mutant molecule[29, 45], on the crystal structures of various HLA alleles[56], and on the ability to coprecipitate TAP and various HLA alleles[37], it has been proposed[10] that the short α2-helix region (positions 138–149), possibly together with the conserved loop (positions 128–138), could form the binding site for one or more components of the peptide-loading complex. Consistent with this hypothesis, mutagenesis of multiple positions (128–136) in the α2 region of the H-2L^d molecule abrogated the binding of TAP, as determined by coprecipitation assay[74].

Although the α3 and/or α2 domains have been convincingly implicated in binding to the TAP complex[6, 26, 29, 45, 62], as discussed above, the precise nature of the ligand is currently unknown. Tapasin or either of the TAP subunits could bind to the α3 and/or α2 HC domains (Fig. 1). A recent report suggested that tapasin binds to the α3 domain region[61]. However, the lack of coprecipitation of mutant MHC class I and tapasin in this case has the alternative interpretation that tapasin binds the TAP heterodimer with greater affinity than the MHC class I and that it preferentially associates with TAP1/TAP2 under conditions of HC/β2m exclusion from the TAP complex, as in the case with α3-domain-mutant MHC class I. Thus, direct association of tapasin, TAP1 or TAP2 with the identified regions in the α3 and/or α2 HC domains remains to be established. In addition, there may be other yet unidentified binding sites for TAP complex in the MHC class I HC. β2m, too, may contain a docking site, since tapasin promoted association of β2m with TAP in the absence of the heavy chain[58] (see Fig. 1). Future experiments should address these important issues.

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