How a tailor achieves the perfect fit

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The adaptive immune system relies on the presentation of antigenic peptides by MHC molecules on the cell surface. This display allows CD8 T lymphocytes to identify and eliminate abnormal cells that are producing “foreign” proteins, such as occur in viral infections or in cancers harboring mutations (1). Understanding how the immunopeptidome—the overall collection of MHC I–presented peptides—is generated, presented, and detected is therefore of great importance to understanding host defense, cancer immunotherapy, and vaccines. However, many details of the underlying mechanisms are still unknown.

The majority of peptides bound to MHC I molecules are of a relatively uniform length, typically 8–10 residues, depending on the particular MHC I molecule. This length matches MHC I’s peptide-binding groove, which has pockets that bind a peptide’s N-terminal α amino group at one end and its C-terminal carboxyl group at the other. These interactions contribute significantly to the binding affinity, thereby fixing the length of bound peptides, as well as the resultant MHC I stability, and the subsequent immune response. But where do these peptides come from? All cells continually hydrolyze their endogenous proteins into oligopeptides through the ubiquitin-proteasome pathway. A fraction of the resulting peptides are transported into the endoplasmic reticulum (ER), where MHC I molecules are located. Although proteasomes generate some peptides of the correct length to bind MHC I molecules, the majority of their products are either too long or too short for stable binding. The peptides that are too long can be trimmed to the optimal size by aminopeptidases in cells. But how is trimming controlled? Early on it was hypothesized that long polypeptides might be trimmed to optimal size while bound to MHC I molecules (2), and in fact MHC I molecules have some capacity to bind long protruding peptides, albeit often more weakly than peptides of optimal length. Whether this model is correct is still not fully resolved (3).

The aminopeptidase responsible for trimming peptides in the ER is ERAP1 (and in humans, also the closely related ERAP2) (4). One of the unusual and fascinating properties of ERAP1 is that it trims using a “molecular ruler.” The enzyme slows and/or stops trimming many peptides when they are 9 or 8 residues in length (the optimal size for binding many MHC I molecules), and in vitro this occurs in the absence of MHC I molecules. How is this accomplished? Structural and enzymatic activity studies (5) have revealed that ERAP1 can adopt an open conformation thought to be largely inactive, in which a cavity is exposed that allows peptide substrates to enter and reach the catalytic site. As ERAP1 closes on its substrate, the active site residues are reoriented so that they become active. This allosteric transition occurs when the substrate’s distal residues interact with a site on ERAP1 that is 8–9 residues away from the active site. Through this mechanism, peptides of <8–9 residues cannot simultaneously reach both the allosteric and catalytic sites and therefore are not further trimmed. Based on observed structures, steric constraints make it hard to model how an MHC I-bound peptide with 6 or fewer extra N-terminal residues could reach ERAP1’s catalytic site (5), and it is also unclear what would trigger the allosteric transition when the peptide’s C-terminal region is bound in the MHC I’s binding groove. Moreover, in the ER, MHC I molecules are in a peptide-loading complex wherein they are densely surrounded by other molecules that might also sterically hinder interactions with ERAP1 (6). As a result, the simplest explanation was that ERAP1 adjusts peptide length prior to MHC I loading. However, recent biochemical and structural evidence showed that ERAPs could trim long peptides on MHC I molecules down to 14 residues or less, particularly for HLA-B*08 (3, 7), suggesting that perhaps ERAP1 can adopt conformations that have not yet been observed.

To investigate this ambiguity, Mavridis et al. (8) generated complexes of long peptides bound to recombinant MHC I molecules, incubated them with purified ERAP1 or ERAP2, and then quantified the reaction products over time by MS or SDS-
Figure 1. Models for ERAP generation of MHC I–presented peptides. Top, ERAP1 trimming of long peptides to mature (optimal) size in solution, followed by peptide binding to MHC I molecules. Bottom, using MHC I molecules as a size template for ERAP to trim MHC I–bound long peptides to mature size. The dashed arrows indicate that this pathway is unlikely, based on results from Mavridis et al. (8).

PAGE. A total of five different antigenic peptides and three different MHC I molecules were examined. When free in solution, all of the long peptides <25 residues were good substrates for the ERAPs and were rapidly trimmed (Fig. 1, top). In contrast, and with only one exception, the MHC I–bound long peptides either were not hydrolyzed by the aminopeptidases, or the trimming was very slow or limited (Fig. 1, bottom). Remarkably, ERAP1 trimmed a peptide with a very long (13 residues) N-terminal extension rapidly when free but not at all when bound to an MHC I molecule. These results indicated that many long peptides bound to MHC I molecules are protected from hydrolysis by the ERAP enzymes.

One exception found by Mavridis et al. (8), and additional exceptions reported by others (7), was for long peptides bound to HLA-B*08. Mavridis et al. found that both ERAP1 and -2, as well as an aminopeptidase unrelated to antigen processing, trimmed an HLA-B*08–bound long peptide reasonably well, albeit still substantially slower than when the peptide was in solution (8). The thermodynamic and kinetic stability of the peptide-HLA-B*08 complexes was analyzed by differential scanning fluorimetry, CD, peptide-exchange, and molecular dynamic modeling. These assays revealed that the rate of trimming of the “susceptible” peptide-MHC I complex was actually slower than the rate of peptide dissociation from the complex. Thus, this may be the exception that proves the rule, as the results suggested that for this susceptible peptide-MHC I complex, peptide trimming was occurring after the peptides dissociated into solution rather than when they were still bound to MHC molecules (Fig. 1). Peptide dissociation may also explain the small amount of trimming that was observed for some of the other peptide-MHC complexes, although this was not investigated. Other experiments failed to find binding of ERAP to either long peptide-MHC I complexes by themselves or within the peptide-loading complex, even when using MHC I complexes loaded with a long pseudopeptide trap that covalently binds ERAP1’s active site; therefore, a physical interaction between ERAP1 and peptides bound to MHC I complexes has not been detected (8).

The Mavridis et al. study, together with the ERAP1 structural information and enzymatic properties discussed above, largely puts to rest models wherein peptides bound to MHC I molecules are trimmed to optimal size, at least as a general mechanism, while providing a satisfying explanation as to how those contradictory data could have been collected. Instead, ERAP1 and -2 by themselves trim most long peptide precursors, which, when the optimal size, are then bound and stabilized by MHC I molecules. Another important implication of these findings is that despite MHC I molecules being intimately associated with the cytosol-to-ER peptide transporter TAP and peptide editor tapasin in the peptide-loading complex, many TAP-transported peptides are not delivered directly into MHC I’s peptide-binding groove, but rather into fluids within the ER lumen, where the ERAPs can trim them. This notion nicely fits with an earlier study that found that a soluble anti-peptide antibody in the ER, which recognizes free but not MHC I–bound peptides, inhibited the formation of peptide-MHC complexes (9). It was previously shown that ERAP1-deficient mice have a substantially altered immunopeptidome, and in one reported case, CD8 immune responses were impaired to a degree where these mice succumb to infection. Moreover, in humans, polymorphisms in ERAP1 and -2 affect enzyme activity and have been associated with autoimmunity, inflammatory diseases, immunity to viruses, and risk of some cancers (10). These studies, combined with the new insights from Mavridis et al., indicate that ERAPs truly are the master tailors in antigen presentation and CD8 T cell immune responses.

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