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Proteases in MHC Class I Presentation and Cross-Presentation

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Cells that have mutated their genes or are virally infected are a potential threat to a host. Consequently, the immune system has evolved mechanisms for CD8 T lymphocytes to identify such cells and eliminate them. The generation of CD8 T cell responses occurs in two phases, both of which critically involve the process of Ag presentation. In the first phase, sentinel cells gather Ags present in tissues and then present them to naive CD8 T cells in ways that stimulate their maturation into effectors. In the second phase, these effector cells seek out and eliminate the pathological cells. The abnormal effectors. In the second phase, these effector cells seek out and eliminate the pathological cells. The abnormal effector cells are identified through their presentation of immunogenic Ags that they are producing. The Ag presentation mechanisms used by the sentinel cells can be different from those in other cells. This article will review these mechanisms with a focus in each case on how antigenic peptides are generated for presentation. *The Journal of Immunology*, 2010, 184: 9–15.

Classical (direct) pathway of MHC class I Ag presentation

All cells transcribe a subset of their genes and translate them into proteins. In parallel, they also continuously degrade these proteins to regulate their levels and eliminate damaged ones (1). In this process, cellular proteins are initially cleaved in the cytoplasm and nucleus into oligopeptide fragments.

To monitor what cells are producing, the immune system has evolved mechanisms to sample the library of peptides produced by cellular catabolism and display them on the cell surface for scrutiny by CD8 T cells (2). In this process, a fraction of the peptides present in the cytosol are transported into the endoplasmic reticulum (ER) by the TAP. In this location, peptides that are of the right length and sequence bind to newly synthesized MHC class I molecules, which then transport them to the cell surface. Through these mechanisms, cells display on their surface a sampling of their expressed gene products. In healthy cells, all of these presented peptides come from normal autologous genes and are ignored because the immune system is tolerant to them. However, if cells are synthesizing mutant proteins or ones from viruses, then peptides from these gene products will also be displayed, and this allows effector CD8 T cells to recognize the abnormal cells and eliminate them.

Proteases that make the initial cleavages to generate a presented peptide

Peptidase (lysosomal) proteases are responsible for degrading a majority of cellular proteins (6) (Fig. 1). At its core is a 20S cylinder that is composed of two outer α rings and two inner β rings with a central channel into which protein substrates enter and are cleaved (3, 6). The β ring contains three active sites, each of which is formed by a different subunit: B1, B2, and B5 (3). All three of these subunits work through the same catalytic mechanism wherein an N-terminal threonine residue makes a nucleophilic attack on a peptide bond of the substrate. However, the three active sites each have different specificities, cleaving preferentially on the carboxyl side of either hydrophobic residues (B5), basic residues (B1), or acidic ones (B2) (3, 6).

The strongest evidence that the proteasome plays a major role in the generation of most presented peptides comes from studies using highly specific inhibitors of the proteasome’s threonine-active sites. In living cells, these agents block completely the presentation of peptides from Ags that require proteolysis, but have no effect on ones that do not need cleavage (e.g., when epitopes are expressed directly from minigenes) and markedly limit the overall supply of peptides to MHC class I molecules (6).

Whereas the immune system has used this phylogenetically older pathway for a source of peptides, it also evolved modifications that are thought to play a special role in Ag presentation. One modification is an alternate set of active site subunits (B1i/MHC-linked low molecular weight protein 2, B2i/MHC-linked low molecular weight protein 1, B5i/MHC-linked low molecular weight protein 3). These subunits are sensitive to cathepsin B inhibitors, but resistant to proteasome inhibitors.

Abbreviations used in this paper: BH, bleomycin hydrolase; Cat, cathepsin; ER, endoplasmic reticulum; ERAP, ER aminopeptidase; IRAP, insulin-regulated aminopeptidase; LAP, leucine aminopeptidase; PA28, 28-kDa proteasome activator; PA700, 700-kDa proteasome activator; PSA, puromycin-sensitive aminopeptidase; RNAi, RNA interference; TPPII, tripeptidyl peptidase II.

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Brief Reviews
B2i/multicatalytic endopeptidase complex-like 1, B5i/MHC-linked low molecular weight protein 7) that, when expressed preferentially, incorporate into newly assembling proteasomes in place of the “standard” B1, B2, and B5 subunits to form so-called “immunoproteasomes” (3, 6). These are constitutively expressed in dendritic cells, lymphocytes, and thymic epithelium and are induced in all cells by proinflammatory cytokines such as IFN-γ.

The net effect of changing these β subunits is to alter the catalytic activity of the proteasome’s active sites. This changes the cleavages made in protein substrates both quantitatively and qualitatively and thereby results in the production of a different set of peptides (7). Where it has been examined for individual Ags, immunoproteasomes sometimes make more or less of a particular epitope (3, 8). It has been predicted that immunoproteasomes generate peptides that are more favorable for Ag presentation; however, how often this is the case and how important immunoproteasomes are to MHC class I Ag presentation is not fully resolved (6, 7). Mice that lack one or two of the immunoproteasome subunits have defects in the presentation of selected epitopes and in some cases partial reductions in the generation of presented peptides overall (7). However, a more complete understanding of the contribution of immunoproteasomes to Ag presentation and maybe even to nonimmune functions awaits the generation of mice that lack all three inducible subunits.

Thymic medullary epithelium also constitutively expresses another B5 subunit (B5i) that preferentially incorporates with B1i plus B2i into proteasomes called thymoproteasomes (9). Mice that lack either B2i or especially B5i show marked reductions in the generation of CD8 T cells and defects in the T cell repertoire, presumably because changes in the peptide repertoire lead to altered T cell selection in the thymus. Another apparent immune modification of the proteasome is the 28-kDa proteasome activator (PA28) complex that is constitutively expressed in dendritic cells and is induced in other cells by IFN-γ. PA28 is a heterohexameric (α3β3) ring that binds to one or both ends of the 20S proteasome. Upon binding, it increases the catalytic activity of all three of the proteasome’s active sites (3) and may do so at least in part opening the ends of the 20S particle allowing substrates to enter this particle. This change in activity can lead to altered cleavages in substrates (10) and increases or decreases in the generation of at least some MHC class I-presented peptides by purified proteasomes (11, 12). Similarly, transfection of PA28 into cells enhanced the presentation of some but not all epitopes (3) and increased the surface expression of some MHC class I molecules while decreasing the levels of other ones (12). Consistent with these findings, PA28−/− mice have a defect in presenting some Ags but not others (13).

So-called “hybrid” proteasomes can form with PA28 on one end and a 700-kDa proteasome activator (PA700) complex on the other. PA700 confers on the proteasome complex the ability to degrade polyubiquitinated substrates (10, 14). Where examined, hybrid proteasome particles make up ~20% of proteasomes in cells (14). Whether PA28 exerts its effects on Ag presentation through proteasomes with or without PA700 is presently not clear.

**Proteasome-independent pathways of Ag processing**

Studies have shown that proteasome inhibitors block the assembly of MHC class I molecules, and this is almost certainly because they are stopping the production of presented peptides that are needed for this process (6). However, the degree of inhibition of MHC class I assembly is less marked for some MHC class I molecules (15). Moreover, there are some examples of epitopes whose presentation is not blocked by proteasome inhibitors or is actually enhanced by this treatment (16, 17). These findings have been interpreted to indicate that this proteasome inhibitor-resistant presentation is being mediated by other proteases. This may indeed be the case; however, caution is warranted in interpreting these results because, at the concentrations used in these studies, most proteasome inhibitors do not block all of the proteasome’s active sites (18). Therefore, in the presence of these inhibitors, proteasomes may still be making cleavages that generate the presented peptides.

One candidate for a protease that might generate presented peptides independently from the proteasome is tripeptidyl peptidase II (TPPII). TPPII was initially suggested as the major mechanism by which cells chronically exposed to proteasome inhibitors survived and generated presented peptides (19), although it is now thought that the proteasomes were not completely inhibited in these treated cells (18). However, purified TPPII can help generate an epitope (20), and the presentation of another epitope was blocked when cells were treated with TPPII inhibitors but not proteasome blockers (16, 20). However, TPPII-null mice have been generated, and their cells do not show any reduction in the expression of MHC class I molecules (suggesting that peptide supply is intact) (21, 22). A role for TPPII in trimming some proteasomal products will be discussed below.

**The size of MHC class I-presented peptides and the products of proteasomes**

MHC class I-presented peptides are of a remarkable uniform size, the vast majority being either 8, 9, or 10 residues, depending on the particular MHC class I molecule (2, 3, 6). This is because the ends of the Ag binding groove of the class I molecule are closed and have pockets that bind the N and C
terminus of the presented peptide. Consequently, only peptides of the exact right size stably bind to this complex.

When purified proteasomes were incubated with Ags, the majority of peptides produced were too short (<7 residues) for Ag presentation. In contrast, only a small minority (<5%) of the peptides generated were of the correct size to bind to any particular MHC class I molecule, whereas a substantially larger fraction (~20%) of products were too long for stable binding (23). In the one case that has been analyzed, the peptides produced by immunoproteasomes were on average longer those made from standard proteasomes (24). When such long peptides are loaded into the cytosol of cells or expressed from minigenes, they are trimmed and presented, indicating that they can serve as precursors of presented peptides.

What trims these long precursors into presented peptides? One protease involved in this process is the proteasome, which can re cleave these peptides into epitopes (25). Interestingly, proteasome inhibitors block the presentation from precursors that have even a single extra C-terminal residue, indicating that these particles are the only activity in cells that can make the proper C-terminal cleavage to generate an epitope (6, 25). Consistent with these findings, the cytosol of cells has little if any carboxypeptidase activity (25). In contrast, precursors that just have extra N-terminal residues are presented, but in this case, proteasome inhibitors do not block this process (6, 25). This suggested that there must be other activities in cells that can remove N-terminal residues. It turns out that this N-terminal trimming of peptides can occur in the cytosol, where they are first produced, or in the ER after they are transported into this location.

**Peptidases and trimming of peptides in the cytosol**

N-extended peptides that are loaded or expressed in the cytosol of cells are trimmed and presented on MHC class I molecules (6, 25). Importantly, this can occur to some extent in cells that are unable to cleave peptides in the ER (discussed further below) indicating that some trimming of precursors occurs in the cytosol. This trimming is mediated by aminopeptidases because precursors that are resistant to hydrolysis by these peptidases (due to a chemically blocked N terminus) fail to be trimmed and presented (26, 27). However, which specific aminopeptidases contribute to MHC class I Ag presentation is not well understood.

One aminopeptidase that may play a special role in trimming peptides is TPPII. TPPII is relatively unique because it trims longer peptides as well as shorter ones, whereas most other aminopeptidases only cleave peptides <14 aa in length. Consequently, inhibition of TPPII significantly reduces the presentation of peptides with long N-terminal extensions but not shorter ones (22, 28). How important is TPPII to Ag presentation? Although an initial report suggested that TPPII was required for the generation of most presented peptides (29), subsequent studies with inhibitors (30), RNA interference (RNAi)-silenced cells (28), and TPPII−/− mice (21, 22) found no major defects in Ag presentation; these latter results are consistent with the finding that proteasomes generate relatively few very long peptides (see above). However, TPPII inhibitors reduce the presentation of a few individual epitopes (16), increase the presentation of others (31), and do not affect responses to many others (32), suggesting that this peptidase may play a role in the presentation of selected sequences.

Cells contain many other cytosolic aminopeptidases that can trim peptides. Three of these, leucine aminopeptidase (LAP) (33), bleomycin hydrolase (BH), and puromycin-sensitive aminopeptidase (PSA) (34), can generate mature epitopes from precursors at least in cell-free systems. However, the contribution of these peptidases to trimming in cells is less clear. Inhibitors of BH and PSA reduced the presentation of a few epitopes; however, these agents were not highly specific ones (34). On the other hand, BH−/−, PSA−/− and LAP−/− mutant mice or BH−/−LAP−/− double-deficient animals show no defect in generating and presenting mature epitopes from N-extended precursor peptides or Ag presentation generally (33, 35, 36). These negative results may indicate that there is substantial redundancy among these enzymes, so that trimming activity is not reduced by the loss of only one or a few peptidases. Alternatively, it is possible that there are other peptidases not yet identified that play a more important role in trimming epitopes. It is also possible that LAP, BH, and/or PSA are more important for the trimming of epitopes other than the ones thus far examined.

**Peptide trimming in the ER**

It is possible to target peptides directly into the ER after they are synthesized by fusing a signal sequence to the N terminus of the peptide (6). When such constructs are expressed from minigenes in cells, the signal sequence targets the peptide into the ER through the SEC61 translocon and is then removed by the signal sequence peptidase. This delivers the peptide into the ER in a manner that is independent of the TAP.

When N-extended peptides were targeted into the ER via SEC61, their extra N-terminal residues were removed and the peptides were presented on MHC class I molecules (25, 27, 37). This indicated that aminopeptidases must exist in the lumen of the ER, and, indeed, microsomes were found to have such trimming activity (38, 39). These activities were purified and identified as two members of the oxytocinase family of aminopeptidases. Although these peptidases have been given several different names, they are now most commonly referred to as ER-associated aminopeptidase or ER aminopeptidase (ERAP) 1 and 2. Mice have only ERAP1, whereas humans and some other mammalian species have both ERAP1 and ERAP2.

**ERAP1**

Most aminopeptidases continued to hydrolyze a substrate until it is converted entirely to amino acids. However, ERAP1 is unique in that it trims long polypeptides rapidly until they reach a size of nine or eight residues and then its hydrolysis stops (40, 41). Therefore, ERAP1 seems to be specialized for generating peptides of the proper size for Ag presentation. This is not to say that ERAP1 always stops trimming when it has generated a mature peptide. Indeed, it trims ~50% of 9-mer peptides to 8-mers and, in so doing, will ultimately destroy some 9-mer epitopes (40) and limit their presentation (41, 42). It has been suggested that ERAP1 might also trim the protruding ends of long peptides when they are bound to MHC class I molecules (43); however, it is not known whether this actually occurs and if ERAP1’s active site could even access such bound peptides.

ERAP1 plays an important role in MHC class I Ag presentation. Cells in which ERAP1 is eliminated through mutation or RNAi silencing (and also naturally lack ERAP2) are
unable to trim N-extended peptides in the ER (41). Therefore, in mouse cells and some human cells, ERAP1 is the only trimming enzyme in the ER, at least for these sequences. ERAP1-deficient cells have reduced surface levels of MHC class I molecules and the peptide-MHC complexes that are made are less stable than on wild-type cells (44–46). These results suggest that ERAP1 makes an important contribution both to the quantity and quality of peptides available for Ag presentation.

Interestingly, loss of ERAP1 decreases the presentation of some epitopes from full-length proteins (ones presumably made as N-extended precursors), does not affect others, and increases the presentation of yet others (41, 42, 46). Consequently, ERAP1 deficiency markedly changes CD8 T cell responses to various epitopes in vivo and alters immunodominance hierarchies (46). Remarkably, ERAP1−/− T cells can recognize and respond against cells from wild-type mice (44), suggesting that loss of ERAP1 may alter the overall repertoire markedly. Presumed polymorphisms in ERAP1 may similarly affect responses because ERAP1 has been linked genetically to the autoimmune disease ankylosing spondylitis (47, 48) and cancer progression (49) in humans.

Based on these findings, it is clear that the Ag processing pathway generates many N-extended peptide precursors and that trimming by ERAP1 has specificity. This specificity has not yet been fully elucidated, but studies with purified ERAP1 and cells expressing ERAP1 have shown that although it can remove all residues from the N terminus of peptides, it does so at different rates (50) and is also affected by sequences in the epitope itself (40, 51, 52).

**ERAP2**

ERAP2 also trims residues from the N terminus of peptides and appears to do so with different specificity from ERAP1, being more active in cleaving N-terminal basic residues from at least some substrates (53). Therefore, the combined actions of ERAP1 and ERAP2 are likely to enhance the hydrolytic capacity of the ER and trim a broader set of sequences. Interestingly, ERAP2 appears to form a complex with ERAP1, and it has been suggested that this combination of two aminopeptidases may trim peptides in a cooperative manner (53). In contrast to ERAP1, ERAP2 does not stop trimming peptides when they are the size of mature epitopes (52).

Silencing of ERAP2 with RNAi reduced the presentation of an epitope (53). However, the contribution of ERAP2 to overall ER trimming is not yet clear. Silencing of ERAP2 only reduced overall MHC class I expression by ~10% (53). Moreover, the hierarchy of specificity in removing any of the 20 aa flanking an epitope was not different in cells expressing only ERAP1 or both ERAP1 and ERAP2 (50). Furthermore, ERAP2 is poorly expressed or not expressed in human brain, liver, thymus, testes, and small intestine (54), and it is not present in mice. Although additional studies are needed, these findings suggest that ERAP2 may play a more limited or specialized role in peptide trimming.

**Cleavage of peptides after the ER**

Ags that exit the ER may be cleaved by proteases in the exocytic pathway. One such protease is furin, a member of the subtilisin family of serine proteases that is resident in the Golgi apparatus. Furin has been implicated in the generation of peptides from a few proteins that are transported via the exocytic route (55, 56). Because the presentation of these peptides does not require TAP, it is thought that furin may generate these peptides from proteins that transit the transgolgi network. However, this is unlikely to be a major source of presented peptides because TAP-negative cells have very low levels of presented peptides, among which some are derived from signal sequences that are hydrolyzed in the ER, and others are not (57). Whether furin contributes to the trimming of peptides that are generated in the cytosol and transported into the ER by TAP is not known.

**Peptide destruction**

Proteases can also cleave within epitopes, destroying them, and this is a process that can limit Ag presentation. In fact, outside of their role in Ag presentation, most peptides are not useful in cells, and their accumulation would be deleterious. As a result, cells have evolved robust catabolic activities to further degrade peptides down to amino acids, which are useful to cells. Consequently, when peptides are added to cell extracts, they are rapidly degraded (58) and when injected into the cytoplasm of living cells have a half-life of only seconds (26).

All of the proteases that may be involved in the generation of peptides can and probably do destroy some epitopes. Thus, the presentation of some epitopes is enhanced by proteasome inhibitors (16). Moreover, increases in the presentation of selected epitopes or in the overall surface expression of class I molecules were seen in cells in which PSA (33), BH+LAP (35), TPPII (22, 28) and ERAP1 (41, 42) were absent or inhibited. Another protease that has been implicated in this process is thimet oligopeptidase, a cytosolic metalloprotease (59). The increased Ag presentation and higher levels of MHC class I molecules after inhibiting these proteases suggest that they normally limit the presentation of some peptides by destroying them.

The fact that cytosol is such a hostile environment for peptides has raised the question of how peptides destined for Ag presentation escape destruction. It may be that most are destroyed [it has been suggested that this is the fate of 99.9% of peptide (60)] and that the ones that do get presented are rescued by rapid transport through TAP and then binding to MHC class I molecules. However, it has also been proposed that there may be mechanisms that protect peptides from degradation. One idea is that cytosolic chaperones may bind peptides and prevent their degradation. Indeed, peptides can be found bound to these proteins (61–63). However, whether binding to chaperones is important for the presentation of most peptides is not clear. In fact, when thimet oligopeptidase was overexpressed in cells, there was almost complete inhibition of the presentation of peptides generated in the cytosol, but no effect on the ones targeted into the ER (59). These results indicate that presented peptides are susceptible to destruction in the cytosol; this suggests either that protective mechanisms do not exist, or, if they do, then they must be in kinetic competition with the destruction pathways. The specificity of the destructive (and protective) mechanisms has not been well characterized. This is an important issue because it may be yet another factor that influences what peptides ultimately get presented.

**Cross-presentation**

Dendritic cells and macrophages have the unique ability to present on MHC class I not only peptides from their own endogenous Ags, but also ones from Ags in their external
environment through a process called cross-presentation (64–67). After acquiring these Ags, dendritic cells carry this information to secondary lymphoid tissues and present it to naïve CD8 T cells in ways that initiate immune responses (64). This ability to present exogenous Ags plays a critical role in immune surveillance of tissues by allowing the immune system to monitor tissues for the presence of cancers or viruses that do not infect dendritic cells. This pathway is also thought to allow the immune system to monitor the Ags in phagosomes of dendritic cells and macrophages and thereby allow CD8 T cells to detect phagocytes that are harboring intracellular bacteria and eliminate them (66, 67).

Ags that are internalized by phagocytosis or macropinocytosis are cross-presented several thousand times more efficiently than soluble proteins (66, 67). Presumably because of this, particulate Ags (e.g., dying cells) are a major source of cross-presented Ags. Although still somewhat controversial, there is accumulating evidence that the major source of cross-presented peptides is intact Ags (66–68) or ones that are partially cleaved (e.g., by caspases in dying cells) (69, 70). In this review, we will focus on the two best-characterized mechanisms by which cross-presented peptides are generated from proteins in phagosomes with an emphasis on what is known about the proteases that generate these peptides. The two mechanisms we will focus on are the phagosome-to-cytosol and vacuolar pathways (Fig. 2). It should be noted that there are also additional mechanisms by which cross-presentation occurs (63, 71–73), although their role in vivo is presently unclear.

**Phagosome-to-cytosol pathway**

In the phagosome-to-cytosol pathway of cross-presentation, blocking proteolysis in these phagosomes does not inhibit cross-presentation and may actually enhance it (66, 67). In fact, dendritic cells may promote cross-presentation by raising the pH in phagosomes and thereby reducing the proteolytic destruction of Ags (65). Therefore, in this pathway, cross-presented peptides are not generated in the phagosomes themselves. Instead, the Ags are transferred from phagosomes into the cytosol where they undergo cleavage. This form of cross-presentation is blocked by proteasome inhibitors, indicating that these particles make the initial cleavages to generate a cross-presented peptide (66, 67). The presentation is also dependent on TAP (66, 67) and, in at least some cases, also ERAP1 (74, 75). Thus, once the exogenous Ag is transferred to the cytosol, it is thought to be processed for cross-presentation in much the same manner as endogenous proteins.

The peptides that are generated in the cytosol may be transported by TAP to MHC class I molecules in the ER. In addition, there is evidence that many ER components, including TAP, somehow get incorporated into phagosomes (73, 76). This has suggested that some peptides generated in the cytosol might be transported by TAP to MHC class I molecules in phagosomes. In this location, the peptides could be further hydrolyzed by phagosomal proteases. One vacuolar peptidase that has been implicated in this process is insulin-regulated aminopeptidase (IRAP, also known as placental leucine aminopeptidase), which is a homolog of ERAP1 and ERAP2 (77). Like ERAP1, IRAP can remove most amino acids from the N terminus of peptides; however, unlike ERAP, it can continue to trim peptides to a size smaller than <8 residues in length (E. Stratikos, personal communication).

Dendritic cells lacking IRAP present endogenous Ags normally, but have about a 50% decrease in their ability to cross-present an exogenous Ag (77). This reduction in presentation is of a similar magnitude to that observed in ERAP1-deficient cells. It is presently unknown whether these two peptidases are working together in the phagosome or in separate compartments (ERAP1 in the ER and IRAP in the phagosome).

**The vacuolar pathway of cross-presentation**

In the vacuolar pathway, the generation of cross-presented peptides is inhibited by cysteine protease inhibitors such as leupeptin, but is resistant to proteasome inhibitors, and their presentation is TAP-independent (66, 67). Therefore, it is thought that the cross-presented peptides are generated in the phagosome itself.

One of the vacuolar cysteine proteases that generates cross-presented peptides is cathespin (Cat) S. Cat S-deficient mice and their dendritic cells have a defect in vacuolar but not phagosome-to-cytosol cross-presentation (78, 79). Cat B-, L-, and D-deficient cells showed no defect in cross-presentation (78). In addition, IRAP might be involved in the generation of presented peptides in this compartment in DCs (77).

Why Cat S-deficient plays such an important role is unclear. Perhaps it can make the appropriate cleavages to generate 8- to 9-mer peptides. In fact, recombinant Cat S was shown to generate a presented peptide in at least one case (78); however, whether it can generate other mature peptides is not known (80). Additionally, Cat S is relatively unique in being strongly active at neutral pH, and this may be important because MHC class I molecules may not stably bind peptides at acidic pH.

Because Cat S-deficient mice lack the vacuolar pathway, and TAP-deficient animals lack the phagosome-to-cytosol pathway, it has been possible to use these genetic models to quantify the contribution of these various pathways to cross-presentation. Such analyses have shown that both pathways contribute to the generation of CD8 T cell responses but that the phagosome-to-cytosol pathway is the major route for presentation of endogenous Ags.
pathway contributes to a greater extent than does the vacuolar mechanism (66, 78).

Conclusions
To supply MHC class I molecules with peptides, the immune system used, and, in some cases adapted, phylogenetically older catabolic pathways that normally break down proteins. The proteasome pathway has been used and adapted with new active sites and regulators to make the initial cleavages in proteins for direct presentation and for one of the major cross-presentation pathways. Aminopeptidases in the cytosol and ER trim the proteasomal products, sometimes generating the mature epitope from longer precursors and other times destroying them. Other peptidases can also cleave these peptides in the endocytic compartments have been used to generate peptides for one of the alternate cross-presentation pathways. In this situation, Cat.S has been identified as one of the key enzymes in these processes and their specificity is important because this will determine what peptides get presented and thereby the magnitude and specificity of responses.

Disclosures
The authors have no financial conflicts of interest.

References


