Review

Proteolytic enzymes involved in MHC class I antigen processing: A guerrilla army that partners with the proteasome

Silvia Lázaro 1, David Gamarra 1, Margarita Del Val ∗
Centro de Biología Molecular Severo Ochoa (CSIC-Universidad Autónoma de Madrid), Madrid, Spain

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ABSTRACT

Major histocompatibility complex class I proteins (MHC-I) load short peptides derived from proteolytic cleavage of endogenous proteins in any cell of the body, in a process termed antigen processing and presentation. When the source proteins are altered self or encoded by a pathogen, recognition of peptide/MHC-I complexes at the plasma membrane leads to CD8+ T-lymphocyte responses that clear infections and probably underlie tumor immune surveillance. On the other hand, presentation of self peptides may cause some types of autoimmunity. The peptides that are presented determine the specificity and efficiency of pathogen clearance or, conversely, of immunopathology. In this review we highlight the growing number of peptidases which, as a by-product of their regular activity, can generate peptide epitopes for immune surveillance. These ~20 peptides collectively behave as a guerrilla army partnering with the regular proteasome army in generating a variety of peptides for presentation by MHC-I and thus optimally signaling infection.

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1. Introduction

Protein degradation pathways process misfolded or aberrant proteins, avoid their accumulation in the cell and maintain protein turnover. Side products of these essential pathways are peptides, some of which can bind MHC-I molecules. When peptides derive from pathogen or tumor proteins, peptide-loaded MHC-I molecules at the cell surface induce a proper CD8+ T-lymphocyte immune response; in any cell type, presentation of self peptides should not induce a response. In addition, peptidases can also cleave within peptide epitopes, destroying them, a process that can limit antigen presentation. Which peptides are targeted by the immune response, and which peptidases have generated them, can directly influence the efficacy of naïve T-cell activation by professional antigen presenting cells, as well as the subsequent efficiency of detection and elimination by CD8+ cytotoxic T lymphocytes of infected cells or tumors that are presenting pathogen or altered peptides.

Antigen processing and presentation takes place in homeostasis in any cell of the body. It involves generation of precursor peptides by proteolytic cleavage typically in the cytosol, transport by the transporter associated with antigen processing (TAP) into the lumen of the endoplasmic reticulum (ER), proteolytic trimming to a typical length of 8–10 amino acids (aa), binding to nascent MHC-I molecules, peptide/MHC-I complex display at the cell surface and recognition by the T-cell receptor of CD8+ cytotoxic T lymphocytes. Antigen presentation by professional antigen presenting cells can also include peptides derived from exogenous proteins in a process termed cross presentation.

The major peptidase in the cell is the multicatalytic, multimeric and highly regulated proteasome, located in cytosol and nucleus. Its long-known role in antigen processing and presentation by MHC-I of many epitopes (Kloetzel and Ossendorp, 2004) is highlighted (i) by the marked inhibition of antigen presentation when it is inhibited, specifically of the generation of the carboxyl terminus (Ct) of epitopes, (ii) by its modulation by interferon-γ and thereby of the epitopes presented, and (iii) by its prominent degradation of defective ribosomal products (DRiPs) for fast antigen presentation (Antón and Yewdell, 2014).

We will briefly address in this review the current knowledge about non-proteasomal exopeptidases and endopeptidases and their role in MHC-I antigen presentation to CD8+ cytotoxic T lymphocytes. Additional references can be found in the Supplemental Material.
2. Cytosolic aminopeptidases and endopeptidases involved in antigen processing

The cytosol is a highly degradative environment, and harbors proteasomes as well as many peptidases that rapidly degrade peptides into aa, a key process for cell survival. In living cells, labeled peptides are degraded within seconds by aminopeptidases (Reits et al., 2003). Cytosolic aminopeptidases can trim the Nt of extended epitope precursors, resulting either in the generation of the correct MHC-I epitope Nt, or in its destruction. Most proteasomal products require further proteolytic trimming in order to generate 8–10 aa-long epitopes with the correct Nt, but a 15-aa-long peptide with 6 extended aa on its Nt can also constitute a natural epitope presented to CD8+ T cells (Saminno et al., 2006). Several aminopeptidases, such as leucine aminopeptidase (LAP), bleomycin hydrolase (Blimh) or puromycin-sensitive aminopeptidase (PSA), participate in antigen processing mostly in cell-free assays, while dipeptidyl-peptidase III (DPP-III) interferes with cross-presentation. Tripeptidyl-peptidase II (TPPII) is a peptidase involved in cell division and apoptosis or diseases like obesity and cancer. TPPII can process an epitope independently of the proteasome, as well as 3 more epitopes for which the proteasomal activity is detrimental (Seifert et al., 2003).

As peptides longer than 15 aa are processed mainly by TPPII rather than by other aminopeptidases, TPPII is currently understood as a co-operator, rather than a substitute, for antigen processing of rare long proteasomal products. Results in TPPII-deficient mice suggest a slightly destructive global role of TPPII in MHC-I epitope production and interference with cross presentation of an exogenous antigen.

Several studies describe cytosolic complementary endopeptidases in antigen processing for MHC-I, and these often can process antigens independently of proteasomes. The thimet oligopeptidase (TOP) is an endopeptidase whose main action results in epitope degradation (Saric et al., 2001), to the extent that it prevents cross presentation of necrotic cells, although it can also generate precursor peptides and cleave peptides generating the Ct of an epitope (Kessler et al., 2011). Caspases are inflammatory and apoptotic endopeptidases. Caspases 5 and 10 participate in a proteasome-independent manner in the production of a viral epitope that can also be similarly processed by proteasomes (López et al., 2010). Caspases 3 and 8 generate substrates from self proteins in apoptotic cells for proteasome-dependent cross presentation by dendritic cells. The insulin-degrading enzyme (IDE) endopeptidase cleaves insulin and contributes to the proteasome-independent production of a tumor epitope (Parmentier et al., 2010). Nardilysin endopeptidase participates in the generation of precursor peptides of a tumor protein and in producing the correct Nt of a viral epitope (Kessler et al., 2011).

3. ER exopeptidases and endopeptidases associated with antigen processing

Compared to the cytosol, the ER shows low proteolytic activity. The most prominent ER proteolytic step in the antigen presentation pathway is trimming by aminopeptidases that remove Nt flanking residues from antigenic peptide precursors. The result of the combined sequential aminopeptidase activity in the cytosol and in the ER has been estimated by analyzing the frequency of aa in the Nt flank of many epitopes, to define a ‘Nt processing sequence motif’ (Schatz et al., 2008). ER aminopeptidase ERAP1 is an interferon-γ-inducible enzyme very relevant for antigen processing, emphasized by the existence of ERAP1 inhibitors in cytomegalovirus (Kim et al., 2011). ERAP1 products in vitro are rarely shorter than 8 or 9 aa, the proper length for binding to MHC-I molecules (Serwold et al., 2002; York et al., 2002). This is consistent with the length preference of TAP transporter to translocate peptides to the ER and of MHC-I for peptide binding. Several MHC-I allotypes prefer proline in epitope position 2 as binding motif; however, TAP cannot transport these final peptides, requiring the transport of Nt extended precursors. Among the strongest evidence for ER trimming is the observation

Table. Following the antigen processing route from the cytosol, through the ER via TAP, into the exocytic route, till the plasma membrane, we will find amino exopeptidases, endopeptidases and carboxy exopeptidases. As cell biology goes, most have a primary role in other cellular processes, but can as a side effect also generate peptides for immunosurveillance.

A set of ~15 degradative peptidases localized in the cytosol and the secretory pathway cooperate with proteasomes in antigen processing, by pre- or post-processing antigens from longer proteasomal precursors or products (Table 1, ‘PD’). Due to this cooperative role in processing given epitopes, final epitope presentation is inhibited by proteasome inhibitors; the contribution of these peptidases thus often passes undetected while the role of proteasomes may be overestimated. Interestingly, ~8 additional peptidases process antigens independently of the proteasome and eventually generate MHC-I mature epitopes (Table 1, ‘PI’); they are mostly endopeptidases, and include TPPII, Caspases 5 and 10, IDE, SP, SPP, Furin and Cathepsin S; they are found in cytosol and in secretory compartments, and the latter often contribute to TAP-independent antigen presentation. Around 10 peptidases are involved in cross presentation by MHC-I of antigens from exogenous sources; those that contribute (Caspases 3 and 8, ERAP1, ACE, SP, Furin, Cathepsin S and IRAP) are mostly located in vesicular compartments, while the cytosolic ones (DPP-III, TPPII, TOP) mostly interfere with cross presentation.

Mice deficient for these peptidases have been studied. In some cases, this has not been possible for lack of a mouse ortholog (Caspases 5 and 10, ERAP2) or because the deficiency is lethal (signal peptidase, furin). Most animal models, even with combined deficiencies, do not show global effects in MHC class I expression nor impairment in antigen presentation (LAP, Blmh, PSA, IDE). In some cases, (i) specific antigens have been shown in vivo to require one of these peptidases for direct or cross presentation (ERAP1, ACE, Cathepsin S, IRAP, Caspases 3 and 8) or to be degraded by them (TPPII, ERAP1, ACE, TOP), (ii) the peptidase decreases (PSA in dendritic cells, TPPII, ACE) or increases (ERAP1, SPP, PC7) global MHC-I levels in vivo, or (iii) the peptidase more globally shapes the in vivo peptide and TCR repertoire (ERAP1, ACE).

Evidence for peptidase involvement reaches variable degrees of certainty: trimming of synthetic extended peptides in cell-free assays with cell extracts or with purified peptidases, broadly or very specific interference with function in cell culture (employing chemical inhibitors, interfering RNA or gene deficient cells), cell culture analysis of constructs with cleavage site mutations or miniprotein or other constructs and, finally, in vivo assays. In vivo and cell culture assays provide general proof of involvement, while ascription of potential amino-terminal (Nt) or Ct trimming activities is mainly based solely on testing putative precursor peptides in vitro (Table 1).

Occasional failures in finding a peptidase responsible for the generation of proteasome inhibitor-resistant epitopes have not a unique explanation: (i) the inhibition of the proteasome may not be complete (Kisselev et al., 2006) and the residual activity suffice to generate the epitope, (ii) the altered pattern of the proteasomal activity caused by its inhibition may be able to generate the epitope (Wherry et al., 2006), and (iii) other unknown peptidases may be responsible for epitope generation when the proteasome activity is impaired.
### Table 1

Peptidases involved in antigen processing for presentation by MHC-I to CD8+ T lymphocytes.

<table>
<thead>
<tr>
<th>Peptidases</th>
<th>Peptidase class and specificity</th>
<th>Evidence of involvement in antigen processing for presentation by MHC-I&lt;sup&gt;a,b,c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leucine aminopeptidase (LAP)</td>
<td>Metalloaminopeptidase. It prefers NT hydrophobic aminocids.</td>
<td>(T): IFN-γ induced NT trimming by LAP of synthetic peptide in cell-free assay.</td>
</tr>
<tr>
<td>Bleomycin hydrolase (Blmh)</td>
<td>Cysteine aminopeptidase. It has no specificity for substrate sequence.</td>
<td>(T): Inhibition of antigen presentation in infected cells by a broadly specific compound, AAF-cmk; NT trimming by Blmh of a VSV peptide in vitro; Blmh affects surface expression of certain human MHC-I allotypes.</td>
</tr>
<tr>
<td>Puromycin-sensitive aminopeptidase (PSA)</td>
<td>Zinc-binding metallo-aminopeptidase. It prefers NT Ala, Leu, Phe, Met and basic aa.</td>
<td>(T): NT trimming by PSA of RU1 peptide in cell-free assays sequentially after TPPII; inhibition of antigen presentation by broadly specific compound, AAF-cmk, in infected and tumoral cells; NT trimming of a VSV peptide and a RU1 peptide in vitro; PSA affects surface expression of certain human MHC-I allotypes. (In vivo): harmful for MHC-I surface expression in dendritic cells.</td>
</tr>
<tr>
<td>Dipeptidyl-peptidase III (DPP-III)</td>
<td>Zinc-binding metalloaminopeptidase. It cleaves NT dibasic dipeptides mainly from oligopeptides.</td>
<td>(PD): exogenous addition of DPP-III to necrotic cells prevents cross presentation of an Ova epitope by dendritic cells in vitro and in vivo. DPP-III is not involved in direct presentation.</td>
</tr>
<tr>
<td>Tripeptidyl-peptidase II (TPPII)</td>
<td>Serine aminopeptidase. It removes tripeptides from NT of substrates unless it finds a Pro. It also acts as a tryptain-like endopeptidase.</td>
<td>(PI): Inhibition by selective compound, butabindide, and interfering RNA in cell culture indicates TPPII processes an EBV LMP1 epitope, and other epitopes that are degraded by proteasomes from HIV nef, EBV LMP1 and influenza virus nucleoprotein. (T): It processes large pro teaseomal products. It cooperates with PSA in NT trimming of RU1 peptides in cell-free assays; NT trimming in vitro. (In vivo): TPPII is harmful for direct and for cross presentation of an Ova epitope; harmful for MHC-I surface expression.</td>
</tr>
<tr>
<td>Thimet oligopeptidase (TOP)</td>
<td>Zinc-binding metallo-endopeptidase. It optimally cleaves oligopeptides on the Ct of Phe, Ala, or Arg.</td>
<td>(T): Inhibition by interfering RNA in cell culture indicates TOP processes two epitopes from tumor proteins PRAME and MART, degrades Ova and EBV LMP1 epitope, and is harmful for MHC-I surface expression; TOP generates in vitro Ct of PRAME and MART epitopes, one after nardilysin. (PD, in vivo): in necrotic cells TOP prevents cross presentation of an Ova epitope by dendritic cells.</td>
</tr>
<tr>
<td>Caspases</td>
<td>Cysteine endopeptidases. They cleave at the Ct of Asp.</td>
<td>(PI): Inhibition by selective compound, z-VAD-cmk, and viral inhibitor B13R in cell culture indicates caspases process a CMV epitope; in vitro caspase 5 produces Ct of the epitope and caspase 10 creates Nt and Ct. (PD): Inhibition by selective compounds indicates caspases 3 and 8 generate in apoptotic cells substrates for proteasome-dependent cross presentation by dendritic cells of self antigens.</td>
</tr>
<tr>
<td>Insulin-degrading enzyme (IDE)</td>
<td>Zinc-binding metallo-endopeptidase. It cleaves preferentially insulin and substrates with a β sheet conformation.</td>
<td>(PI): Inhibition by a battery of broadly specific compounds and interfering RNA in cell culture indicates IDE processes an epitope from Mage-A3; in vitro IDE generates Nt and Ct of the epitope.</td>
</tr>
<tr>
<td>Nardilysin</td>
<td>Zinc-binding metallo-endopeptidase. It recognizes dibasic motifs.</td>
<td>(PD): Inhibition by interfering RNA in cell culture indicates Nardilysin processes two epitopes from tumor protein PRAME and EBV EBNA3 C; Nardilysin cooperates in vitro with TOP for PRAME, and generates Nt of the EBNA3 C epitope.</td>
</tr>
<tr>
<td>Endoplasmic reticulum aminopeptidase 1 (ERAP1)</td>
<td>Zinc-binding metallo-aminopeptidase. It prefers NT hydrophobic and aromatic aa, and aa with long aliphatic side chains. It does not cleave Pro. Its optimal substrates are 9–16 aa-long peptides, and its products are rarely shorter than 8 or 9 aa.</td>
<td>(T): Inhibition by selective compound, leucinethiol, by interfering RNA and by gene deletion in cell culture indicates ERAP1 processes many epitopes; destruction of other epitopes; net contribution to MHC-I surface expression; exact generation of Nt of epitopes in cell culture, cell-free assays and in vitro. It cooperates with ERAP2 in trimming proteasome products; it trims precursors produced by other peptidases in this list. (In vivo): it contributes to cross presentation; it shapes the CD8+ T lymphocyte repertoire.</td>
</tr>
<tr>
<td>Endoplasmic reticulum aminopeptidase 2 (ERAP2)</td>
<td>Zinc-binding metallo-aminopeptidase. It prefers NT positively charged aa, followed by aa with short hydrophobic side chains.</td>
<td>(T): Inhibition by selective compound, leucinethiol, and by interfering RNA in cell culture indicates ERAP2 processes epitopes; exact generation of Nt of epitopes and cooperation with ERAP1 in cell culture and in vitro.</td>
</tr>
<tr>
<td>Angiotensin converting enzyme (ACE)</td>
<td>Zinc-binding metallo-carboxypeptidase. It cleaves Ct dipeptides from oligopeptides with wide substrate specificity.</td>
<td>(T): Inhibition by selective compound, ramipril, by interfering RNA and by gene deletion in cell culture indicates ACE processes 3 self epitopes and degrades 3 self epitopes; it shapes the MHC-I peptide repertoire; harmful for rate of MHC-I surface expression; exact generation of Ct of epitopes in vitro; it enhances cross presentation of an Ova epitope. (In vivo): ACE processes 2 epitopes and degrades 1 epitope; it shapes the CD8+ T lymphocyte repertoire.</td>
</tr>
<tr>
<td>Signal peptidase (SP)</td>
<td>Serine endopeptidase. It cleaves at the Ct of small non-polar aa at the Ct of signal sequences.</td>
<td>(PI): mutation of SP cleavage site indicates SP processes an epitope from MHC-I. (PI): mutation of SP cleavage site indicates it contributes to cross presentation of nict-delivered epitopes independently of TAP. (PD): It degrades an epitope from prostate stem cell antigen; mass spectrometry data suggest TAP-independent generation by SP of exact Ct of HLA-A2-presented self peptides.</td>
</tr>
<tr>
<td>Signal peptide peptidase (SPP)</td>
<td>Aspartyl endopeptidase. It cleaves within transmembrane domains and in signal sequences.</td>
<td>(PI): Inhibition by selective compound, z-(LL)3, and by interfering RNA in cell culture indicates SPP processes a Trh4 epitope; involved in MHC-I surface expression. (PD): Inhibition by selective compound, z-(LL)3, in cell culture indicates SPP processes an epitope in preprolactin signal peptide.</td>
</tr>
<tr>
<td>Furin</td>
<td>Serine endopeptidase. It cleaves preferentially after polybasic residues.</td>
<td>(PI): Inhibition by selective compound, dec-RVKR-cmk, in cell culture indicates Furin processes a CMV epitope to final size independently of TAP, probably in cooperation with trimming amino- and carboxy-peptidases; TAP-independent Nt processing of Ova epitope from a fusion protein.</td>
</tr>
</tbody>
</table>
that ERAP1 trimming stops at peptides with proline in position 2 and thus spares them for binding by MHC I. Binding even of long peptides to MHC-I prevents further trimming by ERAP1, although there is an alternative model for post-binding trimming. Different studies in cell culture and in vivo have shown the contribution of ERAP1 not only in the generation but also in the destruction of different epitopes (Serwold et al., 2002; York et al., 2002). There is even an epitope that is only generated when ERAP1 activity is impaired (Nagarajan et al., 2012). ERAP1 can also cooperate with other non-proteasomal peptidases. ERAP1 is also involved in cross presentation. In ERAP1-deficient mice, MHC-I molecules expressions is diminished and the CD8\(^+\) T-lymphocyte repertoire is altered, indicating that trimming of precursors by ERAP1 is already occurring during thymic selection (Hammer et al., 2006).

ERAP2 is interferon-\(\gamma\)-inducible and can form dimeric complexes with ERAP1 increasing the efficiency in cleaving precursors (Saveanu et al., 2005).

Need in the exocytic pathway for carboxypeptidases or for enzymes generating Ct for antigen presentation has been appreciated since 1992 (Gil-Torregrosa et al., 1998; Henderson et al., 1992; Wei and Cresswell, 1992). Ct trimming has been variably estimated for different epitopes to enhance antigen presentation by >1000-fold (Medina et al., 2009), or to contribute poorly (Snyder et al., 1998), or not at all in rat cells (Pawis et al., 1996). Recent data indicate that angiotensin-converting enzyme (ACE), a carboxy-dipeptidase, can generate the Ct of epitopes from peptides pre-processed by the proteasome. As ERAP1, ACE-deficiency causes a new repertoire of unique epitopes and \(T\)-lymphocyte specificities (Shen et al., 2011). ACE can also cross present antigens. Taken together, these studies show an emerging and significant role for ACE in the generation of MHC-I-restricted epitopes.

Apart from these exopeptidasess, ER endoproteolytic activities were the first non-proteasomal peptides identified in antigen processing in 1992 (Henderson et al., 1992; Wei and Cresswell, 1992). Signal sequences of secretory and membrane proteins have a pattern sequence, are 10 to 30 aa in length and their cleavage by signal peptide (SP) yields short peptides that need little further trimming to bind MHC-I. Yet, about half of the MHC-I ligands identified so far within them by immunoproteomics require both Nt and Ct trimming to generate the finally presented epitope. Exceptionally, signal peptide can generate the exact Ct in ~75% of the signal sequence-derived ligands bound to MHC-I HLA-A2, which easily fits alanine in the Ct position. There are several examples of TAP-independent epitopes generated by signal peptide, as well as others transported by TAP, although some epitopes are destroyed.

### 4. Endopeptidasess and aminopeptidase in the distal secretory pathway associated with antigen processing

Unlike the cytosol or lysosomes, the secretory pathway and most vesicular compartments are poorly degradative. However, there are different endopeptidasess that participate in MHC-I antigen processing. As in the cytosol, these endopeptidasess often can process antigens independently of proteasomes and, in this case, also of TAP.

**Furin** is a trans-Golgi network proprotein convertase mediating the maturation of pepticid hormones and factors, and viral and cellular glycoproteins. It participates in antigen processing of TAP-independent epitopes, both after direct presentation from endogenous proteins (Gil-Torregrosa et al., 1998; Medina et al., 2009; Tiwari et al., 2007) and after cross presentation of cell-penetrating peptides. Although more efficient in TAP-deficient cells, furin can contribute to generating around 1/3 of the molecules of a given peptide/MHC-I complex in TAP\(^-\) cells, showing that this peptide takes part in global antigen presentation in the presence of TAP. Furin-processed antigens targeted to the secretory route elicited functional CD8\(^+\) T lymphocytes in TAP\(^-\) mice (Medina et al., 2009).

**Cathepsins** are located in endolysosomal compartments. Together with furin, cathepsins are involved through a vacuolar TAP-independent pathway in generation and direct presentation of an epitope (Tiwari et al., 2007) and specifically cathepsin S in cross presentation of another one in phagosomes (Shen et al., 2004).

**Proprotein convertase 7 (PC7)** is located in the trans-Golgi network and in endocytic vesicles, it globally stabilizes unstable MHC-I molecules that are sub-optimally loaded in the absence of TAP, and processes a short precursor peptide. This provides a second

### Table 1 (Continued)

<table>
<thead>
<tr>
<th>Peptidases</th>
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<th>Evidence of involvement in antigen processing for presentation by MHC-I(^{a,b,c})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cathepsins</td>
<td>Aspartyl endopeptidasess. Each cathepsin has its own specificity. Cathepsin D and S specificity is primarily guided by residues in P2, Leu or Asp for cathepsin D, and aliphatic for cathepsin S.</td>
<td>(PI): Inhibition by selective compound, pepstatin A, in cell culture indicates cathepsins process Ova epitope from fusion proteins independently of TAP; cathepsin S in phagosomes needed for cross presentation of Ova epitope; in vitro Nt and Ct processing of Ova epitope. (In vivo): cathepsin S contributes to cross presentation of Ova epitope independently of TAP by a vacuolar pathway.</td>
</tr>
<tr>
<td>Proprotein convertase 7 (PC7)</td>
<td>Calcium-dependent serine endopeptidase. It cleaves preferentially after dibasic residues. Zn(^2+)-binding metallo-aminopeptidase. It has a broad specificity, roughly combining that of ERAP1 and ERAP2.</td>
<td>Inhibition by interfering RNA indicates PC7 stabilizes MHC-I in cells without TAP and with malfunctioning peptide loading complex; involved in shaping the MHC-I peptide repertoire; it processes an exogenous EBV peptide precursor.</td>
</tr>
<tr>
<td>Insulin responsive aminopeptidase (IRAP)</td>
<td></td>
<td>(T): in vitro Nt trimming by IRAP of synthetic peptide; (PD): IRAP cross presents Ova beads in cell culture. (In vivo): IRAP contributes to cross presentation of particulate and soluble Ova and a SMY male epitope. IRAP is not involved in direct presentation.</td>
</tr>
</tbody>
</table>

\(a\): trimming of precursor peptides. \(b\): ‘proteasome-independent’: antigen processing activity in the presence of proteasome inhibitors. \(c\): ‘proteasome-dependent’: antigen processing activity in cooperation with proteasomes.

\(CMV\): cytomegalovirus, EBV, Epstein–Barr virus, HIV, human immunodeficiency virus, Ova, ovalbumin, VSV, vesicular stomatitis virus.

\(\text{PD}\): Complete set of references can be found in the Supplemental Table.
quality check-point for loading new peptides onto MHC molecules (Leonhardt et al., 2010).

Finally, insulin-regulated aminopeptidase (IRAP), related to ERAPs but located in a specific endosomal compartment, can cross present epitopes in cell culture and in vivo, although it is not involved in direct antigen presentation (Saveanu et al., 2009).

5. Concluding remarks

The nucleo-cytoplasmic proteasome is recognized as a relevant peptidase that generates antigenic peptide precursors for MHC-I, although this is not its major function in the cell. Both assertions apply also to ~20 other peptidases, which are located in the cytosol, ER and more distal secretory route. Among them, exopeptidases trimming Nt or Ct of epitope precursors cooperate with proteasomes, while endopeptidases commonly can generate epitopes independently of proteasomes. Exopeptidases and endopeptidases, especially but not exclusively those located in the ER or vesicular compartments, equally contribute to cross presentation of exogenous antigens. A single recently-identified carboxypeptidase stands out. Deeper knowledge of new non-proteasomal peptidases and carboxypeptidases involved in these processes is likely to attract much research effort for years to come. Use of chemical inhibitors, interference RNA, and CRISPR technology will allow the understanding of their mechanisms and specificity. Knowledge on the generation of epitopes that are presented at the cell surface to CD8+ T cells will contribute to the design of new epitope-based vaccines against infections by viruses and intracellular bacteria. In addition, knowing how to profit from all proteolytic activities also in tumor cells may contribute to potentiating the immunogenicity of tumors, and thus contribute to the exciting success of novel stimulatory T-lymphocyte-based immunotherapies of cancer.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.molimm.2015.04.014

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