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MULTI-AUTHOR REVIEW

Providing ligands for MHC class I molecules

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Presentation of antigenic ligands by MHC class I molecules is a prerequisite for cytotoxic T cell (CTL) recognition and elimination of cells posing a threat to the organism, including infected and transformed cells. Understanding what sources these ligands are derived from and how they are produced has therefore been the subject of intense scrutiny for years. The collective effort of many laboratories has provided a fairly advanced although still incomplete picture of how these ligands are produced by proteolysis, but there is still much uncertainty with regard to the nature of the proteins they are derived from. This collection of review articles intends to present the state of our present knowledge on the origin and the mode of generation of MHC class I ligands, with an emphasis on recent developments. With a few minor exceptions (e.g., discussion of some aspects of the proteasome), there is no overlap between the reviews, so that each review should be a fresh and rewarding experience for the reader.

The series starts with a discussion of the source of class I ligands by Dolan and colleagues [1]. Although still a matter of debate, the concept that rapidly degraded proteins (RDPs) are a major source of viral and cellular class I ligands has by now become very influential. However, many important questions regarding the precise nature of the RDPs, the mechanism of their production and their intracellular location remain unanswered. As emphasized by the authors, the lack of knowledge concerning the biochemical nature of RDPs is a particularly important obstacle to progress in this field. It is also unclear whether RDPs derive from "faulty" normal ribosomal translation or, alternatively, are produced by a special, possibly dedicated mechanism such as nonsense-mediated mRNA decay. Having postulated the existence of a specialized "immunoribosome" for several years, the authors recently reported intriguing evidence for the compartmentalization of cytosolic antigen processing, such that ligands derived from a full length antigen were protected from competition against free cytosolic peptides for class I presentation [2]. One fascinating possibility is that a putative compartment dedicated to production of endogenous MHC class I ligands might somehow be related to compartmentalization of antigen degradation and class I loading in cross-presentation [3]. Whatever the eventual mechanism for the observed compartmentalization, the issue of the source of class I ligands promises to remain among the most exciting topics in the field of antigen processing for years to come.

Starck and Shastri highlight a closely related aspect concerning the source of class I ligands, the role of non-conventional translation products [4]. An impressive list of published examples documents the relevance of such products as antigenic ligands, which for example are recognized by CTL naturally arising in the context of HIV and SIV infection, and by tumor-specific CTL. A number of non-conventional translation mechanisms can give rise to such ligands, including, but not limited to, alternative reading frames, transcripts from "untranslated" gene regions and use of leucine-encoding CUG initiation codons. While some of these mechanisms will give rise to

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peptide products not encoded in standard reading frames, others, such as nonsense-mediated decay, will frequently do so. Large-scale analysis of MHC class I ligands indicates that the former group of ligands clearly plays a minor role, since the vast majority of ligands can be assigned to standard reading frames. The impact of non-conventional translation in production of class I ligands remains to be established and is the subject of intense research by several groups.

A sizeable part of our knowledge not only on the origins of MHC class I ligands, but also on many other aspects of MHC class I antigen processing derives from the study of tumor antigens. Vigneron and colleagues illustrate the numerous contributions made by this research in a review that discusses methods for identifying tumor antigens, the different classes of such antigens and both conventional and non-conventional mechanisms implicated in processing them [5]. Most CTL that recognize conventional translation products derived from tumor antigens, which are self-proteins, will be tolerized during thymic education. As a result, relative to CTL recognizing pathogen determinants, tumor-specific CTL may more frequently recognize epitopes produced by non-conventional mechanisms, which are likely to be upregulated in a transformed cell. The same reasoning should apply to self-antigens recognized by autoreactive CTL, although there is less experimental evidence corroborating this hypothesis. Recent examples of the insight that can be gained from tumor antigen studies include identification of the roles of insulin-degrading enzyme and nardilysin in cytosolic antigen processing [6, 7].

Insight into the source of MHC class I ligands can also be gained through the large-scale biochemical analysis of ligands. Mester and colleagues provide a truly expert discussion on the state-of-the-art methods available to perform such large-scale studies [8]. Mass spectrometric large-scale analysis will gradually complement and replace in vitro binding assays as a reference method for the determination of MHC class I peptide-binding motifs. The required number of about 50 ligands per allele has so far been achieved for 25 alleles, but many more remain to be studied. Mass spectrometry also is a powerful method for identifying post-translational epitope modifications, which are of particular interest in tumor immunotherapy since they occur more often in transformed tissues. Large-scale analysis of the cellular ligandome so far has produced inconclusive results on the relative role of protein versus mRNA abundance in class I ligand production, and more studies with refined methods will be required to identify the parameters governing the recruitment of cellular peptides as class I ligands [8].

Four reviews deal with the different cellular pathways for proteolytic generation of MHC class I ligands. Sijts and Kloetzel [9] discuss the role of the proteasome, which undoubtedly makes the largest contribution to proteolytic ligand generation. Much of the review is devoted to the role of the immunoproteasome, whose function has been elucidated in unexpected ways in recent studies. Thus, immunoproteasome complexes appear to be important in lymphocyte survival and in regulation of pro-inflammatory cytokine production. The immunoproteasome is also required to maintain protein homeostasis under pro-inflammatory conditions and to clear oxidant-damaged proteins that accumulate in cells upon IFN-γ treatment, and may correspond to DRiPs [10]. A second exciting recent development discussed by Sijts and Kloetzel is the discovery of thymoproteasomes incorporating the unique ß5t subunit that fails to cleave after hydrophobic amino acids [11]. It will surely be fascinating to unravel why the absence of hydrophobic C-terminal peptide anchors, present in the majority of MHC class I ligand-binding motifs, is essential for positive selection of $CD8^+$ T cells by thymic epithelial cells.

Proteasome-produced peptides frequently require trimming to be suitable for MHC class I binding. My review in this issue highlights major progress over the last 5 years concerning trimming peptidases and proteasome-independent ligand generation [12]. Such progress first highlights that truly proteasome-independent generation of MHC class I ligands may in fact be very rare or at least very difficult to prove conclusively. Given that tripeptidyl peptidase II, previously suggested to replace the proteasome in some cases, has not been shown to degrade complete antigens, possibly the single published case of a truly "autonomous" alternative protease is insulin-degrading enzyme, which produces a MAGE-1 tumor epitope [7]. Concerning epitope trimming, research in the last years has highlighted both the negligible and/or redundant role of cytosolic aminopeptidases and the substantial impact and requirement of trimming by endoplasmic reticulum (ER) aminopeptidases. The finding that the latter peptidases are associated with several MHC class I-associated autoimmune diseases [13] surely will prompt more studies into the roles of these enzymes in humans.

Two reviews in this issue concern alternative proteolytic pathways that implicate proteases in compartments different from the cytosol and the perinuclear ER. Del Val and colleagues [14] discuss antigen processing in the post-ER secretory pathway and in vesicular compartments. While a few proteases (furin and proprotein convertase 7) with the potential to contribute to epitope processing have been identified in post-ER secretory pathway compartments, other proteases, including a putative carboxypeptidase, remain to be identified. As emphasized by Del Val and colleagues, epitope generation in the secretory pathway likely matters mainly in cells with compromised TAP

function, such as many tumor cells or cells infected with viruses inhibiting TAP transport. However, the relevance of this pathway is highlighted by the existence of CTL-recognizing TAP-independent epitopes in the normal T cell repertoire.

The contribution of autophagy, a cellular mechanism with a role in clearing protein and other aggregates that is triggered upon starvation, to the generation of MHC class I ligands has only very recently been recognized, as discussed by Chemali and colleagues [15]. While most pertinent published reports provide sketchy evidence, the cell biological mechanism involved has been examined in a recent study on herpes simplex virus 1 (HSV-1)-infected cells [16]. Fusion of autophagosomes with lysosomes allows for access of cytosolic antigens to "vacuolar" (i.e., lysosomal) acid proteases. In the cited study, processing of HSV-1 epitopes also required, next to autophagosome formation, active proteasome complexes, suggesting a complex pathway including several transport steps between intracellular vesicles and the cytosol [16]. Many molecular details, including the compartment where class I molecules are loaded with peptides produced in this pathway, remain to be worked out. The overall impact of autophagy in class I processing is also entirely unclear. Chemali and colleagues suggest a number of factors that may promote antigen shuttling to the novel pathway, including high protein expression in the cytosol, stress situations such as heat shock, viral infection and IFN-γ exposure [15]. An interesting hypothesis is that a pathway for class I ligand production involving autophagy may be triggered by infection as an anti-viral defense.

Some readers may note the absence of a review dedicated to proteases involved in cross-presentation of exogenous antigens by MHC class I molecules. This absence is motivated by two reasons. The first is that little in-depth research on this issue has been undertaken. The second is that, if one leaves aside the "vacuolar" pathway of cross-presentation involving exclusively endolysosomal proteolysis, the proteases presumably involved in cross-presentation are identical to those also involved in the standard processing of endogenous antigens. However, there is a single exception to this rule, which concerns insulin-regulated aminopeptidase (IRAP). This trimming peptidase processes cross-presented antigens degraded in a proteasome-dependent manner in endosomal vesicles and is the only protease so far described to be involved exclusively in cross-presentation [17]. IRAP and other proteases involved in cross-presentation (both proteasome-dependent and vacuolar) are briefly discussed in my review in this issue [12].

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