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The Peptide Cargo of Class I Molecules: Not Just Passive Passengers

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The paper by Townsend and colleagues (1) was a major advance in our understanding of Ag presentation by class I MHC products. The structure of a class I MHC product in a complex with peptide had been published at the time (2), but how a class I MHC product acquired its peptide cargo was not known, nor was it clear that surface expression of class I MHC products critically depends on an appropriate source of peptide. The paper will stand as a shining example of the clever use of somatic cell mutants in conjunction with cell biological/biochemical insights, and it is deservedly a classic in the field. What makes the paper particularly noteworthy and of exceptional didactic value are some of the puzzles left unsolved at the time of publication and the experiments that were instigated to resolve them, leading to the discovery and characterization of the MHC-encoded peptide transporter and the peptide-loading complex. The work that followed led to assays, still in use today, for the detection of peptides that bind to class I MHC products. It inspired work that documented a similar key role for peptide in the proper surface display of class II MHC products. Our understanding of MHC-peptide interactions in the context of both structural biology and the cell biology of Ag presentation thus owes a great deal to this landmark discovery.

The authors started with an unusual mutant cell line defective in class I MHC expression. In the course of studying the relationship between class I MHC expression and the killing of susceptible target cells by NK cells, coauthor Kärre and coworkers had conducted a mutagenesis and Ab- and complement-based selection experiment to obtain cells with reduced levels of class I MHC at the cell surface. The resulting cell line, referred to as RMA-S, is highly susceptible to NK killing and was used to confirm one of the foundations of the "missing self" hypothesis (3), but that is a different story. Because *N*-ethylnitrosourea (ENU)-based mutagenesis is random and results in multiple hits per genome, it was essential to demonstrate that the loss of surface expression was not attributable to mutations in the class I MHC genes themselves. Somatic cell hybrids prepared between the class I MHC-deficient RMA-S cell line and a fibroblast of the H-2^k haplotype showed that the defect was not in the structural genes for the class I MHC products themselves. With the parental RMA cell line and its class I MHC-deficient RMA-S mutant in hand, Townsend and colleagues showed that RMA-S cells were resistant to lysis by CTLs specific for flu nucleoprotein when infected with live flu virus. Notwithstanding this defect, RMA-S cells were not intrinsically resistant to CTL killing, because mere incubation of RMA-S cells with a synthetic peptide that mimics the nucleoprotein-derived CTL epitope resulted in levels of lysis comparable to those seen in wild-type cells exposed to peptide. Although the display of peptide-loaded class I MHC molecules was somehow defective in the RMA-S cell line, peptide-receptive class I MHC products were present at the surface nonetheless.

Cytofluorimetry showed that incubation of RMA-S cells with peptide not only sensitized them for lysis but also led to an increase in the surface expression of properly folded class I MHC products. This effect was peptide-specific; only peptides known to contain an H-2^b-restricted epitope would increase surface expression. Furthermore, the effect was restricted to the particular class I MHC product concerned; peptides that specified an H-2K^b restricted epitope would lead to increased expression of H-2K^b but not of H-2D^b, and peptides that specified an H-2D^b-restricted epitope would increase H-2D^b but not H-2K^b surface levels. Peptides incapable of binding either class I MHC product were without effect.

In RMA-S cells not exposed to peptide, almost all class I MHC products retained the *N*-linked glycans diagnostic of their retention in the endoplasmic reticulum (ER).[2] Because assembly of class I MHC products is confined to the ER, it was assumed that peptides added to RMA-S cells would have to find their way back to the ER to induce assembly, allow egress of the newly assembled class I products, and, after negotiating the secretory pathway, be displayed at the cell surface as a class I MHC-peptide complex. All experiments performed since are fully consistent with the notion, first proposed by Townsend and colleagues (1), that class I MHC molecules depend on peptide for their correct and stable assembly. Interruption of a steady peptide supply compromises expression of class I MHC at the cell surface. The Townsend paper provided the impetus for additional experiments that consolidated this concept, but the data shown were interpreted rather differently than what is now the accepted view. Herein lies the didactic value of the paper.

Williams and coworkers (4) had carefully measured the rate of intracellular transport required for the delivery of newly assembled class I MHC molecules from the ER to the cell surface. Their estimates—approximately 60 min from ER to surface for a "fast" class I MHC molecule and much slower for other class I MHC products, notably those derived from the H-2D locus—have not been challenged since. However, Townsend et al. (1) observed that the addition of peptide to RMA-S cells led to an immediate and steady increase in surface expression. This result runs counter to expectations; if indeed ~120 min are required for the H-2D^b product to traverse the secretory pathway, a lag time of minimally ~120 min would be expected before exogenously added peptide could reach the ER, induce assembly of class I MHC products, and

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[2] Abbreviations used in this paper: ER, endoplasmic reticulum; β_2m, β_2-microglobulin.

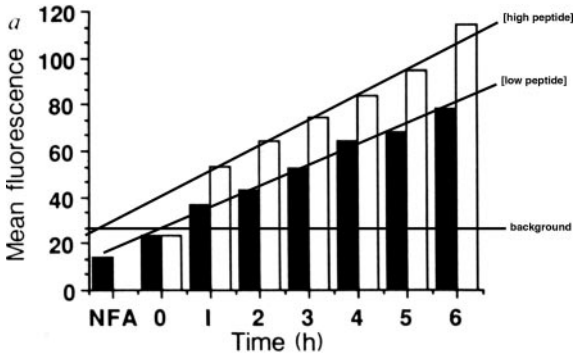

FIGURE 1. The increase in cell surface-exposed class I MHC product, assessed by cytofluorometry, is evident immediately upon the addition of peptide, regardless of peptide concentration, as shown by extrapolation. If H-2D^b molecules require 60–120 min to travel from the ER to the surface and if peptide would have to travel to the ER to induce assembly, then a lag of 60–120 min would have been the expected result. Adapted from Figure 5*A*, Townsend et al. (1) by permission from Macmillan Publishers Ltd. Copyright 1989.

so enhance their surface expression. The data in Fig. 1 (Figure 5*A* in the original publication) tell the story. The intersection of a straight line drawn by connecting the different time points intersects the abscissa at the 0-min time point, if not even earlier; the effect of peptide addition is immediate (Fig. 1). This result is inconsistent with delivery of added peptide to the ER.

By the same token, if indeed peptide reaches the ER and there induces assembly of the free class I MHC subunits, then the newly assembled class I molecule should travel through the secretory pathway and undergo the type of *N*-linked glycan modification diagnostic of passage through the Golgi. The authors assessed the success of their peptide-feeding experiments by scoring for the recovery, by immunoprecipitation, of class I MHC products that retained the L chain β_2-microglobulin (β_2m). In the absence of added peptide little or no β_2m would be recovered, whereas upon the addition of peptide, quantities of β_2m consistent with normal complex formation between the class I MHC H chain and β_2m were recorded. Indeed, provision of a suitable peptide to RMA-S resulted in the recovery of class I MHC molecules with a subunit composition indistinguishable from that in the wild-type RMA cell line, whereas irrelevant peptides failed to do so. However, the extent of class I MHC complex formation induced by feeding peptide to RMA-S cells was not matched by the extent of complex-type *N*-linked glycan modification (Fig. 2; Figure 6*C* in the original publication) as assessed by glycosidase digestion. How is this possible?

We know now that the added peptide accumulates intracellularly at sites other than the ER and is released upon lysis of the RMA-S cells where, in the cell lysate, it combines with class I molecules previously arrested in the ER for lack of peptide. Therefore, in the experiments recorded in Fig. 2, it is unlikely that peptide and "empty" class I MHC products ever met in the ER; they did so only upon detergent lysis of the peptide-loaded cells. This explains the relative lack of success in restoring *N*-linked glycan modifications through the provision of extracellular peptide. In fact, many reports that claim specific associations between proteins of interest lack the controls that distinguish a true intracellular interaction from an interaction that takes place only when the cells are lysed. Mixing experiments can be used to address such concerns.

It has since been determined that the RMA-S cell line harbors a mutation in the TAP transporter and that class I MHC molecules

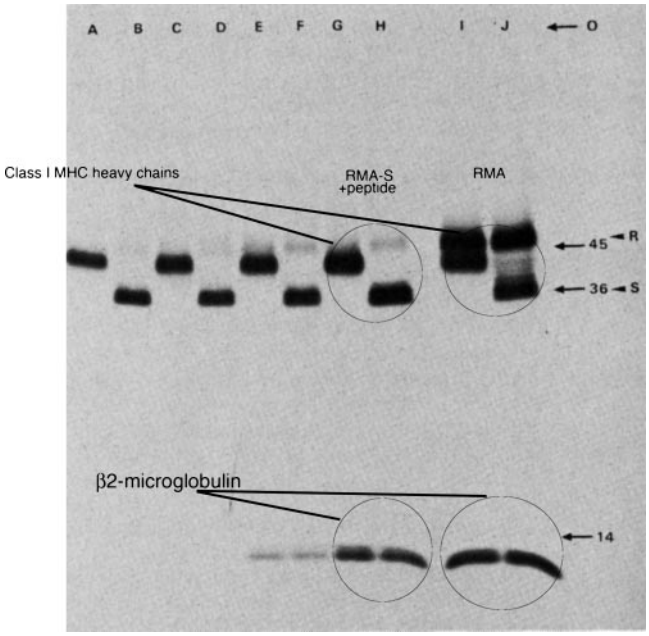

FIGURE 2. Addition of peptide to RMA-S cells results in the recovery of class I MHC products of normal subunit composition compared with RMA cells. The circled areas highlight the H chains and β_2m, respectively, and show comparable subunit ratios for peptide-fed RMA-S and RMA cells. The class I MHC products in peptide-fed RMA-S cells do not undergo terminal glycosylation reactions in proportion to the success of the co-recovery of the class I H chains and β_2m. Adapted from Figure 6*C*, Townsend et al. (1) by permission from Macmillan Publishers Ltd. Copyright 1989.

assembled in the absence of peptide remain stuck in the ER and are more dissociation prone, although their competence for intracellular transport and delivery to the cell surface is not entirely lost. The addition of peptide to intact RMA-S cells allows it to combine with a continuous stream of "empty" class I molecules that arrive at the cell surface. This interaction stabilizes class I MHC products that otherwise would have readily dissociated and be lost from observation and, thus, properly folded class I MHC products are rescued at the cell surface and accumulate over time. This would explain the immediate onset of increased class I MHC expression measured cytofluorometrically (Fig. 1).

Regardless of the details of interpretation revived above, the paper by Townsend et al. should be considered a milestone in the field of Ag presentation. It showed that peptide should truly be considered an essential subunit of a class I MHC product and not a passive passenger that merely rides along to the cell surface. The key insights gleaned from this paper fueled much of the subsequent work on the role of peptide transporters and accessories in peptide loading and thus have given direction to the study of MHC-restricted Ag presentation.

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