Proc. Natl. Acad. Sci. USA
Vol. 92, pp. 341–343, January 1995

Commentary

Getting the inside out: The transporter associated with antigen processing (TAP) and the presentation of viral antigen

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The unique role of antigen presentation by major histocompatibility complex (MHC) class I molecules is to enable the immune system to monitor the internal contents of intact cells. Cytotoxic T cells (CTLs) recognize peptide bound to class I molecules on the cell surface, detect virus infection or malignant transformation, and destroy the diseased cell (1). A key piece of information in the understanding of how the information content of the cytosol is transferred to the cell surface by class I was provided by the study of class I-deficient mutant cell lines, which showed that class I molecules bind peptides in the endoplasmic reticulum (ER) before transit to the cell surface (2–4). The same mutant cells enabled the identification of the molecule responsible for transmitting peptides from the cytosol to the ER–the transporter associated with antigen processing (TAP)–a member of the ABC family of transporters (5–7). In the past 2 yr a series of experimental systems from several laboratories has begun to dissect the function of TAP and to address the role of TAP in the overall process of class I-restricted immunity. The combined effects of the peptides transferred by TAP and the peptide-binding capabilities of the class I molecules themselves must allow the immune system to detect the presence of intracellular parasites, and a diverse array of peptides must be able to be presented to optimize the chance of detecting rapidly mutating viruses. At the same time, functional TAP and class I molecules are necessary to select the repertoire of TCRs capable of recognizing foreign peptide (positive selection) and to eliminate those capable of responding to normal self-cells (negative selection). In this commentary we will discuss how the recently accumulated data on the function of TAP and peptide loading in the ER fit this evolutionary role for TAP. The diverse mechanisms that a number of viruses use to subvert the process of class I-restricted antigen presentation impose an additional selective pressure that may have affected the course of evolution of TAP and the mechanism of peptide loading in the ER.

Despite initial reports of TAP-independent peptide transport in microsomal systems, a recent series of papers has convincingly established techniques for the study of TAP-dependent peptide transport (8–18). Detection of peptide translocation has involved measuring total radiolabeled peptide accumulated in microsomes, measuring peptide bound to class I molecules in semi-permeabilized cells, or capturing radiolabeled glycosylated peptide with lectin-coated Sepharose. The function of human, mouse, and rat TAPs has been examined, and the conclusions drawn from these TAP-dependent systems have been remarkably consistent, confirming that TAP functions as a peptide transporter and beginning to paint the picture of how it serves the needs of class I-restricted antigen presentation. The contribution by Androlewicz *et al.* (9) in a recent issue of this journal now begins a structural analysis of peptide/TAP interaction using photocrosslinkable peptides.

TAP is necessary for efficient peptide transport into the ER (8, 14, 17), and the ability to reconstitute transport in insect cells by expression of TAP suggests that no additional specialized molecule is required (18). TAP is a heterodimer, each subunit of which consists of an ATP-binding domain and a domain that spans the membrane six to eight times (19). Both subunits are encoded in the MHC, and co-expression of both subunits is required for peptide transport (14, 18). Competition studies suggest that there is only a single binding site for peptide (10), and the experiments with photocrosslinkable peptide of Androlewicz *et al.* (9) confirm the previous observation (18) that both subunits are required for the formation of the peptide-binding site. ATP is not required for binding of peptide to TAP (17, 18, 20), but hydrolysis of ATP is required for translocation of peptide to the ER (8, 14, 17, 18).

A major focus of interest has been the substrate specificity of TAPs. As expected, TAPs are able to transport a diverse set of peptides, which is well matched to the peptide-binding requirements of MHC class I molecules, both in length and in the preferred C terminus (12, 13, 16, 18, 20). Peptides longer by a few residues than those found bound to class I can be transported, and there is evidence that longer peptides may be trimmed in the ER and by recycling through the cytosol (21). TAPs from mouse preferentially transport peptides with hydrophobic C termini, whereas the human transporter also transports well those with a basic C terminus, consistent with the broader range of C termini accommodated by human class I molecules (13, 16). In the rat, two TAP alleles transport different sets of peptides: cim^a transports both those with hydrophobic and basic C termini, and cim^b transports preferentially those with a hydrophobic C terminus (10, 13). It is, however, noteworthy that this has only been shown to have functional significance for CTL recognition in the case of laboratory inbred strains, where the linkage between class I and TAP alleles has been disturbed (22).

Does TAP impose any further restriction on the repertoire of peptides available to be presented other than that imposed by their ability to bind to class I? The *cim* phenomenon in the rat indicates that at least in that species TAP can be restricting—clearly more peptides that are capable of binding to class I are produced and made available to TAP than are transported into the ER. It seems possible that the TAPs of other species may impose a similar restriction, and the fact that internal sequence variation can limit the ability of peptides to be transported (11, 13, 17, 18, 20) may be further evidence for a TAP-determined limitation of repertoire. We suggest that such restriction, which seems deleterious given the need to present as wide an array of peptides as possible in the face of new and mutating pathogens, may be advantageous at the time of T-cell receptor (TCR) repertoire selection. If peptide specificity plays a prominent role in negative selection, too diverse an array of self-peptides may unduly limit the selected TCR repertoire. The desirability of diversity in representation of foreign epitopes may be balanced by the need to select an adequate TCR repertoire capable of responding to them. A similar argument has been made to

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explain the limited number of class I alleles expressed by an individual despite the apparent advantage of marked intraspecies polymorphism.

The last few years have also increased our understanding of the handling of class I by the cell during the process of peptide loading. TAP molecules of both mouse and human associate in the ER with class I molecules (23, 24). This association requires that the class I heavy chain be complexed with β_2-microglobulin and can occur with the TAP1 subunit in the absence of TAP2 expression, as shown previously for the mouse and now by Androlewicz *et al.* (9) for the human. The functional significance of this association is not known—but minimally it should ensure the colocalization of TAP and class I in the ER and optimize the exposure of class I to peptide. Immediately after synthesis, class I heavy chain associates with the ER chaperone calnexin (25). Studies in the mouse indicate that TAP, class I heavy chain, β_2-microglobulin, and calnexin may all remain associated until peptide is bound (23, 26). In humans, it has been suggested that class I heavy-chain dissociates from calnexin before associating with β_2-microglobulin (and hence TAP) (27); whether this discrepancy is the consequence of differences in experimental techniques or represents a real difference between the species remains to be determined. It is clear that although peptide, β_2-microglobulin, and class I heavy chain can assemble *in vitro* in solution (28, 29), the association of class I with calnexin and TAP suggests that *in vivo* the event is more controlled. The involvement of other molecules [such as gp94/grp96 (30)] in this process remains to be determined. Each of the systems described for the study of TAP transport has the potential to contribute to this story: the expression of human genes in insect cells will enable definition of the minimal components for peptide loading, the use of photocrosslinkable peptide substrates may help to identify other molecules involved, and the use of β_2-microglobulin-deficient mouse microsomes and cell lines may allow the significance of the class I–TAP association to be determined.

Although the different TAP alleles of the rat encode proteins that clearly transport different sets of peptides, no difference in the peptides transported by mouse TAP variants could be demonstrated (15). There are also several alleles of both human TAP-encoding genes, and although the polymorphism is, in general, limited, one variant of TAP2 (carried by 21% of Caucasians) is longer at the C terminus by 17 amino acids (31, 32). TAP polymorphism has been suggested to account for the MHC linkage of some autoimmune diseases; however, to date there is no evidence for disease association, nor are there any functional data to suggest that the different alleles have a distinct substrate specificity.

The discussion above of the evolutionary pressures on antigen processing concerned the need to cope with the challenge of presenting epitopes from a constantly changing parasite population while maintaining a diverse TCR repertoire. However, some large DNA viruses that are generally antigenically stable impose other pressures on the class I-restricted antigen presentation system. Adenoviruses (33), murine and human cytomegaloviruses (CMVs; refs. 34–36), herpes simplex viruses (HSVs; refs. 37 and 38), and the human papillomavirus (39) each encode proteins that specifically interfere with class I-restricted antigen presentation.

After expression of the early gene products of murine and human CMVs, class I molecules fail to leave the ER. In murine CMV-infected cells, the class I molecules are loaded with peptide but remain in the ER (34). In human CMV-infected cells, class I is rapidly degraded shortly after synthesis (35, 36). Despite the identification of at least one gene responsible for the human CMV effect (Ann Campbell and Thomas R. Jones, personal communication), the mechanisms of these effects and whether or not they involve related viral gene products are not yet determined. Murine CMV and human CMV are thought to have cospeciated with their hosts (40) and have thus coevolved separately with their hosts' antigen-presenting systems for a period of $\approx$30 million years. We think it likely that during this time they (and presumably other pathogens) have not only evolved the means to interact with the peptide loading and class I trafficking systems but may also have influenced the particular development of these systems in each species. If the apparent difference between mouse and human in the point of disengagement of heavy chain from calnexin discussed above is real, it could well reflect a response to such divergent evolutionary pressure.

The mechanism of interference with class I-restricted antigen processing used by HSV may be more closely related to TAP itself. Class I MHC is retained in the ER in cells infected with HSV types 1 and 2 (37, 38), and York *et al.* (38) have recently demonstrated that the HSV protein responsible for this effect is ICP47, an immediate-early gene product that when overexpressed is found in the cytosol and in the nucleus. The ER-retained class I in HSV-infected cells is apparently devoid of peptide, suggesting that ICP47 interferes with the production of peptide or acts somewhere along the path of its delivery to class I. Using a modification of the semipermeabilized cell system described by Neefjes *et al.* (14), we have found that synthetic peptide is not transported into the ER in cells expressing ICP47 (unpublished data). There is recent evidence that ICP47 can interact either directly or indirectly with TAPs (P. Jugovic, I. York, and D. C. Johnson, personal communication). Alternately, the cytosolic distribution of ICP47 and its failure to associate posttranslationally with TAP-expressing membranes raise the possibility that it may interfere with an as-yet-unidentified protein in the antigen-processing pathway, such as a cytosolic chaperone for peptides.

CMVs and HSVs are similar in their host interactions in that their replication strategy involves the establishment of latent infection. The ability to replicate and infect new hosts from latent infection in the face of a fully primed immune system is presumably enhanced by interference with class I-restricted antigen presentation. Other viruses with a similar life-style include the other human herpesviruses: Epstein–Barr virus (where little is known about cell-mediated immunity to the productive phase of infection), varicella-zoster virus, and the recently discovered human herpesviruses 6 and 7. We predict that investigation of antigen presentation in cells infected with these and other viruses will yield further examples of immune viral-evasion strategies, not only for class I- but also for class II-restricted responses.

We see this interaction between the evolution of antigen presentation and the immune-evasion mechanisms of viruses as rather like a prolonged chess game between well-matched players. From the same starting point, different paths are followed, depending on the moves of the opponent. It is clear that the evolution of class I-restricted antigen presentation has affected the evolution of the viruses discussed above, and we contend that the particular immune-evasion strategies developed by viruses have, in turn, influenced the evolution of antigen presentation within each species. If the parallel is not too extravagant, we think that a similar mutual interaction may be beneficial to the studies of both virology and immunology. The study of antiviral immunity has been fundamental in this area in the past. The investigation of antiviral CTLs enabled Doherty, Zinkernagel, and Blanden *et al.* (41, 42) to identify the phenomenon of MHC restriction, and pursuing the unexpected finding that influenza-specific CTLs predominantly recognize intracellular antigens led Townsend *et al.* (43, 44) to discover the mechanism by which this occurs. Conversely, the ability to investigate the mode of action of a viral product such as HSV–ICP47 depends entirely on the recent advances in understanding class I-restricted antigen presentation at the molecular level. Understanding the mechanisms by which CMVs interfere with the process is likely to draw heavily on the cell biology-based studies of class I trafficking, and identification of these viral products might even allow the

development of new therapeutic agents. The understanding of the molecular details of antigen processing contributed by studies such as that of Androlewicz *et al.* (9) is an essential part of this process.

We thank David Johnson, Therese Heemels, and Lynda Tussey for reading the manuscript and for helpful suggestions.

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