Presentation of Numerous Viral Peptides to Mouse Major Histocompatibility Complex (MHC) Class I-restricted T Lymphocytes Is Mediated by the Human MHC-encoded Transporter or by a Hybrid Mouse–Human Transporter

By Jonathan W. Yewdell,* Fernando Esquivel,* Daniele Arnold,† Thomas Spies,‡ Laurence C. Eisenlohr,* and Jack R. Bennink*

From the *Laboratory of Viral Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20892; and the †Dana Farber Cancer Institute, Boston, Massachusetts 02115

Summary

The major histocompatibility complex-encoded transporter associated with antigen processing (TAP) is required for the efficient presentation of cytosolic antigens to class I-restricted T cells. TAP is thought to be formed by the interaction of two gene products, termed TAP1 and TAP2. We find that TAPs consisting either of human subunits, or mouse TAP1 and human TAP2, facilitate the presentation of numerous defined viral peptides to mouse class I-restricted T cells. As human and mouse TAP2 and TAP1 differ in 23 and 28% of their residues, respectively, this indicates that TAP1 and TAP2 can form a functional complex with partners considerably different from those they coevolved with. Moreover, these findings indicate that widely disparate TAPs facilitate delivery of the same peptides to class I molecules. These findings suggest that TAP polymorphism does not greatly influence the types of peptides presented to the immune system.

Cells and Virus. RMA and RMA/s cells (H-2b) were provided by Dr. K. Kärre (Karolinska Institute, Stockholm, Sweden). C1R and T2 cells and their Kk-expressing transfectants were provided by Drs. J. Alexander and P. Cresswell (Yale University, New Haven, CT). 45 cells were provided by R. DeMars (University of Wisconsin, Madison, WI). The expression of Kk molecules by transfectants was confirmed by immunoprecipitation of Kk from detergent extracts of [35S]methionine-radiolabeled cells. Cells and transfectants were maintained in RPMI 1640 supplemented with 7.5% (vol/vol) FCS. The influenza virus A/PR/8/34 and the Sendai parainfluenza type I virus were propagated in the allantoic cavity of 10-d-old chicken eggs. The Indiana strain of vesicular stomatitis virus (VSV) was grown in baby hamster kidney cells. Recombinant vaccinia viruses (rVV) were propagated in thymidine kinase-deficient human 143B osteosarcoma cells. rVV expressing influenza virus gene products nucleoprotein (NP), basic polymerase 2, and hemagglutinin (HA) without its NH2-terminal endoplasmic reticulum (ER) insertion sequence (VV-NP, VV-PB2, and VV-L-HA, respectively) and H-2Kd (VV-Kd) have been described (15-17). rVV expressing H-2Dd (DV-VV), or residues 1-168 of the
A/PR/8/34 NP (VV-NP), were produced by inserting cDNAs encoding the respective genes behind the early/late VV p7.5 promoter into a modified pSC11 plasmid as described (18).

Mice. 6-8-wk-old C57BL/6 (H-2b), CBA/J (H-2k), and BALB/c (H-2d) mice were obtained from The Jackson Laboratory (Bar Harbor, ME). Mice were immunized with influenza virus or β-propiolactone-inactivated Sendai virus by intraperitoneal injection, and with rVV by intravenous injection.

Cytotoxicity Assays. Target cells were infected with viruses as described previously (19, 20). TCD8+ were generated from splenocytes derived from animals immunized with viruses 2-6 wk previously by 7-d in vitro stimulation with virus-infected autologous splenocytes as described (19, 20) or with synthetic peptides provided by the Biological Resources Branch (NIAID). Microcytotoxicity assays were performed as previously described (19, 20). Data are expressed as percent specific release defined as: 100 x [(experimental cpm - spontaneous cpm)/(total cpm - spontaneous cpm)].

Production of Transfected RMA/S Cells. The full-length human TAP2 cDNA (21) was subcloned into RSV.5(neo) plasmid (22) using flanking XbaI sites. RMA/S cells were transfected by electroporation in 0.4-cm cuvettes at 210 V and 960 μFD. Transfectants were selected by growth in 24-well plates in the presence of 1 mg/ml G418. 4 wk after transfection, cells were analyzed by cytofluorography after indirect immunofluorescence using the H-2 D+ specific mAb 28.14.88 (HB-27; American Type Culture Collection, Rockville, MD). Increased levels of surface staining were detected on 22 of 36 G418-resistant populations. The H-2 K+ specific mAb Y3 (HB-176; American Type Culture Collection) was also used to quantitate class I surface expression.

Results and Discussion

We first examined whether TAP consisting of mouse TAP1 and human TAP2 could present peptides to mouse TCD8+. RMA/S cells were transfected with a plasmid containing cDNA encoding the human TAP2 and the neomycin resistance gene. RMA/S is a mouse cell line selected from mutagenized RMA lymphoma cells on the basis of reduced class I expression. The single TAP2 gene present in RMA/S cells has a point mutation at nucleotide position 97 resulting in the introduction of a premature stop codon (23). RMA/S cells demonstrate a diminished capacity to present cytosolic antigens to TCD8+, although the severity of the defect varies among antigens (24-26). Antigen processing and class I cell surface expression is enhanced after transfection with mouse or rat TAP2 genes, which indicates that the antigen processing defect is due largely, if not solely, to the absence of normal TAP2 (27, 28).

Two RMA/S transfecant clones resistant to G418 selection expressing increased levels of cell surface D+ by cytofluorography were selected for further study. Additional cytofluorographic analysis of the clones revealed that both demonstrated increased expression of K+ and D+ class I molecules (Table 1). In the tables, results are shown for only the 6.2 clone, which expressed slightly more class I molecules than 5.2. In most of the functional experiments described below, both 5.2 and 6.2 clones were examined, and 5.2 behaved similarly to 6.2. It is notable that although expression of class I molecules was increased by expression of the human TAP2 gene, it remained lower than in RMA cells. While this could be due to differences between human and mouse TAP2 subunits that limit the effectiveness of human TAP2 in mouse cells, a similar difference was noted between RMA cells and RMA/S cells transfected with the mouse TAP2 gene (27). As noted previously (27), RMA/S cells may be defective in other genes that enhance class I assembly, transport, or stability.

The ability of clone 6.2 to present cytosolic antigens from influenza or Sendai viruses to TCD8+ was investigated next. TCD8+ specific for influenza virus NP were generated by PR8 in vitro stimulation of splenocytes derived from mice primed with a rVV-expressing NP. Such TCD8+ have been shown to recognize a single D+ peptide from NP corresponding to residues 366-374 (28a). TCD8+ specific for Sendai virus NP were generated by Sendai virus stimulation of splenocytes derived from Sendai virus–primed mice. Based on the findings of Kast et al. (29), it is likely that these TCD8+ principally recognize a single K+ restricted peptide corresponding to NP residues 321-328. As seen in Table 2, 6.2 cells demonstrated enhanced presentation of both viral NPs.

The antigen processing capacity of 6.2 cells was further characterized using TCD8+ specific for a peptide corresponding to residues 52-59 from VSV N (30). After infection with VSV, 6.2 cells were lysed at higher levels than RMA/S cells (Fig. 1). Since cytotoxicity assays provide a poor quantitative measure of the amount of peptide–class I complexes displayed on the cell surface, the recognition of cells expressing limiting amounts of peptides was tested. Cells were treated at various times after VSV infection with either a mixture of protein synthesis inhibitors or the fungal metabolite, brefeldin A (BFA), which blocks the exocytosis of peptide–class I complexes formed in the ER (31, 32). These treatments provide, respectively, measures of the efficiency of peptide generation from a limited amount of protein, and the rate at which class I–peptide complexes are delivered to the cell surface in the absence of protein synthesis inhibitors. Addition of protein synthesis inhibitors to RMA/S cells greatly

| Cells          | Control | Anti-D+ | Anti-K+ |
|----------------|---------|---------|---------|
| RMA/S          | 2 (20)  | 100 (233) | 100 (239) |
| RMA/S 6.2      | 2 (20)  | 15 (20)  | 55 (21) |
| RMA/S 6.2      | 1 (20)  | 95 (49)  | 98 (70) |

Table 1. Cytofluorographic Analysis of RMA/S Cells Transfected with the Human TAP2 Gene

Viable cells were incubated with a control mAb specific for VSV G protein, or mAbs specific for D+ (HB-27) or K+ (HB-176). Antibody binding was detected by addition of FITC-conjugated rabbit anti-mouse IgG. Nonspecific staining was gated out of analysis based on their light scattering properties. Data are expressed as percent positive (relative to no first antibody) and mean channel fluorescence of positive cells (in parentheses).
Table 2. Presentation of Individual Influenza and Sendai Virus Antigens by Transfected RMA/S Cells Expressing the Human TAP2 Gene

| Exp. | Cells                  | Antiinfluenza NP | Anti-Sendai |
|------|------------------------|------------------|-------------|
|      |                        | 10:1             | 5:1         | 40:1       | 20:1       |
| A    | RMA                    | 26               | 6           |            |            |
|      | RMA PR8                | 85               | 70          |            |            |
|      | RMA/S                  | 5                | 0           |            |            |
|      | RMA/S PR8              | 31               | 9           |            |            |
|      | RMA/S 6.2              | 4                | 2           |            |            |
|      | RMA/S 6.2 PR8          | 65               | 56          |            |            |
| B    | RMA                    | 3                | 3           |            |            |
|      | RMA Sendai             | 85               | 72          |            |            |
|      | RMA/S                  | 2                | 3           |            |            |
|      | RMA/S Sendai           | 31               | 15          |            |            |
|      | RMA/S 6.2              | 5                | 4           |            |            |
|      | RMA/S 6.2 Sendai       | 83               | 80          |            |            |

Uninfected or virus-infected cells were tested in a 4-h ³⁵Cr release assay at the E/T ratios indicated. TCD₈⁺ specific for individual influenza virus antigens were generated by in vitro PR8 stimulation of splenocytes derived from mice immunized with VV-NP. TCD₈⁺ specific for Sendai virus were generated by in vitro Sendai virus stimulation of splenocytes derived from mice immunized with Sendai virus.

compromised their presentation of antigen to TCD₈⁺ (Fig. 1 A). By contrast, presentation by 6.2 cells was less affected, and control values of lysis were reached by allowing protein synthesis to proceed for 90 min after infection. Indeed, the residual and constant levels of lysis observed at the 0–30-min time points with 6.2 cells probably represents breakthrough biosynthesis of nucleocapsid (N) protein at levels too low to sensitize nontransfected RMA/S cells. Similarly, while BFA completely blocked presentation of N by RMA/S cells even when added as late as 90 min after infection, 6.2 cells transported sufficient peptide-class I complexes to enable recognition of some cells as soon as 60 min after infection (Fig. 1 B). This is similar to the kinetics of presentation of N by RMA cells (24). Thus, we conclude that expression of the human TAP2 gene concomitantly enhances the efficiency of RMA/S cells to produce antigenic peptides from a limited pool of protein, and increases the rate at which class I–peptide complexes can be produced and transported to the cell surface.

Figure 1. Effect of inhibitors on presentation of VSV N by RMA/S and 6.2 cells. (A) Cycloheximide (15 μg/ml) and anisomycin (26 μg/ml) were added to cells at the times indicated after infection and maintained throughout the infection and ³⁵Cr labeling periods until the 4-h microcytotoxicity assay, in which only cycloheximide (15 μg/ml) was present. (B) BFA (5 μg/ml) was added to cells at the times indicated after infection and maintained throughout the infection and ³⁵Cr labeling periods until the 4-h microcytotoxicity assay, in which BFA was present at 1.25 μg/ml. BFA or cycloheximide at these concentrations do not inhibit TCD₈⁺ activity (31). The actual percent specific release values are given within the bar depicting the time point.
Table 3. TAP-dependent Presentation of Mouse Class I-restricted Peptides by Human Cells

| Exp. Cells | Percent specific release |
|------------|-------------------------|
|            | Kk | Kk | Kk | Kk | Kk | Kk |
|            | HA  | HA | HA | HA | HA | HA |
|            | (245–252) | (340–348) | (50–57) | (519–527) | (147–155) | (PR8) |
| A          | C1R Kk + VV | 10 | 3 | 7 |
|            | C1R Kk + VV L–HA | 49 | 37 |
|            | C1R Kk + VV-NP1,168 |  | 62 |
|            | T2 Kk + VV | 5 | 1 | 4 |
|            | T2 Kk + VV-L–HA | 5 | 5 |
|            | T2 Kk + VV-NP1,168 |  | 6 |
| B          | C1R + VV-Kd | 1 | 2 |
|            | C1R + VV-Kd + VV-L–HA | 41 | 50 |
|            | C1R + VV-NP | 0 | 3 |
|            | T2 + VV-Kd | 8 |
|            | T2 + VV-Kd + VV-L–HA | 3 |
|            | T2 + VV-Kd + VV-NP | 0 |
| C          | .45 + VV-Dd | 42 |
|            | .45 + VV-Dd + VV-PB2 | 0 |
|            | T2 + VV-Dd + VV-PB2 | 0 |

Uninfected or virus-infected cells were tested in a 6-h 51Cr release assay at the E/T ratios indicated. Presentation of PB2 was assessed using TcD+ induced by influenza virus in vitro stimulation of splenocytes derived from influenza virus-infected mice. The other TcD+ were generated from splenocytes derived from mice immunized with rVVs expressing individual influenza virus proteins by in vitro stimulation with the synthetic peptide corresponding to the naturally processed determinant. Indirect immunoperoxidase staining using mAbs specific for VV gene products demonstrated that T2 cells expressed equal or greater amounts of VV-encoded proteins than .45 or C1R cells. Additionally, Kk-transfected T2 cells and VV Kk-infected T2 cells are able to efficiently present determinants delivered to the ER through the action of a NH2-terminal leader/signal peptide (39, and our unpublished results).

* Restriction element.
* Specificity of TcD+.
§ Peptide recognized.

These findings demonstrate that human TAP2 can substitute for its mouse homologue in facilitating the presentation of each of three cytosolic viral antigens tested. If TAP1 and TAP2 function as a complex as believed, this would mean that widely different subunits can be substituted for each other without grossly affecting function. This implies that the human TAP functions similarly to mouse TAP.

To test this idea, we examined the capacity of EBV-transformed human B cell lines (C1R and .45) expressing mouse class I molecules to present peptides to mouse TcD+. Mouse class I molecules were expressed from either transfected genes (Kk) or genes inserted into rVVs (Kk and Dd). In either case, influenza virus proteins were expressed by infection with rVVs expressing the relevant influenza virus protein. Mouse TcD+ specific for each of five defined peptide determinants tested from influenza virus HA and NP (33–35) were able to lyse human cells expressing the appropriate class I molecules and viral protein (Table 3). Additionally, an undefined Dd-restricted determinant from PB2 (36) was presented to polyclonal anti-influenza virus mouse TcD+.

To establish the TAP dependence of antigen presentation, antigen processing–deficient T2 cells (10, 37) were included in each of the experiments. These cells possess a single copy of chromosome 6 that contains a 1-Mbp deletion in the HLA region encoding the TAP. Despite expressing similar amounts of VV-encoded proteins (not shown), T2 cells were lysed at or near background levels by each of TcD+ tested. In additional experiments, we found that the presentation of each of these antigens can be reconstituted by the expression of TAP1 and TAP2 genes in the absence of other MHC gene products (our unpublished results). Similar TAP-dependent presentation was found for three defined Kb-restricted determinants from Sendai NP, VSV N, and OVA (38, and not shown), and for undefined VV-derived determinants in association with Kb, Kk, Dd, Kd, and Ld (not shown). Thus, all told, human TAP was able to facilitate the presentation of each of at least 14 viral determinants examined.

Our findings indicate that human and mouse TAPs func-
tation to facilitate presentation of the same set of peptides. As these TAPs are almost certain to exhibit greater differences than TAPs within a given species (human and mouse TAP2 and TAP1 differ, respectively, in 23 and 28% of their residues), this suggests that polymorphism in TAPs does not greatly affect the repertoire of peptides presented by class I molecules to the immune system. The recent findings of Lobigs and Müllbacher (40) lead to the same conclusion. This conclusion conflicts with the observation of the relatively large effect observed on peptides derived from rat RT1.A molecules after the expression of a rat TAP2 allele that differed from the naturally coexpressed allele by as few as 25 residues out of 703 (4% nonhomology) (12). There are a number of potential explanations for this apparent discrepancy. (a) Differences in TAP peptide specificity are of a more quantitative than qualitative nature, and such differences are difficult to detect by the measure of TCD8 recognition. (b) Rat TAP2 is particularly sensitive to amino acid alterations. (c) TAP2 genes can accommodate a large number of changes in many positions without altering its peptide specificity (or changes in different locations balance the effect of one another), while particular changes in certain residues have a large effect on peptide specificity. (d) Amino acid substitutions in TAP2 affect the interaction of the TAP complex with only a subset of peptides, such a subset being preferentially bound by RT1.A molecules.

Distinguishing among these possibilities will require further efforts. Ultimately, to establish the effect of TAP polymorphism on TCD8 responses in vivo, it will be necessary to produce transgenic animals expressing foreign TAP alone, or in combination with their natural TAP.

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Address correspondence to Jonathan W. Yewdell, Department of Health and Human Services, Building 4, Room 213, National Institutes of Health, Bethesda, MD 20892. Laurence C. Eisenlohr’s present address is the Jefferson Cancer Institute, Thomas Jefferson University, Philadelphia, PA.

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References

1. Townsend, A., and H. Bodmer. 1989. Antigen recognition by class I-restricted T lymphocytes. Annu. Rev. Immunol. 7:601.
2. Yewdell, J.W., and J.R. Bennink. 1992. Cell biology of antigen processing and presentation to MHC class I molecule-restricted T lymphocytes. Adv. Immunol. 52:1.
3. Deverson, E., I.R. Gow, W.J. Coadwell, J.J. Monaco, G.W. Butcher, and J.C. Howard. 1990. MHC class II region encoding proteins related to the multidrug resistance family of transmembrane transporters. Nature (Lond.). 348:738.
4. Trowsdale, J., I. Hanson, I. Mockridge, S. Beck, A. Townsend, and A. Kelly. 1990. Sequences encoded in the class II region of the MHC related to the "ABC" superfamily of transporter. Nature (Lond.). 348:741.
5. Spies, T., M. Bresnahan, S. Bahram, D. Arnold, G. Blanck, E. Mellins, D. Pious, and R. DeMars. 1990. A gene in the human major histocompatibility complex class II region controlling the class I antigen presentation pathway. Nature (Lond.). 348:744.
6. Monaco, J.J., S. Cho, and M. Attaya. 1990. Transport protein genes in the murine MHC: possible implications for antigen processing. Science (Wash. DC). 250:1723.
7. Townsend, C., C. Omen, J. Bastin, H.-G. Ljunggren, L. Foster, and K. Kärre. 1989. Association of class I major histocompatibility heavy and light chains induced by viral peptides. Nature (Lond.). 340:443.
8. DeMars, R., R. Rudersdorf, C. Chang, J. Petersen, J. Strandmann, N. Korn, B. Sidwell, and H.T. Orr. 1985. Mutations that impair a posttranscriptional step in expression of HLA-A and -B antigens. Proc. Natl. Acad. Sci. USA. 82:8183.
9. Powis, S.H., I. Mockridge, A. Kelly, L.-A. Kerr, R. Glynne, U. Gileadi, S. Beck, and J. Trowsdale. 1992. Polymorphism of an MHC-linked transporter on the peptides assembled in a class I molecule. Nature (Lond.). 357:211.
10. Colonna, M., M. Bresnahan, S. Bahram, J.L. Strominger, and T. Spies. 1992. Allelic variant of the human putative peptide transporter involved in antigen processing. Proc. Natl. Acad. Sci. USA. 89:3932.
11. Powis, S.H., I. Mockridge, A. Kelly, L.-A. Kerr, R. Glynne, U. Gileadi, S. Beck, and J. Trowsdale. 1992. Polymorphism in a second ABC transporter gene located within the class II region of the human major histocompatibility complex. Proc. Natl. Acad. Sci. USA. 89:1463.
12. Smith, G.L., J.Z. Levin, P. Palese, and B. Moss. 1987. Syn-
thesis and cellular location of the ten influenza polyepitopes individually expressed by recombinant vaccinia viruses. *Virology* 160:336.

16. Townsend, A.R.M., J. Bastin, K. Gould, and G.G. Brownlee. 1986. Cytotoxic T lymphocytes recognize influenza hemagglutinin that lacks a signal sequence. *Nature (Lond.)* 324:575.

17. Eisenlohr, L.C., J.W. Yewdell, and J.R. Bennink. 1992. A transient system for identifying biosynthesized proteins processed and presented to class I MHC restricted T lymphocytes. *J. Immunol. Methods.* In press.

18. Chakrabarti, S., K. Brechling, and B. Moss. 1985. Vaccinia virus expression vector: coexpression of β-galactosidase provides visual screening of recombinant virus plaques. *Mol. Cell Biol.* 5:3403.

19. Bennink, J.R., J.W. Yewdell, G.L. Smith, C. Moller, and B. Moss. 1984. Recombinant vaccinia virus primes and stimulates influenza haemagglutinin-specific cytotoxic T cells. *Nature (Lond.)* 311:578.

20. Yewdell, J.W., J.R. Bennink, G.L. Smith, and B. Moss. 1985. Influenza virus nucleoprotein is a major target antigen for cross-reactive anti-influenza A virus cytotoxic T lymphocytes. *Proc. Natl. Acad. Sci. USA.* 82:1785.

21. Bahram, S., D. Arnold, M. Bresnahan, J.L. Strominger, and T. Spies. 1991. Two putative subunits of a peptide pump encoded in the human major histocompatibility complex class II region. *Proc. Natl. Acad. Sci. USA.* 88:10094.

22. Long, E.O., S. Rosen-Bronson, D.R. Karp, M. Malnati, R.P. Sekaly, and D. Jaracquemada. 1991. Efficient cDNA expression vectors for stable and transient expression of HLA-DR in transfected fibroblast and lymphoid cells. *Hum. Immunol.* 31:229.

23. Yang, Y., K. Früh, J. Chambers, J.B. Waters, L. Wu, T. Spies, and P.A. Peterson. 1992. Major histocompatibility complex (MHC)-encoded HAM2 is necessary for antigenic peptide loading onto class I MHC molecules. *J. Biol. Chem.* 267:11669.

24. Esquivel, F., J.W. Yewdell, and J.R. Bennink. 1992. RMA/S cells present endogenously synthesized cytosolic proteins to class I-restricted cytotoxic T lymphocytes. *J. Exp. Med.* 175:163.

25. Hosken, N.A., and M.J. Bevan. 1992. An endogenous antigenic peptide bypasses the class I antigen presentation defect in RMA-S. *J. Exp. Med.* 175:719.

26. Aldrich, C.J., R. Wüster, E. Hermel, M. Attaya, K.F. Lindahl, J.J. Monaco, and J. Forman. 1992. T cell recognition of Qa-1b antigens on cells lacking a functional Tap-2 transporter. *J. Immunol.* 149:3773.

27. Attaya, M., S. Jameson, C.K. Martinez, E. Hermel, C. Aldrich, J. Forman, K.F. Lindahl, M.J. Bevan, and J.J. Monaco. 1992. Ham-2 corrects the class I antigen-processing defect in RMA-S cells. *Nature (Lond.)* 355:647.

28. Powis, S.J., A.R.M. Townsend, E.V. Deverson, J. Bastin, G.W. Butcher, and J.C. Howard. 1991. Restoration of antigen presentation to the mutant cell line RMA-S by an MHC-linked transporter. *Nature (Lond.)* 354:528.

29a. Lapham, C.K., I. Bacik, J.W. Yewdell, K.P. Kane, and J.R. Bennink. 1993. Class I molecules retained in the endoplasmic reticulum bind antigenic peptides. *J. Exp. Med.* 177:1633.

29b. Kast, W.M., L. Roux, J. Curren, H.J. Blom, A.C. Voor- douw, R.H. Molen, D. Kolakofsky, and C.J.M. Melief. 1991. Protection against lethal Sendai virus infection by in vivo priming of virus-specific cytotoxic T lymphocytes with a free synthetic peptide. *Proc. Natl. Acad. Sci. USA.* 88:2283.

30. Van Bleek, G.M., and S.G. Nathenson. 1990. Isolation of an endogenously processed immunodominant viral peptide from the class I H-2Kb molecule. *Nature (Lond.)* 348:213.

31. Yewdell, J.W., and J.R. Bennink. 1989. Brefeldin A specifically inhibits presentation of protein antigens to cytotoxic T lymphocytes. *Science (Wash. DC).* 244:1072.

32. Nuchtcrn, J.G., J.S. Bonifacino, W.E. Biddison, and R.D. Klaasen. 1989. Brefeldin A implicates egress from endoplasmic reticulum in class I restricted antigen presentation. *Nature (Lond.)* 339:223.

33. Gould, K.G., H. Scotney, and G.G. Brownlee. 1991. Characterization of two distinct major histocompatibility complex class I Kk-restricted T cell epitopes within the influenza A/PR/8/34 virus hemagglutinin. *J. Virol.* 65:5401.

34. Falk, K., O. Rötzschke, K. Deres, J. Metzger, G. Jung, and H.-G. Rammensee. 1991. Identification of naturally processed viral nonapeptides allows their quantification in infected cells and suggests an allele-specific T cell epitope forecast. *J. Exp. Med.* 174:425.

35. Rötzschke, O., K. Falk, K. Deres, H. Schild, M. Norda, J. Metzger, G. Jung, and H.-G. Rammensee. 1990. Isolation and analysis of naturally processed viral peptides as recognized by cytotoxic T cells. *Nature (Lond.)* 348:252.

36. Bennink, J.R., and J.W. Yewdell. 1988. Murine cytotoxic T lymphocyte recognition of individual influenza virus proteins. High frequency of non-responder MHC class I alleles. *J. Exp. Med.* 168:1935.

37. Alexander, J., J.A. Payne, R. Murray, J.A. Frelinger, and P. Cresswell. 1989. Differential transport requirements of HLA and H-2 class I glycoproteins. *Immunogenetics.* 29:380.

38. Hosken, N.A., and M.J. Bevan. 1990. Defective presentation of endogenous antigen by a cell line expressing class I molecules. *Science (Wash. DC).* 248:367.

39. Eisenlohr, L.C., I. Bacik, J.R. Bennink, K. Bernstein, and J.W. Yewdell. 1992. Expression of a membrane protease enhances presentation of endogenous antigens to MHC class I-restricted T lymphocytes. *Cell.* 71:963.

40. Lehigs, M., and A. Müllbacher. 1993. Recognition of vaccinia virus-encoded major histocompatibility complex class I antigens by virus-immune cytotoxic T cells is independent of the polymorphism of the peptide transporters. *Proc. Natl. Acad. Sci. USA.* 90:2676.