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Immunology

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

(antigen presentation/recombinant vaccinia virus)

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ABSTRACT **In the cytotoxic T-cell response to viruses, peptide antigens of cytoplasmic origin are presented at the cell surface by the highly polymorphic major histocompatibility complex (MHC) class I molecules to CD8$^+$ T-lymphocyte receptors. Peptide transporter molecules and other MHC-linked gene products have been implicated in the generation and import of antigenic peptides into the lumen of the endoplasmic reticulum for assembly with MHC class I glycoproteins. These accessory molecules in the antigen-presentation pathway map to a polymorphic region in the class II MHC, and the possibility of their allele-specific selectivity in antigen presentation has been raised. Here we show that additional, functionally polymorphic components are not apparent in an *in vitro* mouse MHC class I-restricted cytotoxic T-cell response to vaccinia and influenza viruses. When the mouse H-2K^d molecule was expressed via a recombinant vaccinia virus in target cells of different mouse MHC haplotypes or cells of rat, Syrian hamster, monkey, and human origin, efficient K^d-restricted and vaccinia virus-specific lysis was observed as measured with bulk effectors and at the clonal level. In addition, human transporters efficiently processed peptides originating from influenza virus nucleoprotein and hemagglutinin antigens as recognized by mouse influenza immune cytotoxic T cells.**

The cytotoxic T (Tc) cell immune response to viruses is directed against virus-derived peptide antigens presented by major histocompatibility complex (MHC)-encoded glycoproteins on the cell surface (1, 2). MHC class I molecules are specialized in the presentation of peptide fragments from endogenous synthesized proteins of intracellular parasites to CD8$^+$ Tc cells and subsequent lysis of infected cells. There is strong evidence that the assembly of MHC class I heavy chain with the β_2-microglobulin light chain and peptides 8–10 amino acids long (3) occurs in the lumen of the endoplasmic reticulum (4–6). Peptide and β_2-microglobulin are essential in stabilizing MHC class I glycoproteins, which confers transport competence of the trimolecular complex to the cell surface (7).

Antigenic peptide determinants originate from proteolytic degradation of polypeptides in the cytoplasmic compartment. For interaction with newly synthesized MHC class I antigens, peptides must traverse the lipid bilayer of the endoplasmic reticulum. This could be mediated by a transporter protein as originally proposed by Townsend and coworkers (6, 8). Two genes mapping to the MHC class II complex were recently identified that have homology with a superfamily of ATP-dependent transporter proteins (9–12). Complementation of a MHC class I expression defect in mutant cell lines following transfection with these MHC class II-linked genes suggests their involvement in antigen processing and presentation (13–17). The protein products of the two genes assemble to form a heterooligomer (13, 17, 18). However, their role as peptide transporters rests on circumstantial evidence, and *in vitro* translation studies indicate that the putative transporter proteins are not involved in shuttling peptides across microsomal membranes but provide a function critical for the assembly of peptides with MHC class I glycoproteins in the lumen of the endoplasmic reticulum (19–22).

Proteolytic processing of cytoplasmic antigens for presentation via MHC class I glycoproteins is apparently also under control of the MHC. An enzyme complex consisting of two subunits of a large cytoplasmic complex of 16 polypeptides, called proteasomes, is encoded by genes in the MHC class II region, in the vicinity of the putative peptide transporter protein genes (23–25). Thus, the MHC of all species examined encodes, apart from the highly polymorphic antigen-presenting molecules, accessory gene products that function in the antigen-processing and -presentation pathway. These MHC class II-linked genes are also polymorphic, which raises the possibility of their allele-specific selective function (9, 26–28). The rat MHC class II-linked gene *cim* may encode such a trans-acting haplotype-specific gene product (15, 29–31). Here we show that a mouse MHC class I antigen can function as a vaccinia virus (VV)-specific restriction element for VV-immune Tc cells in allogeneic and xenogeneic target cells without detectable allele specificity exerted by molecules other than the MHC class I antigens, and we conclude that accessory molecules in the class I pathway of humans and mice have little or no functional polymorphism in antigen presentation.

MATERIALS AND METHODS

Animals. Mouse strains CBA/H (K^{k}D^k) (CBA), C57BL/6 (K^{b}D^b) (B6), B10.A (K^{k}D^d) (5R), C3H.H-2^o(K^{d}D^k) (OH), and BALB/c (K^{d}D^d) (B/c) were obtained from the breeding establishment of the John Curtin School. Only females older than 6 wk were used.

Viruses. The recombinant VV (VV-K^d) coding for the MHC class I K^d molecule, the double recombinants coding for the K^d molecule plus influenza virus nucleoprotein (VV-K^d-NP) or hemagglutinin (VV-K^d-HA), the thymidine kinase-negative (TK$^-$) control VV, and wild-type VV-WR were grown and titrated as described (32, 33). The influenza virus A/WSN (H1N1) was grown and titrated as described (33).

Immunization. Animals were immunized with 10^7 plaque-forming units of VV-WR or 10^4 hemagglutinating units of A/WSN intraperitoneally.

Generation of Effector Cells. Spleen cells from mice immunized 5 days previously were used for primary VV-immune

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Abbreviations: MHC, major histocompatibility complex; Tc cell, cytotoxic T cell; VV, vaccinia virus; BFA, brefeldin A; TK, thymidine kinase; FACS, fluorescence-activated cell sorter.

Tc cells. For generation of alloreactive Tc cells, 8×10^7 5R responder splenocytes were cocultured with 4×10^7 irradiated (2000 rad; 1 rad = 0.01 Gy) B6 allogeneic stimulator cells for 5 days. The generation of secondary influenza immune Tc cells has been described (33).

Target Cells. Thioglycolate-induced peritoneal macrophages (TGM); P815 cells (H-2^d); L929 cells (H-2^k); methylcholanthrene-induced fibrosarcoma cell lines MC57 (H-2^b) and HTG (H-$2K^dD^b$); rat tumor cell line MAT; Syrian hamster melanoma cell line FF (ATCC CRL1479); monkey kidney cell lines CV-1 and Vero; and human cell lines HeLa, 143-B, and 293 were infected with VV and labeled with ^{51}Cr as described in detail elsewhere (33).

^{51}Cr Release Cytotoxicity Assay. The methods used for cell lines and macrophage targets have been described in detail elsewhere (33). The duration of the assays was 6 h. Percentage specific lysis was calculated by the formula % specific lysis = [(experimental release − medium release)/(maximum release − medium release)] × 100. Data given are the means of triplicate determinations. SEM values were always <5%.

Split-Clone Limiting Dilution Analysis. VV-immune Tc cells were generated in cocultures of 5×10^3 or 1×10^4 VV-primed splenocytes with 2×10^5 irradiated (2000 rad) VV-infected (10 plaque-forming units per cell) syngeneic stimulator splenocytes in culture conditions as described (33).

Brefeldin A (BFA) Treatment of Target Cells. The method was in essence that of Yewdell and Bennink (34). In short, 4 $\times 10^6$ target cells were incubated with 5 μg of BFA per ml (Boehringer Mannheim) during ^{51}Cr labeling and VV infection. After 1 h of incubation, 5 ml of medium containing 5 μg of BFA per ml was added and cells were left for a further 2 h. Targets were washed twice and resuspended in medium containing 1 μg of BFA per ml. The final concentration of BFA throughout the 6-h assay period was 0.5 μg/ml.

Fluorescence-Activated Cell Sorter (FACS) Analysis. Human cell lines 143-B and 293 were infected with VV-K^d or VV-TK^- as described for target cells or were left uninfected (HTG). At 4 h after infection the cells were washed, resuspended at 10^7 cells per ml, and labeled at 4°C for 45 min with monoclonal antibody HB-159 (American Type Culture Collection) specific for K^d, followed by fluorescein isothiocyanate-conjugated sheep anti-mouse immunoglobulin (Silenus, Hawthorn, Australia) staining. Cells were examined with a FACScan flow cytometer (Becton Dickinson).

RESULTS

Allogeneic Target Cells Infected with VV Encoding the MHC Class I K^d Molecule Are Lysed by K^d-Restricted VV-Immune Tc Cells. To test the putative functional polymorphism of the accessory gene products in MHC class I antigen processing and presentation, we made use of a VV-K^d recombinant encoding the mouse MHC class I K^d molecule (32, 33, 35). Using two cell lines—L929 (H-2^k) and MC57 (H-2^b)—we tested the ability of K^d-restricted VV-immune effector cells [BALB/c (H-2^d) and C3H.H-2° (K^dD^k)] to lyse specifically VV-K^d-infected cells (Fig. 1*A*). Both effector populations lysed H-2^b and H-2^k cells expressing K^d plus VV-derived peptides.

The situation was similar when macrophages of three independent haplotypes—C57BL/6 (H-2^b), CBA (H-2^k), and DBA-1 (H-2^q)—were used as targets for C3H.H-2° (K^dD^k) VV-immune effector Tc cells. K^d-restricted killing of the macrophages was similar and displayed no haplotype preference (Fig. 1*B*). Thus, within different mouse MHC haplotypes, we could not observe an allele-specific effect of the host H-2 type on H-$2K^d$-dependent killing of target cells transiently expressing the recombinant MHC class I gene.

Mouse MHC Class I Antigens Can Be Expressed in Evolutionarily Distant Target Cells and Can Be Recognized by Mouse VV-Immune Tc Cells. To examine whether trans-acting genes can affect H-$2K^d$-restricted antigen presentation in evolutionarily more distantly related host cells, we used target cells of rat (MAT), Syrian hamster (FF), monkey (Vero, CV-1), and human (HeLa, 143-B, 293) origin and tested them for lysis by mouse VV-immune K^d-restricted Tc cells after infection with the VV-K^d virus. All target cells, irrespective of origin, were efficiently lysed by mouse effectors (Table 1), with the human cell line 293 being the most sensitive. Thus, the class I antigen-processing and -presentation pathway in cells of rat, monkey, and human origin is efficient in the presentation of VV-derived peptides by transiently expressed mouse MHC class I antigen. The Syrian hamster cell line was also sensitive to lysis by mouse effector cells, corroborating the fact that the species, despite expressing essentially monomorphic MHC class I glycoproteins, can mount a class I cytotoxic T-lymphocyte response (36).

Limiting Dilution Split-Clone Analyses on Human VV-K^d-Infected Target Cells. To investigate the lack of transporter polymorphism in cells of human and mouse origin at a clonal level, we undertook split-clone limiting dilution analyses. Fig. 2*A* shows an experiment with 5×10^3 responder cells per well and Fig. 2*B* shows an experiment with 10^4 responders per well. In no case did we find clones that lysed exclusively mouse but not human VV-K^d-infected targets. The overall lower lysis of human targets compared to mouse targets reflects the presence of heterologous adhesion molecules in the mouse effector/human target situation as discussed (33).

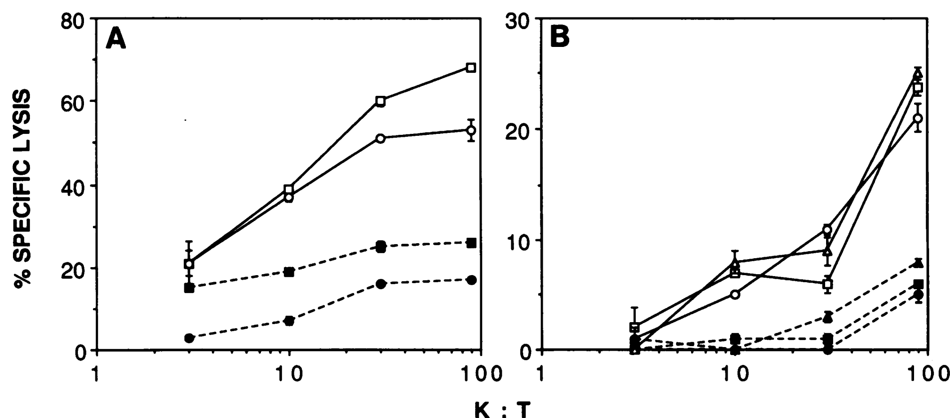

FIG. 1. (*A*) Lysis of MC57 (H-2^b) (●) and L929 (H-2^k) (■) target cells infected with VV-K^d (open symbols) or control VV-TK^- (solid symbols). (*B*) Lysis of thioglycolate-induced peritoneal macrophages from C57BL/6 (H-2^b) (■), CBA (H-2^k) (▲), or DBA/1 (H-2^q) (●) mice infected with VV-K^d (open symbols) or control VV-TK^- (solid symbols) by C3H.H-2° primary *in vivo* VV-immune effector splenocytes. K:T, killer/target cell ratio.

Table 1. Lysis of human, monkey, Syrian hamster, rat, and mouse target cells infected with the K^d recombinant VV by mouse K^d-restricted VV-immune Tc cells

Effector/ target cell	% specific lysis of target cells*																	
	P815		MC57		MAT		FF		Vero		CV-1		HeLa		143-B		293	
	TK^-	K^d	TK^-	K^d	TK^-	K^d	TK^-	K^d	TK^-	K^d	TK^-	K^d	TK^-	K^d	TK^-	K^d	TK^-	K^d
Exp. 1																		
90:1	66	55	NT	NT	7	50	NT	NT	24	56	9	68	5	64	8	66	28	79
30:1	60	48	NT	NT	7	37	NT	NT	21	47	8	45	5	39	6	47	19	72
10:1	50	35	NT	NT	4	19	NT	NT	9	32	2	22	1	19	3	21	8	55
3:1	32	22	NT	NT	2	11	NT	NT	3	16	1	9	0	7	1	9	3	29
Exp. 2																		
15:1			18	66			1	48							1	53		
5:1			7	50			0	21							0	50		
1.7:1			2	28			0	7							0	28		

Effectors were C3H.H-2° splenocytes from animals immunized with WR vaccinia 6 days previously. Target cells were infected with VV-K^d or VV-TK^- for 1 h at a multiplicity of infection of 40 plaque-forming units per cell. NT, not tested.

*Percentage ^{51}Cr release from target cells over a 6-h period with spontaneous release subtracted. Means of triplicate determinations are given with SEM never >4.8%. Cytotoxicity has been described in full elsewhere (33).

BFA Inhibits VV-Encoded MHC Class I Cell-Surface Antigen Expression. To investigate the possibility that VV-encoded MHC class I antigens reach the cell surface via a pathway different from that of endogenously synthesized class I antigens (37), we tested the susceptibility of VV-encoded MHC class I antigens to BFA-mediated inhibition of cell-surface expression (34). MC57 (H-2^b) cells were treated with BFA or were left untreated and infected with VV-K^d or control VV-TK^-. Lysis of VV-infected target cells by H-2^b-restricted VV-immune effector cells was completely inhibited by treatment with BFA (Fig. 3*A*). Using K^d-restricted VV-immune Tc cells, lysis of target cells infected with VV-K^d was inhibited by BFA as was found for H-2^b-restricted killer cells on syngeneic target cells (Fig. 3*B*). Fig. 3*C* demonstrates that under the experimental conditions used Tc cell killing still takes place, as D^b-specific alloreactive Tc cells lysed BFA-treated targets efficiently.

Cell-Surface Expression of VV-Encoded Mouse MHC Class I Antigens on Human Cells. Since target cell recognition by Tc cells requires only low levels of T-cell epitope expression (a MHC class I antigen with the appropriate peptide), we quantitated the efficiency of cell-surface expression of the mouse K^d antigen in two human cell lines (143-B and 293; Fig. 4). Both cell lines expressed high levels of K^d antigen after 4 h of infection with VV-K^d, as shown by surface staining with the K^d-specific monoclonal antibody HB-159 and FACS analysis. The level of surface staining in both human cell lines was similar to that of the endogenous K^d in HTG cells (data not shown). This demonstrates yet again that at least a large proportion of the VV-encoded K^d molecule is assembled and transported to the plasma membrane in a configuration recognizable by an allele-specific monoclonal antibody.

Influenza Virus-Specific Recognition of Human Target Cells by Mouse K^d-Restricted Tc Cells. To test the generality of this peptide transporter nonspecificity, we made use of VV-K^d double recombinants encoding the influenza virus nucleoprotein or hemagglutinin genes. Table 2 shows the result of one such experiment. When expressed in human cells, the mouse K^d MHC class I antigen can present endogenously derived peptides from two other viral antigens distinct from VV-derived peptides, as recognized by mouse influenza-immune K^d-restricted Tc cells.

DISCUSSION

The data presented here clearly show that a recombinant VV-encoded H-$2K^d$ MHC class I molecule functions efficiently in K^d-restricted target cell lysis independent of the haplotype of the host cell. Target cells of human, monkey, Syrian hamster, and rat origin were also subject to VV-

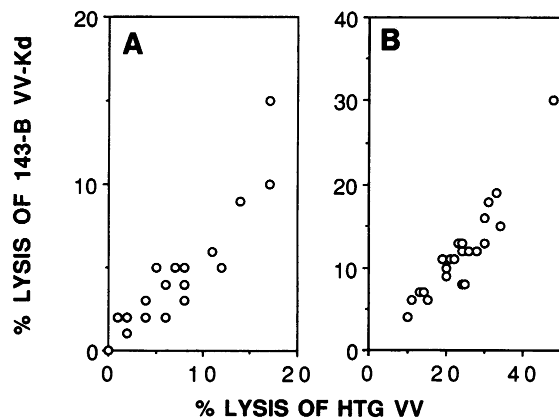

FIG. 2. Limiting dilution split-clone analysis. K^d-specific VV-immune effector cells were generated in microculture wells (24 wells per panel) by coculture of 5×10^3 (*A*) or 1×10^4 (*B*) VV-primed C3H.H-2° responder splenocytes with 2×10^5 irradiated VV-infected syngeneic stimulator splenocytes. Contents of wells were split and tested for lysis on 2×10^4 VV-infected HTG and 2×10^4 VV-K^d-infected human 143-B target cells.

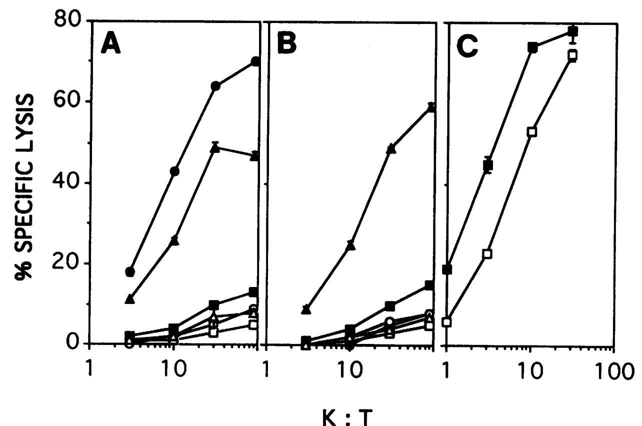

FIG. 3. Effect of BFA on lysis of MC57 (H-2^b) target cells infected with either VV-K^d (▲) or VV-TK^- (●) or left uninfected (■) by B6 (H-2^b restricted) (*A*) or OH (K^d restricted) (*B*) VV-immune Tc cells or 5R anti-B6 (D^b specific) alloreactive Tc cells (*C*). Target cells were either treated with BFA (open symbols) or left untreated (solid symbols). K:T, killer/target cell ratio.

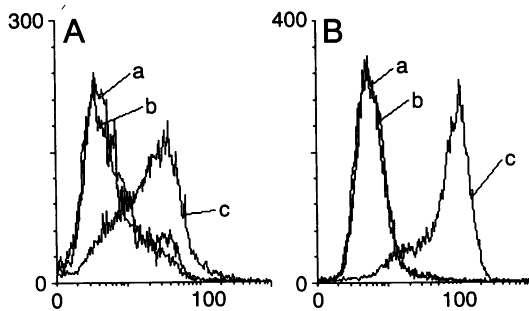

FIG. 4. Cell-surface expression of mouse K^d MHC class I antigen on human 293 (*A*) and 143-B (*B*) cells. Cells were labeled with monoclonal antibody HB-159 specific for K^d (traces a and c), followed by staining with fluorescein isothiocyanate sheep anti-mouse immunoglobulin (traces a, b, and c) after a 4-h infection with either VV-K^d (traces b and c) or VV-TK^- (trace a). Cells were examined with a FACScan flow cytometer. Abscissa, fluorescence; ordinate, cell count.

specific and K^d restriction element-dependent killing when infected with the K^d recombinant virus.

We conclude that the recently identified MHC class II-linked genes encoding putative peptide transporters and subunits of a cytoplasmic protease complex, despite being polymorphic, are not allele specific in antigen presentation via the recombinant K^d MHC molecule. Thus, apparently VV peptides with binding motifs for the recombinant H-2K^d are generated, transported into the lumen of the endoplasmic reticulum, assembled with MHC class I molecules, and presented on the plasma membrane in a process that is not affected by the polymorphism of the accessory molecules involved in this pathway. The same conclusion can be drawn for other viral peptides derived from the nucleoprotein or hemagglutinin molecule of influenza virus and presented via VV-expressed H-2K^d in allogeneic target cells (32). Similarly, we have been able to specifically lyse the human target cell lines 143-B and 293 with K^d-restricted influenza-immune Tc cells after infection with the double VV recombinant constructs encoding either K^d plus the nucleoprotein gene or K^d plus the hemagglutinin gene of influenza virus. Thus, the human peptide transporters are not functionally polymorphic across a species barrier for a variety of mouse K^d-specific peptides. An identical conclusion was drawn by Falk *et al.* (38), who demonstrated that cells of human and mouse origin produced and presented the same peptides via endogenous or transfected H-2K^b. Our results are consistent with the findings that the class I antigen-presentation defect of the mouse mutant cell line RMA-S can be rectified by transfection with the rat peptide transporter gene (*mtp2*) (14) or fusion with a fibroblast cell line of a different haplotype (39). The interpretation that one of the peptide transporters in the mutant line was functional and provided allele specificity after heterooligomerization with the transfected gene product could not be excluded but is unlikely in view of our data.

Table 2. Lysis of human target cells infected with double recombinant VV encoding mouse K^d and influenza virus nucleoprotein (NP) or hemagglutinin (HA) genes by mouse K^d-restricted VV- and influenza virus-immune Tc cells

Effector	Effector/ target cell	% specific lysis of 143-B target cells			
		TK^-	K^d	K^d NP	K^d HA
C3H.H-2°	90:1	21	63	44	47
anti-VV	30:1	17	56	41	38
	10:1	7	34	23	21
	3:1	5	15	9	10
BALB/c	30:1	7	11	38	69
anti-A/WSW	10:1	3	5	18	38
	3:1	1	1	7	17
	1:1	0	0	2	6

Effectors were C3H.H.2° splenocytes from animals immunized with WR vaccines 6 days previously or BALC/c influenza-immune effector cells generated *in vitro* as described in full elsewhere. Percentage specific lysis and target cells are as described in Table 1.

Our results do not agree with the interpretation that attributes changes in MHC class I antigenicity to polymorphic peptide transporter genes. Adaptive polymorphism and coselection of MHC antigens and MHC-linked trans-acting gene products has been proposed as an evolutionary strategy (15, 29–31). It appears to us that the extreme polymorphism of the MHC antigen-presenting molecules (40) is sufficient in antigen processing and presentation without introducing an additional component of functional polymorphism. One consequence of allele-specific peptide transporters and proteases in a highly polymorphic MHC class I environment would be generation of antigen-presentation repertoire defects. A selective effect of two allelic forms of the peptide transporter (mtp2) on the peptide repertoire presented by a rat class I molecule was described recently (9, 15, 26–28) but appears to be an exception related to the rat, which has limited class I polymorphism. This situation is clearly distinct from that in humans and mice. Finally, the putative peptide transporter may not be directly involved in import of peptides into the endoplasmic reticulum (20–22), but it provide cofactors needed for assembly of MHC antigen and peptide in the endoplasmic reticulum, in which case functional polymorphism of the transporter would be an unlikely requirement.

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1. Bjorkman, P. J., Saper, M. A., Samraoui, B., Bennett, W. S., Strominger, J. L. & Wiley, D. C. (1987) *Nature (London)* **329**, 506–511.
2. Townsend, A. R. M., Rothbard, J., Gotch, F. M., Bahadur, G., Wraith, D. & McMichael, A. J. (1986) *Cell* **44**, 959–968.
3. Falk, K., Rötzschke, O., Stevanovic, S., Jung, G. & Rammensee, H.-G. (1991) *Nature (London)* **351**, 290–351.
4. Cox, J. H., Yewdell, J. W., Eisenlohr, L. C., Johnson, P. R. & Bennink, J. R. (1990) *Science* **247**, 715–718.
5. Hosken, N. A. & Bevan, M. J. (1990) *Science* **248**, 367–370.
6. Townsend, A., Öhlén, C., Bastin, J., Ljunggren, H.-G., Foster, L. & Kärre, K. (1989) *Nature (London)* **340**, 443–448.
7. Ljunggren, H.-G., Stam, N. J., Öhlén, C., Neefjes, J. J., Höglund, P., Heemels, M.-T., Bastin, J., Schumacher, T. N. M., Townsend, A., Kärre, K. & Ploegh, H. L. (1990) *Nature (London)* **346**, 476–480.
8. Cerundolo, V., Alexander, J., Anderson, K., Lamb, C., Cresswell, P., McMichael, A., Gotch, F. & Townsend, A. (1990) *Nature (London)* **345**, 449–452.
9. Deverson, E. V., Gow, I. R., Coadwell, W. J., Monaco, J. J., Butcher, G. W. & Howard, J. C. (1990) *Nature (London)* **348**, 738–741.
10. Monaco, J. J., Cho, S. & Attaya, M. (1990) *Science* **250**, 1723–1725.
11. Spies, T., Bresnahan, M., Bahram, S., Arnold, D., Blanck, G., Mellins, E., Pious, D. & DeMars, R. (1990) *Nature (London)* **348**, 744–747.
12. Trowsdale, J., Hanson, I., Mockridge, I., Beck, S., Townsend, A. & Kelly, A. (1990) *Nature (London)* **348**, 742–743.
13. Attaya, M., Jameson, S., Martinez, C. K., Hermel, E., Aldrich, C., Forman, J., Fischer, K. L., Bevan, M. J. & Monaco, J. J. (1992) *Nature (London)* **355**, 647–649.
14. Powis, S., Townsend, A., Deverson, E., Bastin, J., Butcher, G. & Howard, J. (1991) *Nature (London)* **354**, 528–531.
15. Powis, S. J., Deverson, E. V., Coadwell, W. J., Ciruela, A., Huskisson, N. S., Smith, H., Butcher, G. W. & Howard, J. C. (1992) *Nature (London)* **357**, 211–215.
16. Spies, T. & DeMars, R. (1991) *Nature (London)* **351**, 323–324.

17. Spies, T., Cerundolo, V., Colonna, M., Cresswell, P., Townsend, A. & DeMars, R. (1992) *Nature (London)* **355**, 644–646.
18. Kelly, A., Powis, S., Kerr, L.-A., Mockridge, I., Elliott, T., Bastin, J., Uchanska-Ziegler, B., Ziegler, A., Trowsdale, J. & Townsend, A. (1992) *Nature (London)* **355**, 641–644.
19. Lévy, F., Larsson, R. & Kvist, S. (1991) *J. Cell Biol.* **115**, 959–970.
20. Lévy, F., Gabathuler, R., Larsson, R. & Kvist, S. (1991) *Cell* **67**, 265–274.
21. Dobberstein, B. (1992) *Nature (London)* **355**, 109–110.
22. Koppelman, B., Zimmerman, D. L., Walter, P. & Brodsky, F. M. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 3908–3912.
23. Brown, M., Driscoll, J. & Monaco, J. (1991) *Nature (London)* **353**, 355–357.
24. Glynne, R., Powis, S., Beck, S., Kelly, A., Kerr, L.-A. & Trowsdale, J. (1991) *Nature (London)* **353**, 357–360.
25. Robertson, M. (1991) *Nature (London)* **353**, 300–301.
26. Powis, S. H., Mockridge, I., Kelly, A., Kerr, L.-A., Glynne, R., Gileadi, U., Beck, S. & Trowsdale, J. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 1463–1467.
27. Parham, P. (1990) *Nature (London)* **348**, 674–675.
28. Colonna, M., Bresnahan, M., Bahram, S., Strominger, J. L. & Spies, T. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 3932–3936.
29. Livingstone, A., Powis, S., Diamond, A., Butcher, G. & Howard, J. (1989) *J. Exp. Med.* **170**, 777–795.
30. Livingstone, A., Powis, S., Günther, E., Cramer, D., Howard, J. & Butcher, G. (1991) *Immunogenetics* **34**, 157–163.
31. Powis, S., Howard, J. & Butcher, G. (1991) *J. Exp. Med.* **173**, 913–921.
32. Andrew, M. E., Coupar, B. E. H., Boyle, D. B. & Blanden, R. V. (1987) *Eur. J. Immunol.* **17**, 1515–1518.
33. Müllbacher, A., Hill, A., Blanden, R., Cowden, W., King, N. & Tha Hla, R. (1991) *J. Immunol.* **147**, 1765–1772.
34. Yewdell, J. W. & Bennink, J. R. (1989) *Science* **244**, 1072–1075.
35. Coupar, B., Andrew, M., Boyle, D. & Blanden, R. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 7879–7882.
36. Bogaerde, J. v. d. & Howard, J. C. (1991) *Int. Immunol.* **3**, 49–56.
37. Cairns, J. (1960) *Virology* **11**, 603–623.
38. Falk, K., Rotzschke, O. & Rammensee, H.-G. (1992) *Eur. J. Immunol.* **22**, 1323–1326.
39. Öhlén, C., Bastin, J., Ljunggren, H.-G., Imreh, S., Klein, G., Townsend, A. & Kärre, K. (1990) *Eur. J. Immunol.* **20**, 1873–1876.
40. Klein, J. & Figueroa, F. (1981) *Immunol. Rev.* **60**, 22–57.