

Cytomegalovirus Prevents Antigen Presentation by Blocking the Transport of Peptide-loaded Major Histocompatibility Complex Class I Molecules into the Medial-Golgi Compartment

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Summary

Selective expression of murine cytomegalovirus (MCMV) immediate-early (IE) genes leads to the presentation by the major histocompatibility complex (MHC) class I molecule L^d of a peptide derived from MCMV IE protein pp89 (Reddehase, M. J., J. B. Rothbard, and U. H. Koszinowski. 1989. *Nature (Lond.)* 337:651). Characterization of endogenous antigenic peptides identified the pp89 peptide as the nonapeptide ¹⁶⁸YPHF¹⁷⁶MPTNL (del Val, M., H.-J. Schlicht, T. Ruppert, M. J. Reddehase, and U. H. Koszinowski. 1991. *Cell* 66:1145). Subsequent expression of MCMV early genes prevents presentation of pp89 (del Val, M., K. Münch, M. J. Reddehase, and U. H. Koszinowski. 1989. *Cell* 58:305). We report on the mechanism by which MCMV early genes interfere with antigen presentation. Expression of the IE promoter-driven bacterial gene *lacZ* by recombinant MCMV subjected antigen presentation of β -galactosidase to the same control and excluded antigen specificity. The L^d-dependent presence of naturally processed antigenic peptides also in nonpresenting cells located the inhibitory function subsequent to the step of antigen processing. The finding that during the E phase of MCMV gene expression the MHC class I heavy chain glycosylation remained in an Endo H-sensitive form suggested a block within the endoplasmic reticulum/*cis*-Golgi compartment. The failure to present antigenic peptides was explained by a general retention of nascent assembled trimolecular MHC class I complexes. Accordingly, at later stages of infection a significant decrease of surface MHC class I expression was seen, whereas other membrane glycoproteins remained unaffected. Thus, MCMV E genes endow this virus with an effective immune evasion potential. These results also indicate that the formation of the trimolecular complex of MHC class I heavy chain, β_2 -microglobulin, and the finally trimmed peptide is completed before entering the medial-Golgi compartment.

T lymphocytes recognize short peptides derived from antigenic proteins. These peptides are presented at the cell surface by specialized molecules encoded in the MHC (reviewed in reference 1). There are two classes of MHC molecules that present peptides at the cell surface. MHC class II molecules present peptides that are derived from proteins degraded in endosomal vesicles to CD4⁺ T lymphocytes, whereas MHC class I molecules present peptides from proteins degraded in the cytosol to CD8⁺ T lymphocytes. For cytosolic antigen degradation a nonlysosomal proteinase complex encoded in the MHC represents a candidate (2-4). Although translocation of peptides into the rough endoplasmic reticulum (ER)¹ is

believed to require ATP-dependent peptide transporters (5-7), recent evidence indicates that peptide translocation can occur independently of ATP (8). x-ray crystallographic analysis of an MHC class I protein has revealed a cleft where peptides are bound (9). A block in the secretory pathway prevents antigen presentation (10, 11). Indirect evidence points to the ER or *cis*-Golgi reticulum/salvage compartment as the place of peptide binding (12-14), but subsequent compartments cannot be excluded. Studies on cell lines defective in antigen presentation suggest that only after assembly of the MHC class I heavy chain with the peptide and β_2 -microglobulin (β_2m) is a stable trimolecular complex exported to the plasma membrane (15, 16). MHC class I molecules without peptide have a different conformation (14, 17, 18) and are deficient with respect to surface transport and stability (15, 19).

CD8⁺ T lymphocytes that recognize peptides derived from viral proteins synthesized in infected cells play a deci-

¹ Abbreviations used in this paper: Act D, actinomycin D; β -gal, β -galactosidase; β_2m , β_2 -microglobulin; CH, cycloheximide; ER, endoplasmic reticulum; HCMV, human cytomegalovirus; IE, immediate-early; MCMV, murine cytomegalovirus; MEF, mouse embryo fibroblasts; p.i., postinfection.

sive role in the antiviral defence. Yet, some viruses have found the means to interfere with immune recognition (listed in reference 20). Extensively studied is the interference strategy of adenoviruses. In the case of the tumorigenic adenovirus type 12, the viral protein E1A blocks MHC class I expression already at the level of transcription (21, 22). In contrast, the E3/19K protein of adenovirus type 2 binds to the MHC class I molecule and, by retaining it in the ER/*cis*-Golgi compartment, blocks cell surface expression and antigen presentation (23–26).

Cytomegaloviruses (CMV) are species-specific herpes viruses, which persist in the host for life after infection. Viral persistency can take the form of chronic disease, asymptomatic productive infection, or even viral latency. MHC class I-restricted T lymphocytes play a prominent protective role (reviewed in reference 27). Accordingly, infection of the immature or immunocompromised host can result in severe CMV disease and death. Thus, CMV often causes disease after bone marrow transplantation and represents the major viral problem during the final stages of AIDS. In permissively infected cells, CMV gene expression is regulated in a cascade fashion characteristic for herpes viruses. Viral proteins encoded by genes expressed in the immediate-early (IE) phase of infection control the activation of early (E) phase genes. E proteins are required for viral DNA synthesis, which is followed by the synthesis of structural proteins during the late phase of infection. Studies on murine CMV (MCMV) have revealed that CMV also has the capacity to interfere with antigen presentation (28). Recognition by CTL of the IE protein pp89, a nonapeptide of which is presented by the MHC class I molecule L^d in the BALB/c strain of mice (29–31), is abolished after expression of E genes (28). The failure of pp89 presentation appeared to be selective in that MCMV E antigens could be presented. Since pp89 synthesis, stability, and nuclear transport remained unchanged in the E phase, we hypothesized that characteristics of the protein or its regulatory function affected pp89 processing during the E phase.

Here we define the step at which MCMV E gene products interfere with the natural pathway of pp89 processing and peptide presentation. First, the inhibition of further glycosylation indicates that the transport of MHC class I molecules through the Golgi compartment is generally inhibited by MCMV E gene functions. Second, because cells with arrested transport of MHC class I molecules contain already the correctly processed nonapeptide of pp89, these results suggest the ER/*cis*-Golgi compartment represents the site of antigenic peptide binding to MHC class I molecules.

Materials and Methods

Mice. BALB/cJ (H-2^d) and BALB/cJ-H-2^{dm2} (H-2^{dm2}) mice were bred in our own colony under specific pathogen-free conditions.

Cells. Mouse embryo fibroblasts (MEF) prepared from either strain of mice, were used after three *in vitro* passages for virus infection and extraction of naturally processed peptides. In some experiments the SV40-transformed MEF cell line, BALB.SV, was used (31). The cell line P13.1 originates from the mastocytoma P815 cells that were transfected with the *lacZ* gene encoding β -gal (32).

Viruses. MCMV of the strain Smith (VR-194; American Type Culture Collection [ATCC], Rockville, MD) was employed as tissue culture-grown virus. The MCMV recombinant MCMV lac1 was constructed essentially as described for RM408 (33), but using DNA of the wild-type strain Smith. The recombinant vaccinia viruses MCMV-iel-VAC, expressing pp89, and vSC8, expressing β -gal, have been described before (34, 35).

Antibodies. The following mAbs were used: 28-14-8s (anti-L^d α 3, reactive with all L^d molecules) (18); 64-3-7 (anti-L^d α 2, specific for unassembled L^d) (18); 34-5-8s (anti-D^d) (HB 102; ATCC); R17 217.1.3 (anti-murine transferrin receptor) (TIB 219; ATCC); IM7-8.1E4 (anti-pgp1) (36).

Infection Conditions. Selective expression of MCMV IE gene products was achieved by infection of cells with MCMV in the presence of cycloheximide (CH), which was replaced 3 h later by actinomycin D (Act D). Controlled transition to the E phase was achieved by incubating the cells in the absence of drugs for varied periods of time after removal of CH; IE proteins translated in this period activate the transcription of E genes, until Act D blocks further transcription (see Fig. 2 A). Infection was performed by centrifugation at 800 g for 30 min, to enhance the efficiency by a factor of 10–40.

Isolation of Endogenously Processed Peptides. Processed peptides were extracted with TFA from whole cells ($\sim 10^8$ cells per extraction) and tested in a CTL assay as described (31). The protocol for peptide extraction from infected cells mimicked the preparation of target cells. Cells were trypsinized at 7 h postinfection (p.i.), incubated in suspension for 90 min at 37°C, and then subjected to acid extraction.

Cytolytic Assays. For the production of target cells, cells were labeled with ⁵¹Cr for 90 min. Incubation of labeled P815 cells for 1 h at 37°C with HPLC fractions to be tested for the presence of biologically active naturally processed peptides preceded the addition of polyclonal pp89-specific CTL (31). L^d-restricted and β -gal-specific CTL were generated by *in vitro* restimulation of spleen cells from mice *in vivo* primed with vSC8 (10^7 cells/ml in culture medium) with 30 Gy-irradiated P13.1 cells (10^5 cells/ml in culture medium), CTL were propagated by weekly restimulations and addition of recombinant human IL-2 (100 U/ml). Graded numbers of effector cells in threefold dilution steps were used in a standard 3-h cytolytic assay. Data represent the mean percentage of specific lysis from three replicate cultures. The E/T ratio that gave plateau lysis with either CTL usually ranged from 50:1 to 5:1, and in some of the experiments only the values of plateau lysis are shown.

Metabolic Labeling and Immunoprecipitation. Cells were washed three times in methionine-free medium before incubation with [³⁵S]methionine (1,200 Ci/mmol; Amersham, Braunschweig, Germany) at a concentration of 500 μ Ci/ml at 37°C for the time indicated in the figure legends. In pulse-chase experiments, labeled cells were washed in prewarmed complete medium with 10 mM nonlabeled methionine and incubated further for the indicated time. After washing with PBS, cells (2×10^6) were lysed in 1 ml lysis buffer (1% NP-40, 5 mM MgCl₂, 140 mM NaCl, 20 mM Tris, pH 7.6, and 0.2 mM PMSF). Lysates were cleared by centrifugation at 13,000 g for 30 min and supernatants were stored at -20°C . After preclearing of lysates with protein A-Sepharose (75 μ l of a 1:1 buffer/Sepharose slurry), the addition of 2 μ l of ascitic fluid for 45 min, and the addition of 50 μ l of protein A-Sepharose for 30 min, resulted in quantitative precipitation of the respective antigen. To assure quantitative retrieval of immune complexes, the lysates were incubated two more times with protein A-Sepharose before adding the next mAb. The Sepharose beads were washed twice with the first washing buffer (0.2% NP-40, 10 mM Tris-HCl, pH

7.6, 140 mM NaCl, 2 mM EDTA) followed by two additional washing steps with the second (0.2% NP-40, 10 mM Tris-HCl, pH 7.6, 500 mM NaCl, 2 mM EDTA) and third washing buffer (10 mM Tris-HCl, pH 7.6). Immune complexes were eluted by incubation with sample buffer and analyzed by 10–15% polyacrylamide gradient gel electrophoresis.

Endoglycosidase Treatment. For Endo H (Boehringer, Mannheim, Germany) digestion, the immunoprecipitates bound to protein A-Sepharose beads were resuspended in 50 mM phosphate buffer, pH 5.5, with 0.02% SDS, 0.1% NP-40, and 0.2 mM PMSF, and heated to 95°C for 4 min. Thereafter, 100 mM 2-ME and 2 mU of Endo H were added, and digests were analyzed by SDS-PAGE after incubation for 18 h at 37°C.

Flow Cytometry. Trypsinized BALB.SV cells were preincubated in 5% goat serum and then stained with hybridoma supernatants. Bound antibodies were visualized by addition of fluoresceinated goat anti-mouse isotype-specific (Medac, Hamburg, Germany) or anti-rat IgG antibodies (Southern Biotechnology, Birmingham, AL). As negative controls, served cells incubated with the second antibody alone. 10⁴ cells were analyzed for each fluorescent profile on a FACScan IV® (Becton Dickinson & Co., San Jose, CA).

Results

Lack of Selectivity in the Inhibition of Antigen Presentation by MCMV E Gene Products. During the E phase of MCMV replication, the presentation of the IE1 antigen pp89 by the MHC class I molecule L^d is prevented. We reasoned that if pp89 was the only target of inhibition, then intrinsic properties of pp89 should account for the lack of presentation.

Other MCMV IE proteins besides pp89 encoded by gene *ie1* are IE2 and IE3, encoded by genes *ie2* and *ie3*, respectively (37, 38). The corresponding continuous open reading frames were cloned in recombinant vaccinia viruses (M. Meserle, unpublished data). No CTL could be induced by the established procedures (34), and it was concluded that IE2 and IE3 do not represent antigens for BALB/c CTL. An unrelated protein of proven antigenicity for the BALB/c strain is the bacterial protein β -galactosidase (β -gal), which is presented by the MHC class I molecule L^d (32). The *lacZ* gene has been successfully integrated into the MCMV genome (33). We constructed an equivalent MCMV-Smith strain recombinant. A short deletion encompassing the *ie2* promoter was created and the *lacZ* gene under control of the *ie1* promoter was inserted into this position, resulting in the expression of *lacZ* as an IE gene by recombinant MCMV lac1 (Fig. 1).

Presentation to CTL of pp89 and β -gal was tested in cells infected with MCMV lac1 under the conditions described before (28), and is represented schematically in Fig. 2 A. Infection of cells in the presence of the protein synthesis inhibitor CH results in enhanced transcription of IE genes, including gene *ie1* encoding pp89. After replacement of CH by the inhibitor of transcription Act D, IE mRNA is translated, leading to enhanced and selective IE protein synthesis and to enhanced recognition of IE antigens by CTL. Under conditions of selective IE expression, pp89 was presented to CTL in cells infected with either wild-type MCMV or MCMV lac1 (Fig. 2 B, time point 0, absence of E transcrip-

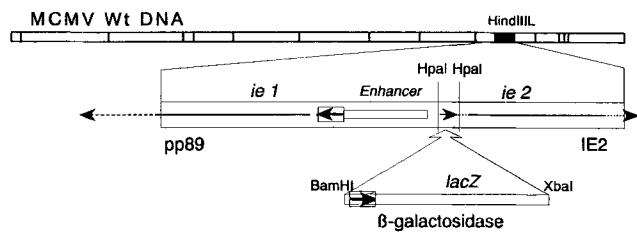


Figure 1. Construction of the MCMV recombinant MCMV lac1. The HindIII cleavage map of the 235-kb double-stranded DNA genome of MCMV is shown at the top and the position of the plasmid cloned 7.3-kb HindIII L fragment is indicated as a black box (39). The genes and regulatory elements contained in this region are shown below in a schematic fashion to indicate the position of the enhancer sequence (open box), the *ie1* gene promoter (boxed arrow), the transcription direction of genes *ie1* and *ie2* that start in this fragment, and to indicate their protein products pp89 (IE1) and IE2 (37, 38, 40). The two HpaI sites flanking the *ie2* promoter (arrow) were used to excise this regulatory element and to replace it by a 3.5-kb fragment consisting of the *ie1* promoter upstream of the bacterial *lacZ* gene open reading frame coding for β -gal. The resulting plasmid was then used to isolate the MCMV lac1 recombinant generated by homologous recombination after cotransfection of cells with linear DNA of the modified HindIII fragment and MCMV strain Smith DNA.

tion). The same conditions, as expected from the *ie1* promoter control of the *lacZ* gene in MCMV-lac1, resulted in β -gal recognition by β -gal-specific CTL (Fig. 2 B, right, time point 0).

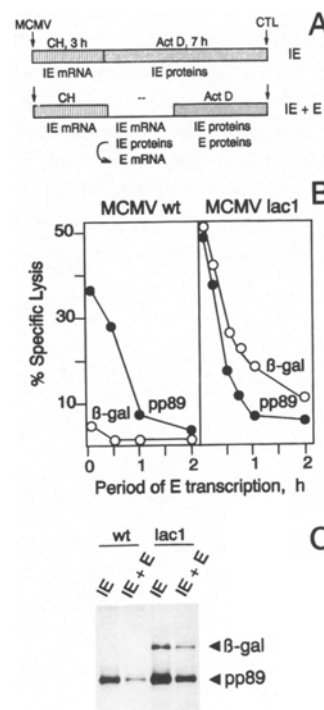


Figure 2. Presentation of both pp89 and β -gal is prevented in the E phase. (A) Experimental design. (Top) Infection of cells in the presence of the protein synthesis inhibitor CH results in enhanced transcription of IE genes. After replacement of CH by the inhibitor of transcription Act D, IE mRNA is translated, leading to selective IE protein synthesis and to presentation of antigen IE. (Bottom) After removal of CH, the synthesized IE proteins activate the transcription of E genes until Act D is added. Translation of E mRNA leads to the synthesis of E proteins. This protocol therefore defines the period of E gene transcription required for synthesis of proteins that inhibit antigen presentation (28). (B) Recognition by pp89-specific (filled circles) or β -gal-specific (open circles) CTL of MEF cells infected with wild-type MCMV (left) or with the recombinant MCMV lac1 coding β -gal (right) under the conditions schematically shown in A. Selective

IE expression (A, top) corresponds to the time point 0 h of E gene transcription. (C) Rate of pp89 and β -gal protein synthesis. MEF cells infected under the two experimental conditions depicted in A with wild-type MCMV or recombinant MCMV lac1, as marked, were labeled with [³⁵S]methionine for 40 min at 6 h p.i. Whole-cell lysates were analyzed by PAGE.

If Act D is not added immediately after removing CH but at later times (see Fig. 2 A, bottom), the synthesized IE proteins activate the transcription of E genes. The duration of E gene transcription is defined by the time at which Act D is added. Translation of E mRNA leads to the synthesis of E proteins. With this protocol, conditions of limited E gene expression after enhanced IE gene expression are achieved. Note that conditions permissive for E gene expression (IE + E conditions depicted in Fig. 2 A), were associated with inhibited presentation of not only pp89 as described before (28), but also of β -gal, and, with comparable kinetics, presentation was eventually abolished (Fig. 2, B, right).

In the case of pp89, protein synthesis continues during MCMV E gene expression. When the rate of β -gal and pp89 synthesis was analyzed in cells infected with MCMV lac1, the decreasing rate of synthesis was found comparable between both proteins under IE and under E conditions (Fig. 2 C). A comparison between cell lines producing significantly different amounts of pp89 had revealed that quantitative differences do not account for the inhibition of antigen presentation (28). Because two completely unrelated antigens are subjected to the same inhibition of antigen presentation after expression as MCMV IE genes, we reasoned that a common step in the MHC class I processing and presentation pathway is affected by MCMV E gene functions.

Correct Processing of pp89 in Absence of Antigen Presentation. The MHC class I processing and presentation pathway can be subdivided into: (a) proteolytic processing, (b) peptide transport into the ER, (c) complex formation of MHC class I heavy chain, β_2m and peptide, and (d) transport of the trimolecular complex to the cell surface. Proteolytic processing and peptide transport were not amenable to testing. Therefore, the latter steps were analyzed. H-2^d cells infected with the vaccinia recombinant MCMV-ieI-VAC express pp89, and the naturally processed nonapeptide ¹⁶⁸YPHFMPTNL¹⁷⁶ is found in cell lysates after acid extraction (31). It coelutes

with the synthetic nonamer as a single peak in fraction number 25 of our standard reverse-phase HPLC runs. The biological activity of extracted peptides is demonstrated in a standard CTL assay, where pp89-specific CTL lyse H-2^d cells that were incubated with the relevant eluted peptide fraction. Cells infected with MCMV under IE conditions contained biological activity in the expected nonapeptide peak at fraction 25 and, in addition, also in fraction 23 (Fig. 3 A, top) (Del Val et al., manuscript in preparation). No biological activity could be recovered from uninfected cells (data not shown). Remarkably, when natural peptides were retrieved from E phase-infected cells that did not present the antigen, essentially the same elution profile of biological activity was observed (Fig. 3 A, bottom). Thus, there are no qualitative changes in the natural peptide composition of infected cells that could account for the differences in antigen presentation.

In addition, the serial dilution of the fractions representing the two antigenic peaks revealed no quantitative differences between cells that did or did not present antigen regarding the content of endogenously processed peptide (Fig. 3 B, top and bottom, respectively). Thus, accurate processing of pp89 did not result in peptide presentation at the cell surface. We concluded that MCMV E gene products interfere with antigen presentation at a step later than processing.

Naturally Processed Peptides Are Bound to the MHC Class I Heavy Chain L^d. Current evidence indicates that only peptides bound by MHC class I molecules are protected from complete degradation (41). Accordingly, peptides can be retrieved only from cells that express the presenting MHC class I molecule(s). The conclusion, however, that the processed peptides of pp89 were already transported into the ER and associated with L^d was premature, because, at least after exogenous addition to cells, the nonapeptide can effectively bind also to the MHC class I molecule K^d (42). Thus, it was conceivable that during the E phase most of the processed peptides were in fact bound by K^d and not by L^d, and therefore

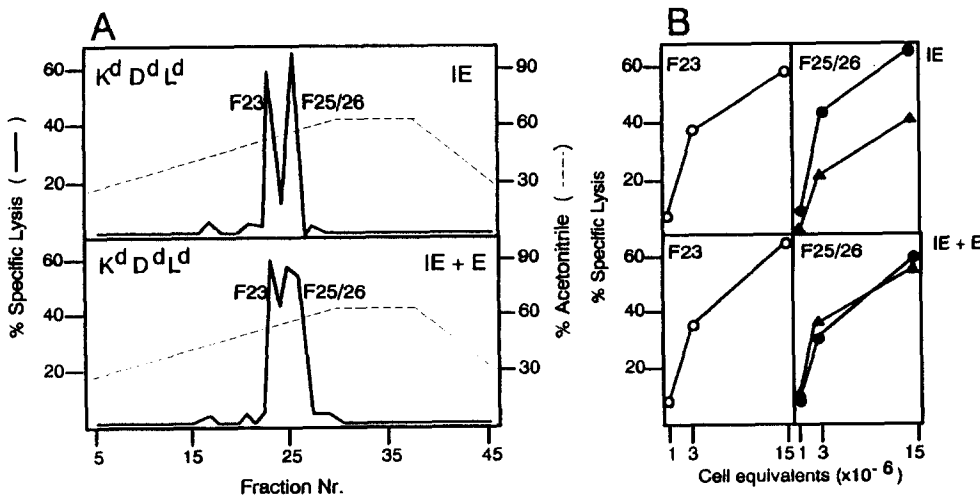


Figure 3. Processing and yield of processed peptides is not affected by MCMV E genes. (A) BALB/c MEF were infected with MCMV under IE (top) or IE + E (bottom) conditions, as defined in Fig. 2 A. At 10 h p.i., acid-soluble molecules were extracted and separated first by gel filtration chromatography and then by reverse-phase HPLC, using the indicated acetonitrile gradient for elution. HPLC fractions were tested in triplicate with pp89-specific CTL for their content of antigenic peptides. Fractions where major biological activities eluted are indicated. (B) The antigenic peptides that were detected in A were quantified by testing serial dilutions of the corresponding HPLC fractions in the cytolytic assay. The calculated number of infected cells from which peptides were recovered is given on the horizontal axis, and percent specific lysis is given on the vertical axis as a measure of the amount of the relevant antigenic peptide. (Left) Fraction 23, open circles; (right) fraction 25, filled circles; fraction 26, filled triangles.

not available for CTL from BALB/c mice, which do not generate K^d-restricted pp89-specific CTL (28). According to this explanation the biological activity of naturally processed peptides from E phase-infected cells would become only apparent, because extracted peptides, liberated from the assumed binding to K^d, could now be bound by the L^d molecules of the target cells after external addition.

To settle this point, peptides were acid extracted from MCMV-infected cells of the H-2^{dm2} mouse strain. This strain differs from BALB/c in that it lacks the gene encoding L^d (43). As shown in Fig. 4, no peptides with biological activity detectable by pp89-specific CTL could be released from H-2^{dm2} cells expressing IE genes of MCMV, and there was also no activity extractable from H-2^{dm2} cells at the E phase of infection (not shown). It was therefore concluded that the detection of pp89-derived peptide activity reflects quantitative peptide binding to L^d, even after E gene expression.

Correct Assembly of MHC Class I Complexes. Peptides play two cooperative roles during the assembly of MHC class I molecules, the folding of the heavy chain that can occur in absence of β_2m , and the stabilization of preformed complexes of heavy chain and β_2m (13, 14). The isolation of peptide from cells that express E genes indicated correct processing, transport into the ER, and association with L^d heavy chains, but not necessarily the formation of the stable trimolecular complex. The MHC class I molecule L^d is known for its reduced affinity for β_2m and its delayed transit to the cell surface, where it is expressed at a lower density (44). In addition, many L^d heavy chains remain in a conformation typical for the lack of β_2m association, suggesting a critical susceptibility of L^d heavy chains during protein folding (18).

Therefore, the amount and conformational properties of synthesized L^d molecules were compared after metabolic labeling of MCMV-infected cells representing both experimental conditions (Fig. 5, top). Quantitative immunoprecipitations were performed sequentially, starting with the mAb 64-3-7, which detects only L^d heavy chains in a conformation that is not associated with β_2m (18). This antibody precipitated ~90% of the L^d molecules. No quantitative differences associated with the different phases of MCMV

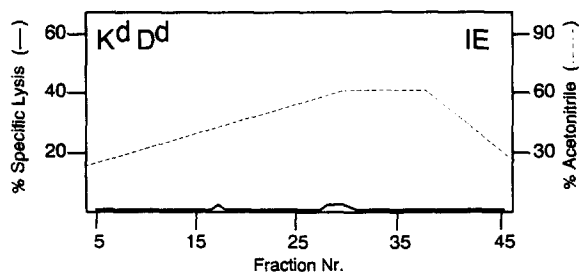


Figure 4. Absence of processed pp89 peptides in cells lacking L^d. H-2^{dm2} MEF lacking the L^d gene were infected with MCMV under IE conditions, and the acid-soluble molecules were extracted at 10 h p.i. and analyzed by HPLC. Individual fractions were tested in triplicate with pp89-specific CTL in a cytolytic assay.

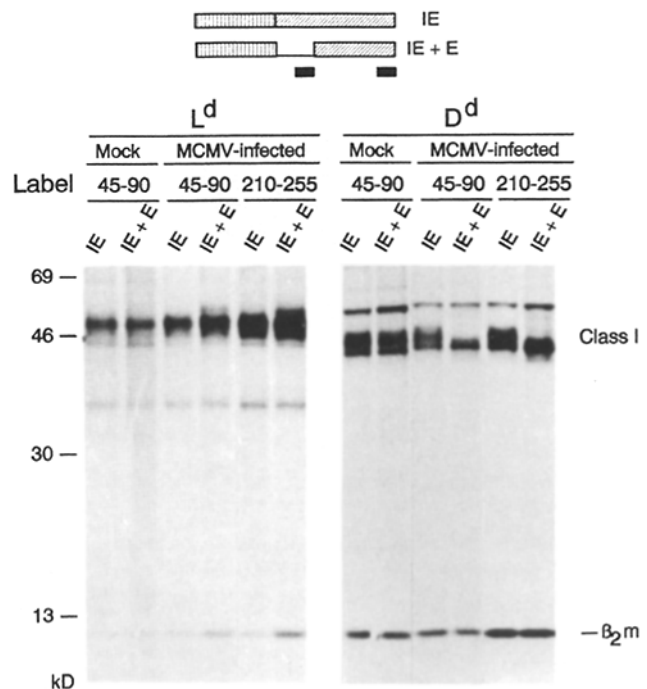


Figure 5. Correct assembly of MHC class I heavy chain/ β_2m complexes during E gene expression. MEF cells were MCMV-infected or mock-infected under the two experimental conditions indicated (top). Metabolic labeling with [³⁵S]methionine was carried out at the two time points marked (filled box). The period of labeling is indicated below in minutes after removal of CH. Cell lysates were immunoprecipitated sequentially, first with mAb 64-3-7 (data not shown), then with mAb 28-14-8s (left), and finally with mAb 34-5-8s (right). Each precipitate was subjected to gel electrophoresis.

gene expression were observed (data not shown). After clearing of the cell lysates from excess antibody, L^d molecules were precipitated with the $\alpha 3$ domain-specific mAb 28-14-8s, which detects the remaining β_2m -complexed L^d molecules, and separated by SDS-PAGE (Fig. 5, left). L^d molecules migrated with a relative electrophoretic mobility of 46 kD as a broad band that probably included differently glycosylated forms. Despite the fact that 64-3-7⁺ L^d molecules were removed in the first precipitation step, the amount of precipitated β_2m was still low, perhaps due to the instability of the L^d/ β_2m complex during the precipitation procedure. Note, however, that L^d/ β_2m complexes were found in comparable amounts under both infection conditions. Considering the L^d dependence of peptide isolation, we thus concluded that correct trimolecular class I complexes are also formed in cells that are unable to present the antigen.

In view of the particular properties of L^d, the MHC class I molecule D^d was also studied, which represents a MHC class I molecule that quantitatively and firmly associates with β_2m , and matures faster. Again, the amount of D^d complexes remained constant after transition of MCMV infection into the E phase (Fig. 5, right). Remarkably, and different from L^d, the D^d molecules synthesized at different times of MCMV gene expression exhibited a different electrophoretic

mobility. Under mock and IE conditions, several bands were found, probably reflecting stages of maturation to forms of complex glycosylation. Transition to the E phase, however, restricted this pattern to a major band of fast mobility, probably representing the glycoprotein precursor. The coprecipitation of β_2m indicated that intracellular assembly of the MHC class I complex was not affected. The fact that not only antigen presentation by L^d but also the glycosylation of D^d was affected suggested a general effect of MCMV E gene products on MHC class I heavy chain maturation.

Defective Glycosylation of MHC Class I Complexes. To locate the effect on the posttranslational modification, the susceptibility of MHC class I molecules to Endo H digestion was determined. As it is the case for other glycoproteins, MHC class I molecules cotranslationally acquire a high-mannose core of N-linked oligosaccharides in the ER. Endo H preferentially cleaves immature N-linked oligosaccharides characteristic of glycoproteins that have not reached the medial-Golgi compartment (45). Further processing of the oligosaccharide chain by enzymes located in the medial-Golgi compartment leads to the fully mature glycoproteins and renders the glycan structure resistant to Endo H digestion (46).

Maturation of MHC class I molecules was studied by pulse-chase experiments. A long pulse of 90 min was required to follow the fate of newly synthesized L^d molecules because the expression of L^d in embryonic fibroblasts is very low (see also Fig. 7, top left). In mock-infected cells and also in IE-infected cells (Fig. 6, top left), the L^d heavy chains, in agreement with a previous report (44), slowly matured to Endo H-resistant forms. This was apparent from comparing the relative amount of Endo H-susceptible and -resistant forms after the pulse at different time points of the chase period. Even after a chase period of 270 min, some L^d heavy chains remained Endo H susceptible. The decrease in the overall content of L^d molecules at that time reflects the instability of this molecule and explains the poor surface expression of L^d . The comparison between L^d molecules from cells representing both infection conditions revealed a clear difference in the acquisition of Endo H resistance. In contrast to the IE-infected cells, the majority of molecules synthesized during the E phase (Fig. 6, top right) remained in the Endo H-sensitive form throughout the chase period, which indicated that they had not gained access to the Golgi enzymes. Note, however, that in spite of defective maturation, there was no difference in the overall stability of L^d molecules.

Essentially the same result was obtained for D^d heavy chains. Although this molecule is already completely processed during the 90-min pulse to Endo H-resistant forms under mock and IE gene expression conditions (Fig. 6, bottom left), E gene expression prevented glycoprotein maturation with an efficiency comparable with L^d without affecting protein stability (Fig. 7, bottom right). Similar results were also obtained for K^d (data not shown). It has been reported that full glycosylation is not a prerequisite for MHC class I molecule transport and cell surface expression (47). Thus, we concluded that after expression of MCMV E genes, the correctly assembled MHC class I complexes remain Endo H sensitive,

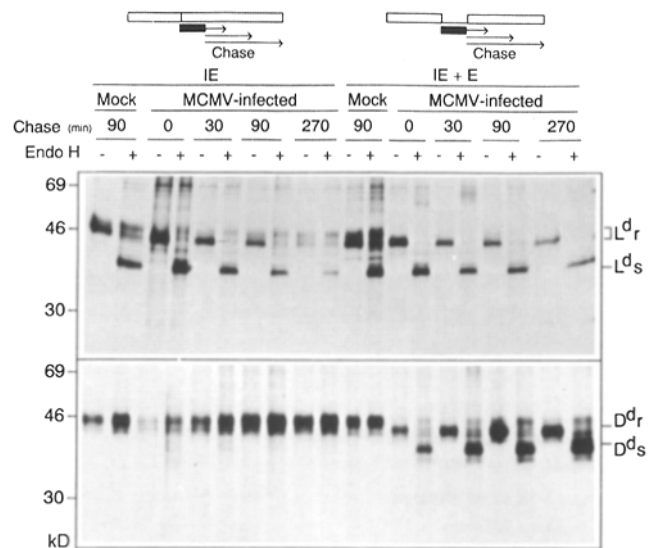


Figure 6. E gene expression prevents acquisition of Endo H resistance by MHC class I molecules. Mock-infected or MCMV-infected cells were treated with CH and Act D as indicated (top). Cells were pulse labeled with [35 S]methionine for 90 min as indicated (filled box), and then chased (arrows) for the indicated times. Immunoprecipitations were performed sequentially first with mAb 64-3-7 (data not shown), then mAb 28-14-8s (top), and finally with mAb 34-5-8s (bottom). Before gel electrophoresis, the marked samples (+) were incubated with Endo H. The bands resulting from enzymatic digestion are indicated on the right with s (for susceptible), or with r (for resistant).

either because they do not reach the medial-Golgi compartment or because they complex with other molecules that prevent correct glycosylation, as shown for the different glycosylation of the CD3 δ chain in cells expressing different forms of the TCR (48).

Defective Transport of MHC Class I Molecules. The surface expression MHC class I molecules is not affected during the

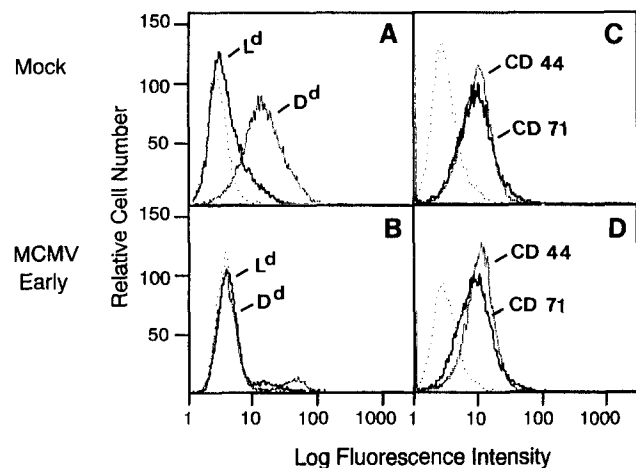


Figure 7. FACS[®] analysis of surface glycoprotein expression in the E phase of infection. BALB.SV cells were incubated with phosphonoacetic acid (A and C) or MCMV infected (B and D) for 18 h in the presence of phosphonoacetic acid to prevent late-phase gene expression, and then stained with mAb 28-14-8s (anti- L^d) and 34-5-8s (anti- D^d) (A and B), or with mAb IM7-8.1E4 (anti-CD44), and R17 217.1.3 (anti-CD71) (C and D).

first hours of the E phase (28), and complex formation between an MHC class I molecule and an MCMV E protein could be compatible with transport to the cell surface. The surface density of the MHC class I molecules L^d and D^d was determined by cytofluorometric analysis at a later stage of the E phase, 18 h postinfection, in order to reveal the fate of nascent MHC class I molecules. The E phase arrest was achieved by infection of cells with MCMV in the presence of phosphonoacetic acid, which blocks viral DNA replication and the subsequent expression of late-phase genes. A decrease of L^d and D^d molecules to almost undetectable levels was observed, impressive in particular for D^d, which is more abundantly expressed (Fig. 7, compare A and B). Similar results were obtained also for the H-2 proteins K^d, L^a, K^b, D^b, K^k, and D^k (data not shown). In contrast, other glycoproteins, like CD71 (transferrin receptor) and the adhesion molecule CD44 (pgp-1), essentially remained unaffected by infection (Fig. 7, C and D). Under the premise that CD44 and CD71 have a turnover rate comparable to that of MHC class I molecules, these data would imply that MHC class I glycoproteins represent a preferential target for the MCMV E gene effect. We concluded that after expression of MCMV E genes the correctly assembled MHC class I complexes do not reach the medial-Golgi compartment and remain Endo H sensitive.

Discussion

We demonstrate that the mechanism of interference by MCMV E gene products with antigen presentation to CTL (28) can be described as a block in the intracellular transport of MHC class I complexes through the Golgi compartment. The dramatic inhibitory effect of MCMV E gene products on viral antigen presentation provides a paradigm for the potential of a herpes virus to interfere with the recognition of infected cells by CTL. CMV persists for life in the infected host, and CD8⁺ T lymphocyte function is the hallmark of host control of CMV (reviewed in reference 27). For a virus that is under control of the cellular immune response, the interference with MHC class I molecule transport is clearly the most effective evasion mechanism that can be envisaged. Provided that the glycosylation studies correctly define the location at which the Golgi transport of MHC class I molecules is blocked, the results imply that binding of peptides to MHC class I molecules occurs in the ER/*cis*-Golgi compartment, and not in subsequent compartments along the secretory pathway.

When the cascade of MCMV gene expression is restricted to IE genes, MCMV-specific CTL detect processed peptides that are derived from the MCMV IE protein pp89 and presented by the MHC class I molecule L^d. Subsequent expression of E genes for a short time prevents pp89 presentation. Yet, E gene expression has little effect on pp89 synthesis, the surface expression of MHC class I molecules appears unchanged when antigen presentation is prevented, and infected cells are still recognized by CTL that detect a viral E antigen that we could not define in molecular terms. This suggested a selective effect on pp89 antigen processing (28).

In the light of the new evidence provided in this paper, however, the mechanism of inhibition is much more general than previously thought.

We report on the following findings. (a) Antigen selectivity was excluded by insertion of the bacterial gene *lacZ* coding for the enzyme β -gal into the MCMV genome under control of the *ie1* promoter. Although both proteins, pp89 and β -gal, differ with regard to primary sequence, intracellular distribution, and function, presentation of both antigens was blocked by MCMV E gene expression. (b) Naturally processed peptides extracted from cells that synthesized pp89 in presence or absence of E proteins had the same biochemical properties, and the same amount of biochemical activity was retrieved in both cases. Therefore, antigen processing was correct in nonpresenting cells, and peptide competition could not explain the failure of antigen presentation. (c) The L^d-dependent presence of peptides in nonpresenting cells revealed peptide transport into the ER and peptide association with the heavy chain, and studies on the conformational properties of the heavy chain provided evidence for correct formation of the trimolecular complex. (d) The observation that during the E phase of viral infection the nascent heavy chains did not acquire resistance to Endo H digestion and did not reach the cell surface revealed a block in the normal maturation and transport of MHC class I molecules. This transport block affects selectively MHC class I molecules.

The fact that we failed to see the general effect on MHC class I molecule maturation in the earlier communication (28) is easily explained. We investigated the effect on L^d at the time it became apparent. Thus, we determined only the resident population of surface-expressed L^d molecules that appeared normal, whereas the fate of nascent molecules was not controlled. The thoroughness of the antigen presentation blockade, however, may be due to characteristic features of the L^d surface molecules. It is expressed on the cell surface at levels three to four times lower than K^d and D^d, the association with β_2m is delayed, and the slower maturation is documented by a delayed processing of the N-linked oligosaccharide (44). L^d molecules that are not occupied by peptide ligands appear at the cell surface, and external addition of peptides to cells can alter the conformation and enhance surface expression (17, 18). Data of Christinck et al. (49) demonstrate that as few as 200 MHC class I-peptide complexes suffice for recognition by CTL in vitro. Therefore, if the transport blockade were incomplete, the number of exported complexes might not fall below the detection threshold for other L^d-presented antigens or for other peptide-MHC class I molecule complexes. By such a mechanism we now explain the escape of an MCMV E antigen from the regulatory effect (28). We have observed that a 10-fold difference in the amount of naturally processed and L^d-presented peptides can be decisive for target formation in vitro and for recovery from lethal viral infection in vivo (31).

A transport block of nascent MHC class I molecules to the cell surface, either by treatment with brefeldin A (10, 11), or by the action of the adenovirus protein E3/19K, prevents presentation of endogenous antigens. Reversal of the

brefeldin A treatment and removal of the ER/*cis*-Golgi retention signal of E3/19K (26), respectively, restores presentation. In the antigen presentation-deficient cell mutant T2, an ER translocation signal rescues presentation of an endogenous peptide (50). Indirect evidence derived from *in vitro* systems suggests that MHC class I molecules bind synthetic peptides in the same compartment where they bind to β_2m (12–14). These data demonstrate that nascent MHC class I molecules are required for peptide presentation, but do not define the place of peptide charging because naturally processed peptides have not been isolated under conditions of inhibited MHC class I molecule transport. If the lack of Endo H resistance correctly defines the location of the transport block by MCMV, then MHC class I molecules residing in the ER/*cis*-Golgi compartment already contain the processed peptides.

Viral effects on MHC expression and on other cell membrane proteins relevant for immunosurveillance have been discussed for a number of viruses. Yet, with the exception of the adenoviruses, there has been no precise molecular analysis (for listing, see reference 20). The adenoviruses provide the example that viruses belonging to the same family can use related, but different strategies, such as an effect on MHC class I mRNA processing (21, 22) or on the intracellular transport of MHC class I molecules (23–25).

For the understanding of CMV infection and disease, it has been a matter of debate whether, in addition to profiting from host conditions of defective cellular immune control, the virus itself has evolved strategies to evade immunosurveillance even in the immunocompetent host. Grundy et al. (51) reported that human CMV (HCMV) binds exogenous β_2m , perhaps masking the virus against the detection by antibodies. Remarkably, HCMV contains an open reading frame (UL18) encoding a glycoprotein with sequence similarity to MHC class I molecules (52). Upon expression by a recombinant vaccinia virus, the UL18 gene product can complex with β_2m (53). The latter authors observed that in HCMV-infected cells no synthesis of mature HLA class I molecules occurred despite unchanged mRNA levels. Although expression of the UL18 gene product has not yet been detected in HCMV-infected cells, this led them to speculate that a function of the HLA homologue could be to sequester β_2m , making it unavailable to nascent cellular class I heavy chains. Sequestration of β_2m would result in a block of peptide pre-

sentation by MHC class I molecules and predict an escape from cellular immune control. In MCMV the mechanisms are clearly different. We did not see an inhibition of MHC class I molecule assembly, and only at a later stage was the maturation of the trimolecular complex inhibited. In this context it is worth mentioning that it was recently observed in HCMV-infected cells that HLA class I molecules were synthesized but remained retained intracellularly (J. E. Grundy, personal communication).

Why is the full glycosylation of assembled ternary complex impaired subsequent to the expression of MCMV E genes? A retention signal is contained within the six COOH-terminal amino acids of the E3/19K protein of adenovirus 2 (56) and mediates the ER/*cis*-Golgi retention of complexes between E3/19K and MHC class I molecules (23) reflected by the Endo H sensitivity of MHC molecules (24, 25). Although representing an attractive speculation, the inhibitory MCMV protein must not necessarily represent a functional homologue of the E3/19K protein. Unlike in adenovirus, no complex formation could be detected under comparable experimental conditions (24) between an MCMV E protein and the nascent MHC molecules. The other possibility, that MCMV E gene products interact with glycosyl transferases, was considered unlikely because other glycoproteins reach the surface and are correctly glycosylated (H. Hengel, unpublished data). The rate-limiting step of glycoprotein transport appears to be the acquisition of the correct tertiary and quaternary structure, and improperly folded molecules and proteins in intermediate stages of folding are retained in the ER or in *cis*-Golgi compartment (57–59). Particularly, unassembled MHC class I molecules are retained in a recycling pathway between the ER and *cis*-Golgi compartment (60). With the available antibodies we have not detected any effect of MCMV on the assembly of the ternary complex. Degen and Williams (61) recently described an 88-kD protein that participates in the biogenesis of MHC class I molecules. This protein binds to the heavy chains and dissociates when the assembled heavy chain- β_2m complex reaches the medial-Golgi compartment and acquires resistance to Endo H digestion. It is worth studying whether the 88-kD protein is still bound to this complex, because its dissociation should not be triggered by peptide binding but by some other, later event.

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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. Ortiz-Navarrete, V., A. Seelig, M. Gernold, S. Frentzel, P.M. Kloetzel, and G.J. Hämmerling. 1991. Subunit of the 20S proteasome (multicatalytic proteinase) encoded by the major histocompatibility complex. *Nature (Lond.)* 353:662.
3. Martinez, C.K., and J.J. Monaco. 1991. Homology of proteasome subunits to a major histocompatibility complex-linked LMP gene. *Nature (Lond.)* 353:664.
4. Kelly, A., S.H. Powis, R. Glynn, E. Radley, S. Beck, and J. Trowsdale. 1991. Second proteasome-related gene in the human MHC class II region. *Nature (Lond.)* 353:667.
5. 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 ABC superfamily of transporters. *Nature (Lond.)* 348:741.
6. Spies, T., M. Bresnahan, S. Bahram, D. Arnold, G. Blanck, E. Mellus, 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.
7. Powis, S., 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.
8. Levy, F., R. Gabathuler, R. Larsson, and S. Kvist. 1991. ATP is required for in vitro assembly of MHC class I antigens but not for transfer of peptides across the ER membrane. *Cell* 67:265.
9. Bjorkman, P.J., M.A. Saper, B. Samraoui, W.S. Bennet, J.L. Strominger, and D.G. Wiley. 1987. Structure of the human class I histocompatibility antigen, HLA-A2. *Nature (Lond.)* 329:506.
10. Nuchtern, J.G., J.S. Bonifacio, W.E. Biddison, and R.D. Klausner. 1989. Brefeldin A implicates egress from endoplasmic reticulum in class I restricted antigen presentation. *Nature (Lond.)* 339:223.
11. Yewdell, J.W., and J. Benninck. 1989. Brefeldin A specifically inhibits presentation of protein antigens to cytotoxic T lymphocytes. *Science (Wash. DC)* 244:1072.
12. Kvist, S., and U. Hamann. 1990. A nucleoprotein peptide of influenza A virus stimulates assembly of HLA-B27 class I heavy chains and β_2 -microglobulin translated in vitro. *Nature (Lond.)* 348:446.
13. Townsend, A., T. Elliott, V. Cerundolo, L. Foster, B. Barber, and A. Tse. 1990. Assembly of MHC class I molecules analyzed in vitro. *Cell* 62:285.
14. Elliott, T., V. Cerundolo, J. Elvin, and A. Townsend. 1991. Peptide induced conformational change of the class I heavy chain. *Nature (Lond.)* 351:402.
15. Townsend, A., C. Öhlén, 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.
16. 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.
17. Lie, W.-R., N.B. Myers, J. Gorka, R.J. Rubocki, J.M. Conolly, and T.H. Hansen. 1990. Peptide ligand induced conformation and surface expression of the L^d class I MHC molecule. *Nature (Lond.)* 344:439.
18. Lie, W.-R., N. Myers, J.M. Conolly, J. Gorka, D.R. Lee, and T.H. Hansen. 1991. The specificity binding of peptide ligand to L^d class I major histocompatibility complex molecules determines their antigenic structure. *J. Exp. Med.* 173:449.
19. Schumacher, T.N.M., M.-T. Heemels, J.J. Neefjes, W.M. Kast, C.J.M. Melief, and H.L. Ploegh. 1990. Direct binding of peptide to empty MHC class I molecules on intact cells and in vitro. *Cell* 62:563.
20. Maudsley, D.J., and J.D. Pound. 1991. Modulation of MHC antigen expression by viruses and oncogenes. *Immunol. Today* 12:429.
21. Schrier, P.I., R. Bernards, T.M.J. Vaessen, A. Houweling, and A.J. Van der Eb. 1983. Expression of class I histocompatibility antigens switched off by highly oncogenic adenovirus in transformed rat cells. *Nature (Lond.)* 305:771.
22. Vaessen, R.T.M.J., A. Houweling, and A.J. Van der Eb. 1987. Posttranscriptional control of class I MHC mRNA in adenovirus 12-transformed cells. *Science (Wash. DC)* 235:1486.
23. Signas, C., M.G. Katze, H. Persson, and L. Philipson. 1982. An adenovirus binds heavy chains of class I transplantation antigens from man and mouse. *Nature (Lond.)* 299:175.
24. Burgert, H.-G., and S. Kvist. 1985. An adenovirus type 2 glycoprotein blocks cell surface expression of human histocompatibility class I antigens. *Cell* 41:987.
25. Andersson, M., S. Pääbo, T. Nilsson, and P.A. Peterson. 1985. Impaired intracellular transport of class I MHC antigens as a possible means for adenoviruses to evade immune surveillance. *Cell* 43:215.
26. Cox, J.H., J.R. Benninck, and J.W. Yewdell. 1991. Retention of adenovirus E19 glycoprotein in the endoplasmic reticulum is essential to its ability to block antigen presentation. *J. Exp. Med.* 174:1629.
27. Koszinowski, U.H., M. Del Val, and M.J. Reddehase. 1990. Cellular and molecular basis of the protective immune response to cytomegalovirus infection. *Curr. Top. Microbiol. Immunol.* 154:189.
28. Del Val, M., K. Münch, M.J. Reddehase, and U.H. Koszinowski. 1989. Presentation of cytomegalovirus immediate-early antigens to cytolytic T lymphocytes is selectively blocked by viral genes expressed in the early phase. *Cell* 58:305.
29. Reddehase, M.J., J.B. Rothbard, and U.H. Koszinowski. 1989. A pentapeptide as minimal antigenic determinant for MHC class I restricted T lymphocytes. *Nature (Lond.)* 337:651.
30. Del Val, M., H.-J. Schlicht, H. Volkmer, M. Messerle, M.J. Reddehase, and U.H. Koszinowski. 1991. Protection against

- lethal cytomegalovirus infection by a recombinant vaccine containing a single nonameric T-cell epitope. *J. Virol.* 65:3641.
31. Del Val, M., H.-J. Schlicht, T. Ruppert, M.J. Reddehase, and U.H. Koszinowski. 1991. Efficient processing of an antigenic sequence for presentation by MHC class I molecules depends on its neighboring residues in the protein. *Cell.* 66:1145.
 32. Rammensee, H.-G., H. Schild, and U. Theopold. 1989. Protein specific cytotoxic T lymphocytes. Recognition of transfectants expressing intracellular membrane-associated or secreted forms of β -galactosidase. *Immunogenetics.* 30:296.
 33. Manning, W.C., and E.S. Mocarski. 1988. Insertional mutagenesis of the murine cytomegalovirus genome: one prominent α gene (*ie2*) is dispensable for growth. *Virology.* 167:477.
 34. Volkmer, H., C. Bertholet, S. Jonjic, R. Wittek, and U.H. Koszinowski. 1987. Cytolytic T lymphocyte recognition of the murine cytomegalovirus nonstructural immediate-early protein pp89 expressed by recombinant vaccinia virus. *J. Exp. Med.* 166:668.
 35. 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.
 36. Budd, R.C., J.C. Cerottini, and H.R. McDonald. 1987. Selectively increased production of interferon-gamma by subsets of Lyt-2⁺ and L3T4⁺ T cells identified by expression of Pgp-1. *J. Immunol.* 138:3583.
 37. Messerle, M., G.M. Keil, and U.H. Koszinowski. 1991. Structure and expression of the murine cytomegalovirus immediate-early gene 2. *J. Virol.* 65:1638.
 38. Messerle, M., G.M. Keil, B. Bühler, and U.H. Koszinowski. 1992. Structural organization, expression, and functional characterization of the murine cytomegalovirus immediate-early gene 3. *J. Virol.* 66:27.
 39. Ebeling, A., G.M. Keil, E. Knust, and U.H. Koszinowski. 1983. Molecular cloning and physical mapping of murine cytomegalovirus DNA. *J. Virol.* 47:421.
 40. Keil, G.M., A. Ebeling-Keil, and U.H. Koszinowski. 1987. Sequence and structural organization of murine cytomegalovirus immediate-early gene 1. *J. Virol.* 61:1901.
 41. Falk, K., O. Rötzschke, and H.-G. Rammensee. 1990. Cellular peptide composition governed by major histocompatibility complex class I molecules. *Nature (Lond.)* 348:248.
 42. Romero, P., G. Corradin, I.F. Luescher, and J.L. Maryanski. 1991. H-2K^d-restricted antigenic peptides share a simple binding motif. *J. Exp. Med.* 174:603.
 43. Hansen, T.H., S.E. Cullen, R. Melvold, H.I. Kohn, L. Flaherty, and D.H. Sachs. 1977. Mutation in a new H-2 associated histocompatibility gene closely linked to H-2^d. *J. Exp. Med.* 145:1550.
 44. Beck, J.C., T.H. Hansen, S.E. Cullen, and D. Lee. 1986. Slower processing, weaker β_2m association, and lower surface expression of H-2L^d are influenced by its amino terminus. *J. Immunol.* 137:916.
 45. Kobata, A. 1979. Use of endo- and exoglycosidases for structural studies of glycoconjugates. *Anal. Biochem.* 100:1.
 46. Kornfeld, R., and S. Kornfeld. 1985. Assembly of Asparagine-linked oligosaccharides. *Annu. Rev. Biochem.* 54:631.
 47. Ploegh, H.L., H.T. Orr, and J.L. Strominger. 1981. Biosynthesis and cell surface localization of nonglycosylated human histocompatibility antigens. *J. Immunol.* 126:270.
 48. Krangel, M.S., B.E. Bierer, P. Devlin, M. Clabby, J.L. Strominger, J. McLean, and M.B. Brenner. 1987. T3 glycoprotein is functional although structurally distinct on human T-cell receptor γ T lymphocytes. *Proc. Natl. Acad. Sci. USA.* 84:3817.
 49. Christinck, E.R., M.A. Luscher, B.H. Barber, and D.B. Williams. 1991. Peptide binding to class I MHC on living cells and quantitation of complexes required for CTL lysis. *Nature (Lond.)* 352:67.
 50. Anderson, K., P. Creswell, M. Gammon, J. Hermes, A. Williamson, and H. Zweerink. 1991. Endogenously synthesized peptide with an endoplasmic reticulum signal sequence sensitizes antigen processing mutant cells to class I-restricted cell-mediated lysis. *J. Exp. Med.* 174:489.
 51. Grundy, J.E., J.A. McKeating, and P.D. Griffith. 1987. Cytomegalovirus strain AD169 binds beta 2 microglobulin in vitro after release from cells. *J. Gen. Virol.* 68:777.
 52. Beck, S., and B.G. Barrell. 1988. Human cytomegalovirus encodes a glycoprotein homologous to MHC class I antigens. *Nature (Lond.)* 331:269.
 53. Browne, H., G. Smith, S. Beck, and T. Minson. 1990. A complex between the MHC class I homologue encoded by human cytomegalovirus and beta 2 microglobulin. *Nature (Lond.)* 347:770.
 54. Rothman, J.E. 1987. Protein sorting by selective retention in the endoplasmic reticulum and Golgi stack. *Cell.* 50:521.
 55. Swift, A.M., and C.E. Machamer. 1991. A Golgi retention signal in a membrane spanning domain of coronavirus E1 protein. *J. Cell Biol.* 115:19.
 56. Nilsson, T., M. Jackson, and P.A. Peterson. 1989. Short cytoplasmic sequences serve as retention signals for transmembrane proteins in the endoplasmic reticulum. *Cell.* 58:707.
 57. Gething, M.-J., K. McCammon, and J. Sambrook. 1986. Expression of wild-type and mutant forms of influenza hemagglutinin: the role of folding in intracellular transport. *Cell.* 46:939.
 58. Copeland, C.S., R.W. Doms, E.M. Bolzau, R.G. Webster, and A. Helenius. 1986. Assembly of influenza hemagglutinin trimers and its role in intracellular transport. *J. Cell Biol.* 103:1179.
 59. Williams, D.B., F. Boriello, R.A. Zeff, and S.G. Nathenson. 1988. Intracellular transport of class I histocompatibility complex molecules. Influence of protein folding on transport to the cell surface. *J. Biol. Chem.* 263:4549.
 60. Hsu, V., L.C. Yuan, J.G. Nuchtern, J. Lippincott-Schwartz, G.J. Hämmerling, and R.D. Klausner. 1991. A recycling pathway between the endoplasmic reticulum and the Golgi apparatus for retention of unassembled MHC class I molecules. *Nature (Lond.)* 352:441.
 61. Degen, E., and D.B. Williams. 1991. Participation of a novel 88-kD protein in the biogenesis of murine class I histocompatibility molecules. *J. Cell Biol.* 112:1009.