Human cytomegalovirus inhibits peptide translocation into the endoplasmic reticulum for MHC class I assembly

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Human cytomegalovirus (HCMV) genes expressed in the early phase of infection mediate the destabilization of nascent major histocompatibility complex (MHC) class I molecules in infected cells and thus prevent antigen presentation to CD8⁺ T lymphocytes. We report that HCMV genes interfere with the MHC class I pathway of antigen presentation by at least two mechanisms. Firstly, permissive infection of fibroblasts is characterized by a continuous decline in the capacity to translocate peptides from the cytosol into the endoplasmic reticulum (ER) by the transporter associated with antigen processing (TAP). Inactivation of peptide transport is operative despite augmented TAP expression during HCMV infection. Secondly, TAP molecules fail to associate with MHC class I heavy chains indicating that HCMV early gene expression also interferes with MHC class I maturation. A temperature-sensitive mutant of HCMV, ts9, which lacks 15 kb of DNA encoding the genes US1-US15 of HCMV, had lost the capacity to interfere with MHC class I assembly and to inhibit the peptide translocation function of TAP. One of the genes deleted in ts9, US11, which was reported to downregulate the expression of MHC class I molecules, does not affect peptide transport by TAP. Therefore, we conclude that HCMV encodes at least two early gene functions that interfere with the MHC class I antigen presentation pathway.

Introduction

Recognition of antigen by CD8⁺ T lymphocytes requires the processing of proteins into peptides that are subsequently presented by major histocompatibility complex (MHC) class I molecules on the cell surface (Townsend & Bodmer, 1989). Intracellular antigens are processed by cytoplasmic proteases among which the proteasome, a large multicatalytic protease complex, plays a major role (Driscoll & Finley, 1992). Peptides are delivered to the endoplasmic reticulum (ER) through the transporter associated with antigen processing (TAP), which is composed of the two integral ER membrane proteins, TAP1 and TAP2 (Androlewicz et al., 1993; Kelly et al., 1992; Kleijmeer et al., 1992; Neefjes et al., 1993a; Spies et al., 1992). MHC class I heavy chains assemble with β₂-microglobulin (β₂m) and peptide in the ER (Krangel et al., 1979; Nuchtern et al., 1989; Townsend et al., 1989). The formation of heterotrimeric MHC class I complexes is sequentially assisted by transient interactions with the chaperon calnexin and with TAP, requires correct folding of the heavy chains, stable association with β₂m, and subsequent binding of the antigenic peptide that is held in the polymorphic peptide binding groove (Degen & Williams, 1991; Ortmann et al., 1994; Suh et al., 1994; Neefjes et al., 1993a). MHC class I molecules lacking peptide display a different conformation and are deficient with respect to surface transport and stability (Baas et al., 1992; Bluestone et al., 1992). Mutant cell lines that lack functional peptide transporters, such as RMA-S or LCL721.174, are characterized by deficient peptide loading onto class I molecules, consequently reduced surface expression of class I molecules and an impaired capacity to present antigen (Cerundolo et al., 1990; Ljunggren et al., 1990), demonstrating the crucial role of TAP for the function of class I molecules.

CD8⁺ T lymphocytes that recognize peptides derived from viral proteins synthesized in infected cells play a decisive role in antiviral defence. Yet, some viruses have evolved strategies to interfere with immune recognition at the post-translational
level (reviewed by Hill & Ploegh, 1995). For instance, the E3/19K protein expressed by group C adenoviruses directly interacts with MHC class I molecules in the ER and blocks their egress to the Golgi compartment (Burgert & Kvist, 1985). The ICP47 protein of herpes simplex virus (HSV) inhibits antigen presentation by binding to TAP, thus preventing peptide translocation into the ER (Früh et al., 1995; Hill et al., 1995; York et al., 1994).

Cytomegaloviruses (CMVs) constitute the beta-subfamily of herpesviruses and establish both acute and chronic infections. In permissively infected cells, CMV gene expression is regulated in a cascade fashion characteristic for herpesviruses. Viral proteins encoded by genes expressed in the immediate-early phase of infection control the activation of early phase genes. Early proteins are required for viral DNA synthesis, which is followed by the synthesis of structural proteins, the bulk of which are expressed during the late phase of infection. Primary infection is followed by lifelong persistence in a latent state from which reactivation can occur. While infection of the immunocompetent host is usually asymptomatic, the virus causes profound disease manifestations in immunologically immature or immunodeficient individuals such as organ transplant recipients or AIDS patients (Ho, 1982). Adjuvant immunotherapy of CMV infections in mice and humans with T lymphocytes has demonstrated a prominent prophylactic role for MHC class I-restricted immunity (Reddehase et al., 1987; Riddell et al., 1992).

CMVs have evolved functions to avoid the cellular immune response. Both human CMV (HCMV) and mouse CMV (MCMV) interfere with surface expression of class I MHC and antigen presentation to CD8+ T lymphocytes (Barnes & Grundy, 1992; Del Val et al., 1989). In MCMV-infected cells, correctly assembled peptide-charged MHC class I complexes are retained in the ER/cis-Golgi compartment by an early gene function leading to abrogation of cytotoxic T lymphocyte recognition (Del Val et al., 1992). In contrast to MCMV, in HCMV-infected fibroblasts MHC class I assembly is reduced and degradation of class I molecules is enhanced (Beersma et al., 1993; Warren et al., 1994; Yamashita et al., 1993). The HCMV UL11 encoded 32 kDa glycoprotein, which is sufficient for MHC class I downregulation (Jones et al., 1995), misdirects MHC class I heavy chains from the ER to the cytosol where they are degraded by the proteasome (Wiertz et al., 1996). Common to both viruses, the antigen presentation function of infected cells is restored by IFN-γ (Hengel et al., 1994, 1995).

Here we report on the dissection and genomic location of two different functions, expressed during the early phase of infection, by which HCMV interferes with the MHC class I antigen presentation pathway. Firstly, HCMV inhibits TAP-dependent transport of peptides from the cytosol into the ER by genes encoded in the region between US11–US15. Secondly, the individually expressed US11 gene does not affect TAP function but downregulates the amount of MHC class I molecules.

**Methods**

1. **Cells.** Human fetal lung fibroblasts, MRC-5 (Bio-Whittacker), in passage 6–20, human skin fibroblasts in passage 6–12 and 293 human neonatal kidney cells (ATCC CRL 1573) were grown in DMEM supplemented with 10% fetal calf serum, penicillin, streptomycin and 2 mm-glutamine. Mouse embryonic fibroblasts (MEF) were prepared from BALB/cj mice and used after three in vitro passages.

2. **Viruses and infection conditions.** Virus stocks of HCMV strain AD169 were prepared as described previously (Hengel et al., 1995). HCMV mutant ts9, a temperature-sensitive mutant generated by nitrosoguanidinium mutagenesis of wild-type strain AD169 (Yamanishi & Rapp, 1979), was kindly provided by K. Radsak, Marburg, Germany. Stocks of ts9 were prepared from cultures grown at the permissive temperature of 33 °C. Titres of ts9 were comparable to titres of HCMV AD169. For infections, subconfluent monolayers of fibroblasts were incubated with HCMV at an m.o.i. of 10 and centrifuged at 800 g for 30 min to increase the efficiency of infection. Control settings showed more than 97% of cells positive for HCMV early gene expression as assessed by immunofluorescence microscopy (Hengel et al., 1995). Late phase gene expression was prevented by treatment with phosphonoacetic acid (PAA, 250 μg/ml), which arrests CMV-infected cells in the late phase. MCMV strain Smith (ATCC VR-194) was used as tissue culture grown virus. MEF were infected at an m.o.i. of 20 and centrifuged at 800 g for 30 min.

3. **Reagents and antibodies.** The following mouse monoclonal antibodies (MAbs) and polyclonal rabbit antisera were used: W6/32 (anti-HLA class I complexes) (ATCC HB95); MAb anti-HCMV 72 kDa nuclear antigen (Du Pont NEN); anti-TAP-1 MAb TAP1.28 (Nijenhuis et al., 1996); anti-TAP-2 MAb TAP2.70 (Nijenhuis et al., 1996); rabbit anti-human heavy chain antisera that recognizes β2m-free class I heavy chains but not β2m-associated MHC class I molecules (provided by Hidde Ploegh, Massachusetts Institute of Technology, Cambridge, Mass., USA); a rabbit antisera recognizing actin (Sigma); goat anti-mouse IgG (Dianova); rabbit anti-HCMV US11 peptide antisera.

4. **Metabolic labelling and immunoprecipitation.** MRC-5 cells were labelled with [35S]methionine and [35S]cysteine (1200 Ci/mmol; Amersham) at a concentration of 700 μCi/ml and lysed in 1% NP40 lysis buffer as described (Del Val et al., 1992; Hengel et al., 1995) or in 1% digitonin lysis buffer (140 mM-NaCl, 20 mM-Tris pH 7.6, 5 mM-MgCl2, 0.2 mM-PMSE, leupeptin and pepstatin). Anti-actin antibodies were used in some experiments to remove actin molecules. Subsequently, immuno-precipitations were performed as described previously (Hengel et al., 1995). Between precipitation steps, excess antibodies were removed by repetitive clearing of the lysates with Protein A-Sepharose. Immune complexes were eluted with sample buffer and analysed by 12–15% polyacrylamide gradient gel electrophoresis. The gels were treated with Amplify (Amersham), dried, and exposed to Kodak X-Omat AR films at −70 °C for 1–7 days.

5. **Cloning and expression of HCMV US genes.** The open reading frames of US11 were cloned after PCR amplification of HCMV AD169 DNA with primers (see below) into the polylinker of the pCDNAneo expression vector (Invitrogen) under the control of the HCMV immediate-early enhancer-promoter. A Kozak sequence (GCC GCC AATG) was introduced to ensure optimal expression. The sequences were cloned using the 5' HindIII and 3' XhoI sites. Primers: US11 forward 5' CGCGGGAAAGCTTGCCTGCGCTGATGCTAATGTTGTTGCTATATATCACC- ACTGCTCCG 3'; resulting in an amplification product of 691 bp.

6. **Transfection of DNA and selection of transfectants.** Human 293 cells were transfected with plasmid DNA (10 μg per dish) by calcium phosphate precipitation and grown in DMEM supplemented with 10% fetal calf serum, penicillin, streptomycin and 2 mm-glutamine. Mouse embryonic fibroblasts (MEF) were prepared from BALB/cj mice and used after three in vitro passages.
phosphate precipitation or using the DOTAP transfection reagent (Boehringer Mannheim). Cell clones were selected in the presence of 0·5 mg/ml G418 (Sigma).

- **Flow cytometry.** Trypsinized cells were washed with PBS supplemented with 2% fetal calf serum and 0·3% NaN3, preincubated in 5% goat serum and then stained with hybridoma supernatants. Bound antibodies were visualized by addition of fluoresceinated goat anti-mouse IgG antibody (Dianova). Cells incubated with the second antibody alone served as negative controls. A total of 105 cells was analysed for each profile of Fig. 4 on a FACScan IV (Becton Dickinson).

- **Peptide translocation assay.** The transport assay was performed essentially as described (Momburg et al., 1994a; Neeffes et al., 1993b). The peptides used had the consensus sequence **RYWANATRSX** (R.X) with X being F, Y, V, I, R, K, T or P (Momburg et al., 1994b). The NAT sequn allows for N-linked glycosylation upon ER translocation. Peptides were radiolabelled with [125I] by chloramine-T catalysed iodination. After trypsinsization and washing, 2.5 x 106 cells were resuspended in incubation buffer (130 mM-KCl, 5 mM-HEPES pH 7·3, 10 mM-NaCl, 1 mM-CaCl2, 2 mM-EGTA, 2 mM-MgCl2). For plasma membrane permeabilization, cells were suspended in streptolysin O solution (1·25 IU/ml incubation buffer; Wellcome) and incubated at 37 °C for 15 min. After three washes, the permeabilized cells were incubated with 10 mM-ATP and 2·5 μl radioiodinated peptide (approx. 0·5 μg) in a final volume of 100 μl incubation buffer for 20 min at 37 °C. Following lysis in 1% NP40, nuclei were removed by centrifugation and the glycosylated peptide fraction was isolated with 30 μl Concanaavalin A (Con A)-Sepharose slurry (Pharmacia) at 4 °C, and quantified by 125I-counting. Input c.p.m. were determined for each peptide and each sample. Con A-recovered c.p.m. were expressed as percentage of input c.p.m. The transport rates shown (Fig. 2) represent means of duplicate samples.

### Results

**The HCMV deletion mutant ts9 lacks MHC class I downregulating gene functions**

HCMV AD169 has been reported to destabilize nascent MHC class I complexes without affecting cell heavy chain mRNA levels (Browne et al., 1990; Beersma et al., 1993; Warren et al., 1994). In the HCMV genome, conserved gene blocks shared between alpha-, beta- and gammaherpesviruses lie approximately between kb 50 and 170 of the 230 kb genome (Chee et al., 1990; Gompels et al., 1995). Gene regions unique for CMV flank the conserved gene blocks. They are nonessential for viral replication but may harbour functions that modulate the host–virus interaction (Chee et al., 1990; Kollert-Jøns et al., 1991; Jones & Muzithras, 1992; Mockenhaupt et al., 1994). To address the genetic basis for down-regulation of MHC class I heavy chains in HCMV-infected cells, we investigated the fate of class I molecules in fibroblasts infected with an HCMV AD169-derived temperature-sensitive mutant, ts9. This mutant lacks a 15 kb spanning genomic region within the unique short region (Us) corresponding to the genes **US1–US15** (Kollert-Jøns et al., 1991; Mockenhaupt et al., 1994; Yamanishi & Rapp, 1979). As reported by several laboratories (Barnes & Grundy, 1992; Beersma et al., 1993; Hengel et al., 1995; Warren et al., 1994; Yamanishi et al., 1993), fibroblasts infected with AD169 for 72 h express drastically reduced steady state levels of MHC class I complexes as determined by MAb W6/32, which recognizes properly assembled MHC/β2m complexes (Fig. 1, left panel). Cytofluorometric analysis revealed, in permissively ts9-infected cells, an almost tenfold increase of MHC class I molecules on the cell surface compared to mock control fibroblasts (data not shown). Culturing mock-infected MRC-5 cells at 33 °C did not affect the level of MHC expression. The increased MHC class I expression in ts9-infected cells was confirmed by metabolic labelling and quantitative immunoprecipitation of MHC I complexes (Fig. 1, right panel) and contrasted markedly to the reduced amounts of heavy chain/β2m complexes found in AD169-infected cells. Transferrin receptor molecules were expressed at comparable densities on the surface of AD169- and ts9-infected cells indicating a selective effect on MHC class I (data not shown). The abundance of free MHC class I heavy chains was only moderately reduced in AD169-infected cells (Fig. 1, left panel), while ts9-infected MRC-5 cells showed unaffected levels of unassembled class I molecules. The induction of MHC class I molecule expression in ts9-infected cultures was due to the paracrine action of IFN-β released upon CMV infection since anti-IFN-β antibodies reduced MHC I surface expression. Comparable expression of viral proteins was seen after infection with both viruses and excluded different kinetics of viral gene expression in ts9-infected cells (data not shown). We conclude that the HCMV deletion mutant ts9 clearly lacks the gene functions that downregulate MHC class I complex formation during permissive infection.

**Peptide translocation into the ER is reduced by early gene functions in HCMV-infected fibroblasts**

Reminiscent of the situation in TAP-deficient cells (Ljunggren et al., 1990; Townsend et al., 1989), MHC class I complexes are unstable in AD169-infected fibroblasts expressing HCMV early genes whereas the abundance of free MHC class I heavy chains is significantly less affected (Beersma et al., 1993; Yamashita et al., 1993; Hengel et al., 1995; see Fig. 1). We therefore reasoned that HCMV may interfere with the transport of peptides into the ER. To quantify TAP activity in MRC-5 fibroblasts, 125I-labelled peptides containing the consensus sequence for N-linked glycosylation, Asn–X–Ser/Thr, were added together with ATP to streptolysin O-permeabilized cells. Following ER translocation, the glycosylated peptide fraction was recovered with Con A–Sepharose (Neeffes et al., 1993b). As depicted in Fig. 2a, two different input peptides were efficiently transported by MRC-5 cells into the ER in an ATP-dependent manner. The peptides RF and RR were transported at similar rates by MRC-5 cells, which is in accordance with the previously reported substrate specificity of human TAP (Momburg et al., 1994b). However, in HCMV-infected cells the efficiency of peptide translocation significantly declined (Fig. 2a). The kinetics shown in Fig. 2 (b) revealed that the transport rates decreased to background levels 96 h post-infection (p.i.) with AD169. Peptide transport was also tested in cells infected with AD169 for 72 h in the...
presence of PAA, a viral DNA polymerase inhibitor that restricts infection to the early phase. Under these infection conditions peptide transport was inhibited to a similar extent compared to permissively AD169-infected MRC-5 cells (Fig. 2a). Essentially the same results were also seen in AD169-infected skin fibroblasts (data not shown). Since the peptide translocation function of TAP was reduced in PAA-treated cells infected with HCMV we concluded that the viral gene responsible for this effect is expressed in the early phase of virus replication.

Like HCMV-infected cells, MCMV-infected fibroblasts expose, at the cell surface, reduced levels of MHC class I molecules associated with the expression of early genes, but the assembly of MHC class I complexes is maintained (Del Val et al., 1992). In contrast to HCMV-infected cells, the efficiency of peptide translocation in MEF in the late phase of MCMV infection 24 h p.i. was not reduced (Fig. 2d). This result demonstrates that HCMV and MCMV interfere with the MHC class I pathway of antigen presentation at different steps and is in agreement with previous findings that document a destabilization of class I complexes in fibroblasts infected with human, but not with mouse, CMV.

**Peptide transport is intact in ts9-infected fibroblasts**

We also tested the transport of peptide R.F during the course of permissive infection with the AD169-derived deletion mutant ts9. Notably, peptide transport by TAP was not affected nor even found to be enhanced in ts9-infected MRC-5 cells (Fig. 2c) and skin fibroblasts (data not shown). Similar results were obtained with the peptides R.R and R.I. These data indicate that a viral gene function essentially involved in the shut-off of TAP-mediated peptide translocation is deleted in ts9 and is most likely localized in the Us region US1-US15.

**HCMV infection induces expression of the TAP1/2 heterodimer that lacks associated MHC class I heavy chains**

Adenovirus 12 interferes with the synthesis of TAP molecules, while HSV-1 turns off the peptide translocation function of TAP (Rotem-Yehudar et al., 1994; Früh et al., 1995; Hill et al., 1995). To decide whether the low amount of glycosylated peptide recovered from HCMV-infected cells was due to decreased levels of TAP synthesis or an inhibition of TAP function, TAP molecules were immunoprecipitated with TAP1- and TAP2-specific antibodies from cell lysates. Precipitation of mock-treated MRC-5 fibroblasts with anti-TAP antibodies resulted in faint bands representing TAP1 and TAP2 chains migrating as 70 and 72 kDa proteins, respectively, in our gel system, and in a significant amount of coprecipitating MHC class I molecules (Fig. 3b, lane 1) devoid of β₂m, which is in contrast to findings in lymphoid cells (Ortmann et al., 1994). Mock-infected MRC-5 cells stimulated with 500 U IFN-γ/ml for 72 h expressed high levels of TAP complexed with MHC heavy chains (Fig. 3b, lane 3). On the other hand, from cells infected with HCMV AD169 for 72 h in the presence of PAA, significantly higher levels of TAP1 and TAP2 were precipitated than from mock-treated fibroblasts, while TAP-associated MHC class I molecules were almost absent (Fig. 3b, lane 2). As expected, assembled MHC class I complexes were significantly diminished in AD169-infected cells also (Fig. 3c). Cells infected with ts9 (Fig. 3, lane 4) expressed elevated levels of TAP molecules which were associated with moderately elevated levels of MHC class I. We
Fig. 2. Expression of HCMV early genes results in an inhibition of peptide transport assayed in vitro with peptides containing a glycosylation signal. (a) Peptide R..F or peptide R..R were translocated for 15 min in the absence or presence of 10 mM-ATP by streptolysin O-permeabilized MRC-5 cells as indicated. Cells were mock-infected or infected with HCMV AD169 (m.o.i., 10) for the periods indicated. Translocated glycosylated peptide was isolated with ConA-Sepharose, quantified, and expressed as the percentage of input peptide. The data represent means of duplicate values. PAA (250 µg/ml) was present during infection to arrest cells in the early phase of infection. (b) A, Kinetics of TAP function assessed with peptide R..F during permissive infection of MRC-5 fibroblasts with AD169 (m.o.i., 10); △, values in the absence of ATP. Transport rates are mean values of duplicate samples. (c) △, Kinetics of TAP function as assessed with peptide R..F during permissive infection of MRC-5 fibroblasts with ts9 (m.o.i., 10) (lane 4) (lane 4) for 72 h. Mock-infected cells shown in lane 3 were exposed to IFN-γ (500 U/ml) for 72 h. Metabolic labelling with [35S]methionine was carried out for 6 h prior to lysis in 1% digitonin lysis buffer. After preclearing of lysates with anti-actin antibodies, the HCMV 72 kDa early (E) protein (a) was precipitated, followed by precipitation with anti-TAP1 MAb 1.28 and anti-TAP2 MAb 2.70 (b). Finally, MHC class I complexes were recovered with MAb W6/32 from the lysates (c).

conclude that HCMV infection does not inhibit but rather enhances the expression of TAP1 and TAP2 molecules, which is in line with data showing augmented mRNA levels for TAP1 and TAP2 in AD169-infected cells (Warren et al., 1994). As demonstrated in Fig. 1 (a), under steady-state conditions free MHC class I heavy chains are clearly present in AD169-infected cells 72 h p.i., although at lower levels than in mock
controls (Beersma et al., 1993; Yamashita et al., 1993; Hengel et al., 1995). The addition of PAA (Fig. 3) somewhat diminishes the downregulation of MHC class I (Hengel et al., 1995). Thus, this result suggests that the TAP/MHC heavy chain association was reduced not only because of a lower overall amount of heavy chains but, in addition, a putative HCMV early gene function which might operate immediately before or at the step of association of MHC class I molecules with TAP during MHC class I maturation.

Expression of the HCMV US11 gene results in MHC class I downregulation but does not affect TAP function

The differences in MHC class I maturation found in ts9 cells compared with AD169-infected cells guided our search for individual functions of HCMV genes located in the gene region that is deleted in ts9 (Kollert-Jöns et al., 1991; Mockenhaupt et al., 1994). Interestingly, Jones et al. (1995) reported that a gene lost in ts9, US11, is able and sufficient to downregulate MHC class I expression. Therefore, we cloned US11 into the expression vector pcDNAI and selected stably transfected clones of human 293 cells. A representative number of cell clones was analysed by FACS for MHC class I surface expression. US11-transfected 293 cells expressed transferrin receptor molecules or ICAM-1 at levels identical to parental 293 cells, indicating that US11 exerts a selective effect on MHC class I (data not shown). Cytofluorometric analysis of 293-US11 transfectants revealed a significant reduction of MHC class I surface expression ranging between 50% and 90% (Fig. 4). Next, we tested whether expression of US11

![Graphical representation of fluorescence intensity values](image-url)
affects the transport of peptides by TAP. Parental 293 cells transported peptides with a moderate efficiency compared to MRC-5 fibroblasts (Fig. 5a). For the different 293–US11 clones tested, minor clonal variations of transport rates were observed, but no impairment of peptide transport occurred as seen after infection with HCMV AD169 (Fig. 5b–e). Thus, US11 is sufficient to downregulate MHC class I surface expression, but does not affect the peptide transport function of TAP.

Discussion

During HCMV infection, the assembly of MHC class I complexes is downregulated and antigen presentation is abolished (Beersma et al., 1993; Warren et al., 1994; Yamashita et al., 1993; Hengel et al., 1995). This immuno-evasive action is under the control of HCMV early genes. Recently, one mechanism by which the MHC class I pathway of antigen processing and presentation is affected has been shown to be mediated by US11 (Jones et al., 1995). The US11 gene product has the remarkable function of mediating selective proteolysis of MHC class I heavy chains by exporting them from the ER back to the cytosol where they are degraded by the proteasome (Wiertz et al., 1996). Here we demonstrate that the strategy of interference by HCMV with the MHC class I pathway of antigen presentation is more complex and includes a second and independent mechanism. During the course of permissive HCMV infection the peptide translocation function of TAP1/2, as measured by an in vivo assay, is progressively downregulated by viral early gene functions that operate at the post-translational level. This inhibition of TAP restricts the supply of cytosolic peptides to the ER and can consequently destabilize MHC class I molecules and limit the formation of stable ternary MHC class I complexes available for transport to the plasma membrane. The US11-encoded early glycoprotein reduces MHC class I expression by a mechanism independent of peptide translocation. Neither function is expressed by the HCMV AD169-derived deletion mutant ts9.

The redundancy of mechanistically different MHC class I reactive genes is a remarkable trait of CMV biology. Based on a number of HCMV deletion mutants (Jones & Muzithras, 1992), Jones et al. (1995) recently identified two regions within the HCMV genome that affect the level of MHC class I heavy chain expression. One region is represented by US11; the other region is within the US2–US5 open reading frames of HCMV AD169. Our data confirm this mapping of MHC class I-specific effects since both regions identified by Jones et al. are also deleted in ts9. Together with US11 and the putative inhibitor of peptide translocation, they downregulate MHC class I during the course of permissive HCMV infection. This may explain the different kinetics observed for the reduction of MHC class I compared to the kinetics of reduced peptide translocation by TAP1/2 in AD169-infected cells. At 72 h p.i., the degradation of free MHC class I heavy chains in the ER is incomplete and inhibition of TAP reaches a maximum. It is tempting to speculate that in this situation a second, US11-independent mechanism is essential for the virus to keep MHC class I assembly under control.

Translocation of peptides into the ER of ts9-infected cells was found to be intact. This locates the interfering viral function in AD169 most likely to the gene region deleted in ts9, i.e. between US1–US15. The generation of ts9 by nitrosoguanidinium mutagenesis, however, does not exclude the possibility that the gene function responsible may reside outside of US1–US15, although extensive restriction pattern analysis of the ts9 genome did not suggest further loss of genes in ts9 (Kollert-Jøns et al., 1991; Mockenhaupt et al., 1994).

According to current concepts of MHC class I assembly as studied in lymphoid cells (Degen & Williams, 1991; Neefjes et al., 1993 a, b; Nößner & Parham, 1995; Ortmann et al., 1994; Suh et al., 1994) the chaperon calnexin mediates class I heavy chain/α1m dimerization. The subsequent association of the MHC heterodimer with TAP molecules may then facilitate capture of TAP-translocated peptides. In the MHC class I-deficient lymphoblastoid cell line 721.220 a genetic defect causes certain class I heterodimers to fail to associate with TAP without affecting the peptide transport function of TAP. This is due to a loss of function of an unidentified gene linked to the MHC (Grandea et al., 1995). It therefore appears that TAP is involved in the formation of ternary MHC class I complexes at two steps. Firstly by the association of MHC class I heterodimers and secondly by translocating peptides into the ER. Obviously, the sequence of events leading to ternary MHC class I complexes differs in fibroblasts from B cells as MHC class I heavy chains directly associate with TAP1/2 without prior association with β2m (see Fig. 3b). In digeitinin extracts of AD169-infected fibroblasts TAP molecules are only weakly, if at all, associated with MHC heavy chains. The failure of MHC class I to associate with TAP could be explained by the action of the US11 glycoprotein and/or further MHC-reactive proteins of HCMV. By reducing the steady state levels of both unassembled and complexed MHC class I molecules in 293 cells (H. Hengel, unpublished observation) the US11 gene product may also reduce the number of class I heavy chains available for binding to TAP. Given the premise that the unknown gene deleted in 721.220 cells is also required for the MHC/TAP interaction in fibroblasts, this function may represent a potential target for a HCMV protein. Finally, our data do not rule out the possibility that the HCMV-encoded unknown inhibitor of peptide translocation may also interfere with the association of MHC class I to TAP. If correct, it is a remarkable feature of HCMV to focus on both functions of TAP, namely the transport of peptides as well as the chaperon function for MHC class I stabilization. Both functions of the virus may synergize in their effect on MHC class I destabilization and thus prevent the assembly of ternary class I complexes.
For a virus that is under the control of the MHC class I-restricted cellular immune response (Reddehase et al., 1987; Riddell et al., 1992), interference with the formation of MHC class I complexes by independent but synergistic mechanisms is clearly the most effective strategy that can be envisaged. Interestingly, multiple independent early gene functions are also used by the closely related MCMV to interfere with the MHC class I pathway (Thäle et al., 1995). Despite the high degree of genomic and biological similarity, the immunoevasive mechanisms active in the two viruses are distinct, although both target MHC class I function. While HCMV targets assembly of MHC class I complexes in the ER, MCMV has evolved functions that operate at a later step. In MCMV, the formation of ternary class I complexes is retained while intracellular transport and cell surface residence of MHC class I are affected at a number of steps (Del Val et al., 1992; Thäle et al., 1995). The fact that MCMV-infected cells, unlike HCMV-infected cells, translocate peptide in constant amounts into the ER confirms and supports our previous finding. Thus, CMVs modulate the MHC class I antigen presentation pathway by different means. Nevertheless, the intervention mechanisms of both CMVs eventually destroy the unique function of MHC class I molecules to present endogenous peptides (Del Val et al., 1989; Hengel et al., 1995). Again, it is common to both viruses that the defective presentation function of infected fibroblasts can be restored by IFN-γ (Hengel et al., 1994, 1995). This is also true for the distinct step of inhibited TAP-mediated peptide transport which can be rescued by preincubation of cells with IFN-γ (H. Hengel and F. Momburg, unpublished observation).

Viruses use diverse mechanisms to subvert the process of class I-restricted antigen presentation (Hill & Ploegh, 1995). Recently developed techniques for the study of TAP-dependent peptide translocation established that the essential function of TAP is to translocate antigenic peptides to the ER (Androlewicz et al., 1993; Momburg et al., 1994a; Momburg et al., 1994b; Shepard et al., 1993). Therefore, it is not surprising that TAP is a preferred target of certain viruses that limit presentation to CD8+ T lymphocytes and establish chronic infections. Examples of such viruses are herpes simplex virus type-1 (Hill et al., 1995; Früh et al., 1995) and adenovirus type 12 (Rotem-Yehudar et al., 1994). Unexpectedly, human fibroblasts like MRC-5 are very efficient regarding their in vitro TAP activity compared to lymphoid tumour cells (Momburg et al., 1994a; Momburg et al., 1994b; F. Momburg, unpublished observation). Therefore, it might be a selective advantage for human herpesviruses like HSV and HCMV, which replicate in this cellular compartment in vivo, tocounter TAP. While adenovirus affects TAP gene or protein expression, HSV-1 interferes by means of the US12-encoded cytosolic immediate-early protein ICP47, which binds physically to TAP (Hill et al., 1995; Früh et al., 1995). Interaction of ICP47 with TAP prevents peptide translocation across the ER membrane; the resulting MHC class I molecules are retained in the ER, but can be stabilized by addition of peptides to detergent extracts (York et al., 1994). The assay we used to quantify the peptide translocation efficacy of TAP depends on the N-linked glycosylation of peptides in the ER. N-linked glycosylation is not generally affected in HCMV-infected cells, and peptides linked to a large glycan are known to reside stably in this compartment (Momburg et al., 1994a), implying that lower rates of translocated peptide cannot be attributed to a putative export of peptides in HCMV-infected cells. Permeabilization of HCMV-infected cells and repeated washes prior to the peptide translocation assay did not abrogate the inhibitory viral function. A putative cytosolic inhibitor should therefore possess a high binding affinity for TAP. Alternatively, the TAP inhibitor could be an ER-resident glycoprotein like US11 (Jones et al., 1995), and thus be resistant to cytosol depletion by washing. The identification of this viral regulator of peptide translocation will not only contribute to our knowledge of the particular CMV-host relationship but could also be instrumental for our understanding of TAP1/2.

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