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The Varicellovirus UL49.5 Protein Blocks the Transporter Associated with Antigen Processing (TAP) by Inhibiting Essential Conformational Transitions in the 6+6 Transmembrane TAP Core Complex

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TAP translocates virus-derived peptides from the cytosol into the endoplasmic reticulum, where the peptides are loaded onto MHC class I molecules. This process is crucial for the detection of virus-infected cells by CTL that recognize the MHC class I-peptide complexes at the cell surface. The varicellovirus bovine herpesvirus 1 encodes a protein, UL49.5, that acts as a potent inhibitor of TAP. UL49.5 acts in two ways, as follows: 1) by blocking conformational changes of TAP required for the translocation of peptides into the endoplasmic reticulum, and 2) by targeting TAP1 and TAP2 for proteasomal degradation. At present, it is unknown whether UL49.5 interacts with TAP1, TAP2, or both. The contribution of other members of the peptide-loading complex has not been established. Using TAP-deficient cells reconstituted with wild-type and recombinant forms of TAP1 and TAP2, TAP was defined as the prime target of UL49.5 within the peptide-loading complex. The presence of TAP1 and TAP2 was required for efficient interaction with UL49.5. Using deletion mutants of TAP1 and TAP2, the 6+6 transmembrane core complex of TAP was shown to be sufficient for UL49.5 to interact with TAP and block its function. However, UL49.5-induced inhibition of peptide transport was most efficient in cells expressing full-length TAP1 and TAP2. Inhibition of TAP by UL49.5 appeared to be independent of the presence of other peptide-loading complex components, including tapasin. These results demonstrate that UL49.5 acts directly on the 6+6 transmembrane TAP core complex of TAP by blocking essential conformational transitions required for peptide transport.

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5 Abbreviations used in this paper: ER, endoplasmic reticulum; ΔN, N-terminally truncated; β₂m, β₂-microglobulin; huTAP, human TAP; MDR, multiple drug resistance; NGFR, nerve growth factor receptor; PLC, peptide-loading complex; TM, transmembrane.

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herpesvirus 1 (BHV-1), strong CD8⁺ T cell responses directed against various glycoproteins were found in BHV-1-infected calves (21). Following reactivation of varicella-zoster virus, high frequencies of circulating T cells recognizing a variety of structural and regulatory proteins were found (22, 23). Strong CD8⁺ T cell responses to the tegument phosphoprotein 65 and the immediate early protein 1 were found for the β-herpesvirus human CMV (24, 25). In healthy carriers of the γ-herpesvirus EBV, a considerable proportion of the peripheral T cell repertoire is directed against EBV-encoded Ags, predominantly derived from latent and immediate early viral proteins (26). Thus, despite the presence of a fully competent immune system mounting a powerful response against herpesvirus infections, these viruses fail to be eliminated. Most likely, this is related to the expression of specific viral immune evasion proteins, efficiently preventing detection and elimination of the virus-producing cells by the immune system. TAP appears to represent a favorite target for herpesvirus immune evasion strategies. The α-herpesviruses HSV-1 and HSV-2 encode the ICP47 protein, which blocks peptide binding to the cytosolic side of TAP (27–31). The US6 protein encoded by human CMV inhibits TAP by preventing ATP binding to TAP (32–36). The EBV-encoded BNLF2a, which is unrelated to ICP47 and US6, inhibits TAP by blocking both ATP and peptide binding (37).

Koppers-Lalic et al. (38) identified the UL49.5 gene product of BHV-1 as a powerful inhibitor of TAP. Although all herpesviruses encode a homologue of the UL49.5 protein (also called glycoprotein N), inhibition of TAP by UL49.5 appears to be restricted to a subset of the genus Varicelloviruses, including bovine herpesvirus 1 (BHV-1), pseudorabies virus, equine herpesvirus 1 and 4, and canine herpesvirus (38, 39). ICP47, US6, BNLF2a, and UL49.5 are structurally unrelated, and the molecular mechanisms through which they block TAP are different. The BHV-1-encoded UL49.5 protein does not interfere with ATP or peptide binding to TAP. Instead, UL49.5 causes a conformational arrest of the TAP complex, thereby preventing translocation of peptides over the ER membrane (38). Additionally, TAP1 and TAP2 are targeted for degradation via the ubiquitin-proteasome system (38). The direct interaction partner of UL49.5 within the PLC has still to be defined. Tapasin may play a role in UL49.5-related instability of TAP, because this molecule contributes essentially to the stability of TAP.

To identify the proteins within the PLC that are targeted by UL49.5, the function of the viral protein was evaluated in cells lacking various components of the PLC, including TAP1, TAP2, and tapasin. Efficient interaction with the PLC and inhibition of peptide transport required the presence of both TAP1 and TAP2; tapasin was dispensable. To study the molecular interaction between TAP and UL49.5 in more detail, the TAP-deficient T cells were reconstituted with recombinant TAP proteins carrying N-terminal deletions. The inhibition of TAP by UL49.5 appeared to involve the minimal functional entity of TAP, including the C-terminal 6 TM helices of TAP1 and TAP2. Inhibition was, however, most efficient in the presence of full-length TAP2 next to truncated TAP1. The coexpression of UL49.5 with TAP1 and TAP2 in insect cells resulted in inhibition of peptide transport, indicating that all other constituents of the PLC were dispensable for UL49.5-mediated inhibition of TAP.

**Materials and Methods**

**DNA constructs**

UL49.5 was amplified from viral DNA and cloned into the pLZRS vector, as described (38). The pLZRS vector information can be found at www.stanford.edu/group/nolan/retroviral_systems/retsys.html.

**Cell lines and recombinant viruses**

The human melanoma cell line Mel JuSo has been transfected to stably express human TAP (hTAP)-1-GFP (40), as described. The EBV-transformed lymphoblastoid cell line 721.220 lacks a functional tapasin protein (13, 41). The 721.220 cells transfected to stably express HLA-B44.05 (referred to as as.220) or to stably express HLA-B44.05 and human wild-type tapasin (referred to as as.220 tapasin) were used in this study (cell lines were provided by A. Williams, Cancer Sciences Division, University of Southampton School of Medicine, Southampton, U.K.). T2 cells do not express TAP1, TAP2, and MHC class II due to a deletion within the region of chromosome 6 carrying the loci for MHC class II and related genes (42). T2 cells were transfected to express TAP constructs of rat origin (20, 43). The cell lines were maintained in RPMI 1640 medium supplemented with 10% FCS and antibodies.

STF1.169 cells are immortalized fibroblasts isolated from a TAP2-deficient patient (44). The cells were immortalized using a plasmid expressing T and t SV-40 Ags (Stu-BamHI fragment from the SV40 genome) under the control of the EIIA promoter of adenovirus 2 (Sma-HindIII fragment), human telomerase under the control of long terminal repeat of Abelson leukemia virus (fragment Nhel-Vsp1 from pGRN45; provided by Geron), and a hygromycin resistance gene. Immortalized cells were selected using 25 µg/ml hygromycin. The STF1.169 cells were complemented with a TAP2 gene of human origin cloned into pRRES-neo (BD Clontech). Transfected cells were selected with 250 µg/ml G418. STF1.169 cells and STF1.169 cells reconstituted with TAP2 (referred to as STF1 and STF1-TAP2, respectively) were maintained in DMEM supplemented with 10% FCS and antibodies.

Recombinant viruses were made using the Phoenix amphotropic packaging system, as described before (www.stanford.edu/group/nolan/retroviral_systems/retsys.html). STF-1 cells, 220 cells, MJS TAP1-GFP cells, and the various T2 cell lines were transfected with recombinant retroviruses to generate the following stable cell lines: STF1 UL49.5 and STF1-TAP2 UL49.5 (containing BHV-1 UL49.5, GFP⁺⁻); MJS TAP1-GFP UL49.5 (containing BHV-1 UL49.5, Δ nerve growth factor receptor [NGFR⁺⁻]); 220 UL49.5, 220 tapasin UL49.5, T2 rat TAP1 UL49.5, T2 rat TAP1 rat TAP2 UL49.5, T2 rat TAP1 rat TAP2 Δ tapasin UL49.5, T2 rat TAP1ΔTAP2 UL49.5, T2 tandem rat TAP1 rat TAP2 Δ tapasin UL49.5, and T2 tandem rat TAP1ΔTAP2 Δ tapasin UL49.5 (all containing BHV-1 UL49.5, GFP⁺⁻). Transduced cells expressing GFP or ANGFR⁺⁻ were selected using a FACS Vantage cell sorter (BD Biosciences). The T2 cells were transfected with a retroviral vector encoding a mutant form of UL49.5, in which the two lysine residues within the cytoplasmic tail were replaced by alanines. This mutation did not affect UL49.5-mediated inhibition and degradation of TAP, but stabilized the UL49.5 protein (M. Verweij, D. Koppers-Lalic, and E. Wiertz, unpublished observation). Moreover, exchange of both lysines to arginines led to the same phenotype in S9 cells (S. Loch, J. Koch, and R. Tampé, unpublished observation). The enhanced expression of mutated UL49.5 is particularly useful in T2 cells, because these cells are difficult to transfect or transduce.

Insect cells (Spodoptera frugiperda, S9) were grown in S9000 medium (Life Technologies). S9 cells were infected with recombinant baculoviruses following standard procedures to stably express full-length lysines to arginines in the ErbB protein and the biotin receptor (45). For coinfections, a multiplicity of infection of 3 was used for the baculoviruses encoding the TAP constructs and UL49.5. The cloning of truncated TAP variants and the production of recombinant baculoviruses expressing the TAP constructs have been described before (7).

**Antibodies**

The following Abs were used: anti-human MHC class I H chain mAb HC-10 (a gift from H. Ploegh, Whitehead Institute for Biomedical Research, Cambridge, MA), anti-human MHC class II mAb StuI-III fragment (46), anti-HLA A2 mAb BB7.2 (47), human anti-HLA Cw1 mAb VP6G3 (48), human anti-HLA B5 mAb GVX1007 (the product of a human hybridoma established from the B lymphocytes of a multiparous woman with anti-HLA B5 serum Abs, by methodology described in Ref. 48), anti-GFP (49), anti-huTAP1 mAb 148.3 (50, 51), anti-huTAP2 mAb 435.3 (52) (provided by P. van Endert, Faculté de Médecine René Descartes, Paris, France); goat anti-rat TAP1 M18, anti-rat TAP2 M9 (Santa Cruz Biotechnology, Inc., CA); rabbit-anti-rat TAP1 D90 (53) (used for immunoprecipitation), anti-rat TAP2 mAb MAC394 (54) (used for Western blotting), sheep anti-rat TAP2 S635 (provided by S. Powis, Bute Medical School, University of St. Andrews, St. Andrews, United Kingdom; used for immunoprecipitation), rat anti-human tapasin mAb 7F6 (55), rabbit-anti-human ERp57 (a gift from S. High, Faculty of Life Sciences, University of St. Andrews, St. Andrews, U.K.), and anti-β-m mAb BBM.1 (55) (provided by J. Neeffjes, Department of Tumor Biology, The Netherlands Cancer Institute, Amsterdam, The Netherlands). In addition, the conjugated Abs W6/32-PE
(Serotec), anti-MHC class II L243-PE (BD Pharmingen), and anti-NGFR-biotin (BD Pharmingen) were used. For detection of UL49.5, a rabbit polyclonal anti-serum (H11) was raised using a synthetic peptide derived from the N-terminal domain of BHV-1 UL49.5 as an Ag (56). mAbs against human transferrin receptor (CD71; BD Biosciences) and human transferrin receptor (H68.4; Roche Diagnostics) were used as controls.

Flow cytometry

Surface levels of MHC class I molecules and control proteins were determined using the specific primary Abs indicated. Bound Abs, if not conjugated to PE, were stained using rabbit anti-human Ig-PE or goat anti-mouse Ig-allophycocyanin. NGFR-biotin was detected using streptavidin-allophycocyanin (BD Pharmingen). Stained cells were measured using a FACSCalibur (BD Biosciences) and analyzed using CellQuest software.

Immunoprecipitations and Western blotting

For immunoprecipitations, cells were dissolved in lysis buffer containing 1% (w/v) digitonin, 50 mM Tris-HCl (pH 7.5), 5 mM MgCl₂, 150 mM NaCl, 1 mM leupeptin, and 1 mM 4-(2-aminoethyl)benzenesulfonyl fluoride, and lysates were incubated with specific Abs, as indicated, and protein G- and A-Sepharose beads (GE Healthcare) were used to isolate the immune complexes. Precipitated immune complexes and 1% Nonidet P-40 lysates of the cells were separated by SDS-PAGE and transferred to polyvinylidene difluoride membranes (GE Healthcare). UL49.5 was separated using 16.5%-tricine PAGE. The blots were incubated with the indicated Abs, followed by HRP-conjugated secondary Abs (DakoCytomation and Jackson ImmunoResearch Laboratories). Bound HRP-labeled Abs were visualized using ECL Plus (GE Healthcare).

Peptide transport assay

Cells were permeabilized using Streptolysin-O (Murex Diagnostics) at 37°C for 10 min. Permeabilized cells were incubated with the fluorescein-conjugated synthetic peptide CVNKTERAY (N-core glycosylation site underlined) in the presence or absence of ATP (10 mM final concentration) at 37°C for 10 min. Peptide translocation was terminated by adding 1 ml of ice-cold lysis buffer (1% Triton X-100, 500 mM NaCl, 2 mM MgCl₂, and 50 mM Tris-HCl (pH 8.0)). After lysis, cell debris was removed by centrifugation. Supernatants were collected and incubated with 100 μl of Con A-Sepharose beads (Amersham Pharmacia) at 4°C for 2 h to isolate glycosylated peptides. After extensive washing of the beads, peptides were eluted with elution buffer (500 mM mannopyranoside, 10 mM EDTA, and 50 mM Tris-HCl (pH 8.0)) by vigorous shaking at 25°C for 1 h. The fluorescence intensity was measured using a fluorescence plate reader (CytoFluor, Applied Biosystems or Berthold Technologies; excitation 485 nm/emission 530 nm). Peptide transport is expressed as percentage of translocation, relative to the translocation observed in control cells (set at 100%).

Peptide transport in microsomes derived from Sf9 cells

Microsomes were prepared, as described (45), and incubated with the fluorescein-(F)-labeled peptide RRYQNSTCFL (N-linked glycosylation site is underlined) in the presence or absence of ATP for 3 min at 32°C. Peptide transport was terminated with stop buffer (PBS and 10 mM EDTA (pH 7.0)) on ice. After centrifugation, membranes were lysed, and the supernatant was incubated with 100 μl of Con A-Sepharose beads (Sigma-Aldrich) overnight at 4°C. The samples were treated according to the procedure described above. Fluorescence intensity was measured using a fluorescence plate reader (Polarstar Galaxy; BMG Labtech).

Results

Consequences of UL49.5 interaction with the MHC class I PLC

UL49.5 blocks the import of peptides into the ER by interfering with essential conformational transitions of TAP required for coprecipitating proteins were analyzed by SDS-PAGE and Western blotting (WB) using Abs against TAP1, TAP2, MHC class I H chain (HC), β₂m, tapasin, Erp57, and UL49.5. The relative amount of the PLC compounds detected in UL49.5-containing cells was expressed relative to the amount in control cells (set at 100%). C, UL49.5 was immunoprecipitated from the MJS TAP1-GFP cells, and the resulting complexes were analyzed for the presence of TAP1, TAP2, tapasin, and UL49.5 using specific Abs. One representative experiment of two independent experiments is shown.
translocation of peptides over the ER membrane (38). Additionally, both TAP subunits are degraded by the ubiquitin-proteasome system (38). In cells expressing a TAP1-GFP fusion protein, degradation of the TAP complex is not observed, but peptide transport is still blocked (38). To study the interactions of UL49.5 with the PLC, MJS cells expressing TAP1-GFP were used to prevent UL49.5-mediated degradation of TAP1 and TAP2. The expression of UL49.5 in the MJS TAP1-GFP cells resulted in inhibition of peptide transport and down-regulation of MHC class I molecules at the cell surface (Fig. 1A, left panel). The expression of MHC class II molecules was not affected (Fig. 1A, right panel). The composition of the MHC class I PLC was evaluated in these cells. A proper composition of the PLC is essential for the stability of its individual components. For example, dissociation of TAP1 from the complex will result in destabilization of TAP2 (57). Alternatively, if UL49.5 would expel tapasin from the PLC, this would destabilize both TAP subunits (15–18). PLCs were isolated from digitonin-solubilized MJS TAP1-GFP cells using Abs against GFP. The presence of TAP1, TAP2, MHC class I complexes, tapasin, ERp57, and UL49.5 was evaluated by Western blot analysis. Similar amounts of TAP1, TAP2, and ERp57 appeared to be present in PLCs from control cells and UL49.5-expressing cells (Fig. 1B). In the latter, UL49.5 was part of the PLCs. The levels of MHC class I H chain and β2m molecules were ~1.5 times higher in UL49.5-containing complexes compared with control complexes (Fig. 1B). This increase is probably resulting from the reduction of peptide supply to MHC class I molecules caused by UL49.5, which leads to reduced maturation and prolonged association of MHC class I complexes with the PLC. The levels of tapasin seemed slightly lower in the UL49.5-containing complexes (Fig. 1B, panel 5). This reduction was repeatedly observed. The role of tapasin in UL49.5-induced inhibition of TAP is investigated in more detail below. Theoretically, UL49.5 could coimmunoprecipitate with incomplete PLCs, whereas the complete set of PLC components could be coisolated with PLCs lacking UL49.5. To exclude this possibility, UL49.5-containing PLCs were isolated using Abs directed against UL49.5 and probed for the presence of PLC components. TAP1, TAP2, and tapasin were found to coprecipitate with UL49.5 (Fig. 1B, panel 5). This reduction was repeatedly observed.

**FIGURE 2.** Tapasin stabilizes the TAP complex, but is not involved in UL49.5-mediated inhibition of TAP function. A, Surface MHC class I expression on tapasin-deficient .220 and the tapasin-reconstituted .220 tapasin cells was assessed after staining with the HLA-Cw1-specific Ab VP 6G3; .220 cells incubated with secondary Ab only (graph 1), .220 control cells stained with VG 6G3 (graph 2), and UL49.5-expressing .220 cells stained with VG 6G3 (graph 3). B, Transport activity of TAP was analyzed in .220 cells using the peptide CVNKTERAY, of which the cysteine was labeled with fluorescein. The assay was performed in the presence of 10 mM ATP (■) or EDTA (□). Peptide transport is expressed as percentage of translocation, relative to the translocation observed in control cells (set at 100%). C, Degradation of TAP is not observed in tapasin-deficient .220 cells. The influence of UL49.5 on expression levels of TAP1, TAP2, and transferrin receptor (TfR) in .220 and .220 tapasin cells was assessed by SDS-PAGE and Western blotting using specific Abs. D, UL49.5 can interact with the TAP complex independent of the presence of tapasin. TAP1 was immunoprecipitated (IP) from both .220 cell lines, and precipitated complexes were analyzed for the presence of TAP1 and UL49.5. One representative experiment of two independent experiments is shown.
tapasin might play a role in the UL49.5-induced inhibition of TAP and, consequently, the down-regulation of surface MHC class I expression. To investigate this possibility, we made use of cells lacking a functional tapasin protein, the lymphoblastoid cell line 721.220 (referred to as .220) (13, 41) and a .220 cell line reconstituted with tapasin (referred to as .220 tapasin). Both .220 cell lines were retrovirally transduced to express UL49.5, and the influence of the viral protein on MHC class I surface expression was assessed using flow cytometry. A clear UL49.5-induced reduction of MHC class I surface expression was detected (Fig. 2A). Interestingly, the MHC class I level on the .220 cells reconstituted with tapasin is visibly higher compared with the level on tapasin-deficient .220 cells (Fig. 2A; compare right and left panels). This probably reflects tapasin-induced stabilization of TAP and, consequently, enhanced peptide-loading and cell surface expression of MHC class I molecules. The influence of tapasin on the stabilization of TAP is reflected by enhanced TAP transport in .220 tapasin cells (Fig. 2B). UL49.5 was found to block peptide transport in both .220 and .220 tapasin cells (Fig. 2B). These results imply that tapasin is dispensable for UL49.5-mediated inhibition of TAP function.

To investigate whether UL49.5 mediates TAP degradation in the absence of tapasin, steady-state levels of TAP1 and TAP2 were assessed in .220 and .220 tapasin cells through Western blot analysis. The presence of tapasin resulted in elevated levels of TAP1 and TAP2 (Fig. 2C, panels 1 and 2; compare lanes 1 and 3). Despite the apparent presence of UL49.5 (Fig. 2C, panel 3, lane 2), no degradation of TAP was detected in .220 cells (Fig. 2C, panels 1 and 2; compare lanes 1 and 2). However, the UL49.5 protein did induce TAP degradation in .220 tapasin cells (Fig. 2C, panels 1 and 2; compare lanes 3 and 4). To control for equal loading, all lysates were stained for transferrin receptor (Fig. 2C, panel 4). The absence of TAP degradation in the UL49.5-expressing .220 cells lacking tapasin suggests that tapasin is needed for UL49.5 to induce degradation of TAP. However, the TAP levels in the .220 cells are much lower than the TAP levels in .220 tapasin cells (Fig. 2C, panels 1 and 2; compare lanes 1 and 3), because TAP is unstable in the absence of tapasin. Thus, it is also possible that the degradation of TAP occurring in tapasin-negative cells cannot be further enhanced by the UL49.5 protein, and therefore, no difference in TAP degradation is observed between UL49.5-expressing and wild-type .220 cells. Note that peptide transport is inhibited in the wild-type .220 cells, despite the fact that UL49.5 does not affect TAP steady-state levels in these cells. This is in agreement with previous observations in cells expressing the TAPI-GFP fusion protein or UL49.5Δtail, which does not cause degradation of TAP, but still inhibits peptide transport (38).

To assess whether UL49.5 binds to TAP in the absence of functional tapasin, TAP complexes were isolated from .220 and .220 tapasin cells solubilized in digitonin and stained for the presence of UL49.5 (Fig. 2D). The viral protein was found to interact with the TAP complex in both cell lines, indicating that the interaction between UL49.5 and TAP is independent of the presence of functional tapasin.

In conclusion, functional tapasin is dispensable for UL49.5-induced inhibition of peptide transport and the reduction of MHC class I surface expression. Even though UL49.5 is still able to interact with the TAP complex, degradation of TAP1 and TAP2 is not accelerated in cells lacking tapasin.

The TAP1-TAP2 heterodimer is required for the association of UL49.5 with the PLC

UL49.5 interacts with the PLC (Fig. 1) (38), but its interaction partner within this multimeric complex has still to be identified. To investigate whether UL49.5 interacts with one of the TAP subunits, we made use of STF1 cells. STF1 cells are immortalized fibroblasts isolated from an MHC class I-deficient patient (44). The cells carry a mutation within the TAP2 gene, resulting in the deletion of the C-terminal 413 aa residues. STF1 cells and TAP2-reconstituted STF1-TAP2 cells were retrovirally transduced to express the UL49.5 protein. The influence of UL49.5 on MHC class I levels in these cells was evaluated using flow cytometry. In the retroviral vector encoding UL49.5, the viral protein was placed in front of an internal ribosomal entry site that is followed by enhanced GFP. As a result, UL49.5-expressing cells can be detected as a GFP-positive population. The UL49.5/GFP-expressing cells were mixed with untransduced control cells. Thus, MHC class I surface expression can be compared between UL49.5-expressing and control cells in one assay (Fig. 3A). Surface MHC class I expression on UL49.5-expressing STF1 cells was significantly up-regulated when

FIGURE 3. The TAPI-TAP2 heterodimer is required for association of UL49.5. A, UL49.5-induced down-regulation of surface MHC class I can only be detected in cells expressing full-length TAP1 and TAP2. The TAP2-deficient STF1 cells and the reconstituted STF1-TAP2 cells were transduced with UL49.5/GFP-encoding retrovirus. The transduced cells were mixed with untransduced, GFP-negative cells to facilitate comparison of expression of MHC class I molecules. Surface MHC class I was stained using the Ab W6/32 and analyzed using flow cytometry. B, UL49.5 does not interact with TAP in STF1 cells lacking TAP2 expression. TAPI or UL49.5 was immunoprecipitated (IP) from cell lysates, and coprecipitating TAP1 or TAP2 was assessed using Western blotting (WB) using the Abs indicated. One representative experiment of two independent experiments is shown.
FIGURE 4.  UL49.5 inhibits the rat TAP complex expressed in T2 cells.  A, Transport activity of TAP was assessed in T2 cells expressing rat TAP1 and rat TAP2, in the presence or absence of UL49.5.  Translocation of the peptide CVNKTERAY, of which the cysteine was labeled with fluorescein, was evaluated in the presence of 10 mM ATP (■) or EDTA (□).  Peptide transport is expressed as percentage of translocation, relative to the translocation observed in control cells (set as 100%).  B, UL49.5-mediated down-regulation of MHC class I expression is not allele specific.  T2 rat TAP1 rat TAP2 cells, with (graph 4) or without UL49.5 (graph 3), were stained for surface MHC class I molecules using the Abs indicated.  Graph 2, MHC class I levels on untransduced T2 cells.  Graph 1, Background staining in the presence of secondary Ab only.  C, The expression levels of rat TAP1 and UL49.5 in T2 cells.  T2 cells expressing TAP1 or both TAP1 and TAP2, in the presence of absence of UL49.5, were analyzed for steady-state levels of TAP1 and UL49.5 by SDS-PAGE and Western blotting (WB) using specific Abs.  D, UL49.5 interacts with rat TAP1 in the absence of TAP2.  TAP1 was immunoprecipitated (IP) from the same cells, and coprecipitating proteins were analyzed for the presence of TAP1 and UL49.5.  E, Steady-state levels of rat TAP2 and UL49.5 in T2 cells.  T2 cells expressing rat TAP2 or both rat TAP1 and rat TAP2, in the presence or absence of UL49.5, were lysed, and the levels of rat TAP1, rat TAP2, and UL49.5 were determined by SDS-PAGE and Western blotting (WB) using specific Abs.  F, UL49.5 interacts with rat TAP2 in the absence of TAP1.  TAP2 was immunoprecipitated (IP) from cell lysates, and coprecipitating proteins were analyzed using specific Abs.  For the cells expressing TAP2 in isolation, 4 times more cells were used for IP to compensate for lower expression levels of TAP2.  The ratio between UL49.5 and TAP was expressed relative to the amount of UL49.5 coprecipitating with rat TAP1/rat TAP2 complexes (D and F, panel 4, set at 100%).  Tpsn, Tapasin.
TAP2 was coexpressed (Fig. 3A; compare the GFP-negative cell populations in left and right diagrams). UL49.5-induced down-regulation of surface MHC class I was only detected on cells expressing both TAP1 and TAP2 (Fig. 3A, right diagram).

To evaluate the ability of UL49.5 to interact with TAP, cells were lysed in the presence of digitonin. TAP1 and UL49.5 were immunoprecipitated, and the immune complexes were probed for the presence of UL49.5 or TAP1 by Western blot analysis. In the absence of TAP2, TAP1 steady-state protein levels were similar in control and UL49.5-expressing STF1 cells (Fig. 3B, first panel, lanes 1 and 2). This indicates that UL49.5 does not destabilize TAP1 in the absence of full-length TAP2. In STF1 cells expressing TAP1 and TAP2, TAP1 levels were considerably reduced in the presence of UL49.5, reflecting UL49.5-induced degradation of TAP (Fig. 3B, first panel, lanes 3 and 4). Despite the low levels of TAP in the UL49.5-expressing STF1-TAP2 cells, an interaction between TAP and UL49.5 could be detected in these cells (Fig. 3B, second panel, lane 4). This interaction was absent in STF1 cells (Fig. 3B, second panel, lane 2). The lack of interaction was not due to lower levels of UL49.5 in STF1 cells, because similar amounts of UL49.5 were detectable in STF1 and STF1-TAP2 cells (Fig. 3B, third panel). Again, an interaction between TAP1 and UL49.5 was only found in the STF1-TAP2 cells (Fig. 3B, fourth panel, lane 4). These data indicate that the presence of TAP1 and TAP2 is necessary for UL49.5 to efficiently interact with the TAP complex.

UL49.5 efficiently interacts with rat TAP and inhibits peptide transport

Based on previous N-terminal truncations of huTAP1 and huTAP2, leading to the identification of a functional 6 + 6 TM core complex (7), a unique collection of rat TAP proteins has been constructed that are very suitable to characterize the interaction sites for UL49.5 within the TAP complex (20, 59). The UL49.5 protein is able to prevent peptide transport by murine TAP as well as huTAP (38, 60). Therefore, it is likely that UL49.5 can also block rat TAP. Indeed, expression of UL49.5 in a rat cell line (Rat-2) resulted in inhibition of peptide transport (data not shown). In agreement with this observation, UL49.5 also inhibited peptide transport in T2 cells expressing rat TAP1 and rat TAP2 (Fig. 4A). The ability of UL49.5 to reduce MHC class I surface expression within these cells was assessed by flow cytometry using various MHC class I-specific Abs. Compared with control cells, UL49.5-expressing cells showed a clear down-regulation of MHC class I surface expression, as detected by the mAb W6/32 (Fig. 4B, first panel). This Ab recognizes a wide range of MHC class I locus products. Because the withdrawal of peptides differentially affects the various MHC class I locus products, the cells were also stained with Abs specific for HLA-A2, HLA-B5, and HLA-Cw1. UL49.5 reduced the cell surface expression of each of these MHC class I haplotypes (Fig. 4B). The down-regulation was specific, because transferrin receptor was expressed equally on all cell lines (Fig. 4B, lower right panel). In conclusion, UL49.5 effectively inhibits MHC class I surface expression by blocking peptide transport by TAP of rat origin.

Efficient interaction of UL49.5 with TAP requires both TAP subunits

To investigate the ability of UL49.5 to bind to the individually expressed TAP subunits, we made use of T2 cells stably expressing rat TAP1, rat TAP2, or, as a control, both subunits. The rat TAP1 and UL49.5 steady-state protein levels were comparable in T2 rat TAP1 and T2 rat TAP1 rat TAP2 cells (Fig. 4C). Note that the rat TAP proteins are not degraded in the presence of UL49.5, again illustrating that peptide loading can be inhibited in the absence of TAP degradation. To study the interaction of UL49.5 with rat TAP1 in the absence of rat TAP2, the rat TAP1 protein was immunoprecipitated from digitonin-solubilized T2 rat TAP1 cells (Fig. 4D, upper panel). T2 cells expressing both TAP subunits were included as a positive control. The resulting immune complexes were analyzed for the presence of UL49.5. Whereas similar amounts of rat TAP1 were precipitated from all cell lines, only a small amount of UL49.5 was detectable in immunoprecipitates from cells expressing rat TAP1 alone (Fig. 4D, middle panel; compare lanes 2 and 4; see also quantification). The immune complexes were also probed for the presence of tapasin. This molecule was detectable at comparable levels in all rat TAP1 immunoprecipitates, regardless of the presence of UL49.5 (Fig. 4D, lower panel). Thus, when rat TAP1 is expressed in the absence of rat TAP2, the interaction between UL49.5 and rat TAP1 is highly inefficient.

huTAP2 is extremely unstable in the absence of TAP1. Consequently, huTAP2 cannot be detected when expressed in isolation (P. Lehner, M. Verweij, and E. Wiertz, unpublished observation). Interestingly, rat TAP2 appears less unstable when expressed in the absence of rat TAP1. Thus, T2 cells expressing rat TAP2 could be used to study its interaction with UL49.5. Expression levels of TAP2 and UL49.5 were verified by Western blotting. The rat TAP2 levels were much lower in T2 rat TAP2 cells compared with T2 cells expressing both TAP subunits (Fig. 4E, upper panel; compare lanes 1 and 2 with lanes 3 and 4). Despite these low levels of TAP2 in the T2 rat TAP2 cells, a considerable amount of rat TAP2 could be immunoprecipitated from these cells (Fig. 4F, upper panel, lanes 1 and 2). UL49.5 was detectable in the rat TAP2 immune precipitates from both cell lines (Fig. 4F, middle panel, lanes 2 and 4; see also quantification). These results indicate that UL49.5 can interact with TAP2 alone, albeit with lower efficiency. Even though twice as much UL49.5 precipitated with TAP2 expressed in isolation compared with TAP1, this does not necessarily imply that UL49.5 binds better to TAP2. Due to different expression levels of TAP1 and TAP2 and the use of different Abs for immunoprecipitations and immunoblotting, it is very difficult to assess whether equal amounts of TAP were isolated from the T2 rat TAP1 and T2 rat TAP2 lysates. The combined results show that optimal binding of UL49.5 to the TAP complex requires both TAP subunits.

The N-terminal domains of TAP1 or TAP2 are nonessential for UL49.5-mediated inhibition of TAP

The N-terminal 4 TM helices of TAP1 and the first 3 TM helices of TAP2 (rat TAP1 rat TAP2) was analyzed. T2 cells expressing wild-type rat TAP1 and rat TAP2 were taken along as a control. The cell lines were transduced to express UL49.5 and subjected to Western blot analysis to determine the steady-state levels of TAP1, TAP2, and UL49.5. These proteins appeared to be expressed at comparable levels in all cell lines tested (Fig. 5B).
FIGURE 5. The N-terminal domains of TAP1 and TAP2 are dispensable for UL49.5-mediated inhibition of TAP. A, Schematic diagrams of TAP constructs. NBD, nucleotide binding domain. B, Steady-state levels of TAP1, TAP2, and UL49.5 were assessed for T2 cells expressing wild-type or truncated forms of rat TAP. Rat TAP1ΔN is deficient of the first four N-terminal TM domains of rat TAP1. Rat TAP2ΔN lacks the first three N-terminal TM domains of rat TAP2. C, UL49.5 inhibits heterodimers of N-terminal deletion mutants of TAP. MHC class I surface expression was assessed for T2 TAP1ΔN TAP2 cells, in the presence (graph 4) or absence (graph 3) of UL49.5, using the Abs indicated. Graph 1, Background staining in the presence of secondary Ab only; graph 2, untransduced T2 cells. D, Interaction between UL49.5 and TAP complexes deficient for the N terminus of TAP1 or TAP2. TAP2 was immunoprecipitated (IP) from the cells, and the resulting complexes were stained for UL49.5 and tapasin. The ratio between UL49.5 and TAP was expressed relative to the amount of UL49.5 coprecipitating with rat TAP1/rat TAP2 complexes (D, panel 4, set at 100%). One representative experiment of three independent experiments is shown. Tpsn, Tapasin.
To investigate the capacity of UL49.5 to interfere with peptide transport in cells expressing ΔN TAP1 or TAP2 proteins, the cells were screened for inhibition of MHC class I expression by UL49.5. Reduced MHC class I surface staining was found on all UL49.5-expressing cells lacking the N-terminal domains of rat TAP2 or rat TAP1 (Fig. 5C, upper and lower panels, respectively). The HLA-A, B, and C locus products were all sensitive to this effect. The MHC class I down-regulation observed in cells expressing truncated rat TAP2 was slightly less efficient (Fig. 5C, upper panel). To evaluate the effect of the N-terminal deletions of TAP1 and TAP2 on UL49.5 interaction, coimmunoprecipitation experiments were performed. TAP complexes were isolated from digitonin-solubilized cells using anti-TAP2 Abs (Fig. 5D, upper panel). The presence of UL49.5 in the immunoprecipitates was evaluated by Western blotting. UL49.5 was found to interact with all constructs (Fig. 5D, middle panel). The amount of UL49.5 coprecipitating with the TAP complex was reduced by 56% when the N-terminal part of rat TAP2 was lacking (Fig. 5D, middle panel, lane 4). This is reflected by the results obtained in the flow cytometry experiments (Fig. 5C, upper panel).
The interaction between UL49.5 and the TAP1ΔN TAP2 complex was reduced by 24% compared with the interaction between UL49.5 and the full-length TAP complex. However, this does not seem to affect the UL49.5-induced down-regulation of MHC class I surface expression (compare Fig. 5C, lower panel, with Fig. 5B). Tapasin was found to coprecipitate with TAP to a similar extent in control cells and UL49.5-expressing cells (Fig. 5D, lower panel, compare lane 3 with lane 4 and lane 5 with lane 6). Thus, the interaction between tapasin and TAP was not affected by UL49.5. Taken together, UL49.5 is still able to cause down-regulation of MHC class I surface expression and interact with the TAP complex in the absence of the N-terminal domains of TAP1 or TAP2. However, the deletion of the N-terminal part of TAP2 negatively influences the binding of UL49.5 and the efficiency of TAP inhibition.

The 6+6 TM core of the TAP heterodimer is sufficient for UL49.5-mediated inhibition of TAP

To investigate whether the core complex of TAP comprising the C-terminal 6 TM helices and the cytoplasmic domain of TAP1 and TAP2 are sufficient for UL49.5 interaction, the ability of UL49.5 to bind to the rat TAP1ΔN/rat TAP2ΔN heterodimer was evaluated. Separate expression of the two truncated subunits did not result in sufficiently high expression. Therefore, the two subunits were expressed as a fusion protein (20) (depicted in Fig. 6A). The N terminus of TAP1 was linked to TM domain 4 of TAP2 in a head-to-tail fashion, via the connector region of the murine multidrug resistance protein, multiple drug resistance (MDR)1b (20). The functionality of the fusion proteins has been demonstrated in peptide translocation assays (20). To control for the influence of this tandem formation, T2 cells coexpressing the individual full-length rat TAP1 and rat TAP2ΔN constructs and T2 cells expressing the fused form rat TAP1-TAP2ΔN were compared. The cells were transduced with retrovirus to express UL49.5. Steady-state levels of the TAP subunits and UL49.5 were assessed by Western blotting. UL49.5 was similarly expressed in all cell lines (Fig. 6B, lower panel). When the TAP subunits were fused, the expression levels were lower than that of free TAP2 (Fig. 6B, upper panel; compare lanes 1 and 2 with lanes 3 and 4 and 5 and 6). This was particularly obvious when the various TAP complexes were immunoprecipitated from digitonin-solubilized cells (Fig. 6C, upper panel). The presence of UL49.5 in the complexes was assessed by Western blotting. A significant amount of UL49.5 was found to coprecipitate with the TAP complex when its subunits were expressed as separate proteins (Fig. 6C, second panel, lane 2). When the same TAP subunits were expressed as a fusion protein, the amount of coprecipitating UL49.5 was much less (Fig. 6C, second panel; compare lanes 2 and 4; see also quantification). Apparently, the fusion of the TAP subunits and their lower expression levels resulted in reduced detection of UL49.5. Yet, UL49.5 was found to interact with the rat TAP1ΔN-rat TAP2ΔN fusion construct to a similar extent (Fig. 6C, second panel, lanes 4 and 6). This result indicates that UL49.5 is capable of interacting with the 6+6 TM core complex of TAP as efficiently as with the TAP1 TAP2ΔN complex. To control for nonspecific binding of UL49.5 during the coimmunoprecipitation experiments, an irrelevant protein, transferrin receptor, was immunoprecipitated from all cell lines. The resulting immune complexes were stained for UL49.5. Nonspecific interaction was not observed between the transferrin receptor and UL49.5 (Fig. 6C, third panel, lanes 1–6). When an aliquot of the cell lysate was loaded onto the gel directly, UL49.5 was readily detectable (Fig. 6C, third panel, control lysate). Staining of the TAP2 immune precipitates for tapasin showed that the interaction of tapasin with TAP was not affected by the presence of UL49.5 in the translocation assays, in the presence of 10 mM ATP (Fig. 6D, third panel). Tapasin was not detectable in the immunoprecipitates containing the rat TAP1ΔN-rat TAP2ΔN fusion protein, confirming the 6+6 TM core TAP complex to be devoid of tapasin binding sites (Fig. 6C, lower panel, lanes 3 and 6) (7, 20).

Sf9 insect cells infected with recombinant baculoviruses to express TAP1 and TAP2 provide an ideal opportunity to study the TAP heterodimer in the absence of other components of the MHC class I PLC (7). High, stable levels of TAP are well-known advantages of this system. In this study, Sf9 cells were infected with baculoviruses to coexpress UL49.5 with wild-type and recombinant forms of huTAP (61). To investigate whether UL49.5 was able to inhibit the function of the 6+6 TM huTAP core complex, Sf9 cells expressing huTAP1ΔN and huTAP2ΔN
or, as a control, full-length huTAP1 and huTAP2 were prepared (7). Expression of all constructs was verified by Western blot analysis (Fig. 7A). In the presence of UL49.5, a reduction of TAP levels was observed, reflecting degradation of huTAP by UL49.5 (Fig. 7A). However, the observed degradation is not as pronounced as in, for example, STF1 cells (Fig. 3B). This might be related to suboptimal degradation of huTAP by the insect ubiquitin-proteasomal system. Subsequently, the ability of UL49.5 to interact with full-length and truncated TAP complexes expressed in S9 cells was verified. TAP was isolated from digitonin-solubilized cells using TAP-specific Abs, and the resulting complexes were stained for TAP1, TAP2, and UL49.5. UL49.5 was found to interact with both TAP complexes. This confirms the results shown in Fig. 6C and proves that UL49.5 is able to interact with the 6+6 TM core domain of TAP. To assess whether this interaction leads to a functional block of TAP, transport activity of the full-length and truncated TAP complex was evaluated in the absence or presence of UL49.5. Peptide transport by the full-length TAP subunits was found to be inhibited efficiently by UL49.5 (Fig. 7C, left panel). Also, in cells expressing the human 6+6 TM core TAP complex, UL49.5 inhibited peptide transport, albeit less effectively (Fig. 7C, right panel).

In conclusion, these experiments show that the 6+6 TM core complex of TAP is sufficient for UL49.5 interaction and for inhibition of peptide transport, in the absence of other components of the PLC.

Discussion

The results presented in this study show that the BHV-1-encoded UL49.5 protein acts directly on the TAP heterodimer to inhibit loading of peptides onto MHC class I molecules. The interaction of UL49.5 with the TAP complex appears to be most efficient when both TAP1 and TAP2 are present. The N-terminal domains of TAP1 and TAP2 are dispensable for UL49.5 binding and inhibition of TAP function, thereby identifying the 6+6 TM TAP core complex as the primary target for UL49.5. However, complete inhibition of TAP by UL49.5 is only observed when full-length TAP2 is present in addition to TAP1N.

To investigate whether UL49.5 binds to TAP1 or TAP2, the interaction with UL49.5 was studied in cells lacking one of the TAP subunits. In the TAP2-deficient STF1 cells, interaction of UL49.5 with TAP1 was not detectable (Fig. 3B). In T2 cells expressing either rat TAP1 or rat TAP2, a weak interaction of UL49.5 with the individual TAP subunits was observed (Fig. 4, D and F). However, huTAP1 expressed individually in insect cells showed a clear association with UL49.5 (61). Most likely, the relatively high expression levels of the individual proteins in S9 insect cells promote interactions between UL49.5 and the TAP subunits. In addition, overexpression of the individual TAP subunits in the T2 cells or insect cells might result in the formation of homodimers adopting a conformation similar to that of TAP heterodimers, thereby creating a structure that facilitates the interaction with UL49.5. In MJS TAP1-GFP cells, endogenous TAP1 has been found to interact with TAP1-GFP (M. Verweij, D. Koppers-Lalic, and E. Wiertz, unpublished observation), showing the possibility of homodimer formation. Homodimerization of the individual TAP1 and TAP2 subunits has also been observed in the presence of chemical cross-linkers (62) or in a system in which TAP subunits were overexpressed (63). In case of the STF-1 cells, homodimers of TAP1 might not exist because of lower TAP1 levels compared with the recombinant T2 or S9 cells. Additionally, the presence in the STF-1 cells of an incomplete TAP2 gene product, comprising its N-terminal 5 TM helices, might interfere with TAP1 homodimer formation in these cells. This might explain the inability of UL49.5 to interact with TAP1 in the STF1 cells.

The γ-2 herpesvirus 68-encoded mK3 protein causes evasion of CTL recognition by inducing the degradation of ER-resident MHC class I molecules via ubiquitination. mK3 requires an interaction with TAP and tapasin to target MHC class I H chains and to preserve its own stability. The protein appears to bind to TAP1 alone, but the interaction with both TAP1 and TAP2 is needed for mK3 to function properly (64–71). The observation that UL49.5 needs both TAP1 and TAP2 to efficiently block peptide transport resembles the reciprocity of mK3 with the PLC. Both proteins are likely to interact with both subunits, but heterodimerization is needed for optimal binding and interference with MHC class I loading.

TAP1 and TAP2 carry 10 and 9 TM helices, respectively. The 6+6 TM core complex containing TM helices 5–10 of TAP1 and TM helices 4–9 of TAP2 appeared to be sufficient for dimerization of the complex and efficient binding and translocation of peptides (7). Using T2 cells expressing ΔN forms of rat TAP1 and TAP2, UL49.5 was found to interact with the 6+6 TM core complex of TAP. Accordingly, TAP function was inhibited by UL49.5 in S9 cells expressing the minimal TAP heterodimer. The inhibition was not as efficient as in S9 cells expressing full-length TAP1 and TAP2. This finding is in agreement with the results obtained in T2 cells expressing core TAP2 (TAP2ΔN) next to full-length TAP1. UL49.5-mediated TAP inhibition was found to be less pronounced in these cells compared with cells expressing full-length TAP2 and TAP1. These results indicate that the N terminus of TAP2 contributes to the efficiency of UL49.5 binding and function. Probably, UL49.5 primarily targets the 6+6 TM core of TAP, but optimal inhibition of TAP also requires the N-terminal region of TAP2, presumably because it stabilizes the TAP complex in a conformation that favors UL49.5 interaction.

The results discussed raise the question as to how a type I membrane protein of only 9 kDa can block TAP function and ultimately induce the degradation of this large heterodimeric transporter complex. Based on the results obtained, the following model can be proposed. The interaction of the ER-luminal and TM domains of UL49.5 with the 6+6 TM core complex may cause a conformational arrest of the transporter that results in the inhibition of peptide transport (38). Both the TM helix and the ER-luminal domain of UL49.5 are required for this effect (38, 61). Insertion of the TM domain of UL49.5 within the core complex of TAP might prevent essential rearrangements of the TAP TM helices and may thus freeze TAP in a translocation-incompetent state. At the same time, the ER-luminal part of UL49.5 might bend over the ER-luminal regions of the transporter. The amino acid proline is known to allow a stable flexure in a protein structure. The N-terminal part of UL49.5 contains three prolines at positions close to the membrane that might facilitate the positioning of the ER-luminal domain of UL49.5 over the luminally exposed regions of TAP. The interaction between the ER-exposed domains of UL49.5 and TAP may further obstruct TAP function. Data from our laboratories indicate that UL49.5 occurs in the TAP complex as a disulfide-linked homodimer (61). Therefore, the interference with TAP function described above might be conducted by two UL49.5 molecules simultaneously, with one UL49.5 molecule interacting with TAP1 and one with TAP2. This might explain the observation that UL49.5 is able to interact with both TAP1 and TAP2. Interestingly, a UL49.5 recombinant lacking this ER-luminal cysteine appeared to be equally active (61).
In addition to the conformational arrest of TAP induced by the ER-luminal and TM parts of UL49.5, the cytoplasmic C-terminal domain of the viral protein mediates proteosomal degradation of UL49.5 and TAP (38). A recombinant form of UL49.5 lacking its cytoplasmic domain is incapable of inducing degradation of TAP (38). The cytoplasmic domain of UL49.5 contains two lysine residues representing potential targets for ubiquitination (72). However, substitution of these lysines for alanines did not prevent degradation of TAP (M. Verweij, D. Koppers-Lalic, and E. Wiertz, unpublished observation). Degradation of huTAP by UL49.5 was clearly observed in T2 cells. The lack of degradation of rat TAP in T2 cells expressing UL49.5 might result from an incompatibility between human components of the ubiquitin-proteasome pathway and the rat TAP proteins. Because of the absence of UL49.5-induced degradation of rat TAP in T2 cells, we cannot draw any conclusions on the importance of the N-terminal parts of TAP1 and TAP2 for this mode of action.

Remarkably, degradation of rat TAP1 and TAP2 was not observed in T2 cells expressing UL49.5. Rat TAP can be targeted for degradation by UL49.5, as seen in the rat embryo-derived fibroblast cell line Rat2 (M. Verweij, D. Koppers-Lalic, and E. Wiertz, unpublished observation). Degradation of huTAP by UL49.5 was clearly observed in T2 cells. The lack of degradation of rat TAP in T2 cells expressing UL49.5 might result from an incompatibility between human components of the ubiquitin-proteasome pathway and the rat TAP proteins. Because of the absence of UL49.5-induced degradation of rat TAP in T2 cells, we cannot draw any conclusions on the importance of the N-terminal parts of TAP1 and TAP2 for this mode of action.

Tapasin is an essential component of the PLC, facilitating the loading of MHC class I molecules with high affinity peptides. In addition, tapasin is required for stable TAP expression. Splenocytes derived from mice deficient for tapasin showed a 100-fold up-regulation of steady-state levels of TAP upon tapasin transfection (15, 17). In addition, in .220 cells transfected to express full-length tapasin, TAP levels were enhanced 3- to 10-fold (16, 18). Therefore, UL49.5-induced degradation of TAP might involve interference with the stabilizing function of tapasin. Although the presence of UL49.5 did affect the levels of tapasin within the PLC to some extent (Fig. 1B), this effect seems too modest to explain the complete degradation of the TAP complex in UL49.5-expressing cells. Moreover, if UL49.5 would expel tapasin molecules from one of the N-terminal parts of the TAP complex, a clear reduction in tapasin levels should have been observed in either TAP1 TAP2ΔN or TAP1ΔN TAP2-containing PLCs (Fig. 5D).

This, however, has not been found. Surprisingly, UL49.5-induced destabilization of TAP was not observed in .220 cells deficient of tapasin. This could indicate that tapasin is required for the degradation of TAP by UL49.5. Alternatively, this finding may be related to the fact that TAP is already very unstable in the .220 cells. Possibly, degradation cannot be increased further by UL49.5. UL49.5 did destabilize TAP1 and TAP2 in SF9 insect cells expressing TAP1, TAP2, and UL49.5 in the absence of tapasin (Fig. 7B). The combined results favor the conclusion that tapasin is dispensable for UL49.5-mediated TAP inhibition and destabilization, and identify the TAP core complex as the functional entity targeted by UL49.5.

In summary, this study has identified the TAP1-TAP2 heterodimer as the prime target of UL49.5. The 6+6 TM core complex of TAP1 and TAP2 is sufficient for UL49.5 to mediate its inhibitory activity. These findings are particularly interesting in view of the fact that TAP forms part of a large family of transporters to which also the MDR proteins belong. Some members of this MDR transporter family confer drug resistance to malignant cells and microbial organisms (75, 76). Inhibitory molecules targeting the core domain of such transporters might help to overcome MDR of these tumor cells and microorganisms.

In addition, UL49.5 can be exploited as an immunosuppressive agent, for example, in HLA-mismatched transplantations and autoimmune diseases. The expression of UL49.5 by transplanted cells will inhibit loading of MHC class I molecules with self Ags, resulting in reduced priming of autoreactive CTLs. The feasibility of this approach has been shown in vitro for minor histocompatibility Ags (77). Finally, the expression of UL49.5 has been shown to elicit an unusual T cell repertoire, directed against T cell epitopes independent of peptide processing, demonstrating cytolytic activity against tumor cells (60, 78). A profound understanding of the mechanism of TAP inhibition by UL49.5 will be instrumental in the development of novel therapies exploiting this viral immune evasion protein.

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References


