Immune evasion by varicelloviruses : the identification of a new family of TAP-inhibiting proteins

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CHAPTER 8

J Immunol. 2007; 178:657-662

The Varicellovirus-Encoded TAP Inhibitor UL49.5 Regulates the Presentation of CTL Epitopes by Qa-1^b

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Impairment of MHC class I Ag processing is a commonly observed mechanism that allows viruses and tumors to escape immune destruction by CTL. The peptide transporter TAP that is responsible for the delivery of MHC class I-binding peptides into the endoplasmic reticulum is a pivotal target of viral-immune evasion molecules, and expression of this transporter is frequently lost in advanced cancers. We recently described a novel population of CTL that intriguingly exhibits reactivity against such tumorimmune escape variants and that recognizes self-peptides emerging at the cell surface due to defects in the processing machinery. Investigations of this new type of CTL epitopes are hampered by the lack of an efficient inhibitor for peptide transport in mouse cells. In this article, we demonstrate that the varicellovirus protein UL49.5, in contrast to ICP47 and US6, strongly impairs the activity of the mouse transporter and mediates degradation of mouse TAP1 and TAP2. Inhibition of TAP was witnessed by a strong reduction of surface MHC class I display and a decrease in recognition of conventional tumor-specific CTL. Analysis of CTL reactivity through the nonclassical molecule Qa-1^b revealed that the presentation of the predominant leader peptide was inhibited. Interestingly, expression of UL49.5 in processing competent tumor cells induced the presentation of the new category of peptides. Our data show that the varicellovirus UL49.5 protein is a universal TAP inhibitor that can be exploited for preclinical studies on CTL-based immune intervention.

Cytotoxic T lymphocytes are important for the immune control of viral infections and have been also shown to exhibit the capacity to eradicate established tumors (1–4). The efficacy and safety of CTL-based immunotherapy are currently being evaluated in experimental clinical trials (5–7). An important complication in this respect is the finding that viruses and tumors display diverse mechanisms by which they can evade CTL responses. Viruses that cause lifelong persistence in the host, such as the herpesviruses EBV, CMV, varicella-zoster virus, and HSV, especially have developed sophisticated immune evasion strategies (8, 9). Reactivation of these viruses is a clinical problem in immune-compromised patients, illustrating the delicate balance between viral persistence and elimination by the CTL immune system. Impairment of Ag presentation via MHC class I, which renders these cells resistant to killing by effector CTL, is also frequently found in tumors (10 –13). In general, these impairments are more frequently observed in advanced stages and metastases than in early-stage cancer lesions, suggesting that natural CTL immunity imposes a selective pressure on the progression of tumor-immune escape variants (14, 15).

One effective mechanism of MHC class I down-modulation is the impediment of the function of the TAP, which mediates the delivery of intracellular peptides for binding to MHC class I molecules in the endoplasmic reticulum (ER) (16). Defects in TAP expression are observed in cancers of diverse origin, including breast, lung, and colon, as well as cervical carcinomas and melanomas (11, 13, 17). Interestingly, dedicated viral proteins that target the peptide transport process have been demonstrated in CMV (US6), HSV (ICP47), gammaherpesvirus 68 (mK3), and varicelloviruses (UL49.5) (18 –23).

Recently, we identified the existence of a unique category of CTL that selectively eradicates cells with MHC class I-processing defects, such as TAP deficiency (24). Normal cells with intact Ag-processing machinery were not recognized. These CTL detect a novel repertoire of peptide Ags that emerges on the surface due to TAP, tapasin, or proteasome impairments. Although the peptides are derived from widely distributed self-proteins, they are not presented by normal processing proficient cells and therefore the immune system considers them as immunogenic neo-Ags (24). We refer to this set of peptides as T cell epitopes associated with impaired peptide processing (TEIPP).

Part of the TEIPP-specific CTL population was restricted by the nonclassical MHC class I molecule Qa-1^b. Qa-1^b and its functional homolog in the human HLA-E are widely expressed, nonpolymorphic class I molecules and present a conserved repertoire of peptides due to their narrow peptide-binding grooves (25, 26). These nonclassical class I molecules have mainly been studied as ligands for the inhibitory NK receptor CD94/NKG2A; however, it has become clear that the population of CTL comprises TCRs restricted by Qa-1^b (27–32). The findings on Qa-1^b-mediated CTL reactivity against TEIPP Ags prompted us to study the presentation of these Ags using an inhibitor of peptide transport. Our attempts were hampered by the fact that the described TAP-inhibiting viral proteins US6, ICP47, and mK3 are ineffective in murine cells (ICP47 and US6) (33–36) or target MHC class I H chains as well (mK3) (37, 38).

In this study, we demonstrate that the UL49.5 molecule from the bovine herpesvirus 1 (BHV1) inactivates TAP function in mouse cells and we show that this protein is a versatile tool for the study of Ag presentation. Introduction of UL49.5 in mouse colon carcinomas reduced surface display of MHC class I and allowed these tumors to avoid recognition by conventional tumor-specific CTL. We furthermore studied the effect of UL49.5 on the Qa-1^b-binding peptide repertoire and found that the presentation of TEIPP Ags was strongly promoted by UL49.5, suggesting that immune-evading viruses might be sensitive to TEIPP-directed CTL immune intervention. Our data suggest that this viral protein might be exploited for preclinical testing of murine TEIPP-directed CTL and for selective expansion of human T cells with equivalent specificity.

Materials and Methods

Cell lines The tumor cell lines used in this study have been generated by chemical carcinogens in different mouse strains. Colon carcinomas C26 and CC36 were derived from the BALB/c stain and MC38 was derived from the C57BL/6 strain (39). Introduction of the UL49.5 gene from BHV1 was established by retroviral gene transduction with the LZRS vector containing an internal ribosome entry site and GFP, as described before (21). Cells with the highest GFP expression were positively sorted by FACS. Fibrosarcoma cells induced by 3-methylcholanthrene (MCA) were generated in the $TAP^{-/-}$ mouse on a C57BL/6 background (24). TAP1 restoration in this cell line was performed with a retroviral construct encoding the mouse TAP1 gene, as described (24).

CTL clone E/88 recognizes the H-$2L^d$-binding peptide SPSYVYHQF comprised in an endogenous retroviral gp70 gene product and was provided by Dr. M. Colombo (40). These CTL were restimulated weekly with irradiated C26 tumor cells along with 10 Cetus units

of recombinant human IL-2 (Chiron). CTL clone B12i recognizes the H-2D^b-derived leader peptide AMAPRTLLL in the context of Qa-1^b and was provided by Dr. J. Dyson via Dr. C. Brooks (41). These CTL were generated in B6-Tla^a mice that harbor the Qa-1^a allotype and we propagated this line by weekly restimulation with irradiated B6 spleen cells and IL-2. TEIPP-specific Qa-1^b-restricted CTL have been described before (24) and were generated by immunization of C57BL/6 mice with syngeneic TAP-deficient, CD80-expressing RMA-S cells and weekly restimulation with a mixture of RMAS. CD80 cells, CD80-expressing EC7.1.Qa-1^b cells, irradiated spleen cells, and IL-2 (24).

All cell lines were cultured in IMDM (Cambrex), supplemented with 8% heat-inactivated FCS (Invitrogen Life Technologies), 2 mM L-glutamine (Valeant Pharmaceuticals), 100 IU/ml of penicillin (Astellas Europe), and 30 μM 2-ME (Merck) at 37°C in humidified air with 5% CO_2.

CTL activation assay and flow cytometry Graded amounts of target cells were incubated with 5 x 10^3 CTL in roundbottom 96-well plates. After 18 h of coculture, supernatants were measured for IFN-γ content using sandwich ELISA, as described before (42).

Surface expression of MHC class I molecules was determined using mouse anti-Qa-1^b mAb (clone 6A8; BD Pharmingen) and mouse anti-L^d mAb (clone 28-14-8; BD Pharmingen) followed with allophycocyanin labeled goat anti-mouse Ig and analyzed on a FACSCalibur machine (BD Biosciences).

Peptide transport assay Mouse colon carcinoma cells (2.5 x 10^6 cells/assay) were semipermeabilized with saponin (0.05% (w/v)) in 50 μl of buffer (PBS with 5 mM $MgCl_2$) for 1 min at room temperature. Cells were washed twice with buffer. Peptide transport assays were performed with 0.46 μM fluorescein-labeled peptide (RRYQNSTC^{f}L, N-core glycosylation site underlined) in buffer (total volume of 100 μl/assay) in the presence of 10 mM ATP for 3 min at 32°C. Apyrase (1 U; Sigma-Aldrich) was added to deplete ATP in the control samples. The transport reaction was terminated by addition of 1 ml of stop buffer (PBS with 10 mM EDTA). Cells were then collected by centrifugation and lysed in buffer (50 mM Tris-HCl, 150 mM NaCl, 5 mM KCl, 1 mM $CaCl_2$, 1 mM $MnCl_2$, and 1% Nonidet P-40 (NP-40) (pH 7.5)) for 20 min on ice. The N-core glycosylated peptides were recovered with Con A-Sepharose beads (Sigma-Aldrich) overnight at 4°C. After washing with lysis buffer, peptides were eluted from the Sepharose beads with 200 mM methyl α-D-mannopyranoside and quantified with a fluorescence plate reader ($\lambda_{ex/em}$ / 485/520 nm, POLARstar Galaxy; BMG).

Immunoblotting Cells (5 x 106 cells) were lysed in NP-40 lysis buffer (1% NP-40 in 50 mM Tris-HCl, 50 mM NaCl, and 5 mM MgCl2 (pH 7.4)) and mixed with SDS sample buffer without boiling. Proteins were separated by SDS-PAGE and transferred to nitrocellulose membranes (Schleicher & Schuell Microscience). Membranes were saturated with skimmed milk powder (2% w/v) and then probed with a β-actin-specific Ab (Sigma-Aldrich), a mouse TAP2-specific serum (TAP2.688, a gift from Dr. F. Momburg, German Cancer Research Center, Heidelberg, Germany), or a mouse TAP1-specific mAb (clone SC-11465; Santa Cruz Biotechnology). After washing with TBS (0.1% Tween 20 in PBS), bound primary Abs were detected using peroxidase-conjugated Abs: goat anti-mouse Ig (Southern Biotechnology Associates), goat anti-rabbit Ig (Southern Biotechnology Associates), and rabbit anti-goat Ig (DakoCytomation), respectively. After washing with TBS, peroxidase activity was visualized by chemiluminescence imaging (Lumi-Imager F1; Roche).

Results

UL49.5 protein impairs TAP function in mouse cells We previously reported that the BHV1-derived molecule UL49.5 is accountable for the inactivation of TAP through inducing conformational changes and active breakdown of this peptide transporter (21). These studies were performed in human cell lines. In this study, we introduced UL49.5 in cells of mouse origin to examine whether this protein also inactivates mouse TAP. Expression of UL49.5 resulted in marked reduction of MHC class I surface display, irrespective of the MHC haplotype or the tissue origin of the cells (H-2^b, H-2^d, and H-2^k) (Fig. 1A and data not shown). These findings suggested that UL49.5, in addition to bovine and human TAP, also inhibits peptide transport by mouse TAP.

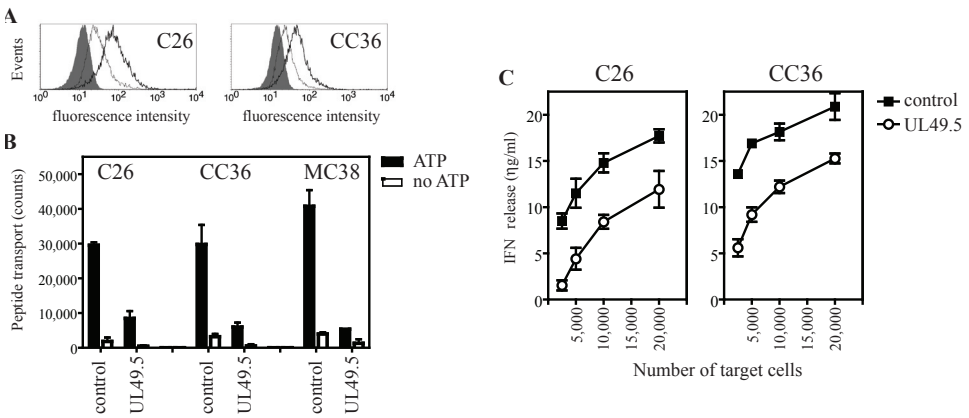

Fig. 1. The viral UL49.5 protein impairs TAP-dependent peptide transport in murine cells. (A) The UL49.5 gene was introduced into C26 and CC36 murine colon carcinoma cell lines through retroviral transduction, and surface MHC class I expression was measured with flow cytometry. Staining of allele-specific anti-H-2L^d mAb was detected by fluorescently labeled anti-mouse Ig on control cells (bold histogram lines) and UL49.5-expressing cells (thin histogram lines). Filled histograms correspond to cells stained with secondary mAb alone. (B) The efficiency of peptide transport was measured in semipermeabilized cells using the fluorescently labeled peptide RRYQNSTC'L. ATP-dependent TAP transport was determined for control cells and UL49.5-expressing cells. Apyrase was applied to deplete endogenous ATP to determine the TAP-independent background transport. Equal amounts of cells were used in all samples as assessed by β-actin protein in immunoblots (data not shown). Means and SDs of triplicates of a representative experiment out of three are depicted. (C) The tumor-specific CTL clone E/88 was used to detect the H-2L^d-mediated presentation of the cognate peptide SPSYVYHQF. This CTL epitope is derived from an endogenous retroviral gp70 product expressed in colon carcinoma C26 and CC36 cells. CTL and tumor cells were coincubated and IFN-γ release by CTL was measured by ELISA. Mean and SD of one of three experiments with comparable results are depicted.

Inhibition of TAP function was more directly assessed in a peptide transport assay using a labeled prototypic peptide. Indeed, a strong reduction in peptide transport activity was observed in cells that expressed the viral UL49.5 gene (Fig. 1B). Compared with control transfected cells, TAP activity was 3- to 5-fold decreased.

Finally, the influence of the TAP inhibition on the processing and presentation of a characterized peptide epitope was evaluated. The surface presentation of the L^d-binding peptide SPSYVYHQF was determined using a peptide-specific CTL clone (40). This peptide is derived from an endogenous tumor Ag that is expressed in colon carcinomas (40). IFN-γ release by the CTL was measured upon coincubation with the L^d-expressing colon carcinoma cell lines C26 and CC36 expressing UL49.5 or a control construct. Four to six times more UL49.5-positive target cells were needed to reach similar IFN-γ levels, showing that UL49.5-

mediated inhibition of TAP has functional consequences for Ag presentation to CTL (Fig. 1C). Collectively, these data show that the BHV1 UL49.5 protein inhibits peptide transport by TAP in mouse cells.

Inactivation of TAP is exerted through degradation Our previous studies in human cells revealed that UL49.5 inactivates TAP via two mechanisms. The binding of UL49.5 to TAP results in a translocation-incompetent state of the transporter complex. Ultimately, UL49.5 mediates the degradation of both TAP subunits via the proteasome (21). UL49.5 breakdown coincides in this process. This mechanism is clearly different from that of other viral proteins that disturb the peptide transport process. To determine whether mouse TAP1 and TAP2 are similarly destabilized by UL49.5, we examined their steady-state levels in immunoblots (Fig. 2). Reduced levels of both TAP1 and TAP2 were observed in the cells that expressed UL49.5 compared with their counterparts (Fig. 2), indicating that UL49.5 destabilizes both subunits. A comparable decrease was observed in all three colon carcinoma cell lines analyzed, explaining the observed general reduction in MHC class I display at the cell surface (see Fig. 1A). These results are in line with our previous findings in human cells and argue that UL49.5 interacts with human and mouse TAP1/TAP2 heterodimers at a region that is structurally homologous.

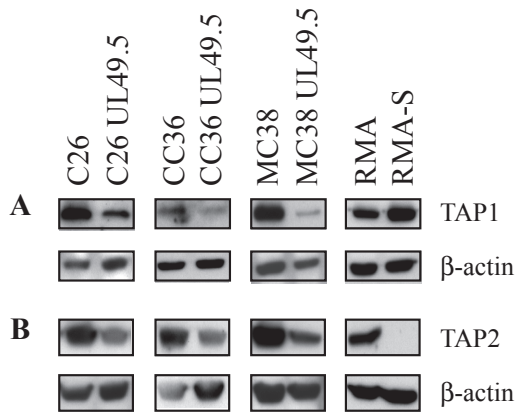

Fig. 2. UL49.5 mediates down-regulation of TAP1 and TAP2. UL49.5-expressing and control colon carcinoma cell lines (C26, CC36, and MC38) were lysed and TAP proteins were detected by immunoblotting using Abs specific for TAP1 (A) or TAP2 (B). Membranes were washed and reprobed with anti-β-actin mAb. Bands were visible at 74 kDa (TAP1), 75 kDa (TAP2), and 42 kDa (β-actin). RMA-S cells harbor an unstable, truncated form of TAP2 (57) and were taken along as a control.

Peptide presentation by the nonclassical MHC Class I molecule Qa-1^b To date, UL49.5 is the first protein that can efficiently inhibit TAP function in multiple species, including mouse. We anticipate that this feature of UL49.5 will make it a very suitable research tool for application in diverse mouse systems of Ag processing and presentation. We examined the influence of UL49.5 on the peptide repertoire that is presented by the nonclassical class I molecule Qa-1^b. We analyzed the Qa-1^b-mediated presentation of a TAP dependent leader peptide (AMAPRTLLL) that is derived from the classical MHC class I molecule H-2D^b (43) and the recently determined TEIPP peptides (24) using Qa-1^b-restricted CTL clones. TEIPP represents a novel set of CTL epitopes that are selectively presented by cells with Ag-processing defects, such as TAP-deficient tumors (24).

MCA-induced fibrosarcoma cells from a TAP$^{-/-}$ mouse failed to trigger AMAPRTLLL-specific CTL (Fig. 3, left panel). Gene transfer of mouse TAP1 restored the presentation of this peptide, whereas IFN-treatment in addition to TAP1 expression further augmented the CTL reactivity (Fig. 3, left panel). In contrast, Qa-1^b-restricted CTL with TEIPP specificity were activated by the TAP-deficient variant and TAP restoration decreased the CTL response (Fig.

3, right panel). Promotion of Ag processing by pretreatment with IFN-γ resulted in even lower CTL responses. This pattern of CTL recognition is in line with our previous results on H-2D^b- and H-2K^b-restricted TEIPP CTL, as target cells with impaired Ag processing efficiently stimulate TEIPP CTL (24). Thus, these data on genetic TAP-deficient cells revealed opposing requirements of these two Qa-1^b-presented peptides for the intracellular processing machinery, in that the leader peptide AMA PRTLLL depends on TAP function and TEIPP peptides benefit from TAP deficiency.

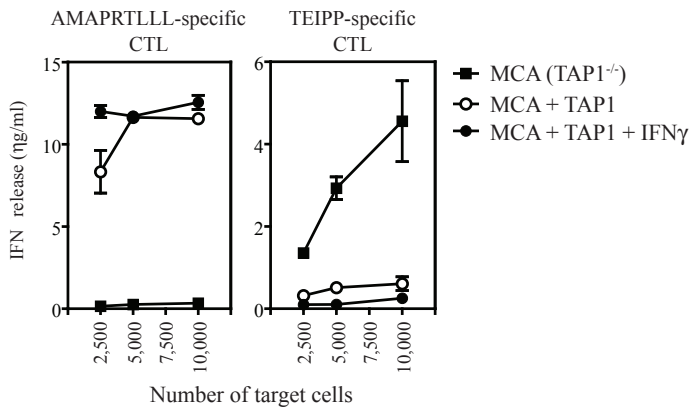

Fig. 3. Qa-1^b-restricted CTL with reactivity against TEIPP Ags. The reactivity of two types of Qa-1-restricted CTL was analyzed against a TAP1-deficient MCA-induced fibrosarcoma cell line. CTL clone B12i (left panel) recognizes the AMAPRTLLL peptide derived from the signal sequence of H-2D^b (41) and CTL clone 12 (right panel) recognizes Qa-1-presented TEIPP peptides (24). Restoration of TAP1 function of MCA cells was achieved by gene transfer (+ TAP1). TAP1-expressing MCA cells were pretreated with IFN-γ before coincubation with CTL to promote Ag-processing capacity of the cells. Two independent Qa-1^b-restricted CTL clones with TEIPP specificity gave similar results. Means and SDs of triplicates from one of two comparable experiments are depicted.

The presentation of Qa-1^b binding TAP-independent epitopes is promoted by UL49.5

Next, we analyzed Qa-1^b-mediated Ag presentation by the three TAP-positive colon carcinoma cell lines (C26, CC36, and MC38, see Fig. 2). All three cell lines display the same Qa-1 allele (Qa-1^b), although they are derived from different mouse strains (BALB/c and C57BL/6) (39). Qa-1 genes display very limited polymorphism; in fact, only two different allele families have been described thus far (44). This allowed us to use the same Qa-1^b-restricted CTL clones for the analysis of the Qa-1 peptide repertoire. In accordance with the TAP1-deficient fibrosarcoma experiments (Fig. 3), the recognition by the AMAPRTLLL peptidespecific CTL was clearly decreased upon expression of UL49.5 (Fig. 4A). The TEIPP-specific CTL did not respond against the parental C26, CC36, or MC38 cells (Fig. 4B), suggesting that the TAP function in these cells precluded the presentation of TEIPP epitopes. Interestingly, UL49.5 expression induced the emergence of TEIPP at the cell surface of the colon carcinomas and strongly promoted activation of the TEIPP-specific T cells (Fig. 4B). These findings imply that, in the absence of functional TAP, peptides other than MHC class I-derived leader peptides substitute the Qa-1^b-binding peptide pool. Overall, Qa-1^b surface levels were not affected by the UL49.5 protein (data not shown), underlining the notion that UL49.5 selectively attacks the Ag-processing machinery and does not limit the availability of Qa-1 H chains.

IFN-γ strongly enhances the class I Ag-processing and presentation machinery. We assessed whether pretreatment of IFN-γ would reduce the UL49.5-mediated display of TEIPP Ags by Qa-1^b. This is of interest because the UL49.5 protein seems to block peptide transport and subsequent presentation only partially (Fig. 1, B and C, and compare Fig. 3 with Fig. 4A). Treatment of CC36 cells with IFN-γ resulted in improved presentation of the TAP-dependent AMAPRTLLL peptide (Fig. 5, left panel). Similar CTL recognition patterns were observed against targets that had not been pretreated with IFN-γ (Fig. 5, left panel). The impact of UL49.5 was comparable with that of nontreated target cells. Strikingly, the reactivity of TEIPP-specific CTL was not affected by IFN-γ treatment of the target cells, indicating that UL49.5

function was sufficient to counteract the augmented Ag processing. Together, our data show that the varicellovirus-derived protein UL49.5 is an efficient TAP inhibitor in mouse cells and may be exploited as a versatile tool for the induced presentation of TEIPP Ags.

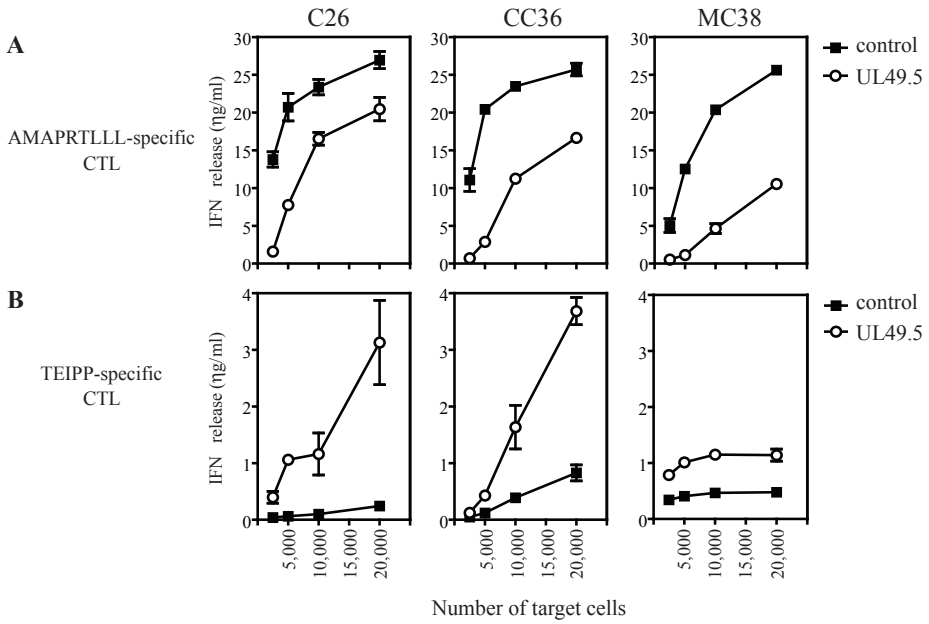

Fig. 4. UL49.5 induces the presentation of TEIPP peptides by Qa-1. (A) Qa-1-restricted CTL (B12i) specific for the AMAPRTLLL leader peptide of H-2D^b were coincubated with C26, CC36, and MC38 cells expressing UL49.5. (B) Qa-1-restricted CTL (clone 12) specific for TEIPP peptides selectively recognizes UL49.5-expressing cells. Two independent Qa-1^b-restricted CTL clones with TEIPP specificity gave similar results. Means and SDs of triplicates of a representative experiment are shown.

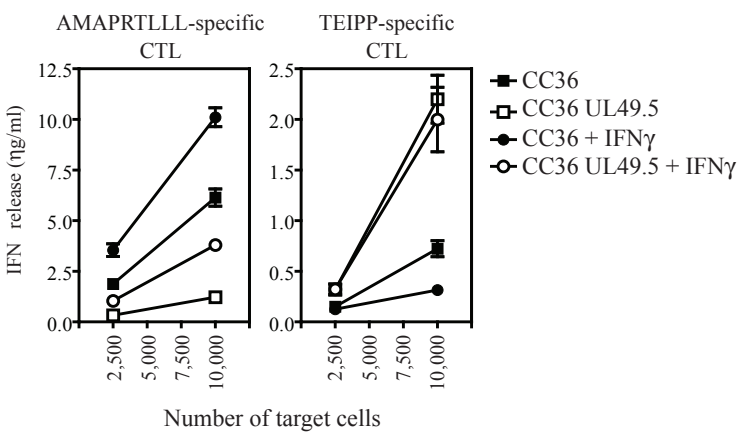

Fig. 5. Stimulation of Ag processing by IFN-γ does not impair UL49.5 function. The reactivity of AMAPRTLLL-specific CTL (B12i, left panel) and TEIPP-specific CTL (clone 12, right panel) was analyzed against TAP-inhibited and control cells that were pretreated with IFN-γ. Colon carcinoma cells were cultured for 48 h in the presence of IFN-γ or normal culture medium. Cells were washed before usage as targets in CTL assays. Means and SDs of triplicates of one representative experiment of three are shown.

Discussion

Immune evasion mechanisms are frequently found in virus-infected cells and tumor cells and allow them to escape from CTL-mediated immune control (8, 9, 14, 15). One of the most frequently observed mechanisms is impairment at the bottleneck: the peptide transporter TAP that delivers peptides into the ER for loading onto MHC class I molecules (45). This leads to a strong and general decrease of class I expression at the cell surface, allowing these aberrant cells to become resistant to effector CTL. We recently discovered a CTL population that is able to selectively react against targets that display deficiencies in their MHC class I-processing pathway (24). These CTL recognize immunogenic neoepitopes derived from commonly expressed self-proteins. The fact that these self-peptides are not presented by cells with normal processing status and only emerge at the cell surface due to defects in the MHC class I Ag-processing machinery explains the observation that these self-peptides are immunogenic and induce strong CTL responses (24). In our current study, we embarked on this novel type of CTL specificity and analyzed the Qa-1^b-mediated presentation of these Ags, which we refer to as TEIPP. To examine the Qa-1^b-binding peptide pool in processing proficient and deficient cells, we set out to search for a versatile tool that could inhibit TAP function in mouse cells. The well-studied viral proteins ICP47, US6, and mK3, which are known for their effective suppression of TAP activity, do not impede mouse TAP function (ICP47 and US6) or target MHC class I molecules (mK3) (18 –20, 22, 23, 33–38, 46–49). In contrast, application of antisense strategies was hampered by unstable expression of the constructs and low efficiency. We considered the recently identified immune evasion molecule UL49.5 derived from the BHV1 as a likely candidate to inhibit mouse TAP. This protein was demonstrated in human cells to induce a conformational change of TAP and, in addition, to actively target TAP to the proteasome for breakdown (21). In this study, we show that this protein is also capable of inhibiting peptide transport by mouse TAP and constitutes a versatile tool for Ag-processing studies.

Interestingly, each herpesvirus that inactivates TAP employs this stealth technology through entirely different mechanisms. ICP47 is a small, soluble cytosolic protein that acts as a highaffinity competitor for peptide binding to TAP and thereby competes for transport of peptide ligands (48). US6 binds to TAP in the ER lumen and prevents ATP binding through a conformational change (49). The mK3 protein carries a cytoplasmic RING finger that catalyzes ubiquitination of TAP (38). Finally, the UL49.5 protein that we recently identified efficiently blocks peptide transport by inducing conformational changes of TAP and by simultaneously shuttling TAP to the proteasome for degradation (21). Whereas the first two viral proteins exhibit very poor activity on mouse TAP (33–36), we show in this study that UL49.5 exerts its function in mouse cells, in addition to human and bovine cells (Fig. 1 and Ref. 21). Apparently, the TAP molecules of these species display enough structural similarity to allow for binding of UL49.5, but not ICP47 or US6. Although the UL49.5-mediated decrease in TAP protein appeared to be less dramatic in mouse cells than in human cells (Fig. 2 and Ref. 21), the functional blockade of TAP activity appears comparable (Fig. 1 and Ref. 21). A plausible explanation for this paradox is provided by our previous findings that deletion mutants of UL49.5, which fail to shuttle TAP to the proteasome, still block TAP transport through conformational changes of TAP (21). Interestingly, UL49.5 proteins encoded by two other varicelloviruses (pseudorabies virus and equine herpesvirus 1) also efficiently inactivate TAP function in the absence of strong TAP breakdown (D. Koppers-Lalić and E.J.H.J. Wiertz, unpublished observations).

Qa-1^b and its human functional homolog HLA-E belong to the group of nonclassical MHC class I molecules and have a wide tissue distribution (32). The main differences with the classical class I proteins are the low expression levels, the very limited polymorphism in the population, and the limited variability in the peptide repertoire they bind (32).

The peptide-binding groove of Qa-1^b and HLA-E seem to be optimized for accommodation

of leader peptides derived from classical MHC class I molecules, which are, in the mouse, encoded by H-2D genes (50), although some viral and bacterial peptides have been shown to bind as well (28 –30, 51, 52). Qa-1^b-restricted CTL responses against several intracellular pathogens have indeed been documented (27–31), demonstrating a physiological role for this nonclassical class I molecule in the CTL immune defense of the host. Strikingly, knowledge on Qa-1^b-mediated presentation of endogenous self-peptides is largely focused on the class I-derived leader peptides. Our finding that the surface presentation of this AMAPRTLLL peptide depends on transport activity of TAP (Figs. 3 and 4) is in agreement with the previous work of others (53), demonstrating that even some leader peptides, which route proteins to the ER, need to gain access to the peptide-loading complex via TAP. Importantly, in this article we show that impairment of the processing of this leader peptide results in the presentation of other self-peptides that normally do not make it to the surface. Peptide elution studies from cells deficient for class I leader peptides have revealed that a Hsp60-derived peptide can substitute for binding to Qa-1^b (54). The fact that our TEIPP-specific Qa-1^b-restricted CTL were not reactive against this mammalian Hsp60-derived peptide (data not shown) indicates that the Qa-1^b-binding peptide pool is broader than initially postulated. The observation that Qa-1^b surface levels are relatively unaffected by TAP1 deficiency (55) underscores the notion that other peptides can compensate for the absence of the class I leader peptide. Identification of this alternative peptide repertoire will enable development of immunotherapeutic approaches for immune-evading viruses and processing deficient tumors. Importantly, due to the very limited polymorphism of Qa-1^b/HLA-E in the population (56), identified peptides could be population-wide Ags, independent of the differences in HLA typing. Our future research is now geared toward the elucidation of these yet-unknown TEIPP peptides that are presented by Qa-1^b and the exploitation of the UL49.5 inhibitor that greatly facilitates this type of study.

In conclusion, our data demonstrate that TAP deficiency leads to the presentation of Qa-1-binding CTL epitopes that are normally not displayed at the cell surface, suggesting that CTL with this novel specificity might be recruited for the immune control of tumors or viruses that use immune evasion strategies. Furthermore, we propose that UL49.5 can be exploited as a versatile tool for the inactivation of peptide transport by TAP in preclinical models for immune silencing, e.g., to prevent CTL destruction against transplants, or immune intervention, e.g., to expand CTL populations with TEIPP specificity to target tumor-immune escape variants.

Acknowledgements

We are grateful to Dr. F. Momburg for the supply of antiserum and to Dr. M. Colombo and Dr. C. Brooks for providing cell lines. Dr. F. Ossendorp is acknowledged for critically reading this manuscript and Dr. N. Fu is acknowledged for technical assistance. This project was financially supported by the Dutch Cancer Society (Grant UL2002-2709 to T.v.H. and S.L.), the Socrates European Student Exchange Program (to C.P.), and the Dutch Diabetes Research Foundation (to D.K.L).

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