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An Early Pseudorabies Virus Protein Down-Regulates Porcine MHC Class I Expression by Inhibition of Transporter Associated with Antigen Processing (TAP)

Aruna P. N. Ambagala,1 Susanne Hinkley, and Subramaniam Srikumaran2

The objectives of this study were to identify the mechanism(s) of pseudorabies virus (PrV)-induced down-regulation of porcine class I molecules and the viral protein(s) responsible for the effect. The ability of PrV to interfere with the peptide transport activity of TAP was determined by an in vitro transport assay. In this assay, porcine kidney (PK-15) cells were permeabilized with streptolysin-O and incubated with a library of 125I-labeled peptides having consensus motifs for glycosylation in the endoplasmic reticulum (ER). The efficiency of transport of peptides from the cytosol into the ER was determined by adsorbing the ER-glycosylated peptides onto Con A-coupled Sepharose beads. Dose-dependent inhibition of TAP activity was observed in PrV-infected PK-15 cells. This inhibition, which occurred as early as 2 h postinfection (h.p.i.), reached the maximum level by 6 h.p.i., indicating that TAP inhibition is one of the mechanisms by which PrV down-regulates porcine class I molecules. Infection of cells with PrV in the presence of metabolic inhibitors revealed that cycloheximide a protein synthesis inhibitor, but not phosphonoacetic acid a herpesvirus DNA synthesis inhibitor, could restore the cell surface expression of class I molecules, indicating that late proteins are not responsible for the down-regulation. Infection in the presence of cycloheximide followed by actinomycin-D, which results in accumulation of the immediate-early protein, failed to down-regulate class I, indicating that one or more early proteins are responsible for the down-regulation of class I molecules. 

Pseudorabies or Aujeszky’s disease is a highly infectious disease of pigs that is prevalent in many countries causing multimillion dollar losses each year to the pig industry (1). The causative agent, pseudorabies virus (PrV), is a member of the subfamily Alphaherpesvirinae of the family Herpesviridae (2). The disease in pigs is characterized by respiratory, neurological, and reproductive disorders (3). The animals that overcome the primary infection carry the virus in a latent form in several tissues, and reproductive disorders (3). The animals that overcome the primary infection carry the virus in a latent form in several tissues, but mainly in the trigeminal ganglia (4). Intermittent reactivation of the latent virus is followed by virus excretion, which results in infection of the susceptible animals in contact (5). The currently used vaccines against this disease elicit a very good Ab response. The Ab response, however, does not correlate well with protection (6), indicating that cell-mediated immunity plays a major role in protection against this disease.

CTLs play a critical role in controlling viral infections, especially in the case of herpesvirus infections (7), where cell-to-cell spread occurs earlier than the hematogenous spread (8). The majority of CTLs are of the CD8+ phenotype and recognize antigenic peptides presented by MHC class I molecules (9). Presentation of viral peptides by class I molecules is the result of a highly intricate multistep pathway of Ag processing and presentation. It involves the cotranslational translocation of class I heavy chain and β2-microglobulin into the lumen of the endoplasmic reticulum (ER), degradation of viral proteins into short peptides by the cytosolic proteasome, transport of these peptides from the cytosol into the ER by the TAP, binding of the peptides by class I molecule, and egress of this stable trimolecular complex from the ER via the Golgi apparatus to be expressed on the cell surface (10). As much as the complexity of this pathway provides great efficiency in elimination of viral pathogens, it also provides numerous opportunities for the viral proteins to interfere with the individual steps of this pathway, as a means of thwarting the CTL response of the host. Thus, many viruses have evolved mechanisms to interfere with the cell surface expression of class I molecules as a means of evasion of CTL recognition (11). Because PrV primarily infects the epithelial cells of the mucosal surfaces and spreads by cell-to-cell contact, this attenuation of surface class I expression may provide an opportunity to escape CTL recognition until sufficient number of virus progeny is generated to invade the surrounding tissues including neurons. Although, PrV has been shown to induce attenuation of MHC class I expression on porcine cells (12), the mechanism(s) of class I down-regulation, or the viral proteins responsible for the effect, have not been identified. In this study, we demonstrate that inhibition of peptide transport activity of the TAP is one of the mechanisms by which PrV down-regulates class I expression, and that one or more early proteins of this virus may be responsible for this effect.

Materials and Methods

Cell lines and viruses

Clones of porcine kidney (PK-15) and Madin Darby bovine kidney (MDBK) cells were obtained from American Type Culture Collection.
AN EARLY PrV PROTEIN DOWN-REGULATES PORCINE TAP

(ATTCC, Manassas, VA). The cells were expanded and maintained in RDG medium (5 g/L each of RPMI and DMEM, 4.5 g/L of glucose, and 2.85 g/L of NaHCO₃) supplemented with 10% FBS, 2 mM l-glutamine, and 20 μg/ml gentamicin (all from Life Technologies, Grand Island, NY). Indiana Funkhauser (IND-F) strain of PrV was obtained from Dr. Andrew Cheung (National Animal Disease Center, U.S. Department of Agriculture, Ames, IA). The virus was propagated in PK-15 cells and was purified over a potassium tartrate gradient. PrV modulated live virus (MLV) vaccine strains PRV/Marker Gold (SyntroVet, Lexena, KS) and Suvaxyn Herdfend PrV ggp (SOLVAY Animal Health, Mendota Heights, MN) were also propagated in PK-15 cells.

IND-F strain of PrV, at 4 × 10² PFU, was diluted in 1.5 ml of RDG medium in petri dishes and irradiated under a UV source (Fotodyne trans-illuminator, Fotodyne, New Бwelwin, WI) from a distance of 6 inches for 6 min. The efficiency of UV inactivation was assessed by absence of cytopathology following adsorption of the irradiated virus onto permissive PK-15 cells.

Antibodies

The monomorphic anti-porcine class I mAb, PT85A (IgG2a; Ref. 13), was purchased from VMRD (Pullman, WA), and the polymorphic anti-porcine class I mAb, 74.11.10 (IgG2b; Ref. 14), was kindly provided by Dr. Joan Lunney (U.S. Department of Agriculture, Beltsville, MD). Both mAbs PT85A and 74.11.10 recognize peptide-bound class I molecules. The anti-porcine pan-tissue marker mAb, 103h01-1-19 (IgM) was purchased from Phoenix Genetix (San Diego, CA). The anti-PrV glycoprotein mAbs were kindly provided by Dr. F. Zuckermann (University of Illinois, Urbana-Champaign). The anti-bovine class I mAb, IL-88S (IgG2a), was obtained from International Livestock Research Institute (Nairobi, Kenya). The anti-human class I mAb, W6/32 (IgG2a), which cross-reacts with bovine class I molecules, was obtained from the ATCC. The mAb W6/32 binds peptide-bound class I molecules only, whereas IL-88S binds class I molecules irrespective of peptide binding. The anti-PrV convalescent pig serum was a gift from Dr. Fernando Osorio (University of Nebraska, Lincoln). mAb MM113 (IgG2a) specific for BHV-1 glycoprotein D (15), mAb 6G11 (IgM) specific for BHV-1 glycoprotein gB (16), and normal pig serum were purchased from Kirkegaard & Perry Laboratories (Gaithersburg, MD).

Infection of cells

Cells (PK-15 or MDBK) grown to subconfluency in tissue culture flasks (25 cm²) or tissue culture dishes (100 × 20 mm) were infected at an appropriate multiplicity of infection (moi). Mock-infected cells were incubated with a similar volume of medium without the virus. Infection was allowed to proceed up to indicated time periods in a humidified incubator, containing 5% CO₂ at 37°C.

Metabolic inhibition experiments

Cycloheximide (ChX) (Sigma, St. Louis, MO) was used at a concentration of 100 μg/ml to inhibit the viral protein synthesis in PrV-infected (10 moi) PK-15 cells, and the cells were subjected to flow cytometry by 6 h post-infection (h.p.i.) (17). To inhibit PrV late protein synthesis, cells were infected at 10 moi in the presence of phosphonoacetic acid (PAA) at 300 μg/ml to inhibit the viral protein synthesis in PrV-infected (10 moi) PK-15 cells, and the cells were subjected to flow cytometry by 6 h.p.i. ChX reversal experiment was performed to allow accumulation of large amounts of PrV IE180 protein in infected cells (19). Briefly, PK-15 cells were either mock infected or infected at 1 moi in the presence of ChX at 50 μg/ml, and infection was allowed to proceed for 5 h. Then the cells were extensively washed and incubated with growth medium containing actinomycin-D (Act-D) at 2.5 μg/ml (Sigma) to prevent further transcription of mRNA, while allowing the translation of already accumulated IE180 mRNA. At 20 h.p.i. the cells were subjected to flow cytometry.

Flow cytometry

PK-15 and MDBG cells, either mock-infected or infected with PrV at the indicated moi, were trypsinized and immediately suspended in FACS buffer (PBS containing 3% horse serum and 0.01% sodium azide) at selected times postinfection. Cells (2 × 10⁶) were dispensed in duplicates into 96-well round-bottom plates in 50 μl volumes and were incubated with relevant mouse mAbs. After 45 min of incubation at 4°C, the cells were washed four times in FACS buffer and stained with FITC-conjugated goat anti-mouse IgG, IgM, and IgG (2.5 μg/ml) at 4°C for 30 min. After three subsequent washes in FACS buffer, the fluorescence exhibited by the cells was assessed using a Becton Dickinson FACScan flow cytometer (Mountain View, CA). For dual-labeling experiments, the cells were infected at 5 moi for 12 h. Mouse mAbs (1:1000) specific for porcine class I molecules were added, followed by porcine anti-PrV convalescent serum (1:500 dilution). PE-conjugated goat Abs to mouse IgG (5 μg/ml) and FITC-conjugated goat Abs to pig IgG (H+L) were added (2.5 μg/ml) in that order. All the incubations in the dual-labeling experiments were conducted for 30 min at 4°C. To exclude the dead cells, propidium-iodide (Calbiochem-Novabiochem, San Diego, CA) was added at a concentration of 1 μg/ml to all the samples before analysis (20).

In vitro transport assay

PK-15 or MDBG cells (6 × 10⁴), either mock-infected or infected with PrV, were trypsinized and washed twice in propagation medium and twice in ice cold transport buffer [130 mM KCl, 10 mM NaCl, 1 mM CaCl₂, 2 mM EGTA, 2 mM MgCl₂, and 5 mM HEPES (pH 7.3) with KOH]. Then the cells were incubated at 37°C with 400 μl of transport buffer containing streptolydin-O (Murex Diagnostics, Dartford, UK.) at a concentration of 1–2 U/ml, until 80% permeabilization was achieved, as judged by trypan blue (0.25%) permeation into cells (21). Subsequently the cells were washed with 10 ml of ice cold transport buffer to wash away the unbound streptolydin-O. The cells were resuspended in 400 μl of transport buffer, divided among four 1.5-ml ultra tubes, and treated with 0.5 μl of ATP (200 mU) (Sigma) and/or apyrase (20 U/ml) (Sigma) as indicated in Fig. 4. Then the cells were incubated for 10 min at 37°C with 10 μl of 1³-labelled peptide library (2 μM final concentration) composed of 2304 different peptides each containing a unique primary radio-iodination motif (NXT/S) (22, 23). The peptide transport into the ER was terminated by the addition of 1 ml of “stop buffer” (transport buffer containing 10 mM EDTA and 0.01% NaOH) at 4°C. For the “0 min” samples, stop buffer was added immediately after the addition of the radiolabeled peptides. The samples were centrifuged at 15,000 × g for 5 min and the supernatant fluid was removed. Then the cells were incubated in ice cold lysis buffer (0.5% Nonidet P-40, 5 mM MgCl₂), and 50 mM Tris-HCl (pH 7.5)). Thirty minutes later, the samples were centrifuged at 15,000 × g for 10 min and the clarified lysate was incubated with 100 μl of Con A-Sepharose beads (Pharmacia Biotech, Piscataway, NJ) for 1 h at 4°C with gentle agitation. Finally, the Sepharose beads were washed four times with ice cold lysis buffer and the bound radioactivity was counted using an automated gamma-counter (ICN Micronemed Systems, Huntsville, AL).

Results

PrV down-regulates cell surface expression of MHC class I molecules on PK-15 cells

PrV has been shown to down-regulate the expression of MHC class I molecules on PK-15 cells (12). To confirm these findings, PK-15 cells infected at 5 moi of IND-F strain of PrV were subjected to flow cytometry at different h.p.i. Cell surface expression of the porcine pan-tissue marker protein was measured as an internal control for cellular protein synthesis in PrV-infected cells. There was a progressive down-regulation of class I expression in PrV-infected PK-15 cells, whereas the expression of the pan tissue marker remained unaffected (Fig. 1). Flow cytometric analysis of class I expression on infected cells by this method, however, does not ensure that a particular cell examined for class I expression is in fact infected by the virus. Therefore, dual-labeling analysis for simultaneous detection of class I molecules and virus proteins was performed on PK-15 cells infected at 5 moi for 12 h. Results of the dual-labeling analysis confirmed the results of the previous experiment. At an moi of 5, almost 80% of the cells analyzed were productively infected with PrV by 12 h.p.i. (Fig. 2). The infected cells expressed lower levels of class I molecules. A bovine cell line, MDBG, that is permissive to PrV infection was also subjected to the same conditions, but no detectable class I down-regulation was observed (data not shown).

PrV MLV vaccine strains also down-regulate cell surface class I expression

To determine whether PrV MLV vaccine strains also are capable of down-regulating class I molecules on infected cells, PK-15 cells were infected at 5 moi with PrV/Marker gold or Suvaxyn vaccine
strains. As shown in Fig. 3, both the MLV vaccine strains induced a pronounced down-regulation of expression of class I molecules on PK-15 cell surface.

**PrV inhibits peptide transport activity in porcine cells**

To understand the mechanism(s) of class I down-regulation, we tested the possibility of TAP inhibition by PrV. The peptide transport activity of porcine TAP was measured by an in vitro transport assay. The first set of experiments were conducted to ascertain that the transport assay measured TAP-mediated transport of peptides. Previous studies have shown that the peptide transport activity of TAP is ATP-dependent (21). As expected, addition of exogenous ATP increased the transport of peptides, as shown by the increased recovery of glycosylated peptides (Fig. 4A). Furthermore, addition of apyrase, which results in the hydrolysis of ATP, profoundly impaired the peptide transport activity (Fig. 4A), indicating that the transport assay measured TAP-mediated transport of peptides. The next set of experiments were conducted to determine the effect of PrV on the transport of peptides. In PrV-infected PK-15 cells, inhibition of peptide transport activity was observed as early as 2 h.p.i., which reached the maximum level by 6 h.p.i. (Fig. 4B). In contrast, peptide transport activity in MDBK cells was not inhibited even at 8 h.p.i. (data not shown) suggesting that the TAP inhibition by PrV may be specific for porcine cells. To determine

**FIGURE 1.** PrV down-regulates cell surface expression of MHC class I molecules on PK-15 cells. PK-15 cells, mock-infected or infected with the IND-F strain of PrV at an moi of 5, were subjected to flow cytometric analysis at indicated h.p.i. Histogram overlays of MHC class I expression detected by the mAb PT85A (A), and the porcine pan-tissue marker protein expression detected by the mAb1030h-1-19 (B) are shown. The mean channel fluorescence of infected cells, expressed as a percentage of that of mock-infected cells (which was arbitrarily set as 100%), are given in parentheses. The mAb MM113 and mAb 6G11 were used as the isotype-matched controls. Dead cells were gated using propidium iodide, and 10,000 viable cells were analyzed for each sample. Results of one representative experiment of three are shown.

**FIGURE 2.** Simultaneous detection of MHC class I molecules and viral proteins on PrV infected PK-15 cells. PK-15 cells, mock-infected or infected with PrV at an moi of 5, were dual-labeled to detect class I molecules and PrV protein expression. The expression of class I molecules was detected by the murine mAb PT85A followed by PE-conjugated anti-murine IgG Abs (y-axis). The expression of viral proteins was detected by porcine anti-PrV polyclonal serum followed by FITC-conjugated anti-porcine IgG (H+L) Abs (x-axis). The isotype-matched control mAb, MM113, and normal pig serum were used to set the gates for PE and FITC fluorescence, respectively. Dot plots show class I expression in mock-infected cells (left panels) and PrV-infected cells (right panels). The percentage of positive cells is also given in the respective quadrants. Results of one representative experiment of three are shown.

**FIGURE 3.** PrV MLV vaccine strains also down-regulate class I expression on PK-15 cells. PK-15 cells, mock-infected or infected with the wild-type virus (○), PrV/Marker Gold (△), or Suvaxyn (■) vaccine virus strains at an moi of 5, were subjected to flow cytometric analysis at indicated h.p.i. with the anti-class I mAb PT85A. MHC class I expression by infected cells is shown as a percentage of that of mock-infected cells. The mAb MM113 was used as the isotype-matched control. Dead cells were gated using propidium iodide, and 10,000 viable cells were analyzed for each sample. The results represent the means of three independent experiments.
the specificity of inhibition of peptide transport activity by PrV, a
dose titration was performed in the next set of experiments. A
direct correlation between the viral moi and the degree of TAP
inhibition was observed (Fig. 4).

PrV early protein(s) are responsible for the down-regulation of
class I molecules

To identify the viral protein(s) responsible for the down-regulation of
class I molecules, we first determined whether de novo viral
protein synthesis was necessary for class I down-regulation. The
cells were treated with UV-inactivated virus at 20 moi, and class
I expression was compared with that of the cells infected with live
virus at 2 moi. Although 2 moi of live PrV induced down-regulation
of class I molecules, even 20 moi of UV-inactivated PrV did not
affect the expression of class I molecules (Fig. 5). To confirm
these findings, cells were infected at 10 moi in the presence of the
protein synthesis inhibitor, ChX. As expected, no class I down-
regulation was observed on PK-15 cells infected in the presence of
ChX (data not shown). Taken together, the above findings con-
firmed the requirement for newly synthesized viral proteins for the
down-regulation of class I molecules. The next set of experiments
was designed to infer the class of PrV proteins (immediate early,
early, or late) involved in the down-regulation. First, to ascertain
the role of viral proteins in class I down-regulation, the cells were
infected in the presence of PAA, an inhibitor of herpesvirus DNA
synthesis. As shown in Fig. 6, PAA was unable to restore class I
expression, ruling out the involvement of late proteins in class I
down-regulation. To determine whether PrV IE protein, IE180,
was responsible for this effect, a ChX reversal experiment was
conducted. Even in the presence of large quantities of IE180, class
I expression was not affected suggesting that one or more early
proteins are responsible for the down-regulation (Fig. 7).

Discussion

The phenomenon of class I down-regulation on PrV-infected
PK-15 cells and the murine cells L929 and Neuro-2A has been
demonstrated previously (12). In addition to confirming this ob-
servation, we have elucidated the mechanism of down-regulation
of class I molecules by PrV. Down-regulation of class I molecules
on the infected cell surface was observed as early as 4 h.p.i (Fig.
1). Class I expression was reduced to 50% of that of mock-infected
cells by 8 h.p.i. Histogram overlays of MHC class I ex-
pression detected by the mAb PT85A are shown. The mAb MM113 was
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FIGURE 4. PrV inhibits peptide transport activity in infected PK-15
cells. A, Peptide transport observed in permeabilized cells was TAP-de-
dependent. To ascertain that the transport assay measured TAP-mediated
transport of peptides, permeabilized PK-15 cells were treated with ATP
and/or apyrase before the addition of radiolabeled peptides. The percent
peptide transport was obtained using the following formula: [{cpm of
Con-A bound peptides/cpm of input peptides}] × 100. Results shown are
the means of duplicates. B, PrV inhibits peptide transport activity in in-
fected PK-15 cells as early as 2 h.p.i. The cells, either mock-infected or
infected with IND-F strain of PrV at 5 moi, were subjected to an in vitro
transport assay at the indicated h.p.i. as described in Materials and Meth-
ods. C, PrV inhibits porcine TAP activity in a dose-dependent manner.
PK-15 cells were either mock-infected or infected at the indicated input
moi. The infection was allowed to proceed for 4 h, and the cells were
subjected to an in vitro transport assay as described in Materials and
Methods.

FIGURE 5. UV-inactivated PrV does not down-regulate MHC class I
expression. PK-15 cells, mock-infected or infected at 2 moi of live PrV
IND-F strain or 20 moi of UV-inactivated virus, were subjected to flow
cytometric analysis at 12 h.p.i. Histogram overlays of MHC class I ex-
pression detected by the mAb PT85A are shown. The mAb MM113 was
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on the infected cell surface was observed as early as 4 h.p.i (Fig.
1). Class I expression was reduced to 50% of that of mock-infected
cells by 8 h.p.i (Fig. 3). Continued expression of the porcine pan-
tissue marker protein on the infected cells indicated that the down-
regulatory effect of the virus was specific for class I molecules. In
addition, dual-labeling experiments, in which expression of both
class I molecules and viral proteins was measured simultaneously,
confirmed that the cells which exhibited attenuated class I expres-
sion were in fact infected by the virus (Fig. 2). These findings,
therefore, confirm that PrV specifically down-regulates class I ex-
pression on PK-15 cells.
PrV IE180 is not responsible for class I down-regulation. MLV vaccines may explain, at least partially, the failure of cur-wild-type virus (Fig. 3). Down-regulation of class I expression by the vaccine strains was almost identical to that observed with the vaccine strains that we tested decreased the expression of class I but also against other viruses and intracellular pathogens. Both the cines would result in defective CTL priming, not only against PrV population of class I molecules, immunization of pigs with MLV vac-sively. If the MLV vaccine strains are also capable of down-reg-

Currently, MLV vaccines against PrV are being used exten-sively. If the MLV vaccine strains are also capable of down-reg-ulation of class I molecules, immunization of pigs with MLV vac-cines would result in defective CTL priming, not only against PrV but also against other viruses and intracellular pathogens. Both the vaccine strains that we tested decreased the expression of class I molecules. In fact, the extent of class I down-regulation by both the vaccine strains was almost identical to that observed with the wild-type virus (Fig. 3). Down-regulation of class I expression by MLV vaccines may explain, at least partially, the failure of cur-

FIGURE 6. PrV late proteins are not responsible for class I down-regulation on PK-15 cells. PK-15 cells, mock-infected or infected with the IND-F strain of PrV at an moi of 5 in the presence or absence of PAA, were subjected to flow cytometric analysis at 6 h.p.i. Histogram overlays of MHC class I expression detected by the mAb PTSSA are shown. The mAb MM113 was used as the isotype-matched control. Dead cells were gated using propidium iodide, and 10,000 viable cells were analyzed for each sample. Results of one representative experiment of three are shown.

FIGURE 7. PrV IE180 is not responsible for class I down-regulation. PK-15 cells were either mock-infected or infected with the IND-F strain of PrV at an moi of 1, in the presence or absence of ChX. The infection was allowed to proceed for 5 h, then the cells were washed thoroughly and incubated in the presence of Act-D. The samples were subjected to flow cytometric analysis at 20 h.p.i. Histogram overlays of MHC class I expression detected by the mAb PTSSA are shown. The mAb MM113 was used as the isotype-matched control. Dead cells were gated using propidium iodide, and 10,000 viable cells were analyzed for each sample. Results of one representative experiment of three are shown.

Recently used MLV vaccines to induce complete protection against this disease. In contrast to the findings with the PK-15 cells, infection of the LI14 cell line, an immortalized B cell line originating from inbred miniature pigs has been reported to result in an up-regulation of class I expression (24). The reasons for this discrep-ancy are not clear. Because porcine B cells are less permissive to PrV infection (25), the importance of this finding in the pathogen-esis of this virus is questionable.

Mellencamp et al. (12), who reported the down-regulation of expression of class I molecules by PrV, did not elucidate the mech-anisms by which the virus causes the observed effect in the porcine epithelial cells, the natural target cells of this virus. In this study, we have demonstrated that PrV inhibits the peptide transport activity of porcine TAP. The increase in the transport of peptides with the addition of exogenous ATP, and the decrease in the trans-port of peptides with the addition of apyrase (Fig. 4A), confirmed that the transport assay that we utilized in this study measured the TAP-mediated transport of peptides. TAP inhibition was detected as early as 2 h.p.i. and reached a maximum by 6 h.p.i. (Fig. 4B). Direct correlation between the viral moi and the degree of TAP inhibition indicated the specificity of inhibition of TAP by PrV (Fig. 4C).

Previous studies in our laboratory have shown that bovine her-pesvirus (BHV)-1 interferes with the TAP activity in MDBK cells (26). Although, MDBK cells (a bovine cell line) are fully permis-sive to PrV infection, no reduction in TAP activity was observed in infected cells, indicating that this inhibition of TAP by PrV may be species-specific. The inability of PrV to inhibit the TAP activity in MDBK cells also could explain the absence of down-regulation of expression of class I molecules on the surface of these cells.

HSV, human cytomegalovirus (HCMV), and BHV-1 also in-hibit the TAP activity in infected cells (26, 27). Infected cell protein (ICP) 47, an IE protein of HSV, has been shown to bind to the peptide binding site of TAP1/2 heterodimer and inhibit its peptide transport activity (28–31). ICP47 binds human TAP with a 100 fold more affinity than murine TAP (30, 31). Recent studies by Jugovic et al. (32) have shown that ICP47 inhibits porcine TAP more efficiently than the human TAP. However, we could not find any ICP47 homologue among the PrV protein sequences available to date. HCMV US6 gene product also interferes with the TAP activity, but by a different mechanism (33, 34). This ER-resident glycoprotein has been shown to associate with the TAP molecules from the ER luminal side and not interfere with peptide binding (35). Until the PrV-encoded putative protein responsible for the inhibition of peptide transport is precisely identified, the mecha-nism of TAP inhibition cannot be addressed.

In addition to the herpesviruses, other viruses have been shown to interfere with class I Ag presentation pathway. The highly oncogenic adenovirus 12 interferes with the transcription of most genes associated with Ag processing including TAP1 and TAP2 (36). Although we cannot exclude this possibility, this phenomen-on may not discord our findings due to following reasons. Even though the precise half life of TAP is not known, it is believed to have a long half life (T. Elliot, personal communication). We ob-served the inhibition of peptide transport activity as early as 2 h.p.i. Therefore, even if PrV interferes with transcription and/or trans-lation of TAP, it does not preclude our finding that PrV inhibits the transport of peptides from the cytosol into the lumen of the ER.

The inhibition of TAP by PrV may not be the only mechanism by which this virus down-regulates the cell surface expression of class I molecules. Mellencamp et al. (12) found reduced levels of class I molecules in PrV-infected mouse cells, which may be due to reduced synthesis or increased degradation of the newly syn-thesized class I molecules in the ER.
It is possible that PrV, like the cytomegaloviruses, may have evolved multiple mechanisms to perturb class I Ag presentation pathway. The immunoprecipitation experiments that we conducted with the PK-15 cells revealed reduced levels of class I molecules in PrV-infected cells (data not shown). However, the immunoprecipitation experiments were performed with an Ab that recognizes peptide-bound class I molecules only. Therefore, it is not possible to differentiate whether the synthesis or assembly (or both) are affected by the virus. Until an anti-porcine class I mAb that binds class I molecules irrespective of peptide binding becomes available, it is difficult to further elucidate these mechanisms. The failure of the anti-porcine pan-tissue marker mAb to function in immunoprecipitation experiments compounded this problem. The possibility of degradation of newly synthesized class I molecules also cannot be ruled out. Comparison of mRNA levels of virus-infected and mock-infected cells would shed light on this issue.

The second objective of this study was to identify the viral protein(s) responsible for class I down-regulation. Like in other herpesviruses, PrV gene expression occurs in a cascade fashion (37). The IE or α genes are expressed first, which induce the expression of early or β genes. The expression of early genes induces the onset of the viral DNA replication which is followed by late or γ gene expression. PrV encodes a single IE protein, IE180, which is a regulatory protein that is essential for virus replication (38). Early gene products are mostly involved in genome replication, whereas the late gene products are mainly structural. Lack of down-regulation of class I molecules in cells treated with UV-inactivated virus or live virus in the presence of ChX, a protein synthesis inhibitor, clearly indicated that virus replication was essential for class I down-regulation. The involvement of PrV IE protein, IE180, was ruled out by using the ChX reversal experiment. The cells infected in the presence of ChX accumulates large amounts of IE180 mRNA in the cytoplasm. Once ChX is removed and Act-D is added, the already accumulated IE180 mRNA undergoes translation and IE180 protein is synthesized in large amounts, whereas further mRNA synthesis is inhibited. Failure to observe class I down-regulation in these cells indicates that IE180 is highly unlikely to be responsible for the effect. The suppression of late protein expression by PAA could not restore the expression of class I to normal levels, indicating that the late proteins are highly unlikely to be responsible for class I down-regulation. Based on these data, we conclude that one or more early PrV protein(s) may be responsible for the down-regulation of class I expression on the cell surface. Additional evidence for our conclusion is provided by the kinetic studies of PrV protein expression. The early proteins are expressed as early as 1 h.p.i. and reach the maximum levels by 2 h.p.i., which accounts for the onset of down-regulation of cell surface class I expression by 4 h (37). Furthermore, since TAP inhibition was seen as early as 2 h.p.i., it is likely that an early protein(s) may be responsible for the inhibition of TAP. PrV IE180 shows a high degree of homology with ICP4 of HSV-1, IE140 of varicella-zoster virus, IE1 of equine herpesvirus, and p180 of BHV-1. It contains a nuclear localization signal and accumulates in the nuclei of the infected cells (39). In contrast, both the proteins that are known to interfere with the TAP activity are localized either in the cytosol (HSV ICP47) or in the ER (HCMV US6 gene product), where they have easy access to the TAP. Furthermore, PrV encodes only a single IE protein; therefore, at least some of the functions of HSV IE proteins have to be fulfilled by PrV early proteins (37). Therefore, it is likely that PrV encodes an early protein(s) which has functional similarity to either HSV-encoded ICP47 or HCMV-encoded US6 gene product.

PrV encodes several early proteins. Identification of early protein(s) that inhibits the transport of peptides by porcine TAP (and possibly other steps in the Ag presentation pathway), necessitates the development of transfectants expressing the individual early proteins, or the development of mutants from which individual early proteins are deleted. There are not many PrV mutants lacking early genes available, and the few that are available have not helped to identify the gene(s) responsible for the down-regulation of the expression of class I molecules (L. W. Enquist, and T. Mettlenleter, personal communication). Alternatively, anti-porcine TAP Abs could be used to coprecipitate the TAP binding PrV early protein(s) along with the TAP. Again, anti-porcine TAP Abs are not available, and the currently available anti-human TAP and anti-murine TAP Abs do not cross-react with porcine TAP. Abs that recognize porcine class I molecules irrespective of peptide binding would help in further elucidation of the mechanisms of class I down-regulation by PrV. Production of anti-porcine TAP and anti-porcine class I Abs is underway in our laboratory.

**Note.** During the review of this manuscript, Sparks-Thissen and Enquist (40) reported the results of their study of class I down-regulation by PrV in the murine system. They observed a differential regulation of Kβ and Kε molecules on the surface of L929 cells. Their study also suggested an early PrV protein to be responsible for the down-regulation of class I molecules during the early phase of infection. They also showed that the down-regulation of class I molecules during the early phase of infection was not due to inhibition of synthesis or transport of the complex through the secretory system implying that PrV might interfere with the transport of peptides, which supports our finding in the porcine system.

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**References**


