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

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CHAPTER 3

Arch Virol. 2003; 148:2023-2037

Bovine herpesvirus 1 interferes with TAP-dependent peptide transport and intracellular trafficking of MHC class I molecules in human cells

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Summary

Bovine herpesvirus 1 (BoHV-1), the cause of infectious bovine rhinotracheitis and infectious pustular vulvovaginitis in cattle, establishes a lifelong infection, despite the presence of antiviral immunity in the host. BoHV-1 has been shown to elude the host immune system, but the viral gene products responsible for this interference have not yet been identified. Studies aiming at the identification of BoHV-1-encoded immune evasion genes have been hampered by the lack of bovine-specific immunological reagents. Some of the immune evasion molecules identified for other herpesviruses are host species specific; others can act across the species barrier. In this study, experiments were performed to investigate whether BoHV-1 can infect human cells and interfere with antigen processing and presentation in these cells.A human melanoma cell line, Mel JuSo, appeared to be permissive for BoHV-1 infection. BoHV-1 induced expression of major viral glycoproteins at the surface of these cells and produced progeny virus up to 10^5 plaque forming units per ml. BoHV-1 infection resulted in impaired intracellular transport of human MHC class I molecules and inhibition of human TAP. These data indicate that the BoHV-1-encoded molecule(s) that block antigen presentation in bovine cells are able to interact with homologous components of the human MHC class I presentation pathway. The fact that immune evasion by BoHV-1 can be studied in human cells will facilitate the identification of the BoHV-1 gene products involved in this process. Moreover, the data presented here suggest that the BoHV-1 encoded inhibitors of antigen presentation represent potential immune suppressive agents for use in humans.

Bovine herpesvirus 1 (BoHV-1) is a pathogen of cattle that causes infectious bovine rhinotracheitis (IBR) and infectious pustular vulvovaginitis (IPV). BoHV-1 belongs to the subfamily *Alphaherpesvirinae*. Other members of this subfamily are e.g. *Herpes simplex virus* (HSV) types 1 and 2, *Pseudorabies virus* (PRV), and *Varicella zoster virus* (VZV). After a short acute phase, BoHV-1 persists in its host for life, mostly in a state of latency. Periodically the virus reactivates, despite the presence of antiviral immunity (13, 30, 41). The fact that BoHV-1 can survive in a fully immunocompetent host indicates that this virus is capable of escaping from elimination by the immune system.

Among the strategies employed by herpesviruses to elude the immune system are those

that interfere with antigen presentation to cytotoxic T lymphocytes (CTLs). CTLs recognize antigenic peptides in the context of major histocompatibility complex (MHC) class I molecules (48, 54]. Peptides presented by MHC class I molecules are mostly generated through degradation of cytosolic proteins by proteasomes (39, 42). The Transporter associated with Antigen Processing, TAP, translocates the resulting peptides from the cytosol into the endoplasmic reticulum (ER), where they associate with MHC class I heavy chains and β_2-microglobulin (β_2m) to form stable heterotrimeric complexes. The MHC class I complexes are then transported to the cell surface where they display their peptide content to CTLs.

Herpesviruses have acquired highly effective strategies that pecifically interfere with detection by MHC class I-restricted CTLs (15, 47, 50). Examples include HSV types 1 and 2 (3, 13, 18, 45, 55), Murine cytomegalovirus (MCMV; (27, 40, 56, 57)), Human cytomegalovirus (HCMV; (2, 4, 17, 24, 25, 32, 52, 53)), Human herpesvirus-8 (12, 23), VZV (1, 11), Epstein-Barr virus (33) and Murine gamma herpesvirus-68 (44). In addition, BoHV-1 (16, 29, 35), PRV (5, 43) and Marek's disease virus (MDV 1, 2 & 3; (22)) have been found to downregulate MHC class I expression. For these viruses however, the gene products responsible for MHC class I downregulation have not yet been identified.

Previously, we have reported that BoHV-1 employs multiple strategies to block MHC class I-restricted antigen presentation (29). Attempts to identify the BoHV-1 proteins responsible for altered intracellular trafficking of MHC class I molecules and inhibition of TAP-dependent peptide transport have been hampered by the limited availability of bovine-specific immunological reagents.The use of a human cell line permissive for BoHV-1 infection would allow the use of a substantial collection of immunological reagents specific for human molecules involved in assembly of MHC class I complexes. Antibodies against for example MHC class I and class II molecules, β2 microglobulin, TAP1 and TAP2, tapasin, ERp57, calnexin, and calreticulin will be instrumental to investigate how BoHV-1 blocks TAP and interferes with the formation of peptide-MHC class I complexes. At present, however, it is unknown whether the BoHV-1-encoded molecule(s) that inhibit antigen presentation are functional in human cells.

In this study,wereport thatBoHV-1 is able to establish a productive infection in a human cell line, Mel JuSo. Biochemical experiments performed with BoHV-1-infected Mel JuSo cells indicate that BoHV-1 interferes with the assembly of human MHC class I complexes and efficiently blocks peptide transport by human TAP.

Materials and Methods

Cells and viruses Madin-Darby bovine kidney (MDBK) cells (American Type Culture Collection [ATCC]) and the human melanoma cell line Mel JuSo (obtained from Dr. J. Neefjes, The Netherlands Cancer Institute, Amsterdam, the Netherlands) were maintained in RPMI-1640 medium, supplemented with 25mM HEPES buffer and 2mML- glutamine (Invitrogen). Both cell lines were grown in the presence of 10% heat-inactivated fetal bovine serum (Greiner), 140 IU/ml Benzylpenicillin and 140µg/ml Streptomycin. The Dutch BoHV-1.1 field strain Lam (BoHV-1^{wt}) and a virion host shutoff (vhs) deletion mutant (BoHV-1^{vhs-}) from this strain (29) were propagated on MDBK cells to obtain stocks with titers of 10^8 plaque forming units/ml, which were stored at -80^0 C until use.

Antibodies Human MHC class I molecules were immunoprecipitated using the monoclonal antibody W6/32, which is directed against a conformation-dependent epitope on MHC class I heavy chains (38). 66Ig10, a monoclonal antibody specific for human transferrin receptor, was obtained from The Netherlands Cancer Institute (Amsterdam, the Netherlands). DF1513, a monoclonal antibody against human transferrin receptor, was purchased from Santa Cruz Biotechnology, Inc. Monoclonal antibodies directed against bovine MHC class I molecules

(IL-A19), MHC class II molecules (IL-A21), and bovine transferrin receptor (IL-A165) were a gift from Dr. J. Naessens (ILRAD, Nairobi, Kenya). Bovine immune serum specific for BoHV-1 was purchased from DAKO. Monoclonal antibodies against BoHV-1 glycoprotein B (MAb 14), glycoprotein D (MAb 40), glycoprotein E/glycoprotein I complex (MAb 75), and glycoprotein C (MAb 71) were obtained from ID-Lelystad (Lelystad, the Netherlands).

Cloning procedures and transfectants The HSV1 ICP47 was excised from pcDNA3-ICP47 (a gift from Dr. K. Früh, OHSU, Beaverton, Oregon, USA) and inserted into the EcoRI site of the pcDNA3-IRES-NLS GFP vector (a gift from Dr. E. Reits, NKI, The Netherlands). Mel JuSo cells were transfected with plasmid DNA using the FuGENE™6 transfection reagent according to instructions of the manufacturer (Roche Molecular Biochemicals). Cell clones were selected in the presence of 1 mg/ml G418 and tested for GFP and ICP47 protein expression.

Flow cytometry Cells were trypsinized and resuspended in phosphate-buffered saline (PBS) containing 1% BSA and 0.05% sodium azide. The cells were incubated with specific antibodies on ice for one hour. After washing, the cells were incubated with PE-conjugated anti-mouse antibody for 45 min. Stained cells were analyzed by flow cytometry (FACScan, Becton Dickinson). To exclude the dead cells, 7-aminoactinomycin D (7-AAD, Sigma) was added at a concentration of 0.5µg/ml to all samples before analysis.

Viral infection of cells In flow cytometry experiments, cell lines were infected with BoHV-1^{wt} at a multiplicity of infection (m.o.i.) of 10. After 2 h, the inoculum was removed and cells were incubated in the presence of complete RPMI-1640 medium for 10 h. In biochemical experiments, confluent Mel JuSo cells were washed once with PBS and infected with BoHV-1^{vhs-} at an m.o.i. of 10 in serum-free RPMI-1640 medium at 37^0 C. After 2 h, complete RPMI-1640 medium was added. In all experiments, mock-infected cells were treated under the same conditions as infected cells.

Plaque assay Mel JuSo and MDBK cells were seeded in six-well tissue culture dishes and infected with serial dilutions of BoHV-1. After incubation at 37^0 C for 1 hour, the inoculum was removed; cells were washed with PBS and overlaid with medium containing 1% methylcelullose.The cultures were incubated for 3 to 5 days and cells were fixed with 4% ice-cold paraformaldehyde. Plaques were visualized by immunostaining with anti-BoHV-1-gB monoclonal antibody as described (51).

One-step growth analysis Mel JuSo and MDBK cells cultured in M12 multi well plates were infected with BoHV-1^{wt} at an m.o.i. of 2 and incubated at 4^0 C for 1 hour. After removal of the inoculum, warm medium was added and cultures were incubated at 37^0 C for 2 hours. Virus penetration was stopped by treatment with low pH buffer for 3 min. Cells and supernatant were collected separately immediately after infection and 4, 8, 12, and 36 h after infection. The cell samples were lysed by freezing and thawing. Extracellular and intracellular (cell-associated) virus titers were determined by titration on MDBK cells.

Biochemical experiments BoHV-1^{vhs-}-infected and mock-infected Mel JuSo cells were incubated for one hour in RPMI-1640 medium lacking methionine and cysteine, followed by a 30 min pulse with 200µCi/ml of [^{35}S] methionine/cysteine (Redivue PRO-MIX, Amersham Life Science, UK). For pulse-chase analysis, pulse-labeled cells were either lysed (chase time 0) or incubated in complete medium, supplemented with cold methionine and cysteine at a final concentration of 1mM for the chase times indicated.

Cells were lysed in NP-40 lysis mix containing 50mM Tris-HCl, [pH 7.4], 5mM $MgCl_2$ and

0.5% NP-40, supplemented with 1mM AEBSF (4-(2-Aminoethyl)-benzenesulfonyl fluoride), 1mM leupeptin and 20μM Cbz-L3 (Carbobenzoxy-l-Leucyl-l-Leucyl-l-Leucinal-H; Peptides International, Inc.). Samples were kept on ice throughout the experiment. Immunoprecipitations and SDS polyacrylamide gel electrophoresis were carried out as described (29). 10% SDS-polyacrylamide gels were dried and exposed to Phosphor Imaging screens, which were scanned in a Personal Molecular Imager FX (BIO-RAD Laboratories). The analysis and quantification of radiolabelled products was performed using Quantity One software (BIO-RAD Laboratories).

Endoglycosidase H (Endo H) digestion was performed following the instructions of the manufacturer (New England Biolabs). Mock-treated samples served as reaction controls.

Peptide transport assay BoHV-1^{wt}-infected and mock-infected Mel JuSo cells werewashed twice with incubation buffer (130mM KCl, 10mM NaCl, 1mM $CaCl_2$, 2mM EGTA, 2mM MgCl2, 5mM HEPES, [pH 7.3]) at 4^0 C. Cells were permeabilized in incubation buffer containing 2 IU/ml of Streptolysin O (Murex Diagnostics Ltd.) for 10 min at 37^0 C. Permeabilization was assessed by trypan blue exclusion. Permeabilized cells (3×10^6 cells/sample) were incubated with 10μl (~100 ng) of the radioiodinated peptide TVNKTERAY in the presence or absence of 10μl of ATP (100mM solution, 1MTris [pH 7.6]) in a total volume of 100 μl at 37^0 C for 10 min (36). Peptide translocation was terminated by adding 1 ml of ice-cold lysis buffer (1% Triton-X100, 500mM NaCl, 5mM $MgCl_2$, 50mM Tris-HCl [pH 7.5]) and samples were left on ice for 20 min. After centrifugation at 12,000×g, supernatants were collected and incubated with 100μl of ConcanavalinA (ConA)-Sepharose to isolate the glycosylated peptides (Amersham Biosciences) at 4^0C for 1 hr. The beads were washed four times with lysis buffer and the amount of radioiodinated peptide associated was determined by gamma counting.

Results

Infection of human Mel JuSo cells by BoHV-1 The melanoma-derived cell line Mel JuSo was used to investigate whether BoHV-1 interferes with antigen presentation in human cells. Mel JuSo cells were selected on the basis of their permissiveness for BoHV-1. BoHV-1^{wt} infection was monitored by evaluation of one-step growth kinetics, plaque formation, and cell surface expression of five BoHV-1-encoded glycoproteins. Growth kinetics of BoHV-1 were comparable for Mel JuSo cells and MDBK cells, although lower infectious virus titers were obtained from Mel JuSo cells throughout a time course of 36 hours (Fig. 1A). BoHV-1 formed plaques in monolayers of Mel JuSo cells and the cells were found to express viral glycoprotein B (Fig. 1B). Flow cytometry analysis of Mel JuSo cells infected at MOI of 10 revealed that over 95% of the cells expressed BoHV-1 gB, gE, gI, gD, and gC at the surface. The levels of viral proteins detected in Mel JuSo cells were comparable to the levels observed on MDBK cells (Fig. 1C). While the majority of Mel JuSo cells expressed uniform levels of these viral glycoproteins, BoHV-1 infection of MDBK cells resulted in heterogeneous surface expression of gD, gB and gC as indicated by the broad profiles obtained.

These data show that BoHV-1 productively infects Mel JuSo cells, replicates in these cells, and is able to express virus-encoded glycoproteins, including the product of a late gene coding for glycoprotein C.

BoHV-1 downregulate cell surface expression of human MHC class I complexes To analyze whether BoHV-1 is able to interfere with MHC class I surface expression on Mel JuSo cells, the following experiments were performed. Mock-infected and BoHV-1-infected Mel JuSo and MDBK cells were collected 12 hours after infection and were stained for cell

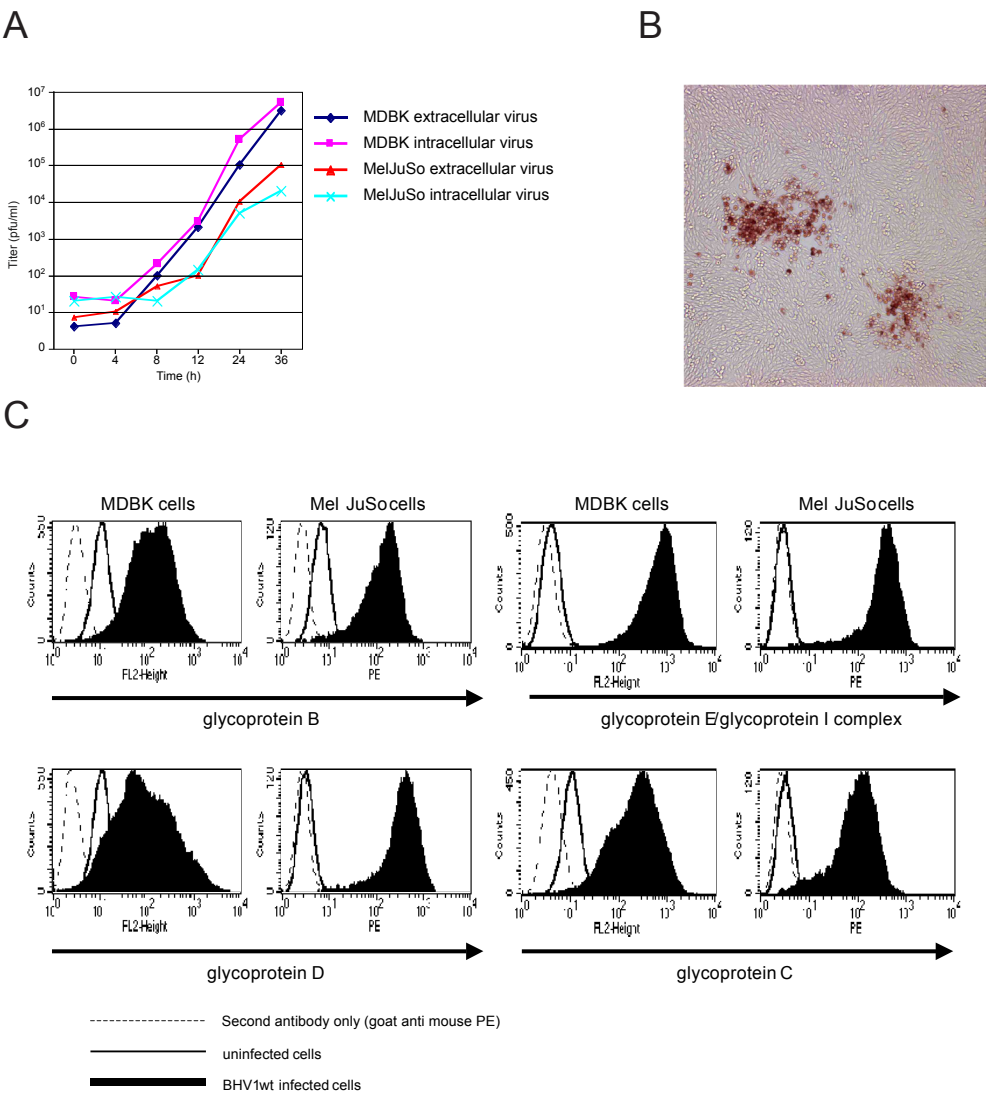

Fig. 1. Infection of Mel JuSo cells by BoHV-1. (A) One-step growth curves of BoHV-1 in Mel JuSo and MDBK cells. (B) Plaque formation on Mel JuSo cells infected with BoHV-1^{wt} for 3 days. Plaques were stained for gB expression (magnification 40×) (C) Surface expression of major BoHV-1 glycoprotein species on infected (bovine) MDBK and (human) Mel JuSo cells. Both cell lines were mock infected (unfilled black lines) or infected with wild type BoHV-1 at MOI of 10 (dark filled lines). At 12 h after infection, cells were stained for the surface expression of gB, gE/gI complexes, gD and gC, followed by PE-conjugated goat anti-mouse antibody. Surface expression was measured using flow cytometry. Background levels (second antibody only) are shown (dotted line)

surface expression of MHC class I, transferrin receptor and MHC class II molecules. The expression levels of these proteins were determined using flow cytometry. Dead cells were excluded from analysis using a gate based on 7-AAD staining and forward scatter. Over 95% of the MDBK and Mel JuSo cells were infected with BoHV-1 (Fig. 1C). Staining for antibodies against host cell proteins is presented as histograms (Fig. 2).

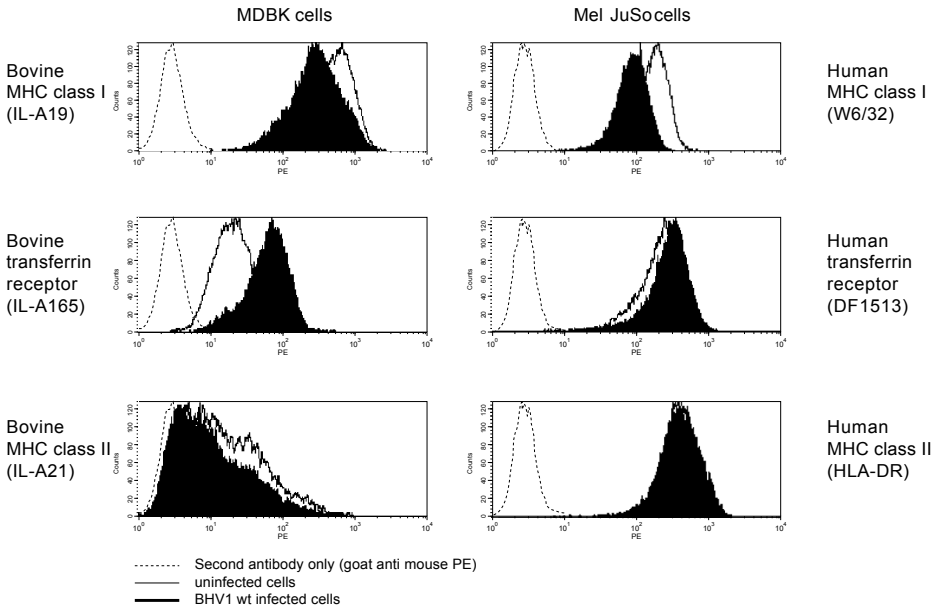

Fig. 2. BoHV-1 infection reduces cell surface expression of bovine and human MHC class I molecules.MDBKand Mel JuSo cells were infected with wild type BoHV-1 (dark filled lines) or mock infected (light filled lines). At 12 h post infection, cells were stained using antibodies specific for bovine or human cell surface molecules. Surface expression was measured by flow cytometry. Background levels (PE-conjugated anti-mouse antibody only) are shown (dotted line)

Mel JuSo cells showed a decrease in MHC class I surface density following BoHV-1 infection (Fig. 2, right row). MHC class I molecules were selectively downregulated, since the expression of other molecules at the cell surface, e.g.MHCclass II complexes,was not affected by BoHV-1 infection of Mel JuSo cells. At 12 hrs after infection, the decrease in MHC class I expression detected in the two cell lines was comparable in the presence BoHV-1^{wt} and BoHV-1^{vhs-} virus (for MDBK cells, see ref. 29).

BoHV-1^{wt} infection of MDBK cells caused a decrease in MHC class II surface expression. Downregulation of MHC class II has been observed in MDBK cells in multiple independent experiments, but interestingly this effect was only detected in cells infected with wild type BoHV-1 and not in cells infected with the BoHV-1 deletion mutant that lacks the virion host shutoff protein. Apparently, bovine MHC class II expression is relatively sensitive to the effects of the vhs protein (20, 29). A similar observation has been reported for HSV1 (49).

Upregulation of transferrin receptor has been observed consistently in the presence of both BoHV-1^{wt} and BoHV-1^{vhs-} virus in MDBK and Mel JuSo cells. At present, we are investigating this phenomenon.

BoHV-1 affects intracellular trafficking of newly synthesized MHC class I molecules in human cells To investigate the cause of reduced surface expression of MHC class I molecules in BoHV-1-infected cells, intracellular transport of MHC class I molecules was monitored in Mel JuSo cells by pulse-chase analysis. MHC class I molecules isolated at different time points were subjected to digestion with endoglycosidase H (Endo H), which

preferentially cleaves immature N-linked glycans found on ER-resident proteins as well as on glycoproteins that have not reached the medial-Golgi compartment. In mock infected Mel JuSo cells, the majority of MHC class I molecules showed conversion to an Endo H-resistant form in the course of the chase, indicating migration of these glycoproteins from the ER-cis-Golgi network to the medial Golgi (Fig. 3B). In contrast, in BoHV-1^{vhs-} infected Mel Juso cells

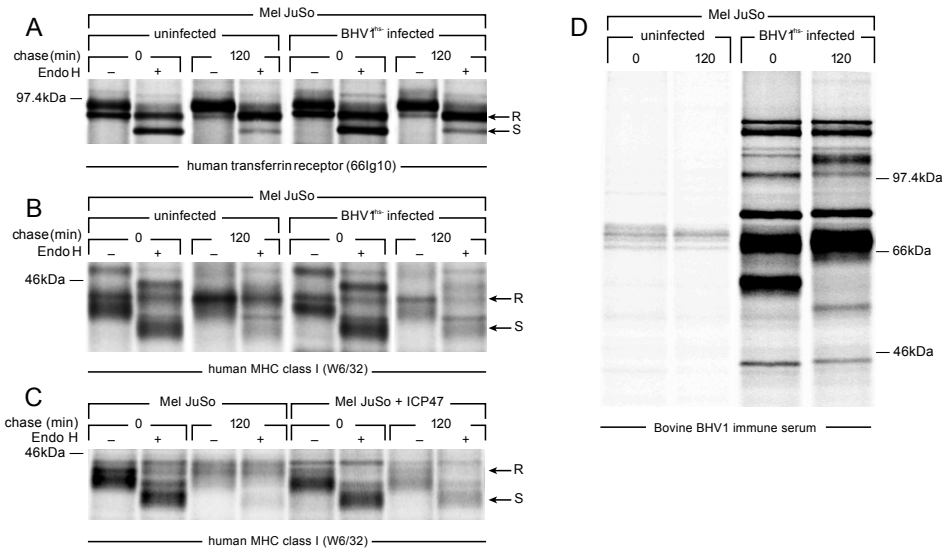

Fig. 3. BoHV-1 infection affects maturation of human MHC class I molecules. Mel JuSo cells were mock infected or infected with BoHV-1^{vhs-} at m.o.i. of 10. At 4.5 h post infection, cells were metabolically labeled for 30 min (chase point 0) and chased for 120 min. Transferrin receptor and MHC class I molecules were isolated from cell lysates using monoclonal antibodies 66Ig10 and W6/32, respectively (A and B). Immunoprecipitates were mock-treated (−) or treated (+) with Endo-H. Indicated are transferrin receptor molecules and MHC class I heavy chains resistant (R) and susceptible (S) to Endo-H treatment. Virus-specific proteins were immunoprecipitated using bovine anti-BoHV-1 immune serum (D). In C, MHC class I molecules were immunoprecipitated from Mel JuSo cells stably transfected with control vector (pcDNA$_3$-IRES-NLS GFP) or with ICP47 (pcDNA$_3$-ICP47-IRES-NLS GFP)

maturation of MHC class I molecules was inhibited. MHC I heavy chains synthesized 4 1/2 to 5 h after infection remained Endo H-sensitive after 120 min of chase (Fig. 3B). In addition, the MHC class I complexes appeared to be less stable as they were recovered less efficiently by the conformation-dependent antibody W6/32 (Fig. 3B, 120 min chase). Intracellular trafficking of a control glycoprotein, transferrin receptor, remained unaffected during the indicated chase times (Fig. 3A). BoHV-1 infection of Mel JuSo cells was demonstrated by the isolation of BoHV-1-specific proteins from the cell lysates using bovine BoHV-1 immune serum in a second round of immunoprecipitations (Fig. 3D).

In conclusion, these experiments show that BoHV-1 causes instability and intracellular retention of MHC class I molecules in human cells.

TAP activity is inhibited in BoHV-1-infected Mel JuSo cells The observed dissociation of MHC class I complexes and their retention in ER-cis-Golgi compartments may be related to a lack of peptides. Peptides are an essential component of MHC class I complexes and the effect of peptide depletion on stability of human MHC class I molecules can be demonstrated using a known inhibitor of TAP, the HSV-encoded ICP47 (14, 18, 55). ICP47 has been shown to interfere with peptide binding to cytosolic domains of the transporter complex (3, 7, 18, 37, 45). In Mel JuSo cells stably transfected with ICP47, maturation of MHC class I molecules was impaired (Fig. 3C). The migration pattern of Endo H-digested MHC class I heavy chains

was very similar in ICP47-expressing and BoHV-1^{vhs-}-infected Mel JuSo cells (Fig. 3B and C). Moreover, MHC class I complexes were unstable in both cell lines (compare W6/32 reactivity at 120 min chase). Combined with the observation that BoHV-1 blocks peptide transport by TAP in bovine cells, these data strongly suggest that BoHV-1 also inhibits the human TAP complex. To investigate this possibility, TAP activity was measured in Mel JuSo cells at 2, 3 and 4 hrs after infection with BoHV-1^{wt} virus (Fig. 4). Already after 2 hrs of infection, Mel JuSo cells displayed some decrease in peptide transport. Four hrs after infection an 86% reduction in recovery of TAP translocated peptides was observed. In conclusion, these data demonstrate that BoHV-1 encodes a potent inhibitor of TAP that not only blocks the activity of the bovine peptide transporter but also its human counterpart.

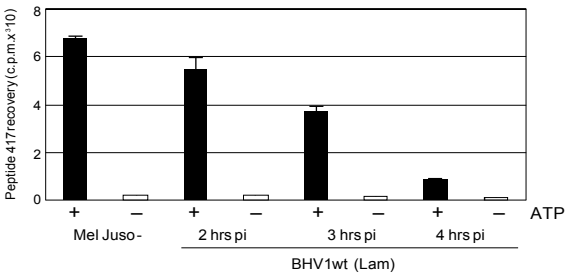

Fig. 4. BoHV-1 inhibits ATP-dependent peptide transport by TAP in human cells. Mel JuSo cells were mock infected or infected with BoHV-1^{wt} at m.o.i. of 10. At 2, 3 and 4 h post infection, cells were collected, permeabilized with Streptolysin O and translocation of the iodinated peptide 417 (TVNKTERAY) into the ER of the cells was measured. Transport rates in the presence (+) of ATP and absence (−) of ATP are indicated. The bars represent means of triplicate values, expressed as counts per minute (c.p.m.)

Discussion

BoHV-1 was found to infect the human melanoma cell line Mel JuSo and perform a complete replication cycle in these cells. The overall patterns of one-step growth curves of both intracellularly and extracellularly produced virus were remarkably similar in Mel JuSo cells and MDBK cells. The onset of excretion of infectious BoHV-1 virus took place in Mel JuSo cells as early as in MDBK cells, namely at 8 hrs after infection. Although virus titers were 10 to 100 times lower in Mel JuSo cells than in MDBK cells, these data show that the human cells can support the replication of BoHV-1 and excrete infectious BoHV-1 virus with titers up to 10^5 p.f.u./ml. Similar observations were done when growth characteristics were evaluated in human fetal lung cells (34). This suggests that virus-host interactions essential for virus replication are similar in human and bovine cells. Several BoHV-1 genes coding for major viral glycoproteins were expressed at comparable levels and at similar time points in both cell types, i.e. the early glycoproteins gB, gD, gE and gI, and the late glycoprotein C. Although these data do not imply that BoHV-1 infection in Mel JuSo cells is identical to BoHV-1 infection in MDBK cells, the observed replication characteristics justified the use of Mel JuSo cells for the assessment of the impact of BoHV-1 infection on MHC class I-restricted antigen presentation.

BoHV-1-infected Mel JuSo cells, identified by the presence of viral glycoproteins at the cell surface, displayed a reduced expression of MHC class I complexes at their cell surface. Downregulation of human MHC class I molecules was comparable in Mel JuSo and MDBK cells. BoHV-1 encodes a virion host shutoff (vhs) protein, which causes a rapid inhibition of host cell protein synthesis as early as 3 hours after infection. This virus-induced inhibition of protein synthesis may also be responsible for reduced cell surface expression of MHC class I molecules in BoHV-1 infected Mel JuSo cells. Using a BoHV-1^{vhs-} mutant we could show that the vhs effect is only partially responsible for downregulation of MHC class I molecules (29). In BoHV-1^{vhs-} infected Mel JuSo cells, synthesis of cellular proteins, including MHC class I

molecules, was unaffected. Newly synthesized MHC class I proteins, however, were retained in an ER/cis-Golgi compartment. Intracellular trafficking of the transferrin receptor was not influenced.

In BoHV-1-infected Mel JuSo cells, MHC class I heavy chain-β2m complexes were formed, but they appeared to be unstable. Dissociation of the MHC class I complexes may be related to the absence of peptides. The effect of peptide depletion on stability of human MHC class I molecules could clearly be demonstrated using the HSV-encoded inhibitor of TAP, ICP47 (3, 14, 18, 45, 55). Intracellular trafficking and stability of MHC class I was impaired in a similar fashion in BoHV-1-infected cells and cells transfected with ICP47 (Fig. 3B and 3C).

When TAP-dependent peptide transport was evaluated in Mel JuSo cells, TAP function appeared to be inhibited throughout the time course of BoHV-1^{wt} infection (Fig. 4). These data suggest that the viral protein responsible for TAP inhibition is synthesized at early stages of infection. Similar results were obtained with BoHV-1^{wt} infected MDBK cells, which is in accordance with previous reports (19, 29). Based on these findings we can now conclude that there must be one or more BoHV-1 early gene products that specifically interfere with the function of both bovine and human TAP. A comparison of TAP function in BoHV-1 ^{wt} and vhs−infected cells revealed a very similar reduction of peptide transport by these two viruses (29). This may be a reflection of the very long half-life of TAP. Compared to TAP, MHC class I molecules, chaperones such as ERp57 and proteasome subunits have a much shorter life span. At 3–5 h post infection the expression of these molecules is reduced in the presence of BoHV-1 wt but not the vhs− virus (29).

The recently identified bovine TAP2 (BoTAP2) subunit showed 80.6% shared identity to the human TAP2 subunit (6). The BoHV-1-encoded inhibitor(s) of TAP may target homologous sequences within the bovine and human transporters. The HSV-encoded ICP47 is also capable of interacting with TAP transporters of other host species, as it blocks bovine, porcine, and canine TAP. It has, however, a minor or no effect on mouse, rat, guinea pig or rabbit TAP (26, 46). Despite the high degree of genomic and biological similarity of BoHV-1 and herpes simplex viruses, searches of genomic and protein databases have not yielded obvious homologues of ICP47 within the BoHV-1 genome.

Several other herpesviruses have been found to inhibit TAP-dependent peptide transport. In addition to HSV types 1 and 2 and BoHV-1, PRV encodes a yet unidentified early gene product that inhibits the peptide transporter (5). The betaherpesvirus HCMV blocks TAP through US6, an ER-resident type 1 membrane glycoprotein that interacts with luminal domains of TAP (4, 17, 20, 21, 28, 32). The fact that viruses target a similar step in antigen presentation, but do so in a different manner, is a frequently observed phenomenon (49).

At present, it is unclear whether the ER retention of MHC class I molecules in BoHV-1-infected cells is solely due to a lack of peptides. The observed retention of MHC class I molecules could involve additional viral protein(s) specifically binding to MHC class I molecules and retaining them in the ER, analogous to the adenovirus E3/19K protein (10), the MCMV gp40 (56, 57), or the HCMV gpUS3 (25, 31). The BoHV-1-encoded inhibitor of peptide translocation may also interfere with the interaction of MHC class I molecules with TAP. Interference with MHC class I–TAP association has been described for adenoviruses and may cause a delay in peptide loading of MHC class I molecules (8).

In conclusion, evidence has been presented here that BoHV-1 infection of human melanoma cells selectively affects MHC class I-restricted antigen presentation. A biochemical analysis of the biosynthesis and intracellular trafficking of MHC class I molecules revealed specific ER retention of newly synthesized MHC I heavy chains, probably as a result of strong decrease in TAP-dependent peptide transport at early time points of infection. The BoHV-1 permissive Mel JuSo cells should facilitate identification and characterization of the gene product(s) involved in BoHV-1-mediated inhibition of MHC class I-restricted antigen presentation. A better understanding of these mechanisms will be instrumental in the development of vaccines and

anti-viral drugs. Based on their activity in human cells, the BoHV-1-encoded inhibitors of antigen presentation may find applications as immune suppressive agents in humans. Moreover, viral immune evasion genes may be useful in the context of gene therapy involving viral vectors. Herpesvirus-derived immune evasion genes have been introduced into adenovirus vectors and were shown to significantly prolong their survival in the host (9).

Acknowledgements

We would like to thank Dr. J. Naessens (ILRAD, Nairobi) for the monoclonal antibodies IL-A19 and IL-A165 and Jan Beentjes for preparing the figures. This work was supported by grant no. RUL 1998–1791 from the Dutch Cancer Society (to M.E.R.).

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