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Short communication

Bovine herpesvirus-1 infection affects the peptide transport activity in bovine cells

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Abstract

Infection of cattle with bovine herpesvirus-1 (BHV-1) impairs the cell-mediated immune response (CMI) of the affected host. We investigated the location of interference of BHV-1 with the major histocompatibility complex (MHC) class I antigen presentation pathway by employing an assay that allows assessment of the peptide transport activity of the Transporter associated with Antigen Presentation (TAP) from the cytoplasm into the endoplasmic reticulum (ER). We found a considerable down-regulation of the peptide transport activity in bovine epithelial cells, taking place as early as 2 h after virus infection. This down-regulation was also dose-dependent, and, at high multiplicities of infection (moi), led to an almost complete shutdown of TAP. By inhibiting peptide transport into the ER, the virus impairs loading of MHC class I molecules and their subsequent egress from the ER to the cell surface. This may lead to defective priming of cytotoxic T lymphocytes. Thus, BHV-1 is yet another member of its family Herpesviridae that selectively interferes with the host's antigen presentation machinery to evade the host's immune response in vivo

Keywords: Bovine herpesvirus 1; MHC class I down-regulation; TAP

Bovine herpesvirus 1 (BHV-1), a member of the Alphaherpesvirinae subfamily, is an economically important pathogen causing considerable losses for the cattle industry in the United States (National Agricultural Statistics Service, 1996). This virus is one of the major pathogens in 'Bovine Respiratory Disease', a disease complex of multiple etiology also known as 'shipping fever' (Yates, 1982). Clinical symptoms of BHV-1 infection involve predominantly the upper respiratory tract (rhinotracheitis) as well as the genital tract (vulvo-

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vaginitis), and pregnant cows frequently abort the fetus (reviewed in Wyler et al., 1989). Most importantly, however, viral infection appears to render the host immunosuppressed in that the cell-mediated immune response (CMI) is depressed (Bielefeldt Ohmann and Babiuk, 1985; Carter et al., 1989). Live BHV-1, as well as the inactivated virus, induce apoptosis in mitogen-stimulated peripheral mononuclear cells, thus inhibiting the proliferative response to antigenic stimulation (Hanon et al., 1996). Additionally, the anti-BHV-1 humoral immune response is not fully protective since infection can proceed in the presence of neutralizing antibodies (Marshall and Letchworth, 1988). This status predisposes infected animals to multiple viral infections, and often fatal secondary bacterial infections, most commonly with *Pasteurella haemolytica* in a viral-bacterial synergism (Yates et al., 1983).

Efficient cytotoxic T lymphocyte (CTL) response to a viral infection is the result of a highly intricate multiple-step pathway of antigen processing and presentation. It involves synthesis of the MHC molecule, assembly with its peptide ligands generated in the cytosol, subsequent egress of the complex from the ER, and transport to the cell surface (reviewed in York and Rock, 1996). As much as the complexity of this pathway provides great efficiency in elimination of viral pathogens invading the cell, it also provides abundant opportunities for viral gene products to interfere with the individual steps of the pathway.

The concept of viral interference with the host's CTL response has been intensively investigated in herpes simplex virus (HSV), an alphaherpesvirus like BHV-1 (Hill et al., 1994; York et al., 1994; Frueh et al., 1995; Hill et al., 1995; Tomazin et al., 1996). Additionally, several studies have contributed to the elucidation of the mechanism of interference with the CMI by human cytomegalovirus (HCMV; van Endert et al., 1994; Jones et al., 1995; Gilbert et al., 1996; Hengel et al., 1996; Jones et al., 1996; Machold et al., 1997), murine cytomegalovirus (MCMV; del Val et al., 1992; Campbell and Slater, 1994; Thaele et al., 1995; Ziegler et al., 1997), and adenoviruses (Feuerbach et al., 1994; Flomenberg et al., 1994; Rotem-Yehudar et al., 1994; Schouten et al., 1995; Rotem-Yehudar et al., 1996). Previously, we have shown that BHV-1 down-regulates the surface expression of MHC class I on bovine epithelial cells (Nataraj et al., 1997). In an attempt to identify the location of viral interference with the antigen presentation pathway, we investigated if BHV-1 exerts an inhibitory effect on the heterodimeric protein responsible for peptide transport into the ER, the TAP. For this purpose we employed an assay that allows direct assessment of the translocation activity of TAP (Neefjes et al., 1993; Hill et al., 1995). The assay takes advantage of the fact that peptides, once translocated into the ER, are glycosylated on a glycosylation consensus motif (Asn-X-Thr/Ser, $X \neq Pro$), and subsequently stably retained in the ER (Heemels et al., 1993). Glycosylated peptides are then recovered on concanavalin A-Sepharose beads.

For the peptide translocation assay, Madin Darby Bovine Kidney (MDBK) cells were grown to subconfluency, and mock-infected or infected at different input multiplicities of infection (moi) with the plaque-purified Cooper strain of BHV-1. Infection was allowed to proceed for various periods of time before cells were trypsinized, and washed twice in propagation medium (1:1 mixture of DMEM and RPMI, 10% fetal bovine serum, 2 mM L-glutamine, 20 μg/ml gentamycin), and twice at 4°C in transport buffer (130 mM KCl, 10 mM NaCl, 1 mM $CaCl_2$, 2 mM EGTA, 2 mM $MgCl_2$, 5 mM HEPES, pH 7.3 with KOH). Streptolysin O, at a concentration of 1–2 U/ml in transport buffer, was added, and appropriate permeabilization of the cells was assessed microscopically with 0.25% Trypan Blue solution. A peptide library comprising 2304 different peptides (Heemels et al., 1993), each containing a Y for radioiodination and a consensus glycosylation motif (NXT), was labeled with ^{125}I in a chloramine-T catalyzed reaction. Ten microliters of labeled peptide (from an approximately 30 μM solution) was added to 10^6 permeabilized cells. Incubation for 10 min at 37°C allowed for transport of the peptides into the ER before addition of 1 ml of 'stop buffer' at 4°C (transport buffer, 10 mM EDTA, 0.01% NaN_3) which terminates the transport. In 0-min control samples, stop buffer was added immediately after addition of the peptide

solution. The cells were then lysed (0.5% NP-40, 5 mM $MgCl_2$, 50 mM Tris–HCl, pH 7.5), and the clarified lysate was incubated with 100 μl of concanavalin A-Sepharose with gentle shaking for 1 h at 4°C. After four washes of the beads with lysis buffer, the radioactivity associated with the beads was counted in a γ-counter. All samples were run in duplicates, and results shown are the mean of three independent experiments.

For the first set of experiments, cells were infected at 10, 25, 50 and 100 input moi, and infection was allowed to proceed for 4 h before assaying the cells. Fig. 1 shows the percentage of peptide transport in BHV-1 infected cells compared to mock-infected cells. The peptide translocation activity of TAP in those mock-infected cells was arbitrarily set as 100%. In infected cells the transport activity of the TAP was down-regulated in a dose-dependent manner. Whereas at 10 moi the TAP activity was down-regulated by 65% compared to mock-infected cells, infection at 100 moi led to an almost complete shutdown of the peptide transport (93% down-regulation). In the second set of transport assays, we studied the kinetics of the BHV-1 induced down-regulation of TAP activity. Cells were infected at 20 input moi and incubated for the times indicated. As shown in Fig. 2, infected cells could maintain the integrity of peptide transport up to 1.5 h post-infection (p.i.). Between 2 and 4 h, however, there was a rapid and considerable down-regulation of the TAP activity (transport down-regulated by 65%). After infection with BHV-1 at 100 input moi (data not shown) we observed a slight down-regulation of TAP activity as early as 1 h p.i. The most prominent decrease in TAP activity, however, was also observed between 2 and 4 h p.i., and the TAP activity remained at this low level during the time period examined (6 h p.i.). Since BHV-1 expresses its viral proteins in a temporally tightly regulated fashion (Misra et al., 1981), these data could be interpreted as an immediate-early protein of the virus being responsible for the observed effect on TAP activity. Additionally, the

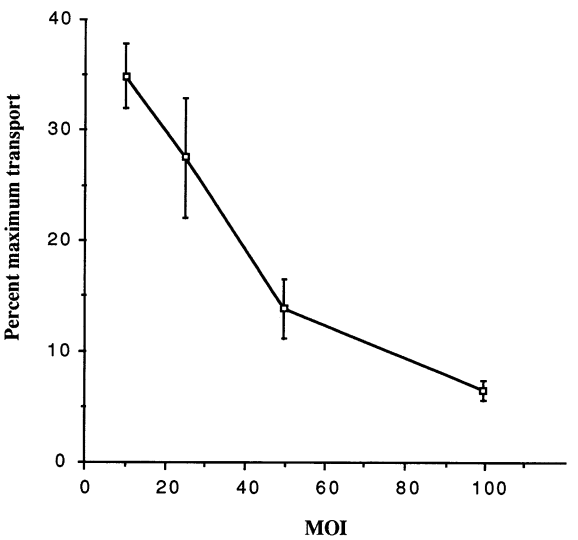

Fig. 1. BHV-1 inhibits TAP-dependent peptide transport in a dose-dependent manner. MDBK cells were either mock-infected or infected at the indicated input moi. Four hours p.i., cells were subjected to the transport assay to assess their capacity of TAP-dependent peptide transport. The x-axis represents the input viral moi, and the y-axis represents the transport of peptides in infected cells expressed as a percentage of the transport in mock-infected cells, which was arbitrarily set as 100%. All samples were run in duplicates, and the results shown are the mean of three independent experiments.

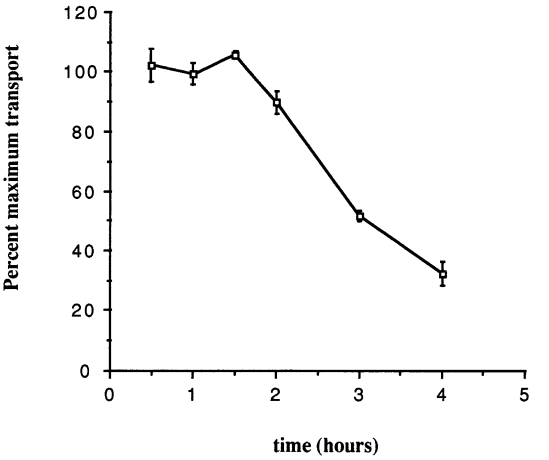

Fig. 2. Kinetics of down-regulation of peptide transport activity in BHV-1-infected cells. MDBK cells were either mock-infected or infected with BHV-1 at 20 moi. At the indicated hours p.i., cells were subjected to the transport assay to assess their capacity of TAP-dependent peptide transport. The x-axis represents the hours p.i., and the y-axis represents the transport of peptides in infected cells expressed as a percentage of the transport in mock-infected cells, which was arbitrarily set as 100%. All samples were run in duplicates, and the results shown are the mean of three independent experiments.

down-regulation persists over at least 6 h p.i. Therefore, it is tempting to speculate that the viral protein responsible might be expressed at both immediate–early and early time points in the viral replication cycle, to assure decreased class I surface expression during the time when the virus has yet to make progeny inside the cell. This would be consistent with the observation that the peptide transport in cells infected at 100 input moi showed a slightly decreased transport activity as early as 1 h p.i. (data not shown), which would coincide with the onset of expression of immediate-early genes. Between 3 and 4 h p.i., which can be considered the peak of immediate–early gene expression, the TAP activity is considerably impaired. The fact that the observed down-regulation occurred in a dose-dependent manner indicates a specific viral interference with TAP activity. In addition, a control experiment where MDBK cells were infected with an equivalent input moi of pseudorabies virus, as well as bovine viral diarrhea virus, showed no down-regulation of TAP activity (data not shown). Therefore, the possibility of TAP being down-regulated by the presence of a heavy virus load in the cytoplasm can be ruled out.

This study indicates that the highly specialized peptide transporter TAP is a cellular target for BHV-1. Since peptide loading is required for ultimate surface expression of MHC class I molecules (Townsend et al., 1989), this interference could explain the previously observed down-regulation of the surface expression of class I molecules (Nataraj et al., 1997). The fact that TAP is not completely shut off, even in the later stages of infection and at a very high input moi, is consistent with our finding that the down-regulation of class I molecules on the surface is considerable but not complete (Nataraj et al., 1997).

Since the cytosolic immediate–early protein ICP47 of HSV has been identified to selectively bind to TAP and abrogate peptide transport (Frueh et al., 1995; Hill et al., 1995; Tomazin et al., 1996; Ahn et al., 1996), the HCMV gene product US6 also has been shown to interfere specifically with the TAP activity (Hengel et al., 1996; Ahn et al., 1997; Hengel et al., 1997). Additionally, the gene products from two early genes, US11 and US2, accelerate class I heavy chain turnover (Jones et al., 1995; Machold et al., 1997), and the immediate–early US3 gene product impairs egress of the class I heavy chain from the ER (Jones et al., 1996). An HCMV matrix protein, pp65, is involved in selectively restricting access of the viral immediate–early proteins to the proteolytic machinery of the cell (Gilbert et al., 1996). In MCMV infected cells, there are multiple mechanisms that appear to function independently, however in concert, to down-regulate the class I expression (Thaele et al., 1995; Ziegler et al., 1997). Intriguingly, TAP has been reported to be a target for adenovirus 12 also (Rotem-Yehudar et al., 1994, 1996), in that both TAP-1 and TAP-2 mRNA synthesis is down-regulated in infected cells. Additionally, interference of the virus with the processing of a NFκB1 precursor down-regulates the transcription of class I mRNA (Schouten et al., 1995).

In light of those findings providing evidence for the presence of multiple independent mechanisms to evade the host's CTL response, it is not surprising that BHV-1 also appears to employ two approaches: down-regulation of MHC class I synthesis (Nataraj et al., 1997) and inhibition of peptide transport. Thus, BHV-1, similar to HCMV, MCMV and adenovirus 12, seems to also have evolved multiple and/or sequential mechanisms to dwarf the expression of class I molecules.

Although there are several details that await further elucidation, BHV-1, like other prominent members of its family, appears to specifically and efficiently target the MHC class I antigen presentation pathway. This strategy, taking place very early in infection, might impair CTL recognition and elimination of infected cells, thus allowing efficient immediate–early and early viral gene expression. In light of the recent findings of the presence of redundant mechanisms in related viruses to evade host CTL responses, it is likely that more than one gene product of the virus may be responsible for interference with class I expression on the cell surface.

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