Unmasking the masters of evasion : TAP inhibition by varicellovirus UL49.5 proteins

Verweij, M.C.

General discussion

10

General discussion

An inefficient virus kills its host. A clever virus stays with it. – James Lovelock

The large majority of the human population carries one or more herpesviruses. These viruses are often encountered early in life and are never cleared. Herpesviral infections are often asymptomatic, but when the surveilling immune system weakens, the virus might reactivate. A well-known example of an occasionally reactivating virus is herpes simplex virus 1 that can cause cold sores whenever its host is weakened by illness or stress. The herpesvirus-host relationship is usually balanced through anti-viral immune responses and the counteracting immune evasion strategies employed by the virus. This thesis focuses on the immune evasion molecule UL49.5 encoded by a subgroup of varicelloviruses. This protein inhibits the transporter associated with antigen processing (TAP), thereby obstructing a key event in the processing and presentation of virus-derived antigens.

In addition to BoHV-1 UL49.5, seven other UL49.5 proteins have been identified as potent TAP inhibitors (**chapter 5 and 6**). These include BoHV-5, bubaline herpesvirus 1 (BuHV-1), cervid herpesvirus 1 (CvHV-1), pseudorabiesvirus (PRV), equid herpesvirus (EHV) 1 and 4, and feline herpesvirus 1 (FeHV-1) UL49.5. BoHV-1, PRV, EHV-1 and EHV-4 UL49.5 inhibit peptide transport by introducing a conformational arrest within the TAP complex. The capacity of BoHV-1 UL49.5 to destabilize TAP is shared by the homologs of BoHV-5, BuHV-1, and CvHV-1, which are all varicelloviruses infecting ruminants. Mutational studies have shown that amino acid residues Arg72 and Arg74 of the C-terminal RGRG motif are required for BoHV-1 UL49.5-initiated TAP degradation (**chapter 4**). The RGRG motif is conserved amongst the UL49.5 proteins of BoHV-5, BuHV-1, and CvHV-1, suggesting that this domain also mediates TAP degradation induced by these homologs. EHV-1 and EHV-4 UL49.5 prevent ATP binding to the TAP transporter, in addition to the induction of a conformational arrest. EHV-1 UL49.5 deficient of its cytosolic tail retained the capacity to block ATP binding, which implies that this protein is not directly occupying the ATP binding site of TAP. PRV and, most likely, FeHV-1 UL49.5 only cause conformational rearrangements within TAP, without interfering with the stability or ATP-binding capacity of the complex. This is probably the common basis of TAP inhibition by varicellovirus UL49.5 proteins. The fact that, so far, only EHV-1 and EHV-4 UL49.5 interfere with ATP binding is presumably associated with these conformational changes as well.

BoHV-1 UL49.5 exhibits the remarkable feature of inhibiting human, in addition to bovine TAP. The crosstalk between BoHV-1 UL49.5 and the proteins involved in MHC I peptide presentation could therefore be studied in using a wealth of reagents specific for human proteins and in cell lines that lack particular components of the MHC I antigen presentation pathway (**chapter 2**). These studies revealed that BoHV-1 UL49.5 targets the '6+6 TM' core domain of TAP directly, other members of the peptide loading complex are

dispensable. The conformation of TAP was comparably affected in cells expressing BoHV-1, PRV or EHV-1 UL49.5, suggesting that these homologs target the same region. BoHV-1 inhibits the function of bovine, human, mouse, rat, porcine, and equine TAP (our unpublished observations), which indicates that the viral inhibitor associates with a domain within the '6+6 TM' core complex of TAP that is conserved between these host species. This assumption is strengthened by the fact that all known TAP-inhibiting homologs are capable of blocking human TAP in addition to their host TAP (**chapter 5 and 6**).

Studies aimed to functionally dissect the BoHV-1 UL49.5 protein revealed that essentially all domains of the protein are indispensable and function as a whole (**chapter 3 and 4**). The ER luminal domain of the protein by itself retains partial TAP inhibition capacity, although the transmembrane domain is required to fully stabilize this interaction. The cytosolic tail domain of BoHV-1 contains two functional motifs, a yet unknown motif that contributes to TAP inhibition and the RGRG motif that mediates UL49.5-induced degradation of TAP. However, the cytosolic tail only functions in the presence of the ER luminal domain. Thus, BoHV-1 UL49.5 contains multiple functional motifs that rely on each other's presence, this is summarized in Fig. 1. Comparison the amino acid sequence of BoHV-1 and other TAP-inhibiting homologs revealed that regions of homology are limited and scattered throughout the sequence. These observations obscure the definition of the sequence domains or individual amino acid residues that are responsible for TAP inhibition and the mechanism exploited.

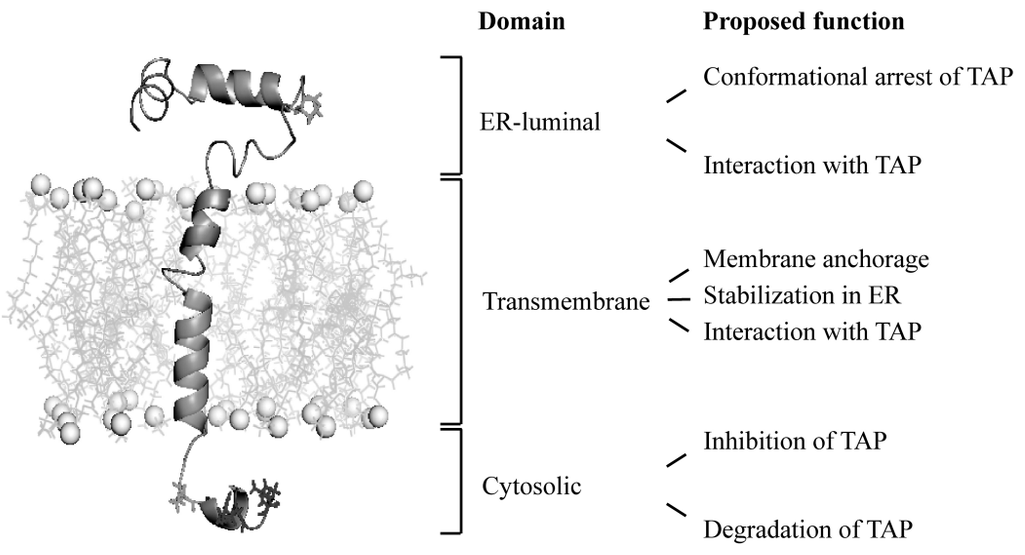

Fig. 1, the predicted 3D structure of BoHV-1 UL49.5. Predicted tertiary structure of UL49.5 (ribbon) inserted in a lipid bilayer (sticks) as determined using the I-TASSER server. The different domains of the UL49.5 protein together with their proposed function are indicated. The proline residues 48 and 87 are shown in ball-and-stick in light gray, the arginine residues 93 and 95 are shown in ball-and-stick in dark gray.

Stable expression of BoHV-1 UL49.5 in various mouse cell lines revealed that this protein is also capable of strongly inhibiting mouse TAP, which is reflected by the downregulation of MHC I molecules encoded by different alleles (**chapter 7**). The efficacy of BoHV-1 UL49.5 was gratefully applied to effectively induce cytotoxic T cell responses directed against tumor cells in which TAP function is comprised. Several tumors have been shown to limit the presentation of tumor-derived antigens on MHC I molecules by downregulating TAP expression (Chang et al., 2003; Hicklin et al., 1999). The absence of conventional antigen processing, results in the presentation of T cell epitopes associated with impaired peptide processing or 'TEIPP' antigens (van Hall et al., 2006). Previously, BoHV-1 UL49.5 was used to inhibit TAP-dependent antigen presentation in mouse colon carcinoma cells, thereby selectively enhancing the presentation of TEIPP on these cells (van Hall et al., 2007). In **chapter 8** we describe the establishment of three TEIPP-specific human T cell clones using BoHV-1 UL49.5-expressing antigen presenting cells. Such clones can be used to specifically target TAP-inhibited tumor cells. In addition, these results imply that BoHV-1 UL49.5-expressing dendritic cells can be utilized as a vaccine to induce tumor-specific immune responses.

So far, seventeen different herpesviruses have been assigned to the genus *Varicellovirus*, eleven of which have been screened for UL49.5-mediated TAP inhibition (**chapter 5 and 6**) (Davison et al., 2009). The six remaining varicelloviruses include caprine or goat herpesvirus 1 and CvHV-2 or reindeer herpesvirus, which share a high degree of homology with the other ruminant infecting varicelloviruses and are therefore expected to code for a TAP-inhibiting UL49.5 protein (Ros and Belak, 2002). This also counts for EHV-3, EHV-8 or asinine herpesvirus 2 (infecting donkey), and EHV-9 or gazelline herpesvirus (infecting gazelle) that are closely related to EHV-1 and EHV-4 (Hartley et al., 1999; Browning et al., 1988; Fukushi et al., 1997). Phocid herpesvirus 1 (PhHV-1) or harbor seal herpesvirus is interesting with respect to UL49.5-induced TAP inhibition. This virus is closely related to canine herpesvirus 1 (CaHV-1) and FeHV-1 (Martina et al., 2003). The latter varicelloviruses code for a UL49.5 protein that potently inhibits TAP, while CaHV-1 UL49.5 only affects TAP transport by ~20 percent. It would be interesting to see whether PhHV-1 UL49.5 behaves like FeHV-1 or CaHV-1 UL49.5. Nevertheless, it is clear that the UL49.5 proteins encoded by carnivore-infecting varicelloviruses are not on the same page. Another weak TAP inhibitor is the UL49.5 protein of simian varicellovirus (SVV) UL49.5, this protein reduces TAP function by only 15 percent. However, its human counterpart encoded by varicella-zoster virus (VZV) does not affect peptide transport at all. Surprisingly, this UL49.5 homolog is able to interact with the TAP transporter.

Outside the genus *Varicellovirus* no TAP-inhibiting UL49.5 proteins were identified. Herpes simplex virus (HSV) 1 and 2, members of the simplexviruses, code for the TAP

inhibitor ICP47 (York et al., 1994; Hill et al., 1995; Fruh et al., 1995). Homologs of this protein are only found in simplexviruses, suggesting that its development is a late evolutionary event that occurred after simplexviruses and varicelloviruses separated (Fig. 2). In accordance, evidence exists that the ICP47 gene arose *de novo* and was not captured from elsewhere (McGeoch and Davison, 1999).

The betaherpesviruses human cytomegalovirus (HCMV) and rhesus cytomegalovirus (RhCMV) code for the TAP inhibitor US6 (Ahn et al., 1997; Lehner et al., 1997; Hengel et al., 1997; Pande et al., 2005). Other Old World primates, namely chimpanzee and simian or African green monkey cytomegalovirus (CCMV and SCMV, respectively) carry homologs of US6 that are likely to inhibit TAP as well. Viruses that infect New World primates, including aotine or owl monkey cytomegalovirus (AoCMV) and squirrel monkey cytomegalovirus (SqCMV) code for proteins that are structurally related to US6, but no obvious sequence homology is found (our unpublished observations). No US6-like genes are apparent in cytomegaloviruses with non-primate hosts, including mouse cytomegalovirus and rat cytomegalovirus (Rawlinson et al., 1996). Thus, it seems that the US6 gene family probably evolved during early primate evolution, with the TAP-inhibiting function arising in the Old World primate lineage (Fig. 2).

The gammaherpesvirus Epstein-Barr virus (EBV) specifically inhibits TAP function via the BNLF2a protein (Hislop et al., 2007; Horst et al., 2009). The subfamily *Gammaherpesvirinae* includes the genus *Lymphocryptovirus*, to which EBV belongs, and the genus *Rhadinovirus*, which includes Kaposi's sarcoma-associated herpesvirus and murine gammaherpesvirus-68. No homologs of EBV BNLF2a have been found among the rhadinoviruses, and so far no TAP inhibitor has been identified for these viruses. Among the lymphocryptoviruses, homologs of EBV BNLF2a are present in viruses that infect Old World primates, but not those that infect New World primates. BNLF2a homologs expressed by rhesus, chimpanzee, baboon and gorilla lymphocryptoviruses (RLV, CLCV, BLCV and GoLCV) demonstrate between 53% and 63% similarity to EBV BNLF2a and display a similar disposition of hydrophilic and hydrophobic regions. Moreover, these homologs are capable of downregulating cell surface expression of MHC I molecules (Hislop et al., 2007), pointing towards conservation of the TAP-inhibiting properties of BNLF2a among lymphocryptoviruses of Old World primates, and indicating that the BNLF2a gene arose after the divergence of Old World and New World primate gammaherpesviruses (Fig. 2).

Immune evasion molecules that specifically inhibit TAP are identified for all *Herpesviridae* subfamilies. UL49.5, ICP47, US6, and BNLF2a are non-related proteins that independently evolved to serve one goal: inhibition of transport of viral peptides into the endoplasmic reticulum. As described above, the proteins ICP47, US6 and BNLF2a developed after certain points of divergence late in evolution, as homologs of these

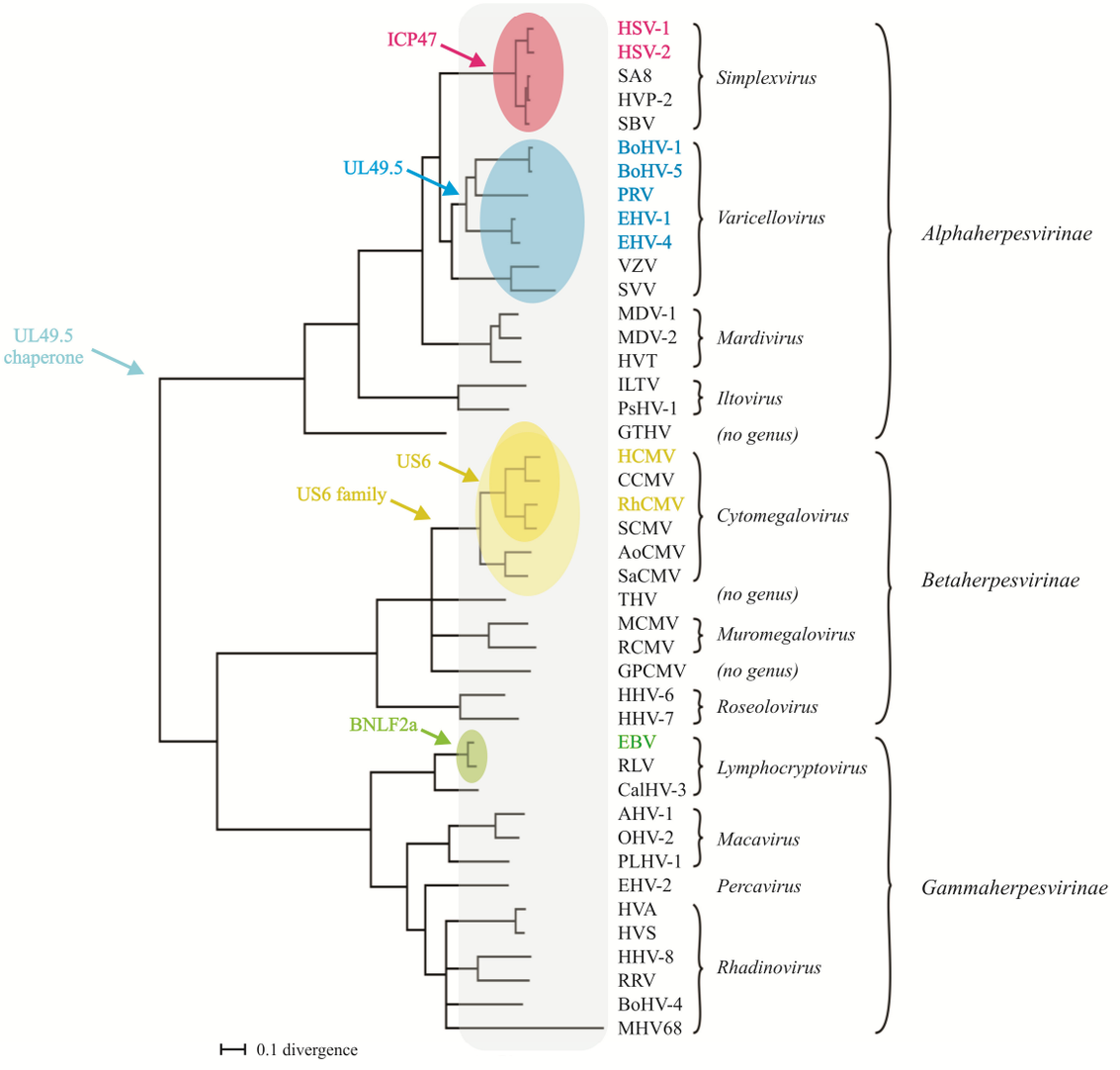

Fig. 2, phylogenetic tree for the family *Herpesviridae*. The Bayesian tree is based on amino acid sequence alignments for six large, well-conserved genes, namely the orthologs of HSV-1 genes UL15, UL19, UL27, UL28, UL29 and UL30, and is derived from McGeoch et al., 1999. Assignments to genera and subfamilies are shown on the right. Abbreviations not mentioned in the text are: MDV-1, Marek's disease virus type 1; MDV-2, Marek's disease virus type 2; HVT, herpesvirus of turkey; ILTV, infectious laryngotracheitis virus; PsHV-1, psitticid herpesvirus 1; GTHV, green turtle herpesvirus; THV, tupaia herpesvirus; GPCMV, guinea pig cytomegalovirus; CalHV-3, callitrichine herpesvirus 3; AHV-1, alcelaphine herpesvirus 1; OHV-2, ovine herpesvirus 2; PLHV-1, porcine lymphotropic herpesvirus 1; HVA, herpesvirus ateles; HVS, herpesvirus saimiri; HHV-8, human herpesvirus 8; RRV, rhesus rhadinovirus; and MHV68, murine gammaherpesvirus 68. Red, blue, orange and green shading show viruses that have the ICP47, UL49.5, US6 or BNLF2a TAP inhibitor genes, and corresponding coloring of virus abbreviations show viruses in which these genes have been shown to be functional as TAP inhibitors. Light orange shading shows that all members of the genus *Cytomegalovirus* have members of the US6 gene family. Light blue shading shows that all members of the family *Herpesviridae* have a UL49.5 ortholog, which presumably preserves a more ancient function as a chaperone for gM.

proteins are only found for subgroups of viruses within one genus. In contrast, homologs of UL49.5 are encoded by all herpesviruses sequenced to date (Davison et al., 2009; McGeoch et al., 2006). The ability to strongly inhibit TAP is only preserved for some varicellovirus-encoded proteins. However, the existence of weakly inhibiting UL49.5 homologs (encoded by CaHV-1 and SVV) and of the non-inhibiting, but interacting VZV UL49.5 protein implies that these proteins once belonged to the family of TAP-inhibiting UL49.5 homologs. It is possible that the TAP inhibitory function was gained after the varicelloviruses branched off, and was largely lost in the VZV lineage and by CaHV-1 (Fig. 2). Whatever the truth may be, a subgroup of varicelloviruses retained the ability to block TAP through UL49.5, providing us with a valuable tool to employ in both fundamental and applied research.

Reference List

Ahn,K., Gruhler,A., Galocha,B., Jones,T.R., Wiertz,E.J., Ploegh,H.L., Peterson,P.A., Yang,Y., Fruh,K., 1997. The ER-luminal domain of the HCMV glycoprotein US6 inhibits peptide translocation by TAP. Immunity 6**,** 613-621.

Browning,G.F., Ficorilli,N., Studdert,M.J., 1988. Asinine herpesvirus genomes: comparison with those of the equine herpesviruses. Arch. Virol. 101**,** 183-190.

Chang,C.C., Campoli,M., Ferrone,S., 2003. HLA class I defects in malignant lesions: what have we learned? Keio J. Med. 52**,** 220-229.

Davison,A.J., Eberle,R., Ehlers,B., Hayward,G.S., McGeoch,D.J., Minson,A.C., Pellett,P.E., Roizman,B., Studdert,M.J., Thiry,E., 2009. The order Herpesvirales. Arch. Virol. 154**,** 171-177.

Fruh,K., Ahn,K., Djaballah,H., Sempe,P., van Endert,P.M., Tampe,R., Peterson,P.A., Yang,Y., 1995. A viral inhibitor of peptide transporters for antigen presentation. Nature 375**,** 415-418.

Fukushi,H., Tomita,T., Taniguchi,A., Ochiai,Y., Kirisawa,R., Matsumura,T., Yanai,T., Masegi,T., Yamaguchi,T., Hirai,K., 1997. Gazelle herpesvirus 1: a new neurotropic herpesvirus immunologically related to equine herpesvirus 1. Virology 227**,** 34-44.

Hartley,C.A., Drummer,H.E., Studdert,M.J., 1999. The nucleotide sequence of the glycoprotein G homologue of equine herpesvirus 3 (EHV3) indicates EHV3 is a distinct equid alphaherpesvirus. Arch. Virol. 144**,** 2023-2033.

Hengel,H., Koopmann,J.O., Flohr,T., Muranyi,W., Goulmy,E., Hammerling,G.J., Koszinowski,U.H., Momburg,F., 1997. A viral ER-resident glycoprotein inactivates the MHC-encoded peptide transporter. Immunity 6**,** 623-632.

Hicklin,D.J., Marincola,F.M., Ferrone,S., 1999. HLA class I antigen downregulation in human cancers: T-cell immunotherapy revives an old story. Mol. Med. Today 5**,** 178-186.

Hill,A., Jugovic,P., York,I., Russ,G., Bennink,J., Yewdell,J., Ploegh,H., Johnson,D., 1995. Herpes simplex virus turns off the TAP to evade host immunity. Nature 375**,** 411-415.

Hislop,A.D., Ressing,M.E., van Leeuwen,D., Pudney,V.A., Horst,D., Koppers-Lalic,D., Croft,N.P., Neefjes,J.J., Rickinson,A.B., Wiertz,E.J., 2007. A CD8+ T cell immune evasion protein specific to Epstein-Barr virus and its close relatives in Old World primates. J. Exp. Med. 204**,** 1863-1873.

Horst,D., van Leeuwen,D., Croft,N.P., Garstka,M.A., Hislop,A.D., Kremmer,E., Rickinson,A.B., Wiertz,E.J., Ressing,M.E., 2009. Specific targeting of the EBV lytic phase protein BNLF2a to the transporter associated with antigen processing results in impairment of HLA class I-restricted antigen presentation. J. Immunol. 182**,** 2313-2324.

Lehner,P.J., Karttunen,J.T., Wilkinson,G.W., Cresswell,P., 1997. The human cytomegalovirus US6 glycoprotein inhibits transporter associated with antigen processing-dependent peptide translocation. Proc. Natl. Acad. Sci. U. S. A. 94**,** 6904-6909.

Martina,B.E., Harder,T.C., Osterhaus,A.D., 2003. Genetic characterization of the unique short segment of phocid herpesvirus type 1 reveals close relationships among alphaherpesviruses of hosts of the order Carnivora. J. Gen. Virol. 84**,** 1427-1430.

McGeoch,D.J., Davison,A.J., 1999 The molecular evolutionary history of the herpesviruses. In: Domingo,E., Webster,R., Holland,J. (Eds.), In Origin and Evolution of Viruses. Academic Press, Londen, pp. 441-465.

McGeoch,D.J., Rixon,F.J., Davison,A.J., 2006. Topics in herpesvirus genomics and evolution. Virus Res. 117, 90-104.

Pande,N.T., Powers,C., Ahn,K., Fruh,K., 2005. Rhesus cytomegalovirus contains functional homologues of US2, US3, US6, and US11. J. Virol. 79, 5786-5798.

Rawlinson,W.D., Farrell,H.E., Barrell,B.G., 1996. Analysis of the complete DNA sequence of murine cytomegalovirus. J. Virol. 70, 8833-8849.

Ros,C., Belak,S., 2002. Characterization of the glycoprotein B gene from ruminant alphaherpesviruses. Virus Genes 24, 99-105.

van Hall,T., Laban,S., Koppers-Lalic,D., Koch,J., Precup,C., Asmawidjaja,P., Offringa,R., Wiertz,E.J., 2007. The varicellovirus-encoded TAP inhibitor UL49.5 regulates the presentation of CTL epitopes by Qa-1b1. J. Immunol. 178, 657-662.

van Hall,T., Wolpert,E.Z., van,V.P., Laban,S., van,d., V, Roseboom,M., Bres,S., Grufman,P., de,R.A., Meiring,H., de,J.A., Franken,K., Teixeira,A., Valentijn,R., Drijfhout,J.W., Koning,F., Camps,M., Ossendorp,F., Karre,K., Ljunggren,H.G., Melief,C.J., Offringa,R., 2006. Selective cytotoxic T-lymphocyte targeting of tumor immune escape variants. Nat. Med. 12, 417-424.

York,I.A., Roop,C., Andrews,D.W., Riddell,S.R., Graham,F.L., Johnson,D.C., 1994. A cytosolic herpes simplex virus protein inhibits antigen presentation to CD8+ T lymphocytes. Cell 77, 525-535.