Possible TAP inhibitors in HHV-6 and HHV-7 based on known characteristics of TAP inhibitors and their evolutionary development in herpesviruses.

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Abstract

Herpesviruses are well-known because of their latency and immune evasion strategies. The MHC class I antigen presentation pathway is an important target, especially MHC class I downregulation. Downregulation of MHC molecules can be accomplished in different ways. One of those ways extensively used by herpesviruses, tumors and even cowpoxvirus is to inhibit peptide loading onto the MHC class I molecules by blocking the transporter associated with antigen presentation (TAP). All subfamilies of the *herpesviridae* encode for different TAP inhibitors. As for the human herpesviruses, only varicella zostervirus (VZV), human herpesvirus (HHV-) 6 and HHV-7 have no known TAP inhibitors. In this thesis a list is generated with possible TAP inhibitors for the β-herpesviruses HHV-6 and HHV-7. The list is based on several characteristics shared by the known TAP inhibitors. More importantly, the evolutionary development of herpesviruses and their immune evasion proteins suggests that relatively new genes will be located near the ends of the genomes. In this study a clear picture has been drawn to show that this is true for the TAP inhibitors and that this might be a great tool to predict the location of immune evasion proteins.

Introduction

The main goal of viruses is to replicate. A balance between virus production and the survival of its host is a requisite for the survival of their population. The population proceeds to replicate, infect and continue to exist as a group. Therefore it can be said that virologists are really population biologists, an aspect that should always be kept in mind while studying viruses. Any virus population needs a balance between virus production and the surviving of their host, because without their host they will not be able to replicate at all. Not only should their host survive the viral infection, the viruses should also survive the host's counteractions against their infection; another balance to be reached. Many RNA viruses counter the host's immune system with high mutation rates coupled with short generation times leading to mutations that give the opportunity to change and thereby to mislead or hide from the host's immune system. One very well known event occurring with especially RNA viruses is the rapid change of antigens. When these are changed it will be more difficult for the immune system to recognize them. The large DNA viruses however, have longer generation times because of the replication of their large genomes, large capsids and proofreading of the DNA polymerase. Proofreading makes sure that there are fewer mistakes during genome replication, which leads to genomes with lower mutation rates. This means that they cannot alter the antigenic profile to circumvent the immune system and that they need other actions against their host's immune system. During evolution viruses have been able to gain survival advantages by interfering with the host's immune system.

Viruses can exchange parts of their genomes within their own population, different populations, different viruses, and their host (1). Viruses have left their traces for millions of years in our DNA, for example, human retroviruses and their elements make up about 5 to 8% of our DNA (2). This indicates the big impact that genetic exchange between viral DNA and their hosts has. With the exchange of genomic information viruses can gain all sorts of new (parts of) genes, and the viruses gaining proteins with the highest fitness advantages will be selected. However, it is possible that this fitness advantage does not compensate for disadvantages which could come along with the new gene, such as a bigger genome, loss of (parts of) important genes by insertion of new genes and a long translation time of the new gene product, which can all lead to longer replication times or hampering the virus production

in another way. Therefore, new genes might contribute to the diversity of a population, but there will also be a cost-profit selection, filtering the least profitable genes from the population. Hereby, a diverse collection of gene sets can evolve. The bigger the diversity within the surviving limits, the bigger the chance for a population to survive. A bottleneck will diminish a population's strength, because it decreases the population's diversity (3). Virus evolution is the constant change of a viral population in the face of selective pressure. One of the biggest selective pressures for viruses is the immune system of their host. Viruses will keep on adapting to this system, consequently, they will co-evolve with (the immune system of) their hosts (4).

One arm of the immune system is the innate immune system. Viruses are known to affect this arm by multiple strategies, such as interfering with a central player in this part of the immune system, the natural killer (NK) cells. These cells can rapidly secrete cytokines, chemokines and can directly kill an infected host cell. Several viruses produce proteins that evade recognition by NK cells (2, 5, 6). NK cells will be activated by different sets of cell surface receptors that allow them to distinguish between normal cells and transformed or infected cells (7). MHC class I molecules can serve as inhibitory ligands for NK cells, thus when there are no MHC class I molecules on the surface of a cell, NK cells are activated and attack those cells (8).

Another important part of the immune system against viral infections is the adaptive arm of the immune system. One of its most important players is the major histocompatibility complex (MHC) class I presentation pathway. This pathway ensures that intracellular events can be monitored on the outside of the cell. In the cytosol, proteasomal degradation of proteins produce peptides. These peptides are sampled and presented on the cell surface by MHC class I molecules. These peptides need to enter the endoplasmic reticulum (ER), where they are loaded onto MHC class I molecules. The transporter associated with antigen processing (TAP) actively transports peptides into the ER. TAP is a TAP1/TAP2 heterodimer and belongs to the ATP-binding cassette family of transporters (9). Tapasin links TAP with MHC class I and β_2-microglobulin dimer, resulting in a high concentration of peptides in close proximity of the MHC class I molecules. This helps forming the MHC class I-peptide heteromer more easily (10). Calnexin and calreticulin help stabilizing newly synthesized MHC class I molecules. The disulfide isomerase ERp57 is recruited by calreticulin to stabilize several protein-protein interactions within the peptide loading complex (11). After peptide loading, MHC class I molecules dissociate from TAP and leave the ER to move to the surface of the cell, where MHC class I molecules present a range of peptides to an important part of the adaptive immune system, the $CD8^+$ cytotoxic T lymphocytes (CTLs). CTLs have been selected during their development to bind with different affinity to self proteins than to non-self proteins. In this way they recognize cells which present non-self peptides on the surface after which different responses can be initiated, generally resulting in the elimination of the infected cells.

DNA viruses cannot change antigens as easily as RNA viruses because of the proofreading of DNA polymerase, hence they need other, more elaborate, mechanisms to escape from CTLs. DNA viruses have therefore developed many different strategies to prevent CTL recognition, where the MHC class I pathway plays a major role. Retroviruses, adenoviruses, herpesviruses and poxviruses all encode immune evasion proteins (12-16), the immune evasion strategies of herpesviruses being the best studied. Herpesviruses are among the largest viruses with a virion of 200-250 nm in diameter and consisting of a linear double-stranded DNA genome of 120 – 240 kbp containing ~70 to ~170 open reading frames (ORFs). The three subfamilies of the *herpesviridae*, that cause lifelong infections in theirs hosts, are the α-, β- and γ- herpesviruses. α-herpesviruses are neurotropic and all known avian herpesviruses belong to this subfamily. β-herpesviruses are ubiquitous, cause little or no

disease in immunocompentent hosts and are higly species-specific. γ- herpesviruses are divided into two groups, γ1- and γ2- herpesviruses which encode oncogenes and are associated with malignant tumors. Humans are the natural host of eight different herpesviruses: herpes simplex virus (HSV) types 1, 2 and varicella-zoster virus (VZV) (all α-herpesviruses), Epstein-Barr virus (EBV, a γ1-herpesvirus), human cytomegalovirus (HCMV) and human herpesvirus 6 (HHV-6), HHV-7 (all three β-herpesviruses) and HHV-8 (a γ2-herpesvirus).

The three subfamilies have diverged from a common ancestor around 400 million years ago (17, 18). The vigorous phylogenetic tree that has been constructed for herpesviruses shows evidence of synchronous development of virus and host lineages over large evolutionary timespans (18). The development of herpesviral genomes during evolution has been related to that of their hosts. The points where herpesviral sequences had the chance to diverge happen together with well-established points of divergence of the host in their evolution. Development of the viruses seems to be integrated with the development of their host (4). The phylogenetic tree was originally based upon biological criteria such as the characteristic virion architecture, but with the accumulation of DNA sequence data for herpesvirus genomes only a few virus species were reassigned among subfamilies, indicating that both were good measures for phylogeny. Herpesviruses show extensive adaptation to the immune systems of all their different and always very specific hosts (19).

TAP inhibitors of herpes viruses.

Most human herpesvirus genomes contain one or more genes which encode for immune evasion proteins. A part of these proteins is directed against the previously described MHC class I presentation pathway. By interfering with this pathway, the host's immune system will not recognize infected cells as easily as without this interference. This is one of the aspects that give herpesviruses the possibility to return from their latency and have the opportunity to produce sufficient viruses to infect a new host. It also gives the initial infecting viruses more time to replicate larger amounts of viruses when they first enter the host, because the immune evasion proteins usually are synthesized during the first few hours of infection. In this way the immune evasion proteins can make sure that there is no antigen presentation in the first stages of infection, while other strategies will need to evade the immune system later on during infection.

The MHC class I presentation pathway has different players, such as the proteasome, TAP, tapasin and β_2-microglobulin (20). A number of herpesviral genes evade the proteasome, including EBV nuclear antigen 1 (EBNA1), and the latency-associated nuclear antigen 1 (LANA-1) encoded by HHV-8, which escape degradation by the proteasome (21-23). The mechanisms involved in proteasomal degradation are still poorly understood and the discovery of more proteasome inhibitors in viruses might help to better understand this process (24).

There are multiple other mechanisms by which viral proteins keep the MHC class I molecules from presenting peptides on the cell surface. One of these mechanisms is retaining MHC class I molecules in the ER. The US3 protein encoded by HCMV can retain certain MHC alleles that are tapasin-dependent, as US3 directly binds tapasin and inhibits tapasin-dependent peptide loading (24-26). MHC class I molecules that do not efficiently bind a peptide will not be stable enough to travel out of the ER. Two HCMV proteins, US2 and US11, use yet another mechanism to dispose of the MHC class I molecules (27, 28). They dislocate newly synthesized MHC class I heavy chains to the cytosol, where the proteins are degraded by the proteasome (29). Not all MHC class I locus products are equally sensitive to US2 and US11-mediated degradation. In this case very small changes in amino acid residues can make a MHC class I molecule more or less sensitive to downregulation by US2 and U11

(30). The mouse hepatitis virus (MHV) 68 E3 ligase mK3 uses a similar mechanism to target MHC class I molecules for proteasomal degradation. This protein is only found in the presence of its main binding partner; TAP (31). Homologues of mK3 are also found in HHV-8, but they use a different pathway for the degradation of MHC class I (32).

A number of other herpesviral gene products block MHC I antigen presentation by targeting TAP. TAP is a heterodimer, composed of the two multimembrane-spanning subunits TAP1 and TAP2. A pore is formed across the ER membrane by the TAP1 and TAP2 helices, that allows antigenic peptides to transport from the cytosol to the ER lumen. This process is regulated by ATP hydrolysis at the C-terminal nucleotide binding domains. Upon peptide binding to the C-terminal domains of TAP, a conformational change is driven by the hydrolysis of ATPs bound to TAP. The six C-terminal transmembrane domains together with the nucleotide binding domain form the TAP core complex. The N-terminal transmembrane domains are not essential for peptide transport, they are however involved in binding of tapasin (33).

Peptide transport by TAP is blocked by an array of different viral proteins with different structures and different approaches. HCMV glycoprotein US6 is inhibiting peptide translocation with its ER-luminal domain by inhibition of ATP binding to the cytosolic nucleotide-binding domain (34-37). EBV BNLF2a blocks TAP function by preventing the binding of peptides and ATP (38). Although US6 and BNLF2a bind to different parts of TAP, they exclude each other from binding to TAP. This might be due to the fact that both factors arrest distinct conformations of the TAP complex, leaving it impossible for the other to bind such conformation (39). While UL49.5 of different varicelloviruses use different strategies to inhibit TAP, they all seem to arrest TAP in a translocation-incompetent state. The UL49.5 protein of BoHV-1 reduces the steady state level of TAP and targets TAP for proteasomal degradation, while equine herpesvirus type 1 (EHV-1) and EHV-4 UL49.5 interfere with ATP binding (40-42). The TAP inhibitor of HSV-1 and HSV-2, ICP47, competes with peptides for the peptide-biding site (43, 44). The fact that TAP inhibitors use such different strategies suggests that the TAP inhibitors are not diverged from a common ancestral protein. This could mean that viruses gained those different TAP inhibitor genes independent of each other during evolution.

There are many differences in the functional features of the TAP inhibitors. There is also no sequence homology between those proteins, neither in their gene sequence, nor in their protein sequence. Nevertheless, there are some common structural features. All proteins are transmembrane proteins except for ICP47 (37, 45, 46). This latter protein was primarily described as being a soluble protein, but the protein is most stable when bound to a lipid bilayer (45). The active TAP inhibition sites are variably ER-luminal (US6 and BNLF2a) or cytosolic (UL49.5 and ICP47). UL49.5 uses its ER-luminal domain for conformational arrest, while the cytosolic domain is responsible for inhibition and degradation. The inhibitory domains of the TAP inhibitors BNLF2a and UL49.5 are located at the N-terminal tail. ICP47 has the active site in the C-terminal residues (residues 3-34) (45). The inhibitory domain of US6 is located in the centre of the protein. An overview of the mode of action of the four TAP inhibitors described here is depicted in figure 1.

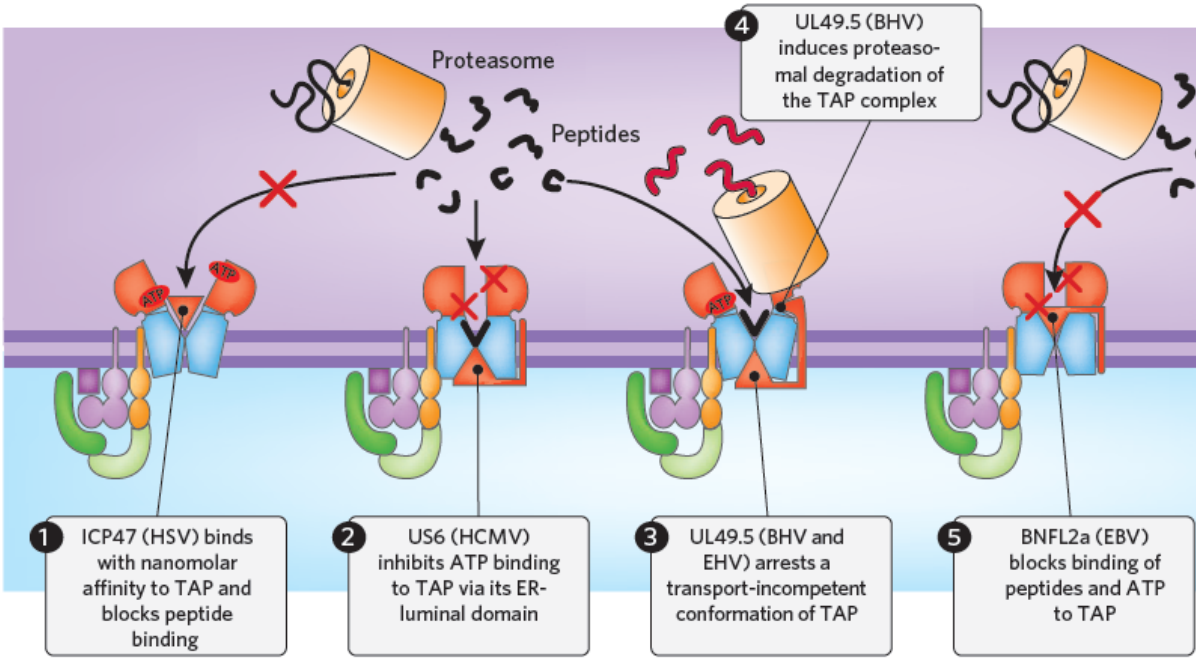

Figure 1. TAP inhibitors; immune evasion proteins in herpesviruses. (**1**) ICP47 binds to the cytosolic site of TAP and thereby prevents peptide binding (**2**) US6 binds to the ER-luminal domain of TAP to retain TAP in an ATP binding- incompetent conformation, thereby indirectly blocks ATP binding. (**3**, **4**) UL49.5 arrests TAP in another specific conformation, resulting in inhibition of peptide translocation. In addition UL49.5 targets the peptide loading complex for proteasomal degradation. (**5**) BNLF2a binding inhibits peptides and ATP binding to TAP. (HSV herpes simplex virus, HCMV human cytomegalovirus, BHV bovine herpesvirus, EHV equin herpesvirus, EBV Epstein-Barr virus) Adjusted from (33, 47).

Orthologues of the TAP inhibitors might give further insight in the different modes of action of the different TAP inhibitors, as well as in the evolutionary development of these genes. HCMV US6 orthologues have only been found among primate cytomegaloviruses. The orthologue in the rhesus cytomegalovirus (RhCMV), called gRh185, blocks TAP in both rhesus as well as human cells. Peptide transport is downregulated to similar percentages by gRh185 as by US6 (48). In viruses infecting other Old World primates homologues of US6 have been found, but no functional inhibition of TAP has been discovered yet. BNLF2a orthologues have also only been found among viruses infecting Old World primates, such as rhesus lymphocryptovirus, chimpanzee (pan) herpesvirus, orang-utan herpesvirus and gorilla herpesvirus. MHC class I molecules were downregulated by these BNLF2a orthologues. Considering their relatively high sequence homology (53 to 63%) one might expect them to inhibit TAP in similar way as described for BNLF2a, however, this has not been confirmed (38).

The only functional orthologue of ICP47 that was found is expressed by HSV-2, which has 42% amino acid sequence homology with ICP47 from HSV-1. This orthologue was found to be able to block TAP at similar concentrations and it binds TAP with virtual identical affinity (49). Other ICP47 homologues seem to have conserved some domains of the ICP47 gene, those are encoded by simian agent 8, herpesvirus papio 2 and herpes B virus, a macaque herpesvirus (50). Despite the homology none of them block TAP. For ICP47 of herpes B virus the lack of a TAP binding domain might explain this (51).

Homologues of UL49.5 are encoded by all herpesviruses and are known as glycoprotein N, which forms a heterodimer with glycoprotein M. This protein has gained a TAP inhibitory function in some of the herpesviruses families. TAP-blocking orthologues have been identified only among the α-herpesviruses, of which some have already been described above (40, 41). TAP-inhibiting UL49.5 homologues have been found among the

varicelloviruses, although not all UL49.5 homologues of varicelloviruses are able to block TAP. For example, VZV UL49.5 is capable of interacting with TAP, but does not inhibit its activity. This might indicate that the VZV protein encoded by UL49.5 has lost part of its TAP inhibiting function, or that the other gene products have evolved differently by not only binding, but also blocking TAP. This latter possibility is supported by the fact that the UL49.5 homologues have distinct mechanisms by which they block TAP (40). Hence they gained their TAP-inhibiting function independently of each other. The discovery of these homologues helps unravelling the evolution of the TAP inhibitors and where to find them on the genome.

An interesting correspondence between all described TAP inhibitors is that their genes are located near the terminus of the viral genomes, as depicted in figure 2. The genes located near the end of the genome are always found on the negative strand and the genes located near the beginning of the genome are found on the positive strand. This is also true for all homologues found for the human TAP inhibitors described above. A genome organization where the newer and often less conserved genes are located near the ends of the genome and the strongly conserved genes are located in the centre of the genome is common among viruses in general. The ancestral virus of the herpesviruses contributed 43 genes to modern α-, β-, and γ-herpesviruses. 9 of these genes are found to be related to cellular genes (52). These 43 genes are usually located in the central region of the genome, with the extremities containing the most lineage-specific genes. A similar genome arrangement is found in other viruses, such as adenoviruses (53) and poxviruses (54). Those conserved genes are involved in vital aspects of the viral life cycle, for example, cell entry, DNA replication, packaging and formation of the virion and genes involved in host-virus interaction (55). Many genes involved in control of these vital aspects appear to have developed independently in the subfamilies and genera. Some genes are found in the same positions in the genomes of viruses from different subfamilies, but because of divergence the amino acid sequences are not always obviously related (19). The order of the conserved genes is not always the same in the genomes of the different subfamilies, let alone the order of the lineage-specific genes. The immediate early and intermediate genes are more prone to be located in the extremities of the genome, therefore this might be an example of time and spatial organization. The location of the genes not only indicates when it has been added to the viral genome during evolution, but, to some extent, it will also gives a prediction about when it will be transcribed during viral infection(19, 56-58). As shown in figure 2 all TAP inhibitors are located near the ends of the genome and thus seem to belong to the genes that have been acquired later during evolution.

Besides the TAP inhibitors, there are many viral immune evasion proteins discovered and many have the same target, but they show little to no structural similarities (59). This could suggest that all these immune evasion proteins have been gained independently into the viral genomes, as discussed above for the UL49.5 homologues. Genetic recombination is one of the important sources of genetic variation in populations of DNA viruses. HSV-1 uses the formation of replication compartments for the concentration of cellular proteins that participate in recombination and repair with viral genomes (4). The genome of HSV-1 is organized in two covalently linked segments, both segments are composed of a unique sequence flanked by inverted repeats. During replication of the genome homologous recombination events occur between the inverted repeats. This will give opportunities to gain new genes (60). Another example is of the γ-Herpesviruses which use the packaging of DNA for virus-host recombination when viral DNA is cleaved. Viral genes with sequence homology to host immune defence genes are often virulence genes acquired in ways as described in these examples (4).

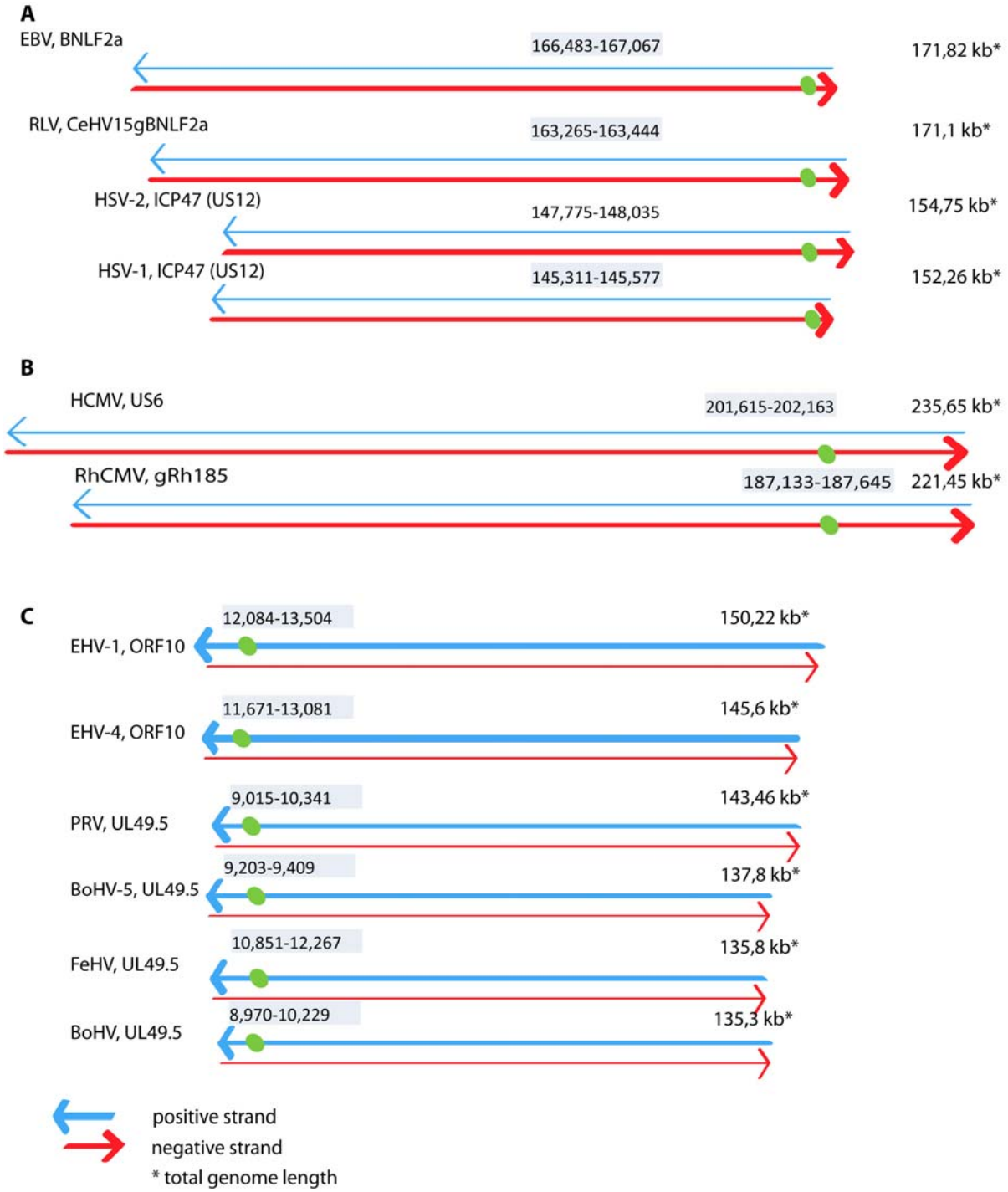

Figure 2. Genomes of viruses encoding a TAP inhibitor. The green spot indicates where the TAP inhibitor is located in the genome and on which strand, with corresponding sequence. (**A**) γ_1- and α- herpesviruses BNLF2a and ICP47 orthologs respectively. (**B**) β-herpesviruses US6 orthologs. (**C**) α-herpesviruses UL49.5 orthologs. (**A**) The gene locations of the TAP inhibitors of the γ_1- and α- herpesviruses are very similar, even though they are from different herpesvirus families. The genomes are aligned using the location of the TAP inhibitor as reference. The sizes depicted here are calculated to be a scale-model for the genomes and the alignments of the genomes. Information about the genomes and location of genes is found on the website of NCBI (61). For a list of the virus strains in this picture see supplementary data 1. The genomes of the γ_1- herpesviruses chimpanzee, baboon and gorilla lymphocryptoviruses were not available, therefore they are not displayed here. (EBV Epstein-Barr virus, RLV rhesus lymphocryptovirus, HSV-2 herpes simplex virus type 2, HSV-1 herpes simplex virus type 1, HCMV human cytomegalovirus, RhCMV rhesus cytomegalovirus, EHV-1 equine herpesvirus type 1, EHV-4 equine herpesvirus type 4, PRV pseudo rabiesvirus, BoHV-5 bovine herpesvirus type 5, FeHV feline herpesvirus, BoHV-1 bovine herpesvirus type 1).

As immune evasion proteins have co-evolved with their host, these are relatively new genes in the viral genomes and they are known to be translated during the first few hours of infection. This is in correspondence with the fact that the genes are located in the extremities of the genomes.

HHV-6 and HHV-7 immune evasion

The fact that so many different TAP inhibiting proteins with different strategies have developed and, moreover, many of them independent of each other, suggests that TAP inhibition is important to the survival of herpesviruses. Recognition and thereafter elimination of the virus infected cell can be avoided when activation of the CTLs is inhibited. The lack of a T cell response suggests that there should be inhibition of the MHC class I presentation pathway. A recent paper on transplantation and herpesviruses (62) points to the importance of cell-mediated immunity in controlling human herpesvirus infection. The development of severe herpesvirus-associated clinical complications in immunosuppressed individuals is often seen in individuals whose T cell immunity is compromised. This indicates that a T cell response is important for the control of viral infections. T cells of patients infected with β-herpesviruses show function impairment and there is acquisition of markers of T cell exhaustion. Patients infected with Roseoloviruses HHV-6 or HHV-7 lack antigen-specific T cells. For HHV-6 and HHV-7 suppression of the helper T cell response is also noted. For a few of these immune dysfunctions the proteins involved have been identified, but for many there is need of further research (62).

A big difference between HHV-6 and 7 and the other herpesviruses is that HHV-6 and HHV-7 infect cells that play a key role in the normal, cellular immune response against viruses, namely the $CD4^+$ T cells (63). Nevertheless, one should not forget that all nucleated cells express MHC class I molecules, including the immune cells. Next to infecting $CD4^+$ cells HHV-6A also infects cytotoxic effector cells such as $CD8^+$ T cells, γδ T cells and NK cells (64). HHV-6A has been shown to express a protein which downregulates the T cell receptor/CD3 complex, U24. By downregulating CD3 the U24-expressing T cells can no longer be activated by antigen-presenting cells (65). Accordingly only the infected T cells can no longer be *activated*, however this does not mean that MHC class I expression of viral antigens will not be recognized by the immune system. The Roseoloviruses will therefore still need to express immune evasion proteins directed against the MHC class I presentation pathway to evade recognition and destruction by CTLs. One strategy conducted by HHV-6 is the impairment of helper T cells, this inhibits the generation of new HHV-6-specific CTLs (66). The mechanisms underlying the impairment of helper T cells are still unknown.

Some other immune evasion strategies of HHV-6 and 7 have been described. HHV-6 inhibition of the innate antiviral response is conducted via inhibition of transcription of the β-interferon gene by the immediate-early 1 (IE-1) protein (67). U20 of HHV-6B inhibits tumor necrosis factor receptor-dependent signalling and apoptosis (68). Functional impairment of antigen presentating cells, such as macrophages and dendritic cells and suppression of IL-12 secretion by those cells, as well as suppression of IL-2 secretion has been described for HHV-6 (64). U21 of both HHV-6 and HHV-7 binds to and diverts MHC class I molecules to an endolysosomal compartment, effectively removing them from the cell surface (69, 70). Although most herpesviruses only downregulate certain MHC class I alleles HHV-7 seems to downregulate all types of MHC class I molecules through one single protein: U21(71). In most herpesviruses there is not one single immune evasion protein that completely shuts off all antigen presentation by MHC I, however HHV-7 might be an exception. In most herpesviruses some MHC class I alleles might escape inhibition and a TAP independent MHC class I presentation pathway has been described (72). Taking this together it suggests that all herpesviruses need at least one, but probably more than one, protein inhibiting MHC class I

antigen presentation to CTLs (69, 73, 74). For example; HCMV is encoding at least four immune evasion proteins, while EBV encodes three and KSHV at least two inhibitors of MHC class I antigen presentation (22, 23, 29, 30, 46, 75-78). The data about the immune evasion protein U21 of HHV-7 suggest that HHV-7 is an exception hereof and therefore might not need other proteins inhibiting the MHC class I presentation pathway. The HLA classes that are not downregulated by the immune evasion proteins of the other herpesviruses might play a role in the inhibition of NK cell activation. As for HHV-7, it has been shown that the same protein that downregulates all HLA types, U21, also downregulates NK-activating ligands (79). Hereby HHV-7 evades recognition of both CTLs and NK cells through one single protein.

On the other hand, co-evolution of viruses with their host suggests that there should be MHC class I inhibitors in all herpesviruses, as many herpesviruses encode for those specific immune evasion proteins. A widespread feature among herpesviruses is not only inhibition of the MHC class I presentation pathway, but more specific inhibition of TAP. The fact that it is so widespread and gained in independent manners of each other, suggests that the TAP inhibitors of HHV-6 and HHV-7 have just not been found yet.

Possible TAP inhibitors in HHV-6 and HHV-7

The data described in this review have been used to select some candidate genes which might encode a TAP inhibitor. Characteristics to help chose those candidates have been selected by comparing the known TAP inhibitors from other herpesviruses. The list is depicted in table 1. The basis for the selection of these candidates is shown in figure 1. As depicted in this figure, all known TAP inhibitors are located near the ends of the viral genomes. For the β-herpesviruses this is in the 15% most extreme parts of the genome, and for the location of the other TAP inhibitors it is found that they are even more extremely located in the last 5% of the genome. Therefore the first step in the assortment procedure was to select the genes located in the first or last 20% of the genome. The size of the TAP inhibitors ranges between 59 and 280 amino acids, and for the β-herpesviruses this is 246 to 280 amino acids. Therefore only proteins smaller than 300 amino acids, being located within the range in the genome, have been selected.

As seen in figure 2, TAP inhibitors expressed from the minus strand of the genome are located at the far *end* of the genome. Only the genes encoding UL49.5 in the α-herpesviruses are located on the plus strand and those are located at the *beginning* of the genome. This combination should be kept in mind, like the fact that most TAP inhibitors have been found on the minus strand of the viral genomes. There is still too little data to be sure if there is a preference for genes encoding TAP inhibitors to be located on the minus strand, or that this is a coincidence. Most TAP inhibitors seem to have orthologues among other herpesviruses, although not all of them are functional as TAP inhibitor. UL49.5 of VZV is an example hereof, as it is able to bind, but not to block TAP (80). Some other characteristics considering the further selection of potential TAP inhibitors in HHV-6 and HHV-7 are sometimes more difficult to apply, because certain knowledge of the protein is required. One important aspect that all TAP inhibitors seem to have in common is that they are membrane-associated, with the majority being membrane proteins. In general, except for ICP47, the N-terminal part of the TAP inhibitors contain the active inhibitory domain of the protein. So, in summary, a candidate gene product in the search for the next TAP inhibitor should at least be located on the ends of the genome and should not be longer than 300 amino acids. Features which have a preference are: being located on the minus strand of the genome, having orthologues within the herpesvirus subfamily and being a membrane protein.

Most genes encoding smaller proteins have been found in the beginning of the HHV-6 and HHV-7 genomes, only a few locate near the end of the genome. U18 is one of the

proteins that stands out in both HHV-6 and HHV-7. It has the right size, seems to be conserved among all herpesviruses, is a type I membrane glycoprotein, located on the minus strand and no function in HHV-6 and HHV-7 has been described yet. A homologue of U18 named UL37 in HCMV and other herpesviruses is found, but no homologues where found in other virusfamilies. UL37 is best studied in HSV-1 where it is identified as a tegument protein and essential in the viral life cycle, even in cell culture (81, 82). and the UL37 of HCMV is an antiapoptotic protein (83). Both proteins are known to travel through the ER (84, 85). If there is some structural similarity between the different homologues, this indicates that U18 will also have access to the ER, where it has the possibility to interact with TAP.

Although for some of the genes listed in table 1 a function has already been described, this does not have to mean that they can not also have a TAP inhibiting function. UL49.5 is a binding partner to glycoprotein M in all herpesviruses, but also block TAP (40, 41). As previously described HHV-6A U24 downregulates the T cell receptor (65). The same protein, U24, has been found to also downregulate an early endosomal recycling receptor and is implicated as a protein mimicking myelin basic protein in the disease multiple sclerosis (86, 87). HHV-6B U12 encodes a functional β-chemokine receptor(88).

The list table will be a good start for further research. The next steps to be taken would be to find out more about the homology between these genes and genes from other herpesviruses as well as the homology between the products of those genes.

Discussion

Among human herpesviruses HHV-6, HHV-7 and VZV are the ones for which no TAP inhibitors have been discovered. Here we propose that, although they have not been discovered yet, these viruses will have TAP inhibitors. An interesting question now is if other (DNA) viruses are also likely to encode for TAP inhibitors or at least inhibitors of the MHC class I presentation pathway.

Like herpesviruses, adenoviruses and the retrovirus HIV also inhibit the MHC class I presentation pathway (12, 13, 89). Another feature these viruses have in common is that they have a long lasting relationship with their host upon infection. Other DNA viruses which establish such infections are Hepatitis B virus, Papilomavirus, Polyomavirus and lymphocytic choriomeningitis virus (90). These viruses might also benefit from inhibition of the MHC class I antigen presentation pathway. One of the most important requirements for persistent or latent infection is the ability to hide from the immune system. Herpesviruses are well known and well studied in this aspect, but there are probably many more immune evasion proteins to be discovered among DNA viruses, especially among the ones hiding from the immune system during latency.

As for the RNA viruses, they can hide more easily from the immune system by rapidly changing their epitopes. This rapid change of epitopes relies upon high production rates of the viral genomes. So, when a RNA virus is persistent or latently present in the cells it might need other measures to evade recognition by CTLs. Measles virus is an example of a RNA virus from the paramyxoviridae that establishes a persistent infection in its host. Another member of the paramyxoviridae is the respiratory syncitial virus (RSV). The CTL response is very important in controlling RSV infection. In mice, one important epitope is restricted to a certain MHC class I allele, H-2(b), and is presented on the cell surface in a TAP-independent manner (91, 92). Severity of disease progression in mice infected with RSV depends on which MHC allele is being expressed(91). This implies the importance of the MHC class I presentation for the survival of this virus and it suggests that a TAP inhibitor might be discovered amongst Paramyxoviridae.

Cowpoxvirus is not a latent nor a persistent infection, but it does encode a TAP inhibitor (15).

Table 1: (61, 93, 94)

	Gene	Size	Strand	Location	Functions	Structure	Homology	Conserved
HHV-6A	U12	351	+	5'-end		GPCR	UL33 HSV and HCMV	HHV-6,7 and HCMV
HHV-6A	U13	106	+	5'-end		Unknown	UL34 HCMV?	HHV-6,7 and HCMV
HHV-6A	U15	191	-	5'-end		Helical membrane domain	UL25/UL35 HCMV	HHV-6,7 and HCMV
HHV-6A	U18	293	-	5'-end		Type I membrane	UL37 HCMV	Herpesviruses
HHV-6A	U22	202	-	5'-end		Membrane protein	/	/
HHV-6A	U23	236	-	5'-end		Membrane protein	/	/
HHV-6A	U24	87	-	5'-end	Downregulation TCR a.o.	Unknown	/	/
HHV-6A	U24a	57	-	5'-end		Potential TM domain	/	/
HHV-6B	B1	158	+	5'-end		Unknown	/	/
HHV-6B	B2	75	+	5'-end		Unknown	/	/
HHV-6B	B3	59	+	5'-end		Unknown	/	/
HHV-6B	B4	193	-	5'-end		Unknown	/	/
HHV-6B	B5	79	+	5'-end		Membraneprotein	/	/
HHV-6B	U12	205	+	5'-end	β-chemokine R	Unknown	/	/
HHV-6B	U13	107	+	5'-end		Unknown	/	/
HHV-6B	U15	191	-	5'-end		Unknown	/	/
HHV-6B	U18	294	-	5'-end		Type I membrane	UL37 HCMV	/
HHV-6B	U22	202	-	5'-end		Glycoprotein	/	/
HHV-6B	U23	299	-	5'-end		Glycoprotein	/	/
HHV-6B	U24	88	-	5'-end		Potential TM domain	/	/
HHV-6B	U24a	57	-	5'-end		Glycoprotein	/	/
HHV-6B	B8	265	-	3'-end		Unknown	/	HHV-6A/6B/7
HHV-6B	B9	106	-	3'-end		Unknown	/	/
HHV-7	U13	98	+	5'-end		Unknown	UL34 HCMV?	/
HHV-7	U15	191	-	5'-end		Unknown	/	/
HHV-7	U18	295	-	5'-end		Type I membrane	UL37 HCMV	/
HHV-7	U23	171	-	5'-end		Type I membrane	/	/
HHV-7	U24	82	-	5'-end		Potential TM domain	/	/
HHV-7	U24a	56	-	5'-end		Potential TM domain	/	/

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