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Review

Viral interference with antigen presentation: Trapping TAP☆

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ABSTRACT

Following primary infection, herpesviruses persist for life in their hosts, even when vigorous anti-viral immunity has been induced. Failure of the host immune system to eliminate infected cells is facilitated by highly effective immune evasion strategies acquired by these herpesviruses during millions of years of co-evolution with their hosts. Here, we review the mechanisms of action of viral gene products that lead to cytotoxic T cell evasion through interference with the function of the transporter associated with antigen processing, TAP. The viral TAP inhibitors impede transport of peptides from the cytosol into the ER lumen, thereby preventing peptide loading onto MHC class I complexes. Recent insights have revealed a pattern of functional convergent evolution. In every herpesvirus subfamily, inhibitors of TAP function have been identified that are, surprisingly, unrelated in genome location, structure, and mechanism of action. Recently, cowpox virus has also been found to encode a TAP inhibitor. Expanding our knowledge on how viruses perturb antigen presentation, in particular by targeting TAP, not only provides information on viral pathogenesis, but also reveals novel aspects of the cellular processes corrupted by these viruses, notably the translocation of peptides by the ATP-binding cassette (ABC) transporter TAP. As the various TAP inhibitors are anticipated to impede discrete conformational transitions it is expected that crystal structures of TAP-inhibitor complexes will reveal valuable structural information on the actual mechanism of peptide translocation by TAP. Viral TAP inhibitors are also used for various (clinical) applications, for example, as effective tools in antigen presentation studies and as immunomodulators in immunotherapy for cancer, heterologous vaccination, and transplant protection.

1. Introduction

T cells contribute crucially to the control of viral infection, detected through the recognition of viral peptides presented at the cell surface in the context of MHC molecules. Large DNA viruses, such as herpesviruses and poxviruses, express from 70 to over 200 viral proteins during their replicative cycle, giving rise to many peptide antigens displayed on infected cells for detection by and subsequent activation of immune T cells. Thus, antigen presentation to T cells provides an attractive target for viral interference. Immune escape through epitope loss is limited for these viruses, because, unlike RNA virus-encoded RNA polymerases, (viral) DNA polymerases exert proof-reading activity leading to a strong reduction of mutations acquired by these potential antigens over time. Yet, the large coding capacity of the herpesvirus and poxvirus genomes allows for incorporation of genes encoding proteins dedicated to immune evasion. Over the past decades, viral immune evasive functions have been identified that target every step along the MHC class I (MHC I) antigen presentation pathway (Horst et al., 2011). The translocation of peptides from the cytoplasm into the ER lumen by TAP is a critical step in the MHC I presentation pathway and, as such, TAP appears to be a favorite target for viral immune evasion.

2. Current status

Up until now, five viral genes have been identified to encode inhibitors of peptide transport mediated by TAP (Table 1). Herpes simplex virus (HSV)-1 and -2 encode a cytosolic protein, ICP47, which acts as a pseudosubstrate for TAP and prevents binding of peptides to the transporter. The UL49.5 proteins of several varicelloviruses impede essential conformational transitions of TAP required for the translocation of peptides over the ER membrane. Additionally, some TAP-inhibiting UL49.5 proteins target TAP for proteasomal degradation or interfere with ATP binding to TAP. The latter strategy is also employed by the human cytomegalovirus (HCMV) protein US6, a type I membrane protein that acts on TAP through its ER luminal domain. Epstein-Barr virus (EBV) encodes a tail-anchored protein, BNLF2a, which effectively inhibits TAP function predominantly through its cytosolic domain by interfering with the binding of both ATP and peptides to TAP. All the

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Table 1
Comparison of the structural features and mechanisms by which virus-encoded proteins interfere with TAP-mediated peptide transport (reviewed in Horst et al., 2011).

Inhibitor	Virus	Structure	# aa	Degradation of TAP1/2	Interference with peptide binding	Interference with ATP binding	Conformational alterations
ICP47	HSV-1/2	Cytosolic	88/86	No	Yes	No	Yes
UL49.5	BoHV-1[a]	Type I mp	96	Yes	No	No	Yes
UL49.5	EHV-1/4	Type I mp	100	No	No	Yes	Yes
UL49.5	PRV	Type I mp	98	No	No	No	Yes
UL49.5	FeHV-1	Type I mp	95	No	Not tested	No	Not tested
US6	HCMV	Type I mp	182	No	No	Yes	Yes
BNLF2a	EBV	TA protein	60	No	Yes	Yes	Not tested
CPXV12	CPXV	Type II mp	69	No (TAP1)[b]	Not tested	Not tested	Not tested

[a] The UL49.5 proteins of the related varicelloviruses BoHV-5, BuHV-1 and CvHV-1, all infecting ruminants, were also found to inhibit TAP and induce its degradation.
[b] The effect of the CPXV12 protein on TAP2 stability has not been tested yet.
Abbreviations: aa, amino acid; mp, membrane protein; and TA, tail-anchored.

above-mentioned TAP inhibitors are encoded by herpesviruses (Horst et al., 2011). Interestingly, the latest viral TAP inhibitor discovered, the type II membrane protein CPXV12, is encoded by cowpox virus (CPXV), a member of the poxvirus family (reviewed in Wilkinson and Lehner, 2009). The mechanism of TAP inhibition by CPXV12 remains to be determined.

When expressed alone in cells, the viral TAP inhibitors described above were proven to inhibit peptide transport by TAP, resulting in reduced surface display of MHC I-peptide complexes, and failure of cytotoxic T cell activation. It is not known what happens during natural infection of cells by these viruses. *In vitro*, the role of TAP inhibitors encoded by HSV-1, EBV, and CPXV has been shown. Infection of cells with either ICP47-deficient HSV-1 (Todo et al., 2001) or BNLF2a-deficient EBV (Croft et al., 2009) led to enhanced $CD8^+$ T cell activation compared to cells infected with the corresponding wild-type viruses. Deletion of the TAP inhibitor CPXV12 slightly improved $CD8^+$ T cell recognition of cells infected with CPXV (reviewed in Wilkinson and Lehner, 2009).

In the EBV study, BAC-generated recombinant EBV viruses that lack the BNLF2a gene enhanced T cell recognition of EBV antigens presented by lytically infected B cells (Croft et al., 2009). Interestingly, this effect was only observed for T cells that were specific for viral antigens expressed during immediate-early or early phases of productive EBV infection. Activation was still hampered when late antigen-specific T cells were used to monitor antigen presentation, irrespective of BNLF2a expression; this observation indicates that other immune evasion molecules were active at late time points. In addition to the TAP inhibitor BNLF2a, EBV codes for at least two other proteins obstructing MHC I antigen presentation: BILF1 increases endolysosomal degradation of MHC I molecules and BGLF5 causes reduced MHC I synthesis as a consequence of global mRNA degradation.

It was recently discovered that very early and transient expression of lytic cycle proteins occurs during primary EBV infection of B cells (Jochum et al., 2012). BNLF2a was one of the mRNAs detected in the B cells within hours after infection and is thought to be transduced directly from viral particles. Following immediate synthesis, BNLF2a protected the newly infected B cells from T cell elimination, increasing chances to result in latent infection. It is likely that also other (herpes)viruses carry mRNAs within the viral particles, capable of influencing the host cell immediately upon infection. This provides a versatile exploitation of immune evasion, especially relevant for quick inhibition of TAP-mediated peptide transport.

3. Future perspectives

Interesting new developments will result from thorough *in vivo* studies in animals infected with viruses that differ in their expression of viral TAP inhibitor(s). The first experiments reported suggest an important role for TAP inhibition during infection. *In vivo* experiments from the laboratory of W. Yokoyama et al. showed that a double knock-out cowpox virus lacking both CPXV12 and CPXV203 is highly attenuated in mice, indicating that evasion of $CD8^+$ T cell recognition contributes to the virulence of CPXV (reviewed in Wilkinson and Lehner, 2009). To determine the specific role of the TAP inhibitor, the single mutant should be studied.

Future efforts should focus on elucidation of the exact mechanism of action of the viral TAP inhibitors and, thereby, TAP function. Although several aspects have been resolved and led to the insight that all inhibitors act differently (Table 1), the exact details of peptide transport by TAP, and how viral proteins can interfere with this process, remain enigmatic. TAP is a heterodimer, composed of TAP1 and TAP2, with an architecture common to all ATP-binding cassette (ABC) transporters. Each half-transporter contains a cytosolic nucleotide-binding domain (NBD) and a transmembrane domain (TMD). One of the current models for TAP function proposes that peptide binding to the cytosolic side of the TMDs induces dimerization of the NBDs. This, in turn, results in a conformational 'outward facing' rearrangement of the TM helices and release of peptide into the ER lumen. Hydrolysis of ATP subsequently separates the NBDs and brings the TM helices back to an 'inward facing' position.

Experimental support for this model could come from resolving crystal structures of TAP complexes in various conformations. Several structures of ABC transporters have been elucidated and, based on homology with the crystallized Sav1866 exporter of *S. Aureus*, a structural model has been constructed for TAP, at least for the NBDs together with the core 6 TM helices, in a single conformation (Parcej and Tampe, 2010). Since the viral TAP inhibitors block TAP by unique mechanisms, each inhibitor might trap TAP in a specific conformation. Crystallization of TAP in complex with these inhibitors will not only result in the structural elucidation of several TAP conformations, but will also give a better insight into the exact mechanisms of TAP inhibition. Additionally, extrapolation of such findings may lead to a better understanding of other ABC transporters, including the multidrug-resistance transporters that are associated with chemotherapy failure.

From an evolutionary point, elucidation of viral (TAP) immune evasion molecules also reveals interesting conceptual advances. Members of the herpesvirus family have co-evolved with their hosts over millions of years, causing a fascinating relationship of these viruses with their hosts. The observation that multiple viral gene products interfere with MHC I-restricted antigen presentation to $CD8^+$ T cells testifies to the importance of this effector arm of the immune system in antiviral immunity. The fact that the immune evasion proteins inhibiting TAP do so in different ways represents a striking example of functional convergent evolution.

Based on the discoveries made so far, novel immune evasion mechanisms are expected to be elucidated for other herpesviruses, such as varicella zoster virus (VZV) and human herpesvirus 6 and 7, viruses carried by over 90% of the human population but poorly studied in the context of immune evasion. It will also be interesting