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Review

The macromolecular peptide-loading complex in MHC class I-dependent antigen presentation

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Abstract. A challenging task for the adaptive immune system of vertebrates is to identify and eliminate intracellular antigens. Therefore a highly specialized antigen presentation machinery has evolved to display fragments of newly synthesized proteins to effector cells of the immune system at the cell surface. After proteasomal degradation of unwanted proteins or defective ribosome products, resulting peptides are translocated into the endoplasmic reticulum by the transporter associated with antigen processing and loaded onto major histocompatibility complex (MHC) class I molecules. Peptide-MHC I complexes are transported via the secretory pathway to the cell surface where they are then inspected by cytotoxic T lymphocytes, which can trigger an immune response. This review summarizes the current view of the intracellular machinery of antigen processing and of viral immune escape mechanisms to circumvent destruction by the host.

Keywords. ABC transporter, antigen processing, MHC class I, peptide-loading complex, tapasin, translocation pore, TAP.

Introduction

Higher eukaryotic organisms must defend themselves against invaders and cancer to survive. Therefore, jawed vertebrates have evolved the adaptive immune system, which allows for specific recognition and elimination of antigens that are abundant in body fluids or within cells [1]. The major effector cells of the adaptive immune response are B and T lymphocytes. B lymphocytes express and secrete antibodies, which target and neutralize humoral antigens for later destruction, whereas cytotoxic T lymphocytes (CD8+, CTLs) recognize and destroy infected or malignant cells. Since CTLs cannot access the interior of body cells for inspection, a sophisticated machinery is needed to present intracellular antigens on the surface of nucleated cells. Here, breakdown products of intracellular proteins are displayed on major histocompatibility complex (MHC) class I carrier proteins at the cell surface. If the peptides are derived from normal cellular proteins, the CTLs remain silent, except in autoimmune diseases. In contrast, peptides derived from foreign or mutated proteins trigger apoptosis of the antigen-presenting cell (APC) [2]. As few as 10–100 antigen-MHC class I complexes on a cell can induce a CTL response [3]. To escape of the immune system, viruses have evolved sophisticated strategies preventing the generation and presentation of antigenic peptides, in many cases leading to lifelong persistence and repeated reactivation within the host [reviewed in refs. 4–7].

The MHC class I-dependent pathway of antigen processing

A main task of the MHC class I-dependent pathway of antigen processing is the selection of highly abundant and/or high-affinity peptides for MHC class I loading and presentation to the immune system. Endogenous proteins

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are constantly degraded by the proteasome, a multicatalytic protease complex in the cytosol [8]. After polyubiquitination of unwanted proteins and defective ribosome products (DRiPs), which both represent the major substrate for proteasomal degradation [3, 9], these proteins are fragmented to peptides of 3–22 residues [10]. More than 99% of these peptides are destroyed within 1 min after their generation and are lost for presentation at the cell surface [11]. The remaining peptides are processed by cytosolic peptidases like the two aminopeptidases puromycin-sensitive aminopeptidase (PSA) and bleomycin hydrolase (BH) [12]. Below the length of 15 amino acids, peptides are exclusively trimmed by tripeptidyl peptidase-II (TPP-II), which displays both exo- and endopeptidase activity [13], and are subsequently translocated into the endoplasmic reticulum (ER) lumen by the transporter associated with antigen processing (TAP). After further trimming of the peptides by the ER-luminal aminopeptidases 1 and 2 (ERAP1/2) [14–16] to a length of 8–11 residues [17, 18], they are loaded onto MHC class I molecules, a complicated and tightly regulated process, which requires a macromolecular peptide-loading complex (PLC) comprised of TAP1, TAP2, tapasin, MHC class I heavy chain, β_2-microglobulin, calreticulin, and ERp57 [19, 20]. Following peptide binding, MHC-peptide complexes are released from the PLC and shuttled via the Golgi apparatus to the cell surface where they are scanned by CTLs, which may trigger an immune response. Antigen processing and presentation are far from being quantitative. Less than 0.1% of the generated and matured MHC class I-loaded peptides are finally presented to CTLs at the cell surface [3, 21]. The MHC class I-dependent pathway of antigen processing and presentation is illustrated in Figure 1.

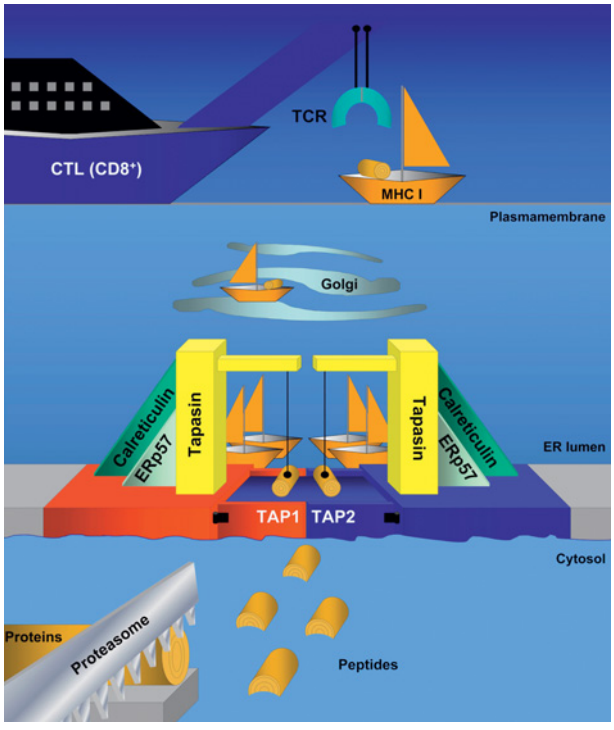

Figure 1. The MHC class I-dependent pathway of antigen processing. Intracellular proteins are degraded by the proteasome and resulting peptides are translocated by the ABC transporter TAP into the ER lumen. Mediated by a macromolecular peptide-loading complex, these peptides are subsequently transferred onto empty MHC class I molecules and shuttled via the secretory pathway to the cell surface, where the antigen-MHC class I complexes are scanned by CTLs.

The transporter associated with antigen processing

The ER-resident TAP represents a crucial checkpoint in the MHC class I-dependent pathway of antigen presentation of the adaptive immune system in vertebrates. Peptides derived from proteasomal degradation of polyubiquitinated endogenous proteins are translocated into the ER lumen by TAP and, after loading onto MHC class I molecules, shuttled to the plasma membrane for inspection by CTLs. Many of the proteasome-digested peptides are poor substrates for TAP-dependent translocation into the ER lumen since they are either too long or too short and therefore lost for ER translocation [22]. TAP preferentially binds peptides with a length of 8–16 amino acids, but transports peptides of 8–12 amino acids most efficiently [23–25], although longer peptides (up to 40 amino acids) or sterically restricted peptides can also be transported [25–28]. For efficient peptide presentation, the antigen-processing machinery has to ensure that every subsequent component in the cascade is provided with the favored peptide species. Upon interferon-γ (IFN-γ) stimulation, the three active proteasomal subunits are replaced by specialized $\beta 1_i$, $\beta 2_i$, and $\beta 5_i$ subunits forming so-called immunoproteasome subunits [reviewed in ref. 29]. The immunoproteasome generates peptides with C-terminal residues, which are favored by both human TAP and MHC class I molecules. However, the preference for N-terminal residues differs considerably between TAP and MHC class I molecules. To cope with this 'out-of-register' problem, ER-resident aminopeptidases are needed to further trim the translocated peptides for efficient loading onto MHC class I molecules [14, 16]. By employing combinatorial peptide libraries, the peptide-binding motif of human TAP was deciphered [30, 31]. In addition to free N and C termini, the first three N-terminal and the C-terminal residue of the peptide are important for binding to TAP. Peptides with a hydrophobic or basic residue at the C terminus are favored. Although peptide generation, transport, and loading are tightly regulated, the theoretical pool of peptides which might be displayed at the cell surface is enormous ($>10^7$), since the sequence between both termini of the

peptide varies with respect to binding to TAP and MHC class I molecules. This concept of diversity and specificity is highly beneficial to the host since the cellular proteome can be monitored as a 'peptidome' displayed by MHC I molecules on the cell surface.

TAP is a heterodimeric ATP-binding cassette (ABC) transporter composed of TAP1 and TAP2 [32–34]. Both subunits can be subdivided into a transmembrane domain (TMD) that binds peptides and forms the translocation pore within the ER membrane, and a cytosolic nucleotide-binding domain (NBD), which energizes peptide translocation across the membrane by ATP hydrolysis [35]. Peptide binding, ATP hydrolysis and peptide transport are tightly coupled by large structural rearrangements within the transporter [28, 36]. The TMDs include 10 and 9 transmembrane segments (TMs) for TAP1 and TAP2, respectively [37, 122]. The core domain of 6 + 6 TMs, a common building block for most ABC transporters, is essential and sufficient for ER targeting, membrane insertion, heterodimerization, peptide binding, and transport of the heterodimeric TAP complex, whereas the unique N-terminal domains are important for binding to tapasin [38, 39] (Fig. 2). Studies with photo-active peptides showed that both TAP subunits contribute to peptide binding [23]. The peptide-binding pocket was localized in the cytosolic loops between H4 and H5 of TAP1, H4 and H5 of TAP2, and a stretch of 15 amino acids C-terminal of H6 of TAP1 and TAP2, respectively (Fig. 2) [40, 41].

To date, only the high-resolution X-ray crystal structure of the TAP1-NBD has been solved [42]. However, demonstrated that single-particle electron microscopy (EM) analysis the TAP1/2 complex has a diameter of approximately 10 nm with a central cavity of about 3 nm [43].

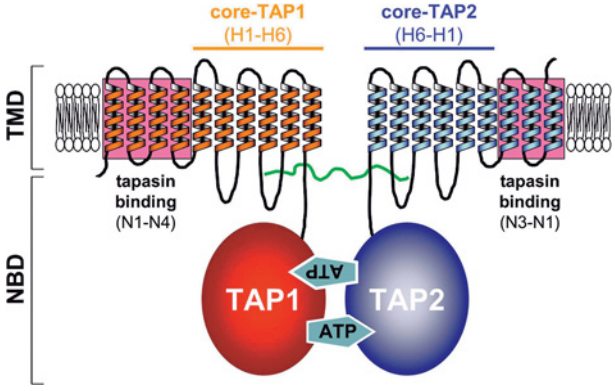

Figure 2. Structural organization of TAP. TAP comprises a heterodimer of TAP1 and TAP2. The transmembrane domains (TMDs) are subdivided into a core domain (H1–6), which contains the peptide (green) binding site and forms the translocation pore. The N-terminal transmembrane segments of TAP1 and TAP2 (N1–4 and N1–3) recruit the adapter protein tapasin. The nucleotide-binding domains (NBDs) energize peptide translocation across the ER membrane by ATP hydrolysis.

The TAP-associated protein tapasin

Tapasin is a central component of the PLC and has diverse functions such as: (i) recruitment of ERp57 and MHC class I molecules at TAP [20, 44]; (ii) stabilization of TAP, since restoration of tapasin in tapasin-negative cells resulted in a threefold increase in the expression level of TAP [45–48]; (iii) increase in the efficiency of MHC class I surface presentation and the amount of MHC molecules associated with high-affinity peptides, which is strongly impaired in tapasin-deficient cells [20, 44, 49–52]; (iv) exchange of high-k_{off} by low-k_{off} peptides in the binding groove of MHC class I molecules [53], and (v) coordination and facilitation of peptide loading onto MHC class I molecules [52–54]. Interestingly, the binding affinity of peptides to TAP is not influenced by tapasin [45]. Very recently, it was further suggested that tapasin acts as a regulator of ERp57 in blocking its oxidoreductase escape pathway [55].

Tapasin is a type I membrane glycoprotein with a molecular mass of 48 kDa. Structurally, it is comprised of an ER-luminal domain (residues 1–392), a transmembrane helix (residues 393–417), and a short cytosolic tail (residues 418–428), which bears an ER retention signal (consensus sequence KKxx) (Fig. 3). The ER-luminal domain can be subdivided into at least two domains of which the C-terminal domain has an immunoglobulin (Ig) fold. Each domain is stabilized by a disulfide bond (C_7–C_{71} and C_{292}–C_{362}). Another cysteine within the ER-luminal domain (C_{95}) is essential for the disulfide formation to ERp57 [44, 55, 56], promoting retention of ERp57 in the PLC [44]. The asparagine residue (N_{233}) between the two globular domains is the site of N core glycosylation leading to slower electrophoretic mobility in SDS-PAGE [51, 57]. Mutational studies have shown that the N-terminal 50 amino acids of tapasin as well as part of the C-terminal Ig fold are important for the recruitment of MHC class I molecules [45, 57, 58].

MHC class I molecules – a shuttle and display device for peptides

MHC class I molecules are composed of a type I membrane anchored heavy chain (HC, 44 kDa) and a non-covalently associated soluble protein termed β_2-microglobulin (12 kDa). This complex is unstable, but can be stabilized by association with tapasin or antigenic peptide. The ER-luminal domain of the MHC class I HC can be subdivided into three domains (α1–α3) of which the distal two domains (α1 and α2) form the binding groove for antigenic peptides [59, 60]. The human HC is N core-glycosylated at N_{86} in the α1 domain providing the binding site for calreticulin [61, 62]. The length preference for MHC class I-associated peptides is 8–11 residues [17, 18]. Like

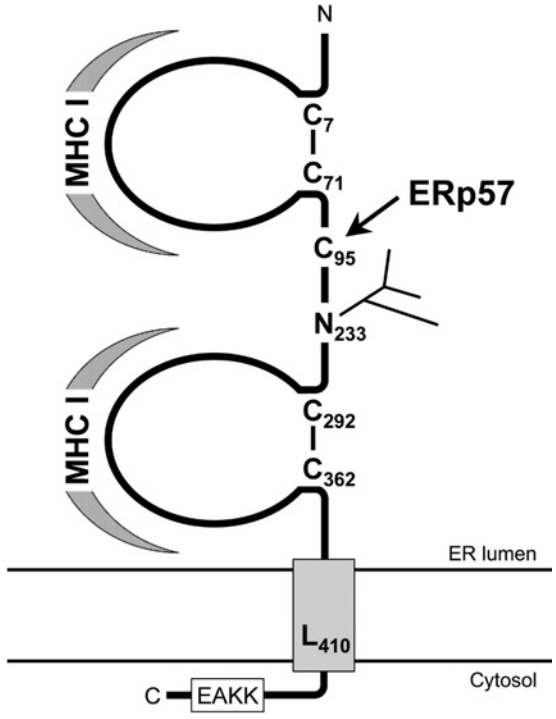

Figure 3. Structural organization of the type I transmembrane protein tapasin. The sites which are involved in binding to MHC class I and ERp57 are indicated.

TAP, MHC class I molecules recognize the N- and C-terminal residues of antigenic peptides, which flank a variable sequence region for T cell recognition.

The maturation of MHC class I molecules within the ER is a complex process, which is regulated in time and space by a variety of auxiliary factors, like the chaperones calnexin (CNX) and calreticulin (CRT), and the oxidoreductase ERp57 [62–66]. Following cotranslational translocation of MHC class I HC and β_2-microglobulin into the ER via the Sec61 complex, both polypeptide chains mature and assemble accompanied by sequential folding cycles [67, 68]. Disulfide bond formation in the ER, which parallels folding within the MHC class I loading complex, has a strong impact on the efficiency of peptide loading [67, 69].

Auxiliary factors

The ER quality control system involves the immunoglobulin-binding protein (BiP), CRT and CNX as well as the cochaperone ERp57, which is an oxidoreductase of the protein disulfide isomerase (PDI) family. Both CRT and CNX form complexes with ERp57 [70, 71]. Nascent polypeptide chains are cotranslationally translocated into the ER and N core-glycosylated ($Glc_3Man_9GlcNAc_2$). After trimming of the core oligosaccharide to the monoglycosylated form ($Glc_1Man_9GlcNAc_2$), CRT and CNX interact with the sugar moieties, preferentially within the first 50 residues from the N terminus of the polypeptide chain [72]. In a second step, ERp57, which forms transient intermolecular disulfide bonds with the newly synthesized glycoproteins, is recruited to the complex. Most probably, ERp57 forms 1:1 complexes with both CRT and CNX [70, 73]. Interestingly, a partial loading complex lacking ERp57 is deficient in MHC peptide loading [44].

CRT, CNX, and ERp57 promote folding of a variety of nascent proteins designated for the cell surface or for secretion [71, 74]. CRT and CNX interact with monoglucosylated N-linked glycans of nascent glycoproteins within the ER and facilitate folding of MHC class I molecules [70, 71, 74, 75], whereas ERp57 forms disulfide intermediates with nascent substrate proteins for folding via two CxxC motifs ($C_{57}xxC_{60}$ and $C_{406}xxC_{409}$), which are common for the thioredoxin protein family [76–79].

CNX (65 kDa; apparent molecular mass 90 kDa [20]) is a type I ER membrane protein, whereas CRT (46 kDa) is a soluble protein in the ER lumen. Interestingly, the ER-luminal domains of CNX and CRT share a high degree of homology [reviewed in ref. 69]. The high-resolution X-ray structure (2.9 Å) of the ER-luminal domain of CNX (residues 47–468) has been solved [80]. As characteristic structural elements, CNX possesses a proximal globular and a 14-nm arm domain, which might physically constrain the substrate glycoprotein and prevent diffusion from the cavity formed by the globular and the arm domain [80, 81]. Moreover, the tips of the arm domains of both CNX and CRT are likely to bind to ERp57 [81, 82]. However, the globular domains might also play a role in the interaction, presumably in a Zn^{2+}-dependent fashion [81].

Assembly of the PLC

After synthesis, MHC class I HCs rapidly interact with BiP and CNX in the ER lumen [83–85]. Subsequently, the MHC class I HCs dissociate from CNX and associate with β_2-microglobulin, CRT, ERp57, and a preassembled complex of TAP1, TAP2, and tapasin [62, 86, 87]. The fully assembled PLC is essential for selection of high-affinity antigens, and their transfer onto empty MHC class I molecules for later presentation to effector cells of the immune system.

The extra N-terminal domains of TAP are crucial for tapasin binding and thus for assembly of the PLC [39]. Importantly, tapasin interacts with both TAP and MHC class I molecules [20, 45, 57, 58, 65, 88]. The interaction network at the PLC is highly dynamic. While in TAP-deficient cells, tapasin interacts stably with MHC class I molecules for several hours, this intermolecular cross-talk becomes dynamic and productive in the presence of TAP and the other components of the PLC. Tapasin then assists in the sequential assembly of many peptide-MHC class I

complexes [56]. The finding that, in TAP-deficient cells, tapasin interacts stably with MHC class I molecules is further supported by the observation that tapasin confers ER retention on MHC class I molecules in invertebrate cells [89]. Interestingly, the stability of tapasin is not affected by interaction with TAP or MHC class I molecules [56].

By use of a monoclonal antibody against a C-terminal peptide of human TAP1, components of the PLC were copurified and the stoichiometry of subunits within the complex was determined [20]. The results showed that the heterodimeric TAP complex binds four tapasin molecules and the ratio of tapasin to MHC class I within the macromolecular PLC is about 1:1 [20, 56]. Consequently, the molecular mass of the PLC (1×TAP1, 1×TAP2, 4×tapasin, 4×MHC I-HC, 4×β_2-microglobulin, 4×ERp57, and 4×calreticulin) can be estimated at approximately 1 MDa. However, it remains puzzling how four tapasin-MHC sub-complexes are organized by the two TAP subunits. This is especially challenging since tapasin can bind independently to singularly expressed TAP1 and TAP2 subunits [19, 48] as well as to TAP1 and TAP2 within a functional, heterodimeric complex [J. Koch, R. Guntrum and R. Tampé, unpublished results]. Based on these data, it is tempting to speculate how a virtually symmetric PLC is assembled around a rather asymmetric TAP heterodimer. Biochemical and structural data on P-glycoprotein and MsbA [90–93] imply that the N-terminal extensions of TAP1 and TAP2 point to opposite sites of the core transporter and most likely provide two separate platforms for the assembly of the macromolecular MHC PLC (Fig. 4A). Moreover, it appears unlikely that tapasin forms higher-order multimers, although the authors cannot completely rule out the possibility [56], suggesting that tapasin associates with TAP at four independent sites.

Although the N-terminal domains of TAP1 and TAP2 were identified to mediate tapasin binding [39], the precise binding sites for tapasin have not been mapped. Previous studies suggest that TAP and tapasin interact through their TMs since soluble human tapasin variants and a transmembrane domain point mutant of human tapasin (L410F) were impaired in MHC class I surface expression [54]. Moreover, the L410F mutant was defective in TAP association, but bound to class I molecules, CRT and ERp57. The authors further discuss the possibility that the interaction of TAP and tapasin could be mediated by a putative leucine-zipper formed by the leucine residues 396, 403, 410, and 417 within the transmembrane domain of tapasin [54].

Dick and colleagues could demonstrate that ERp57 (C_{57}) and tapasin (C_{95}) form a disulfide-linked intermediate, which might be essential for the generation of correctly folded and oxidized MHC class I molecules [44]. Since cysteines exposed in the ER mediate assembly, retention, and degradation of proteins, the disulfide network within the PLC might have an impact on the general stability of the components and the assembled complex [67, 94, 95]. The current structural view of the PLC within the ER membrane is illustrated in Figure 4.

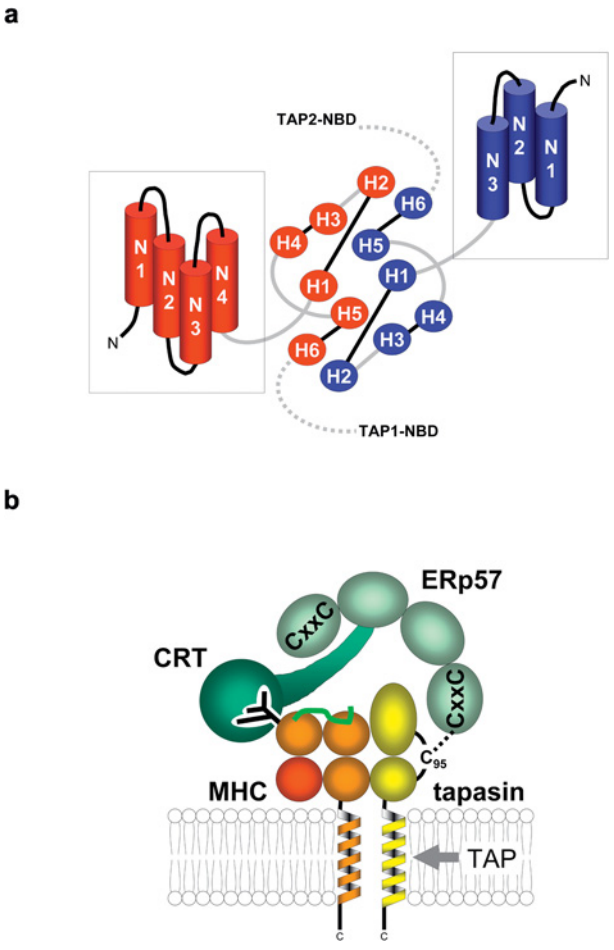

Figure 4. Current view of the PLC. (*a*) Superposition of the transmembrane domains of TAP and MsbA from *Vibrio cholerae* (top view) [93]. The core domain of TAP (H1–6) contains the substrate-binding site and forms the translocation pore, whereas the N-terminal domains (N1–4 and N1–3), which presumably point in opposite directions, provide assembly platforms for the PLC. (*b*) Schematic representation of the interactions between the proteins which contribute to the PLC. Here, tapasin (yellow) acts as an adapter protein between TAP and the complex comprised of MHC class I (heavy chain, orange; β_2-microglobulin, red), calreticulin (CRT, dark green), and ERp57 (light green). Based on the available data, four of these complexes are recruited at the N-terminal domains of the TAP complex.

Modulators of the PLC

The fact that mainly DRiPs or unwanted proteins are degraded by the proteasome enables the host to prevent successfully the spread of pathogens, which employ the host cell for protein synthesis [3, 96]. The complexity of the pool of peptides presented at any one time is biased by highly abundant peptides derived from ribosomes and

heat-shock proteins [3, 97]. After virus infection, viral proteins are expressed at very high levels and, most importantly, many viruses utilize virus host shut-off factors to reprogram the host's machinery to synthesize viral proteins. Consequently, the complexity of the pool of presented peptides is shifted toward peptides of viral origin [3].

In particular, herpes- and adenoviruses have evolved sophisticated strategies to prevent the generation or presentation of antigenic peptides, leading to a blockage in immune surveillance and, in many cases to lifelong persistence and repeated reactivation within the host. Basically, all steps of the antigen presentation pathway are targeted. Some of the viral proteins involved have been identified. A major aim for these immune evasion proteins is the macromolecular PLC. To date, four herpesvirus proteins have been identified, which use quite different mechanisms to interfere with processes at the PLC.

The immediate early protein ICP47 of herpes simplex virus (HSV-1 and HSV-2, 10 kDa) binds to the heterodimeric TAP complex from the cytosol and prevents peptide but not ATP from binding to TAP [98, 99]. In the absence of a functional TAP transporter (within 3 h of infection with HSV), MHC class I molecules are not loaded with peptides and, as a consequence, are retained in the ER and ultimately directed for proteasomal degradation [86, 100, 101]. By systematic truncations, the region of ICP47 sufficient for TAP inhibition was assigned to residues 3–34 [102]. Moreover, by alanine scanning mutagenesis, three regions (8–12, 17–24, and 28–31) were identified within the active domain of ICP47, which are critical for TAP inhibition [103]. ICP47 is largely unstructured in aqueous solution. However, in a lipid-like environment, the active domain of ICP47 adopts a helix-loop-helix structure [104, 105]. The binding site of ICP47 at TAP has not been mapped. Chemical cross-linking experiments indicate that ICP47 and the peptide substrate most likely occupy different, maybe overlapping sites at TAP [98, 99, 103]. Both the competition for binding site(s) and structural rearrangements are possible inhibition mechanisms, which cannot be distinguished.

Another prominent example is the human cytomegalovirus (HCMV) early protein US6 (21 kDa) that binds to TAP from the ER lumen [106–108]. The ER-luminal domain of US6 is sufficient to inhibit peptide translocation by specifically blocking ATP binding to the cytosolic NBDs of TAP at the opposite side of the membrane [109, 110]. The molecular mechanism of US6 has not yet been elucidated. However, the data suggest that US6 interferes with the cross-talk between the TMDs and the NBDs of TAP1 and TAP2, possibly accompanied by structural rearrangements within the transport complex since a direct interaction of US6 with the NBDs across the membrane appears unlikely. A 6 + 6 TM core-TAP complex lacking the N-terminal domains of TAP1 and TAP2 was still inhibited by US6, demonstrating that US6 interacts with the ER-luminal loops of the core translocation complex [39].

Recently, two other viral immune evasion proteins were identified, the MK3 protein of the murine γ-herpesvirus-68 [111, 112] and the UL49.5 protein of varicelloviruses [113, 114]. The ER membrane protein MK3 leads to polyubiquitination of the cytosolic tails of newly synthesized MHC class I HCs and directs them to proteasomal degradation [113]. Moreover, tapasin and TAP are targeted for degradation, a process which involves an MK3 RING finger domain [115, 116]. However, a ubiquitinated TAP intermediate could not be trapped. Coimmunoprecipitation experiments with TAP1 and MK3 in the absence of tapasin and TAP2 suggest that MK3 associates with the PLC via TAP1 [116]. Interestingly, the MK3 protein interferes with the IFN-γ-induced upregulation of surface MHC class I. However, the underlying mechanism has not yet been elucidated.

The type I membrane protein UL49.5 (9 kDa, also termed gN) of varicelloviruses is a non-essential envelope protein involved in maturation of the glycoprotein gM [117–119], which causes a drastic suppression of MHC class I cell surface expression by blocking TAP function [113, 114, 120]. So far, an inhibitory effect was demonstrated for the bovine and equine herpesvirus 1 (BHV 1, EHV 1) as well as for the pseudorabies virus (PrV). Interestingly, two mechanisms are used by UL49.5 to inhibit TAP-dependent peptide translocation: (i) arrest of the TAP complex in a functionally incompetent conformation and (ii) proteasomal degradation of components of the PLC [114]. Here, the C-terminal cytosolic tail of UL49.5 is important to mediate the degradation process [114].

Perspectives

Very little is known about the structural organization of the macromolecular PLC. Up to now, only the X-ray crystal structures of MHC class I molecules, and the NBD of TAP1 have been solved. In addition, more structural information is needed. To understand the function of the macromolecular assembly of all components of the PLC, many mechanistic questions remain open. (i) How are peptide translocation, peptide loading, and dissociation of the peptide-loaded MHC complex synchronized? (ii) How does tapasin catalyze peptide binding to the MHC? (iii) How does the peptide transfer onto MHC class I molecules work (diffusion vs chaperone assisted transfer)? (iv) Where is the precise interaction site of tapasin within the N-terminal domains of TAP1 and TAP2? (v) The heterodimer of TAP1 and TAP2 contains 19 cysteine residues, which are not essential for function [121]; however, specific ERp57-mediated disulfide bond rearrangements were identi-

fied within the PLC. Therefore, it is tempting to elucidate the stable and especially the transient disulfide-mediated interactions during PLC assembly.

A variety of viral proteins have been identified, which promote escape from the immune system of the host. Several of these interfere directly with antigen processing at the PLC in the ER membrane. These viral factors provide powerful tools to decipher functional interactions within the MHC class I-dependent pathway of antigen presentation in general and of the PLC in particular. Moreover, they provide the basis for the development of novel viral therapies; here, the recently identified family of varicellovirus proteins (UL49.5) is of special interest.

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