Antigen degradation or presentation by MHC class I molecules via classical and non-classical pathways

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Abstract

Major histocompatibility complex (MHC) class I molecules usually present endogenous peptides at the cell surface. This is the result of a cascade of events involving various dedicated proteins like the peptide transporter associated with antigen processing (TAP) and the ER chaperone tapasin. However, alternative ways for class I peptide loading exist which may be highly relevant in a process called cross-priming. Both pathways are described here in detail. One major difference between these pathways is that the proteases involved in the generation of peptides are different. How proteases and peptidases influence peptide generation and degradation will be discussed. These processes determine the amount of peptides available for TAP translocation and class I binding and ultimately the immune response.

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1. Introduction

The primary function of the immune system is to protect the individual against intra- and extracellular pathogens such as viruses and bacteria. Two sets of specialized cells have evolved to recognize antigens: B and T lymphocytes. Each B and T lymphocyte has its individual antigen receptor which, upon antigen recognition, undergoes clonal expansion to form effector and memory cells providing specific acquired immunity. The B lymphocytes are concerned with the humoral immune response. These cells bind native antigens with their B cell receptor (BCR), which, upon maturation of the B cell, will be converted into a secreted form which is then called immunoglobulin (Ig) or antibody. The cellular immune response is executed by cytotoxic and helper T lymphocytes. T cells recognize through their T cell receptor (TCR) small antigenic peptide fragments in association with molecules of the major histocompatibility complex (MHC), a name derived from their ability to evoke powerful transplant rejections between individuals of the same species. Currently, it is appreciated that MHC molecules enable the immune system to monitor the internal contents of intact cells by binding peptides inside the cell and transporting them to the cell surface.

The MHC gene complex encodes two major classes of peptide receptors, the MHC classes I and II molecules. MHC class I molecules are cell surface glycoproteins expressed on virtually all nucleated mammalian cell types. CD8+ cytotoxic T cells (CTL) recognize antigen in the context of MHC class I and will kill a 'target' cell upon proper recognition. MHC class II molecules are expressed on the surface of professional antigen presenting cells (APC) and recognized by CD4+ T helper (Th) cells. Differences in assembly, intracellular transport and usage of specialized chaperones allow MHC classes I and II molecules to present peptides from different intracellular compartments and thereby from different sources. However, the dogma that endogenous antigens are presented by MHC class I molecules, while exogenous antigens are displayed by MHC class II molecules does not always hold, as will be outlined later. This review will focus on several aspects of conventional and alternative MHC class I antigen processing and presentation and highlight the role of intracellular proteases in generating and eliminating presentable antigens.

2. Conventional MHC class I antigen processing and presentation

In general, MHC class I molecules present peptides derived from endogenous antigens. The peptides originate mostly from cytosolic or nuclear proteins and are generated...
Fig. 1. Schematic model of the conventional MHC class I antigen processing and presentation pathway. Endogenous antigens are degraded by the proteasome into short peptides. These peptides are transported into the ER lumen by TAP. In the ER, newly synthesized MHC class I heavy chains assemble with β2m and a peptide. This assembly process involves transient interactions with calnexin, calreticulin, Erp57, tapasin and TAP. Upon peptide binding, the MHC class I heterotrimeric complex is released from the ER and transported to the cell surface via the constitutive secretory pathway.

3. Peptide generation by proteasomes

Cytosolic or nuclear antigens first need to be degraded into small peptides before they can be transported by TAP and bound by MHC class I molecules. The bulk of proteins in eukaryotic cells are targeted for degradation by conjugation with ubiquitin. In the ubiquitin-conjugation process, which requires ATP and several enzymes, multiple ubiquitin moieties are covalently attached to the ε-amino group of lysine residues in the protein substrate (reviewed by Jentsch, 1992). This polyubiquitin chain marks the protein for rapid degradation by the proteasome. Proteins containing “destruction” boxes or PEST sequences, which consist of particular stretches of conserved amino acids, are also degraded by the proteasome. Alternatively, they are recognized by ubiquitin ligases that transfer ubiquitin to a target protein. Many ubiquitins are subsequently added to form a polyubiquitin tail that is recognized by the 19S cap of the proteasome. This cap also performs the unfolding reaction that precedes degradation by the proteasome (Kloetzel, 2001). The proteasome is the main proteolytic system in the nucleus and cytosol of all eukaryotic cells (Fig. 2). The crystal structures of archaebacterial and yeast proteasomes have revealed the complex architecture of proteasomes (Lowe et al., 1995; Groll et al., 1997). The 20S proteasome is a multicatalytic complex of ~700 kD composed of two heptameric outer rings of α subunits and two heptameric inner rings of β subunits, which together form a hollow cylinder. The β subunits are encoded as inactive precursors, which are activated by autocatalytic cleavage of the peptide bond preceding the catalytic residue (Seemuller et al., 1996). This autocatalytic processing results in proteolytically active β subunits with an N-terminal threonine as active-site nucleophile (Seemuller et al., 1995). The shortest distance between the active-sites of two β subunits is 28 Å, which corresponds roughly to the length of an extended peptide chain of eight amino acids (Lowe et al., 1995). Human proteasomes probably contain only three distinct active β subunits (X, Y and Z) while the other four β subunits seem to lack active-sites (Seemuller et al., 1995). All three active β subunits are, upon stimulation with interferon (IFN)γ, replaced by another set of active β subunits: MECL1, LMP2 and LMP7 (Belich et al., 1994;
Fig. 2. Structural features of proteasome complexes in eukaryotic cells. Left, schematic view of subunit arrangement in the 19S regulator, the 20S proteasome core particle and the 11S regulator. The 19S regulator contains approximately 20 different subunits including six ATPases. The 20S proteasome is a barrel-shaped structure of four stacked rings, each comprising seven subunits. The outer rings are formed by the structural α subunits and the two central rings by the catalytically active β subunits. The 11S regulator or PA28 is a hexameric complex consisting of two kinds of IFN-α-inducible subunits, PA28α and β. Middle, from top to bottom: the 26S, 19S-20S-PA28 and PA28-proteasome complexes. Right, schematic cross-section of the thermoplasma acidophilum 20S proteasome core particle, showing the location of the α subunits, the β subunits and peptidase active-sites (white dots). Adapted from (Rubin and Finley, 1995).

The 20S proteasome is involved in degradation of unfolded proteins and polypeptides (Wenzel and Baumeister, 1995) and can function independently or in association with two regulatory subunits. The 26S proteasome consists of the 20S proteasome core with at both ends 19S (PA700) regulatory subunits and is involved in ATP-dependent degradation of ubiquitin-conjugated proteins. Alternatively, the 20S proteasome can associate with an 11S (PA26; REG) regulator complex, which is composed of two IFN-α-inducible subunits PA26α and β, forming the PA28-proteasome complex (Dubiel et al., 1992; Ma et al., 1992; Ahn et al., 1996; Knowlton et al., 1997). Recently, simultaneous binding of the 19S and 11S subunits to 20S proteasomes has been reported (Hendil et al., 1998). It is plausible that upon IFN-γ stimulation, a 19S-20S-PA28 complex is formed, in which the 19S part de-ubiquitinates and PA28 activates the degradation by the 20S particle.

All three proteasomal complexes have been implicated in the generation of peptides for MHC class I antigen presentation. Ubiquitin-conjugation and degradation by the 26S proteasome plays a role in the generation of MHC class I peptide epitopes for some antigens (Townsend et al., 1988; Michalek et al., 1993; Grant et al., 1995). The presence of PA28 in the 20S proteasome complex markedly increases peptide hydrolysis (Dubiel et al., 1992; Ma et al., 1992) and leads to an enhanced production of double-cleavage products (Groettrup et al., 1995; Groettrup et al., 1995; Dick et al., 1996; Dick et al., 1994). These double-cleavage products are generated through coordinated consecutive or simultaneous cleavage of protein substrates by two active-sites in the proteasome. This enhanced dual-cleavage activity of the PA28-proteasome increases, the amount of peptides of optimal length for TAP transport (see later), which may also be capable of binding to MHC class I molecules. Proteasomes can cleave peptides at the C-terminal side of basic (trypsin-like activity), hydrophobic (chymotrypsin-like activity), acidic, branched chain and small neutral amino acids (Orlowski et al., 1993; Cardozo et al., 1994). These multiple proteolytic activities have been defined using short fluorogenic peptides. However, when longer peptides or proteins are used as substrates, the very complex cleavage patterns do not correlate with the aforementioned specificities (Wenzel et al., 1994; Ehring et al., 1996; Dolenc et al., 1998). Obviously, definition of proteasomal cleavage specificity based on the residue directly adjacent to the cleavage site does not correspond with reality. Proteasomes are able to generate class I epitopes in vitro and in vivo from various proteins and polypeptides (Groettrup et al., 1995; Boes et al., 1994; Dick et al., 1994; Wenzel et al., 1994; Eggers et al., 1995; Kuckelkorn et al., 1995; Anton et al., 1997; Niedermann et al., 1996; Goth et al., 1996). The efficiency of class I presentation of a particular epitope depends on the proteasomal cleavage patterns within and adjacent to this epitope (Eggers et al., 1995; Niedermann et al., 1995; Yellen-Shaw et al., 1997; Ossendorp et al., 1996; Yellen-Shaw and Eisenlohr, 1997), and cleavage sites can be predicted.
with some accuracy (Kessler et al., 2001; Toes et al., 2001).

The IFNγ-inducible proteasome subunits LMP2 and LMP7 are encoded in the MHC region of the genome (Brown et al., 1991; Glynté et al., 1991; Kelly et al., 1991; Martinez and Monaco, 1991; Ortiz-Navarrete et al., 1991). This suggested a role for proteasomes in MHc class I antigen processing and presentation, like the TAP genes which are encoded within the same region (see later). LMP2 and LMP7 are dispensable for overall antigen presentation (Andrews et al., 1992; Momburg et al., 1992; Yewdell et al., 1994). However, mice carrying a targeted deletion in LMP2 (Van Kaer et al., 1994) or LMP7 (Feeling et al., 1994) exhibit defects in MHc class I-specific T cell responses. Incorporation of LMP2 and LMP7 into the proteasome is likely to have a subtle effect on the generation of certain antigenic peptides (Cerundolo et al., 1995; Kuckelkorn et al., 1995; Sibille et al., 1995; Stöhwasser et al., 1996; Schmadke et al., 1998). Whether this effect is due to enhanced trypsin- and chymotrypsin-like activity of LMP2/LMP7 containing proteasomes (Driscoll et al., 1993; Gacynska et al., 1993; 1994; Van Kaer et al., 1994; Aki et al., 1994; Kuckelkorn et al., 1996; Ehring et al., 1996), it is still a matter of debate (Boes et al., 1994; Grootert et al., 1995; Ustrell et al., 1995; Schmadke et al., 1998). After IFNγ stimulation, both trypsin- and chymotrypsin-like proteasome activities become rate limiting for MHc class I peptide generation (Benham and Neefjes, 1997). Intriguingly, these activities generate peptides with a basic or hydrophobic C-terminal residue, matching the requirements for efficient transport by TAP and binding with high affinity to MHc class I molecules (see later). It has been proposed that the ability of proteasomes to generate potentially immunocompetent peptides evolved well before the vertebrate immune system (Niedermann et al., 1997), which is not unlikely.

Additional evidence for the involvement of proteasomes in class I epitope generation comes from the use of various membrane permeable proteasome inhibitors. Peptide aldehydes are reversible inhibitors of the proteasome and reduce cytosolic antigen degradation, resulting in impaired MHc class I assembly and peptide presentation to CTLs (Roch et al., 1994; Harding et al., 1995; Yang et al., 1996; Hughes et al., 1996; Niedermann et al., 1997; Vinitsky et al., 1997; Anton et al., 1998; Luckey et al., 1998). The fact that peptide aldehydes can also inhibit cysteine proteases, possibly in the ER, complicates the interpretation of results obtained with these inhibitors (Hughes et al., 1996; Bai and Forman, 1997). The fungal metabolite lactacystin (Fenteany et al., 1995; Dick et al., 1996, 1997; Grau et al., 1997) and peptide vinyl sulfones (reviewed in [Bogoy et al., 1997a,b, 1998]) covalently bind to β subunits and are irreversible inhibitors with higher specificity for the proteasome. These inhibitors did not reduce antigen degradation and presentation by MHc class I molecules (Bai and Neefjes, 1997; Cerundolo et al., 1997; Vinitsky et al., 1997; Anton et al., 1998; Niedermann et al., 1997; Benham et al., 1998; Glas et al., 1998; Luckey et al., 1998). These studies demonstrate an important role for proteasome-dependent degradation in the generation of MHc class I presentable peptides. However, assembly of MHc class I molecules with certain peptides persists even in the presence of proteasome inhibitors (Hughes et al., 1996; Sijts et al., 1996; Benham et al., 1998; Cerundolo et al., 1997; Grau et al., 1997; Vinitsky et al., 1997; Anton et al., 1998; Glas et al., 1998; Luckey et al., 1998). This could mean that either proteasome inhibition is not complete or a small fraction of antigenic peptides is generated by other mechanisms in the cell, including by ER peptideases.

4. Peptide generation: old or new targets

Proteins are degraded at the end of their natural life after recognition by the E3 system (for review (Deshazes, 1999)). This system first adds one and later multiple ubiquitins to the proteins which subsequently become a target for proteasomal breakdown. Peptides are then generated. This implies that antigens can only be presented to the immune system after degradation of relative old proteins. Viruses could respond by producing stable proteins and the immune system can not immediately recognize infected cells. Although this certainly happens, the immune system is still able to respond almost immediately after translation of (viral) antigens. Using both biochemical and biophysical techniques it has been shown that a relative large fraction of proteins is degraded into peptides almost immediately after translation. This included viral proteins (Schubert et al., 2000; Reits et al., 2000). This fraction constitutes more than 30% of all proteins synthesized (Schubert et al., 2000) and may approach up to 80% (Reits et al., 2000). This high number may be caused by incorrect translation (the so-called DriPs) (Yewdell, 2001; Schubert et al., 2000), misfolding after translation, misassembly and/or proteins with a natural short half-life and probably affects all proteins although to a different extent. Still the consequence is that class I molecules present peptides from new targets thus allowing the immune system to respond immediately to a novel antigen but also to old targets since class I can not discriminate between the two pools.

5. Peptide trimming or destruction in the cytosol

A recent calculation by Yewdell (Yewdell, 2001) suggested that many more proteins are degraded than presented by MHc class I molecules. In fact, he calculated that only 1 peptide is binding to an MHc class I molecule from 10^4 proteins degraded. A similar inefficiency was experimentally found (Montoya and Del Val, 1999). Since neither loading of MHc class I molecules (Benham and Neefjes, 1997) nor peptide translocation by TAP (Reits et al., 2000) does occur at a saturating rate, the inefficiency is generated at a point
before TAP but after degradation by the proteasome. This suggests that many peptides are lost before they are able to associate with TAP. The half-life of peptides is very short due to aminopeptidase activity (Reits et al., submitted for publication). In fact, this peptidase activity is that intense that many peptides are degraded before they can ‘escape’ by binding to TAP and enter in the ER lumen where peptidase activity is relatively low (Roelse et al., 1994). This explains the relative inefficiency of antigen presentation by class I molecules and furthermore suggests that low copy proteins may not be presented because the few peptides derived from such proteins usually do not survive this cytosolic peptidase activity. The specificity of cytosolic peptidases is known for individual peptides like leucine aminopeptidase (LAP). However, it is unclear whether cytosolic peptidases skew the outcome of an immune response. Next to the specificity of the proteasome, the peptide transporter TAP and MHC class I molecules, the specificity of cytosolic peptidases should be taken into account to arrive at more accurate predictions of peptides presented by MHC class I molecules.

6. Peptide generation by other mechanisms in the cytosol and secretory pathway

Presentation of an epitope with C-terminal, but not N-terminal flanking residues can be blocked by proteasome inhibitors. This indicates involvement of an aminopeptidase activity in MHC class I antigen processing (Crau et al., 1997). Recently, in vitro studies have implicated the cytosolic leucine aminopeptidase in MHC class I antigen presentation (Beninga et al., 1998). Other peptidases have been claimed to be involved in this process as well. These include TPP II (Geier et al., 1999), LAP (Beninga et al., 1998), TOP (Saric et al., 2001), bleomycynydrodase and puromycin-sensitive aminopeptidase (Stolze et al., 2000). The relative importance of these peptidases is as yet unclear.

Antigenic peptides can also be produced by peptidases in the secretory pathway, as is shown by the presentation of peptides derived from signal sequences by certain MHC class I molecules (Wei and Creswell, 1992; Henderson et al., 1992). In general, these fragments are usually longer than nine amino acids, suggesting that they are not efficiently trimmed in the ER. Interestingly, a peptide aldehyde proteasome inhibitor has been shown to inhibit the generation of signal sequence derived peptides (Hughes et al., 1996). Although degradation rates of free peptides in the ER are slow as compared to those in the cytosol (Roelse et al., 1994), MHC class I binding peptides can be generated by aminopeptidases in the ER (Elliott et al., 1995; Snyder et al., 1994, 1997, 1998). Carboxypeptidases or endopeptidases (other than the signal sequence peptidase) have not been defined in the ER. This suggests that the proteasome should make peptide fragments with the correct C-terminus but N-terminally extended. The N-terminally extended peptides can then be trimmed to the correct size by amino peptidases. Since TAP translocates peptides with a Pro at position 2 only very poorly, this mechanism may be critical for those class I molecules containing a Pro at position 2 as an anchor residue. It has been proposed that trimming of peptides can occur even when bound to MHC class I molecules (Falk et al., 1990), but experimental data to support this hypothesis are still lacking. To date, the identity of the ER-resident proteases or peptidases is unknown, only some inhibitors have been reported (Hughes et al., 1996; Komlosh et al., 2001). In addition, inhibitors of the trans-Golgi network (TGN) resident protease furin were recently found to interfere with presentation of a viral protein (Gil-Torregrosa et al., 1998). In principle, any mechanism that leads to the generation of peptides in the cytosol or the ER could contribute to MHC class I binding peptides.

7. Peptide translocation by the peptide transporter TAP

Peptides produced in the cytosol have to be translocated over the ER membrane before they can associate with MHC class I molecules in the ER. The first indication that this transfer into the ER required a specific transporter came from mutant cell lines that were deficient in antigen presentation. The murine T lymphoma cell line RMA-S and the human lymphoblastoid LCL721.174 (0.174) and its derivative T2 cell lines all have very low MHC class I cell surface expression (DeMars et al., 1984, 1985; Saltar et al., 1985). Unstable complexes of class I heavy chain-β2m heterodimers are largely retained in the ER, suggesting that these mutant cell lines are defective in the transport of peptides from the cytosol to the ER. The genes responsible for this defect map to the MHC class II region (Monaco et al., 1990; Deverson et al., 1990; Trowsdale et al., 1990; Spies et al., 1990) and encode two proteins called TAP1 and 2. The 0.174 and T2 cell lines have a large deletion encompassing both transporter genes (Spies et al., 1990). The defect in the RMA-S cell line results from a single base change in TAP2 resulting in a premature stop codon (Yang et al., 1992). Transfection of the cDNAs of TAP1 and/or TAP2 restores class I cell surface expression in these mutant cell lines. Conversely, mice with a targeted deletion in the TAP1 gene showed a drastically reduced expression of MHC class I molecules (Van Kaer et al., 1992) and consequently failed to develop a normal CD8+ T cell repertoire (Ashton-Rickardt et al., 1993; Aldrich et al., 1994). In addition, an inherited mutation in the human TAP2 gene has been described, also resulting in low MHC class I surface expression and a low number of CD8+ T cells (de la Salle et al., 1994). In some human tumors and transformed cell lines, down-regulated expression of the peptide transporter genes correlates with reduced levels of cell surface class I, which presumably reduces the immunogenicity of these tumor cells (Restifo et al., 1993; Cromme et al., 1994; Rotem-Yehudar et al., 1994). Combined, these data illustrate the essential role of TAP as the principal supplier.
of peptides for class I molecules. For murine and human TAP genes only a limited sequence polymorphism is found (Colonna et al., 1992; Carrington et al., 1993; Pearce et al., 1993; Szafer et al., 1994). The rat TAP2* and TAP2* alleles however, differ by 25 amino acids (Powis et al., 1993). This polymorphic variation is responsible for translocation of distinct sets of peptides into the ER for loading by MHC class I molecules (Powis et al., 1993; Momburg et al., 1994a; Heemels and Ploegh, 1994). Further analysis shows that at least seven amino acids that localize to the putative peptide binding site of TAP are involved in this differential substrate specificity (Momburg et al., 1996; Deverso et al., 1998).

TAP1 and TAP2 are members of the ATP binding cassette (ABC) family of transport proteins (Higgins, 1992). These TAP complexes are confined to the ER (Kleinjans et al., 1992) (Fig. 3). Each TAP subunit has an N-terminal hydrophobic region with multiple predicted transmembrane domains. The first transmembrane segments of both TAP1 and TAP2 are sufficient for ER localization (Vos et al., 1999). The precise membrane topology of TAP1 and TAP2 is still a matter of debate. Most recent studies indicate that TAP1 has eight and TAP2 seven transmembrane domains (Vos et al., 1999). Both TAP1 and TAP2 contain a cytosolic C-terminal hydrophilic domain that contains the ATP binding site. TAP1 and TAP2 have been shown to form a heterodimer and expression of both subunits, as well as functional ABCs, are required for TAP function (Powis et al., 1991; Kelly et al., 1992; Spies et al., 1992; Attaya et al., 1992). A recent low resolution 3D-structure of TAP confirms various aspects of these data. TAP appears as a compact structure with a clear pore (Velarde et al., 2001) Velarde.

The initial step in the translocation process is peptide binding to TAP. This does not require ATP hydrolysis (Shepherd et al., 1991; van Endert et al., 1994; Androlewicz and Cresswell, 1994). A cooperative peptide binding site is formed by sequences of both TAP1 and TAP2 at a site between the putative pore and the ABCs (Androlewicz et al., 1994; Nijenhuis et al., 1996; Nijenhuis and Hammerling, 1996). Translocation of peptides from the cytosol into the ER lumen by TAP requires hydrolysis of ATP (Neefjes et al., 1993a; Shepherd et al., 1993; Androlewicz et al., 1993; Meyer et al., 1994).

The ABC domains hydrolyze ATP to induce the conformation changes required for substrate transfer, as can be visualized by alterations in the lateral mobility of TAP-GFP by FRAP studies (Reits et al., 2000). In living cells, TAP is moving fast when inactive and slow when in the process of pumping peptides. This has been used to visualize the pool of peptides in living cells (Reits et al., 2000). How does TAP pump peptides (for review see (Reits et al., 2000))? Briefly, the two ABC domains are not free but interact with each other (Lapinski et al., 2000) employing a so-called signature loop. This loop interacts with the γ-phosphate of the ATP of the opposing ABC domain and is released after hydrolysis of this phosphate (for review, (Reits et al., 2000)). Inactivation of the ABC domains of TAP suggest that hydrolysis by one ABC domain is required for conformational changes for peptide translocation whereas ATP hydrolysis of the other ABC domain completes the translocation cycle (Karttunen et al., 2001; Alberts et al., 2001; Saveau et al., 2001). This should result in conformational transitions that could be rather major as shown for MDR1 (Rosenberg et al., 2001) and somehow induce unilateral transport of peptides.

8. Substrate specificity of TAP

The specificity of the TAP peptide binding site has been studied by direct binding assays using human TAP molecules expressed in insect cells (van Endert et al., 1994, 1995; Meyer et al., 1994; Uebel et al., 1995) and by in vitro translocation or competition assays with either permeabilized cells (Neefjes et al., 1993a; Androlewicz et al., 1993) or microsomes (Shepherd et al., 1993; Heemels et al., 1993). The entry of peptides into the ER lumen of permeabilized cells has been monitored by peptide binding to MHC class I molecules in the ER (Androlewicz et al., 1993) or by accumulation of glycosylated peptide in the ER (Neefjes et al., 1993a). For the latter method, model peptides were designed containing an N-linked glycosylation consensus sequence N-X-T/S (where X is any amino acid except proline) and a tyrosine residue that can be radioiodinated. These peptides will become glycosylated upon arrival in the ER lumen (Kornfeld and Kornfeld, 1985). Following detergent lysis, this 'ER-specific label' can be used to recover translocated peptides, by the lectin concanavalin A coupled to sepharose beads. The N-linked glycan also protects the peptides...
against degradation in and release from the ER (Roelse et al., 1994; Momburg et al., 1994b). The earlier mentioned methods (reviewed in (Neisig et al., 1997)) have allowed definition of the substrate specificity of TAP. TAP most efficiently translocates peptides of 8–16 amino acids in length (Momburg et al., 1994b), similar to the size of peptides binding to MHC class I molecules (see later). Shorter and longer peptides can be translocated, albeit with considerable lower efficiency. The translocation efficiency decreases with increasing peptide size, but there does not seem to be a clear upper limit (Kooymann et al., 1996). In addition to the size of the peptide, its sequence also affects the efficiency of translocation by TAP. The C-terminal residue of a peptide is a key determinant for efficient translocation by TAP. Marine TAP and the rat TAP2 allele prefer peptides with a hydrophobic C-terminal residue. The rat TAP2 allele and human TAP show a broader selectivity in that they efficiently translocate peptides with hydrophobic or basic C-termini (Heemels and Ploegh, 1994; Schumacher et al., 1994; Momburg et al., 1994a). Acetylation or methylation of the N-terminus or conversion of the C-terminal carboxyl group of a peptide to an amide decreases its translocation efficiency considerably (Momburg et al., 1994a; Schumacher et al., 1994). The influence on translocation efficiency of amino acid substitutions at other positions in a peptide appears to be only minor. One clear exception is proline at position 2 or 3, which strongly affects translocation (Heemels and Ploegh, 1994; Neisig et al., 1995; van Endert et al., 1995; Uebel et al., 1995). Addition of flanking residues to the N-terminus of a peptide with a proline at position 3 significantly increases its translocation rate (Neisig et al., 1995). It has been speculated that particular immunodominant epitopes may be translocated as slightly longer precursors, which are then trimmed in the ER to the optimal size for MHC class I binding. However, ER peptides involved in this process have not been identified yet.

Additional studies have concentrated on the introduction of unnatural amino acids and chemically modified peptide substrates to gain further insight into substrate selectivity and translocation by TAP. Inclusion of α-amino acids in a peptide decreases its affinity for TAP (Wang et al., 1996; Grommé et al., 1997; Uebel et al., 1997). Introduction of two α-amino acids at opposite ends of a 9-mer peptide completely abrogates transport. A retro-inverso peptide, containing exclusively α-amino acids in which the spatial topochemistry of the side chains is maintained but the C and N atoms of the backbone are reverted, is not a substrate for TAP (Grommé et al., 1997; Uebel et al., 1997). Thus, proper orientation of peptide side chains toward the backbone is essential for interaction with TAP, indicating that substrate recognition by TAP is strictly stereospecific. Circular peptides, like cyclosporin A are not substrates for TAP, probably because they do not contain free N- and C-termini. Hydrogen bonds between MHC molecules and the peptide bond of bound peptide contribute most of the binding energy required for this interaction. An analogous situation was expected for peptide recognition by TAP. However, exchanging normal peptide bonds with isosteric peptide bonds, which maintain the conformation of the backbone but can not form hydrogen bonds, enhanced peptide translocation (Grommé et al., 1997). The reason for improved translocation of peptides with isosteric peptide bonds is not entirely clear, but may be the result of the decreased rotational freedom of these isosteric peptide bonds. Extending the size of the peptide substrate side chains by conjugating a bulky hydrophobic group larger than naturally occurring side chains, still allowed efficient translocation by TAP (Uebel et al., 1995; Grommé et al., 1997; Rock et al., 1991). Side chain extensions up to ~70 Å by sequential addition of lysines to the ε-amino group of lysine incorporated at position 2 or 7 in a nonameric peptide does not obstruct translocation. However, when this lysine side chain is extended further, translocation efficiency drops severely (Grommé et al., 1997). This suggests that the pore of TAP has a remarkable flexibility and is not restricted to translocation of normal side chain containing peptides only. In conclusion, although large side chains are tolerated, proper stereochemical conformation as well as free amino and carboxyl groups are important for the interaction of peptide substrates with TAP and subsequent translocation into the ER.

9. Inhibitors of TAP

Viruses have evolved several mechanisms to interfere with MHC class I antigen processing and presentation in order to evade CTL-mediated immune responses. These will be described in detail later. Here, obstruction of TAP function and the consequent retention of MHC class I molecules in the ER are discussed. The herpes simplex virus (HSV) types 1 and 2 encoded immediate early protein ICP47 blocks transport of class I molecules from the ER (York et al., 1994; Hill et al., 1994; Schust et al., 1996) and renders infected fibroblasts resistant to lysis by CD8+ CTL (York et al., 1994). ICP47 is an 87 amino acid (9 kDa) cytosolic polypeptide that binds TAP, thereby inhibiting binding and translocation of peptides from the cytosol into the ER lumen (Hill et al., 1994; Früh et al., 1995; Ahn et al., 1996; Tomazin et al., 1996) without interfering with ATP binding (Ahn et al., 1996; Tomazin et al., 1996). Blocking of TAP is species-specific, in that human, but not murine TAP is efficiently inhibited by ICP47 (Früh et al., 1995; Ahn et al., 1996; Jugovic et al., 1998; Tomazin et al., 1998). In aqueous solutions, ICP47 appears to be mainly unstructured, but in the presence of negatively charged lipid membranes, it adopts an α-helical structure (Beinert et al., 1997). However, the TAP-binding conformation of ICP47 remains to be established. The N-terminal 54 amino acids are conserved between HSV-1 and HSV-2. ICP47 and contain the core region of residues 1-35 that is responsible for TAP-binding and subsequent inhibition of TAP function (Galocha et al., 1997; Neumann et al., 1997). The N-terminus of ICP47 contacts both TAP subunits, whereas
residue Y21 binds only to TAP1 (Galocha et al., 1997). The binding mode of ICP47 to TAP is probably different from that of peptides, as peptides stabilize the TAP heterodimer, whereas ICP47 has a destabilizing effect on TAP in cross-linking studies (Lacaille and Androlewicz, 1998). In addition, N-acetylated peptides cannot bind to TAP (Neefjes et al., 1993b), whereas N-acetylation has no effect on the ability of ICP47 to inhibit TAP (Galocha et al., 1997).

The human cytomegalovirus (HCMV) US6 glycoprotein also inhibits TAP-mediated peptide loading of class I molecules. The open reading frame US6 encodes an 184 amino acid (21 kD) transmembrane glycoprotein that is localized to the ER (Ahn et al., 1997; Hengel et al., 1997). The luminal domain of US6 is responsible for ER retention and inhibition of TAP activity (Ahn et al., 1997). Unlike ICP47, US6 does not interfere with peptide binding to TAP (Ahn et al., 1997; Hengel et al., 1997). Interestingly, ICP47 binding to TAP is not affected by US6 (Ahn et al., 1997). In the ER US6 associates with the TAP/MHC class I complex (see later) (Ahn et al., 1997; Hengel et al., 1997; Lehner et al., 1997) in which class I heavy chains and tapasin (see later) are dispensable (Hengel et al., 1997). The exact mode of TAP inhibition by US6 still needs to be elucidated, but it is clear that it differs from that of ICP47 which interferes with the first step of TAP-mediated translocation of peptides. US6 induces a conformational change in TAP and maintains that conformation as measured by FRAP (Reits et al., 2000), and traps TAP in a state inhibiting all ATP binding (Hewitt et al., 2001; Kyritsis et al., 2001).

Recently, the first chemical TAP inhibitors have been designed (Grommé et al., 1997). These inhibitors are peptides with elongated side chains larger than 125 Å. The modified side chain is generated through coupling of 20 lysines to the ε-amino group of a lysine residue in the backbone of the peptide. These peptides can bind to, but not be translocated by TAP. The interaction of these peptides with extended side chains with TAP is not stable, but readily reversible. Since these inhibitors compete with normal peptides for TAP binding, their binding mechanism may be similar. Probably, side chain size limitations differ for peptide binding and actual translocation (Reits et al., 2000). Further insight into the molecular mechanisms of TAP inactivation should facilitate the design of TAP inhibitors which might be useful as immune suppressive agents in tissue transplantation or in treatment of MHC class I-related autoimmune diseases.

10. Structure of MHC class I molecules

The function of MHC class I molecules, the binding and presentation of peptides, is inextricably related to their structure. MHC class I molecules consist of a glycosylated transmembrane heavy chain (45 kD) noncovalently associated with a 12 kD soluble protein, β2m, and a short peptide usually of 8–10 amino acids. Murine class I heavy chains are encoded at chromosome 17 by the H-2K, -D and -L loci. In humans, heavy chains are encoded in the MHC region at chromosome 6 by three loci called human leukocyte antigen (HLA)-A, -B and -C. The HLA class I loci are highly polymorphic: more than 80 alleles for the HLA-A, 180 for the HLA-B and 40 for the HLA-C loci have been identified (reviewed in (Bodmer et al., 1997)). Both alleles of each locus are expressed, resulting in the expression of up to six different MHC class I molecules. The heavy chain harbors three domains (α1–α3) of which the α1 and α2 domain, consisting of two α-helices resting on a sheet of eight β-strands, form the peptide binding groove (Fig. 4).
The peptide binding groove contains pockets that accommodate particular peptide side chains termed anchor residues, which anchor the peptide in the groove. These pockets vary in depth and chemical nature between allelic variants and thus determine the set of peptides that can be bound by a particular class I allele. In the MHC class I peptide binding groove six pockets, A–F, have been identified. The A and F pockets interact with the N- and C-termini of the bound peptide, while the pockets B–E interact with peptide side chains (e.g. pocket B interacts with the side chain of the residue at position 2 relative to the N-terminus of the peptide). Since the shape of the pockets is dependent on the MHC class I allele, allele-specific peptide binding motifs, consisting of two or sometimes three anchor residues, can be defined. For peptides associated with human class I molecules, the C-terminal anchor may be either hydrophobic or basic. In the case of murine class I molecules, all bound peptides analyzed to date have a hydrophobic side chain at their C-termini. Thus, selectivity of peptide translocation by TAP (see earlier) is mirrored by peptide binding by MHC class I molecules, with respect to the C-terminal residue. So far, no peptides with acidic C-termini were found to bind human MHC class I molecules. Among class I molecules sharing structural similarity in the peptide binding pockets, the associated peptide motifs also have similarities and can be grouped into supermotifs. For example, the HLA-A301, -A1101, -A3101, -A3301 and -A6801 subtypes bind structurally similar peptides (Falk et al., 1994; Sidney et al., 1996) (Table 1).

<table>
<thead>
<tr>
<th>Peptide binding motif for HLA-A3-like molecules</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anchor</td>
<td>A, L, I, V, M, T, S</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>K, R</td>
</tr>
</tbody>
</table>

Adapted from (Sidney et al., 1996).

The N- and C-terminal residues of bound peptides produce multiple hydrogen bonds and salt bridges with conserved residues in two pockets located at opposite ends of the peptide binding groove (pockets A and F). These interactions are essential for stable association and constrain the length of bound peptides to about 8–10 residues. Few exceptions have been reported of class I molecules that accommodate peptides longer than 10 amino acids. In those cases, the central portion of the peptide bulges out of the peptide binding groove, while the N- and C-termini are correctly anchored (Guo et al., 1992). In addition, other exceptions to this tight binding of peptide termini have been reported wherein one or both ends of the peptide extend outside the peptide binding groove (Collins et al., 1994; Urban et al., 1994). In summary, the peptide forms an integral part of the MHC class I structure and plays an important role in assembly, stability and transport of MHC class I complexes to the cell surface.
proper glycan (Neefjes and Ploegh, 1988) suggests that calreticulin is dispensable for this step in folding as well. The calreticulin heavy chain-$\beta$-m complex is then able to interact with TAP (Suh et al., 1994; Ortman et al., 1994). This interaction is mediated by tapasin (TAP-associated glycoprotein), which appears to be a specialized chaperone critical for class I but not other folding proteins (Sadasivan et al., 1996; Ortmann et al., 1994). MHC class I molecules, that normally efficiently bind to TAP, fail to do so in tapasin deficient cells and cell surface expression of MHC class I molecules is decreased (Grandea et al., 1996). Tapasin is likely performing a role similar to that of HLA-DM in MHC class II loading (Broeke et al., 2002). It probably stabilizes the peptide free form. The recruitment of MHC class I to TAP may not be critical since soluble tapasin lacking the ER retention signal also supports peptide loading of class I molecules (Lehner et al., 1998). The affinity of the interaction of the heavy chain-$\beta$-m heterodimer with TAP is dependent on the class I allele. Many of the $\beta$ locus products associate poorly or not at all with TAP, whereas most HLA-A and -C locus products associate with high affinity (Neissig et al., 1996). This interaction with TAP may ensure efficient capture of translocated peptides by class I complexes. How non-TAP-binding class I molecules fold and capture peptides, is as yet unclear. Recently, another member of this multicomponent "loading complex" has been identified: Erp57, which can act as a thiol-dependent reductase and cysteine protease. Erp57 may have a role in disulfide bond formation in class I molecules or in trimming of peptides in the ER (Lindquist et al., 1998; Hughes and Creswell, 1998; Morrice and Powis, 1998). Thus, various chaperones are involved in different stages of folding, most of which are probably dispensable or redundant except for tapasin. Interestingly, peptides bind efficiently to various ER chaperones, including gp96, gp170, Erp72, and calnexin (Spée et al., 1999; Spée and Neefjes, 1997). The chaperone PDI was most efficient in binding peptides. The function of these interactions is unclear but they may be a simple consequence of the fact that chaperones interact with unfolded sequences that are mimicked by peptides. Still, some of these chaperones are employed in specific immunizations (Srivastava et al., 1998). Whether these chaperones are involved in peptide loading of MHC class I molecules or in peptide release from the ER lumen, is unclear.

Upon binding of peptide, the MHC class I complex is released from TAP (Ortman et al., 1994; Suh et al., 1994). At physiological temperature, binding of a peptide is essential for stability and transport of MHC class I molecules from the ER to the cell surface. "Empty" human MHC class I heavy chain-$\beta$-m heterodimers are unstable and will be retained in the ER. Eventually, these incompletely assembled subunits are translocated to the cytoplasm, through the translocon, and subsequently degraded by the proteasome (Wiertz et al., 1996; Hughes et al., 1997). Analogously, peptides that do not bind to MHC class I molecules are exported from the ER in an ATP-dependent manner (Schumacher et al., 1994; Roelse et al., 1994). This retrograde transport of peptides into the cytosol may serve to remove excess peptides and is not mediated by TAP (Roelse et al., 1994) but by the SEC61 translocon (Koopmann et al., 2000). This system is used both for ER export of malfolded proteins and peptides, in fact peptides can compete with proteins for ER exit via the translocon.

Following peptide binding and release from the ER, MHC class I complexes are transported through the Golgi apparatus and the TGN to the cell surface. In the Golgi apparatus, N-linked glycosylation is completed and in the TGN sialic acids are added to the complex (Neefjes and Ploegh, 1988). Transport to the cell surface completes the biosynthetic pathway of class I molecules where various class I alleles arrive with different kinetics probably due to different efficiencies of peptide loading (Neefjes and Ploegh, 1988). At the plasma membrane, MHC class I molecules display their antigenic peptide for immune surveillance by CTLs. Under normal circumstances, CTLs do not recognize complexes containing 'self' peptides with their TCR. However, during viral infection or malignant transformation, a different spectrum of peptides is displayed by MHC class I molecules. T cells bearing the appropriate TCR will then recognize the peptide-MHC class I complex and eliminate the diseased cell.

Thus, presentation of peptides by MHC class I molecules plays a central role in the cellular immune response to virally infected cells. As mentioned previously, viruses employ sophisticated mechanisms to avoid detection by the host immune system. Later, some of the mechanisms by which viruses specifically interfere with every step of MHC class I antigen processing and presentation are discussed. Adenovirus type 12 coordinates represses the transcription of LMP, TAP, and to a lesser extent MHC class I genes (Rottem-Yehudar et al., 1994, 1996; Schnier et al., 1993; Bernards et al., 1983; Lassam and Jay, 1989). Epstein-Barr virus-encoded nuclear antigen 1 (EBNA-1) contains a glycine-alanine repeat that inhibits, in cis, antigen processing for MHC class I-restricted antigen presentation (Levitskaya et al., 1995). By an unknown mechanism, the HCMV matrix protein pp65 interferes with presentation of another immediate early HCMV protein, the 72 kD transcript factor (Gilbert et al., 1996). Another example of viral immune escape by interference with antigen processing is provided by Friend/Moloney/Rauscher type murine leukemia virus, in which a single amino acid substitution prevents a dominant viral CTL epitope from being processed correctly by the proteasome (Oeserdoop et al., 1996). The strategies by which HSV and HCMV interfere with TAP-mediated peptide translocation have been discussed previously. Adenovirus types 2 and 5 express a 19 kD ER-resident glycoprotein (E3/19K) that binds to MHC class I molecules, causing their retention in the ER/cis-Golgi (Andersson et al., 1985; Burgert and Kvist, 1985).
Comparably, the HCMV encoded glycoprotein US3 retains MHC class I heavy chain-β2m heterodimers in the ER (Ahn et al., 1996; Jones et al., 1996). Although HCMV US3 is functionally similar to adenovirus E3/19K, there is no homology between these two viral proteins. In addition, HCMV expresses glycoproteins US2 and US11 which dislocate newly synthesized MHC class I heavy chains from the ER by export through the Sec61 translocon back into the cytosol. In the cytosol, the class I heavy chains are deglycosylated and degraded by the proteasome. HIV-1 Nef expression causes down-modulation of cell surface MHC class I molecules. Nef induces internalization, accumulation and degradation of MHC class I molecules in endosomal vesicles allowing escape from MHC class I-mediated CTL killing (Schwartz et al., 1996; Mangasarian et al., 1997; Collins et al., 1998). As is the case for virally infected cells, the immune system may exert a similar selective pressure on tumor cells. This results in escape of immune surveillance by cells that have a functional deficiency in one or more components of MHC class I antigen presentation. It has been reported that tumor cells can also transcriptionally down-regulate expression of LMP, TAP and MHC class I genes (Doyle et al., 1985; Restifo et al., 1993; Cromme et al., 1994; Gabathuler et al., 1994). Thus it appears that virtually every mechanism possible to abrogate the normal class I antigen presentation pathway is utilized by viruses. Many surprises may await us.

Fig. 5. Model for the phagosome-to-cytosol pathway for MHC class I-restricted presentation of exogenous antigens. Exogenous antigens are internalized by phagocytosis or endocytosis and are degraded in the phagolysosome. Some of the intact antigens and/or peptides are released into the cytosol where they enter the conventional MHC class I antigen processing and presentation route involving processing by the proteasome, translocation by TAP and assembly with newly synthesized MHC class I molecules in the ER followed by transport to the cell surface via the constitutive secretory pathway. This pathway is dependent on functional TAP and newly synthesized MHC class I molecules.

12. Alternative MHC class I antigen processing and presentation of exogenous antigens

The vast majority of exogenous antigens are presented by MHC class II molecules which are expressed on professional APC like macrophages, monocytes, dendritic cells and B cells. Intracellular proteins that target to endocytic compartments and internalized transmembrane proteins arrive in endosomes. In addition, receptor-mediated, endocytic or phagocytic uptake introduces microorganisms, soluble or particulate exogenous antigens into endocytic compartments. Subsequently, these exogenous antigens are degraded in acidic endocytic compartments by proteases which are active at low pH. This type of processing is inhibited by lysosomotropic agents that prevent endosomal acidification (chloroquine, ammonium chloride) or inhibitors of acid proteases (leupeptin). MHC class II molecules associate in the ER with the invariant chain (Ii) which targets this complex to a specialized endosomal compartment, termed MIIC for MHC class II-containing compartment. The invariant chain also serves to block peptide binding to MHC class II molecules in the ER, which is the canonical site where MHC class I molecules sample their antigenic peptides. In the endocytic pathway, the invariant chain is cleaved and removed from class II molecules in exchange for antigenic peptides in a process supported by a ‘lysosomal’ chaperone protein termed HLA-DM. Following
binding of peptides, the resulting MHC class II-peptide complexes are deposited at the cell surface for surveillance by CD4+ T cells (reviewed in (Castellino et al., 1997)).

While the conventional MHC class I antigen processing and presentation pathway may have evolved primarily to enable presentation of endogenous proteins, a variety of exogenous proteins can also be presented in association with class I molecules. This raises an interesting topological problem because MHC class I molecules assemble in the ER, while exogenous antigens are targeted to the endocytic compartment. At least two fundamentally different pathways for presentation of exogenous antigens by MHC class I molecules have been described (Figs. 5 and 6). One involving access of exogenous antigen to the conventional MHC class I loading pathway, and another involving unusual post-Golgi loading of MHC class I molecules. The following section gives an overview of these different pathways.

13. The phagosome-to-cytosol pathway

The failure of chloroquine to inhibit MHC class I presentation of some exogenous antigens indicated that the antigenic peptides were produced in a nonlysosomal compartment. In addition, the requirement for newly synthesized MHC class I molecules, functional TAP and proteasome activity suggested that intact or peptide fragments of phagocytosed exogenous antigens gain access to the cytosol and thereby to the conventional MHC class I processing pathway (Jin et al., 1988; Kovacsics-Bankowski and Rock, 1995; Liu et al., 1995; Norbury et al., 1995; Sousa and Germain, 1995; Shen et al., 1997; Brossart and Bevan, 1997). Transfer of a diverse set of compounds from endocytic compartments into the cytosol without disruption of the endosomal membrane has been shown (Isenman and Dice, 1993; Kovacsics-Bankowski and Rock, 1995; Norbury et al., 1995).

Alternatively, exogenous antigens may be released into the cytosol as a result of phagocytic overload or ‘indigestion’, leading to a loss of phagosomal membrane integrity (Sousa and Germain, 1995). In contrast, some types of antigen have a unique and well-characterized ability to be delivered to the cytosol and evoke CTL responses. This is the case for bacteria that produce toxins that disrupt membranes (Brunt et al., 1990; Darji et al., 1995; Mazzaccaro et al., 1996) and fusogenic viruses (Yewdell et al., 1988). Heat shock proteins (HSP) derived from virus-infected or tumor cells, when injected in a host, have been shown to elicit virus- or tumor-specific CTL responses, a phenomenon also known as cross-priming (Udono et al., 1994; Roman and Moreno, 1996; Arnold et al., 1995). The immunogenicity of HSP preparations has been attributed to peptides bound to HSPs.
which are taken up by cells mainly of monocytic origin. Peptides from endocytosed gp160-peptide complexes appear to be loaded onto newly synthesized MHC class I molecules in a chloroquine-resistant manner (Suto and Srivastava, 1995) but the mechanism by which this occurs is unclear. Thus, for some antigens, it is still unknown whether specific transport or non-specific transfer from phagosomes to the cytosol is involved (Suto and Srivastava, 1995; Mazzacaro et al., 1996; Brossart and Bevan, 1997; Norbury et al., 1997; Shen et al., 1997; Rescigno et al., 1998). Ultimately, these exogenous proteins or peptides, that access the cytosol from phagosomes, follow the conventional endogenous antigen processing pathway for MHC class I presentation. An important implication is that antigen presented through this pathway will be processed in the same manner as endogenously synthesized antigens, allowing the same epitopes to be presented. Therefore, a practical implication of this pathway may be found in the development of vaccines against viral infections or tumors. However, first the exact mechanism by which these exogenous antigens enter the conventional MHC class I processing pathway needs to be established before this system can be efficiently manipulated to our own advantage. This system is supposedly inefficient in vivo but may be strongly improved when understood.

14. Post-golgi loading of MHC class I molecules

A proteasome- and TAP-independent pathway for loading of exogenous antigens on MHC class I molecules has also been reported (Liu et al., 1995; Martinez-Knader et al., 1995; Schirmbeck et al., 1995; Schirmbeck and Reimann, 1996; Song and Harding, 1996; Wick and Pfeifer, 1996). This pathway may involve processing of peptides in an endosomal or phagosomal compartment, as lysosomotropic agents block presentation (Schirmbeck and Reimann, 1994; Martinez-Knader et al., 1995; Schirmbeck et al., 1995; Liu et al., 1997). These peptides, that are generated in endosomal compartments, may be regenerated into the extracellular fluid followed by recapture on peptide-receptive cell surface MHC class I molecules (Pfeifer et al., 1993; Song and Harding, 1996; Harding and Song, 1994). Other studies have excluded peptide regeneration as a mechanism of class I loading (Kovacsovics-Bankowski et al., 1993; Kovacsovics-Bankowski and Rock, 1995; De Brujin et al., 1995; Albert et al., 1998; Schirmbeck and Reimann, 1996).

An alternative explanation for the TAP- and proteasome-independent presentation of exogenous antigens by MHC class I is that loading of MHC class I molecules occurs actually within endosomal compartments. Newly synthesized class I molecules may reach this compartment by direct transport from the ER (De Brujin et al., 1995). This may involve association with the invariant chain (Segata and Brenner, 1995; Vigna et al., 1996), although the in vivo relevance of this interaction on the intracellular routing of MHC class I molecules in murine cells was not substantiated (Tourne et al., 1996). Alternatively, MHC class I molecules may be present in endosomal compartments due to internalization of cell surface class I complexes. B and T cells, monocytes and macrophages but also melanoma, may spontaneously or upon induction internalize class I molecules (Tse and Permis, 1984; Machy et al., 1987; Dasgupta et al., 1988; Capps et al., 1989; Vega and Strominger, 1989; Reid and Watts, 1992; Hochman et al., 1991; Abdel Motal et al., 1993, 1995; Grommé et al., 1999). In addition, upon association with HIV-1 Nef, cell surface MHC class I molecules are rapidly internalized and targeted to endosomes (Schwartz et al., 1995). These internalized MHC class I molecules are likely to be already loaded with peptides (Abdel Motal et al., 1995). It has been speculated that at low pH, peptides dissociate from class I complexes allowing exchange by other peptides present in the endosomes (Hochman et al., 1991; Styrbom et al., 1996). In fact, this has been validated in in vitro experiments (Grommé et al., 1999). Interestingly, particular peptides bind with high affinity to class I molecules at acidic pH (Styrbom et al., 1996). Following peptide exchange in an endosomal compartment, these MHC class I molecules could recycle back to the cell surface (Grommé et al., 1999). This pathway will utilize other proteases than those used in the conventional pathway for peptide generation for class I molecules. Probably, certain peptides are generated in both systems whereas others only in one of the pathways or not at all. An important role for recycling MHC class I molecules in CTL responses to exogenous antigens is illustrated in data from transgenic mice (Abdel Motal et al., 1995). These mice are transgenic for glycosylphosphatidylinositol (GPI)-linked MHC class I molecules which can not be recycled because they are lipid anchored to the cell membrane and lack a cytoplasmic tail with potential internalization signals. These transgenic mice can not generate a CTL response against a particular epitope, in contrast to wild-type mice with normal transmembrane MHC class I heavy chains.

In summary, MHC class I molecules are generally concerned with the presentation of endogenous antigens. Exogenous antigens, such as soluble proteins, usually fail to enter the classical pathway of antigen processing and presentation. However, several studies showed that antigenic epitopes derived from exogenous antigens can be presented by MHC class I molecules and induce CTL responses in vitro. Importantly, processing and presentation of these exogenous antigens for MHC class I-restricted T cell recognition also occurs in vivo (Staerz et al., 1987; Carbone and Bevan, 1990; Grant and Rock, 1992; Kovacsovics-Bankowski et al., 1993; Schirmbeck et al., 1994; Faló et al., 1995; Wiberg et al., 1996). The ability to internalize and present exogenous antigens may be a special feature of particular professional APCs. In addition, the physical nature of an exogenous antigen may influence its uptake by APC and its intracellular processing route (reviewed in Jondal et al., 1996). Further studies are needed to elucidate the mechanisms by which the exogenous antigenic peptides are processed and loaded onto MHC class I molecules. Particularly,
the proteases that are involved in the generation of exogenous peptides require more study. These alternative pathways may play an important role in the immune surveillance for vacuolar pathogens. Indeed, CTL responses against endosomal bacteria are essential in controlling some parasitic infections (reviewed in (Kunzmann et al., 1992)). In addition, presentation of exogenous antigens by MHC class I resulting in CTL cross-priming offers new perspectives for immunotherapy. Successful targeting of exogenous antigens into this pathway in vivo could potentially prime protective CTL responses to tumors or virally infected cells.

15. Concluding remarks

The presentation of peptides derived from intracellular antigens by MHC class I molecules to cytotoxic T lymphocytes plays an important role in the cellular immune response. Conventional MHC class I antigen processing involves proteolytic degradation, predominantly by the proteasome, of cytosolic or nuclear proteins into short peptide fragments. Those peptides escaping cytosolic degradation are translocated from the cytosol across the ER membrane by a heterodimeric transporter complex formed by two MHC-encoded proteins, TAP1 and TAP2. The antigenic peptide is an essential part of the MHC class I molecule. Inhibition of the proteasome significantly decreases the amount of peptides available for binding to MHC class I molecules. This is reflected in a direct reduction of the amount of peptides available for binding to MHC class I molecules, the rest is degraded to amino acids.

Despite the presence of MHC class I alleles, MHC class I-mediated CTL responses to exogenous antigens have been observed in vitro and in vivo. Concepts of the processes underlying this alternative MHC class I presentation pathway are only just emerging. It is important to place this alternative route in proper context relative to the well-established conventional pathway of class I loading. The alternative pathway seems to function primarily in cells that are endowed with the capacity to internalize exogenous antigens, such as professional APC. Even in these cells, the contribution of the alternative pathway to the formation of stable cell surface MHC class I-peptide complexes is probably only minor. Nevertheless, this alternative MHC class I antigen processing and presentation pathway may play an important role in protective immunity against intracellular bacteria and in induction of specific CTL responses. We thank Marieke van der Velde for support in the preparation of this manuscript.

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