Review

MHC Class I Antigen Presentation- Recently Trimmed and Well Presented

Barry Flutter[1] and Bin Gao[1, 2]

Presentation of antigenic peptide to T cells by major histocompatibility complex (MHC) class I molecules is the key to the cellular immune response. Non-self intracellular proteins are processed into short peptides and transported into endoplasmic reticulum (ER) where they are assembled with class I molecules assisted by several chaperone proteins to form trimeric complex. MHC class I complex loaded with optimised peptides travels to the cell surface of antigen presentation cells to be recognised by T cells. The cells presenting non-self peptides are cleared by CD8 positive T cells. In order to ensure that T cells detect an infection or mutation within the target cells the process of peptide loading and class I expression must be carefully regulated. Many of the cellular components involved in antigen processing and class I presentation are known and their various functions are now becoming clearer. *Cellular & Molecular Immunology*. 2004;1(1):22-30.

Key Words: antigen presentation, MHC class I, T cell epitope, peptide loading

Introduction

CD8 positive cytotoxic T lymphocytes (CTLs) play an important role in non-self immune recognition. Each CD8 positive cytotoxic T lymphocyte produced in the thymus carries a different T cell receptor (TCR) capable of recognising peptide antigen complexed with a molecule known as the major histocompatibility complex class I molecule (MHC class I). MHC class I molecules are found on the surface of virtually all nucleated cells and when TCR recognition of a MHC class I peptide complex occurs, the CTLs can become activated and take action against the target cells. In this way CTLs can eradicate cells carrying foreign, mutated or in some cases over-expressed proteins.

The importance of MHC molecules was first noticed in 1974 when Zinkernagel and Doherty found that virus specific cytotoxic T lymphocytes only attacked infected cells bearing the same MHC class I molecules as the CTL itself (1). This phenomenon is known as MHC restriction and the implication behind the finding was that MHC class I molecules had a role in CTL recognition of infected cells. However, the mechanism behind MHC restriction remained unclear for some years. In 1982, Alain Townsend at Oxford University showed that CTL clones specific for influenza A proteins do not require expression of complete viral glycoproteins in the target cells (2) and then later they showed that specific CTLs could kill non-infected cells simply by pulsing the cell with specific peptides from proteins of the flu virus (3). From this it was deduced that the antigenic determinant of the virus was not full length protein but short peptides derived from proteins expressed in the cell. These findings along with the concept of MHC restriction suggested that MHC class I molecules play a role in CTL recognition by facilitating the presentation of peptide antigens.

In 1987, Pamela Bjorkman and Don Wiley solved the crystal structure of the MHC class I complex and noticed an area of electron dense material in the groove of MHC class I molecules (4). By refolding the class I complex with homogenous peptide they later confirmed that this electron dense region was bound peptide. Shortly after naturally processed viral peptides were isolated from infected cells by acid extraction and sequenced, it was shown that the peptides eluted from the cells depended upon the MHC class I alleles present (5).

MHC class I

Three different highly polymorphic genes code for the MHC class I heavy chain (HC) inherited from each parent and therefore each individual carries up to 6 different HC alleles. In contrast, the light chain or β_2-microglobulin (β_2m), as it is more commonly known, is relatively conserved and is invariant within the complex. The final component of the trimeric MHC class I complex expressed at the cell surface is the bound peptide; this is the antigenic element of the complex and can potentially be derived from any protein resident within the cell. The crystal structure of the class I molecule has greatly enhanced our understanding of how peptides bind to class I and helped to explain how each allele of class I can interact with so many peptide antigens (4). There are 4 protein domains in the MHC class I heterodimer, three of which, the α1, α2 and α3 domains are formed by the heavy chain while β_2m forms the 4th domain.

[1]Infection and Immunity, Institute of Child Health, University College London, UK.

[2]Corresponding to: Dr. Bin Gao, Infection and Immunity, Institute of Child Health, University College London, 30 Guilford Street, London WC1N 1EH, UK. E-mail: bgao@ich.ucl.ac.uk.

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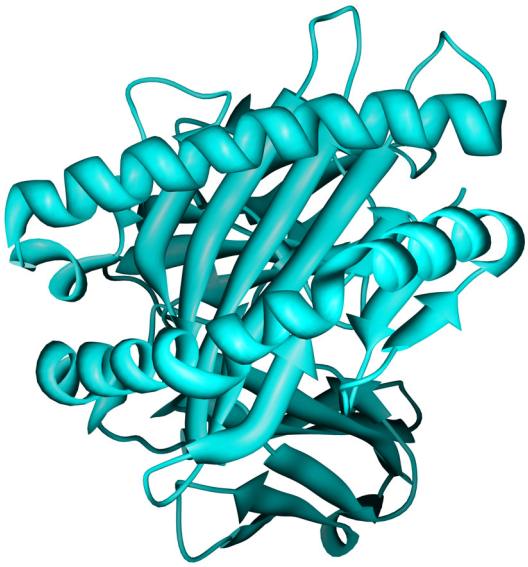

Figure 1. Top view of HLA-A2 showing the peptide groove formed at its base by the β-sheet and at the sides by the α-helices of the α1 and α2 domains. Picture courtesy of Chimera (6).

The α1 and α2 domains share a very similar structure consisting of 4 antiparallel β-strands followed by one long α-helix. The two domains interact in such a way that the 8 β-strands form one large β-sheet crossed by two α-helices which leave a long groove down the centre. This long groove, bounded by the β-sheet at its base and α-helices at either side forms the peptide binding site (Figure 1).

The bound peptide is generally 8-12 amino acids in length and is bound to the groove at both its N-terminus and its C-terminus and also at certain key anchor residues. The anchor residues are determined by the shape of the peptide groove and vary from one HC allele to another; although binding to the HC requires certain anchor points in the peptide sequence often more than one amino acid can act as the anchor residue. There is much more flexibility in the other amino acids of the peptide meaning that each HC allele can bind to a very wide range of peptide sequences. It is no surprise to find that the majority of the polymorphic residues of class I are found around this peptide groove allowing variation in the shape and electrostatic properties of the groove so that each HC allele can bind a different array of peptides by means of different anchor residues. This maximises the number of peptide antigens which an individual can present as each of the six alleles carried will be able to bind and present a different repertoire of peptides (7).

TCR recognition of class I

The stimulation of the T cells by an antigen requires not only interaction between the TCR and the bound peptide but also key interaction with the class I molecule itself. The importance of these interactions was highlighted by Ajitkumar in 1988 who showed by site directed mutagenesis that unlike antibodies to the mouse class I

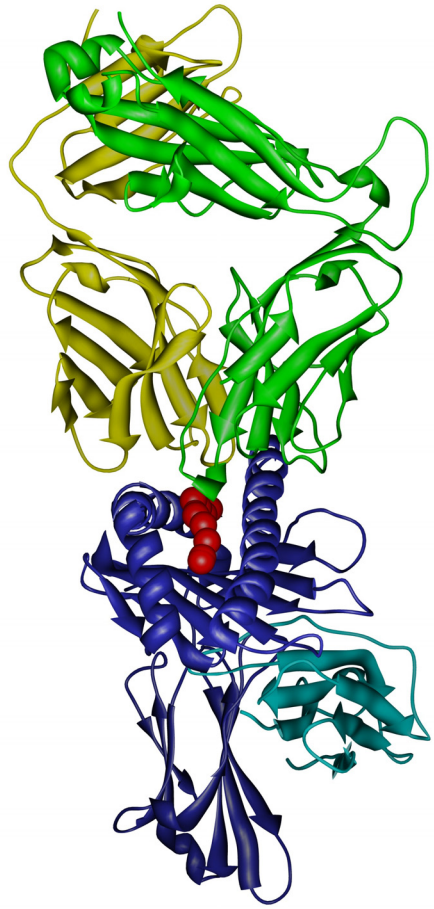

Figure 2. Crystal structure of HLA-A2 (HC - dark blue, β_2m - cyan) bound with tax peptide (red) docking with antigen specific TCR (α chain - yellow, β chain - green). Picture created in Chimera (6).

molecule K^b which recognise randomly distributed residues on the class I surface, TCR's recognition multiple residues located exclusively on the surface of the α-helices which surround the peptide antigen (8).

In 1996, two separate groups published crystal structures of the docking of an MHC class I peptide complex and the TCR confirming the multiple interactions between the TCR and both the antigenic peptide and the surrounding residues of the class I molecule (9, 10).

MHC class I presentation failure

In 1986, Peter Creswell's laboratory reported a mutant cell line 174xCEM.T2 in which MHC class I HC and β_2m are expressed normally but fail to form dimers and are not processed or transported to the cell surface. They proposed that an additional molecule absent from 174xCEM.T2 and encoded by a HLA-linked gene is necessary for efficient assembly of class I antigen subunits (11). Cerundolo and Townsend demonstrated that 174, a parent cell line of 174xCEM.T2 has lost a function required for presentation of intracellular viral antigens with class I molecules of the major histocompatibility complex (MHC), but retains the capacity to present defined epitopes as extracellular peptides.

They mapped the genetic defect to a position within the MHC locus on human chromosome 6 (12). Townsend in collaboration with Powis and colleagues first linked the deficiency to a MHC linked transporter (13). TAP or the transporter associated with antigen presentation is a heterodimeric protein necessary for class I antigen presentation. The genes for both TAP1 and TAP2 map to the MHC locus (12) and TAP mutant cell lines have a low cell surface expression of class I despite normal expression of both HC and β_2m. Peptides targeted to the endoplasmic reticulum (ER) by means of a signal sequence restore cell surface expression of class I indicating that the mutant cell suffers because of an absence of available peptides in the ER (14). These results suggest that the function of TAP is to transport peptides from the cytoplasm where they are produced during normal protein degradation to the ER where new class I molecules are synthesised.

Interestingly not only is TAP necessary for the provision of peptides to class I molecules but it also immunoprecipitates with both HC and β_2m (15). *In vitro* this interaction is lost upon binding of peptide to class I suggesting that the HC- β_2m heterodimer is in complex with TAP before it is loaded with peptide but that on peptide binding it is released to traffic to the cell surface (16). More detailed immunoprecipitation work has shown that at least three other proteins which are also found bound to TAP and class I prior to peptide loading. Together these proteins form what is known as the peptide loading complex (PLC). The PLC is centred on the TAP1-TAP2 dimer and contains the HC-β_2m class I dimer and tapasin (TAP associated protein, Tpn) at a stoichiometry of 1 TAP : 4 Tpn : 4 HC (17). As well as this other components including the chaperones calreticulin (Crt), ERp57 and some calnexin are also found in the complex (Figure 3C).

Intuitively we would expect that the loading of peptides onto class I for antigen presentation must be a balance of two main factors. On the one hand the peptides bound to class I should have a high enough binding affinity for the heavy chain alleles present to avoid extensive peptide dissociation from class I, whilst on the other hand a very wide range of peptides must bind to class I so that the cell is able to present any antigen from almost any cellular protein to the passing T cells.

A correlation has been found between the length of time that class I molecules spend in the ER and the extent to which they are loaded with peptide implying that this balance must be carefully controlled (18). This is probably one of a number of functions carried out by the proteins in the PLC which clearly has a role in the quality control of class I exported from the ER.

The trimeric class I complex

The formation of the trimeric class I complex of β_2m, HC and peptide occurs in the ER and involves three crucial stages: synthesis and folding of the heavy chain and β_2m, provision of peptides for class I loading, and the loading onto HC of peptide with high affinity for the peptide groove. Only once these processes have occurred can the loaded class I molecules be exported to the cell surface for surveillance by T cells (Figure 3).

Folding of the peptide receptive HC-β_2m heterodimer

Shortly after or during synthesis in the ER, HC is bound by calnexin (19, 20), which may act to stabilise and protect it from degradation (21) or facilitate folding and association with β_2m. Calnexin is a membrane bound molecular chaperone containing a lectin site which recognises monoglucosylated N-oligosaccharides (22, 23). Human class I molecules contain one N-glycan site whilst mouse class I molecules have two or three such sites and this may explain differences in the observed binding of calnexin to developing class I molecules. In human cells free HC is predominantly found associated with calnexin, however, upon binding of β_2m calnexin is dropped in favour of calreticulin (24). On the other hand in mouse cells the interaction with calnexin can persist beyond β_2m binding and possibly right up until class I export from the ER (19). Although most heavy chain molecules appear to associate with calnexin after synthesis in human cells and for longer in mouse cells it is important to note that in both human and mouse cells with calnexin deficiency class I peptide loading and cell surface expression are normal (23, 24). This leads to the conclusion that calnexin is dispensable in class I processing, probably owing to some ER chaperone redundancy through calreticulin or BiP. What does seem to be clear is that only once the heavy chain has been assembled into a dimer with β_2m can it bind to calreticulin (Figure 3B) and progress to the PLC (25).

Production and provision of peptides for class I

Peptides for class I loading are produced from proteins circulating in the cytoplasm, which are constitutively degraded by the proteasome at variable rates. These degradation products are dependent on the proteasome involved in protein cleavage. IFN-γ stimulation alters the components of the 26S proteasome by incorporating the subunits LMP2, LMP7 and MECL-1 and producing an "immunoproteasome" (26). This immunoproteasome tends to produce longer peptides and cleaves after hydrophobic and basic residues, which are transported more easily by TAP and are more suitable for binding to MHC class I. In this way the carboxyl terminus of peptides which bind to class I is produced directly by the proteasome. In contrast the proteasome does not seem to cut peptides exactly to size at their amino terminus. Since peptide epitopes vary, this presumably allows the proteasome to produce a wide range of peptide precursors where it would be much more difficult to produce the exact peptide for class I binding. Using a modified ovalbumin molecule the relative quantities of the peptide epitope SIINFEHL and its amino-extended precursors have been analysed (27). It was shown that although the peptide which loads onto class I and presented to T cells is the 8-mer SIINFEHL the levels of this peptide within the cell are very low. The 9-mer KSIINFEHL is present at significantly higher levels but localises only to the ER and its production is dependant on functional TAP. The 10-mer and 11-mer precursors of the peptide on the other hand are found at higher levels still and mainly reside in the cytoplasm. These findings suggest that the proteasome

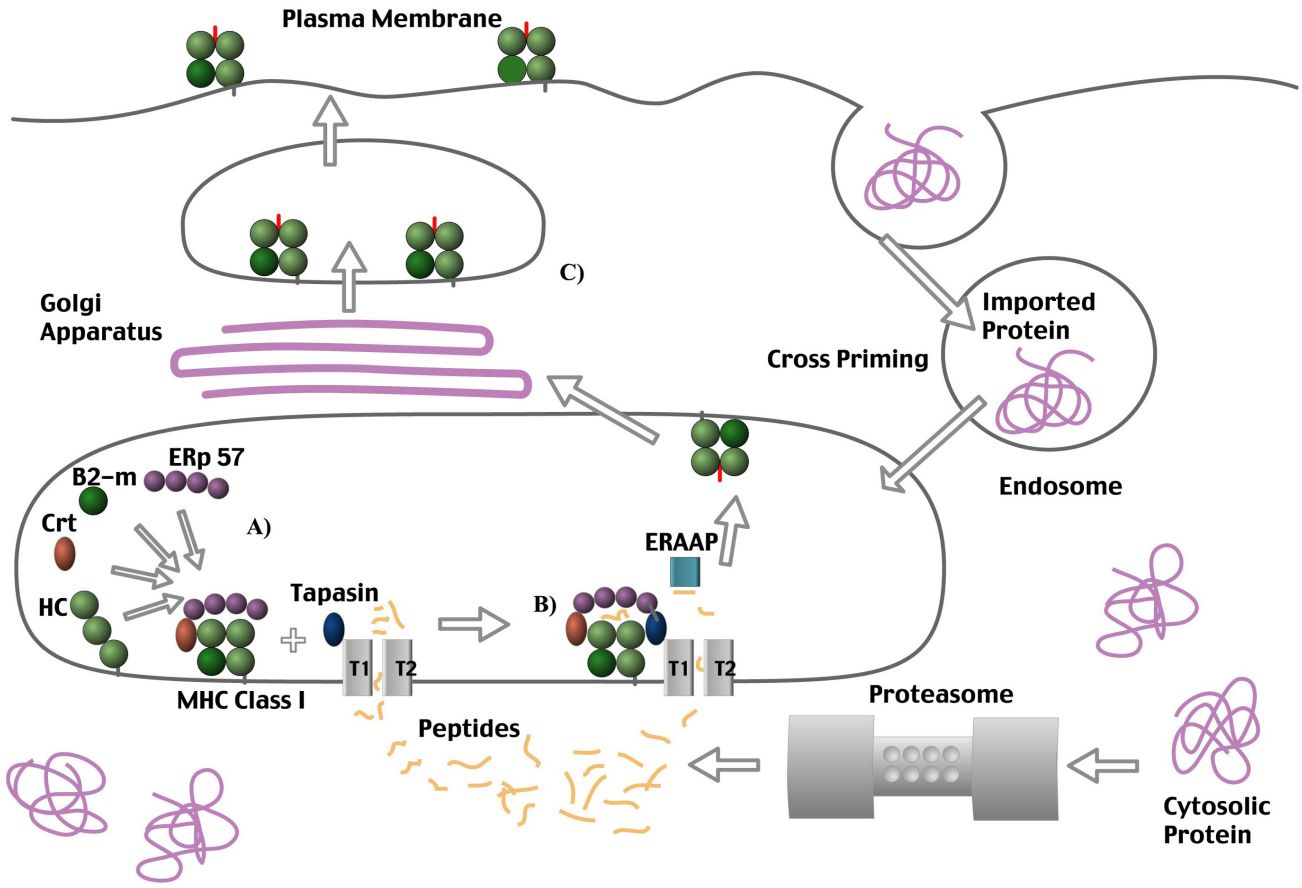

Figure 3. Class I antigen presentation pathway. A) Heavy chain (HC) disulfide bridges form and association with β_2m occurs allowing calreticulin (Crt) to bind. B) The class I molecule enters the mature complex and binds to peptides provided by TAP (T1 and T2). Tapasin is found disulfide bonded to ERp57. C) Once loaded with peptide, class I is released from the loading complex and traffics to the cell surface via the Golgi network. Picture refined by Yuan Gao.

produces amino-extended versions of the peptide which are subsequently trimmed at their amino terminus to produce peptides for class I binding.

ERAAP

Recent work has shown that the main enzyme involved in peptide trimming is located within the ER (28-30). This enzyme, the ER aminopeptidase associated with antigen processing (ERAAP or ERAP1), was isolated from mouse spleen cells by ion exchange chromatography and its activity was assayed using leucine *p*-nitroanilide (28). The isolated protein had a molecular mass of roughly 100 kD and its aminopeptidase activity was inhibited by leucinethiol. By trypsin digestion and mass spectrometry the peptide fingerprint was determined and used to search the National Centre for Biotechnology Information Database of proteins. The matched protein was a murine leucine aminopeptidase. Serwold et al. went on to show that this protein was endoglycosidase H (Endo H) sensitive, co-localised with BiP and gp96 and that the level of protein in different tissue types correlated well with MHC class I. Not only this but like heavy chain, tapasin, TAP and other components of the class I pathway, expression of ERAAP was also increased 10-fold in fibroblasts when cultured with IFN-γ. Concomitant with this increase in expression is an increase in aminopeptidase activity (29). Assays on the activity of ERAAP show that it cleaves peptides of ≥ 10 at a high rate, cleaves 9 amino acids (a. a.) peptides at a reduced rate and has little or no activity for peptides of ≤ 8 a. a. This range of cleavage specificity ought to be ideal for the production of the 8-9 a. a. peptides which form the majority of epitopes presented by class I.

Using RNA interference (RNAi) two groups have recently succeeded in suppressing the activity of ERAAP. This work has confirmed that ERAAP is important in producing the peptide repertoire for class I presentation. Interestingly although in some cases suppression of ERAAP correlates with the loss of peptide presentation and specific CTL response, certain epitopes appear to be produced and presented more efficiently in the absence of ERAAP (28). This may reflect the fact that some peptides are produced independently of ERAAP and consequently they may face less competition for class I binding when ERAAP is suppressed and presented better.

York et al. noticed that surface expression of K^b in HeLa cells was actually increased when ERAAP was suppressed using RNAi. They also showed by pulse chase experiments that the rate of assembly of HLA-A, B and C was increased under RNAi. They attributed this to the destruction of peptides by ERAAP that bind to class I molecules. In this

situation provision of peptide is presumably limiting the rate of class I trafficking and hence, loss of any peptides which can bind to class I will decrease surface expression. In fact a low level of aminopeptidase either from the remaining ERAAP or from other aminopeptidases might be ideal as it will slowly trim precursors that require processing without destroying epitopes which already bind to class I. Conversely when cells are stimulated with IFN-γ, the proteasome seems to produce little or no more peptides of the correct length for binding, but 2-4 fold higher rates of amino-extended versions of the peptides (31). Peptides now cease to be limiting and may frequently require trimming before being loaded onto class I. ERAAP will trim peptides that are imported by TAP and produce the correct epitopes to bind to class I. Although some epitopes may be destroyed and presented at a lower level (28) the overall amount of surface class I is now as high as or higher than when ERAAP is suppressed (30). Crucially, the range of peptides presented may also be increased which is an important factor in development of immune response during inflammation, when IFN-γ is high.

The fact that Paz et al. (27) found trimming of the K^b peptide precursor KSIINFEHL to be strongly increased in the presence of K^b but not with the irrelevant K^d, along with evidence from Saric (29) that ERAAP is responsible for trimming amino-extended versions of the very similar SIINFEKL peptides leads us to the intriguing possibility that ERAAP may cleave peptides which are already in association with class I. This suggests that ERAAP may be recruited to the loading complex in order to trim peptides with a high affinity for the specific class I alleles present in the complex. The presence of ERAAP in the complex may only be transient but on the other hand it would be no surprise to find that ERAAP interacts in some way with the proteins of the complex.

TAP - the peptide transporter

Androlewicz et al. have revealed that the translocation of peptides from the cytoplasm to the ER is in an ATP dependent fashion (32). Pulse chase analysis shows that without provision of peptides by TAP, class I molecules do not traffic to the Golgi body but instead are slowly degraded in the ER (33). TAP associates with HC via tapasin and although its presence in the complex is topologically convenient (as it allows peptides to enter the ER in close proximity to HC) it does not appear to be an essential component of the peptide loading complex. TAP mutants which still function as peptide transporters but do not associate with tapasin are not found in association with heavy chain yet class I molecules appear to become loaded with peptide and dissociate from tapasin in the normal way (34). Much is now known about the structural and functional properties of TAP which has been well reviewed previously (35).

Peptide loading and optimisation

Tapasin

By immunoprecipitation of TAP1 it was discovered that class I molecules form a complex with TAP and in the same experiment the molecule tapasin was discovered also present in this complex (36). The mutant cell line LCL721.220 (37) shows significant defects in antigen presentation and the overall cell surface expression of class I is reduced (38). The mutation responsible for this defect is a simple base substitution two nucleotides from the 5′ end of intron two of the gene coding for tapasin (37). The GT which is invariant in 5′ splice sites has thus been altered to GC and as a result exon 3 splices directly to exon 1 excising exon 2 from the messenger RNA in the process. When tapasin is reintroduced in to .220 cells the phenotype is rescued, cell surface expression of class I returns and the association between heavy chain and TAP is restored (17). It is apparent from this and other work that tapasin is required for interaction between the HC-β_2m dimer and TAP (17, 39) and this highlights the importance of tapasin in the formation of the loading complex by acting as a bridge between TAP and class I.

Unlike in TAP negative cells, where the loss of cell surface expression arises from a lack of peptide translocation into the ER rather than from the absence of TAP in the loading complex, it is essential that tapasin is both expressed and that it incorporates into the peptide loading complex with HC to facilitate peptide loading. Mutant class I HC molecules known as T134K in which threonine is replaced by lysine at position 134, are unable to interact with tapasin (18). As a result, T134K forms a dimer with β_2m which is not found in association with tapasin or TAP. T134K traffics to the cell surface at an increased rate either in a peptide receptive state or with a low affinity peptide bound. Hence, it is unable to present endogenous viral epitopes to T cells.

Since the discovery of tapasin and the demonstration of its importance in MHC class I antigen presentation our understanding of its role has been complicated by the finding that cell surface levels of class I in the tapasin knockout are somewhat allele specific (40). Whereas HLA-B44 alleles are almost completely lost from the cell surface in LCL721.220 cells, HLA-B27 alleles seem to be normally expressed at the cell surface even in the absence of tapasin. This allelic difference has been pinpointed to residue 114 of heavy chain (41). Alleles with a histidine residue at position 114 express in the absence of tapasin whereas alleles with a glutamic acid residue at position 114 are dependant on tapasin. Because of this, HLA-B27 and a number of other alleles which still express at the cell surface in tapasin deficient cell lines, are often referred to as "tapasin independent" alleles. But it is interesting to note that even the heavy chain B27 is found in the loading complex in normal tapasin proficient cells and hence, although B27 does not require tapasin association per se for cell surface expression, tapasin is still involved in its normal trafficking and may still play some role, for instance in the loading of certain peptide epitopes.

There are two main reasons postulated for the loss of surface class I in the absence of tapasin. One possibility is that class I cannot become properly loaded with peptide in the absence of tapasin and as a result does not traffic to the cell surface but is degraded in the ER. This is supported by the finding that in .220 cells HLA HC-β_2m dimers are retained for longer in the ER before dissociation and presumably degradation (42). Alternatively, it may be that class I dimers leave the ER as normal but that in the absence of tapasin, instead of loading with high affinity peptides, many of the class I molecules leave the ER loaded with low affinity peptides which dissociate easily either

during trafficking or at the cell surface. When class I molecules become unloaded, heavy chain quickly dissociates from β_2m and the empty heavy chain molecules are internalized and marked for degradation and this would account for the low surface levels of class I. This latter situation appears to be the case in mice experiments where, as expected overall class I expression is low in the tapasin mutant but trafficking of K^b and D^b molecules measured by acquisition of Endo H resistance is similar in spleen cells from $Tpn^{-/-}$ and $Tpn^{+/-}$ mice (43, 44). It is likely that both mechanisms play a part in the low cell surface expression of class I in the tapasin mutant and we already know that there are some allelic differences in the interaction of class I with tapasin. It is also possible that the function of tapasin is subtly different in mice and in human and this could account for some of the observed differences.

An artificial soluble tapasin without transmembrane and cytoplasmic domains has been able to rescue the tapasin deficiency of .220 cells although there are no association between TAP and class I molecules observed in the rescued cells (45). However, Gao et al. have shown that a similar, naturally truncated form of tapasin restores normal class I surface expression otherwise impaired in .220 cells while the peptides loaded on the surface of rescued cells are suboptimal, which indicates that transmembrane and cytoplasmic fragments of tapasin involved in association of TAP with class I molecules are required for full function of tapasin (46).

ERp57

ERp57 is a member of protein disulfide isomerase family whose functions include disulfide bond isomerisation, reduction and oxidation (47-49). The catalytic activity of these proteins is dependent on the cysteine residues in two CXXC motifs (50, 51). ERp57 forms complexes with either calnexin or calreticulin and together they ensure the correct folding and disulphide bond formation of monoglucosylated polypeptides in the ER (52, 53). It is not surprising then that ERp57 is found in both the early complex with heavy chain and calnexin and also in the mature loading complex with TAP, class I, tapasin and calreticulin (54-56). There are three disulphide bonds within the folded heavy chain molecule one of which is found inside the peptide groove of class I. ERp57 is a likely candidate for the correct formation of these bonds, and there is now evidence that the presence of ERp57 within the complex may be crucial to correct peptide loading. Firstly, it has been shown that ERp57 can specifically reduce partially folded peptide receptive MHC class I molecules while showing little or no activity for peptide loaded class I molecules (57). It is possible then that ERp57 may be important in the unfolding of MHC class I molecules by reduction of heavy chain disulfide bonds when they remain in a peptide receptive state for a prolonged period for example when peptide supply is poor. More interestingly it was shown last year that ERp57 forms an intermediate in which tapasin is disulfide bonded to ERp57 (58). Mutation of cysteine residue 95 to alanine in tapasin abolishes formation of this disulfide bond intermediate and also seems to prevent complete oxidation of class I. As a result the C95A tapasin mutant is only able to partially restore class I expression in LCL721.220 (tapasin deficient) cells compared with a full recovery when normal tapasin is transfected into these cells. The implication of this is that formation of this last disulfide bond in the loading complex may require both ERp57 and tapasin and that this step is crucial somehow in the quality control process of peptide loading onto class I.

Calreticulin

The ER chaperone calreticulin has two well characterised basic functions. Firstly it has a vital function in intracellular calcium homeostasis and as a result deletion of calreticulin causes a lethal impairment of cardiac development during embryogenesis (59). Secondly it is an important ER chaperone in the folding of newly synthesised glycoproteins such as class I molecules. However, the role of calreticulin in class I antigen processing is much less well characterised than that of tapasin or TAP. It has been established by immunoprecipitation that calreticulin is present in the PLC and that this interaction relies on the presence of TAP and tapasin (24, 54, 60).

The calreticulin deficient mice fibroblast cell line K42 has allowed some assessment of the role of calreticulin in the PLC. Although loss of calreticulin does not appear to be as severe in antigen processing as a loss of tapasin K42 cells show a 50-80% loss in cell surface expression of class I as compared to normal K41 cells. When either exogenous peptide or β_2m is added to K42 cells surface expression is increased 4.5 fold compared to just 1.2 fold in K41 cells. This suggests that loss of class I surface expression in K42 is due to loss of unstable class I molecules at the surface which can be rescued by high affinity peptides or by an excess of β_2m. Pulse chase analysis shows that in the absence of calreticulin HC traffics to the cell surface at an increased rate rather like the mutant T134K HC this increased rate of trafficking could result in poor peptide loading within the ER. There is more evidence too that peptide loading within the ER is defective in K42; conformation dependant antibodies show that a much greater proportion of class I molecules in the ER remains in the peptide receptive state in K42 than in K41. Not only this but impaired T cell recognition was observed for 3 out of 4 peptide epitopes tested in K42. Despite the absence of calreticulin in K42 heavy chain is still found in association with TAP, tapasin and ERp57 in the loading complex (61). The exact role of calreticulin therefore, remains unclear; it may be that the presence of calreticulin in the loading complex stabilises interactions between the other molecules and consequently, that HC is retained for longer allowing better optimisation of peptide binding. One other attractive possibility is that calreticulin chaperones peptides from TAP to the peptide groove of class I. Cross-linking experiments have shown that calreticulin can bind to peptides delivered to the ER via TAP (62). It is known that peptides are found bound to chaperones such as heat shock proteins in the cytosol (63) and it is possible that calreticulin could fulfill such a role within the ER.

Export of class I to the cell surface

It has been convincingly shown that premature export of class I from the ER to the cell surface results in poor surface expression and reduced antigen presentation to cytotoxic T lymphocytes (18, 61). However, the mechanism for the export of class I from the ER is not entirely clear.

The Bulk Flow model of ER export postulates that ER

resident proteins are maintained in the ER by means of retention and retrieval signals, other proteins which do not have such signal sequences traffic from the ER by default to the Golgi (64). Under this model class I would be retained in the ER whilst in the PLC because of the presence of chaperone molecules with retention signals, the rate of export would depend on the rate of release of class I from the PLC. This would explain nicely the poor loading of class I molecules which bind poorly to the PLC such as T134K mutants or in tapasin deficient cell lines. However, recent studies show that an increase in the rate of release of peptide loaded class I from the PLC induced by provision of an excess of high affinity peptide has no effects on ER to Golgi transport (65, 66). Furthermore, it has also been shown that peptide loaded class I molecules accumulate at ER exit sites and that they associate with a putative cargo molecule BAP31 (67). These findings point towards a selective model of class I export involving recruitment of peptide loaded class I into COPII vesicles. In this situation the export is limited by the rate of incorporation of class I molecules into COPII vesicles and not on release of class I from the PLC. In this model it is still important that the PLC retain poorly loaded class I. Although the overall rate of export of loaded class I does not rise concomitantly with release of class I from the PLC, the ratio of poorly loaded to well loaded class I will increase if the PLC does not retain class I until it becomes loaded with high affinity peptide. As a result the proportion of stable class I being released from the ER will fall and surface expression of class I and antigen presentation will fall.

Paulsson et al. (68) propose a further level of export control where poorly loaded class I can be returned to the ER from the Golgi. In their work they show that tapasin is present in the Golgi body and that it is able to associate with both the α and β chains of the coatamer COPI. The COPI coatamer mediates retrograde Golgi to ER transport (69) and could therefore provide a mechanism for class I molecules which have lost their peptide during ER to Golgi transport to be returned to the ER where they can be reloaded with peptide. They argue that the building up of class I molecules in the ER in TAP mutant cells is not due to lacking of export of class I from the ER to the Golgi but because of return of class I to the ER from the Golgi. This hypothesis is supported by the observation that levels of class I in the Golgi body of TAP negative are similar to those in wild type cells (70).

Conclusion

It is clear that the loading complex is essential in ensuring that peptide loading is carried out correctly. In some way the molecules present provide the necessary peptides and facilitate their binding to class I. The complex is then able to selectively release class I binding high affinity peptide and hence provides a quality control mechanism. The exact functions of the components of the complex remain somewhat mysterious and this is probably partly because the molecules cooperate with each other in complex ways. Hence when you knockout one component of the complex you also weaken the interactions of other molecules present in the complex and so you see overlapping phenotypes in for instance the tapasin knockout and the calreticulin knockout.

Further work may provide useful information on the importance of disulfide bridge formation and the role of ERp57 in peptide loading. It may also be possible to assess in more detail how the loading complex is important in presenting a good repertoire of peptides on the class I molecules and hence generating good T cell responses. The technique of RNA interference demonstrated in the recent work on ERAAP will almost certainly provide a useful tool for the knockout of multiple genes in the complex and allow a systematic analysis of the function of the components and how they interact.

References

1. Zinkernagel RM, Doherty PC. Restriction of *in vitro* T cell-mediated cytotoxicity in lymphocytic choriomeningitis within a syngeneic or semiallogeneic system. Nature. 1974; 248:701-702.
2. Townsend AR, Skehel JJ. Influenza A specific cytotoxic T-cell clones that do not recognize viral glycoproteins. Nature. 1982;300:655-657.
3. Townsend AR, Rothbard J, Gotch FM, Bahadur G, Wraith D, McMichael AJ. The epitopes of influenza nucleoprotein recognized by cytotoxic T lymphocytes can be defined with short synthetic peptides. Cell. 1986;44:959-968.
4. Bjorkman PJ, Saper MA, Samraoui B, et al. Structure of the human class I histocompatibility antigen, HLA-A2. Nature. 1987;329:506-512.
5. Rotzschke O, Falk K, Deres K, et al. Isolation and analysis of naturally processed viral peptides as recognized by cytotoxic T cells. Nature. 1990;348:252-254.
6. Huang CC, Couch GS, Pettersen EF, Ferrin TE. Chimera: an extensible molecular modeling application constructed using standard components. Pac Symp Biocomput. 1996;1:724.
7. Bjorkman PJ, Saper MA, Samraoui B, Bennett WS, Strominger JL, Wiley DC. The foreign antigen binding site and T cell recognition regions of class I histocompatibility antigens. Nature. 1987;329:512-518.
8. Ajitkumar P, Geier SS, Kesari KV, et al. Evidence that multiple residues on both the alpha-helices of the classI MHC molecule are simultaneously recognized by the T cell receptor. Cell. 1988;54:47-56.
9. Garcia KC, Degano M, Stanfield RL, et al. An alphabeta T cell receptor structure at 2.5 A and its orientation in the TCR-MHC complex. Science. 1996;274:209-219.
10. Garboczi DN, Ghosh P, Utz U, Fan QR, Biddison WE, Wiley DC. Structure of the complex between human T-cell receptor, viral peptide and HLA-A2. Nature. 1996;384:134-141.
11. Salter RD, Cresswell P. Impaired assembly and transport of HLA-A and -B antigens in a mutant TxB cell hybrid. EMBO J. 1986;5:943-949.
12. Cerundolo V, Alexander J, Anderson K, et al. Presentation of viral antigen controlled by a gene in the major histocompatibility complex. Nature. 1990;345:449-452.
13. Powis SJ, Townsend AR, Deverson EV, Bastin J, Butcher GW, Howard JC. Restoration of antigen presentation to the mutant cell line RMA-S by an MHC-linked transporter. Nature. 1991; 354:528-531.
14. Anderson K, Cresswell P, Gammon M, Hermes J, Williamson A, Zweerink H. Endogenously synthesized peptide with an endoplasmic reticulum signal sequence sensitizes antigen processing mutant cells to class I- restricted cell-mediated lysis. J Exp Med. 1991;174:489-492.
15. Ortmann B, Androlewicz MJ, Cresswell P. MHC class I/beta 2-microglobulin complexes associate with TAP transporters before peptide binding. Nature. 1994;368:864-867.
16. Suh WK, Cohen-Doyle MF, Fruh K, Wang K, Peterson PA, Williams DB. Interaction of MHC class I molecules with the

transporter associated with antigen processing. Science. 1994; 264:1322-1326.
17. Ortmann, B, Copeman J, Lehner PJ, et al. A critical role for tapasin in the assembly and function of multimeric MHC class I-TAP complexes. Science. 1997;277:1306-1309.
18. Lewis JW, Neisig A, Neefjes J, Elliott T. Point mutations in the alpha 2 domain of HLA-A2.1 define a functionally relevant interaction with TAP. Curr Biol. 1996;6:873-883.
19. Degen E, Williams DB. Participation of a novel 88-kD protein in the biogenesis of murine class I histocompatibility molecules. J Cell Biol. 1991;112:1099-1115.
20. David V, Hochstenbach F, Rajagopalan S, Brenner MB. Interaction with newly synthesized and retained proteins in the endoplasmic reticulum suggests a chaperone function for human integral membrane protein IP90 (calnexin). J Biol Chem. 1993;268:9585-9592.
21. Williams DB. Calnexin leads glycoproteins into the fold. Glycoconj J. 1995;12:iii-iiv.
22. Hammond C, Braakman I, Helenius A. Role of N-linked oligosaccharide recognition, glucose trimming, and calnexin in glycoprotein folding and quality control. Proc Natl Acad Sci U S A. 1994;91:913-917.
23. Scott JE, Dawson JR. MHC class I expression and transport in a calnexin-deficient cell line. J Immunol. 1995;155:143-148.
24. Sadasivan BK, Cariappa A, Waneck GL, Cresswell P. Assembly, peptide loading, and transport of MHC class I molecules in a calnexin-negative cell line. Cold Spring Harb Symp Quant Biol. 1995;60:267-275.
25. Farmery MR, Allen S, Allen AJ, Bulleid NJ. The role of ERp57 in disulfide bond formation during the assembly of MHC class I in a semipermeabilized cell translation system. J Biol Chem. 2000;275:14933-14938.
26. Gaczynska M, Rock KL, Goldberg AL. Gammainterferon and expression of MHC genes regulate peptide hydrolysis by proteasomes. Nature. 1993;365:264-267.
27. Paz P, Brouwenstijn N, Perry R, Shastri N. Discrete proteolytic intermediates in the MHC class I antigen processing pathway and MHC I-dependent peptide trimming in the ER. Immunity. 1999;11:241-251.
28. Serwold T, Gonzalez F, Kim J, Jacob R, Shastri N. ERAAP customizes peptides for MHC class I molecules in the endoplasmic reticulum. Nature. 2002;419:480-483.
29. Saric T, Chang SC, Hattori A, et al. An IFN-gamma-induced aminopepti-dase in the ER, ERAP1, trims precursors to MHC class I-presented peptides. Nat Immunol. 2002;3:1169-1176.
30. York IA, Chang SC, Saric T, et al. The ER aminopeptidase ERAP1 enhances or limits antigen presentation by trimming epitopes to 8-9 residues. Nat Immunol. 2002;3:1177-1184.
31. Cascio P, Hilton C, Kisselev AF, Rock KL, Goldberg AL. 26S proteasomes and immunoproteasomes produce mainly N-extended versions of an antigenic peptide. EMBO J. 2001; 20:2357-2366.
32. Androlewicz MJ, Anderson KS, Cresswell P. Evidence that transporters associated with antigen processing translocate a major histocompatibility complex class I-binding peptide into the endoplasmic reticulum in an ATP-dependent manner. Proc Natl Acad Sci U S A. 1993;90:9130-9134.
33. Knittler MR, Alberts P, Deverson EV, Howard JC. Nucleotide binding by TAP mediates association with peptide and release of assembled MHC class I molecules. Curr Biol. 1999;9:999-1008.
34. Paulsson KM, Anderson P, Chen S, et al. Assembly of tapasinassociated MHC class I in the absence of the transporter associated with antigen processing (TAP). Int Immunol. 2001;13:23-29.
35. Lankat-Buttgereit B, Tampe R. The transporter associated with antigen processing: function and implications in human diseases. Physiol Rev. 2002;82:187-204.
36. Androlewicz MJ, Ortmann B, van Endert PM, Spies T, Cresswell P. Characteristics of peptide and major histocompatibility complex class I/beta 2-microglobulin binding to the transporters associated with antigen processing (TAP1 and TAP2). Proc Natl Acad Sci U S A. 1994;91:12716-12720.
37. Copeman J, Bangia N, Cross JC, Cresswell P. Elucidation of the genetic basis of the antigen presentation defects in the mutant cell line. 220 reveals polymorphism and alternative splicing of the tapasin gene. Eur J Immunol. 1998;28:3783-3791.
38. Grandea AG 3rd, Androlewicz MJ, Athwal RS, Geraghty DE, Spies T. Dependence of peptide binding by MHC class I molecules on their interaction with TAP. Science. 1995; 270:105-108.
39. Sadasivan B, Lehner PJ, Ortmann B, Spies T, Cresswell P. Roles for calreticulin and a novel glycoprotein, tapasin, in the interaction of MHC class I molecules with TAP. Immunity. 1996;5:103-114.
40. Peh CA, Burrows SR, Barnden M, et al. HLA-B27-restricted antigen presentation in the absence of tapasin reveals polymorphism in mechanisms of HLA class I peptide loading. Immunity. 1998;8:531-542.
41. Park B, Lee S, Kim E, Ahn K. A single polymorphic residue within the peptide-binding cleft of MHC class I molecules determines spectrum of tapasin dependence. J Immunol. 2003; 170:961-968.
42. Tan P, Kropshofer H, Mandelboim O, Bulbuc N, Hammerling GJ, Momburg F. Recruitment of MHC class I molecules by tapasin into the transporter associated with antigen processing-associated complex is essential for optimal peptide loading. J Immunol. 2002;168:1950-1960.
43. Grandea AG 3rd, Golovina TN, Hamilton SE, et al. Impaired assembly yet normal trafficking of MHC class I molecules in Tapasin mutant mice. Immunity. 2000;13:213-222.
44. Garbi N, Tan P, Diehl AD, et al. Impaired immune responses and altered peptide repertoire in tapasin-deficient mice. Nat Immunol. 2000; 1:234-238.
45. Lehner PJ, Surman MJ, Cresswell P. Soluble tapasin restores MHC class I expression and function in the tapasin-negative cell line. 220. Immunity. 1998;8:221-231.
46. Gao B, William T, Sewell A, Elliott T. Generation of a functional, soluble Tapasin protein from an alternatively spliced mRNA. Genes Immun. 2003; online publication doi: 10.1038/sj.gene. 6364043.
47. Gilbert HF. Protein disulfide isomerase and assisted protein folding. J Biol Chem. 1997;272:29399-29402.
48. High S, Lecomte FJ, Russel SJ, Abell BM, Oliver JD. Glycoprotein folding in the endoplasmic reticulum: a tale of three chaperones? FEBS Lett. 2000;476:38-41.
49. Ellgaard L, Helenius A. ER quality control: towards an understanding at the molecular level. Curr Opin Cell Biol. 2001;13:431-437.
50. Hirano N, Shibasaki F, Sakai R, et al. Molecular cloning of the human glucose-regulated protein ERp57/GRP58, a thiol-dependent reductase. Identification of its secretory form and inducible expression by the oncogenic transformation. Eur J Biochem. 1995;234:336-342.
51. Urade R, Oda T, Ito H, Moriyama T, Utsumi S, Kito M. Functions of characteristic Cys-Gly-His-Cys (CGHC) and Gln-Glu-Asp-Leu (QEDL) motifs of microsomal ER-60 protease. J Biochem. 1997;122:834-842.
52. Oliver JD, Roderick HL, Llewellyn DH, High S. ERp57 functions as a subunit of specific complexes formed with the ER lectins calreticulin and calnexin. Mol Biol Cell. 1999;10: 2573-2582.
53. Tan L, Andersen MH, Elliott T, Haurum JS. An improved assembly assay for peptide binding to HLA-B*2705 and H-2K (k) class I MHC molecules. J Immunol Methods. 1997;209:25-36.
54. Hughes EA, Cresswell P. The thiol oxidoreductase ERp57 is a component of the MHC class I peptide-loading complex. Curr Biol. 1998;8:709-712.
55. Lindquist JA, Jensen ON, Mann M, Hammerlin GJ. ER-60, a

chaperone with thiol-dependent reductase activity involved in MHC class I assembly. EMBO J. 1998;17:2186-2195.
56. Morrice NA, Powis SJ. A role for the thiol-dependent reductase ERp57 in the assembly of MHC class I molecules. Curr Biol. 1998;8:713-716.
57. Antoniou AN, Ford S, Alphey M, Osborne A, Elliott T, Powis SJ. The oxidoreductase ERp57 efficiently reduces partially folded in preference to fully folded MHC class I molecules. EMBO J. 2002;21:2655-2663.
58. Dick TP, Bangia N, Peaper DR, Cresswell P. Disulfide bond isomerization and the assembly of MHC class I-peptide complexes. Immunity. 2002;16:87-98.
59. Mesaeli N, Nakamura K, Zvaritch E, et al. Calreticulin is essential for cardiac development. J Cell Biol. 1999;144:857-868.
60. Lewis JW, Sewell A, Price D, Elliott T. HLA-A*0201 presents TAP-dependent peptide epitopes to cytotoxic T lymphocytes in the absence of tapasin. Eur J Immunol. 1998;28:3214-3220.
61. Gao B, Adhikari R, Howarth M, et al. Assembly and antigen-presenting function of MHC class I molecules in cells lacking the ER chaperone calreticulin. Immunity. 2002;16:99-109.
62. Spee P, Neefjes J. TAP-translocated peptides specifically bind proteins in the endoplasmic reticulum, including gp96, protein disulphide isomerase and calreticulin. Eur J Immunol. 1997; 27:2441-2449.
63. Udono H, Srivastava PK. Heat shock protein 70-associated peptides elicit specific cancer immunity. J Exp Med. 1993;178:1391-1396.
64. Wieland FT, Gleason ML, Serafini TA, Rothman JE. The rate of bulk flow from the endoplasmic reticulum to the cell surface. Cell. 1987;50:289-300.
65. Suh WK, Mitchell EK, Yang Y, Peterson PA, Waneck GL, Williams DB. MHC class I molecules form ternary complexes with calnexin and TAP and undergo peptide-regulated interaction with TAP via their extracellular domains. J Exp Med. 1996;184:337-348.
66. Marguet D, Spiliotis ET, Pentcheva T, Lebowitz M, Schneck J, Edidin M. Lateral diffusion of GFP-tagged H2Ld molecules and of GFP-TAP1 reports on the assembly and retention of these molecules in the endoplasmic reticulum. Immunity. 1999; 11:231-240.
67. Spiliotis ET, Manley H, Osorio M, Zuniga MC, Edidin M. Selective export of MHC class I molecules from the ER after their dissociation from TAP. Immunity. 2000;13:841-851.
68. Paulsson KM, Kleijmeer MJ, Griffith J, et al. Association of tapasin and COPI provides a mechanism for the retrograde transport of major histocompatibility complex (MHC) class I molecules from the Golgi complex to the endoplasmic reticulum. J Biol Chem. 2002;277:18266-18271.
69. Harter C, Reinhard C. The secretory pathway from history to the state of the art. Subcell Biochem. 2000;34:1-38.
70. Baas EJ, van Santen HM, Kleijmeer MJ, Geuze HJ, Peters PJ, Ploegh HL. Peptide-induced stabilization and intracellular localization of empty HLA class I complexes. J Exp Med. 1992;176:147-156.