Physical and Functional Association of the Major Histocompatibility Complex Class I Heavy Chain \(\alpha_3\) Domain with the Transporter Associated with Antigen Processing

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Summary

C D8\(^+\) T lymphocytes recognize antigens as short, M HC class I-associated peptides derived by processing of cytoplasmic proteins. The transporter associated with antigen processing translocates peptides from the cytosol into the ER lumen, where they bind to the nascent class I molecules. To date, the precise location of the class I-TAP interaction site remains unclear. We provide evidence that this site is contained within the heavy chain \(\alpha_3\) domain. Substitution of a 15 amino acid portion of the H-2D\(^\text{b}\) \(\alpha_3\) domain (aa 219-233) with the analogous MHC class II (H-2\(\text{A}^\text{d}\)) \(\beta_2\) domain region (aa 133-147) results in loss of surface expression which can be partially restored upon incubation at 26\(^\circ\)C in the presence of excess peptide and \(\beta_2\)-microglobulin. Mutant H-2D\(^\text{b}\)(D\(\text{b}^{219-233}\)) associates poorly with the TAP complex, and cannot present endogenously-derived antigenic peptides requiring TAP-dependent translocation to the ER. However, this presentation defect can be overcome through use of an ER targeting sequence which bypasses TAP-dependent peptide translocation. Thus, the \(\alpha_3\) domain serves as an important site of interaction (directly or indirectly) with the TAP complex and is necessary for TAP-dependent peptide loading and class I surface expression.

The M HC class I molecule is a heterotrimeric complex comprised of a 44-kD heavy chain, \(\beta_2\)-microglobulin (\(\beta_2\m; 12-kD\) light chain),\(^1\) and a peptide of 8–10 residues (1-4). This complex is recognized by C D8\(^+\) T cells when displayed on the surface of cells. Assembly of class I molecules occurs in the endoplasmic reticulum (ER) when the newly synthesized heavy chain associates with resident ER chaperone calnexin, which facilitates folding and disulfide bridge formation of the heavy chain and promotes its binding to \(\beta_2\m\) (5, 6). Class I-\(\beta_2\m\) dimers then associate with a heterodimeric, ER membrane protein called TAP (for transporter associated with antigen processing), which consists of TAP1 and TAP2. TAP transports peptides which are predominantly derived from cytosolic proteins into the ER lumen in an ATP-dependent manner (7, 8).

Physical association of class I heavy chain-\(\beta_2\m\) dimers with TAP as determined by coprecipitation studies (9-12) suggests a specific role of TAP in delivering peptides directly to the M HC class I. It is not clear at present whether TAP associates with M HC class I directly or via an adaptor molecule. A recently described protein, tapasin, is required for class I interaction with TAP (13-17) and has more recently been shown to be necessary for \(\beta_2\m\) association with TAP (18). Thus, tapasin can be described as a molecular bridge between class I and TAP molecules. Studies on the role of tapasin have been carried out using human cell lines and although tapasin seems to be required for proper class I assembly and subsequent expression in these cell lines, a murine counterpart for tapasin remains to be identified.

Peptide loading of M HC class I can also occur in a TAP-independent manner, as evidenced by the surface expression on TAP-deficient cells of class I molecules that are loaded with signal sequence-derived peptides (19, 20). However, this TAP-independent peptide loading seems to be a minor pathway as it is relevant for a limited set of

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1Abbreviations used in this paper: \(\beta_2\m\), \(\beta_2\)-microglobulin; ER, endoplasmic reticulum; ES, E3/19K leader/signal sequence; NEPHGE, nonequilibrium pH-gradient gel electrophoresis; NP, nucleoprotein; R P10, RPMI 1640/10% FCS; TAP, transporter associated with antigen processing; VV, vaccinia virus; wt, wild type.
MHC class I alleles that can bind signal sequence peptides, and the diversity of the bound peptides is very limited (19, 20). Once localized to the ER lumen, peptides can bind to and thereby stabilize nascent class I molecules. Peptide binding results in the release of the class I molecule from the ER (9, 10) and subsequent transport to the cell surface via the exocytic pathway. The majority of misfolded, incompletely assembled, or empty class I molecules are retained in the ER from where they are removed to the cytosol and degraded by the proteasome (21).

Thus, association of class I heavy chain-β2m with the TAP complex (TAP1, TAP2, and possibly tapasin) appears to be a critical event in MHC class I assembly. The location of the site of interaction on class I with TAP complex remains uncertain. Both the extracellular (22) and the transmembrane region/cytoplasmic tail (23) have been implicated in this interaction. Point mutations introduced in the α3 domains of both H-2Ld and H-2Dd resulted in the loss of TAP coprecipitation with the class I heavy chain (11, 22). However, these same point mutations do not affect the ability of these molecules to be expressed at the cell surface (24–26) and to present endogenous peptides (26), in contrast to mutations in either TAP or β2m that drastically affect both cell surface expression and antigen presentation of MHC class I (27–30). Evidence is presented here that physical association with the TAP complex, TAP-dependent peptide loading, and cell surface expression of class I is completely abolished by a 15-amino acid substitution made in the H-2Dd α3 domain. Thus, this region could define an interaction site on the murine class I heavy chain with the TAP complex.

Materials and Methods

Generation of Chimeric H-2Dd Constructs. PCR overlap extension was used to create H-2Dd cDNA with substitutions in the α3 domain. For the 62-amino acid replacement mutant, class I α3 domain base pairs 666–849 (cDNA) were replaced with class II β2 domain base pairs 2830–3013 (genomic DNA). The latter fragment was obtained using the genomic H-2IA H-2Dd cDNA with substitutions in the α3 domain base pairs 666–849 (cDNA) were replaced with class II β2 domain base pairs 2830–3013 (genomic DNA). The latter fragment was obtained using the genomic H-2IA 1–750 and 736–1097 base pair gene fragments. The 1–750 and 736–1097 base pair gene fragments were then sewn using sense = 5’-GGATCCAGATGGG-3’ and antisense = 5’-GGA-

TCCACGTTTACA-3’ primers.

PCR products were inserted into the pGEM vector (Promega, Madison, WI) from which they were sequenced using the following primers: sense = 5’-ACCGAGGTGTCTAGGACT-TCTTGGCC-3’, antisense = 5’-AAAAAGCCACACACGTCCT-

CAATGTAGGGC-3’. The BglII, SacI fragment of pGEM (now containing cDNA for mutant H-2Dd) was used to replace the BglII, SacI fragment from the wild-type (wt) H-2Dd cDNA contained in the BlueScript vector. The NotI fragment from BlueScript-mutant H-2Dd then replaced the corresponding portion of the wt Dd in the pCMU-Dd plasmid (31). The BamHI-digested 1.1-kb fragment from pCMU-Dd was then inserted in the appropriate orientation into the BamHI cloning site of the phoAP-I-neo expression vector and transfected into P815 cells by electroporation as described previously for the wt-2H (32).

Reverse Transcription PCR. Total RNA was isolated from 5–106 cells using TRIzol reagent (GIBCO BRL, Gaithersburg, MD) following the manufacturer’s protocol. cDNA was synthesized using the Superscript premplification system for first-strand cDNA synthesis (GIBCO BRL). PCR was carried out using Taq polymerase (Fisher Scientific, Fairlawn, NJ) and 20 μg/ml of each primer. Amplification was conducted for 30 cycles. Each cycle consisted of 60 s at 94°C, 60 s at 60°C, and 90 s at 72°C. The following primers were used: for β-actin, sense = 5’-GTGGGGCCGCCAGGACCA-3’, antisense = 5’-CTCCTATTGTCACCCAGATTTCC-3’; for H-2Dd, sense = 5’-TGATCCAGTCCATGCTC-3’, antisense = 5’-TGATGCCCAGTCCATGCTC-3’. One fifth of each PCR sample was loaded onto a 0.8% agarose gel and visualized by ethidium bromide staining.

Cell Lines. P815 transfectants were maintained in RPMI 1640/10% FCS (R P10) supplemented with 500 μg/ml Geneticin (GIBCO BRL). The influenza A/PR/8/34 nucleoprotein (NP) peptide 366–374–specific CTL line, PR 8.2 (29) was maintained by weekly restimulations with irradiated C57/BL6 spleen cells pulsed with 10 μM influenza NP peptide (ASENNMETM) in R P10 containing 5% rat con A supernatant.

To generate CTLs specific for endogenous influenza peptide, C57BL/6 mice were immunized with influenza strain A/PR/8/34 (a gift from D. A. Garcia-Sastre, Mt. Sinai Medial Center, New York) by intraperitoneal injection and spleens were harvested after 10 d and stimulated in vitro for 5–6 d with virus-infected, autologous splenocytes. These CTLs were used in cytotoxicity assays using recombinant vaccinia constructs.

Cytotoxicity Assays. Target cells were pulsed with 35Cr labeled with 35Cr iodide in R P10 medium for 1 h at 37°C, washed twice with PBS, and plated at 104 cells/well of a 96-well round-bottomed plate. Influenza NP 366–374 peptide as well as effector cells (CTLS) were then added to the wells to a final volume of 100 μl/well. After a 4-h incubation at 37°C, 10 μl of the supernatants were harvested and 35Cr-release was measured. Where flu peptide concentrations range from 1 nM to 10 μM, the effector to target ratio was kept constant at 10:1.

For induction of class I expression, P815 transfectants (targets) were incubated overnight at 26°C in serum-free medium (0-01 MEM I; GIBCO BRL) in the presence of 10 μM NP 366–374 peptide with or without human β2m (Sigma Chemical Co., St. Louis, MO) at 5 μg/ml. Cells were then pulsed with 35Cr, washed three times with PBS, and plated at 104 cells/well. CTL assays
were carried out as above with effector to target ratios starting at 100:1 with serial, threefold dilutions of effector cells.

Flow Cytometry. 2.5 × 10^6 P815 and P815 transfectants were washed once in PBS/2% FCS followed by incubation with a saturating amount of biotinylated anti-H-2^b^ antibody (KH95; Pharmingen, San Diego, CA) for 30 min at 4°C. Cells were washed twice with PBS/2% FCS and then suspended in 100 μl of a 1:100 dilution of streptavidin-PE (Caltag Labs., South San Francisco, CA). Cells were washed twice and resuspended in 300 μl PBS/1% formaldehyde. All samples were analyzed using a FACScan flow cytometer (Becton Dickinson, Mountain View, CA).

Generation of Various Constructs. To produce the NP 366–374 recombinant vaccinia virus (VV), complementary oligonucleotides were designed and synthesized to insert into a modified pSC11 plasmid (33). The plus strand (+) was composed of the following bases: TGCACCACCATGGCTCCATAGAAATATTGGAGACTATGTGATAGGTACCCGCGG TACCTATCACAGCATTCTATGTCATTGTC. This sequence encoded an insertional SalI site extension (GC). The complementary minus strand (−) was composed of the following bases: GCACCGGGTACCTATCACAGCATTCTATGTCATTGTC. The plus and minus strand oligonucleotides were annealed to create double-stranded DNA with SalI and NotI cloning sites and inserted into the modified version of pSC11 downstream of the early/late VV p7.5 promoter.

The E3/19K leader/signal sequence (ES) NP 366–374 recombinant vaccinia virus was constructed by inserting synthetic oligonucleotides (Styl–N ot1 double-stranded DNA fragment) behind the ES cloned into pSC11 (33, 34). All oligonucleotide insertions into the pSC11 plasmids were confirmed by DNA sequencing. Finally, recombinant vaccinia viruses were generated in CV-1 cells by homologous recombination, plaque-purified at least three times, and propagated in thymidine kinase-deficient human 143B osteosarcoma cells as described (35).

Immunoprecipitations. Metabolic labeling, immunoprecipitation, and 2D nonequilibrium pH-gradient gel electrophoresis (NEPHGE)–PAGE were performed in essence as previously described (36, 37), except that 1% digitonin was used instead of 0.5% NP-40. Antibodies used for precipitation were obtained as follows: the anticalnexin antiserum was purchased from Stressgen (Victoria, Canada), the anti–heavy chain serum (38) was obtained from H. Ploegh (Massachusetts Institute of Technology, Boston, MA), and anti-TAP antisera were produced by immunizing rabbits with purified recombinant mouse TAP1 or TAP2-GST fusion proteins, and will be described in detail elsewhere (N. and D., and J.J. Monaco, manuscript in preparation).

Results

Mutant H-2^d^ Molecules Can Be Stabilized at the Cell Surface. To test whether mutant heavy chains that may be reaching the cell surface in very limited quantities could be captured and stabilized at the cell surface, transfectants were incubated overnight at 26°C in the presence of excess influenza NP 366–374 peptide and β2m. Transfectants were then labeled with 51Cr and used in a 51Cr-release assay where the level of killing of mutant transfectant targets pulsed with peptide was compared with that of P815-wtD^b^ controls. Neither mutant molecule could sensitize P815 cells for lysis in the CTL assay (Fig. 1 D), demonstrating that the steady-state levels of mutant heavy chains available for peptide binding were below the detectable threshold for a CTL assay.

Mutant H-2^d^ Molecules Can Be Stabilized at the Cell Surface. To test whether mutant heavy chains that may be reaching the cell surface in very limited quantities could be captured and stabilized at the cell surface, transfectants were incubated overnight at 26°C in the presence of excess influenza NP 366–374 peptide and β2m. Transfectants were then labeled with 51Cr and used in the influenza peptide-specific cytotoxicity assay. The results show that P815-D^b^ 219–233 was lysed comparably to P815-wtD^b^, but that P815-D^b^ 196–257 was not specifically lysed (Fig. 2 A). We conclude that the 15–amino acid mutant H-2^d^ molecules can be stabilized at the cell surface by addition of exogenous peptide and β2m and that the stabilized molecule can present antigenic peptide to CTLs, suggesting that it is not grossly misfolded. The phenotype of the D^b^ 196–257 is much more severe, however, perhaps due to misfolding of the molecule. Subsequent studies were carried out using only the P815-D^b^ 219–233 transfectant.

To determine whether incubation at 26°C and addition of exogenous peptide and β2m can upregulate surface ex-
pression enough to be detected by FACS® analysis, transfectants treated in this manner were stained with an antibody against the H-2Db molecule. The FACS® results indicate that surface expression of the 15–amino acid mutant molecule can be detected at a level comparable to that of P815-wtDb maintained at 37°C (Fig. 2B). In fact, mere incubation at 26°C in the absence of peptide (but presence of β2m) results in significant upregulation of Dβ219–233 cell surface expression.

To exclude the possibility of a randomly linked mutation related to general antigen processing and/or class I assembly, we examined the surface expression of the H-2Kd, neomycin resistance, and β-actin expression in P815, P815-wtDb, P815-Dβ219–233, and P815-Dβ196–257 cells. Comparable levels of H-2Kd were seen in these cells (data not shown) suggesting that the defect in proper class I assembly is restricted to the mutant heavy chain.

Upregulation of Surface Expression of Mutant H-2Dβ Molecules Requires β2m. The 15-amino acid mutant contains substitutions within the class I α3 domain that could possibly affect the ability of β2m to bind to the heavy chain. Substituted amino acid positions 231 and 233 are thought to be 2 of the 13 contact sites between the α3 domain and β2m (40). However, the α1 and α2 domains contain 11 and 13 potential β2m interaction sites, respectively, so it seems unlikely that a change in only two β2m contact sites would abrogate its interaction with the heavy chain. Still, it is conceivable that substitutions made at these positions could negatively affect the overall interaction between the heavy chain and β2m to a degree such that proper class I assembly in the ER does not occur, resulting in intracellular retention of the molecule. However, the fact that mutant H-2Db molecules are stabilized by addition of peptide and β2m suggests that these molecules are capable of association with β2m. In fact, an appreciable upregulation of surface expression is seen only in the presence of exogenous peptide (data not shown). Lack of upregulation of surface expression by peptide alone is also evident when cells treated in this manner are used as targets in a CTL assay (Fig. 3). These results suggest that the mutant H-2Dβ heavy chain is able to associate with β2m.

Mutant H-2Dβ Molecules Are Deficient in TAP-dependent Peptide Loading. The phenotype of the mutant H-2Dβ transfectants is reminiscent of that of the cell line RMA-S.
The inability of the mutant H-2Db molecule to proper peptide loading and subsequent surface expression of class I molecules.

**TAP Does Not Associate with the 15–Amino Acid Mutant H-2Db219–233**

whereas parental P815 were not lysed after infection with either of the vaccinia constructs (Fig. 4). These results demonstrate that TAP-dependent peptide transport to the mutant H-2Db molecule is specifically impaired. In addition, these results reconfirm the fact that the mutant heavy chain is capable of association with β2m. Thus, the α3 domain of class I must contain important sites of interaction either directly or indirectly with TAP that are critical for proper peptide loading and subsequent surface expression of class I molecules.

**Figure 2.** Low temperature, peptide, and β2m upregulate the D^d219–233 at the cell surface. (A) Cytotoxicity assay using influenza NP 366-374-specific H-2Db^d-restricted CD8^+ cell line as effector and ^35S-labeled P815, P815-wtDb^d, P815-D^d219–233, and P815-D^d196–257 as target cells. All targets were preincubated overnight at 26°C in the presence of 10 μM NP 366-374 and 5 μg/ml human β2m. Effector to target ratio was 10:1. (B) Immunofluorescence analysis of P815, P815-wtDb^d, and P815-D^d219–233 cells P815 and P815-wtDb^d were cultured at 37°C, whereas P815-D^d219–233 cells were cultured at 26°C in the absence or presence of NP 366-374 plus β2m. Cells were stained with biotinylated anti-H-2Db^d-specific monoclonal antibody followed by streptavidin-PE. Bold lines, cells stained with monoclonal antibody; plain lines, cells stained with secondary reagent alone.

**Figure 3.** Presentation of NP 366-374 to specific CD8^+ cells by D^d219–233 requires the presence of β2m. P815, P815-wtDb^d, and P815-D^d219–233 cells were incubated overnight at 26°C in the absence of fetal calf serum, 10 μM NP 366-374 and/or 5 μg/ml human β2m were added as indicated. Cells were then labeled with ^35S-Cr and used in a cytotoxicity assay with an NP 366-374-specific CD8^+ cell line as effector cells.
Dsb219–233 to present endogenous influenza NP 366–374 peptide only when it is linked COOH terminally to an ER insertion sequence, thus allowing it to bypass the requirement for TAP-dependent peptide loading. Finally, the functional defect in TAP-mediated peptide translocation to Dsb219–233 correlates with the finding that physical association of Dsb219–233 with the TAP complex is drastically reduced. Together, these results argue that amino acids 219–233 of the α3 domain serve as an important docking site for the TAP complex during the assembly of MHC class I molecules.

Although human β2m is clearly binding to Dsb219–233, as evidenced by the requirement for human β2m to stabilize the Dsb219–233 at the cell surface (Fig. 3), this does not necessarily reflect the ability of mouse β2m to bind Dsb219–233. Still, the fact that antigen presentation by Dsb219–233 occurs when peptides are targeted to the ER in a TAP-independent manner (Fig. 4) provides evidence that mouse β2m too is binding to the mutant heavy chain. If the lack of Dsb219–233 surface expression and antigen presentation were due to impaired β2m binding, the phenotype would remain consistent, even when peptides are targeted to the ER by linkage to an ER insertion sequence.

It has been previously suggested that TAP may associate with the α3 domain of the class I heavy chain. This was based on findings that substitution of a single amino acid within the α3 domain (H-2Ld227 or H-2Dd222) can result in the loss of class I association with TAP, as determined in coprecipitation studies (11, 22). However, these molecules are still present at the cell surface at levels detectable by FACS analysis (24–26) and are able to present endogenous peptides (26), suggesting that a true loss of TAP-dependent peptide loading has not occurred. Still, the loss of class I–TAP association as detected in immunoprecipitations using H-2Ld227 and H-2Dd222 hints to the α3 domain as an important site of interaction with the TAP complex. It is known that the association between TAP and class I is very labile in most detergents other than digitonin (10).

**Discussion**

We have shown that substitution of amino acids 219–233 within the α3 domain of H-2Dd results in the loss of its expression at the cell surface. Cell surface expression of Dd219–233 can be rescued by incubation at 26°C with addition of excess peptide and β2m. The rescued molecule is functional in its ability to present exogenous peptide for recognition by CD8+ T cells, suggesting that substitutions introduced into the α3 domain do not grossly affect the conformation of the molecule. P815-Dd219–233 exhibits a phenotype very similar to that of RMA-S cells, which led us to believe that the defect of Dd219–233 expression is due to a lack of TAP-dependent peptide loading. This was demonstrated by the ability of Dd219–233 to present endogenous influenza NP 366–374 peptide only when it is linked COOH terminally to an ER insertion sequence, thus allowing it to bypass the requirement for TAP-dependent peptide loading. Finally, the functional defect in TAP-mediated peptide translocation to Dd219–233 correlates with the finding that physical association of Dd219–233 with the TAP complex is drastically reduced. Together, these results argue that amino acids 219–233 of the α3 domain serve as an important docking site for the TAP complex during the assembly of MHC class I molecules.

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the in vivo function of TAP in loading peptide onto class I. This could explain why H-2L\(^d\)227 and H-2D\(^d\)222 are still expressed and function normally at the cell surface, yet are shown by immunoprecipitation not to associate with TAP. Perhaps caution must be taken when interpreting the results of immunoprecipitations that indicate a lack of TAP association with class I molecules. This is further supported by the findings of allelic variations in the ability of human class I heavy chains to associate with TAP, as HLA-B35 alleles do not coprecipitate with TAP (43) and yet are expressed at the cell surface and present antigenic peptides efficiently (44, 45).

Point mutations of the \(\alpha 2\) domain of the human class I molecule HLA-A0201 (position 134) result in \(-80\%\) reduced surface expression and diminished ability to present endogenous antigens (46, 47), implicating the \(\alpha 2\) domain of the heavy chain in binding to TAP. However, the same mutant molecule is rapidly transported to the cell surface without bound peptides. Apparently, this molecule escapes degradation that normally happens to the majority of partially assembled class I molecules (21). It has therefore been suggested that mutation at position 134 disrupts interaction with an accessory molecule (such as calreticulin) responsible for sorting the peptide-free class I molecules to the degradative pathway and/or ER retention of unloaded molecules (48). Our results do not exclude the role of the \(\alpha 2\) domain in contributing to class I association with TAP. In fact, an \(\alpha 2\) domain contact with the TAP complex could enhance the association necessary for peptide transfer onto the class I molecule. We do show, however, that a net change of 11 amino acids within the \(\alpha 3\) domain is sufficient to dissociate class I from TAP function.

References

1. Schumacher, T. N. M., M. T. H. Heemels, J. J. Neeffes, W. M., Kast, C. J. M. Melief, and H. L. Ploegh. 1990. Direct binding of peptide to empty MHC class I molecules on intact cells and in vitro. Cell. 62:563–567.
2. Townsend, A., C. O. hlen, J. Bastin, H. G. Ljungren, L. Foster, and K. Karre. 1989. Association of class I major histocompatibility heavy and light chains induced by viral peptides. Nature. 340:443–448.
3. Van Bleek, G. M., and S. G. Nathenson. 1990. Isolation of an endogenously processed immunodominant viral peptide from the class I H-2K\(^b\) molecule. Nature. 348:213–216.
4. Falk, K., O. Rotzschke, S. Stevanovic, G. Jung, and H. G. Rammensee. 1991. Allele-specific motifs revealed by sequencing of self-peptides eluted from MHC molecules. Nature. 351:290–296.
5. Tector, M., and R. D. Salter. 1995. Calnexin influences folding of human class I histocompatibility proteins but not their assembly with beta 2-microglobulin. J. Biol. Chem. 270: 19638–19642.
6. Vasilakos, A., M. Cohen-Doyle, P. A. Peterson, M. R. Jackson, and D. B. Williams. 1996. The molecular chaperone calnexin facilitates folding and assembly of class I histocompatibility molecules. EMBO (Eur. Mol. Biol. Organ.) J. 15:1495–1506.
7. Shepherd, J. C., T. N. M. Schumacher, P. G. Ashton-Connor, S. Imaeda, H. L. Ploegh, C. A. Janeway, and S. Tonegawa. 1993. TAP1-dependent peptide translocation in vitro is ATP-dependent and peptide selective. Cell. 74:577–584.
8. Neeffes, J. J., F. Momburg, G. J. Hammerling. 1993. Selective and ATP-dependent translocation of peptides by the MHC-encoded transporter. Science. 261:769–771.
9. Suh, W.-K., M. F. Cohen-Doyle, K. Fruh, K. Wang, P. A. Peterson, and D. B. Williams. 1994. Interaction of MHC class I molecules with the transporter associated with antigen processing. Science. 264:1322–1326.
10. Ortmann, B., M. Andreolwicz, and P. Cresswell. 1994. MHC class I/\(\beta 2\)-microglobulin complexes associate with TAP transporters before peptide binding. Nature. 368:864–867.
11. Carreno, B. M., J. C. Solheim, M. Harris, I. Stroynowski, J. M. Connolly, and T. H. Hansen. 1995. TAP associates with a unique class I conformation, whereas calnexin associates with multiple class I forms in mouse and man. J. Immunol. 155: 4726–4733.
12. Andreolwicz, M. J., B. Ortmann, P. M. van Endert, T. Spies, and P. Cresswell. 1994. 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. USA. 91:12716–12720.
13. Shimizu, Y., and R. Demars. 1989. Production of human cells expressing individual transferred HLA-A, -B, -C genes using and HLA-A, -B, -C null human cell line. J. Immunol. 142:3320–3328.
14. Grandea, A. G. I., M. J. Andreolwicz, R. S. Athwal, D. E. Garey, and T. Spies. 1995. Dependence of peptide binding by MHC class I molecules on their interaction with TAP. Sci-
ence. 270:105–108.

15. Sadasivan, B., P.J. Lehner, B. Oertmann, T. Spies, and P. Cresswell. 1996. Roles of calreticulin and a novel glycoprotein, tapasin, in the interaction of MHC class I molecules with TAP. Immunity. 5:103–114.

16. Li, S., H.-O. Sjogren, U. Hellman, R.F. Pettersson, and P. Wang. 1997. Cloning and functional characterization of a subunit of the transporter associated with antigen processing. Proc Natl Acad Sci USA. 94:8708–8713.

17. Oertmann, B., J. Copeman, P.J. Lehner, B. Sadasivan, J.A. Herberg, A.G.I. Grandea, S.R. Riddell, R.J. Tampe, T. Spies, J. Trowsdale, and P. Cresswell. 1997. A critical role for tapasin in the assembly and function of multimeric MHC class I–TAP complexes. Science. 277:1306–1309.

18. Solheim, J.C., M.R. Harris, C.S. Kindle, and T.H. Hansen. 1997. Prominence of β2-microglobulin, class I heavy chain conformation, and tapasin in the interactions of class I heavy chain with calreticulin and the transporter associated with antigen processing. J. Immunol. 158:2236–2241.

19. Wei, M., and P. Cresswell. 1992. HLA-A2 molecules in an antigen-processing mutant cell contain signal sequence-derived peptides. Nature. 356:443–446.

20. Henderson, R.A., H. Michel, K. Sakaguchi, J. Shabanowitz, E. Appella, D.F. Hunt, and V.H. Engelhard. 1992. HLA-A2.1–associated peptides from a mutant cell line: a second pathway of antigen processing. Proc Natl Acad Sci USA. 89:5058–5062.

21. Hughes, E., C. Hammond, and P. Cresswell. 1997. Misfolded major histocompatibility complex class I heavy chains are translocated into the cytoplasm and degraded by the proteasome. Proc Natl Acad Sci USA. 94:1896–1901.

22. Suh, W.-K., E.K. Mitchell, Y. Yang, P.A. Peterson, G.L. Waneck, and D.B. Williams. 1996. 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. 184:337–348.

23. Lee, N., A.R. Malacko, A. Ishitani, M.-C. Chen, J. Bajorath, H. Marquardt, and D.E. Geraghty. 1995. The membrane-bound and soluble forms of HLA-G bind identical sets of endogenous peptides but differ with respect to TAP association. Immunity. 3:591–600.

24. Potter, T.A., T.V. Rajan, R.F. Dick II, and J.A. Bluestone. 1989. Substitution at residue 227 of H-2 class I molecules abrogates recognition by CD8-dependent, but not CD8-independent, cytotoxic T lymphocytes. Nature. 337:73–75.

25. Connolly, J., T.H. Hansen, A.L. Ingold, and T.A. Potter. 1990. Recognition by CD8 on cytotoxic T lymphocytes is ablated by several substitutions in the class I α3 domain: CD8 and the T-cell receptor recognize the same class I molecule. Proc Natl Acad Sci USA. 87:2137–2141.

26. Kilalleen, N., A. Mioriati, H.-S. Teh, and D.R. Littman. 1992. Requirement for CD8-major histocompatibility complex class I interaction in positive and negative selection of developing T cells. J. Exp. Med. 176:89–97.

27. Williams, D.B., B.H. Barber, R.A. Flavell, and H. Allen. 1989. Role of β2-microglobulin in the intracellular transport and surface expression of murine class I histocompatibility molecules. J. Immunol. 142:2796–2806.

28. Vitteio, A., T.A. Potter, and L.A. Sherman. 1990. The role of β2-microglobulin in peptide binding by class I molecules. Science. 250:1423–1426.

29. Attaya, M., S. Jameson, C.K. Martine, E. Hermel, C. Aldrich, J. Forman, K. Fischer-Lindahl, M.J. Bevan, and J.J. Monaco. 1992. Ham-2 corrects the class I antigen processing-defect in RMA-S cells. Nature. 355:647–649.

30. Spies, T., V. Cerundolo, M. Collona, P. Cresswell, A. Townsend, and R. DeMars. 1992. Presentation of viral antigen by MHC class I molecules is dependent on a putative peptide transporter heterodimer. Nature. 355:644–646.

31. Joly, E., and M.B.A. Oldstone. 1991. Manufacture of a functional cDNA for the H-2Db molecule using a retroviral shuttle vector. Immunogenetics. 34:62–65.

32. Jhaver, K.G., T.D. Rao, A.B. Frey, and S. Vukmanovic. 1995. Apparent split tolerance of CD8+ T cells from β2-microglobulin-deficient (β2m–/–) mice to syngeneic β2m+/+ cells. J. Immunol. 154:6252–6261.

33. Eisenlohr, L.C., I. Bažík, J.P. Bennink, K. Bernstein, and J.W. Yewdell. 1992. Expression of a membrane protease enhances presentation of endogenous antigens to MHC class I-restricted T lymphocytes. Cell. 71:963–972.

34. Bažík, I., J.H. Cox, R. Anderson, J.W. Yewdell, J.R. Bennink. 1994. TAP-independent presentation of endogenously synthesized peptides is enhanced by endoplasmic reticulum insertion sequences when located at the amino but not carboxy terminus of the peptide. J. Immunol. 152:381–387.

35. Chakrabarti, S., K. Brechling, B. Moss. 1985. Vaccinia virus expression vector: coexpression of beta-galactosidase provides visual screening of recombinant virus plaques. Mol. Cell. Biol. 5:3403–3409.

36. Brown, M.G., J. Driscoll, and J.J. Monaco. 1991. Structural and serological similarity of MHC-linked LMP and proteasome (multicatalytic proteasome) complexes. Nature. 353:355–357.

37. Nandi, D., E. Woodward, D.B. Ginsburg, and J.J. Monaco. 1997. Intermediates in the formation of mouse 20S proteasomes: implications for the assembly of precursor β subunits. EMBO (Eur. Mol. Biol. Organ. ) J. 16:5363–5375.

38. Machold, R.P., S. Andree, L. Van Kaer, H.-G. Ljunggren, and H.L. Ploegh. 1995. Peptide influences the folding and intracellular transport of free major histocompatibility complex class I heavy chains. J. Exp. Med. 181:1111–1122.

39. Brown, J.H., T.S. Jardetzky, J.C. Gorga, J.C. Stern, R.G. Urbano, J.L. Strominger, and D.C. Wiley. 1993. Three-dimensional structure of the human class II histocompatibility antigen HLA-DR1. Nature. 364:33–39.

40. Bjorkman, P.J., M.A. Saper, B. Samaurai, W.S. Bennett, J.L. Strominger, and D.C. Wiley. 1987. Structure of the human class I histocompatibility antigen, HLA-A2. Nature. 329:506–512.

41. Anderson, K., P. Cresswell, M. Gammon, J. Hermes, A. Williamson, and H. Zweerink. 1991. Endogenously synthesized peptide with an endoplasmic reticulum signal sequence sensitizes antigen processing mutant cells to class I-restricted cell-mediated lysis. J. Exp. Med. 174:489–492.

42. Eisenlohr, L.C., J.W. Yewdell, and J.R. Bennink. 1992. Flanking sequences influence the presentation of an endogenously synthesized peptide to cytotoxic T lymphocytes. J. Exp. Med. 175:481–487.

43. Neisig, A., R. Wubbolts, X. Zang, C. Melief, and J. Nefes. 1996. Allelic-specific differences in the interaction of MHC class I molecules with transporters associated with antigen processing. J. Immunol. 156:3196–3206.

44. Ooba, T., H. Hayashi, S. Karaki, M. Tanabe, K. Kano, and M. Takiguchi. 1989. The structure of HLA-B35 suggests that it is derived from HLA-Bw58 by two genetic mechanisms.
45. Koziel, M.J., D. Dudley, J.T. Wong, J. Dienstag, M. Houghton, R. Ralston, and B.D. Walker. 1992. Intrahepatic cytotoxic T lymphocytes specific for hepatitis C virus in persons with chronic hepatitis. J. Immunol. 149:3339–3344.

46. Lewis, J.W., A. Neisig, J. Neefjes, and T. Elliott. 1996. Point mutations in the α2 domain of HLA-A2.1 define a functionally relevant interaction with TAP. Curr. Biol. 6:873–883.

47. Peace-Brewer, A.L., L.G. Tussey, M. Matsui, G. Li, D.G. Quinn, and J.A. Frelinger. 1996. A point mutation in HLA-A*0201 results in failure to bind the TAP complex and to present virus-derived peptides to CTL. Immunity. 4:505–514.

48. Elliott, T. 1997. How does TAP associate with MHC class I molecules. Immunol. Today. 18:375–379.