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The Ig-Like Domain of Tapasin Influences Intermolecular Interactions

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Presentation of antigenic peptides to T lymphocytes by MHC class I molecules is regulated by events involving multiple endoplasmic reticulum proteins, including tapasin. By studying the effects of substitutions in the tapasin Ig-like domain, we demonstrated that H-2L\(^*\)/tapasin association can be segregated from reconstitution of folded L\(^*\) surface expression. This finding suggests that peptide acquisition by L\(^*\) is influenced by tapasin functions that are independent of L\(^*\) binding. We also found that the presence of a nine-amino acid region in the Ig-like domain of mouse or human tapasin is required for association with L\(^*\), and certain point substitutions in this sequence abrogate human, but not mouse, tapasin association with L\(^*\). These data are consistent with a higher overall affinity between L\(^*\) and mouse tapasin compared with human tapasin. In addition, we found that other point mutations in the same region of the tapasin Ig-like domain affect MHC class I surface expression and Ag presentation. Finally, we showed that the cysteine residues in the Ig-like domain of tapasin influence tapasin’s stability, its interaction with the MHC class I H chain, and its stabilization of TAP. Mutagenesis of these cysteines decreases tapasin’s electrophoretic mobility, suggesting that these residues form an intramolecular disulfide bond. Taken together, these results reveal a critical role for the tapasin Ig-like domain in tapasin function. The Journal of Immunology, 2004, 172: 2976–2984.

M ajor histocompatibility complex class I molecules present peptides at the cell surface to CTL, triggering cell killing that counters infection or malignancy. The assembly of the MHC class I H chain with its L chain, \(\beta_2\)-microglobulin (\(\beta_2\)-m), a, and peptide occur in the endoplasmic reticulum (ER) (1). The proteins known to participate in the MHC class I peptide-loading complex are tapasin, TAP, calreticulin, and ERp57 (2). TAP transports peptides produced by proteasomal degradation into the ER, where they undergo N-terminal trimming by the ER aminopeptidase associated with Ag processing (3). Once the MHC class I H chain groove folds around peptide, the MHC/\(\beta_2\)-m/peptide heterotrimer is freed from the assembly complex and processed through the Golgi to the cell surface (4–7).

Experiments with the tapasin-deficient 721.220 cell line have revealed that tapasin binds to the MHC class I H chain and that tapasin/MHC binding is required for TAP to associate with the MHC class I H chain (8–10). TAP is stabilized by tapasin transfection, and the stabilization of TAP results in a rise in the overall cytosol—ER peptide translocation level (11, 12). It has also been demonstrated that the absence of functional tapasin in 721.220 reduces the binding of peptide by TAP (13). The importance of tapasin to MHC class I Ag presentation has been confirmed in tapasin knockout mice. The steady state expression of TAP in the splenocytes of tapasin-deficient mice is greatly reduced, and the mice express surface MHC class I molecules that are low in quantity and unstable (14–16).

There is currently no x-ray crystallographic structure of tapasin; however, structural predictions from tapasin sequences and recent biophysical studies have provided relevant information. Translation of the human tapasin cDNA sequence indicates that human tapasin is a 428-aa, proline-rich protein (10, 17). The murine and rat tapasin molecules are slightly longer due to extended C termini and an additional single residue in the middle of rat tapasin (18, 19). The membrane-proximal region of tapasin displays homology to the Ig constant region (Ig-C1) and the Ig-like domains of other proteins (including MHC molecules), thus identifying tapasin as a member of the Ig superfamily (10). Within the Ig-like region of tapasin are two conserved cysteine residues, and there are an additional three conserved cysteine residues that lie N terminal of the Ig homology region (19). Tapasin has a hydrophobic transmembrane region, and the cytoplasmic portion of tapasin contains an ER retention motif. Analytical ultracentrifugation indicates that tapasin is elongated in solution, and controlled proteolysis experiments show that tapasin has two major ER-lumenal domains that are linked by a flexible, solvent-exposed area (20). The first of these two domains consists of the first 85 residues of the protein, and the second tapasin domain encompasses the remainder (~300 residues), which includes the Ig-like domain (20).
There have been a limited number of reports on the influence of structural elements in tapasin on its function. In one such study a tapasin mutant that lacks an N-terminal sequence (Δ1–50) was demonstrated to interact with TAP, but not to associate with HLA-B8 or restore B8 surface expression (12). Thus, the N-terminus of tapasin appears to affect interactions between tapasin and the MHC class I H chain. A soluble tapasin mutant with a deletion of the transmembrane and cytoplasmic domains did not facilitate HLA-B8/TAP association, but did restore Ag presentation by B8, suggesting that physical interaction between HLA class I and TAP might not be necessary for Ag presentation (11). However, a more recent study that examined the function of three soluble human tapasin variants and a tapasin point mutant that binds TAP poorly (tapasin L410F) indicated that loss of TAP interaction may prevent binding of an optimal spectrum of peptides (21). Mutational analysis has also shown that the tapasin cysteines at positions 7 and 71 form an intramolecular disulfide bond, and that the cysteine at position 95 makes a transient intermolecular bond with ERp57 within the assembly complex (22). Based on homology with other proteins, the existence of another intramolecular disulfide bond in tapasin within the Ig-like domain can be postulated, but it has not been experimentally demonstrated.

By analyzing several novel tapasin mutants, we have investigated the influence of the Ig-like domain of tapasin on its interactions with H-2Ld and TAP and on the loading of peptides. Our results indicate that Ld association with tapasin is not required for the partial restoration of Ld surface expression. Furthermore, we discovered that a region of nine amino acids within the tapasin Ig-like domain is crucial for both mouse and human tapasin association with Ld. We found that two amino acid residues within this nine-amino-acid region in the human, but not the mouse, tapasin association with Ld. We found that two amino acid residues within this nine-amino-acid region in the human, but not the mouse, tapasin Ig-like domain influence peptide presentation by Ld to a CTL clone. Finally, we determined that an intramolecular disulfide bond within the Ig-like domain of tapasin is influential in the stabilization of tapasin and in its association with MHC class I and TAP. Thus, our findings emphasize the existence of both similarities and subtle, yet important, functional differences between mouse and human tapasin in their interactions with a murine MHC class I molecule. Overall, our results suggest that the tapasin Ig-like domain plays a role in tapasin association with MHC class I and Ig-like class I/peptide surface expression, and that tapasin intrachain disulfide bonding is necessary for tapasin to be able to engage in normal intermolecular interactions in the peptide-loading complex.

Materials and Methods

Antibodies

The 64-3-7 and 30-5-7 Abs detect open, peptide-free and folded, peptide-bound in complexes. Overall, our results suggest that the tapasin Ig-like domain plays a role in tapasin association with MHC class I and H chain disulfide bonding is necessary for tapasin to be able to engage in normal intermolecular interactions in the peptide-loading complex.

ImmunoMEMs and Western blots

ImmunoMEMs and Western blotting were performed by a method similar to a published procedure (40). For immunoMEMs, the cells were washed in 20 mM iodoacetamide in PBS (Sigma–Aldrich, St. Louis, MO) three times and then lysed in 3-(1-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS) lysis buffer. For each immunoprecipitation, 1 × 10^6 cells were used, and the cells that were compared within each experiment were of matched confluence and high viability. The CHAPS lysis buffer was composed of 1% CHAPS (Sigma–Aldrich) in TBS (pH 7.4) with freshly added 0.2 mM PMSF, 20 mM iodoacetamide, and excess MAb. After 1 h of incubation on ice, the lysates were centrifuged to pellet cell nuclei, and the supernatants were incubated with protein A-Sepharose beads (Amersham Pharmacia Biotech, Piscataway, NJ). The beads were washed with 0.1% CHAPS in TBS (pH 7.4) four times and boiled in 0.125 M Tris (pH 6.8)/2% SDS/12% glycerol/0.02% bromophenol blue to elute the proteins.

The eluted proteins were electroblotted on SDS–PAGE gels (Invitrogen, Carlsbad, CA) and transferred to Immobilon-P membranes (Millipore, Bedford, MA) for Western blotting. After overnight blocking in PBS/0.05% Tween 20% (w/v) dry milk, the membranes were incubated in dilute Ab for 2 h, washed three times with PBS/0.05% Tween 20, and incubated for 1 h in a dilution of biotin-conjugated goat anti-mouse or anti-rabbit IgG (Caltag Laboratories, San Francisco, CA) or anti-Armenian hamster IgG (Jackson ImmunoResearch Laboratories, West Grove, PA). After three PBS/0.05% Tween 20 washes, the membranes were incubated in a dilution of streptavidin-conjugated HRP (Zymed Laboratories, San Francisco, CA) (1:1,000), washed with PBS/0.3% Tween 20, and visualized by soaking briefly in ECL Western blot developing reagents (Amersham Pharmacia Biotech), and exposed to Kodak BioMax film (Eastman Kodak, Rochester, NY).

Flow cytometry

In the flow cytometry assays, cells were suspended at 5 × 10^6/ml in 0.2% BSA/0.1% sodium azide/PBS, and 0.1-mL aliquots were distributed to the wells of a 96-well U-bottom plate. The cells were incubated with excess mAb or with PBS/BSA/sodium azide alone (as a control) at 4°C for 30 min, washed twice, and incubated with a PE-conjugated, Fc-specific F(ab’)2 portion of goat anti-mouse IgG (Jackson ImmunoResearch Laboratories) at 4°C for 30 min. The cells were washed twice, resuspended in PBS/BSA/sodium azide, and analyzed with a FACSCalibur flow cytometer (BD Biosciences, San Jose, CA). CellQuest software (BD Biosciences) was used for statistical analysis.
Results

Analysis of mutations in human and mouse tapasin for their effects on MHC class I association

In a previous study we constructed a human tapasin mutant (Δ334–342) with a deletion of aa 334–342 (HHSDBGVSL) and showed that although it did not bind detectably to Ld, it nevertheless stabilized TAP and increased the proportion of folded Ld molecules at the cell surface (41). To investigate the role of the 334–342 region of tapasin in more detail, we made a corresponding mouse tapasin mutant cDNA with a 334–342 deletion and both human and mouse tapasin mutant cDNAs with specific point substitutions in the 334–342 sequence (Fig. 1). Human tapasin mutants D337A and S341R/L342T were expressed stably in 721.220-Ld (Fig. 2, left panel). Human tapasin D337A and S341R/L342T, like tapasin Δ334–342, did not coprecipitate with Ld (Fig. 2, middle panel). However, like tapasin Δ334–342, the human D337A and S341R/L342T mutants associated with TAP, although slightly more weakly than wild-type tapasin, and stabilized TAP (S341R/L342T more than D337A; Fig. 2, right panel). Furthermore, both human tapasin mutants were able to facilitate Ld assembly and surface expression (Fig. 3). Thus, the phenotypes of these point mutants are consistent with the previously described phenotype of human tapasin Δ334–342 (41) and indicate that positions D337 and S341/L342 are crucial to human tapasin’s association with Ld.

We also made wild-type and mutant mouse tapasin transfectants of a mouse tapasin knockout-Ld cell line and assessed the effect of mutations in mouse tapasin on its binding to Ld and TAP and on the surface expression of Ld. The mouse tapasin mutant Δ334–342 mimics the human tapasin mutant equivalent (41) in that it does not interact with Ld (Fig. 4), but does bind to TAP (data not shown) and increase the surface expression of folded Ld molecules (Fig. 5). In contrast to the corresponding human tapasin mutants, the mouse tapasin mutants D337A and S341R/Q342T were able to bind to Ld (data not shown), which may indicate that the overall affinity of mouse tapasin for Ld is sufficiently great that point substitutions in this region have a negligible effect. As both the human and mouse tapasin Δ334–342 mutants contribute significantly to Ld surface expression without detectable binding to Ld, tapasin’s interactions with other proteins, such as TAP, are evidently important to its facilitation of MHC class I assembly.

Mutations at tapasin positions 334/335 reduce CTL recognition of a known peptide Ag

We also generated a human tapasin mutant with substitutions at positions 334 and 335 in this region (tapasin H334F/H335Y; Fig. 1). Tapasin H334F/H335Y and wild-type tapasin were expressed at similar levels when transfected into 721.220-Ld cells (41). Unlike tapasin Δ334–342, D337A, and S341R/L342T, the H334F/H335Y mutant coprecipitated with Ld in amounts equivalent to wild-type tapasin (data not shown). We have previously shown that the ratio of cell surface open (64-3-7) Ld to folded (30-5-7) Ld is low on 721.220 transfectants expressing the H334F/H335Y tapasin mutant compared with the ratio on cells expressing wild-type tapasin (41) due to a decrease in cell surface open (64-3-7) forms. Expressions of the H334F/H335Y tapasin mutant, relative to the wild type, reduced the surface expression of open Ld.

To assess whether Ag presentation is altered by the H334F/H335Y substitutions, we used a well-characterized CTL clone, 2C, which recognizes a conserved, ubiquitous epitope from 2-oxoglutarate dehydrogenase in the context of Ld (39, 42–44). The octameric p2Ca peptide (LSPFPFDL) was identified as the ligand that is naturally processed and presented to the 2C clone in the context of Ld (42, 43). The 2C clone also recognizes a nonamer variant of p2Ca called QL9 (QLSPFPFDL) (44). The p2Ca peptide has a very low affinity for Ld (45–49), although the QL9 peptide has higher affinity for Ld (47, 48). Recognition of Ld by the 2C CTL clone was depressed by the presence of human tapasin H334F/H335Y in 721.220 cells relative to wild-type tapasin (Fig. 6, top and bottom panels). We confirmed that the low 2C recognition of the human tapasin H334F/H335Y-transfected cells was not due to an overall reduction in folded Ld surface expression on these cells by measuring the surface expression of folded Ld molecules concurrently with the CTL assay (Fig. 3, bottom panel). Thus, in 721.220 cells, human tapasin H334F/H335Y evidently down-regulates initial binding of the 2C epitope to Ld or destabilizes its binding to Ld. In contrast, mouse tapasin H334F/H335Y expressed in mouse tapasin fibroblast knockout cells facilitated presentation of the 2C epitope by Ld to the same extent as wild-type mouse tapasin (data not shown), indicating that there is a difference in the effects of human and murine tapasin on the presentation of this epitope by Ld.

We also compared 2C recognition of 721.220-Ld+tapasin H334F/H335Y with and without culturing the cells with exogenous p2Ca peptide. As shown in the bottom panel of Fig. 6, exogenously delivered p2Ca peptide is able to bind to Ld that has been intracellularly assembled in the presence of human tapasin H334F/H335Y, as indicated by partial up-regulation of 2C recognition. However, after culture of the targets with p2Ca peptide, 2C lysis of the tapasin H334F/H335Y transfectant was still lower than lysis of the wild-type tapasin transfectant, consistent with the availability of fewer peptide-receptive Ld forms on the tapasin H334F/H335Y transfectant.

Conserved cysteines in the Ig-like domain form a disulfide bond

By mutagenesis, an intramolecular disulfide bond in tapasin was demonstrated to exist between cysteine 7 and cysteine 71 (22). The Ig-like domain of tapasin probably also contains a disulfide bond based on the presence of such a bond in other members of the Ig domain.
supergene family, but experimental evidence for this second disulfide bond has not been previously reported. To examine whether this additional disulfide bond exists and, if so, to determine its influence on tapasin’s stability and its binding to MHC class I and TAP, we created human tapasin mutants with substitutions at the Ig-like domain cysteines (C295S, C362S, and C295S/C362S) and expressed them in 721.220-Ld cells (Fig. 1). To make functional comparisons to corresponding mutants with substitutions at other cysteine positions, we also generated human tapasin mutants with substitutions at cysteine positions 7, 71, and 95 (Fig. 1). Mutagenesis of the C295 and/or C362 positions slowed electrophoretic migration of human tapasin (Fig. 7), providing novel, direct evidence that these residues form an intramolecular disulfide bond in the Ig-like domain. We also expressed a murine tapasin C295S mutant in Ld−transfected tapasin knockout cells and found that it exhibited slower gel migration, consistent with the existence of an intramolecular disulfide bond at this position in murine tapasin as well (data not shown).

As previously observed (22), substitution of C7 and C71 in human tapasin slowed electrophoretic migration, indicating disruption of an intramolecular disulfide bond in the N-terminal end. We also found, as previously reported (22), that mutagenesis of C71 caused human tapasin to electrophorese as at least two closely...
Conserved cysteines in the Ig-like domain are necessary for interaction of tapasin with MHC class I H chain and stabilization of TAP

To assess the importance of tapasin cysteines to the association of human tapasin with Ld, we immunoprecipitated Ld from lysates of 721.220-Ld cells transfected with wild-type human tapasin or each of the human cysteine mutants. In Fig. 8, the top left panel verifies the immunoprecipitation of Ld, and the bottom left panel demonstrates the degree of association of each tapasin mutant with Ld. The Ld H chain is strongly associated with wild-type human tapasin, C7S, and C71S. However, minimal quantities of human tapasin mutants C9S, C295S, and C362S coprecipitated with Ld (Fig. 8, left panel). We also have confirmed that murine tapasin C95S and C295S proteins bind relatively weakly to Ld in mouse cells compared with wild-type murine tapasin (data not shown). Interestingly, the poor ability of tapasin C95S to bind to Ld contrasts with the ability of a tapasin C95A mutant to bind to HLA-B44 (22), suggesting that there may be an allele- or species-specific difference in the effect of mutating this position on tapasin interaction with the MHC class I H chain. Tapasin C95S is expressed efficiently in human and mouse cells (Fig. 7 and data not shown); therefore, destabilization of tapasin due to the C95S mutation is not the major factor in the inability of tapasin C95S to associate with Ld. The situation is more complex with tapasin mutants C295S and C362S, which are partially destabilized as a direct result of the substitution at position 295 or 362.

FIGURE 5. The cell surface expression of folded Ld was increased by intracellular expression of mouse tapasin Δ334–342, despite its inability to bind detectably to Ld. The surface expression of Ld on mouse tapasin knockout fibroblast-Ld cells (No Tapasin), tapasin knockout fibroblast-Ld+tapasin (Tapasin), and tapasin knockout fibroblast-Ld+tapasin Δ334–342 (Δ334–342) was assessed by flow cytometry with Abs 30-5-7 and 64-3-7, and the ratios of mean fluorescence values obtained with each Ab is indicated on the bar graph. The mean fluorescence values obtained with the secondary Ab were <10 channels in all cases. The results shown were reproduced in an independent experiment.
immunoprecipitates were electrophoresed on a 4%–20% (for Ld) or 10% acrylamide Tris-glycine gel (for tapasin), transferred to a membrane, and probed with mAb 64-3-7 (for Ld) or anti-tapasin serum. Right panel, Abrogation of the disulfide bond in the tapasin Ig-like domain, but not the N-terminal disulfide bond, interferes with the binding and stabilization of TAP. Immunoprecipitations were performed with mAb 148.3 on lysates of the indicated cell types. The immunoprecipitates were electrophoresed on 10% acrylamide Tris-glycine gels, transferred to membranes, and probed with anti-TAP or anti-tapasin serum. These results were reproduced in a separate assay.

or indirect result of their mutations. Nevertheless, careful comparisons of the quantities of wild-type tapasin and tapasin mutant proteins in the Western blots shown in Figs. 7 and 8 (bottom left panel) suggest that the C295S and C362S mutations also affect tapasin/Ld interaction.

We proceeded to compare two of the human tapasin cysteine mutants, C71S and C295S, to wild-type tapasin to determine their relative abilities to bind and stabilize TAP. As shown in Fig. 8 (right panel), human tapasin C71S coprecipitates with TAP and stabilizes TAP well. In contrast, human tapasin C295S neither coprecipitates with TAP nor stabilizes TAP (Fig. 8, right panel). These results suggest that the binding of human tapasin to TAP is more dependent on the formation of the disulfide bond in the tapasin Ig-like domain than on the formation of the N-terminal disulfide bond. The relatively low expression of the human C295S mutant may be a factor; however, even prolonged exposures of the tapasin Western blot shown in the bottom right panel of Fig. 8 failed to reveal any TAP-associated human tapasin C295S. Thus, the C295S mutation in human tapasin seems to be detrimental to TAP association, in addition to affecting tapasin stability and MHC class I H chain interaction. (Another possibility, which cannot be excluded by our results, is that association of human tapasin with TAP stabilizes the disulfide bond in the human tapasin Ig-like domain.) Compared with human tapasin C295S, murine tapasin C295S is better able to stabilize TAP and to coprecipitate with it, although it is less able than to do so than wild-type murine tapasin (data not shown). This finding is perhaps reflective of the fact that murine tapasin causes a much greater increase in TAP expression in mouse cells (300-fold) than human tapasin does in human cells (3-fold) (11–16).

Ld is expressed at the surface of 721.220-Ld cells, but in the absence of tapasin cotransfection, it is present in an abnormally high proportion of open, peptide-free forms (32, 41) (Fig. 9). Consistent with its inability to stabilize TAP, human tapasin C295S was less able than tapasin C71S to facilitate the expression of stably folded Ld molecules at the surface of transfected 721.220 cells, and both mutants facilitated the expression of stably folded Ld more poorly than wild-type tapasin (Fig. 9). Compared with human tapasin C295S, mouse tapasin C295S was better able to increase the expression of stably folded Ld at the cell surface, probably due to its relatively stronger ability to stabilize TAP (data not shown).
Discussion

Based on the homology of tapasin to MHC molecules and other members of the Ig-like family, a tertiary structure of tapasin has been predicted (10, 50, 51). According to this model, aa 334–342 are predicted to be externally exposed (50, 52, 53) (Fig. 10). As the human and mouse tapasin 334–342 mutants and the human tapasin D337A and S341R/L342T mutants do not associate with L^d (41) (Figs. 2 and 4), this tapasin region probably contacts L^d. Previous studies found evidence for interaction sites on both the MHC class I a2 and a3 domains for tapasin (7, 38, 54, 55). Given that aa 334–342 of tapasin are modeled to be at a membrane-distal position in the three-dimensional structure of the tapasin Ig-like domain (Fig. 10), these amino acids are in position to make contact with either the MHC class I a2 or a3 domain. Tapasin aa 334–342 might associate with MHC class I a2 domain aa 128–136, which form an exposed, conserved loop that apparently interacts with tapasin (54). In this scenario, the binding of peptide would cause a conformational change in the MHC a2 domain loop, freeing it from interaction with aa 334–342 of tapasin and allowing the completed MHC class I heterotrimer to leave the ER.

Tapasin regulates the rate of egress of MHC class I molecules, based on our findings that, relative to wild-type tapasin, mutant tapasin H334F/H335Y delays the migration of MHC class I to the cell surface (41). This delay may be due to tapasin H334F/H335Y having slow on (and off) rates for the open MHC class I H chain or to it having a slow off rate for MHC/peptide complexes. The length of time the MHC class I molecule resides in the ER may influence the quantity of MHC class I molecules ultimately expressed at the cell surface and the presentation of specific peptides. This theory is suggested by our findings that tapasin H334F/H335Y depresses the level of cell surface open L^d forms and reduces the presentation of the 2C epitope on 721.220-L^d cells (41) (Fig. 6).

When exogenous p2Ca peptide was added to 721.220-L^d+tapasin H334F/H335Y cells, recognition of these cells by 2C was increased, but it was still slightly lower at all E:T cell ratios tested compared with 2C recognition of 721.220-L^d+tapasin+p2Ca (Fig. 6, bottom panel). These observations could be due to the presence of a relatively low number of cell surface L^d molecules assembled in the presence of tapasin H334F/H335Y that are able to bind exogenous peptide. This reasoning would be consistent with our previously published data showing that there is a low number of L^d molecules in the open conformation at the surface of H334F/H335Y-expressing cells (41).

Without the intracellular presence of functional tapasin, the production of appropriately folded cell surface L^d is very low (32, 41) (Figs. 3 and 5). However, based on our findings with tapasin A334–342, tapasin D337A, and tapasin S341R/L342T, we propose that tapasin/L^d interaction is not an absolute requirement for facilitating the surface expression of stably folded L^d molecules (41) (Figs. 2–5). Thus, functions of tapasin not involving direct physical association with L^d, perhaps the ability to stabilize TAP (Fig. 2, right panel), are important.

Abrogation of the disulﬁde bond in the Ig-like domain had a much more signiﬁcant impact on human tapasin function than did disruption of the disulﬁde bond between positions 7 and 71. The mutations in human tapasin that prevented disulﬁde bridging in the Ig-like domain aﬀected its stability, its interactions with the MHC class I H chain and TAP, and its capacity to contribute to stable folding of L^d molecules at the cell surface (Figs. 7–9). Notably, human tapasin mutants able to stabilize TAP (C71S, D337A, and S341R/L342T) allowed folded L^d molecules to be expressed in normal quantities at the surface, whereas those that did not stabilize TAP (C295S) did not facilitate stable surface L^d expression.

The impact of abrogating the Ig-like domain disulﬁde bond of murine tapasin was lower in terms of both TAP association and contribution to stable L^d surface expression, which may be related to the relative degree of TAP stabilization provided by mouse vs human tapasin.

It is likely that tapasin destabilization induced by these mutations has secondary eﬀects on interaction with MHC class I and TAP. However, our results do not formally exclude the possibility that the human C295S and C362S mutations cause a primary loss of interaction with MHC class I and TAP, and that the loss of either or both of these interactions contributes to tapasin instability. Consistent with this possibility, we did find evidence of slightly reduced expression of human and mouse tapasin Δ334–342 mutants (lacking MHC class I association), which may be due to some degree of instability (41) (Fig. 4). No reduction in expression was reported for the tapasin ΔN50 mutant, which interacts ineﬃciently with the MHC class I H chain; however, unlike the tapasin Δ334–342 mutant, the ΔN50 mutant does still associate with some MHC class I molecules (12, 41) (Fig. 4). Soluble tapasin, lacking the C-terminal 35 aa, does not bind to TAP, but yet is expressed at the same level as the wild type in 721.220-B8 transfectants, suggesting that the loss of TAP interaction does not cause tapasin instability (11).

Results from some previous studies have suggested that in some, but not all, MHC allotype/tapasin combinations, tapasin exhibits species speciﬁc eﬀects, although conclusions about these eﬀects are somewhat dependent on the type of assay used to analyze MHC class I assembly (14, 32, 56–59). In comparing the assembly of murine MHC class I molecules in mouse and human cells, the degree to which the presence of mouse vs human β_m affects the peptide loading and cell surface expression of murine MHC class I allotypes can also be a factor. It is known that interaction with human β_m greatly up-regulates the recognition of L^d by an anti-α/α2 domain Ab, 34-1-2, suggesting that human β_m leads to changes in L^d structure and/or stability (60, 61). One feature known to diﬀer between mouse and human systems is the degree to which tapasin stabilizes TAP. As mentioned above, tapasin causes a much greater increase in TAP expression in mouse than in human cells (11, 16).

Our results from comparisons of the eﬀects of human vs murine tapasin mutants (expressed in human and murine cells, respectively) on L^d/tapasin interaction and L^d peptide presentation suggest that a shared region on both of these species variants, positions 334–342, interacts with L^d. The substitution of individual positions within this sequence altered human tapasin’s, but not murine tapasin’s, eﬀects on L^d, perhaps due to an overall higher aﬃnity of mouse tapasin for L^d. Some diﬀerences were also observed in our studies in the eﬀects of mutating the C295 position in human vs mouse tapasin with regard to the relative eﬀect on TAP interaction and the ability to facilitate the stable expression of folded L^d molecules at the cell surface. Studies of the eﬀects of human/murine tapasin mutant chimeras may further clarify the structural bases for these observations.

The C95 amino acid in human tapasin has been reported to disulﬁde bond transiently to ERp57 within the assembly complex (22). Our study revealed that tapasin C95S, expressed as either a human tapasin mutant in 721.220-L^d or a mouse tapasin mutant in tapasin knockout ﬁbroblast cells, interacts very ineﬃciently with L^d (Fig. 8 and data not shown). In contrast, a human tapasin mutant with a substitution at the same position bound to HLA-B44 to the same extent as wild-type tapasin (22). Thus, there are allele- or species-speciﬁc diﬀerences in the eﬀects of substitutions at this position in tapasin, and we are currently exploring the implications.
of these differences for ERp57 as well as MHC association with tapasin.

Understanding tapasin’s ability to regulate MHC class I surface expression and peptide selection is integral to the comprehension of Ag presentation. The quantity and quality of cell surface MHC molecules are strongly influenced by tapasin, and thus, tapasin activity is vital to the cellular immune response. Our data demonstrate that mutational analysis of tapasin provides insight into aspects of the tapasin structure that are crucial to its ability to assist MHC class I assembly.

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References


