The Binding of TAPBPR and Tapasin to MHC Class I Is Mutually Exclusive

Clemens Hermann, Lisa M. Strittmatter, Janet E. Deane and Louise H. Boyle

J Immunol published online 25 October 2013
http://www.jimmunol.org/content/early/2013/10/24/jimmunol.1300929

Supplementary Material http://www.jimmunol.org/content/suppl/2013/10/25/jimmunol.1300929.DC1

Subscription Information about subscribing to The Journal of Immunology is online at:
http://jimmunol.org/subscription

Permissions Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts

The Journal of Immunology is published twice each month by
The American Association of Immunologists, Inc.,
1451 Rockville Pike, Suite 650, Rockville, MD 20852
Copyright © 2013 by The American Association of Immunologists, Inc. All rights reserved.
Print ISSN: 0022-1767 Online ISSN: 1550-6606.
The Binding of TAPBPR and Tapasin to MHC Class I Is Mutually Exclusive

Clemens Hermann,* Lisa M. Strittmatter,* Janet E. Deane,† and Louise H. Boyle*

The loading of peptide Ags onto MHC class I molecules is a highly controlled process in which the MHC class I–dedicated chaperone tapasin is a key player. We recently identified a tapasin-related molecule, TAPBPR, as an additional component in the MHC class I Ag-presentation pathway. In this study, we show that the amino acid residues important for tapasin to interact with MHC class I are highly conserved on TAPBPR. We identify specific residues in the N-terminal and C-terminal domains of TAPBPR involved in associating with MHC class I. Furthermore, we demonstrate that residues on MHC class I crucial for its association with tapasin, such as T134, are also essential for its interaction with TAPBPR. Taken together, the data indicate that TAPBPR and tapasin bind in a similar orientation to the same face of MHC class I. In the absence of tapasin, the association of MHC class I with TAPBPR is increased. However, in the absence of TAPBPR, the interaction between MHC class I and tapasin does not increase. In light of our findings, previous data determining the function of tapasin in the MHC class I Ag–processing and presentation pathway must be re-evaluated.

The Journal of Immunology, 2013, 191: 000–000.

Received for publication April 5, 2013. Accepted for publication September 19, 2013.

This work was supported by a Wellcome Trust Career Development Award (Grant 085038 to L.H.B.) and a Wellcome Trust Ph.D. Studentship (Grant 089563 to C.H.). J.E.D. is supported by a Royal Society University Research Fellowship.

Address correspondence and reprint requests to Dr. Louise H. Boyle, Cambridge Institute of Medical Research, Wellcome Trust/Medical Research Council Building, University of Cambridge, Addenbrooke’s Hospital, Hills Road, Cambridge CB2 0XY, United Kingdom.

*Department of Pathology, Cambridge Institute of Medical Research, University of Cambridge, Cambridge CB2 0XY, United Kingdom; and †Department of Haematology, Cambridge Institute of Medical Research, University of Cambridge, Cambridge CB2 0XY, United Kingdom.

Copyright © 2013 by The American Association of Immunologists, Inc. 0022-1767/13/$16.00.

Materials and Methods

Homology modeling of TAPBPR

A model for the structure of TAPBPR was generated using the Fold and Function Assignment System based on a profile–profile matching algorithm (28, 29). Tapasin was identified as the closest structural homolog available in the Protein Data Bank, and its structure (PDB-ID 3F8U) (12) was used as a template to generate a model for TAPBPR using the program SCWRL4 to predict and optimize side-chain conformations (30). The model was built for only the luminal domains of TAPBPR. Figures were generated with PyMOL Molecular Graphics System, Version 1.3 (Schrödinger).

Cell culture

HEK-293T, HeLa, and KBM-7 cells were maintained in DMEM, RPMI 1640, and IMDM media (Life Technologies), respectively, supplemented with 10% FCS, 100 U/ml penicillin, and 100 μg/ml streptomycin at 37˚C and 5% CO2. To induce expression of endogenous TAPBPR, cells were treated with 50 U/ml IFN-γ (Roche) at 37˚C for 48 h.

Abbreviations used in this article: ER, endoplasmic reticulum; shRNA, short hairpin RNA; WT, wild-type.

Address correspondence and reprint requests to Dr. Louise H. Boyle, Cambridge Institute of Medical Research, Wellcome Trust/Medical Research Council Building, University of Cambridge, Addenbrooke’s Hospital, Hills Road, Cambridge CB2 0XY, United Kingdom. E-mail address: lhb22@cam.ac.uk.

The online version of this article contains supplemental material.

Copyright © 2013 by The American Association of Immunologists, Inc. 0022-1767/13/$16.00.
 Constructs
PKI-A2 encoding an N-terminally GFP-tagged HLA-A2 molecule was de-
scribed previously (31). Full-length untagged TAPBPR and untagged HLA-
A2 were cloned into pCR-Blunt II-TOPO. Site-directed mutagenesis was
performed to mutate specific residues in TAPBPR or HLA-A2 using Quik-
Change site-directed mutagenesis (Stratagene) along with the primer pairs
listed in Tables I and II. TAPBPR and its variants were subsequently cloned
into the lentiviral vector pHRSIN-C56W-UHEM, producing TAPBPR under
the SFFV promoter and the GFP derivative protein cellular under a ubiquitin
promoter. GFP-A2 or untagged HLA-A2 and their variants were cloned into
the lentiviral vector pHRSIN-PPT-SGW. For RNA interference, lentiviral
short hairpin RNA (shRNA) plasmid V2LHS_155531 on the pCGPz back-
ground (Open Biosystems) was transfected into HEK-293T cells using Tran-
still Transl (Sigma) in the presence of polyjet (Polyjet (Cal-
king Vector Norwell, MA); rabbit anti-GFP (Ab290; Abcam); mouse anti-GFP (Roche);
rabbit anti-calcnexin (Enzo Life Sciences); mAb HCl10, which recognizes
HLA-A, HLA-B, and HLA-C containing a PxxWDR motif at aa 57–62 in
the α1 domain (33, 34); mAb specific for conformational HLA-A2 and
HLA-A68 (One Lambda); and HLA-A2-specific mAb BB7.2 (35). IgG1 and
IgG2a isotype-control Abs were also used (Dako). Species-specific fluo-
rescent and HRP-conjugated secondary Abs were from Molecular Probes
and Dako, respectively.

 Radioactivity and pulse chase
Cells were starved in methionine and cysteine-free RPMI 1640 for 30 min
at 37°C and labeled with [35S]methionine/cysteine (Amersham Pharmacia)
at 37°C for the indicated time. In TAPBPR half-life experi-
ments, samples were chased at 37°C for 0–72 h in medium containing
analogous methionine/cysteine.

 Immunoprecipitation and gel electrophoresis
Cells were lysed in methionine and cysteine-free RPMI 1640 for 30 min
at 37°C and labeled with [35S]methionine/cysteine Promix (Amersham
Pharmacia) at 37°C for the indicated time. In TAPBPR half-life experi-
ments, samples were chased at 37°C for 0–72 h in medium containing
analogous methionine/cysteine.

 Results
MHC class I binding sites defined on tapasin are conserved on
TAPBPR
A series of tapasin mutants (TN3, TN4, TN5, TN6, TN7) identified an
MHC class I binding site on tapasin (12). To determine whether a similar
MHC class I binding site is conserved on TAPBPR, we compared amino acid sequence alignments of human tapasin with
human TAPBPR (Fig. 1A). Although tapasin and TAPBPR are only
∼22% identical, a number of the key residues on tapasin critical for
binding to MHC class I are well conserved on TAPBPR (Fig. 1A).
MHC class I binding sites defined by the tapasin TN6 mutant (E185, 
R187, Q189, Q261) are completely conserved on TAPBPR, whereas
those identified by the tapasin TN7 mutant (H190, L191, K193) are
relatively conserved, sharing the histidine and a charged residue. A
leucine at residue 250, characterized by the tapasin TN5 mutant,
also contributes to MHC class I binding. This is an isoleucine in
TAPBPR and is localized in a region with high sequence identity
with the two molecules sharing the preceding residues EGTY.

 An additional MHC class I interaction site has been suggested on
the membrane proximal domain of tapasin consisting of residues
333–342 (18–21). The amino acid alignment suggests that, although
R333 and S341 are conserved between the two proteins, there is
considerable variation between the two molecules in this region.
However, it is possible that Q334 and S335 in TAPBPR form similar
hydrogen bonds with MHC class I and, therefore, could contribute to
the binding.

We used the crystal structure of human tapasin obtained by Dong
et al. (12) to create a homology model for TAPBPR. The residues
that were shown to be important for MHC class I binding by tapasin
lie on a well-conserved and highly ordered face of tapasin. Our
tematically conserved model indicates that this face is structurally
conserved in TAPBPR, allowing us to predict that MHC class I will bind
TAPBPR in the same manner as it does tapasin (Fig. 1B, 1C). Many
of these residues are highly conserved in TAPBPR across different
species, supporting the possibility that they make up a functionally
important region of TAPBPR (Supplemental Fig. 1).

Residues in the N-terminal and C-terminal domains of
TAPBPR are involved in the association with MHC class I

To determine whether the conserved residues described above form
a MHC class I binding site on TAPBPR, a panel of eight mutant
TAPBPR molecules was made in which charge alterations were
applied following the same concept as Dong et al. (12) (Table I). This
panel of TAPBPR molecules was cloned into a lentiviral expression
vector with a bicistronic GFP reporter and transduced into HeLa
cells. Because endogenous TAPBPR expression is undetectable in
HeLa cells, this cell line provides an ideal system for testing the
effect of alterations to TAPBPR on its ability to bind to MHC
class I. To produce stable HeLa cells expressing comparable
TAPBPR levels, cell lines were sorted based on emerald expression
encoded bistrionically from the TAPBPR protein. Western blotting
for transduced TAPBPR revealed that all TAPBPR mutant
molecules were stably expressed (Fig. 2A).

To analyze the interaction between the TAPBPR molecules and
MHC class I, TAPBPR was immunoprecipitated from cell lysates
using a TAPBPR polyclonal antiserum, followed by Western blotting
for the MHC class I H chain using HC10. A strong association
between TAPBPR and the MHC class I H chain was observed with
wild-type (WT) TAPBPR (Fig. 2A). The TN4 mutant also bound
strongly to MHC class I (Fig. 2A). However, no association was
observed between TAPBPR and MHC class I using the TN5 or TN6
TAPBPR mutants (Fig. 2A). The TN7 mutant exhibited a reduced
capacity to interact with MHC class I. Therefore, like tapasin, a
conserved patch on the N-terminal domain of TAPBPR constitutes

Flow cytometry
Following trypsinization, cells were incubated at 37°C in RPMI 1640 sup-
plemented with 10% FCS to allow membrane recovery from trypsinization.
Cells were stained at 4°C with MHC class I–specific Abs anti-A68 or BB7.2.
Isotype-control Abs were used as negative controls. Abs were subsequently
detected with species-specific Alexa Fluor 647 secondary Abs (Molecular
Probes). Cells were analyzed on a BD Bioscience FACSCalibur four-color
analyzer.
a major binding domain for MHC class I, with residues I261 (TN5), E205, R207, Q209, Q272 (TN6) and H210, K211, R213 (TN7) on TAPBPR contributing to the interaction.

The C-terminal domain of TAPBPR also appeared to contribute to the interaction with MHC class I. No association was observed between TAPBPR and MHC class I using the TC2 or TC3 mutant TAPBPR molecules in which residue R335 or Q336/S337 was altered (Fig. 2A). In contrast, MHC class I could bind to TAPBPR in which nearby residue A339 (TC4) or S343/I344 (TC5) was mutated (Fig. 2A).

The t_{1/2} of the five TAPBPR mutants that showed a reduction in binding to MHC class I was determined by pulse-chase analysis to determine whether the loss of association was a consequence of TAPBPR instability. Pulse-chase analysis revealed that the TAPBPR mutants TN5, TN7, and TC3 had a similar t_{1/2} to WT TAPBPR when expressed in HeLa cells (Fig. 2B). A difference was observed in radiolabeling efficiency for TN6. However, the labeled TAPBPR TN6 protein also appeared stable over time. The TC2 mutant was the only mutant that appeared to be less stable than WT TAPBPR (Fig. 2B). To further examine protein stability of the TAPBPR

---

**Table 1. Panel of TAPBPR mutant molecules**

<table>
<thead>
<tr>
<th>Name</th>
<th>Residues Mutated</th>
<th>Primers Used for Site-Directed Mutagenesis</th>
<th>Predicted TAPBPR Domain</th>
</tr>
</thead>
<tbody>
<tr>
<td>TN4</td>
<td>K22E, D23R</td>
<td>5'-GGTCTTCTAAGTCTTCTGGTGAGGGCCTCCGAGGCTCTTCTCCTG-3'</td>
<td>N-terminal</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5'-GGTCTTCTAAGTCTTCTGGTGAGGGCCTCCGAGGCTCTTCTCCTG-3'</td>
<td>N-terminal</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5'-GGTCTTCTAAGTCTTCTGGTGAGGGCCTCCGAGGCTCTTCTCCTG-3'</td>
<td>N-terminal</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5'-GGTCTTCTAAGTCTTCTGGTGAGGGCCTCCGAGGCTCTTCTCCTG-3'</td>
<td>N-terminal</td>
</tr>
<tr>
<td>TN5</td>
<td>I261K</td>
<td>5'-GGTCTTCTAAGTCTTCTGGTGAGGGCCTCCGAGGCTCTTCTCCTG-3'</td>
<td>N-terminal</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5'-GGTCTTCTAAGTCTTCTGGTGAGGGCCTCCGAGGCTCTTCTCCTG-3'</td>
<td>N-terminal</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5'-GGTCTTCTAAGTCTTCTGGTGAGGGCCTCCGAGGCTCTTCTCCTG-3'</td>
<td>N-terminal</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5'-GGTCTTCTAAGTCTTCTGGTGAGGGCCTCCGAGGCTCTTCTCCTG-3'</td>
<td>N-terminal</td>
</tr>
<tr>
<td>TN6</td>
<td>E205K, R207E, Q209S, Q272S</td>
<td>5'-GGTCTTCTAAGTCTTCTGGTGAGGGCCTCCGAGGCTCTTCTCCTG-3'</td>
<td>N-terminal</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5'-GGTCTTCTAAGTCTTCTGGTGAGGGCCTCCGAGGCTCTTCTCCTG-3'</td>
<td>N-terminal</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5'-GGTCTTCTAAGTCTTCTGGTGAGGGCCTCCGAGGCTCTTCTCCTG-3'</td>
<td>N-terminal</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5'-GGTCTTCTAAGTCTTCTGGTGAGGGCCTCCGAGGCTCTTCTCCTG-3'</td>
<td>N-terminal</td>
</tr>
<tr>
<td>TN7</td>
<td>H210S, K211E, R213E</td>
<td>5'-GGTCTTCTAAGTCTTCTGGTGAGGGCCTCCGAGGCTCTTCTCCTG-3'</td>
<td>N-terminal</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5'-GGTCTTCTAAGTCTTCTGGTGAGGGCCTCCGAGGCTCTTCTCCTG-3'</td>
<td>N-terminal</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5'-GGTCTTCTAAGTCTTCTGGTGAGGGCCTCCGAGGCTCTTCTCCTG-3'</td>
<td>N-terminal</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5'-GGTCTTCTAAGTCTTCTGGTGAGGGCCTCCGAGGCTCTTCTCCTG-3'</td>
<td>N-terminal</td>
</tr>
<tr>
<td>TC2</td>
<td>R335D</td>
<td>5'-GGTCTTCTAAGTCTTCTGGTGAGGGCCTCCGAGGCTCTTCTCCTG-3'</td>
<td>N-terminal</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5'-GGTCTTCTAAGTCTTCTGGTGAGGGCCTCCGAGGCTCTTCTCCTG-3'</td>
<td>N-terminal</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5'-GGTCTTCTAAGTCTTCTGGTGAGGGCCTCCGAGGCTCTTCTCCTG-3'</td>
<td>N-terminal</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5'-GGTCTTCTAAGTCTTCTGGTGAGGGCCTCCGAGGCTCTTCTCCTG-3'</td>
<td>N-terminal</td>
</tr>
<tr>
<td>TC3</td>
<td>Q336D, S337D</td>
<td>5'-GGTCTTCTAAGTCTTCTGGTGAGGGCCTCCGAGGCTCTTCTCCTG-3'</td>
<td>N-terminal</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5'-GGTCTTCTAAGTCTTCTGGTGAGGGCCTCCGAGGCTCTTCTCCTG-3'</td>
<td>N-terminal</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5'-GGTCTTCTAAGTCTTCTGGTGAGGGCCTCCGAGGCTCTTCTCCTG-3'</td>
<td>N-terminal</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5'-GGTCTTCTAAGTCTTCTGGTGAGGGCCTCCGAGGCTCTTCTCCTG-3'</td>
<td>N-terminal</td>
</tr>
<tr>
<td>TC4</td>
<td>A339D</td>
<td>5'-GGTCTTCTAAGTCTTCTGGTGAGGGCCTCCGAGGCTCTTCTCCTG-3'</td>
<td>C-terminal</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5'-GGTCTTCTAAGTCTTCTGGTGAGGGCCTCCGAGGCTCTTCTCCTG-3'</td>
<td>C-terminal</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5'-GGTCTTCTAAGTCTTCTGGTGAGGGCCTCCGAGGCTCTTCTCCTG-3'</td>
<td>C-terminal</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5'-GGTCTTCTAAGTCTTCTGGTGAGGGCCTCCGAGGCTCTTCTCCTG-3'</td>
<td>C-terminal</td>
</tr>
<tr>
<td>TC5</td>
<td>S343R, I344T</td>
<td>5'-GGTCTTCTAAGTCTTCTGGTGAGGGCCTCCGAGGCTCTTCTCCTG-3'</td>
<td>C-terminal</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5'-GGTCTTCTAAGTCTTCTGGTGAGGGCCTCCGAGGCTCTTCTCCTG-3'</td>
<td>C-terminal</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5'-GGTCTTCTAAGTCTTCTGGTGAGGGCCTCCGAGGCTCTTCTCCTG-3'</td>
<td>C-terminal</td>
</tr>
</tbody>
</table>
All experiments were repeated independently at least three times.

For each of the TAPBPR variants, as indicated in the key. Lines represent signal intensity at the 0-h time point. Symbols indicate specific data points.

The half-life of TAPBPR was plotted as a percentage of the reimmunoprecipitated with TAPBPR mAb raised to aa 23–122 of TAPBPR.

Nuclear supernatants. Following elution and denaturation, TAPBPR was isolated by immunoprecipitation using mAb PeTe4 from precleared post-

WT TAPBPR expressed in HeLa cells via pulse-chase analysis. HeLa cells and TAPBPR immunoprecipitates as indicated. (Fig. 2C–E) showed a strong association with MHC class I, as measured by coinmunoprecipitation with TAPBPR (Fig. 2A). Surprisingly, the TN4 mutant, which appeared to show a strong association with MHC class I, was not quite as efficient as WT TAPBPR (Fig. 2E). However, they were not quite as efficient as WT TAPBPR (Fig. 2E).

The GFP tag was used to differentiate between the endogenous HLA alleles in HeLa cells and the transduced HLA-A2, which runs at ~70 kDa. We reported previously that this N-terminal GFP tag did not significantly alter export rates or surface expression of HLA-A2 compared with untagged HLA-A2 (31). To induce TAPBPR expression, the HeLa cells were treated with IFN-γ (27). In TAPBPR immunoprecipitation experiments, an association was observed between TAPBPR and the GFP-A2 WT molecule (Fig. 3A). In contrast, no association was observed between TAPBPR and GFP-A2–T134K in which a threonine at position 134 was mutated to a lysine. In agreement with previously reported findings (13, 14), mutation of T134K reduced the expression of conformation HLA-A2 detected by BB7.2 to ~30% of WT-A2 in IFN-γ-induced HeLa cells (Fig. 3B). Other single-point mutations (D122R, E128R, W133T) in the loop under the α2-1 helix of MHC class I similarly affected the ability of TAPBPR to associate with HLA-A2 (Supplemental Fig. 3). The lysine residue at position 144, which points outward in the α2-1 helix, was also important for the association between TAPBPR and the MHC class I H chain (Supplemental Fig. 3), whereas the lysine residue at position 146, which points inward toward the peptide-binding groove, was not critical in the association between TAPBPR and MHC class I (Fig. 3A). As controls, immunoprecipitation experiments were performed in parallel for tapasin in the IFN-γ–treated HeLa panel. As expected, GFP-A2 WT bound to tapasin, but GFP-A2–T134K failed to associate (Fig. 3A).

These findings were confirmed in reciprocal immunoprecipitation...
experiments in which GFP-A2 was immunoprecipitated, followed by blotting for tapasin and TAPBPR (Fig. 3C).

**Mutation of residues in MHC class I α3 domain inhibit TAPBPR binding**

The C-terminal domain of tapasin is predicted to bind to residues 222–229 in the α3 domain of the MHC class I H chain (20, 22–25). To determine whether these residues on MHC class I were required for association with TAPBPR, two GFP-A2 constructs were made in which amino acids in this region were mutated (Table II). The GFP-A2 α3-1 mutant, in which four residues in the α3 domain were mutated (E222K, D223R, D227R, E229K), did not bind to TAPBPR, demonstrating that this region is crucial for MHC class I to associate with TAPBPR (Fig. 3A, 3C). The GFP-A2 α3-2 mutant, in which only residues D227 and E229 were mutated, also did not bind to TAPBPR, confirming that this region is crucial for MHC class I to associate with TAPBPR. Two GFP-A2 constructs were made in which the C-terminal domain of the MHC class I H chain (20, 22–25) was predicted to bind to residues 222–229 in the α3 domain of the MHC class I H chain (20, 22–25). To determine whether these residues on MHC class I were required for association with TAPBPR, two GFP-A2 constructs were made in which amino acids in this region were mutated (Table II). The GFP-A2 α3-1 mutant, in which four residues in the α3 domain were mutated (E222K, D223R, D227R, E229K), did not bind to TAPBPR, demonstrating that this region is crucial for MHC class I to associate with TAPBPR (Fig. 3A, 3C). The GFP-A2 α3-2 mutant, in which only residues D227 and E229 were mutated, also did not bind to TAPBPR, refining the binding site further to involve these two amino acids. As expected, mutation to this region of the α3 domain also inhibited binding of tapasin to the MHC class I H chain (Fig. 3A, 3C). Again, cell surface expression of conformational HLA-A2 detected with BB7.2 was severely reduced in these mutants, which were no longer able to bind to tapasin or TAPBPR (Fig. 3B).

**Mutant MHC class I molecules associate with β2m**

To further verify the importance of T134 and residues in the α3 domain of MHC class I in the association with TAPBPR, untagged WT A2, T134K, or α3-2 A2 was expressed in the classical MHC class I α- cell line 721.221. In TAPBPR immunoprecipitation experiments, only untagged WT-A2 was found to associate with TAPBPR (Fig. 3D). Neither untagged T134K nor untagged α3-2 was able to associate with TAPBPR (Fig. 3D). These results using untagged HLA-A2 molecules expressed in 721.221 confirm the finding observed with the GFP-tagged HLA-A2 molecules expressed in HeLa cells.

Because β2m is crucial for the association of both TAPBPR and tapasin with the MHC class I H chain, the ability of the T134K and α3-2 mutant HLA-A2 molecules to associate with β2m was determined. Like WT A2, both T134K and α3-2 mutant HLA-A2 were found to associate with β2m when expressed in 721.221 (Fig. 3D). Therefore, the loss of interaction of the mutant HLA-A2 molecules with TAPBPR is not an indirect consequence of failure to associate with β2m.

**The association of MHC class I with TAPBPR is increased in the absence of tapasin**

Because TAPBPR and tapasin bind in a similar orientation to the same face of MHC class I, it is possible that they compete with each other for MHC class I binding. To investigate whether tapasin competes with TAPBPR for MHC class I, the association of MHC class I with TAPBPR was compared in WT and tapasin-deficient KBM-7 cells. Immunoprecipitation of total cellular TAPBPR revealed a significant increase in the association of MHC class I with TAPBPR in tapasin-deficient cells compared with WT cells (Fig. 4A). This increased association was quantified further in radiolabeled cells, which revealed an approximate 2-fold increase (1.97 ± 0.065) in the association of MHC class I with TAPBPR in the absence of tapasin (Fig. 4B). Therefore, tapasin can compete with TAPBPR for MHC class I binding.

**The association of MHC class I with tapasin does not increase in the absence of TAPBPR**

Next, we determined whether TAPBPR can compete with tapasin for MHC class I binding. If TAPBPR competes with tapasin for MHC class I binding, then the association of MHC class I with tapasin should be regulated negatively by TAPBPR expression (i.e., depletion of TAPBPR should increase the association of MHC I with tapasin, whereas overexpression of TAPBPR should decrease the association of MHC I with tapasin). However, tapasin immunoprecipitation experiments did not reveal an increase in MHC class I binding to tapasin upon TAPBPR depletion in KBM-7 (Fig. 4A) or HeLa (Fig. 4C) cells. In contrast, densitometry in radiolabeled cells revealed that the association between tapasin and MHC class I was reduced slightly (by 6.5 ± 0.02%) in the absence of TAPBPR (Fig. 4B). Supporting this finding, a slight decrease in the association between tapasin and MHC class I was observed in the absence of TAPBPR in steady-state immunoprecipitation experiments in HeLa cells (Fig. 4C). Finally, the inability of TAPBPR to compete with tapasin for MHC class I was confirmed in HeLa cells overexpressing TAPBPR, in which a slight, but consistent, increase in association was observed between tapasin and MHC class I compared with WT HeLa cells (Fig. 4C). Taken together, these results suggest that TAPBPR does not compete with tapasin for MHC class I.

**Discussion**

In this study, we show that TAPBPR binds in a similar orientation to the same face of MHC class I as does tapasin. Therefore, a single MHC class I molecule cannot bind tapasin and TAPBPR at the same time. Thus, TAPBPR and tapasin binding to MHC class I are mutually exclusive. In agreement with this, we showed previously that there was no association between tapasin and TAPBPR in
immunoprecipitation experiments. Furthermore, tapasin bound to MHC class I in the absence of TAPBPR, and TAPBPR bound to MHC class I in the absence of tapasin (27).

FIGURE 3. Residues in the α2 and α3 domain of MHC class I are crucial for associating with TAPBPR. (A) TAPBPR or tapasin was isolated by immunoprecipitation using PeTe4 and Pasta1, respectively, from IFN-γ-treated HeLa cells stably expressing a panel of GFP-tagged HLA-A2 mutant molecules lysed in 1% digitonin–TBS. Western blot analysis was performed for TAPBPR (R021), tapasin (Rgp48N), or GFP-A2 (using anti-GFP) on lysates and immunoprecipitates, as indicated. The last lane on the TAPBPR and tapasin immunoprecipitation gels is a lysate from GFP-A2 WT cells. (B) Cytofluorometric analysis of HLA-A2 on HeLa cells stably transduced with selected GFP-A2 mutant molecules. All cell lines were treated with IFN-γ for 48 h before staining with the HLA-A2 conformational-specific Ab BB7.2. HeLa cells (gray dashed line), GFP-A2 WT (filled graph), GFP-A2–T134K (black line), and GFP-A2 α3-2 (gray line). (C) GFP-A2 was isolated by immunoprecipitation using a GFP-specific Ab from IFN-γ-treated HeLa cells stably expressing the panel of GFP-tagged HLA-A2 mutant molecules. Western blot analysis was performed for TAPBPR, tapasin, or GFP-A2 (using an anti-GFP Ab), as indicated. The last lane on the gel is a lysate from GFP-A2 WT cells. All experiments were repeated independently at least three times. (D) Untagged HLA-A2 WT, A2–T134K, or A2 α3-2 was expressed in the classical MHC class I cell line 721.221. After solubilization in 1% digitonin–TBS, immunoprecipitation was performed for TAPBPR (using mAb PeTe4) or β2m from precleared postnuclear lysates. Western blot analysis was performed for HLA-A2 using HCA2. Nontransfected 721.221 cells were included as a negative control (-).

FIGURE 4. The MHC I:TAPBPR association increases in the absence of tapasin, but the MHC I:tapasin association does not increase in the absence of TAPBPR. (A) Total cellular levels of TAPBPR and tapasin were isolated by immunoprecipitation with PeTe4 and Pasta1, respectively, from IFN-γ-treated WT, tapasin gene trap knockout, or KBM-7 cells depleted of TAPBPR by stable transduction with TAPBPR-specific shRNA lysed in 1% digitonin–TBS. Western blot analysis was performed for TAPBPR (R021), tapasin (Rgp48N), MHC class I (HC10 and HCA2), or calnexin (rabbit anti-calnexin). (B) IFN-γ-treated WT, tapasin gene trap knockout, or TAPBPR-depleted KBM-7 cells were radiolabeled with [35S]cysteine/methionine for 30 min, followed by immunoprecipitation of TAPBPR (using mAb PeTe4) or tapasin (using Pasta1). Signal intensity of the MHC class I H chain bands was determined by densitometry. To calculate the fold change in MHC class I association with tapasin or TAPBPR, the following calculation was used: MHC class I H chain bands for test cell line/MHC class I H chain bands for WT cell line. This was normalized to the fold change in signal intensity of immunoprecipitated protein (i.e., tapasin or TAPBPR). (C) Total cellular levels of TAPBPR and tapasin were isolated by immunoprecipitation with PeTe4 and Pasta1, respectively, from IFN-γ-treated HeLa-S, HeLa-S shTAPBPR, HeLa-M, or HeLa-M overexpressing WT TAPBPR cells lysed in 1% digitonin. Western blot analysis was performed for TAPBPR (R021), tapasin (Rgp48N), MHC class I (HC10 and HCA2), and calnexin (rabbit anti-calnexin). All experiments were repeated independently at least three times.

We previously demonstrated that overexpression of TAPBPR severely reduces surface levels of peptide-loaded MHC class I and increases MHC class I free H chain expression (27). Because overexpression of TAPBPR resembles the phenotype of a tapasin-deficient cell, superficially TAPBPR appears to oppose the function of tapasin in the MHC class I Ag-presentation system. This is highly reminiscent of the opposing effect of HLA-DO on the function of HLA-DM in the MHC class II Ag-presentation system (36–38), raising the possibility that TAPBPR is the equivalent of...
HLA-DO for the MHC class I system. It was shown recently that HLA-DO is a MHC class II mimic and binds tightly to HLA-DM, directly suppressing HLA-DM from interacting with MHC class II (39). Because there is no direct association of TAPBPR with tapasin, mechanistically TAPBPR does not appear to work in the same manner as HLA-DO. However, with TAPBPR and tapasin being oriented on MHC class I in a similar manner, an opposing role of TAPBPR could be envisaged by it competing directly with tapasin for MHC class I binding. However, this also does not appear to be the case because TAPBPR expression does not decrease the interaction between MHC class I and tapasin. Surprisingly, TAPBPR expression slightly increases the tapasin:MHC class I association. Thus, TAPBPR does not compete with tapasin for MHC class I binding but actually may cooperate with tapasin. However, tapasin competes with TAPBPR for MHC class I. Therefore, how can we explain the fact that MHC class I surface expression in HeLa cells overexpressing TAPBPR resembles tapasin deficiency? In both situations, the ratio of tapasin/TAPBPR is altered in favor of TAPBPR, implicating an increased association of MHC class I with TAPBPR in some of the phenotypic effects observed in tapasin-deficient cells.

In light of the discovery of TAPBPR as an additional chaperone oriented on MHC class I in the same way as tapasin, the precise function of tapasin in the Ag-presentation pathway now needs to be re-evaluated. Much of the work examining tapasin function has understandably been performed using either tapasin-deficient cells (6, 8, 40–45) or mutant MHC class I molecules that no longer associate with tapasin (13, 14, 16, 23, 25). However, in tapasin-deficient cells, it is now apparent that TAPBPR is present and still capable of binding to MHC class I. In fact, MHC class I molecules exhibit increased binding to TAPBPR in the absence of tapasin. Therefore, the findings observed in cells lacking tapasin are likely to be highly reflective of TAPBPR function. More alarming is the issue of experiments in which MHC class I mutants were used, such as T134K, because it is clear from our data that it is not only the functional effect of tapasin on MHC class I that is lost, but also that of TAPBPR.

Our discovery of the interaction between TAPBPR and MHC class I helps to explain some of the conflicting data generated with regard to the function of tapasin over the past 2 decades. There is no doubt about the critical role played by tapasin in the loading of peptide onto MHC class I given the severe reduction in MHC class I surface expression in the absence of tapasin (2, 8, 40, 45, 46). However, there have been some discrepancies with regard to the function of tapasin as a peptide editor, a process in which low-affinity peptides are replaced with those exhibiting higher affinity (3, 6, 8–10, 17, 43, 47–49). It is likely that some of the conflicting data generated are due to the influence of TAPBPR on MHC class I. For example, the T134K mutation has a more severe effect on surface expression of conformational HLA-A2 than does the absence of tapasin (T134K-A2 is expressed at only 20% of WT A2 in C1R cells, whereas HLA-A2 in 721.220 cells is expressed at 50–60% of HLA-A2 in 721.221 cells) (13, 14, 41). Such differences are likely to be due to a lack of binding of TAPBPR to HLA-A2–T134K compared with an increased association between MHC class I and TAPBPR in the experiments using 721.220 cells. Our findings also raise the question of why are there two MHC class I–specific chaperones in the Ag-presentation pathway oriented on MHC class I in a similar manner. Are there two alternative pathways of peptide loading or a single pathway that involves sequential engagement of the two related proteins? Is one protein primarily involved in peptide loading and the other in peptide editing? Is one the ER-resident MHC class I chaperone, whereas the other performs a similar function outside the peptide loading complex? We favor the idea of a single pathway for MHC class I peptide loading in which tapasin and TAPBPR represent sequential steps in the same pathway. We speculate that MHC class I first associates with tapasin to be loaded with peptide and then interacts with TAPBPR. An initial interaction of MHC class I with tapasin instead of TAPBPR could be influenced by subtle alterations in the form of MHC class I, accessory proteins, or spatial separation. If MHC class I sequentially engages with the two related proteins in this manner, TAPBPR could monitor the stability of MHC class I as it dissociates from the peptide loading complex. This is consistent with the increased binding of TAPBPR to MHC class I in the absence of tapasin, a condition that produces suboptimally loaded MHC class I. In this way, TAPBPR acts as a second quality control checkpoint or post–peptide loading complex gatekeeper for MHC class I. Given the orientation of TAPBPR on MHC class I, it is conceivable that TAPBPR is capable of peptide editing. In support of an influence of TAPBPR in peptide selection, TAPBPR slows down the anterograde transport of MHC class I, a property that was shown to allow MHC class I to efficiently optimize its peptide cargo (16, 27). Furthermore, TAPBPR prolongs the contact between MHC class I and the peptide loading complex, an event that is highly likely to alter peptide selection by MHC class I (27). Another question under investigation is whether tapasin and TAPBPR exhibit differential preference for specific HLA gene products. Separation of the function of tapasin from that of TAPBPR is needed to further elucidate the molecular mechanism governing peptide selection by MHC class I molecules.

Acknowledgments

We thank Peter Cresswell for Abs and John Trowsdale (University of Cambridge) for discussions and critical reading of the manuscript.

Disclosures

The authors have no financial conflicts of interest.

References


