

# Impaired immune responses and altered peptide repertoire in tapasin-deficient mice

Natalio Garbi<sup>1</sup>, Pamela Tan<sup>1</sup>, Alexander D. Diehl<sup>2</sup>, Benedict J. Chambers<sup>2</sup>, Hans-Gustaf Ljunggren<sup>2</sup>, Frank Momburg<sup>1</sup> and Günter J. Hammerling<sup>1</sup>

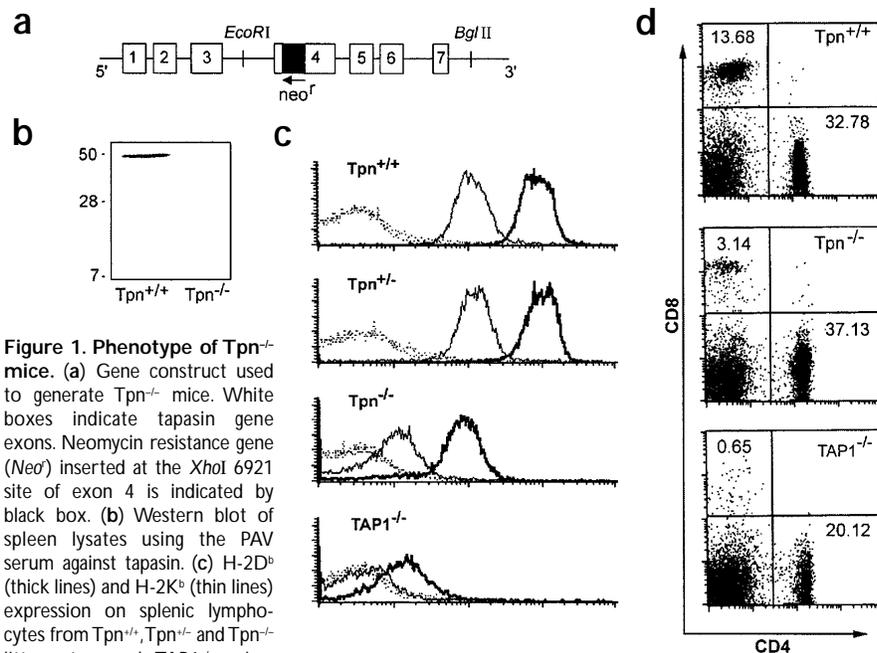
Tapasin is a component of the major histocompatibility complex (MHC) class I antigen-loading complex. Here we show that mice with a disrupted tapasin gene display reduced MHC class I expression. Cytotoxic T cell (CTL) responses to viruses are impaired, and dendritic cells of tapasin-deficient mice do not cross-present protein antigen via the MHC class I pathway, indicating a defect in antigen processing. Natural killer (NK) cells from tapasin-deficient mice have an altered repertoire and are self-tolerant. In addition, the repertoire of class I-bound peptides is altered towards less stably binding ones. Thus tapasin plays a role in CTL and NK immune responses and in optimal peptide selection.

Cytotoxic T lymphocytes (CTLs) recognize peptides from foreign antigens that are presented by major histocompatibility complex (MHC) class I molecules. Antigens are degraded in the cytosol by proteasomes and the resulting peptides are translocated by the transporter associated with antigen presentation (TAP) into the endoplasmic reticulum (ER) where they bind to MHC class I molecules<sup>1</sup>. Only properly assembled MHC class I-peptide complexes are transported to the cell surface. Peptide binding appears to be facilitated by a loading complex consisting of MHC class I, tapasin, TAP, the ER chaperone calreticulin<sup>2</sup> and the thiol oxidoreductase ER60<sup>3-5</sup>. Tapasin is a 48-kD transmembrane protein<sup>2</sup> that is encoded by a gene at the centromeric end of the MHC<sup>6,7</sup>. Tapasin appears to be essential for the loading complex as it forms a bridge between TAP and MHC class I molecules, so that four tapasin class I-calreticulin complexes are bound to each TAP1-TAP2 heterodimer<sup>8</sup>. After peptide loading, class I molecules undergo conformational changes<sup>9</sup>, triggering their release from tapasin and export to the cell surface.

*In vitro* work with the tapasin-mutant B cell line 721.220<sup>10</sup> and with transfected insect cells has suggested a role for tapasin in MHC class I surface expression and peptide loading<sup>8,11-15</sup>, but the requirement for tapasin appears to vary depending on the *HLA* allele investigated<sup>12,16,17</sup>. In insect cells tapasin was found to retain class I molecules in the ER<sup>13</sup>, but this function alone does not explain why tapasin enhances peptide

loading because soluble tapasin devoid of the ER-retention motif can still support loading<sup>18</sup>.

Because most functional data on tapasin have been generated with the use of the above-mentioned insect cells or the human mutant cell



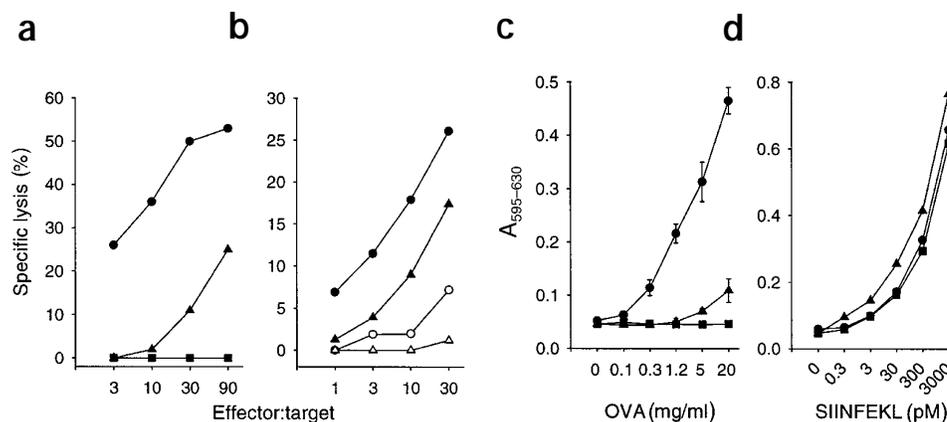
**Figure 1. Phenotype of *Tpn*<sup>-/-</sup> mice.** (a) Gene construct used to generate *Tpn*<sup>-/-</sup> mice. White boxes indicate tapasin gene exons. Neomycin resistance gene (*Neo<sup>r</sup>*) inserted at the *Xho*I 6921 site of exon 4 is indicated by black box. (b) Western blot of spleen lysates using the PAV serum against tapasin. (c) H-2D<sup>b</sup> (thick lines) and H-2K<sup>b</sup> (thin lines) expression on splenic lymphocytes from *Tpn*<sup>+/+</sup>, *Tpn*<sup>+/-</sup> and *Tpn*<sup>-/-</sup> littermates, and *TAP1*<sup>-/-</sup> mice. Controls were isotype-matched antibodies to TNP (dotted lines).

Data shown are representative of four independent experiments. The distinct staining intensity of H-2D<sup>b</sup> and H-2K<sup>b</sup> antibodies is due to different biotinylation. (d) CD4/CD8 ratio of splenic lymphocytes. Staining of *Tpn*<sup>-/-</sup> cells was similar to *Tpn*<sup>+/+</sup> cells (data not shown). Results are representative of several independent experiments.

<sup>1</sup>Division of Molecular Immunology, German Cancer Research Center, Im Neuenheimer Feld 280, D – 69120 Heidelberg, Germany. <sup>2</sup>Microbiology and Tumor Biology Center, Karolinska Institute, S–17177 Stockholm, Sweden. Correspondence should be addressed to G.J.H. ([hammerling@dkfz.de](mailto:hammerling@dkfz.de)).

**Figure 2. Reduced influenza-specific CTL responses and antigen presentation by  $Tpn^{-/-}$  bone marrow-derived DCs.**

(a) Influenza virus-specific CTL responses of  $Tpn^{-/-}$  ( $\blacktriangle$ ),  $TAP1^{-/-}$  ( $\blacksquare$ ) and wild-type ( $\bullet$ ) mice against influenza NP(366–374)-loaded RMA cells. (b) Presentation of influenza antigen by infected DC from  $Tpn^{-/-}$  and wild-type DC to influenza-specific C57BL/6 (B6)-derived CTL. For control, noninfected  $Tpn^{-/-}$  ( $\triangle$ ) and wild-type ( $\circ$ ) DC were used. (c) Cross-presentation of soluble ovalbumin (OVA) by  $Tpn^{-/-}$ ,  $TAP1^{-/-}$  and wild-type DC to H-2K<sup>b</sup>-OVA-specific T cell hybridoma B3Z. Decreased absorbancy values indicate reduced cross-presentation. Symbols as in a. (d) Presentation of exogenous OVA peptide SIINFEKL by DCs to B3Z. Results are representative of several independent experiments. Symbols as in a.



line 721.220, which contains residual expression of a truncated tapasin product<sup>19</sup>, it is still not clear how tapasin would effect the complex interactions of an immune response. To understand the function of tapasin *in vivo* we generated, by homologous recombination, mice with deficient tapasin expression. We show that tapasin-negative mice are not only compromised in their CTL and natural killer (NK) cell responses but also in their ability to optimally select and load peptides.

## Results

### Phenotype of tapasin-deficient mice

The tapasin gene construct used for generation of knockout mice is schematically shown in **Fig. 1a**. Tapasin-deficient ( $Tpn^{-/-}$ ) mice developed and bred normally but tapasin protein was not detectable by immunoblotting (**Fig. 1b**). Cell surface staining of splenocytes revealed a ~90% reduction of MHC class I H-2K<sup>b</sup> and H-2D<sup>b</sup> expression, which indicates a requirement for tapasin in class I assembly, but this reduction was not as profound as the one observed in  $TAP1^{-/-}$  mice<sup>20</sup> (**Fig. 1c**). Similar results were obtained for lymph node cells and peripheral blood lymphocytes (data not shown). The number of CD8<sup>+</sup> T cells in the periphery, but not of CD4<sup>+</sup> T cells, was reduced (**Fig. 1d**) suggesting impaired positive selection due to low class I expression in the thymus. Indeed, the number of single positive CD8<sup>+</sup> T cells in the thymus was reduced (data not shown).

### Impaired CTL responses and antigen presentation by DC

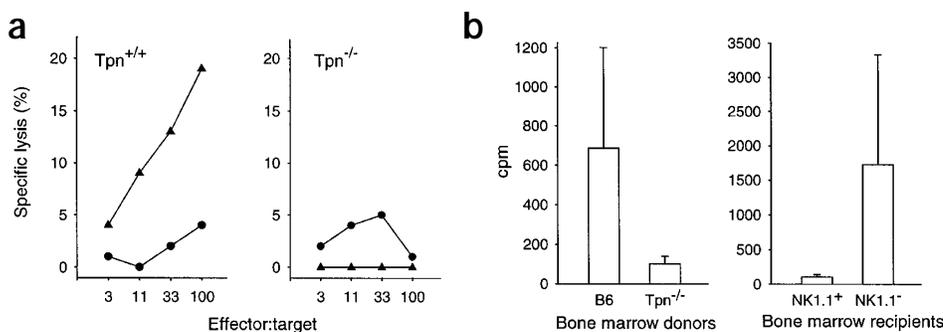
To assess immune responsiveness, CD8<sup>+</sup> T cell responses to virus and protein antigen were investigated.  $Tpn^{-/-}$  mice were infected with influenza virus and CTL activity determined by lysis of peptide-loaded RMA target cells. Influenza-specific CTL activity was reduced in  $Tpn^{-/-}$  mice (**Fig. 2a**). This was probably due to two factors: the diminished frequency of CD8<sup>+</sup> T cells and the inability of  $Tpn^{-/-}$  cells to efficiently

present influenza antigen via the MHC class I pathway. Because dendritic cells (DCs) are more readily infected with influenza virus than lymphocytes, we studied their ability to present influenza antigens. Infected DCs from  $Tpn^{-/-}$  mice showed reduced lysis by influenza-specific CTLs obtained from infected wild-type mice, suggesting a presentation defect (**Fig. 2b**).

We also investigated the ability of DC to cross-present exogenous protein via the MHC class I pathway<sup>21</sup>. There is evidence that this cross-presentation mechanism is crucial for antiviral immunity<sup>22</sup> and tolerance induction to organ-specific antigens<sup>23</sup> thus we investigated the role of tapasin in cross-presentation. After bone marrow-derived DC were pulsed with OVA only wild-type DCs, and not  $Tpn^{-/-}$  or  $TAP1^{-/-}$  DCs, could present OVA via MHC class I molecules to the OVA-H-2K<sup>b</sup>-specific hybridoma B3Z (**Fig. 2c**). There was no difference in presentation of the H-2K<sup>b</sup>-binding OVA peptide SIINFEKL (**Fig. 2d**). Thus, tapasin was required for peptide loading in the cross-presentation pathway of DCs.

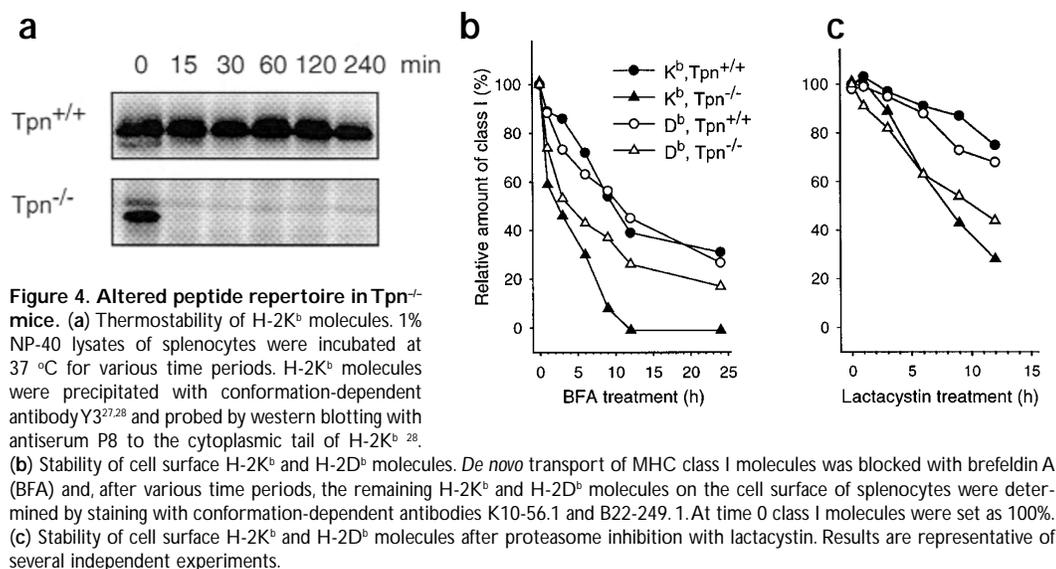
### Altered NK cell repertoire in $Tpn^{-/-}$ mice

Splenic NK cell numbers, as assessed by flow cytometry using the DX5 monoclonal antibody (mAb), were similar in C57BL/6 (B6) and  $Tpn^{-/-}$  mice ( $3.76 \pm 0.93\%$ ;  $3.73 \pm 0.51\%$ , respectively). Interleukin 2 (IL-2)-activated NK cells from B6 mice readily killed  $Tpn^{-/-}$  target cells, whereas B6 targets survived (**Fig. 3a**). In contrast, IL-2-activated NK cells from  $Tpn^{-/-}$  mice did not kill  $Tpn^{-/-}$  target cells indicating that  $Tpn^{-/-}$  NK cells had an altered specificity. The latter result was not a mere consequence of a general impairment of NK cell-mediated cytotoxicity in  $Tpn^{-/-}$  mice, as NK cells from  $Tpn^{-/-}$  mice readily killed YAC-1 targets (58% specific lysis at an effector:target ratio of 100:1). In all respects, NK cells from B6 mice and  $Tpn^{-/-}$  mice displayed similar patterns of reactivity, excluding the possibility that the effects



**Figure 3. Altered NK cell repertoire in  $Tpn^{-/-}$  mice.**

(a) Cytotoxic activity of IL-2-activated NK cells from wild-type (left panel) and  $Tpn^{-/-}$  (right panel) mice against wild-type ( $\bullet$ ) and  $Tpn^{-/-}$ -derived ( $\blacktriangle$ ) Concanavalin (Con A) blast targets. (b) *In vivo* rejection responses against wild-type or  $Tpn^{-/-}$  bone marrow grafted to B6 mice. Rejection of wild-type and  $Tpn^{-/-}$  grafts by B6 mice (left panel). Rejection of  $Tpn^{-/-}$  grafts by untreated (NK1.1+) or NK1.1-depleted (NK1.1-) B6 recipients (right panel). Low levels of incorporated <sup>125</sup>I-5-iodo-2'-deoxyuridine is indicative of rejection of grafted bone marrow.



observed with  $Tpn^{-/-}$  NK cells were merely related to the 129/Sv background genes in the  $Tpn^{-/-}$  mice. In line with the *in vitro* cytotoxicity data,  $Tpn^{-/-}$  bone marrow cells were readily rejected in B6 mice but were not in NK1.1<sup>+</sup> cell-depleted B6 mice (Fig. 3b). This demonstrates an NK cell-dependent rejection response against  $Tpn^{-/-}$  bone marrow grafts *in vivo*.

### Impaired peptide selection in $Tpn^{-/-}$ mice

Tapasin may have a quantitative effect on the peptide loading process as suggested by the impaired antigen presentation by DCs from  $Tpn^{-/-}$  mice (Fig. 2). Consequently we investigated whether or not tapasin could influence the selection of the class I-bound peptides. Because peptides are crucial for stabilization of MHC molecules, their thermostability reflects the average affinity of the spectrum of bound peptides<sup>24,25</sup>. Cell lysates of spleen cells were incubated for various time periods at 37 °C and remaining H-2K<sup>b</sup> molecules precipitated with the Y3 antibody, which binds only to conformationally intact  $\alpha_1$ - $\alpha_2$  domains of H-2K<sup>b</sup>, and not to denatured molecules<sup>26,27</sup>. The amount of precipitated H-2K<sup>b</sup> was determined by western blotting using antiserum to the H-2K<sup>b</sup> cytoplasmic tail<sup>28</sup>. The upper H-2K<sup>b</sup> bands were endoglycosidase H (endoH) resistant and therefore reflected post-Golgi material. The lower bands were endoH sensitive and represented H-2K<sup>b</sup> molecules that were still in the ER (Fig. 4a and data not shown).

The H-2K<sup>b</sup> molecules in lysates of tapasin-deficient cells decayed rapidly at 37 °C and were clearly less stable than wild-type-derived H-2K<sup>b</sup> (Fig. 4a). As T cell recognition depends on MHC peptides displayed at the cell surface, we directly measured the effect of tapasin on the thermostability of class I molecules on the cell. *De novo* transport of MHC class I to the cell surface was blocked with the drug BFA<sup>29</sup> in  $Tpn^{-/-}$  and  $Tpn^{+/+}$  lymphocytes, and cells were stained with H-2K<sup>b</sup> and H-2D<sup>b</sup> antibodies that were specific for intact MHC class I conformation. Surface MHC class I molecules on tapasin-negative cells decayed faster than those of  $Tpn^{+/+}$  cells (Fig. 4b). In several experiments the half-life for H-2K<sup>b</sup> ranged between 0.5–2.5 h in  $Tpn^{-/-}$  cells versus 9–10 h in wild-type cells whereas the half-life for H-2D<sup>b</sup> ranged between 2–4 h in  $Tpn^{-/-}$  versus 10–12 h in wild-type cells.

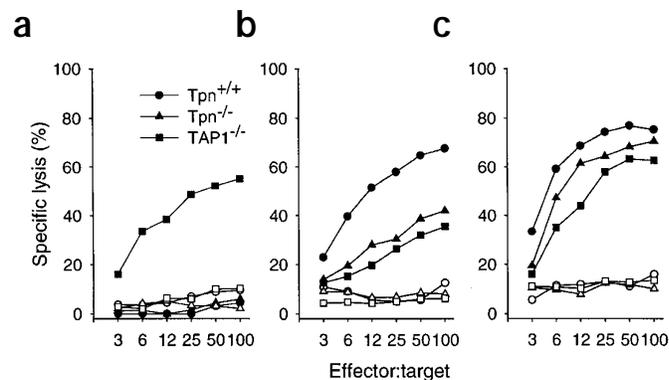
Although conformation-specific antibodies were used for detection of MHC class I molecules, it was not clear from these results whether the H-2K<sup>b</sup> and H-2D<sup>b</sup> molecules on  $Tpn^{-/-}$  cells were empty or

occupied with peptide. To investigate the peptide dependency,  $Tpn^{-/-}$  cells were incubated for various times with lactacystin, an inhibitor of the proteasome that is the major supplier for MHC class I-binding peptides. Lactacystin led to reduced H-2K<sup>b</sup> and H-2D<sup>b</sup> cell surface expression (Fig. 4c). As lactacystin prevents the formation of new peptide-loaded MHC class I molecules<sup>30</sup>, the results should represent the decay of preexisting class I molecules at the cell surface. These decay curves were similar to the ones obtained with BFA (Fig. 4b) and so we concluded that the majority of H-2K<sup>b</sup> and H-2D<sup>b</sup> molecules on

tapasin-negative cells were loaded with peptides that were generated by the proteasome. The reduced half-life of class I molecules on tapasin-negative compared to wild-type cells indicated that tapasin influenced the quality of the peptide repertoire by selecting more stably binding peptides. The amount of class I on tapasin-negative cells was too low for isolation and sequencing of peptides.

### Tolerance to wild-type MHC class I antigens

To see whether the peptide repertoires of  $Tpn^{-/-}$  and wild-type mice were completely or only partially different, we investigated the ability of CTLs from these mice to respond to syngeneic H-2<sup>b</sup> antigens.  $Tpn^{-/-}$  mice failed to generate CTLs after immunization with H-2<sup>b</sup> wild-type cells (Fig. 5a), suggesting tolerance due to partially overlapping peptide repertoires. In contrast, TAP1<sup>-/-</sup> mice mounted a strong CTL response against syngeneic H-2<sup>b</sup> antigens from wild-type mice<sup>31,32</sup> (see Fig. 5a), probably because TAP1<sup>-/-</sup> mice are not tolerant to the set of cytosol-derived and TAP-dependent peptides that normally constitute the major pool of MHC class I-bound peptides. For comparison, the response against H-2<sup>k</sup> alloantigens was measured. The primary



response against H-2<sup>k</sup> alloantigens was measured. The primary

H-2<sup>k</sup>-specific CTL response generated by Tpn<sup>-/-</sup> splenocytes was reduced (Fig. 5b) but after *in vivo* priming the response was comparable to that of Tpn<sup>+/+</sup> splenocytes (Fig. 5c). These results indicate that the few CD8<sup>+</sup> T cells present in tapasin-negative mice were fully functional and could be expanded by stimulation with alloantigens.

## Discussion

The results presented here suggest distinct functions for tapasin. Tapasin appears to be critical for peptide loading onto both class I alleles expressed by the H-2<sup>b</sup> haplotype, H-2K<sup>b</sup> and H-2D<sup>b</sup>. The loading defect leads to reduced selection of CD8<sup>+</sup> T cells and to an impairment of CTL viral responses. Likewise, cross-presentation of protein antigen by DC is severely affected. The few CD8<sup>+</sup> T cells in Tpn<sup>-/-</sup> mice, however, appeared to be fully functional suggesting that tapasin does not directly interfere with T cell function. In other studies, tapasin has been considered to be dispensable for presentation of OVA peptide by the H-2K<sup>b</sup> molecule<sup>13,14</sup>, although this may have been due to peptide overexpression by the vaccinia virus systems used. These studies and our work, as presented here, suggest a quantitative rather than an absolute role for tapasin in loading of particular antigenic peptides.

We have also demonstrated that a deficiency in tapasin expression is sufficient to render target cells susceptible to NK cell-mediated killing *in vitro* and to mediate NK cell-dependent rejection *in vivo*. The results also show that NK cells from Tpn<sup>-/-</sup> mice have an altered specificity and are self-tolerant. This adaptation of the NK cell repertoire most likely occurs during development of NK cells and probably serves to ensure self-tolerance. In this respect, our data resemble results obtained in TAP1<sup>-/-</sup> and  $\beta_2$ -microglobulin-deficient ( $\beta_2M^{-/-}$ ) mice<sup>33</sup>. However, the fine specificity of Tpn<sup>-/-</sup> NK cells may differ from that seen in TAP1<sup>-/-</sup> and  $\beta_2M^{-/-}$  mice as well as that of H-2K<sup>b</sup>-H-2D<sup>b</sup> mice<sup>34</sup>.

In addition to its quantitative role in peptide loading, tapasin appears to be crucial for peptide selection. In the absence of tapasin, peptides bind less stably to H-2K<sup>b</sup> and H-2D<sup>b</sup> molecules, resulting in lower stability of class I molecules. Thus, tapasin influences the peptide repertoire by selecting more stably binding peptides. Reduced class I stability has also been proposed for HLA alleles expressed in 721.220 cells<sup>11,17</sup>. The precise mechanism that tapasin uses for selection of more stably binding peptides is not clear, but it may be comparable to that proposed for the HLA-DM molecule, which influences peptide loading and peptide selection in the MHC class II pathway. It has been suggested that HLA-DM keeps "empty" MHC class II molecules in a peptide-receptive, and probably more open, conformation<sup>35,36</sup>. Likewise, within the MHC class I-loading complex, tapasin may stabilize "empty" MHC class I molecules in a peptide-receptive open conformation. In agreement with this assumption it has been reported that tapasin-associated and peptide-loaded H-2L<sup>d</sup> class I molecules display different conformations<sup>9</sup>. In such a scenario, which may be similar to the quality control model proposed for HLA-DM<sup>35,36</sup>, optimally fitting peptides would more efficiently close the peptide binding groove than weakly binding ones and thereby may initiate dissociation of peptide-loaded MHC class I molecules from tapasin and subsequent transport to the cell surface. Although loading pathways and compartments are different, both class I and class II molecules seem to be similar in their requirement for the assistance of specialized accessory molecules for optimal peptide loading and selection.

## Methods

**Generation of tapasin-deficient mice.** To establish knockout mice, published procedures were followed<sup>37</sup>. A 9-kb fragment containing the mouse tapasin gene was isolated from a strain 129  $\lambda$  phage library by hybridization with a mouse tapasin cDNA. The tapasin gene was disrupted by insertion of the neomycin resistance gene in reverse orientation into the

*Xho*I site of exon 4. Homologous recombination using the strain 129 (H-2<sup>b</sup>)-derived embryonic stem cell line E14.1 and generation of knockout mice was done as described<sup>37</sup>. Identification of Tpn<sup>-/-</sup> mice was done by Southern blotting of *Eco*RI plus *Bgl*II digests, resulting in fragments of 8.4 kb and 6.2 kb from Tpn<sup>+/+</sup> and Tpn<sup>-/-</sup> mice, respectively.

**Antibodies, cytofluorometric analysis and western blotting.** For H-2 cell surface staining, cells were incubated with biotinylated H-2K<sup>b</sup>-specific mAb K10-56 and H-2D<sup>b</sup>-specific mAb B22-249<sup>27</sup>, followed by phycoerythrin-labeled streptavidin (Pharmingen, San Diego). CD4 and CD8 staining was done with fluorescein isothiocyanate-conjugated CD4 and phycoerythrin (PE)-conjugated CD8 antibodies (Pharmingen) using a FACScan (Becton Dickinson, San Jose). NK cells were stained with DX5 antibody (Pharmingen). For assessment of tapasin expression 10<sup>7</sup> splenocytes were lysed in SDS-sample buffer and run through 15% SDS-PAGE. Tapasin was visualized by western blotting using rabbit serum PAV generated against amino acids 2–20 of human tapasin, which is crossreactive with mouse tapasin, following described procedures<sup>38</sup>. For thermostability studies, NP-40 lysates of spleen cells were incubated at 37 °C for various time periods, H-2K<sup>b</sup> molecules were immunoprecipitated with conformation-dependent antibody Y3<sup>26,27</sup> and probed by western blotting using rabbit serum P8 against the cytoplasmic tail of H-2K<sup>b</sup> molecules<sup>38</sup>. Bands were visualized by enhanced chemiluminescence and quantified using the Lumi-Imager system (Roche Diagnostics, Mannheim, Germany). Where indicated, spleen cells were cultured at 37 °C with 20  $\mu$ M lactacystin (Sigma) or BFA (Sigma). Decreasing concentrations of BFA (10, 5, 2.5 and 1.25  $\mu$ g/ml) were used at various time points as described<sup>29</sup>. To increase the staining intensity of surface H-2K<sup>b</sup> molecules for stability analysis, incubation with purified K10-56 antibody was followed by biotinylated goat anti-mouse (Pharmingen) and then PE-labeled streptavidin.

**CTL and T hybridoma responses.** Antiviral CTL responses were performed as previously described<sup>29</sup>. Briefly, mice were immunized intraperitoneally with 400 HAU live A/Japan/305/57 influenza virus. After 10 days, splenocytes were restimulated *in vitro* with 2000 HAU influenza virus. CTL activity was assessed 5 days later using RMA cells loaded with 10 ng/ml of influenza NP(366–374) in a standard <sup>51</sup>Cr-release assay. For target DCs<sup>39</sup>, bone marrow-derived DCs were incubated with 1500 HAU A/Japan/305/57 influenza virus in serum-free medium for 10 min on ice followed by 30 min incubation at 37 °C. After removal of excess virus by washing, bone marrow-derived DCs were <sup>51</sup>Cr-labeled and employed as targets in a standard <sup>51</sup>Cr-release assay using influenza specific CTL. For CTL responses against syngeneic MHC (H-2<sup>b</sup>), spleen cells of mice immunized 8 days earlier and restimulated *in vitro* for 6 days with syngeneic B6 spleen cells were tested against <sup>51</sup>Cr-labeled 129/Sv (H-2<sup>b</sup>) target cells. For allogeneic MHC (H-2<sup>d</sup>) CTL responses, mice were immunized (only for secondary responses) and spleen cells stimulated *in vitro* for 5 days with CBA (H-2<sup>d</sup>) target cells and CTL activity tested by using <sup>51</sup>Cr-labeled CBA target cells. Target cells were splenocytes cultured with 5  $\mu$ g/ml Con A for 48 h. For presentation of soluble OVA, DCs were generated from bone marrow as described<sup>40</sup> and pulsed with soluble OVA, which had been tested for the absence of free peptides. OVA presentation was evaluated using the OVA-H-2K<sup>b</sup>-specific T cell hybridoma B3Z with spectrophotometrical quantification of T cell activity<sup>41</sup>.

**NK cell cytotoxicity and bone marrow transplantation assays.** Generation of IL-2-activated NK cells was performed as previously described<sup>39</sup>. NK cell cytotoxicity was tested in a standard <sup>51</sup>Cr-release assay. Target cells were obtained by culturing single spleen cell suspensions in minimum essential alpha medium supplemented with 10% fetal calf serum, antibiotics and 2.5  $\mu$ g/ml of Con A for 48 h. Bone marrow transplantation experiments were performed essentially as previously described<sup>42</sup>. 10<sup>6</sup> bone marrow cells in PBS buffer were intravenously grafted into groups of six to seven irradiated (800 rad) B6 recipients. On day five the mice were intraperitoneally inoculated with 3  $\mu$ Ci of <sup>125</sup>I-5-iodo-2'-deoxyuridine in 0.2 ml PBS buffer, killed 24 h later, and radioactivity in the spleen measured in a gamma-counter. For depletion of NK cells, 24 h before bone marrow grafting, mice were inoculated with 200  $\mu$ g of NK1.1 PK136 mAb.

### Acknowledgements

We thank A. Klevenz, S. Schmitt and G. Küblbeck for their technical help in generating gene constructs and knock-out mice and B. Vey for secretarial help. P8 antiserum was provided by J. Neeffjes, PAV serum by G. Moldenhauer and B3Z hybridoma by T. Serwold and N. Shastri. Supported by the EC project ERB-4061-PL-95-1079 (G.J.H.), and by the Swedish Medical Research Council and the Swedish Cancer Society (H.-G.L. and B.J.C.).

Received 25 May 2000; accepted 3 August 2000.

- Momburg, F. & Hammerling, G. J. Generation and TAP-mediated transport of peptides for major histocompatibility complex class I molecules. *Adv. Immunol.* **68**, 191–256 (1998).
- Sadasivan, B. *et al.* Roles for calreticulin and a novel glycoprotein, tapasin, in the interaction of MHC class I molecules with TAP. *Immunity* **5**, 103–114 (1996).
- Lindquist, J. A., Jensen, O. N., Mann, M. & Hammerling, G. J. ER-60, a chaperone with thiol-dependent reductase activity involved in MHC class I assembly. *EMBO J.* **17**, 2186–2195 (1998).
- Morrice, N. A. & Powis, S. J. A role for the thiol-dependent reductase Erp57 in the assembly of MHC class I molecules. *Curr. Biol.* **8**, 713–716 (1998).
- Hughes, E. A. & Cresswell, P. The thiol oxidoreductase Erp57 is a component of the MHC class I peptide-loading complex. *Curr. Biol.* **8**, 709–712 (1998).
- Herberg, J. A. *et al.* Genomic analysis of the Tapasin gene, located close to the TAP loci in the MHC. *Eur. J. Immunol.* **28**, 459–467 (1998).
- Grande, A. G., 3rd *et al.* Sequence, linkage to H2-K, and function of mouse tapasin in MHC class I

- assembly. *Immunogenetics* **48**, 260–265 (1998).
8. Ortmann, B. *et al.* A critical role for tapasin in the assembly and function of multimeric MHC class I-TAP complexes. *Science* **277**, 1306–1309 (1997).
  9. Carreno, B. M. *et al.* 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 (1995).
  10. Greenwood, R., Shimizu, Y., Sekhon, G. S. & DelMars, R. Novel allele-specific, post-translational reduction in HLA class I surface expression in a mutant human B cell line. *J. Immunol.* **153**, 5525–5536 (1994).
  11. Grandea III, A. G. *et al.* Dependence of peptide binding by MHC class I molecules on their interaction with TAP. *Science* **270**, 105–108 (1995).
  12. Peh, C. A. *et al.* HLA-B27-restricted antigen presentation in the absence of tapasin reveals polymorphism in mechanisms of HLA class I peptide loading. *Immunity* **8**, 531–542 (1998).
  13. Schoenhals, G. J. *et al.* Retention of empty MHC class I molecules by tapasin is essential to reconstitute antigen presentation in invertebrate cells. *Embo J.* **18**, 743–753 (1999).
  14. Deng, Y. *et al.* Assembly of MHC class I molecules with biosynthesized endoplasmic reticulum-targeted peptides is inefficient in insect cells and can be enhanced by protease inhibitors. *J. Immunol.* **161**, 1677–1685 (1998).
  15. Lauvau, G. *et al.* Tapasin enhances assembly of transporters associated with antigen processing-dependent and -independent peptides with HLA-A2 and HLA-B27 expressed in insect cells. *J. Biol. Chem.* **274**, 31349–31358 (1999).
  16. Neisig, A. *et al.* Allele-specific differences in the interaction of MHC class I molecules with transporters associated with antigen processing. *J. Immunol.* **156**, 3196–3206 (1996).
  17. Lewis, J. W., Sewell, A., Price, D. & Elliott, T. HLA-A\*0201 presents TAP-dependent peptide epitopes to cytotoxic T lymphocytes in the absence of tapasin. *Eur. J. Immunol.* **28**, 3214–3220 (1998).
  18. Lehner, P. J., Surman, M. J. & Cresswell, P. Soluble tapasin restores MHC class I expression and function in the tapasin-negative cell line .220. *Immunity* **8**, 221–231 (1998).
  19. Copeman, J., Bangla, N., Cross, J. C. & Cresswell, P. Elucidation of the genetic basis of the antigen presentation defects in the mutant cell line .220 reveals polymorphism and alternative splicing of the tapasin gene. *Eur. J. Immunol.* **28**, 3783–3791 (1998).
  20. Van Kaer, L., Ashton Rickardt, P. G., Ploegh, H. L. & Tonegawa, S. TAP1 mutant mice are deficient in antigen presentation, surface class I molecules, and CD4<sup>+</sup> T cells. *Cell* **71**, 1205–1214 (1992).
  21. Norbury, C. C. *et al.* Constitutive macropinocytosis allows TAP-dependent major histocompatibility complex class I presentation of exogenous soluble antigen by bone marrow-derived dendritic cells. *Eur. J. Immunol.* **27**, 280–288 (1997).
  22. Sigal, L. J., Crotty, S., Andino, R. & Rock, K. L. Cytotoxic T-cell immunity to virus-infected non-haematopoietic cells requires presentation of exogenous antigen. *Nature* **398**, 77–80 (1999).
  23. Miller, J. F. *et al.* Induction of peripheral CD8<sup>+</sup> T-cell tolerance by cross-presentation of self antigens. *Immunol. Rev.* **165**, 267–277 (1998).
  24. Ljunggren, H. G. *et al.* Empty MHC class I molecules come out in the cold. *Nature* **346**, 476–480 (1990).
  25. Schumacher, T. N. *et al.* Direct binding of peptide to empty MHC class I molecules on intact cells and in vitro. *Cell* **62**, 563–567 (1990).
  26. Jones, B. & Janeway, C. A. Jr Cooperative interaction of B lymphocytes with antigen-specific helper T lymphocytes is MHC restricted. *Nature* **292**, 547–549 (1981).
  27. Ortiz-Navarrete, V. & Hammerling, G. J. Surface appearance and instability of empty H-2 class I molecules under physiological conditions. *Proc. Natl Acad. Sci. USA* **88**, 3594–3597 (1991).
  28. Neeftjes, J. J., Smit, L., Gehrman, M. & Ploegh, H. L. The fate of the three subunits of major histocompatibility complex class I molecules. *Eur. J. Immunol.* **22**, 1609–1614 (1992).
  29. Zhou, X. *et al.* TAP2-defective RMA-S cells present Sendai virus antigen to cytotoxic T lymphocytes. *Eur. J. Immunol.* **23**, 1796–1801 (1993).
  30. Suh, V. K. *et al.* 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 (1996).
  31. Aldrich, C. J. *et al.* Positive selection of self- and alloreactive CD8<sup>+</sup> T cells in Tap-1 mutant mice. *Proc. Natl Acad. Sci. USA* **91**, 6525–6528 (1994).
  32. Sandberg, J. K. *et al.* TAP1-deficient mice select a CD8<sup>+</sup> T cell repertoire that displays both diversity and peptide specificity. *Eur. J. Immunol.* **26**, 288–293 (1996).
  33. Salcedo, M. & Ljunggren, H. G. Natural killer cells in MHC class I deficient mice. *Chem. Immunol.* **64**, 44–58 (1996).
  34. Perarnau, B. *et al.* Single H2K<sup>b</sup>, H2D<sup>b</sup> and double H2K<sup>d</sup>D<sup>d</sup> knockout mice: peripheral CD8<sup>+</sup> T cell repertoire and anti-lymphocytic choriomeningitis virus cytolytic responses. *Eur. J. Immunol.* **29**, 1243–1252 (1999).
  35. Kropshofer, H., Hammerling, G. J. & Vogt, A. B. How HLA-DM edits the MHC class II peptide repertoire: survival of the fittest? *Immunol. Today* **18**, 77–82 (1997).
  36. Kropshofer, H., Hammerling, G. J. & Vogt, A. B. The impact of the non-classical MHC proteins HLA-DM and HLA-DO on loading of MHC class II molecules. *Immunol. Rev.* **172**, 267–278 (1999).
  37. Dubois, B. *et al.* Resistance of young gelatinase B-deficient mice to experimental autoimmune encephalomyelitis and necrotizing tail lesions. *J. Clin. Invest.* **104**, 1507–1515 (1999).
  38. Kropshofer, H. *et al.* A role for HLA-DO as a co-chaperone of HLA-DM in peptide loading of MHC class II molecules. *Embo J* **17**, 2971–2981 (1998).
  39. Chambers, B. J., Salcedo, M. & Ljunggren, H. G. Triggering of natural killer cells by the costimulatory molecule CD80 (B7-1). *Immunity* **5**, 311–317 (1996).
  40. Lutz, M. B. *et al.* An advanced culture method for generating large quantities of highly pure dendritic cells from mouse bone marrow. *J. Immunol. Methods* **223**, 77–92 (1999).
  41. Sanderson, S. & Shastri, N. LacZ inducible, antigen/MHC-specific T cell hybrids. *Int. Immunol.* **6**, 369–376 (1994).
  42. Ljunggren, H. G., Van Kaer, L., Ploegh, H. L. & Tonegawa, S. Altered natural killer cell repertoire in Tap-1 mutant mice. *Proc. Natl Acad. Sci. USA* **91**, 6520–6524 (1994).