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Differential reactivity of residual CD8$^+$ T lymphocytes in TAP1 and β2-microglobulin mutant mice*

TAP1 -/- and β2-microglobulin (β2m) -/- mice (H-2^b background) express very low levels of major histocompatibility complex (MHC) class I molecules on the cell surface. Consequently these mice have low numbers of mature CD8$^+$ T lymphocytes. However, TAP1 -/- mice have significantly higher numbers of CD8$^+$ T cells than β2m -/- mice. Alloreactive CD8$^+$ cytotoxic T lymphocyte (CTL) responses were also stronger in TAP1 -/- mice than in β2m -/- mice. Alloreactive CTL generated in TAP1 -/- and β2m -/- mice cross-react with H-2^b-expressing cells. Surprisingly, such cross-reactivity was stronger with alloreactive CTL from β2m -/- mice than with similar cells from TAP1 -/- mice. The β2m -/- mice also responded more strongly when primed with and tested against cells expressing normal levels of H-2^b MHC class I molecules. Such H-2^b-reactive CD8$^+$ CTL from β2m -/- mice but not from TAP1 -/- mice also reacted with TAP1 -/- and TAP2-deficient RMA-S cells. In contrast, H-2^b-reactive CD8$^+$ CTL from neither β2m -/- mice nor TAP1 -/- mice killed β2m -/- cells. In line with these results, β2m -/- mice also responded when primed and tested against TAP1 -/- cells. We conclude that the reactivity of residual CD8$^+$ T cells differs between TAP1 -/- and β2m -/- mice. The MHC class I-deficient phenotype of TAP1 -/- and β2m -/- mice is not equivalent: class I expression differs between the two mouse lines with regard to quality as well as quantity. We propose that the differences observed in numbers of CD8$^+$ T cells, their ability to react with alloantigens and their cross-reactivity with normal H-2^b class I are caused by differences in the expression of MHC class I ligands on selecting cells in the thymus.

1 Introduction

Mice that are devoid of either TAP1 or β2m genes (H-2^b background) have reduced numbers of class I molecules of the major histocompatibility complex (MHC) on the cell surface [1–3] and thus lack normal ligands involved in T cell selection. Consequently such mice have very few mature CD8$^+$ T cells [1–3]. These properties have been instrumental in the recent analysis of the molecular events underlying the specificity of positive and negative selection [4–7] and have led to the definition of an avidity model for T cell selection [5]. Nonetheless, despite their low numbers, residual CD8$^+$ T cells in β2m -/- and TAP1 -/- mice respond to allogeneic lymphocytes and tumor cells [8–12]. These alloreactive CD8$^+$ T cells are different from alloreactive CD8$^+$ T cells generated in wild-type mice; in particular, they cross-react with cells expressing normal levels of H-2^b class I molecules. TAP1 -/- and β2m -/- mice also generate strong CTL responses when stimulated with, and tested against, cells that express normal levels of H-2^b class I molecules [11, 12]. These findings suggest that TAP1 -/- and β2m -/- mice express class I ligands in the thymus that are capable of positively selecting a limited number of CD8$^+$ T cells reactive with H-2^b class I molecules. However, due to low ligand density of the selecting molecules it is suggested that only T cells that have sufficiently high affinity for the (few) selecting H-2^b class I molecules are positively selected. Given the high affinity for self MHC class I molecules, these CTL respond vigorously against cells expressing normal levels of self (H-2^b) class I molecules. This in contrast to wild-type mice where such T cells are eliminated through negative selection.

Although TAP1 -/- and β2m -/- mice appear superficially similar, expression of MHC class I molecules, and thus T cell selection ligands, differ with respect to quantity as well as quality between the two types of mice. Cells devoid of β2m are likely to express a limited number of MHC class I heavy chains [13–15], which still could be occupied by peptides [14, 15]. Cells devoid of TAP1 express low levels of MHC class I heavy chains complexed with β2m, either devoid of peptide, or occupied by peptides that are

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Abbreviations: **β2m:** β2-Microglobulin **TAP:** Transporter associated with antigen processing

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delivered to the MHC class I molecules independent of the TAP complex [16, 17]. As a consequence of these differences, we reasoned that the reactivity of residual $CD8^+$ T cells in TAP1 -/- and β2m -/- mice may be different.

To address this issue, we compared levels and reactivity of $CD8^+$ T cells in these mice. Numbers of $CD8^+$ T cells in the periphery, the ability to react with alloantigens as well as the ability to cross-react with, or respond to, class I H-2^b were assessed and found to differ between the two types of mutant mice. The differences observed are discussed in the frame of recent studies on T cell development in TAP1 -/- and β2m -/- mice.

2 Materials and methods

2.1 Mice

The generation of TAP1 -/- and β2m -/- mice (H-2^b background, [B6X129/Sv]F_{5-10}) has been described [1–3]. Age-matched TAP1 or β2m mice, homozygous or heterozygous for the TAP1 or β2m mutations, were used in the present study. C57BL/6 (B6, H-2^b) and BALB/c (H-2^d) mice were obtained from the Jackson laboratory (Bar Harbor, ME, USA). β2m -/- mice [2] were generously provided by Dr. B. Koller (University of North Carolina, Durham, NC). MHC class II -/- mice [18] were generously provided by Drs. C. Benoist and D. Mathis (CNRS, INSERM, Strasbourg, France). All mice used were at the age of 4–10 weeks, and maintained at the Animal Department (Center for Cancer Research, Massachusetts Institute of Technology, Cambridge, MA). Animal care was in accordance with institutional guidelines.

2.2 FACS analysis

For FACS analysis of CD8-expressing T cells, approximately 50 µl of tail vein blood was mixed with 10 µl 0.5 M EDTA (pH 8) and incubated with a fluorescein isothiocyanate (FITC)-conjugated anti-CD8 mAb (53-6.7; PharMingen, San Diego, CA), for 30 min at room temperature. FACS brand lysis solution (1 ml; Becton Dickinson, San José, CA), diluted 1:10 according to the manufacturer's protocol, was subsequently added to the cells to lyse the erythrocytes. After 5 min of incubation, the cells were washed twice in phosphate-buffered saline (PBS) and 10000 viable cells were analyzed using a FACScan flow cytometer (Becton Dickinson). Student's *t*-test was used for statistical evaluation of the data.

2.3 Cell lines

RMA wild-type and RMA-S (TAP2-deficient; [19, 20]) cell lines were derived from the Rauscher virus-induced lymphoma line RBL-5 of C57BL/6 (H-2^b) origin. EL-4 is a thymoma line of C57BL/6 (H-2^b) origin. P815 is a mastocytoma of DBA/2 (H-2^b) origin. All cell lines were maintained in RPMI tissue culture medium supplemented with 10% FCS, penicillin-streptomycin and L-glutamine. For the generation of concavalin A (Con A)-activated T cell blasts (referred to as Con A blasts), single-cell suspensions of splenocytes (approximately 40×10^6 cells) were cultured in 30 ml complete tissue culture medium supplemented with 2.5 µg Con A (Sigma, St. Louis, MO) per ml for 48 h.

2.4 Generation of CTL and cytotoxic assay

Mice were used unprimed, or were primed by intraperitoneal injection of 20×10^6 irradiated (2500 rad) spleen cells. One to two weeks after immunization, responder spleen cells (50×10^6) were restimulated with 25×10^6 2500-rad irradiated spleen cells *in vitro* in complete tissue culture medium supplemented with 5×10^{-5} M 2-mercaptoethanol. After 5 days, the cells were used as effectors in a standard ^{51}Cr-release assay (see below). All cytotoxicity assays were performed by ^{51}Cr release. Titrated numbers of effector cells were tested against 10000 ^{51}Cr-labeled target cells. Percentual cytotoxic release was calculated according to the formula: [(experimental release-spontaneous release)/(maximum release-spontaneous release)] × 100.

3 Results

3.1 Different levels of $CD8^+$ T lymphocytes in peripheral blood of TAP1 -/- and β2m -/- mice

When compared side by side, the numbers of $CD8^+$ T cells differ significantly between the TAP1 -/- and β2m -/- mice (Fig. 1). Peripheral blood lymphocytes from TAP1 -/- mice contain on average 0.83% (S.D. 0.32%) $CD8^+$ T cells, while age-matched β2m -/- mice have on average 0.20% (S.D. 0.07%) $CD8^+$ T cells ($n = 10$ for both mice; TAP1 *vs.* β2m $p < 0.0005$). A similar pattern was observed when numbers of $CD8^+$ T cells in the spleen were compared (data not shown).

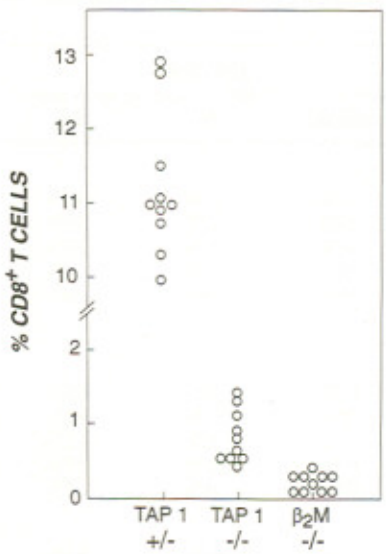

Figure 1. Different levels of $CD8^+$ T cells in peripheral blood of TAP1 -/- and β2m -/- mice. The percentage of $CD8^+$ T cells in the total number of leukocytes in peripheral blood is shown ($n = 10$).

3.2 Stronger alloreactive CTL responses in TAP1 -/- mice

We compared allospecific CTL responses in TAP1 -/- mice and β2m -/- mice to test whether the difference in $CD8^+$ T cell numbers between these mice correlates with their capacity to generate CTL against alloantigens. When primed with, and tested against allogeneic targets (P815 or BALB/c-derived Con A blasts), alloreactive CTL responses from TAP1 -/- mice were significantly stronger than those from β2m -/- mice (Table 1; BALB/c Con A blasts not shown). This difference in reactivity was even more evident in primary mixed lymphocyte cultures (MLC) (Table 1). In contrast to the differences in reactivity against allogeneic targets, allospecific CTL from β2m -/- mice cross-reacted with $H\text{-}2^b$-expressing cells (EL-4- or B6-derived Con A blasts) at levels that were at least equal, and in most experiments, even stronger than the reactivity of allospecific T cells from TAP1 -/- mice (Table 1; B6 Con A blasts not shown).

Table 1. Comparison of T cell responses in TAP1 -/- and β2m -/- mice; Alloreactivity and cross-reactivity with cells expressing normal levels of $H\text{-}2^b$ class I molecules

Targets	E:T ratio	Effectors TAP1 +/− anti-BALB/c	TAP1 −/− anti-BALB/c	β2m −/− anti-BALB/c
Primary MLC				
P815	100:1	71%	53%	12%
P815	50:1	72%	37%	3%
P815	25:1	58%	16%	2%
P815	12:1	32%	6%	0%
P815	6:1	16%	1%	0%
Secondary MLC				
P815	40:1	82%	82%	54%
P815	20:1	82%	77%	40%
P815	10:1	76%	58%	29%
P815	5:1	61%	47%	19%
P815	2.5:1	52%	31%	11%
EL-4	40:1	15%	33%	35%
EL-4	20:1	10%	23%	28%
EL-4	10:1	8%	13%	22%
EL-4	5:1	6%	12%	14%
EL 4	2.5:1	3%	6%	10%

Table 2. Comparison of anti-$H\text{-}2^b$-specific T cell responses in TAP1 -/- and β2m -/- mice

Targets	E:T ratio	Effectors TAP1 +/− anti-B6	TAP1 -/- anti-B6	β2m -/- anti-B6
Primary MLC				
EL-4	100:1	0%	11%	32%
EL-4	50:1	0%	6%	39%
EL-4	25:1	0%	0%	24%
EL-4	12:1	0%	0%	4%
EL-4	6:1	0%	0%	0%
Secondary MLC				
Expt. 1				
EL-4	33:1	10%	34%	100%
EL-4	11:1	7%	21%	86%
EL-4	4:1	1%	8%	62%
EL-4	1:1	0%	2%	27%
EL-4	0.4:1	0%	0%	7%
Expt. 2				
EL-4	33:1	4%	46%	82%
EL-4	11:1	2%	28%	79%
EL-4	4:1	0%	13%	79%
EL-4	1:1	0%	6%	58%
EL-4	0.4:1	0%	2%	29%

3.3 β2m -/- mice generate stronger anti-$H\text{-}2^b$-specific responses than TAP1 -/- mice

Despite lower number of $CD8^+$ T cells, β2m -/- mice respond more strongly than TAP1 -/- mice when immunized with, and tested against, $H\text{-}2^b$-expressing targets (Table 2). Similar results were generated using CTL from primary MLC (Table 2). Furthermore, β2m -/- anti-B6 (anti-$H\text{-}2^b$-reactive) $CD8^+$ CTL also reacted with Con A blasts from TAP1 -/- mice as well as TAP2-deficient RMA-S cells but, as expected, not against β2m -/- target cells (Fig. 2A). This suggests that β2m -/- mice possess CTL that are able to react with class I $H\text{-}2^b$ molecules expressed on TAP-deficient cells as well as cells with normal expression of $H\text{-}2^b$ class I molecules. On the other hand, TAP1 -/- anti-B6 $CD8^+$ CTL failed to kill β2m -/- as well as TAP1 -/- targets (Fig. 2A).

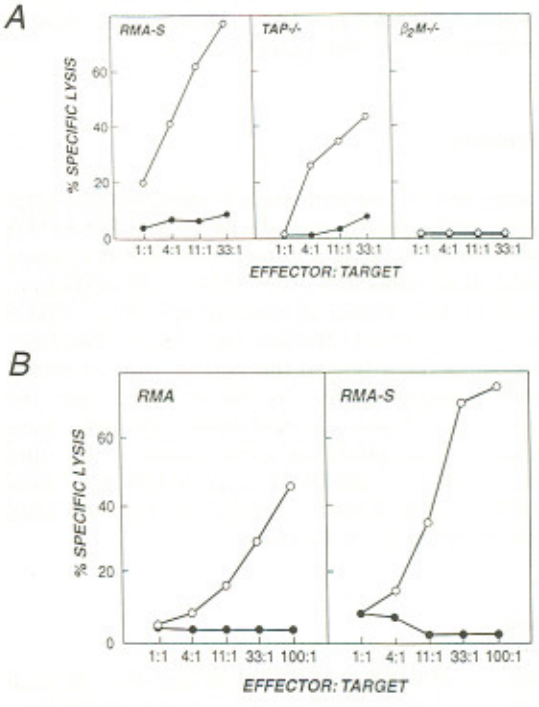

Figure 2. (A) Anti-$H\text{-}2^b$-reactive CTL from β2m -/- mice, but not from TAP1 -/- mice, cross-react with TAP deficient targets. Effectors were TAP1 -/- anti-B6 (●) or β2m -/- anti-B6 (○) MLC. Targets, as indicated, were TAP1 -/- and β2m -/- Con A blasts as well as TAP-defective RMA-S cells. (B) TAP1 -/- cells elicit a CTL response in β2m -/- mice. Effectors were β2m -/- anti-TAP -/- (○) and control TAP1 -/- anti-TAP1 -/- (●) MLC. Targets, as indicated, were TAP-defective RMA-S cells and RMA wild-type cells.

3.4 β2m -/- mice generate a CTL response against TAP1 -/- cells

To directly test whether β2m -/- mice were able to elicit a response against TAP1 -/- cells, β2m -/- and control TAP1 -/- mice were primed with TAP1 -/- cells and CTL tested against TAP1 -/- Con A blasts, RMA-S and RMA wild-type cells. CTL generated from β2m -/- mice, but not from TAP1 -/- control mice, readily killed the TAP-deficient TAP1 -/- or RMA-S target cells as well as wild-type RMA cells (Fig. 2B; TAP1 -/- targets not shown). Interestingly, the killing of wild-type RMA cells was always less than the killing of RMA-S targets. Control experiments revealed that TAP1 -/- mice, primed with either β2m -/- or endogenous TAP1 -/- cells, did not elicit any CLT response under similar conditions (data not shown). Thus, while TAP1 -/- mice were unable to respond to β2m -/- targets, β2m -/- mice responded strongly against TAP1 -/- targets.

4 Discussion

The responses of residual $CD8^+$ T cells in TAP1 -/- and β2m -/- mice differ: (i) Alloreactive CTL responses from TAP1 -/- mice were stronger than those from β2m -/- mice. (ii) Alloreactive CTL from β2m -/- mice cross-reacted with cells expressing normal H-2^b at levels which were at least equal, and in most experiments even stronger, than the cross-reactivity of allospecific CTL from TAP1 -/- mice. (iii) β2m -/- mice responded more strongly than TAP1 -/- mice when immunized with, and tested against, H-2^b-expressing targets. (iv) β2m -/- anti-H-2^b (B6)-reactive CTL reacted with TAP-deficient targets, whereas TAP1 -/- anti-H-2^b (B6) CTL, as expected, were tolerant against the same targets. (v) β2m -/- mice were also able to respond directly to TAP1 -/- cells.

We interpret these differences as a consequence of T cell selection occurring on MHC class I ligands that differ quantitatively and qualitatively. Cells devoid of β2m are likely to express a limited number of class I heavy chains, at least some of which are occupied by peptide ([15]; R. Machold et al., unpublished observations). Cells devoid of TAP1 express low levels of class I heavy chains complexed with β2m, either devoid of peptide, or occupied by peptides, that bind to class I molecules independent of the TAP complex [16, 17]. Though severely reduced, both H-$2K^b$ and H-$2D^b$ molecules on cells derived from TAP1 -/- mice are clearly detectable with a panel of commonly used anti-H-$2K^b$ and H-$2D^b$ mAb, while expression is significantly lower (for K^b, barely detectable) on cells from β2m -/- mice. TAP1 -/- Con A blasts are also readily lysed by anti-H-2^b-specific CTL, while β2m -/- Con A blasts are less efficiently lysed (unpublished observations).

The specific ability of residual $CD8^+$ T cells in TAP1- or β2m-deficient mice to react with class I H-2^b molecules on normal H-2^b-expressing cells, indicates that these T cells have not undergone negative selection [11, 12]. This is most likely a consequence of low ligand density of the selecting MHC class I molecules expressed in the TAP1 -/- and β2m -/- mice, and restrictions in the repertoire of peptides presented by class I molecules could further contribute to this phenomenon. This relative lack of negative selection is more pronounced in β2m -/- mice, as judged by their strong ability to react with H-2^b-expressing cells. One explanation for this may be lower levels of class I molecules detected on the cell surface of β2m -/- cells, as compared to TAP1 -/- cells. This may not only explain why CTL from β2m -/- mice react more strongly against B6-derived target cells but also why T cells from such mice are reactive against TAP1 -/- cells which express relatively higher levels of class I molecules than β2m -/- cells.

Could $CD8^+$ T cells in TAP1 -/- or β2m -/- mice have been selected on I-A^b (class II) molecules? It cannot be excluded that some of the residual $CD8^+$ cells, present in the periphery of TAP1 or β2m -/- mice have been selected on class II molecules. However, several observations make it less likely that (at least a majority of) the $CD8^+$ T cells have been selected on class II molecules in these mice. (i) The number of $CD8^+$ T cells differ in TAP1 -/- and β2m -/- mice (this study), while expression of I-A^b is not affected (unpublished results). (ii) The class I molecules expressed on TAP1 -/- as well as β2m -/- cells are sufficient to allow, at least, $CD8^+$ CTL recognition [14, 15]; unpublished observations), and might likewise suffice for positive selection. (iii) Alloreactive CTL from TAP1 -/- as well as β2m -/- mice show a strong bias towards reactivity with H-2^b class I. Class II -/- target cells of the H-2^b haplotype are killed as efficiently as class II +/− or +/+ target cells (unpublished results). (iv) class II-negative β2m -/- mice may still display residual $CD8^+$ CTL reactivity [21]. The intriguing possibility that positive selection of (some of) these residual $CD8^+$ cells involves the murine CD1 molecule [22] remains to be resolved.

In conclusion, the T cell repertoire in TAP1 -/- mice differs from that in β2m -/- mice. The selecting ligands in these mice are different and most likely affect the outcome of T cell selection. As a result, the numbers of functional $CD8^+$ T cells in the peripheral organs, their ability to react with alloantigens, and their cross-reactivity with class I H-2^b are affected. These differences in the expression of selecting ligands may be of importance in elucidating different aspects of T cells selection as well as their ability to mount $CD8^+$ MHC class I-restricted responses utilizing either of the two mutant mice.

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