

## Reduced Thymic Maturation but Normal Effector Function of CD8<sup>+</sup> T Cells in CD8 $\beta$ Gene-targeted Mice

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### Summary

CD8 is a cell surface glycoprotein on major histocompatibility complex class I-restricted T cells. Thymocytes and most peripheral T cells express CD8 as heterodimers of CD8 $\alpha$  and CD8 $\beta$ . The intestinal intraepithelial lymphocytes (IEL), which have been suggested to be generated extrathymically, express CD8 predominantly as homodimers of CD8 $\alpha$ . We have generated CD8 $\beta$  gene-targeted mice. CD8 $\alpha$ <sup>+</sup> T cell population in the thymus and in most peripheral lymphoid organs was reduced to 20–30% of that in wild-type littermates. CD8 $\alpha$  expression on thymocytes and peripheral T cells also decreased to 44 and 53% of the normal levels, respectively. In contrast, neither the population size nor the CD8 $\alpha$  expression level of CD8 $\alpha$ <sup>+</sup> IEL was reduced. This finding indicates that CD8 $\beta$  is important only for thymic-derived CD8<sup>+</sup> T cells. The lack of CD8 $\beta$  reduces but does not completely abolish thymic maturation of CD8<sup>+</sup> T cells. Our result also reveals the role of CD8 $\beta$  in regulating CD8 $\alpha$  expression on thymic derived T cells. Peripheral T cells in these mice were efficient in cytotoxic activity against lymphocytic choriomeningitis virus and vesicular stomatitis virus, suggesting that CD8 $\beta$  is not essential for the effector function of CD8<sup>+</sup> T cells.

CD8 is expressed on MHC class I-restricted T cells as a disulfide-linked heterodimer of  $\alpha$  and  $\beta$  subunits (1–3). The exception are the intestinal intraepithelial lymphocytes (IEL)<sup>1</sup> that express CD8 predominantly as homodimers of CD8 $\alpha$  (4). Cell surface expression of CD8 $\beta$  requires the coupling with CD8 $\alpha$  as heterodimers, whereas CD8 $\alpha$  can be expressed on the cell surface as homodimers (5–8). CD8 has been suggested to have dual functions. It serves as an adhesion molecule to enhance the affinity of T cells to their target cells (9–11). It is also a coreceptor of TCR and participates in TCR signaling (10, 12–17). The coreceptor function of CD8 requires the coengagement of CD8 and TCR to the same MHC class I molecule (12, 13, 15–17), and the association of CD8 with protein tyrosine kinase p56<sup>lck</sup> (10, 11, 14). The extracellular portion of CD8 $\alpha$  binds to the  $\alpha 3$  nonpolymorphic domain of MHC class I molecules (12, 13, 18–20), and the cytoplasmic portion of CD8 $\alpha$  is associated with the protein tyrosine kinase p56<sup>lck</sup> (21).

CD8 is crucial for thymic maturation of cytotoxic T cells (22–24). Mice bearing a null mutation at the CD8 $\alpha$  gene by gene targeting lack MHC class I-restricted T cells and are defective in cytotoxic effector function (24). T cell response can be synergized by transfected CD8 $\alpha$  (9, 10, 13, 14, 25, 26). However, CD8 $\alpha\beta$  heterodimers have been reported to be more effective than CD8 $\alpha$  homodimers in enhancing T cell response (27, 28). The role of CD8 $\beta$  in T cell ontogeny and function is still largely unknown. A regulatory role of CD8 $\beta$  in altering the structure of CD8 complex upon T cell activation has been suggested (29). A recent report on mice chimerized with CD8 $\beta$  gene-targeted embryonic stem (ES) cells suggested that CD8 $\beta$  is necessary for thymic maturation of CD8<sup>+</sup> T cells (30). We have simultaneously generated CD8 $\beta$  gene-targeted mice. Our study showed that a significant peripheral CD8<sup>+</sup> T cell population was still present in these mice. We also compared the CD8<sup>+</sup> T cells in the peripheral lymphoid organs and in the intestinal epithelium. The result demonstrates that CD8 $\beta$  is important only for CD8<sup>+</sup> T cells from the thymus, but not for IEL. We further examined the cytotoxic effector function of peripheral T cells against lymphocytic choriomeningitis virus (LCMV) and vesicular stomatitis virus (VSV) infection.

<sup>1</sup> Abbreviations used in this paper: ES cells, embryonic stem cells; IEL, intraepithelial lymphocytes; LCMV, lymphocytic choriomeningitis virus; pfu, plaque-forming unit; VSV, vesicular stomatitis virus.

## Materials and Methods

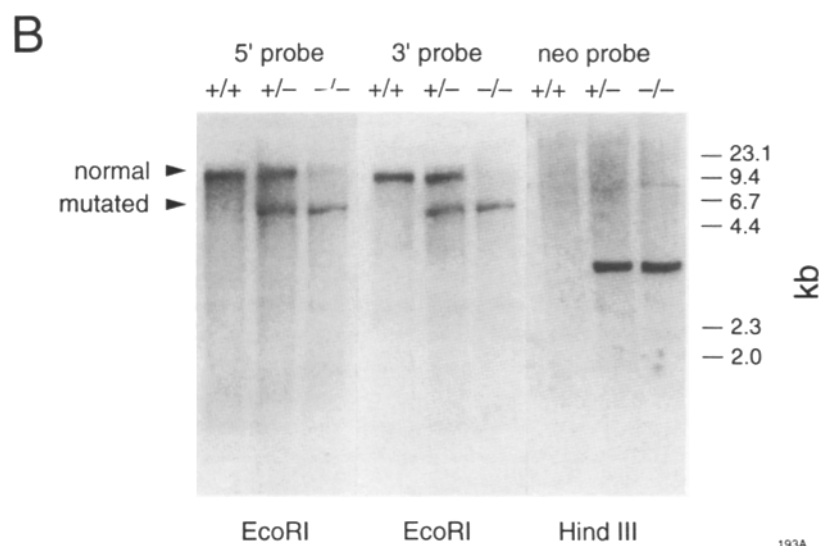
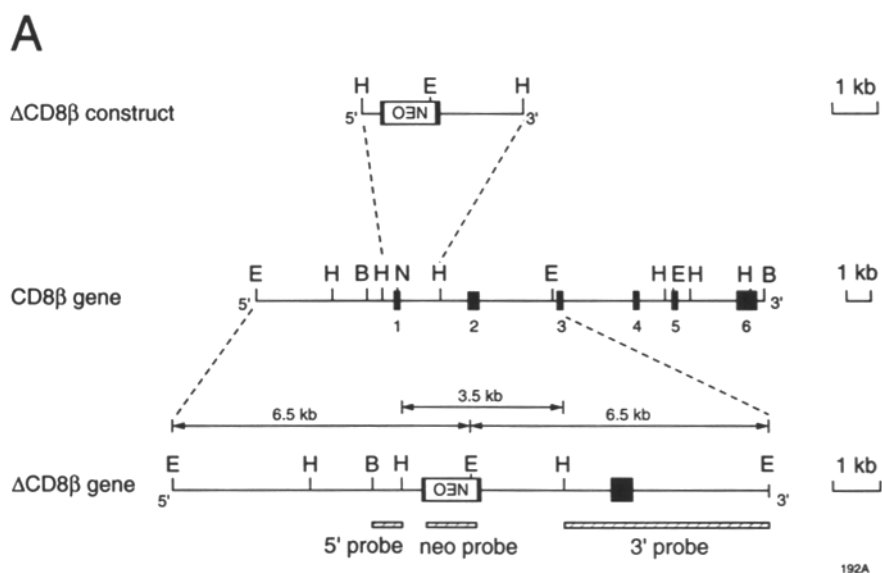
**Disruption of the *CD8 $\beta$*  Gene in Mice by Homologous Recombination.** A 2.5-kb mouse genomic DNA fragment covering the first exon of the *CD8 $\beta$*  gene, 0.4 kb of the 5' flanking region and 2 kb of the intron region was used in the gene-targeting construct (Fig. 1A). The neomycin resistance gene *pMC1neopolA* (Stratagene, La Jolla, CA) was inserted into the *Nco*I site within exon 1 of the gene. The gene-targeting construct was electroporated into D3 ES cells as described previously (24). Transfected cells were selected by G418 and the targeted gene was detected by PCR using primers specific for the neomycin resistance gene and the *CD8 $\beta$*  gene. The targeted *CD8 $\beta$*  gene in cells was eventually confirmed by genomic hybridization. ES cells with the targeted *CD8 $\beta$*  gene were injected into blastocysts to generate chimeric mice. Mice heterozygous for the targeted *CD8 $\beta$*  gene (*CD8 $\beta$*   $+/-$  mice) were obtained from chimeric mice by germline transmission. Mice homozygous ( $-/-$ ) for the disrupted *CD8 $\beta$*  gene were obtained by crossbreeding of *CD8 $\beta$*   $+/-$  mice.

**Flow Cytometric Analysis of Lymphocytes.** Single cell suspensions

from thymus, spleen, and lymph nodes of 6–8-wk-old mice were prepared. IEL were purified from the intestine according to the method described by van der Heijden and Stock (31).  $10^6$  cells were stained with mAb for 30 min at 4°C in 100  $\mu$ l of PBS containing 2% FCS and 0.1% sodium azide. Cells were then washed and analyzed for single-, double-, and triple-color flow cytometry on a FACScan® (Becton Dickinson & Co., Mountain View, CA). mAb used were specific for *CD8 $\alpha$* , *CD8 $\beta$* , *CD4*, and *CD3* (PharMingen, San Diego, CA). Anti-Thy-1 and anti-B cell specific *CD45* mAb (PharMingen) were used for detecting T and B cells, respectively.

**LCMV-induced Footpad Swelling Reaction.** Mice were infected with 500 plaque forming units (pfu) of LCMV-Armstrong (32) by intradermal injection into hind footpads. Footpad thickness was measured daily with a spring-loaded caliper. Footpad swelling is calculated as (actual thickness – thickness before infection)/(thickness before infection).

**Primary Ex Vivo Cytotoxicity against LCMV Infection.** Mice were immunized by intradermal injection of 30  $\mu$ l LCMV-Armstrong (500 pfu). On day 8, spleen cells were tested for LCMV-specific



**Figure 1.** Disruption of the *CD8 $\beta$*  gene by homologous recombination. (A) The 5' region of the mouse *CD8 $\beta$*  gene within the 2.5-kb HindIII-digested DNA fragment was used in the gene-targeting construct. The neomycin resistance gene was inserted in reverse orientation into the *Nco*I site within exon 1 of the *CD8 $\beta$*  gene. The restriction maps of the *CD8 $\beta$*  gene before and after homologous recombination are shown. The 5' and 3' probes, as well as the neo probe used in genomic hybridization are shown. Solid squares, exons; E, EcoRI; H, HindIII; B, BamHI; N, *Nco*I. (B) *CD8 $\beta$*   $+/+$ ,  $+/-$ , and  $-/-$  mice were confirmed by genomic hybridization. The intact *CD8 $\beta$*  gene after EcoRI digestion was hybridized by the 5' and 3' probe as an 11.5-kb DNA fragment, whereas the disrupted *CD8 $\beta$*  gene was split into two 6.5-kb DNA fragments. The disrupted *CD8 $\beta$*  gene was also identified by the neo probe as a 3.5-kb DNA fragment after HindIII digestion.

cytotoxicity in a 4-h  $^{51}\text{Cr}$  release assay. Target cells were EL-4 cells (H-2<sup>b</sup>) that had been incubated with or without the relevant MHC class I-binding LCMV glycoprotein peptide (amino acid residues 33–42, 50 mM) for 1 h at 37°C (33, 34).  $^{51}\text{Cr}$  release was measured in duplicate and mean values are shown. Spontaneous  $^{51}\text{Cr}$  release was <20% for all target cells. Specific  $^{51}\text{Cr}$  release was calculated as (measured  $^{51}\text{Cr}$  release – spontaneous  $^{51}\text{Cr}$  release)/(total  $^{51}\text{Cr}$  release – spontaneous  $^{51}\text{Cr}$  release).

**Primary Ex Vivo Cytotoxicity against VSV Infection.** Mice were immunized intravenously with VSV ( $2 \times 10^6$  pfu serotype Indiana in 200  $\mu\text{l}$ ) on day 0. On day 6, spleen cells were tested for cytotoxicity in a 4-h  $^{51}\text{Cr}$  release assay. The EL-4 target cells were either uninfected or infected with VSV (15 pfu/cell) for 3 h at 37°C.  $^{51}\text{Cr}$  release was measured in duplicate and mean values are shown. Spontaneous  $^{51}\text{Cr}$  release was <20% for all target cells. Specific  $^{51}\text{Cr}$  release was calculated as described above.

**LCMV-specific Cytotoxicity after In Vitro Restimulation.** Mice were immunized with LCMV-Armstrong as described in the above section. On day 8, spleen cells were restimulated in vitro with irradiated peritoneal macrophages that had been infected with LCMV, as described in detail previously (35). Spleen cells were harvested after 5 d and tested for cytotoxicity on LCMV glycoprotein peptide-labeled EL-4 target cells as described above. Spontaneous  $^{51}\text{Cr}$  release was <22% for all target cells studied.

**VSV-specific Cytotoxicity after In Vitro Restimulation.** Mice were immunized intravenously with VSV as described above. On day 6, spleen cells were restimulated with irradiated syngeneic C57BL/6 spleen cells that had been cultured with UV inactivated VSV (15 pfu/cell) for 1 h at 37°C.  $3 \times 10^6$  responder spleen cells were restimulated with  $2 \times 10^6$  stimulator cells in 24-well plates. After 5 d of culture, spleen cells were tested for cytotoxicity on VSV-infected EL-4 target cells as described above. Spontaneous  $^{51}\text{Cr}$  release was <16% for all target cells studied.

## Results

**Generation of the CD8 $\beta$  Gene-targeted Mice.** To understand the role of the CD8 $\beta$  in T cell ontogeny and function, we generated mice with the CD8 $\beta$  gene disrupted by homologous recombination. The CD8 $\beta$  gene was mutated in exon 1 by an insertion of the neomycin resistance gene (Fig. 1A). The site of insertion is 7 nucleotides downstream from the translation start site, which would abolish translation of the CD8 $\beta$  protein. Mice chimerized with CD8 $\beta$  gene-targeted ES cells were generated from embryo injection. CD8 $\beta$  +/– mice were obtained from germline transmission of chimeric mice and CD8 $\beta$  –/– mice were from cross-breeding of CD8 $\beta$  +/– mice. Disruption of the CD8 $\beta$  gene in mice was confirmed by genomic Southern hybridization (Fig. 1B). The CD8 $\beta$  –/– mice were fertile and appeared to be healthy. Cell numbers recovered from thymi, lymph nodes, spleens, and intestinal epithelia of the CD8 $\beta$  –/– mice were similar to those from wild-type littermates (Table 1). The percentages of the T cell and B cell subsets in different peripheral lymphoid organs of CD8 $\beta$  +/– and –/– mice were within normal ranges (Table 2).

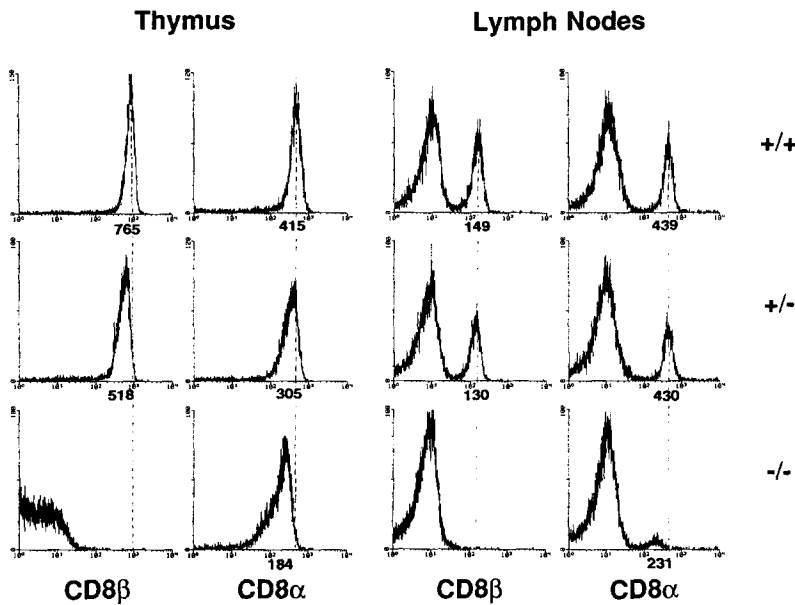
**Reduction in Population Size and in CD8 $\alpha$  Expression of Thymic-derived CD8<sup>+</sup> T Cells.** CD8 $\beta$  was not detected on peripheral T cells or thymocytes from CD8 $\beta$  –/– mice (Figs. 2, 3, and 4). However, CD8 $\alpha$ <sup>+</sup> T cells were present in the peripheral lymphoid organs of CD8 $\beta$  –/– mice (Figs. 2, 3, and Table 3). CD8 $\alpha$ <sup>+</sup> T cells in CD8 $\beta$  –/– mice had a normal level of TCR expression (data not shown), but the population size was smaller than that in wild-type littermates. In lymph nodes, CD8 $\alpha$ <sup>+</sup> T cells were reduced to 30% of

**Table 1.** Cell Numbers Recovered from Different Lymphoid Organs of the CD8 $\beta$  +/+, +/–, and –/– mice.

Mouse genotype	Thymus	Lymph nodes	Spleen	Intestinal IEL
CD8 $\beta$ +/+	$10.0 \times 10^7$	$0.9 \times 10^7$	$4.5 \times 10^7$	$1.2 \times 10^6$
CD8 $\beta$ +/-	$7.1 \times 10^7$	$6.0 \times 10^7$	$5.4 \times 10^7$	$3.0 \times 10^6$
CD8 $\beta$ -/-	$11.5 \times 10^7$	$1.0 \times 10^7$	$10.2 \times 10^7$	$1.4 \times 10^6$

**Table 2.** The Percentages of B Cell and T Cell Populations in Lymphocytes from Different Lymphoid Organs of the CD8 $\beta$  +/+, +/–, and –/– mice

Mouse genotype	Lymph nodes		Spleen		Blood	
	B cell	T cell	B cell	T cell	B cell	T cell
CD8 $\beta$ +/+	26%	74%	49%	51%	27%	73%
CD8 $\beta$ +/-	22%	78%	54%	46%	35%	65%
CD8 $\beta$ -/-	24%	76%	58%	42%	39%	61%

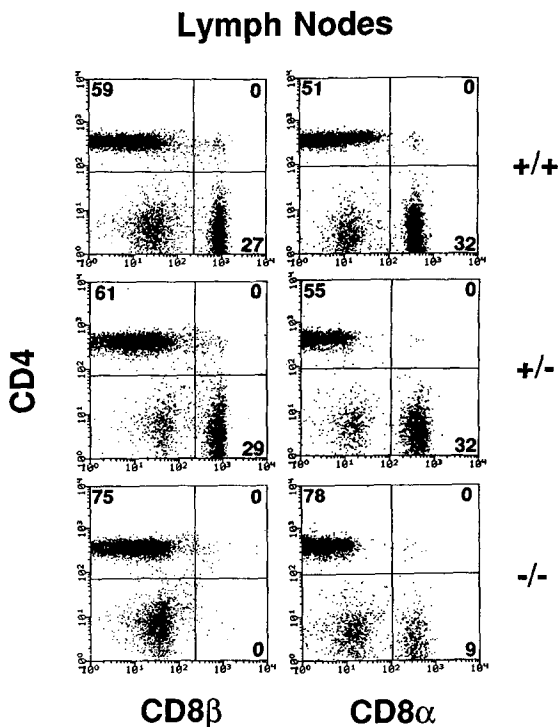


**Figure 2.** Decreased CD8 $\alpha$  expression on CD8 $^+$  T cells from CD8 $\beta$  gene-targeted mice. Histograms of CD8 $\beta$ , CD8 $\alpha$  expression on thymocytes, and lymph node cells from CD8 $\beta$   $+/+$ ,  $+/-$ ,  $-/-$  mice are shown. Mean values of CD8 stainings are shown under the peaks. Mesenteric lymph node cells were stained with mAb against CD8 $\alpha$  and CD8 $\beta$  as described in Materials and Methods. Samples were analyzed using the FACScan<sup>®</sup> program. Machine settings for acquisition of thymus and lymph node samples were different.

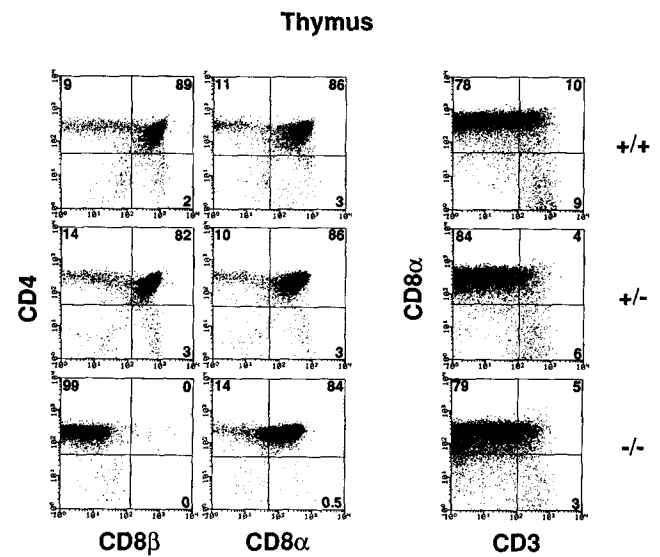
that in wild-type mice (Fig. 3). Ratios of CD4 $^+$  to CD8 $\alpha^+$  T cells in the peripheral lymphoid organs of CD8 $\beta$   $-/-$  mice were about 10:1 in repeated experiments, as opposed to the ratio of about 1.6:1 in wild-type littermates (Fig. 3, Table 3). Immature thymocytes in CD8 $\beta$   $-/-$  mice were

CD4 $^+$  CD8 $\alpha^+$  double positive and were normal in population size and in CD3 expression (Fig. 4). Consistent with the observation in the periphery, the subset of CD8 $\alpha^+$  mature thymocytes in CD8 $\beta$   $-/-$  mice was reduced to 20% of that in wild-type mice (Fig. 4). In contrast, the CD8 $\alpha^+$  IEL population in CD8 $\beta$   $-/-$  mice was comparable with that in wild-type mice (Fig. 5). The IEL cell number harvested from the intestinal epithelia of CD8 $\beta$   $-/-$  mice was also in a normal range (Table 1).

A significant reduction of CD8 $\alpha$  surface expression was



**Figure 3.** Decreased CD8 $\alpha^+$  T cell population in lymph nodes of CD8 $\beta$  gene-targeted mice. Mesenteric lymph node cells from CD8 $\beta$   $+/+$ ,  $+/-$ , and  $-/-$  mice were double stained with mAb specific for CD4, CD8 $\alpha$ , and CD8 $\beta$  and analyzed by flow cytometry.



**Figure 4.** Decreased thymic ontogeny of CD8 $\alpha^+$  T cells in CD8 $\beta$  gene-targeted mice. Thymocytes from CD8 $\beta$   $+/+$ ,  $+/-$ , and  $-/-$  mice were triple stained with mAb specific for CD3, CD4, CD8 $\alpha$ , and CD8 $\beta$  and analyzed by flow cytometry.

**Table 3.** The Ratio of CD4<sup>+</sup> T Cell to CD8<sup>+</sup> T Cell Subsets in Different Lymphoid Organs of the CD8 $\beta$  +/+, +/-, and -/- Mice

Mouse genotype	Thymus	Lymph nodes	Spleen	Blood
CD8 $\beta$ +/+	3.7:1	1.6:1	1.8:1	1.1:1
CD8 $\beta$ +/-	3.3:1	1.7:1	2.6:1	3.6:1
CD8 $\beta$ -/-	14:1	8.7:1	9.5:1	11.5:1

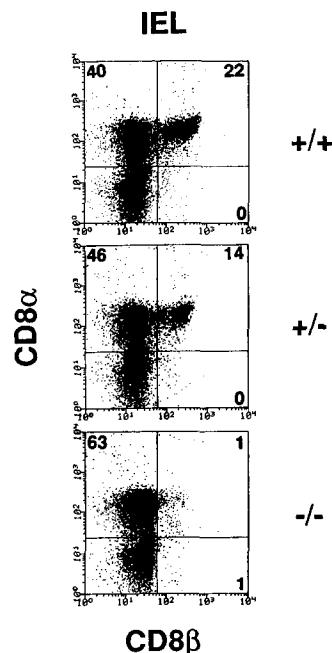
found on peripheral T cells from CD8 $\beta$  -/- mice (53% of the level in wild-type control mice) (Fig. 2). CD8 $\alpha$  expression levels on immature thymocytes from CD8 $\beta$  -/- mice were also low (44% of normal levels) and spanned a wide range (Fig. 4). Low CD8 $\alpha$  expression was particularly obvious for early stage thymocytes which display low levels of CD3 expression. In contrast, CD8 $\alpha$  expression levels on IEL from CD8 $\beta$  -/- mice were unchanged (Fig. 5).

In conclusion, the absence of CD8 $\beta$  only affects thymic derived T cells but has no impact on IEL. CD8 $\beta$  is needed for optimal CD8 $\alpha$  expression and efficient thymic maturation of CD8<sup>+</sup> T cells.

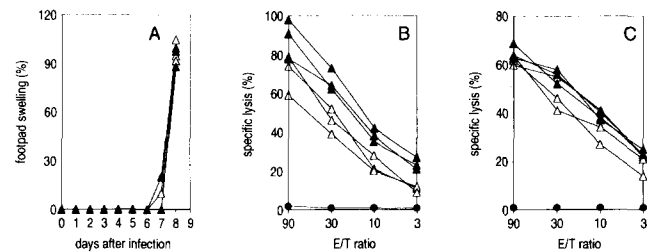
*CD8<sup>+</sup> T Cells without CD8 $\beta$  Are Efficient in Cytotoxic Effector Function against In Vivo Viral Infection.* CD8 $\beta$  -/- mice provide a well-defined animal model for studying the in vivo immune response and effector function of the CD8<sup>+</sup> T cells that lack CD8 $\beta$ . Cytotoxic T cell responses were studied in CD8 $\beta$  -/- mice after infection with LCMV and VSV. LCMV-specific CTL function in vivo was assessed by monitoring footpad swelling after intradermal injection of

LCMV. Due to extensive local virus replication, the T cell response against LCMV causes an immunopathological swelling reaction of the footpad. The early phase of the swelling reaction (starting on day 8) is mediated exclusively by CD8<sup>+</sup> cytotoxic T cells (36–38). This swelling reaction was normal in CD8 $\beta$  -/- mice (Fig. 6 A), demonstrating that an efficient in vivo cytotoxic T cell activity can be triggered in these mice.

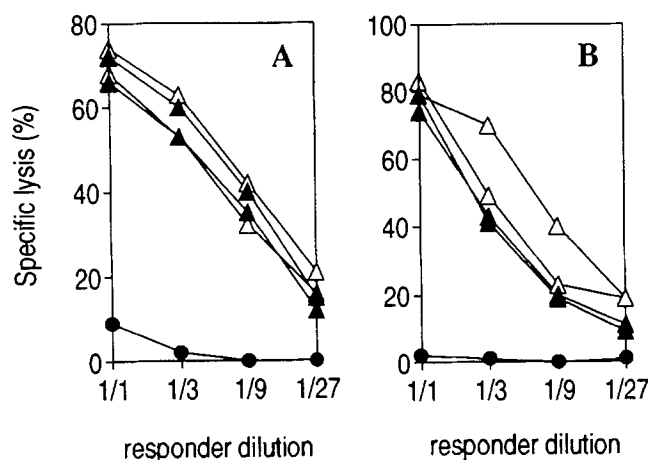
Primary ex vivo cytotoxic T cell function was assessed with <sup>51</sup>Cr release assays. After 8 d of LCMV infection in CD8 $\beta$  -/- mice, peripheral T cells showed a virus-specific cytotoxicity comparable to that in wild-type controls (Fig. 6 B). Similarly, efficient VSV-specific cytotoxic activity was observed in CD8 $\beta$  -/- mice after VSV infection (Fig. 6 C). The



**Figure 5.** Normal CD8 $\alpha$ <sup>+</sup> IEL in CD8 $\beta$  gene-targeted mice. Intestinal IEL cells were purified from CD8 $\beta$  +/+, +/-, and -/- mice as described in Methods and Materials. Cells were stained with mAb specific for CD8 $\alpha$  and CD8 $\beta$  and analyzed by flow cytometry.



**Figure 6.** Normal effector function of cytotoxic T cells in CD8 $\beta$  gene-targeted mice. (A) Mice were infected with LCMV locally into hind footpads as described in Materials and Methods. Footpad swelling is calculated as (actual thickness - thickness before infection)/(thickness before infection). Open triangles, CD8 $\beta$  -/- mice; solid triangles, CD8 $\beta$  +/+ mice. Each curve shows the swelling of the hind footpad of one mouse. (B) Spleen cells from mice immunized with LCMV were tested for cytotoxicity on EL-4 (H-2<sup>b</sup>) target cells that were labeled with the relevant MHC class I-binding LCMV glycoprotein peptide, as described in Materials and Methods. Open triangles, CD8 $\beta$  -/- mice; solid triangles, CD8 $\beta$  +/+ mice; solid circles, unprimed C57BL/6 (H-2<sup>b</sup>) control mice. Nonspecific lysis on EL-4 target cells without the viral peptide was <10% for the different effector cells. (C) Mice were intravenously infected with VSV and spleen cells were tested for cytotoxicity on VSV-infected EL-4 target cells, as described in Materials and Methods. Open triangles, CD8 $\beta$  -/- mice; solid triangles, CD8 $\beta$  +/+ mice; solid circles, unprimed C57BL/6 (H-2<sup>b</sup>) control mice. Nonspecific lysis on uninfected EL-4 target cells was <4% for the different effector cells. For both B and C, each curve represents values taken from one mouse. <sup>51</sup>Cr release was measured in duplicate, mean values are shown (SD <13% for all values). Specific <sup>51</sup>Cr release was calculated as (measured <sup>51</sup>Cr release - spontaneous <sup>51</sup>Cr release)/(total <sup>51</sup>Cr release - spontaneous <sup>51</sup>Cr release).



**Figure 7.** Efficient effector function of cytotoxic T cells in *CD8β* gene-targeted mice. (A) Spleen cells from mice infected with LCMV were restimulated *in vitro* with LCMV-infected irradiated peritoneal macrophages, as described in Materials and Methods. Cytotoxicity of spleen cells on LCMV glycoprotein peptide labeled EL-4 target cells are shown. Open triangles, *CD8β*  $-/-$  mice; solid triangles, *CD8β*  $+/+$  mice; solid circles, unprimed C57BL/6 control mice. Spontaneous  $^{51}\text{Cr}$  release was  $<22\%$  for all targets shown. (B) Spleen cells from VSV-infected mice were restimulated with irradiated syngeneic C57BL/6 spleen cells that had been cultured with UV-inactivated VSV, as described in Methods and Materials. Cytotoxicity of spleen cells on VSV-infected EL-4 target cells was studied. Open triangles, *CD8β*  $-/-$  mice; solid triangles, *CD8β*  $+/+$  mice; solid circles, unprimed C57BL/6 control mice. Spontaneous  $^{51}\text{Cr}$  release was  $<16\%$  for all targets shown.

demonstration of primary *ex vivo* cytotoxicity in *CD8β*  $-/-$  mice suggests that an *in vivo* proliferation of the  $\text{CD8}^+$  T cells must have occurred, because the frequency of antigen specific CTL must be in the range of  $10^{-2}$  to allow detectable primary *ex vivo* cytotoxicity (35, 39).

Although primary *ex vivo* cytotoxicity against LCMV and VSV in the *CD8β*  $-/-$  mice was observed, a slight reduction was noticed when compared with those in wild-type mice. The question as to whether this reduction resulted from a limited number of  $\text{CD8}^+$  T cells or a decreased effector function of the  $\text{CD8}^+$  T cells is clarified by performing  $^{51}\text{Cr}$  release assays after *in vitro* restimulation. The difference in numbers of effector cells is evened out by further *in vitro* proliferation of effector cells during the restimulation. As demonstrated in both LCMV- and VSV-specific responses, the cytotoxic activities of the restimulated effector cells from *CD8β*  $-/-$  mice were as effective as those from wild-type littermate controls (Fig. 7).

In conclusion, peripheral  $\text{CD8}^+$  T cells in *CD8β*  $-/-$  mice were shown to be efficient in cytotoxic effector function against LCMV and VSV infection.

## Discussion

We have demonstrated in *CD8β*  $-/-$  mice that thymic ontogeny of  $\text{CD8}^+$  T cells is reduced but not completely abolished in the absence of *CD8β*. This is evidenced by a significant number of  $\text{CD8}^+$  T cells remaining in the periphery and by a normal CTL activity found in these mice

in response to viral infection. The thymic origin of the peripheral  $\text{CD8}^+$  T cells in these mice is also suggested by the corresponding decrease in *CD8α* expression on thymocytes and peripheral T cells. The result suggests that *CD8α* alone as a homodimer is inefficient for thymic maturation of  $\text{CD8}^+$  T cells.

It is interesting to note that  $\text{CD8}^+$  IEL in *CD8β*  $-/-$  mice is not reduced in population size or decreased in *CD8α* expression. IEL are distributed at the intestinal epithelium and are heterogeneous populations dominated by  $\text{CD8}^+$  T cells with a TCR- $\alpha/\beta$  or  $\gamma/\delta$  (40–42). It has been suggested that IEL are generated extrathymically (42–45). T cells with autoreactive TCR are deleted in the thymus during maturation (46–51), whereas IEL with the forbidden TCR are still present in the intestinal epithelium (44, 45). The observation that IEL are not affected by the absence of *CD8β* in these mice further supports the notion that IEL are derived extrathymically.

The finding of a low *CD8α* expression on thymic-derived T cells is unexpected, because IEL do not express *CD8β* and still retain normal levels of *CD8α* expression. There are two possible explanations for this observation. It could be a consequence of preferential thymic selection for  $\text{CD8}^{\text{low}}$  T cells. Alternatively, *CD8* as  $\alpha/\alpha$  homodimers could be less stable than  $\alpha/\beta$  heterodimers and therefore are expressed less efficiently on the cell surface. The first explanation does not seem to be plausible, because low *CD8α* expression was found on thymocytes at different stages of maturation. In particular, *CD8α* on mature T cells is not lower than that on immature thymocytes, which argues against an enrichment for  $\text{CD8}^{\text{low}}$  T cells after thymic selection. The second explanation is possible but only applies to thymic derived T cells, because the lack of *CD8β* has no impact on *CD8α* expression on IEL. In fact, the specific decrease of *CD8α* expression on thymic-derived T cells reveals a novel regulatory role of *CD8β* in adjusting the *CD8α* expression level on thymic-derived T cells.

We have shown in a previous report that mice heterozygous for the disrupted *CD8α* gene (*CD8α*  $+/-$ ) have a reduced cell surface expression of both *CD8α* and *CD8β* (50% of the normal level) (24). Despite low *CD8* levels, thymic ontogeny of  $\text{CD8}^+$  T cells in these mice appears to be normal (24). On the other hand, the lack of *CD8β* in *CD8β*  $-/-$  mice causes a similar decrease in *CD8α* expression, but results in decreased thymic ontogeny of  $\text{CD8}^+$  T cells. Thus, an overall decrease in *CD8* level may not be critical, but complete ablation of *CD8β* results in impaired thymic ontogeny.

How does *CD8β* affect the function of *CD8* in thymic ontogeny? The role of *CD8β* could be quantitative in enhancing the overall *CD8* expression, or qualitative in modifying *CD8* functions. In *CD8α*  $-/-$  mice carrying different transgenic TCR, we have shown previously that *CD8* is strictly needed in positive selection, but differentially required in negative selection of T cells (52). The reduced  $\text{CD8}^+$  T cell population in *CD8β*  $-/-$  mice could result from an increased negative selection, or a decreased positive selection of thymocytes. Further examination of *CD8β*  $-/-$  mice

bearing transgenic TCR should allow us to dissect the distinct role of CD8 $\beta$  in positive and negative selection.

The role of CD8 $\beta$  in cytotoxic function was examined by infecting CD8 $\beta$   $-/-$  mice with LCMV or VSV. Cytotoxic T cell activity as assessed by primary ex vivo  $^{51}\text{Cr}$  release assays was slightly less than that in wild-type control mice, apparently due to the reduced CD8 $^+$  T cell population. After secondary in vitro stimulation with the same viral antigens, cytotoxicity generated in CD8 $\beta$   $-/-$  mice was efficient and comparable with wild-type controls. Therefore on a cell-to-cell basis the CD8 $^+$  T cells that lack CD8 $\beta$  are as efficient in cytotoxic function as the CD8 $^+$  T cells in wild-type littermate controls. Taken together, the antigen induction, the in vivo proliferation, the differentiation into lytic effector cells and the cytolytic effector function of the CD8 $^+$  T cells in the periphery of the CD8 $\beta$   $-/-$  mice were shown to be normal. The demonstration of CTL functions against the above two nominal antigens in CD8 $\beta$   $-/-$  mice also suggests that the TCR repertoire of CD8 $^+$  T cells in these mice is comparable with that in wild-type mice.

The results obtained from our CD8 $\beta$   $-/-$  mice are different from a recent report on chimeric mice (30). We have provided phenotypic and functional data to suggest that a

significant population of thymic-derived CD8 $^+$  T cells is still present in CD8 $\beta$   $-/-$  mice. The previous report on chimeric mice showed only a minute CD8 $^+$  T cell population in the periphery that, in contrast to our study, was suggested to be derived extrathymically (30). In addition, the change in CD8 $\alpha$  expression was not reported for the chimeric mice. These discrepancies probably arise from the limitation of the chimeric model as compared with CD8 $\beta$   $-/-$  mice. The cells to be analyzed in the chimeric mice represented only a fraction of the total population. Furthermore, the population size of ES cell-derived CD8 $^+$  T cells could be affected by other factors, such as competition between defective ES cell-derived cells and normal host cells during thymic maturation of CD8 $^+$  T cells.

In summary, the phenotypic and functional studies in our CD8 $\beta$   $-/-$  mice show that the absence of CD8 $\beta$  only reduces thymic maturation of functional CD8 $^+$  T cells. The result also reveals a role of CD8 $\beta$  in regulating CD8 $\alpha$  cell surface expression on thymic derived T cells. Despite the lack of CD8 $\beta$  expression, these CD8 $^+$  T cells are functionally comparable to those in wild-type mice, suggesting that CD8 $\beta$  is not crucial for the antigenic response and the effector function of CD8 $^+$  T cells.

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