Reduced Thymic Maturation but Normal Effector Function of CD8+ T Cells in CD8β 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 CD8α and CD8β. The intestinal intraepithelial lymphocytes (IEL), which have been suggested to be generated extrathymically, express CD8 predominantly as homodimers of CD8α. We have generated CD8β gene-targeted mice. CD8α+ T cell population in the thymus and in most peripheral lymphoid organs was reduced to 20–30% of that in wild-type littermates. CD8α 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 CD8α expression level of CD8α+ IEL was reduced. This finding indicates that CD8β is important only for thymic-derived CD8+ T cells. The lack of CD8β reduces but does not completely abolish thymic maturation of CD8+ T cells. Our result also reveals the role of CD8β in regulating CD8α 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 CD8β is not essential for the effector function of CD8+ T cells.

CD8 is expressed on MHC class I–restricted T cells as a disulfide-linked heterodimer of α and β subunits (1–3). The exception are the intestinal intraepithelial lymphocytes (IEL)[1] that express CD8 predominantly as homodimers of CD8α (4). Cell surface expression of CD8β requires the coupling with CD8α as heterodimers, whereas CD8α 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 $p56^{lck}$ (10, 11, 14). The extracellular portion of CD8α binds to the α3 nonpolymorphic domain of MHC class I molecules (12, 13, 18–20), and the cytoplasmic portion of CD8α is associated with the protein tyrosine kinase $p56^{lck}$ (21).

CD8 is crucial for thymic maturation of cytotoxic T cells (22–24). Mice bearing a null mutation at the CD8α 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 CD8α (9, 10, 13, 14, 25, 26). However, CD8αβ heterodimers have been reported to be more effective than CD8α homodimers in enhancing T cell response (27, 28). The role of CD8β in T cell ontogeny and function is still largely unknown. A regulatory role of CD8β in altering the structure of CD8 complex upon T cell activation has been suggested (29). A recent report on mice chimerized with CD8β gene-targeted embryonic stem (ES) cells suggested that CD8β is necessary for thymic maturation of CD8+ T cells (30). We have simultaneously generated CD8β gene-targeted mice. Our study showed that a significant peripheral CD8+ T cell population was still present in these mice. We also compared the CD8+ T cells in the peripheral lymphoid organs and in the intestinal epithelium. The result demonstrates that CD8β is important only for CD8+ 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.

[1] *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.

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Materials and Methods

Disruption of the CD8β Gene in Mice by Homologous Recombination. A 2.5-kb mouse genomic DNA fragment covering the first exon of the CD8β gene, 0.4 kb of the 5′ flanking region and 2 kb of the intron region was used in the gene-targeting construct (Fig. 1 *A*). The neomycin resistance gene *pMC1neopolA* (Stratagene, La Jolla, CA) was inserted into the NcoI 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 CD8β gene. The targeted CD8β gene in cells was eventually confirmed by genomic hybridization. ES cells with the targeted CD8β gene were injected into blastocysts to generate chimeric mice. Mice heterozygous for the targeted CD8β gene (CD8β +/− mice) were obtained from chimeric mice by germline transmission. Mice homozygous (−/−) for the disrupted CD8β gene were obtained by crossbreeding of CD8β +/− 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 μl 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 CD8α, CD8β, 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 μl LCMV-Armstrong (500 pfu). On day 8, spleen cells were tested for LCMV-specific

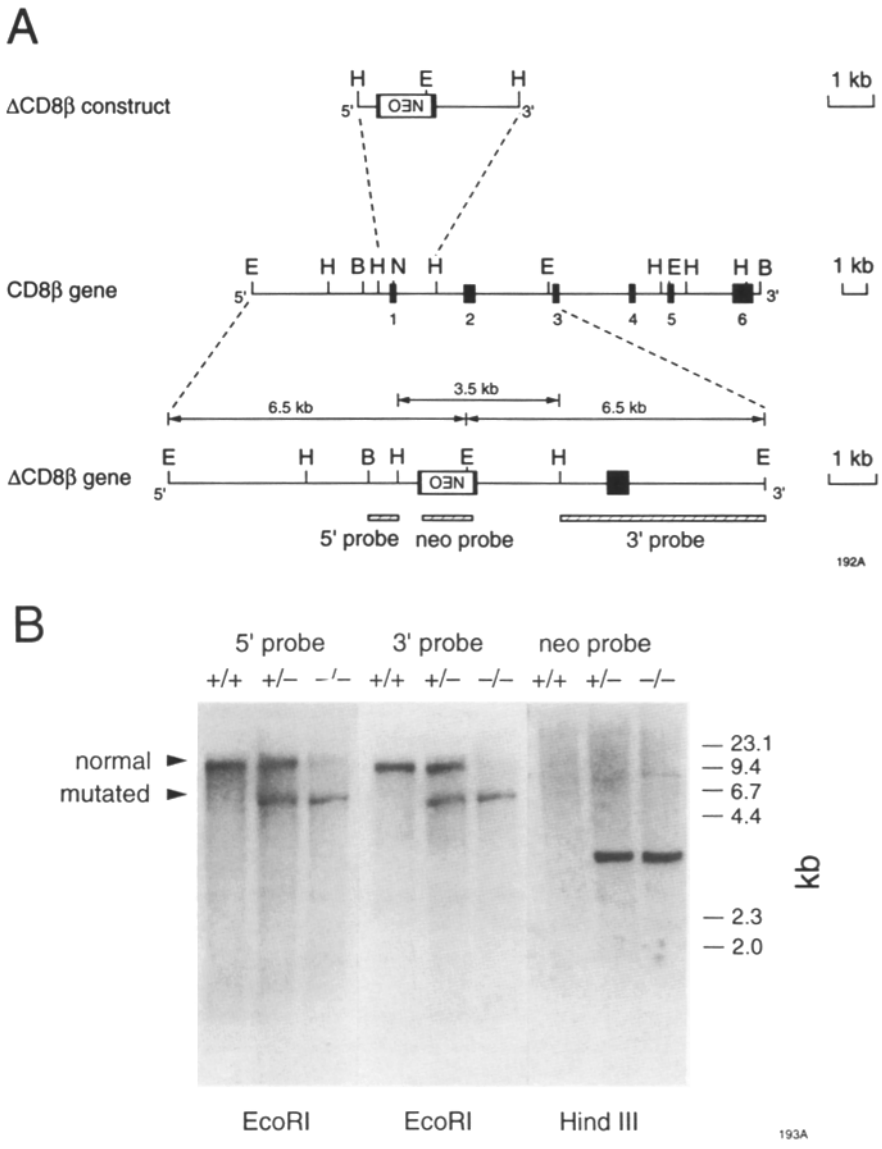

Figure 1. Disruption of the CD8β gene by homologous recombination. (*A*) The 5′ region of the mouse CD8β gene within the 2.5-kb HindIII-digested DNA fragment was used in the gene-targeting construct. The neomycin resistance gene was inserted in reverse orientation into the NcoI site within exon 1 of the CD8β gene. The restriction maps of the CD8β 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*, NcoI. (*B*) CD8β +/+, +/−, and −/− mice were confirmed by genomic hybridization. The intact CD8β gene after EcoRI digestion was hybridized by the 5′ and 3′ probe as an 11.5-kb DNA fragment, whereas the disrupted CD8β gene was split into two 6.5-kb DNA fragments. The disrupted CD8β gene was also identified by the neo probe as a 3.5-kb DNA fragment after HindIII digestion.

cytotoxicity in a 4-h ^{51}Cr release assay. Target cells were EL-4 cells ($H\text{-}2^b$) 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}Cr release was measured in duplicate and mean values are shown. Spontaneous ^{51}Cr release was <20% for all target cells. Specific ^{51}Cr release was calculated as (measured ^{51}Cr release − spontaneous ^{51}Cr release)/(total ^{51}Cr release − spontaneous ^{51}Cr release).

Primary Ex Vivo Cytotoxicity against VSV Infection. Mice were immunized intravenously with VSV (2×10^6 pfu serotype Indiana in 200 μl) on day 0. On day 6, spleen cells were tested for cytotoxicity in a 4-h ^{51}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}Cr release was measured in duplicate and mean values are shown. Spontaneous ^{51}Cr release was <20% for all target cells. Specific ^{51}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}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. 3×10^6 responder spleen cells were restimulated with 2×10^6 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}Cr release was <16% for all target cells studied.

Results

Generation of the CD8β Gene-targeted Mice. To understand the role of the CD8β in T cell ontogeny and function, we generated mice with the CD8β gene disrupted by homologous recombination. The CD8β gene was mutated in exon 1 by an insertion of the neomycin resistance gene (Fig. 1 *A*). The site of insertion is 7 nucleotides downstream from the translation start site, which would abolish translation of the CD8β protein. Mice chimerized with CD8β gene–targeted ES cells were generated from embryo injection. CD8β +/− mice were obtained from germline transmission of chimeric mice and CD8β −/− mice were from cross-breeding of CD8β +/− mice. Disruption of the CD8β gene in mice was confirmed by genomic Southern hybridization (Fig. 1 *B*). The CD8β −/− mice were fertile and appeared to be healthy. Cell numbers recovered from thymi, lymph nodes, spleens, and intestinal epithelia of the CD8β −/− 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 CD8β +/− and −/− mice were within normal ranges (Table 2).

Reduction in Population Size and in CD8α Expression of Thymic-derived $CD8^+$ T Cells. CD8β was not detected on peripheral T cells or thymocytes from CD8β −/− mice (Figs. 2, 3, and 4). However, $CD8\alpha^+$ T cells were present in the peripheral lymphoid organs of CD8β −/− mice (Figs. 2, 3, and Table 3). $CD8\alpha^+$ T cells in CD8β −/− 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, $CD8\alpha^+$ T cells were reduced to 30% of

Table 1. *Cell Numbers Recovered from Different Lymphoid Organs of the CD8β +/+, +/−, and −/− mice.*

Mouse genotype	Thymus	Lymph nodes	Spleen	Intestinal IEL
CD8β +/+	10.0×10^7	0.9×10^7	4.5×10^7	1.2×10^6
CD8β +/−	7.1×10^7	6.0×10^7	5.4×10^7	3.0×10^6
CD8β −/−	11.5×10^7	1.0×10^7	10.2×10^7	1.4×10^6

Table 2. *The Percentages of B Cell and T Cell Populations in Lymphocytes from Different Lymphoid Organs of the CD8β +/+, +/−, and −/− mice*

Mouse genotype	Lymph nodes		Spleen		Blood	
	B cell	T cell	B cell	T cell	B cell	T cell
CD8β +/+	26%	74%	49%	51%	27%	73%
CD8β +/−	22%	78%	54%	46%	35%	65%
CD8β −/−	24%	76%	58%	42%	39%	61%

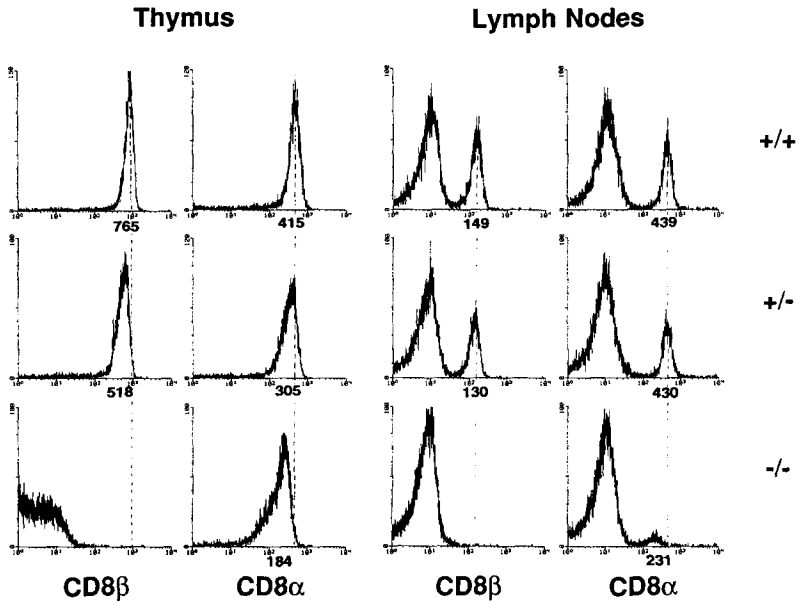

Figure 2. Decreased CD8α expression on CD8⁺ T cells from CD8β gene-targeted mice. Histograms of CD8β, CD8α expression on thymocytes, and lymph node cells from CD8β +/+, +/−, −/− mice are shown. Mean values of CD8 stainings are shown under the peaks. Mesenteric lymph node cells were stained with mAb against CD8α and CD8β as described in Materials and Methods. Samples were analyzed using the FACScan® program. Machine settings for acquisition of thymus and lymph node samples were different.

that in wild-type mice (Fig. 3). Ratios of CD4⁺ to CD8α⁺ T cells in the peripheral lymphoid organs of CD8β −/− 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 CD8β −/− mice were CD4⁺ CD8α⁺ double positive and were normal in population size and in CD3 expression (Fig. 4). Consistent with the observation in the periphery, the subset of CD8α⁺ mature thymocytes in CD8β −/− mice was reduced to 20% of that in wild-type mice (Fig. 4). In contrast, the CD8α⁺ IEL population in CD8β −/− mice was comparable with that in wild-type mice (Fig. 5). The IEL cell number harvested from the intestinal epithelia of CD8β −/− mice was also in a normal range (Table 1).

A significant reduction of CD8α surface expression was

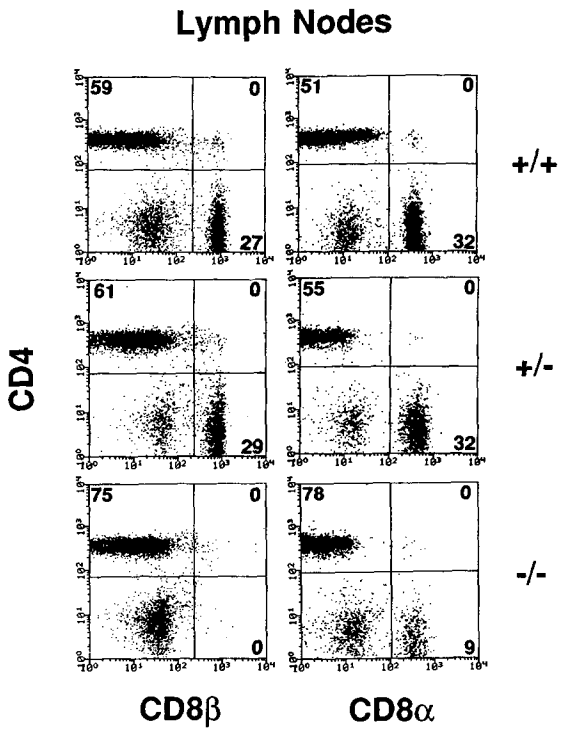

Figure 3. Decreased CD8α⁺ T cell population in lymph nodes of CD8β gene-targeted mice. Mesenteric lymph node cells from CD8β +/+, +/−, and −/− mice were double stained with mAb specific for CD4, CD8α, and CD8β and analyzed by flow cytometry.

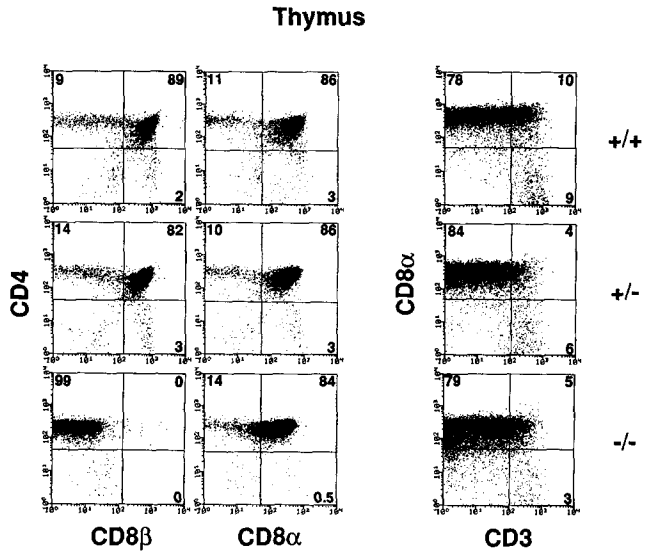

Figure 4. Decreased thymic ontogeny of CD8α⁺ T cells in CD8β gene-targeted mice. Thymocytes from CD8β +/+, +/−, and −/− mice were triple stained with mAb specific for CD3, CD4, CD8α, and CD8β and analyzed by flow cytometry.

Table 3. *The Ratio of CD4⁺ T Cell to CD8⁺ T Cell Subsets in Different Lymphoid Organs of the CD8β +/+, +/−, and −/− Mice*

Mouse genotype	Thymus	Lymph nodes	Spleen	Blood
CD8β +/+	3.7:1	1.6:1	1.8:1	1.1:1
CD8β +/−	3.3:1	1.7:1	2.6:1	3.6:1
CD8β −/−	14:1	8.7:1	9.5:1	11.5:1

found on peripheral T cells from CD8β −/− mice (53% of the level in wild-type control mice) (Fig. 2). CD8α expression levels on immature thymocytes from CD8β−/− mice were also low (44% of normal levels) and spanned a wide range (Fig. 4). Low CD8α expression was particularly obvious for early stage thymocytes which display low levels of CD3 expression. In contrast, CD8α expression levels on IEL from CD8β −/− mice were unchanged (Fig. 5).

In conclusion, the absence of CD8β only affects thymic derived T cells but has no impact on IEL. CD8β is needed for optimal CD8α expression and efficient thymic maturation of CD8⁺ T cells.

CD8⁺ T Cells without CD8β Are Efficient in Cytotoxic Effector Function against In Vivo Viral Infection. CD8β −/− mice provide a well-defined animal model for studying the in vivo immune response and effector function of the CD8⁺ T cells that lack CD8β. Cytotoxic T cell responses were studied in CD8β −/− 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⁺ cytotoxic T cells (36–38). This swelling reaction was normal in CD8β −/− 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 ^{51}Cr release assays. After 8 d of LCMV infection in CD8β −/− 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 CD8β −/− mice after VSV infection (Fig. 6 *C*). The

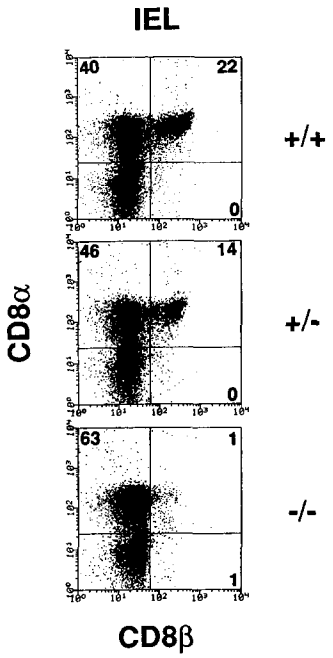

Figure 5. Normal CD8α⁺ IEL in CD8β gene-targeted mice. Intestinal IEL cells were purified from CD8β +/+, +/−, and −/− mice as described in Methods and Materials. Cells were stained with mAb specific for CD8α and CD8β and analyzed by flow cytometry.

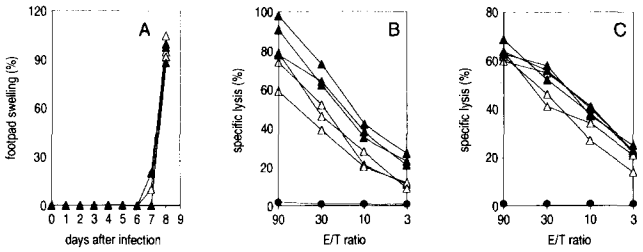

Figure 6. Normal effector function of cytotoxic T cells in CD8β gene-targeted mice. (*A*) Mice were infected with LCMV locally into hind footpads as described in Materials and Methods. Footpad swelling is calculated as (actual thickness − thickness before infection)/(thickness before infection). *Open triangles*, CD8β −/− mice; *solid triangles*, CD8β +/+ 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-2ᵇ) target cells that were labeled with the relevant MHC class I-binding LCMV glycoprotein peptide, as described in Materials and Methods. *Open triangles*, CD8β −/− mice; *solid triangles*, CD8β +/+ mice; *solid circles*, unprimed C57BL/6 (H-2ᵇ) 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*, CD8β −/− mice; *solid triangles*, CD8β +/+ mice; *solid circles*, unprimed C57BL/6 (H-2ᵇ) 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. ^{51}Cr release was measured in duplicate, mean values are shown (SD <13% for all values). Specific ^{51}Cr release was calculated as (measured ^{51}Cr release − spontaneous ^{51}Cr release)/(total ^{51}Cr release − spontaneous ^{51}Cr release).

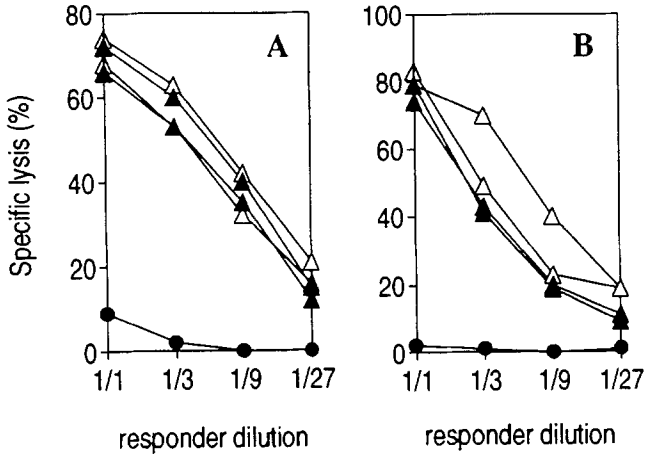

Figure 7. Efficient effector function of cytotoxic T cells in CD8β gene-targeted mice. (*A*) Spleen cells from mice infected with LCMV were restimulated in vitro with LCMV-infected irradiated peritoneal macrophages, as described in Materials and Methods. Cytotoxicity of spleen cells on LCMV glycoprotein peptide labeled EL-4 target cells are shown. *Open triangles*, CD8β −/− mice; *solid triangles*, CD8B +/+ mice; *solid circles*, unprimed C57BL/6 control mice. Spontaneous ^{51}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}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 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 CD8$^+$ T cells or a decreased effector function of the CD8$^+$ T cells is clarified by performing ^{51}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 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 CD8$^+$ T cells is reduced but not completely abolished in the absence of CD8β. This is evidenced by a significant number of 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 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 CD8$^+$ T cells.

It is interesting to note that 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 CD8$^+$ T cells with a TCR-α/β or γ/δ (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 CD8^{low} T cells. Alternatively, CD8 as α/α homodimers could be less stable than α/β 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 CD8^{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 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 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 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 CD8β in positive and negative selection.

The role of CD8β in cytotoxic function was examined by infecting CD8β −/− mice with LCMV or VSV. Cytotoxic T cell activity as assessed by primary ex vivo ^{51}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 CD8β −/− mice was efficient and comparable with wild-type controls. Therefore on a cell-to-cell basis the CD8$^+$ T cells that lack CD8β 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 CD8β −/− mice were shown to be normal. The demonstration of CTL functions against the above two nominal antigens in CD8β −/− 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 CD8β −/− 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 CD8β −/− 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 CD8α expression was not reported for the chimeric mice. These discrepancies probably arise from the limitation of the chimeric model as compared with CD8β −/− 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 CD8β −/− mice show that the absence of CD8β only reduces thymic maturation of functional CD8$^+$ T cells. The result also reveals a role of CD8β in regulating CD8α cell surface expression on thymic derived T cells. Despite the lack of CD8β expression, these CD8$^+$ T cells are functionally comparable to those in wild-type mice, suggesting that CD8β is not crucial for the antigenic response and the effector function of CD8$^+$ T cells.

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References

1. Swain, S.L. 1983. T cell subsets and the recognition of MHC class. *Immunol. Rev.* 74:129.
2. von Boehmer, H., P. Kisielow, H. Kishi, B. Scott, P. Borgulya, and H.-S. Teh. 1989. The expression of CD4 and CD8 accessory molecules on mature T cells is not random but correlates with the specificity of the alpha beta receptor for antigen. *Immunol. Rev.* 109:143.
3. Parnes, J.R. 1989. Molecular biology and function of CD4 and CD8. *Adv. Immunol.* 44:265.
4. Parrott, D.M.V., C. Tait, S. MacKenzie, A. Mowat, M.D. Davies, and H.S. Micklem. 1983. Analysis of the effector functions of different populations of mucosal lymphocytes. *Ann. NY Acad. Sci.* 409:307.
5. Blanc, D., C. Bron, J. Gabert, F. Letourneur, H.R. MacDonald, and B. Malissen. 1988. Gene transfer of the Ly-3 chain gene at the mouse CD8 molecular complex:co-transfer with Ly-2 polypeptide gene results in detectable cell surface expression of the Ly-3 antigenic determinants. *Eur. J. Immunol.* 18:613.
6. Gorman, S.D., Y.H. Sun, R. Zamoyska, and J.R. Parnes. 1988. Molecular linkage of the Ly-3 and Ly-2 genes. Requirements of Ly-2 for Ly-3 surface expression. *J. Immunol.* 140:3646.
7. Schmidt-Ullrich, R., and K. Eichmann. 1990. Transfection of the CD8α gene restores specific target cell lysis: factors that determine the function and the expression of CD8 in a cytotoxic T cell clone. *Int. Immunol.* 2:237.
8. Zamoyska, R., A.C. Vollmer, K.C. Sizer, C.W. Liaw, and J.R. Parnes. 1985. Two Ly-2 polypeptides arise from a single gene by alternative splicing patterns of mRNA. *Cell.* 43:153.
9. Ratnofsky, S.E., A. Peterson, J.L. Greenstain, S.J. Burakoff. 1987. Expression and function of CD8 in a murine T cell hybridoma. *J. Exp. Med.* 166:1747.
10. Letourneur, F., J. Gabert, P. Cosson, D. Blanc, J. Davoust, and B. Malissen. 1990. A signalling role for the cytoplasmic segment of the CD8α chain detected under limiting simulatory conditions. *Proc. Natl. Acad. Sci. USA.* 87:2339.
11. Miceli, M.C., P. von Hoegen, and J.R. Parnes. 1991. Adhesion versus coreceptor function of CD4 and CD8: role of the cytoplasmic tail in coreceptor activity. *Proc. Natl. Acad. Sci. USA.* 88:2623.
12. Salter, R.D., R.J. Benjamin, P.K. Wesley, S.E. Buxton, T.P. Garrett, C. Clayberger, A.M. Krensky, A.M. Norment, D.R. Littman, and P. Parham. 1990. A binding site for the T-cell co-receptor CD8 on the alpha 3 domain of HLA-A2. *Nature (Lond.).* 345:41.

13. Connolly, J.M., T.H. Hansen, A.L. Ingold, and T.A. Potter. 1990. Recognition by CD8 on cytotoxic T lymphocytes is ablated by several substitutions in the class I alpha 3 domain: CD8 and the T-cell receptor recognize the same class I molecule. *Proc. Natl. Acad. Sci. USA.* 87:2137.
14. Zamoyska, R., P. Derham, S.D. Gorman, P. von Hoegen, J.B. Bolen, A. Veillette, and J.R. Parnes. 1989. Inability of CD8 alpha polypeptides to associate with p56lck correlates with impaired function in vitro and lack of expression in vivo. *Nature (Lond.).* 342:278.
15. Aldrich, C.J., R.E. Hammer, S. Jones-Youngblood, U. Koszinowski, L. Hood, I. Stroynowski, and J. Forman. 1991. Negative and positive selection of antigen-specific cytotoxic T lymphocytes affected by the alpha 3 domain of MHC I molecules. *Nature (Lond.).* 352:718.
16. Ingold, A.L., C. Landel, C. Knall, G.A. Evans, and T.A. Potter. 1991. Co-engagement of CD8 with the T cell receptor is required for negative selection. *Nature (Lond.).* 352:721.
17. Killeen, N., A. Moriarty, H.-S. Teh, and D.R. Littman. 1992. Requirement for CD8-major histocompatibility complex class I interaction in positive and negative selection of developing T cells. *J. Exp. Med.* 176:89.
18. Norment, A.M., R.D. Salter, P. Parham, V.H. Engelhard, and D.R. Littman. 1988. Cell-cell adhesion mediated by CD8 and MHC class I molecules. *Nature (Lond.).* 336:79.
19. Potter, T.A., T.V. Rajan, R.F. Dick II, and J.A. Bluestone. 1989. Substitution at residue 227 of H-2 class I molecules abrogates recognition by CD8-dependent, but not CD8-independent, cytotoxic T lymphocytes. *Nature (Lond.).* 337:73.
20. Salter, R.D., A.M. Norment, B.P. Chen, C. Clayberger, A.M. Krensky, D.R. Littman, and P. Parham. 1989. Polymorphism in the α3 domain of HLA-A molecules affects binding to CD8. *Nature (Lond.).* 338:345.
21. Turner, J.M., M.H. Brodsky, B.A. Irving, S.D. Levin, R.M. Perlmutter, and D.R. Littman. 1990. Interaction of the unique N-terminal region of tyrosine kinase p56lck with cytoplasmic domains of CD4 and CD8 is mediated by cysteine motifs. *Cell.* 60:755.
22. Ramsdell, F., and B.J. Fowlkes. 1989. Engagement of CD4 and CD8 accessory molecules is required for T cell maturation. *J. Immunol.* 143:1467.
23. Zúñiga-Pflücker, J.C., L.A. Jones, D.L. Longo, and A.M. Kruisbeek. 1990. CD8 is required during positive selection of $CD4^-/CD8^+$ T cells. *J. Exp. Med.* 171:427.
24. Fung-Leung, W.-P., M.W. Schilham, A. Rahemtulla, T.M. Kundig, M. Vollenweider, J. Potter, W. van Ewijk, and T.W. Mak. 1991. CD8 is needed for development of cytotoxic T cells but not helper T cells. *Cell.* 65:443.
25. Dembic, Z., W. Haas, R. Zamoyska, J. Parnes, M. Steinmetz, and H. von Boehmer. 1987. Transfection of the CD8 gene enhances T-cell recognition. *Nature (Lond.).* 326:510.
26. Gabert, J., C. Langlet, R. Zamoyska, J.R. Parnes, A.M. Schmitt-Verhulst, and B. Malissen. 1987. Recognition of MHC class I specificity by transfer of the T cell receptor and Lyt-2 genes. *Cell.* 50:545.
27. Wheeler, C.J., P. von Hoegen, and J.R. Parnes. 1992. An immunological role for the CD8β chain. *Nature (Lond.).* 357:247.
28. Karaki, S., M. Tanabe, H. Nakauchi, and M. Takiguchi. 1992. β-chain broadens range of CD8 recognition for MHC class I molecule. *J. Immunol.* 149:1613.
29. Casabo, L.G., C. Mamalaki, D. Kioussis, and R. Zamoyska. 1994. T cell activation results in physical modification of the mouse CD8β chain. *J. Immunol.* 152:397.
30. Nakayama, K., K. Nakayama, I. Negishi, K. Kuida, M.C. Louie, O. Kanagawa, H. Nakauchi, and D.Y. Loh. 1994. Requirement for CD8 β chain in positive selection of CD8-lineage T cells. *Science (Wash. DC).* 263:1131.
31. van der Heijden, P.J., and W. Stock. 1987. Improved procedure for the isolation of functional active lymphoid cells from the murine intestine. *J. Immunol. Med.* 103:161.
32. Buchmeier, M.J., R.M. Welsh, F.J. Dutko, and M.B.A. Oldstone. 1980. The virology and immunobiology of lymphocytic choriomeningitis virus infection. *Adv. Immunol.* 30:275.
33. Pircher, H.P., D. Moskophidis, U. Rohrer, K. Burki, H. Hengartner, and R.M. Zinkernagel. 1990. Viral escape by selection of cytotoxic T cell-resistant virus variants in vivo. *Nature (Lond.).* 346:629.
34. Pircher, H.P., U. Rohrer, D. Moskophidis, R.M. Zinkernagel, and H. Hengartner. 1991. Lower receptor avidity required for thymic clonal deletion than for effector T cell function. *Nature (Lond.).* 351:482.
35. Oehen, S., H.P. Waldner, T.M. Kündig, H. Hengartner, and R.M. Zinkernagel. 1992. Cytotoxic T cell memory to lymphocytic choriomeningitis virus is governed by persisting antigen. *J. Exp. Med.* 176:1273.
36. Leist, T.P., S.P. Cobbold, H. Waldmann, M. Aguet, and R.M. Zinkernagel. 1987. Functional analysis of T lymphocyte subsets in antiviral host defence. *J. Immunol.* 138:2278.
37. Fung-Leung, W.-P., T.M. Kündig, R.M. Zinkernagel, and T.W. Mak. 1991. Immune response against lymphocytic choriomeningitis virus infection in mice without CD8 expression. *J. Exp. Med.* 174:1425.
38. Moskophidis, D., and F. Lehmann-Grube. 1989. Virus-induced delayed-type hypersensitivity reaction is sequentially mediated by $CD8^+$ and $CD4^+$ T lymphocytes. *Proc. Natl. Acad. Sci. USA.* 86:3291.
39. Moskophidis, D., U. Assmann Wischer, M.M. Simon, and F. Lehmann-Grube. 1987. The immune response of the mouse to lymphocytic choriomeningitis virus. V. High numbers of cytolytic T lymphocytes are generated in the spleen during acute infection. *Eur. J. Immunol.* 17:937.
40. Bonneville, M., C.A. Janeway, Jr., K. Ito, W. Haser, I. Ishida, N. Nakanishi, and S. Tonegawa. 1988. Intestinal intraepithelial lymphocytes are a distinct set of $\gamma\delta$ T cells. *Nature (Lond.).* 336:479.
41. Goodman, T., and L. LeFrancois. 1988. Expression of the $\gamma\delta$ T cell receptor on intestinal $CD8^+$ intraepithelial lymphocytes. *Nature (Lond.).* 333:855.
42. Guy-Grand, D., N. Cerf-Bensussan, B. Malissen, M. Malassis-Seris, C. Briottet, and P. Vassalli. 1991. Two gut intraepithelial $CD8^+$ lymphocyte populations with different T cell receptors: a role for the gut epithelium in T cell differentiation. *J. Exp. Med.* 173:471.
43. Klein, J. 1986. Ontogeny of the Thy-1-, Lyt-2^+ murine intestinal intraepithelial lymphocyte. Characterization of a unique population of thymus-independent cytotoxic effector cell in the intestinal mucosa. *J. Exp. Med.* 164:309.
44. Rocha, B., P. Vassalli, and D. Guy-Grand. 1991. The Vβ repertoire of mouse gut homodimeric α $CD8^+$ intraepithelial T cell receptor α/β^+ lymphocytes reveals a major extrathymic pathway of T cell differentiation. *J. Exp. Med.* 173:483.
45. Rocha, B., H. von Boehmer, and D. Guy-Grand. 1992. Selection of intraepithelial lymphocytes with CD8 α/α co-receptors by self-antigen in the murine. *Proc. Natl. Acad. Sci. USA.* 89:5336.
46. Teh, H.S., P. Kisielow, B. Scott, H. Kishi, Y. Uematsu, H.

Bluthmann, and H. von Boehmer. 1988. Thymic major histocompatibility complex antigens and the alpha beta T-cell receptor determine the CD4/CD8 phenotype of T cells. *Nature (Lond.).* 335:229.

47. Pircher, H.P., K. Burki, R. Lang, H. Hengartner, and R.M. Zinkernagel. 1989. Tolerance induction in double specific T-cell receptor transgenic mice varies with antigen. *Nature (Lond.).* 342:559.
48. Sha, W.C., C.A. Nelson, R.D. Newberry, D.M. Kranz, J.H. Russell, and D.Y. Loh. 1988. Selective expression of an antigen receptor on CD8-bearing T lymphocytes in transgenic mice. *Nature (Lond.).* 335:271.
49. Berg, L.J., A.M. Pullen, B. Fazekas de St. Groth, D. Mathis, C. Benoist, and M.M. Davis. 1989. Antigen/MHC-specific T cells are preferentially exported from the thymus in the presence of their MHC ligand. *Cell.* 58:1035.
50. Kaye, J., M.L. Hsu, M.E. Sauron, S.C. Jameson, N.R. Gascoigne, and S.M. Hedrick. 1989. Selective development of $CD4^+$ T cells in transgenic mice expressing a class II MHC-restricted antigen receptor. *Nature (Lond.).* 341:746.
51. MacDonald, H.R., R.K. Lees, R. Schneider, R.M. Zinkernagel, and H. Hengartner. 1988. Positive selection of $CD4^+$ thymocytes controlled by MHC class II gene products. *Nature (Lond.).* 336:471.
52. Fung-Leung, W.-P., V.A. Wallace, D. Gray, W.C. Sha, H. Pirchers, H.-S. Teh, D.Y. Loh, and T.W. Mak. 1993. CD8 is needed for positive selection but differentially required for negative selection of T cells during thymic ontogeny. *Eur. J. Immunol.* 23:212.