and 90 μg per milliliter of DNA. We used similar plots to determine the apparent V_{max} and K_m^{DNA} values by varying the concentration of DNA from 7 to 50 μg/ml in 40 mM sodium glycinate (pH 9.9) with 10 mM Ca^{2+} (data not shown). Reactions were initiated by the addition of 20 ng of protein, and we determined the initial velocities by monitoring the increase in absorbance at 260 nm (A_{260}). The numbers reported represent the standard deviations of three data collections obtained for each protein on the same day. Protein concentrations were initially determined by SDS-PAGE analysis of ^{35}S-methionine–labeled samples of wild-type and mutant SNase. The wild-type band was excised and quantitated by scintillation counting; the remaining wild-type sample was assayed for catalytic activity, and the known specific activity was used to determine the concentration (in micrograms per milliliter) of wild-type SNase present. Quantitation of the gel slices containing mutant proteins by scintillation counting was followed by catalytic assay to provide the specific activity of each mutant in units of ΔA_{260} min^{-1} μg^{-1} SNase. For the pH versus rate profiles, assays were done as described above, at 10 mM Ca^{2+} and at DNA concentrations ranging from 6 to 80 μg/ml. Between pH 7.5 and 8.5, 40 mM tris-HCl was used as the buffer; from pH 8.5 to 9.9, assay mixtures were buffered with 40 mM sodium glycinate. Duplicate data points were gathered for each concentration of DNA at each pH; points on the pH versus rate plot represent the average value of two (NABA and ADPA) or three (wild type) of such sets of data. The lines were fit by linear least squares analysis; no weighting was performed.

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21. Both mutants (Glu^{43}→NABA and Glu^{43}→ADPA) and the wild-type enzyme exhibited a significant kinetic isotope effect in D_2O, with V_{max}^H/V_{max}^D = 2 to 3. The solvent isotope effects observed here suggest that chemical steps contribute to the rate-determining step in both the wild type and the mutants; chemistry and loop movement may be coupled, as was recently observed for triosephosphate isomerase (*25*). Kinetic isotope effects were determined as described [R. L. Schowen, in *Isotope Effects on Enzyme Catalyzed Reactions*, W. W. Cleland, M. H. O'Leary, D. B. Northrop, Eds. (University Park Press, Baltimore, MD, 1977), pp. 64–99]. Concentrated buffer stocks (400 mM sodium glycinate) were prepared by addition of solid glycine to H_2O or D_2O and adjustment of the pH with 6 N NaOH (in D_2O, pD = pH + 0.4); the final H_2O content in the D_2O buffers was less than 10% (v/v) after this procedure. Assay buffers were then prepared by dilution of stock solutions into H_2O or D_2O. Values of V_{max} were obtained from Lineweaver-Burk plots of two sets of kinetic data for each protein: for pH or pD values from 9.4 to 10.4, assay buffers contained 40 mM NaGly, 10 mM Ca^{2+}, and 10, 20, 40, or 80 μg of DNA per milliliter; for pD values above 10.4, buffers contained 40 mM NaGly, 10 mM Ca^{2+}, and 80, 100, or 120 μg of DNA per milliliter. The kinetic isotope effects were estimated from the differences in maximal values of V_{max}, which were observed at pH 9.9 in H_2O and pD 10.4 (Glu^{43}→NABA) or 10.6 (wild type and Glu^{43}→ADPA) in D_2O. By monitoring the intrinsic fluorescence of Trp^{140} [D. Shortle and A. M. Meeker, *Proteins* **1**, 81 (1986)] as a function of pH or pD, we have determined the midpoint for denaturation of wild-type SNase to be at pH 10.4 in H_2O and pD 11.2 in D_2O. As such, we do not believe that denaturation effects complicate our analyses.
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Thymic Selection of Cytotoxic T Cells Independent of CD8α-Lck Association

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The CD8α cytoplasmic domain associates with $p56^{lck}$, a nonreceptor protein–tyrosine kinase. The biological relevance of CD8α-Lck association in T cell development was tested with transgenic mice generated to express a CD8α molecule with two amino acid substitutions in its cytoplasmic domain, which abolishes the association of CD8α with Lck. The CD8α mutant was analyzed in a $CD8^{-/-}$ background and in the context of the transgenic 2C T cell receptor. The development and function of $CD8^+$ T cells in these mice were apparently normal. Thus, CD8α-Lck association is not necessary for positive selection, negative selection, or CD8-dependent cytotoxic function.

During the course of thymic development, selection events occur in the thymus to eliminate self-reactive T cells (negative selection) and retain T cells reactive to foreign antigens (positive selection) (*1*). Among the cell surface molecules involved in these processes are the T cell receptor (TCR) and CD8 or CD4 glycoproteins on the T cells and the major histocompatibility complex (MHC) proteins bound to antigenic peptides on antigen-presenting cells in the thymus. T cell precursors are initially $CD4^-CD8^-TCR^-$ double negative (DN) but differentiate to a $CD4^+CD8^+TCR^{lo}$ double positive (DP) stage. These DP cells are thought to be susceptible to selection. Mature T cells that are positively selected are phenotypically $CD4^+CD8^-$ or $CD4^-CD8^+$ single positive (SP).

The CD8 surface glycoprotein is involved in the outcome of thymic selection (*2–4*). In mouse thymocytes, CD8 is a disulfide-linked homodimer of 38-kD α (Lyt2) chains or a heterodimer of α and 30-kD β (Lyt3) chains. In peripheral T cells, CD8 occurs predominantly as an αβ heterodimer (*5*). CD8 functions as an adhesion molecule by binding to the nonpolymorphic α3 domain of MHC class I molecules. CD8 may also function in intracellular signal transduction, because the cytoplasmic domain of CD8α associates noncovalently with $p56^{lck}$, a Src family nonreceptor tyrosine kinase (*6*). Substitution of two cysteines in the CD8α cytoplasmic domain with alanine or serine residues abolishes its association with Lck (*7, 8*).

To assess the role of CD8α-Lck association during T cell development, we analyzed transgenic mice that contained mutations at the two cysteines in the CD8α cytoplasmic domain ($CD8^{CA}$). Peripheral blood T lymphocytes from the founders ($CD8^{CA}8^{+/+}$) demonstrated only $CD4^+CD8^+$ and $CD4^-CD8^+$ cells. The $CD4^+CD8^+$ population consisted of cells that would normally have been $CD4^+$ but now expressed the $CD8^{CA}$ transgene; no $CD4^+CD8^-$ cells were found in the peripheral blood of these mice (*9*). In the founder lines analyzed, $CD8^{CA}$ transgene expression was two to three times that of endogenous CD8α. To study the effects of the $CD8^{CA}$ mutation without endogenous wild-type CD8 molecules, we mated $CD8^{CA}$ mice to CD8-deficient mice [$CD8^{-/-}$ (*3*)] to obtain $CD8^{CA}8^{-/-}$ mice (*10*). The

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thymi of age-matched C57BL/6 ($CD8^{+/+}$), $CD8^{-/-}$, and $CD8^{CA}8^{-/-}$ mice all contained ~5 × 10^7 cells.

Immunoprecipitation and in vitro kinase assays verified that the cytoplasmic domain of $CD8^{CA}$ does not bind Lck (Fig. 1A). In $CD8^{+/+}$ thymocytes, almost equal amounts of Lck activity were associated with CD4 and CD8α. In $CD8^{-/-}$ thymocytes, Lck activity was associated only with CD4. Similarly, in $CD8^{CA}8^{-/-}$ thymocytes, substantial kinase activity was associated with CD4, whereas none was associated with $CD8^{CA}$, as expected from data in transfectants (*7, 8*). The other phosphoproteins (migrating at about 97 and 50 kD) were not reproducibly seen. Excess amounts of acid-denatured enolase were used in the kinase reaction (Fig. 1B) so that even extremely low amounts of coprecipitated Lck could be detected by the phosphorylation of enolase. The conditions of the kinase reaction favored the phosphorylation of enolase over Lck autophosphorylation (*11*). In the $CD8^{+/+}$ sample, Lck autophosphorylation was not evident (although it was detected with longer autoradiography), but Lck activity was detectable indirectly by enolase phosphorylation. In the $CD8^{CA}8^{-/-}$ sample, enolase was not phosphorylated, and thus no detectable Lck activity was associated with CD8. Therefore, mutation of the cysteine motif in the CD8α cytoplasmic domain disrupted CD8α-Lck association in the T cells of $CD8^{CA}8^{-/-}$ transgenic mice.

We analyzed $CD8^{+/+}$, $CD8^{-/-}$, and $CD8^{CA}8^{-/-}$ thymocytes by flow cytometry (Fig. 2A). The percentage of $CD4^+CD8^+$ and $CD4^-CD8^+$ cells was comparable between $CD8^{+/+}$ and $CD8^{CA}8^{-/-}$ thymocytes. The DP population in the $CD8^{CA}8^{-/-}$ thymus consisted of $CD4^+CD8^+$ cells (81%) and cells that would have been $CD4^+CD8^-$ (15%) but now expressed the $CD8^{CA}$ transgene (Fig. 2A). Of the $CD8^{CA}8^{-/-}$ thymocytes, 83% were $CD8\alpha^+\beta^+$ and 16% were $CD8\alpha^+\beta^-$. Therefore, $CD8^{CA}$ co-expressed with CD8β in thymocytes and $CD8^{CA}8^{-/-}$ thymocytes formed a population similar to that seen in $CD8^{+/+}$ thymi.

When Thy1-positive lymph node cells from $CD8^{+/+}$, $CD8^{-/-}$, and $CD8^{CA}8^{-/-}$ mice were analyzed (Fig. 2B), 33% of $CD8^{+/+}$ and 17% of $CD8^{CA}8^{-/-}$ peripheral T cells were $CD4^-CD8^+$. The $CD8^{CA}$ transgene was co-expressed with endogenous CD8β molecules; all $CD4^-CD8^+$ cells were $CD8\alpha^+\beta^+$. Because CD8β can only be expressed on the cell surface with CD8α (*12*), these $CD8\alpha^+\beta^+$ cells originate from $CD4^-CD8^+$ cells that survived positive selection. The $CD4^+CD8\alpha^+\beta^-$ cells (82%) in $CD8^{CA}8^{-/-}$ mice are the result of $CD8^{CA}$ transgene expression on $CD4^+$ cells. Functionally, $CD8^{CA}$ can substitute for CD8α, because positive selection occurred in $CD4^-CD8^+$ cells expressing the $CD8^{CA}$ transgene with CD8β. Therefore, CD8α-Lck association was not necessary in the signaling pathway for $CD8^+$ T cells to differentiate and populate the periphery.

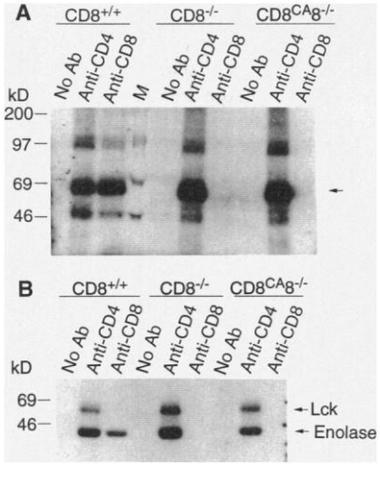

Fig. 1. In vitro (**A**) autophosphorylation and (**B**) transphosphorylation activities of anti-CD8α immunoprecipitates from $CD8^{+/+}$, $CD8^{-/-}$, and $CD8^{CA}8^{-/-}$ thymocyte lysates. In vitro kinase assays were conducted in the absence and presence of enolase (*27*). The sizes of the molecular standards (M) are indicated in kilodaltons. The arrow indicates the position of autophosphorylated Lck, which migrates as a protein of ~60 kD.

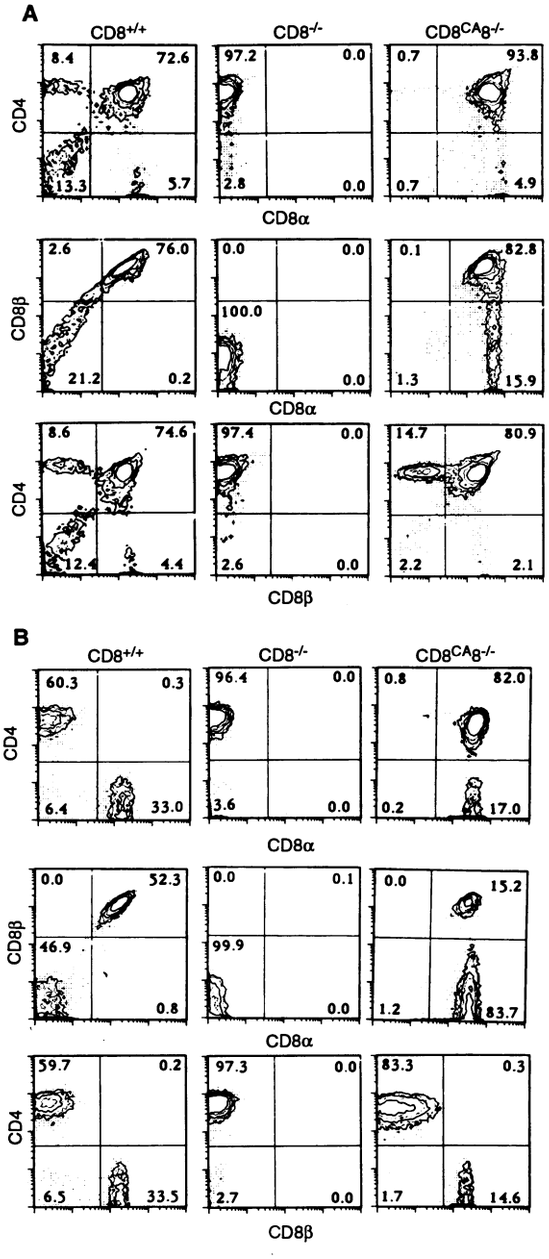

Fig. 2. Expression of the $CD8^{CA}$ transgene on (**A**) thymocytes and (**B**) lymph node cells. Cells from 6- to 12-week-old C57BL/6 ($CD8^{+/+}$), $CD8^{-/-}$, and $CD8^{CA}8^{-/-}$ mice were stained with the following reagents: phycoerythrin (PE)-conjugated anti-CD4 (clone GK1.5, Becton Dickinson), fluorescein isothiocyanate (FITC)–conjugated anti-CD8α (clone 53-6.7, Becton Dickinson), PE- or FITC-conjugated antibody to CD8β (clone 53-5.8, PharMingen), biotinylated antibody to 2C TCR clonotype (1B2, purified from ascites), biotin-, PE-, or FITC-conjugated antibody to Thy1.2 (clone 53-2.1, PharMingen), and streptavidin–Red 613 (Gibco BRL). Dead cells were eliminated from acquisition by propidium iodide gating. Thymic T cells were gated by forward scatter (FSC) and side scatter (SSC) parameters; lymph node T cells were gated by FSC versus SSC parameters and by $Thy1.2^+$ staining. We collected 10,000 to 15,000 events per sample on a FACScan flow cytometer (Becton Dickinson) and analyzed them with FACScan software. In $CD8^{+/+}$ mice, $CD8\alpha^+$ cells expressed endogenous CD8α molecules and in $CD8^{CA}8^{-/-}$ mice, $CD8\alpha^+$ cells expressed the $CD8^{CA}$ transgene. The numbers in each quadrant represent the percentage of cells in each subpopulation (*28*).

We explored positive selection of $CD8^{CA}$ cells in class I MHC–restricted 2C TCR transgenic mice in the *H-2*^{b} MHC haplotype. T cells expressing the 2C TCR are preferentially $CD4^-CD8^+$ cells (*13*) and are detected with the monoclonal antibody (mAb) 1B2, which specifically recognizes the 2C TCR (*14*). Thymocytes from $2C^+$ $CD8^{+/+}$ (*H-2*^{b}) and $2C^+$ $CD8^{CA}8^{-/-}$ (*H-2*^{b}) mice had selective clonotype expression on their $CD8^+$ T cells (*9*). There were no $CD8^+1B2^+$ T cells in the thymus or periphery of $2C^+$ $CD8^{-/-}$ (*H-2*^{b}) mice (*9*, *15*). In the periphery of $2C^+$ $CD8^{CA}8^{-/-}$ (*H-2*^{b}) mice, the population skewed toward mature $CD8^+$ cells (Fig. 3, A and B). The $CD8^+$ cells were 61% $CD8\alpha^+\beta^+1B2^+$ and 32% $CD8\alpha^+\beta^-1B2^+$ (Fig. 3B). Whereas the $CD8\alpha^+\beta^-1B2^+$ cells are the previously observed $CD4^-CD8^-1B2^+$ cells (*16*) expressing the $CD8^{CA}$ transgene, the $CD8\alpha^+\beta^+1B2^+$ cells were positively selected. The CD8α-Lck association was not required for positive selection of 2C TCR–bearing cells and skewing toward the CD8 population.

Negative selection was assessed in $2C^+$ $CD8^{CA}8^{-/-}$ (*H-2*$^{d/b}$) mice in which *H-2L*^{d} functions dominantly as the negative selection element (*13*). In $2C^+$ $CD8^{CA}8^{-/-}$ (*H-2*$^{d/b}$) mice, almost all the $CD8\beta^+1B2^+$ thymocytes (*9*) and $CD8\beta^+1B2^+$ lymph node cells (Fig. 3B) were deleted. The CD8α-Lck association was not required for negative selection of the $CD8\beta^+1B2^+$ cells. This result was expected because negative selection still occurred in $2C^+$ $CD8^{-/-}$ (*H-2*$^{d/b}$) mice (*15*) and was consistent with in vitro studies with tyrosine kinase inhibitors showing that Lck is not involved in the signal for antigen-stimulated clonal deletion (*17*).

Cytotoxic T cells that are CD8-dependent are required for a primary allogeneic response (*18*). We investigated whether CD8α-Lck association was required for cytotoxic function in a mixed-lymphocyte reaction (Fig. 4). Splenocytes from $CD8^{CA}8^{-/-}$ (*H-2*^{b}) and $CD8^{+/+}$ (*H-2*^{b}) mice mediated comparable allogeneic responses against ^{51}Cr-labeled BALB/c (*H-2*^{d}) splenocytes; splenocytes from $CD8^{-/-}$ or BALB/c mice did not lyse the BALB/c targets. The cytotoxic activity of $CD8^{CA}8^{-/-}$ cells was CD8-dependent; more than 90% of the activity was inhibited by first incubating the effectors with a mAb to CD8α. The CD8α-Lck association was not required for CD8-dependent allogeneic responses.

Compared with $CD8^{+/+}$ mice, the efficiency of positive selection and cytotoxic function was decreased in $CD8^{CA}8^{-/-}$ mice. Although twofold overexpression of normal CD8 in transgenic mice does not significantly affect the positive selection of $CD8^+$ cells (*4*), the two- to threefold overexpression of $CD8^{CA}$ may have subtle effects on positive selection that cannot be easily quantitated. Therefore, we cannot rule out the possibility that a minor subset of cells requires CD8α-Lck association for thymic development and cytotoxic function. However, the conclusion that CD8α-Lck association is not essential for thymocyte selection and T cell activation remains the same.

In contrast with our results, others have proposed that CD8α-Lck association is important for positive selection (*19*). In that model, CD4 transgene overexpression affects the positive selection of a class I MHC–restricted TCR by altering the competition between the CD4 and CD8α cytoplasmic domains for Lck. An alternative explanation is that the excess CD4 sequestered a yet unidentified protein from the CD8α cytoplasmic domain that is crucial for efficient $CD8^+$ cell positive selection. In contrast with our conclusion that CD8α-Lck association is not necessary for mature $CD8^+$ cell activation, another group showed that a CD8α cysteine mutant was unresponsive to mAb-induced CD3-CD8 cross-linking (*20*). However, the mixed-lymphocyte reaction may be more physiologically relevant than antibody stimulation of a transfected T cell line.

The Lck protein is important in early T cell development (*21*) and is also required for cytotoxic activity (*22*). However, the association of Lck with CD8α may not be the critical aspect of Lck's involvement in these processes. If intracellular signaling by means of CD8 is important, perhaps an as yet identified protein associates with the CD8α cytoplasmic domain to transduce the signals for T cell development. This putative protein would recognize a distinct motif on the mutated $CD8^{CA}$ cytoplasmic domain. A 32-kD guanosine triphosphate–binding protein, p32, co-immunoprecipitates with CD4-Lck and CD8α-Lck complexes (*23*) and could be a candidate for transducing signals through the CD8α cytoplasmic domain.

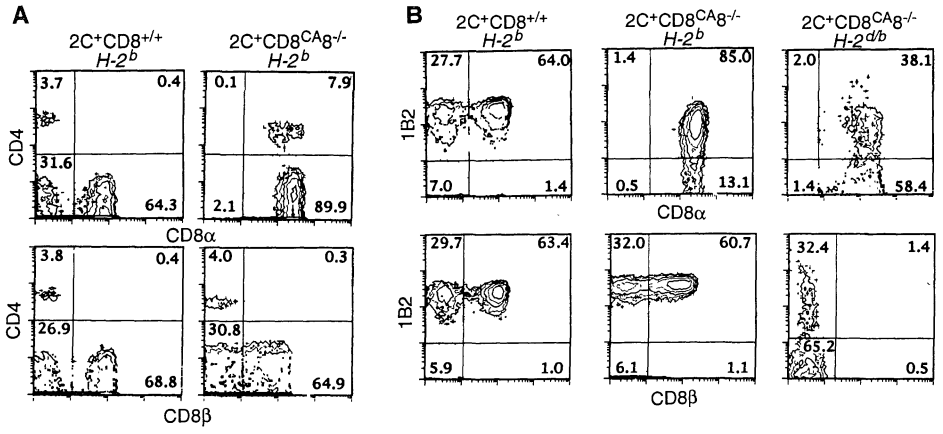

Fig. 3. Expression of the $CD8^{CA}$ transgene (**A**) and the 2C TCR (**B**) on lymph node cells. Cells from 6- to 12-week-old $2C^+$ $CD8^{+/+}$ (*H-2*^{b}) and $2C^+$ $CD8^{CA}8^{-/-}$ (*H-2*^{b} or *H-2*$^{d/b}$) mice were stained with the same procedures and reagents described in Fig. 2. In addition, biotinylated 1B2 antibody (purified from ascites) was used to detect the 2C TCR.

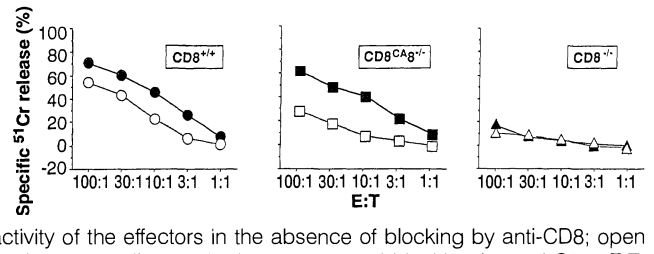

Fig. 4. Mixed-lymphocyte cytotoxicity assay. Splenocytes from $CD8^{+/+}$, $CD8^{-/-}$ and $CD8^{CA}8^{-/-}$ (all in an *H-2*^{b} background) were assayed for in vitro allogeneic response to ^{51}Cr-labeled BALB/C (*H-2*^{d}) targets (*29*). Closed symbols represent cytolytic T lymphocyte (CTL) activity of the effectors in the absence of blocking by anti-CD8; open symbols represent the CTL activity of the effectors in the presence of blocking by anti-CD8. E:T, effector to target ratio.

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10. To ensure T cell–specific transgene expression, we constructed the $CD8^{CA}$ transgene so that it was expressed by the CD3δ promoter and enhancer (*24*). In the transgene construct, we mutated cytoplasmic cysteine residues 200 and 202 (*7*) to alanine by the polymerase chain reaction (PCR) overlap-extension method (*25*) using Lyt2.1 (*26*) as the template. Fertilized eggs from $(C57BL/6 \times C3H)F_1$ females mated to BALB/c males (Harlan Sprague-Dawley) were microinjected with $CD8^{CA}$ transgene DNA. Mice were screened for inheritance of the transgene by PCR and Southern (DNA) hybridization analysis of tail DNA. To confirm the nucleotide substitutions corresponding to the two amino acid changes, we amplified the DNA region containing the mutation by PCR from the tail DNA of the $CD8^{CA}$ founders, subcloned it into pBluescript (Stratagene), and sequenced the region by the dideoxynucleotide termination method (USB). Five founder lines were generated and two were subsequently followed. Lines 14 and 28 were bred to CD8-deficient mice (*3*) and 2C TCR transgenic mice (*13*) to generate $CD8^{CA}8^{-/-}$ ($H\text{-}2^b$) or $2C^+$ $CD8^{CA}$-$8^{-/-}$ ($H\text{-}2^b$ or $H\text{-}2^{d/b}$) mice.
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27. Thymocytes (3×10^7 to 4×10^7) from $CD8^{+/+}$, $CD8^{-/-}$, and $CD8^{CA}8^{-/-}$ mice were lysed in nonionic detergent Brij 96 buffer [1% Brij 96 (w/v) (Sigma), 20 mM tris (pH 8.0), 150 mM NaCl, 50 mM NaF, 100 μM Na_3VO_4, 1 mM phenylmethylsulfonyl fluoride, leupeptin (10 μg/ml), and aprotinin (10 μg/ml)] and precleared with protein A–Sepharose CL-4B beads (Sigma). Precleared thymocyte lysates were added to protein A–Sepharose beads preadsorbed with rabbit antibodies to rat immunoglobulin G (Kappel) along with 5 μg of antibody to CD4 (anti-CD4) (GK1.5, purified from rat ascites or clone RM-4-4, PharMingen), 5 μg of antibody to CD8 (anti-CD8) (clone 53-6.7, PharMingen), or no specific antibody and allowed to form complexes. After extensive washing, the immunoprecipitates were incubated in 30 μl of kinase buffer [25 mM Hepes (pH 7.3), 0.1% Brij 96 (w/v), 10 mM $MgCl_2$, 3 mM $MnCl_2$, 30 μM Na_3VO_4, and 10 μM adenosine triphosphate (ATP) with 10 μCi of [γ-^{32}P]ATP (3000 μCi/mmol, Amersham)] for 45 min at *RT* to allow Lck autophosphorylation. For kinase assays in the presence of 10 μg of acid-denatured enolase (Boehringer Mannheim), one-fourth of the immunoprecipitates (from lysates of 3×10^7 thymocytes) were incubated in 15 μl of kinase buffer for 15 min at room temperature with constant vortexing. We stopped the kinase reactions by adding 2× sample loading buffer to the mixture, boiling it, and applying it to 10% SDS–polyacrylamide gel electrophoresis. Rainbow ^{14}C-methylated protein molecular weight markers (Amersham) were used to estimate the size of the phosphorylated bands. Protein gels were dried and exposed to XAR film (Kodak).
28. Care of mice used in the experiments was in accordance with the institutional guidelines of the Washington University School of Medicine.
29. We cultured 2×10^7 splenocyte effectors from 6- to 12-week-old mice with 2×10^7 irradiated stimulator splenocytes in 20 ml of mixed lymphocyte reaction (MLR) medium [RPMI with 10% heat-inactivated fetal calf serum and 5×10^{-5} M β-mercaptoethanol supplemented with Hepes (pH 7.3), penicillin-streptomycin, L-glutamine, nonessential amino acids, and sodium pyruvate] at 37°C for 5 days. The target splenocytes (5×10^6) were stimulated with concanavalin A (3 μg/ml) in 5 ml of MLR medium at 37°C for 2 days, purified on Histopaque 1077 (Sigma), and washed. The targets (2×10^6 to 5×10^6) were labeled with 0.5 mCi of $Na_2{}^{51}CrO_4$ (Amersham) in 1 ml of MLR medium containing 0.1 M α-methyl D-mannoside (USB) for 90 min at 37°C, washed with 0.1 M α-methyl D-mannoside in MLR medium, and left at 25°C for 30 min for spontaneous release. We plated 10^4 labeled targets in 96-well round-bottom plates (Costar). Effectors were purified on Histopaque 1077, washed, and plated at the indicated effector-to-target ratios in 200-μl reactions. For antibody blocking studies, effectors were preincubated for 30 min with either 2 μg of purified antibody to CD8α (anti-CD8α) (clone 53-6.7, Becton Dickinson) or MLR medium at 37°C before addition of labeled targets. Samples were centrifuged at 500*g* for 3 min, incubated for 3 hours at 37°C for cytolytic activity, and harvested with a supernatant collection system (Skatron). We measured ^{51}Cr release for each sample by γ-counting. The average of duplicate samples was determined and the percent of specific ^{51}Cr release was calculated from the following formula: $100 \times (\text{experimental} - \text{spontaneous release}) \times (\text{total} - \text{spontaneous release})^{-1}$. The data are representative of two experiments.
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Disappearance of the Lymphoid System in Bcl-2 Homozygous Mutant Chimeric Mice

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The *bcl-2* proto-oncogene can prevent the death of many cell types. Mice were generated that were chimeric for the homozygous inactivation of *bcl-2*. Lymphocytes without Bcl-2 differentiated into phenotypically mature cells. However, in vitro, the mature T cells that lacked Bcl-2 had shorter life-spans and increased sensitivity to glucocorticoids and γ-irradiation. In contrast, stimulation of CD3 inhibited the death of these cells. T and B cells with no Bcl-2 disappeared from the bone marrow, thymus, and periphery by 4 weeks of age. Thus, Bcl-2 was dispensable for lymphocyte maturation, but was required for a stable immune system after birth.

The *bcl-2* proto-oncogene was initially isolated from the breakpoint of the t(14;18) chromosomal translocation found in over 60% of human follicular lymphomas (*1*). As a result of the translocation, Bcl-2 expression in B cells is aberrantly increased (*2*). The deregulated Bcl-2 promotes cell survival and prevents programmed cell death in many, but not all, types of cells (*3*). The topographical distribution of Bcl-2 protein in tissues characterized by apoptotic cell death also suggests a potential role for Bcl-2 in programmed cell death (*4*). Cells derived from transgenic mice that have deregulated and overexpressed *bcl-2* displayed extended B and T cell survival (*5*, *6*). These transgenic studies suggested that Bcl-2 may play an important role in B cell and T cell differentiation as well as in the maintenance of B cell memory (*6*, *7*).

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