Brief Definitive Report

Function of CD3ε-mediated Signals in T Cell Development

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Abstract

The T cell antigen receptor (TCR) and pre-TCR complexes are composed of multiple signal-transducing subunits (CD3γ, CD3δ, CD3ε, and ζ) that each contain one or more copies of a semiconserved functional motif, the immunoreceptor tyrosine-based activation motif (ITAM). Although biochemical studies indicate that individual TCR-ITAMs may bind selectively or with different affinity to various effector molecules, data from other experiments suggest that at least some ITAMs are functionally equivalent. In this study, we examined the role of CD3ε ITAM-mediated signals in T cell development by genetically reconstituting CD3ε-deficient mice with transgenes encoding either wild-type or ITAM-mutant (signaling defective) forms of the protein. The results demonstrate that signals transduced by CD3ε are not specifically required for T cell maturation but instead contribute quantitatively to TCR signaling in a manner similar to that previously observed for ζ chain. Unexpectedly, analysis of TCR-transgenic/CD3ε-mutant mice reveals a potential role for CD3ε signals in T cell survival.

Key words: TCR • CD3epsilon • development • mice • cell survival

Introduction

Signals transduced by the TCR initiate a broad range of mature T cell responses. Signaling by the TCR and its precursor, the pre-TCR, are also required for thymocyte development and selection (1–3). The pre-TCR and TCR activate intracellular signaling cascades through semiconserved sequences of amino acids referred to as immunoreceptor tyrosine-based activation motifs (ITAMs). After receptor engagement, phosphorylation of TCR-ITAM tyrosines leads to the recruitment and activation of src homology 2 (SH2) domain–containing kinases (2). The pre-TCR and TCR contain four distinct signal-transducing subunits—CD3γ, CD3δ, CD3ε, and ζ—assembled as dimers: γ/ε, δ/ε, and ζζ. Each of the CD3 subunits contains a single ITAM, whereas ζ chain contains three ITAMs.

Analysis of the function of TCR-ITAMs has revealed that individual motifs bind with different affinities to the same effector (ZAP-70) and bind selectively to other potential effector molecules (4, 5). On the other hand, isolated ITAMs have been shown to be independently capable of initiating a broad range of T cell responses, including proliferation and cytokine synthesis (6, 7).

Experimental data support the idea that at least some TCR-ITAMs are functionally equivalent with respect to the signaling requirements for T cell development. For example, mature T cells are still generated in mice that express TCRs lacking the three ζ chain ITAMs, and these cells appear functionally competent (8–10). In contrast to ζ chain, the importance of signals transduced by the CD3 subunits has not been as rigorously addressed. Chimeric (Tac/ζ or Tac/ε) transgenes were found to be capable of independently mimicking pre-TCR signals and some TCR signals; however, it is unknown if endogenous ζ and CD3ε chains contributed to these results (11). Mice lacking CD3γ, CD3δ, or CD3ε exhibit various degrees of developmental arrest, demonstrating a clear role for these proteins in T cell maturation (12–15). However, the importance of the CD3 chains to the TCR signaling response is difficult to assess in knockout mice, as these proteins are also required for TCR assembly and surface expression (12–15).

Among the CD3 subunits, CD3ε is the most likely candidate for performing a critical role in the TCR signaling

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response. CD3ε is represented twice in the TCR complex, serving as a component of both the γ/ε and δ/ε dimers. In addition, after TCR cross-linking, CD3ε and ζ are the predominant tyrosine-phosphorylated TCR subunits. In this study, we assessed the importance of CD3ε signals during T cell development by genetically reconstituting CD3ε-deficient mice with transgenes encoding either the wild-type (WT) or a mutant (signaling defective) form of the protein. The results demonstrate that signals transduced by CD3ε are not specifically required for T cell maturation but instead contribute quantitatively to TCR signaling. Interestingly, the phenotype of TCR-transgenic/CD3ε-mutant mice suggests a potential role for CD3ε signals in particular or TCR signals in general for the generation and/or survival of mature T cells.

Materials and Methods

Generation of CD3ε$^{\Delta/\Delta}$, CD3ε$^{\Delta/\Delta}$; ε-tg, and CD3ε$^{\Delta/\Delta}$; εM-tg Mice. The generation of mice lacking expression of CD3ε (CD3ε$^{\Delta/\Delta}$) and huCD2-CD3ε transgenic (tg) mice has been described previously (15). The huCD2-CD3εM transgene was generated by mutating the murine CD3ε ITAM sequence in vitro using a synthetic oligonucleotide that substitutes phenylalanine (AAA) for tyrosine (ATA) at position 181. Transgenic founder lines that expressed levels of protein that most closely matched that of endogenous CD3ε were used in the experiments described here. TCR-transgenic mice used in these studies included the MHC class I–restricted TCRs H-Y (16) and P14 (17), which were maintained in the H-2D^b background.

Western Blot Analysis. Thymocytes were enumerated, washed twice in PBS, and resuspended in PBS at a concentration of 10^8 per milliliter. Thymocyte stimulations, immunoprecipitations, Western blotting, and polyacrylamide electrophoresis were performed as described (18, 19). Separated proteins were transferred to polyvinylidene difluoride membranes and blotted with antiphosphotyrosine mAb (4G10; Upstate Biotechnology) or monoclonal anti-CD3ε (HMT3.1) followed by anti–mouse IgG–horseradish peroxidase (Transduction Laboratories) or protein A–horseradish peroxidase (Transduction Laboratories), respectively, and detected by chemiluminescence (ECL; Amersham Pharmacia Biotech).

Multicolor Flow Cytometry and Measurement of Calcium Flux. Single-cell suspensions of thymocytes or lymph node cells were processed, stained, and analyzed as described previously (8). Calcium flux measurements were performed as described (19).

Proliferation and Cytokine Assays. Single-cell suspensions were prepared from thymi or lymph nodes in RPMI plus 10% FCS. CD4$^+$CD8$^-$ T cells were enriched by panning after incubating cells with antibodies to CD8, B220, and MAC-1 for 30 min. Alternatively, CD8$^+$CD4$^-$ thymocytes were enriched by panning after incubating cells with antibodies to CD4, B220, and MAC-1 for 30 min. Cells were washed, resuspended in RPMI plus 10% FCS, and placed on rabbit anti–mouse Ig–coated plates at 25°C, 5% CO_2. Nonadherent cells were collected after 1–2 h. In some experiments, purified cells were labeled with the intracellular fluorescent dye carboxyfluorescein diacetate succinimidyl ester (CFSE) before stimulation. Cells were washed twice in HBSS, resuspended at 4×10^6 cells/ml in HBSS and 50 ng/ml CFSE, incubated for 15 min at 37°C, and then washed twice in HBSS and resuspended in RPMI plus 10% FCS. Accessory cells and APCs were prepared from spleen suspensions of C57BL/6 mice. APCs were depleted of T cells with anti-Thy1.2 + C′ and irradiated with 3,000 rads. For antibody-mediated stimulation, wells of 96-well flat-bottomed plates were coated with antibodies at the indicated concentrations (see Fig. 3) overnight at 37°C and washed three times with sterile media. 10^5 responder T cells were added to the wells, incubated at 37°C for 40 h, and then pulsed with 1 μCi of [^{3}H]thymidine for 8–12 h. For stimulation with peptide, 10^5 responder T cells were combined with 5×10^5 APCs (obtained from female mice) in flat-bottomed 96-well plates in the presence or absence of the peptide. Smcy peptide (KCSRNRQYL) was synthesized in the FDA Core Facility and was added to culture at the indicated concentrations (see Fig. 3). Proliferation and cytokine assays were performed as described (8, 19). For IL-2 production, cells were stimulated as described above and culture supernatants were harvested after 48 h. IL-2 ELISAs were performed using the reagents and protocol obtained from PharMingen.

Results and Discussion

T Cell Development Is Restored in CD3ε$^{\Delta/\Delta}$; εM-tg Mice. T cell development is arrested at the CD4$^-$CD8$^-$ (DN) stage in CD3ε$^{\Delta/\Delta}$ mice, such that the more mature subsets of CD4$^+$CD8$^+$ (DP), CD4$^+$CD8$^-$ (CD4-SP), and CD4$^-$CD8$^+$ (CD8-SP) thymocytes normally present in the thymus of adult mice are absent in CD3ε$^{\Delta/\Delta}$ mice (Fig. 1 A; reference 15). As a consequence of this developmental block, total thymocyte numbers are markedly reduced in CD3ε$^{\Delta/\Delta}$ mice (<10% of normal), and mature T cells are undetectable in the peripheral lymphoid organs (Fig. 1 A; reference 15). As reported previously (15) and shown in Fig. 1 A, expression of transgenic WT CD3ε (ε-tg) in CD3ε$^{\Delta/\Delta}$ mice can rescue this developmental block, as assessed by the normalization of thymus cellularity and the presence of normal numbers of DP thymocytes and mature, TCR^{hi} SP T cells in the thymus and periphery. Significantly, genetic reconstitution with a transgene encoding a mutant form of CD3ε that contains a Y→F mutation of the distal tyrosine residue within the single CD3ε ITAM (εM-tg) also rescued the developmental defect in CD3ε$^{\Delta/\Delta}$ mice (Fig. 1 A). CD3ε$^{\Delta/\Delta}$; ε-tg and CD3ε$^{\Delta/\Delta}$; εM-tg mice contained similar numbers of thymocytes, and these were composed of approximately the same ratio of DP, CD4-SP, and CD8-SP cells as control, CD3ε$^{+/+}$ mice (Fig. 1 A). In addition, normal numbers of TCR^{hi} CD4-SP and TCR^{hi} CD8-SP T cells were present in the periphery of CD3ε$^{\Delta/\Delta}$; εM-tg mice (Fig. 1 B).

The normalization of thymus cellularity in CD3ε$^{\Delta/\Delta}$; εM-tg mice also indicated that the mutant CD3ε protein could restore surface expression of pre-TCR complexes on immature DN thymocytes and that these complexes could transduce signals sufficient to promote the transition of DN thymocytes to the DP stage. To explore this issue further, we examined the phenotype of DN thymocytes obtained from CD3ε$^{\Delta/\Delta}$; εM-tg mice. As shown previously (15) and in Fig. 1 C, DN thymocytes are arrested at the CD44$^-$ CD25$^+$ stage in CD3ε$^{\Delta/\Delta}$ mice. The observation that mutations in several distinct genes that are required either for

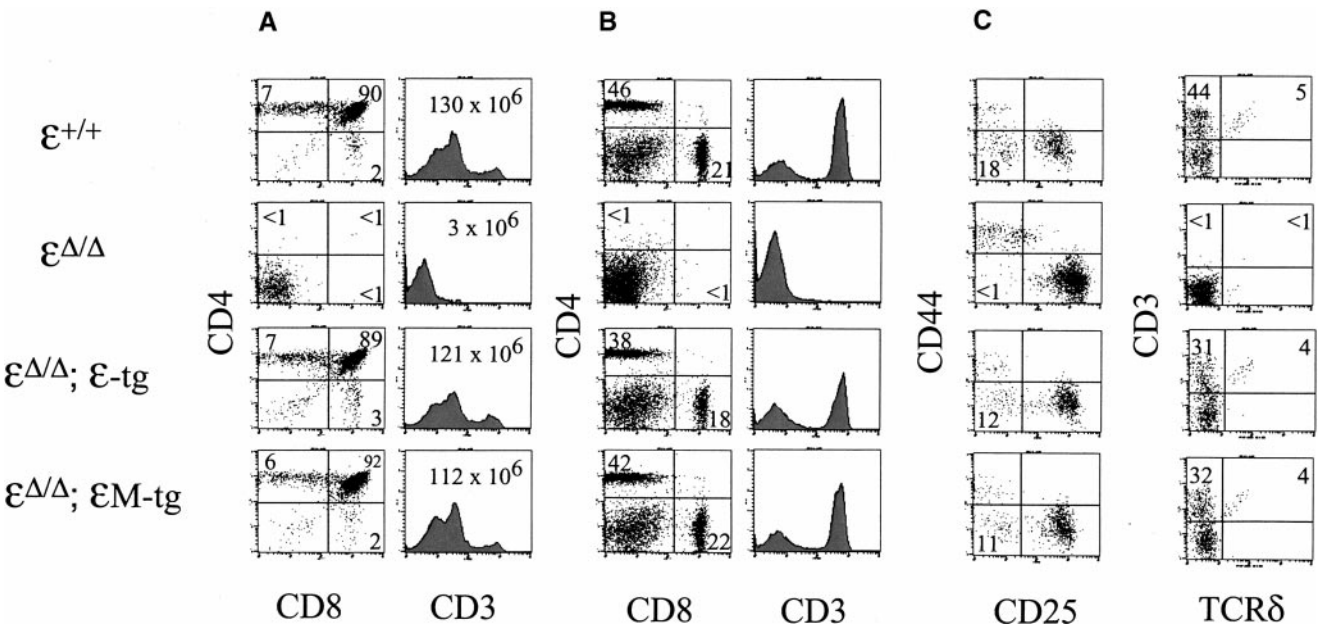

Figure 1. Phenotype of CD3$\epsilon^{\Delta/\Delta}$; ϵ-tg and CD3$\epsilon^{\Delta/\Delta}$; ϵM-tg mice. Comparison of thymocytes (A) and lymph node cells (B) from control ($\epsilon^{+/+}$), $\epsilon^{\Delta/\Delta}$, $\epsilon^{\Delta/\Delta}$; ϵ-tg, and $\epsilon^{\Delta/\Delta}$; ϵM-tg mice by FACS® analysis. Two-color plots show CD4/CD8 staining profiles. Numbers in quadrants indicate the percentage of cells within that quadrant. Histograms show CD3 surface staining on total thymocytes or total lymph node cells. Total thymocyte numbers are provided in the histograms. Results are representative of three separate experiments. (C) Analysis of $CD4^-CD8^-$ thymocyte subsets in CD3$\epsilon^{\Delta/\Delta}$; ϵ-tg and CD3$\epsilon^{\Delta/\Delta}$; ϵM-tg mice. Left panels, two-color (CD25 vs. CD44) staining on gated ($CD4^-CD8^-CD3^-$) thymocytes. Right panels, CD3 vs. TCR-δ staining on gated ($CD4^-CD8^-$) thymocytes.

pre-TCR surface expression or for pre-TCR signaling result in developmental arrest at this identical stage indicates that signaling by the pre-TCR is necessary for the maturation of DN, $CD44^-CD25^+$ thymocytes to the $CD44^-$ $CD25^-$ stage (1). Notably, DN, $CD44^-CD25^-$ thymocytes were present in $\epsilon^{\Delta/\Delta}$; ϵM-tg mice in numbers similar to those observed in CD3$\epsilon^{\Delta/\Delta}$; ϵ-tg mice (Fig. 1 C), indicating that functional pre-TCR complexes are expressed in CD3$\epsilon^{\Delta/\Delta}$; ϵM-tg mice. Finally, the mutant CD3ϵ chain was also capable of rescuing the development of DN-α/β TCR^+ and DN γ/δ TCR^+ T cells when introduced into CD3$\epsilon^{\Delta/\Delta}$ mice, as these cells were detected both in the thymus (Fig. 1C) and periphery (data not shown) of CD3$\epsilon^{\Delta/\Delta}$; ϵM-tg mice.

The ϵM Transgene Encodes a Signaling-defective Protein. Based on the results of in vitro studies, the ϵM transgene is predicted to encode a protein that does not contribute to the TCR signaling response (4, 5, 20, 21). However, in view of the normal phenotype observed in CD3$\epsilon^{\Delta/\Delta}$; ϵM-tg mice, we wished to examine this issue in greater detail in our transgenic system. A hallmark of ITAM function is its ability to become tyrosine phosphorylated upon T cell activation, thereby creating a site for stable association with and activation of ZAP-70 (2). After activation with pervanadate, tyrosine phosphorylation of both CD3ϵ and TCR-ζ is observed in thymocytes from control (CD3$\epsilon^{+/+}$) and CD3$\epsilon^{\Delta/\Delta}$; ϵ-tg mice (Fig. 2 A). In contrast, whereas pervanadate stimulation of thymocytes from CD3$\epsilon^{\Delta/\Delta}$; ϵM-tg mice induced tyrosine phosphorylation of TCR ζ chain, tyrosine phosphorylation of the mutated CD3ϵ chain was nearly undetectable (Fig. 2 A). Similar results were obtained after stimulation of thymocytes by TCR cross-linking (data not shown). Reblotting with anti-CD3ϵ mAb demonstrated the presence of the mutant CD3ϵ protein in the immunoprecipitated CD3 complexes (Fig. 2 A). As the proximal ITAM tyrosine is retained in the ϵM protein, the extremely low level of tyrosine phosphorylation observed after stimulation was unexpected. Interestingly, a TCR ζ chain transgene containing Y→F mutations of the NH_2- (but not the COOH-) terminal tyrosine residues in each of its three ITAMs also failed to become phosphorylated in vivo upon T cell activation (9). Recent data suggest that tyrosine phosphorylation of the TCR ζ chain follows a hierarchical progression in which the phosphorylation of specific ITAM tyrosines proceeds and perhaps facilitates phosphorylation of other ITAM tyrosines (22). Our findings may reflect a similar hierarchy for phosphorylation of the CD3ϵ-ITAM tyrosines. In any event, these results indicate that the ϵM protein is weakly (if at all) tyrosine phosphorylated upon TCR engagement.

Although a defect in thymocyte maturation was not observed in CD3$\epsilon^{\Delta/\Delta}$; ϵM-tg mice, we reasoned that one might be revealed in the presence of another mutation that impairs pre-TCR/TCR surface expression and/or signaling. Mice lacking TCR ζ chain ($\zeta^{-/-}$) exhibit a profound, though incomplete, block in T cell development characterized by reduced TCR surface expression and a reduction in the number of DP and SP thymocytes and mature, peripheral SP T cells (reference 23; Fig. 2 B). The low number of DP thymocytes in $\zeta^{-/-}$ mice indicates that pre-TCR sur-

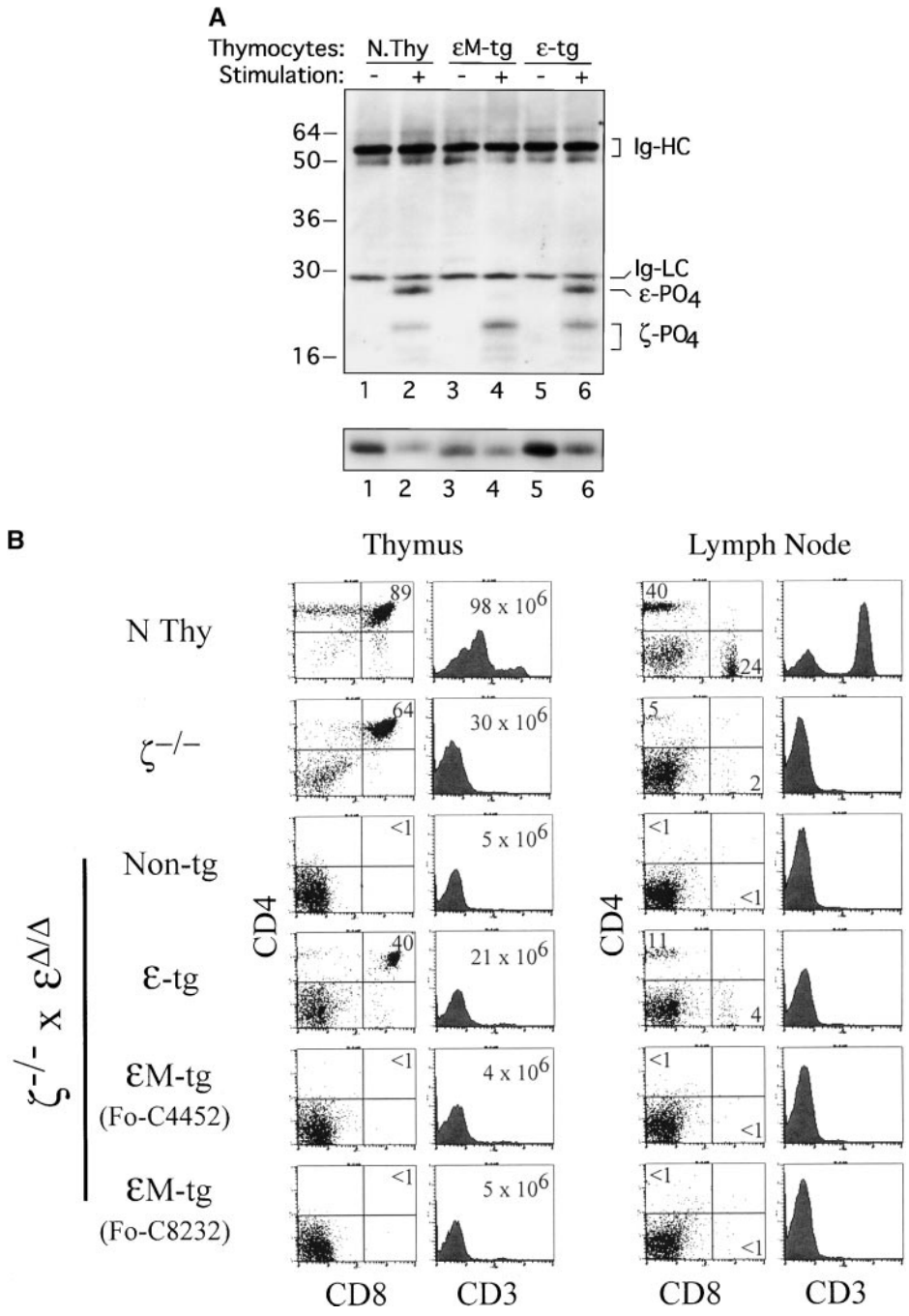

Figure 2. (A) Analysis of CD3ϵ and CD3ϵM protein tyrosine phosphorylation after stimulation of thymocytes with pervanadate. Thymocytes (10^8) from nontransgenic B6 mice (N.Thy), CD3$\epsilon^{\Delta/\Delta}$; ϵ-tg (ϵ-tg), and CD3$\epsilon^{\Delta/\Delta}$; ϵM-tg (ϵM-tg) mice were incubated for 5 min in medium lacking (−) or containing (+) pervanadate. Cell lysates were immunoprecipitated with anti-CD3ϵ (mAb 145-2C11). Immunoprecipitated proteins were resolved by 12% SDS-PAGE, followed by antiphosphotyrosine (mAb 4G10) Western blotting (top blot). Blots were subsequently stripped and reprobed with anti-CD3ϵ (mAb HMT3.1; bottom blot). (B) Genetic reconstitution of mice deficient for ζ and CD3ϵ ($\zeta^{-/-} \times \epsilon^{\Delta/\Delta}$) with transgenes encoding WT or mutant CD3ϵ proteins. Two-color plots show CD4/CD8 staining profiles on total thymocytes or total lymph node cells. Numbers in quadrants indicate the percentage of cells within that quadrant. Histograms show CD3 surface staining on total thymocytes or total lymph node cells. Total thymocyte numbers are provided in the histograms. Results are shown for two independently generated ϵM-tg founder lines with similar transgene copy number.

face expression and/or pre-TCR signaling is compromised in the absence of TCR ζ chain. When mice rendered deficient for both CD3ϵ and TCR-ζ ($\epsilon^{\Delta/\Delta} \times \zeta^{-/-}$) were reconstituted with the WT CD3ϵ transgene, a phenotype essentially indistinguishable from that of $\zeta^{-/-}$ mice was observed (i.e., low numbers of DP and SP thymocytes and peripheral T cells), reflecting the ability of the transgenic CD3ϵ protein to rescue the $\epsilon^{\Delta/\Delta}$ but not the $\zeta^{-/-}$ defect (Fig. 2 B). However, two independently generated ϵM transgenes expressing similar (~1×) levels of ϵM protein were unable to effect a similar partial rescue of T cell development when introduced into $\epsilon^{\Delta/\Delta} \times \zeta^{-/-}$ mice (Fig. 2 B). In addition to demonstrating that the ϵM protein is signaling defective in vivo, these results reveal that a signaling threshold exists for the pre-TCR, below which this complex is incapable of inducing the formation of DP thymocytes. These findings are consistent with those of Ardouin et al. (24) demonstrating a gene dosage effect of CD3ϵ for pre-TCR function in the absence of ζ chain. As both pre-TCR surface expression and signaling are thought to be compromised in $\zeta^{-/-}$ mice, a more complete understanding of pre-TCR signaling requirements awaits the analysis of $\epsilon^{\Delta/\Delta} \times \zeta^{-/-}$ mice reconstituted with signaling-deficient forms of both proteins.

TCR Signaling Responses Are Intact or Mildly Impaired in CD3ε$^{\Delta/\Delta}$; εM-tg Mice. To assess whether the TCR complexes expressed on thymocytes from CD3ε$^{\Delta/\Delta}$; εM-tg mice can transduce signals similar to those normally elicited by TCR cross-linking, we first examined if TCR engagement led to the activation of downstream signaling pathways. Several early events in the TCR activation cascade, such as the phosphorylation of ζ chain (Fig. 2 A), ZAP-70, and LAT (linker for activation of T cells; data not shown), were unaffected in CD3ε$^{\Delta/\Delta}$; εM-tg mice. These results were not unexpected, as ZAP-70 is known to be activated following its association with phosphorylated ζ chain ITAMs after TCR engagement, and ζ chain expression is unaltered in CD3ε$^{\Delta/\Delta}$; εM-tg mice. Activation-induced calcium responses were also indistinguishable in thymocytes from CD3ε$^{\Delta/\Delta}$; ε-tg and CD3ε$^{\Delta/\Delta}$; εM-tg mice (data not shown).

Mature T cell responses in CD3ε$^{\Delta/\Delta}$; εM-tg mice were examined by analyzing the ability of peripheral T cells to proliferate and produce IL-2 after cross-linking of the TCR and CD28. Although no significant differences were observed under conditions of maximal TCR stimulation, T cells from CD3ε$^{\Delta/\Delta}$; εM-tg mice exhibited impaired proliferative responses (Fig. 3 A) and cytokine responses (data not shown) when limiting concentrations of stimulating antibody were used (Fig. 3 A). Impaired proliferative responses were also observed when proliferation was assessed by CFSE staining (Fig. 3 B). Together, these results demonstrate that although CD3ε-mediated signals are not specifically required for T cell maturation or mature T cell functional responses, they contribute quantitatively to TCR signaling.

Thymocyte Selection and T Cell Survival in TCR-tg/CD3ε$^{\Delta/\Delta}$; εM-tg Mice. To examine the role of CD3ε signals in thymocyte selection, we bred the H-Y TCR transgene into the CD3ε$^{\Delta/\Delta}$; ε-tg and CD3ε$^{\Delta/\Delta}$; εM-tg backgrounds. Positive selection of thymocytes in H-Y TCR-tg/H-2D^{b} female mice results in the generation of CD8-SP thymocytes and T cells that express high levels of the H-Y clonotypic TCR (detected by mAb T3.70) (16). Positive selection of H-Y TCR^{+} thymocytes appeared uncompromised in H-Y TCR-tg/CD3ε$^{\Delta/\Delta}$; εM-tg mice when compared with H-Y TCR-tg/CD3ε$^{\Delta/\Delta}$; ε-tg mice, as assessed by total thymocyte numbers and the number and percentage of CD8-SP/T3.70^{hi} cells in the thymus (Fig. 4 A). However, examination of peripheral lymphoid organs revealed a striking paucity of CD8-SP/T3.70^{hi} T cells in H-Y TCR-tg/CD3ε$^{\Delta/\Delta}$; εM-tg mice (Fig. 4 A). Low numbers of CD8-SP/T3.70^{hi} T cells were consistently observed in both the lymph nodes and spleens of H-Y TCR-tg/CD3ε$^{\Delta/\Delta}$; εM-tg mice regardless of the age of the mice tested (range 4 wk to 6 mo). Although CD8-SP/T3.70^{hi} T cell numbers were markedly reduced in H-Y TCR-tg/CD3ε$^{\Delta/\Delta}$; εM-tg mice, CD8-SP/T3.70^{-} and CD4-SP T cell numbers were similar to or greater than those in H-Y TCR-tg/CD3ε$^{\Delta/\Delta}$; ε-tg mice, indicating that the generation and/or survival of CD8-SP/T3.70^{hi} peripheral T cells was selectively compromised in H-Y TCR-tg/CD3ε$^{\Delta/\Delta}$; εM-tg mice.

One possible explanation for the results obtained with H-Y TCR-tg/CD3ε$^{\Delta/\Delta}$; εM-tg mice is that T3.70^{hi} CD8-SP thymocytes fail to complete the maturation process in the thymus. However, arguing against this, H-Y TCR-tg/CD3ε$^{\Delta/\Delta}$; ε-tg and H-Y TCR-tg/CD3ε$^{\Delta/\Delta}$; εM-tg mice

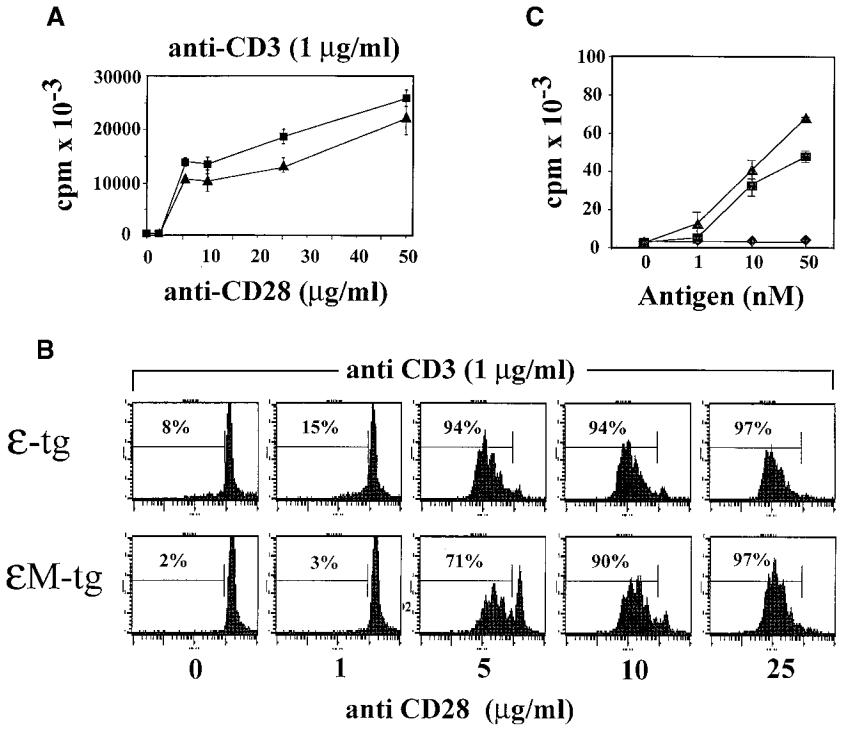

Figure 3. (A) Functional responses of T cells from CD3ε$^{\Delta/\Delta}$; ε-tg and CD3ε$^{\Delta/\Delta}$; εM-tg mice. CD4^{+}CD8^{-} T cells were purified from lymph nodes and cultured on plates precoated with anti-CD3ε and varying amounts of anti-CD28 mAbs as indicated. After 40 h, cells were pulsed with [^{3}H]thymidine and harvested. ■, CD3ε$^{\Delta/\Delta}$; ε-tg. ▲, CD3ε$^{\Delta/\Delta}$; εM-tg. Results shown are representative of those obtained in five of six experiments (in one experiment, the proliferative responses of T cells from CD3ε$^{\Delta/\Delta}$; ε-tg and CD3ε$^{\Delta/\Delta}$; εM-tg mice were not significantly different). (B) Analysis of proliferative responses by CFSE labeling. Experimental conditions were identical to those described in A except that cells were labeled with CFSE before stimulation. Histograms show CFSE staining on live gated CD4^{+} cells. Results shown are representative of three individual experiments. (C) Proliferative responses of CD8-SP thymocytes from H-Y TCR-tg/CD3ε$^{\Delta/\Delta}$; ε-tg and H-Y TCR-tg/CD3ε$^{\Delta/\Delta}$; εM-tg mice. CD4^{-}CD8^{+} thymocytes were purified and cultured on plates with APCs prepared from female mice + peptide antigen (Smcy; KCSRNRQYL). After 40 h, cells were pulsed with [^{3}H]thymidine and harvested. ■, H-Y TCR-tg/CD3ε$^{\Delta/\Delta}$; ε-tg. ▲, H-Y TCR-tg/CD3ε$^{\Delta/\Delta}$; εM-tg. ◆, CD3ε$^{+/+}$.

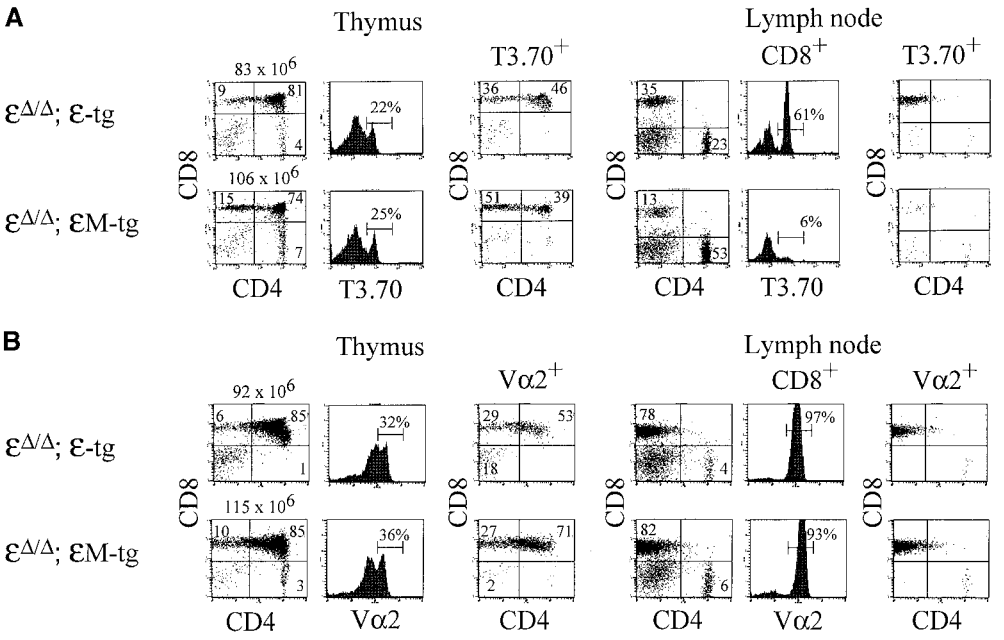

Figure 4. Positive selection of thymocytes from CD3$\epsilon^{\Delta/\Delta}$; ϵ-tg and CD3$\epsilon^{\Delta/\Delta}$; ϵM-tg mice expressing H-Y– or P14-transgenic TCRs. (A) Comparison of thymocytes and lymph node T cells from H-Y TCR–transgenic/CD3$\epsilon^{\Delta/\Delta}$; ϵ-tg and H-Y TCR-transgenic/CD3$\epsilon^{\Delta/\Delta}$; ϵM-tg females. Two-color plots show CD4/CD8 staining profiles on total or T3.70$^+$ (gated) thymocytes and lymph node cells. Numbers in quadrants indicate the percentage of cells within that quadrant. Histograms show staining of total thymocytes or CD8$^+$ lymph node T cells with the H-Y clonotype–specific antibody, T3.70. (The percentage of T3.70^{hi} cells and the gate used for the T3.70$^+$ two-color plots is also shown.) Results are representative of four separate experiments. (B) Comparison of thymocytes and lymph node T cells from P14 TCR–transgenic/CD3$\epsilon^{\Delta/\Delta}$; ϵ-tg and P14 TCR-transgenic/CD3$\epsilon^{\Delta/\Delta}$; ϵM-tg mice. Two-color plots show CD4/CD8 staining profiles on total or Vα2$^+$ (gated) thymocytes and lymph node cells. Numbers in quadrants indicate the percentage of cells within that quadrant. Histograms show staining of total thymocytes or CD8$^+$ lymph node T cells with the P14 TCR α chain–reactive mAb Vα2. (The percentage of Vα2^{hi} cells and the gate used for the Vα2$^+$ two-color plots is also shown.) Results are representative of two separate experiments.

contained similar numbers of phenotypically (Fig. 4 and data not shown) and functionally (Fig. 3 C) mature T3.70^{hi} CD8-SP thymocytes.

To determine if the effect of the ϵM-tg was specific to cells that express the H-Y TCR, we examined development and selection of thymocytes expressing a different MHC class I–restricted TCR, P14. Thymocytes that express the P14 TCR are also positively selected in the H-2D^b background, leading to the generation of CD8-SP/Vα2^{hi} thymocytes and peripheral T cells (17). Examination of thymocytes and peripheral (lymph node) T cells from P14 TCR-tg/CD3$\epsilon^{\Delta/\Delta}$; ϵM-tg mice revealed a phenotype nearly identical to that of P14 TCR-tg/CD3$\epsilon^{\Delta/\Delta}$; ϵ-tg mice (i.e., mice of both genotypes contained similar numbers of CD8-SP/Vα2^{hi} thymocytes and peripheral T cells) (Fig. 4 B). Thus, the generation and survival of CD8-SP T cells that express the P14 TCR did not appear to be compromised in CD3$\epsilon^{\Delta/\Delta}$; ϵM-tg mice.

Although other explanations remain possible, these results are most consistent with the notion that CD3ϵ signals are required for the survival of naive H-Y TCR$^+$ but not P14 TCR$^+$ T cells. Recent studies have shown that self-MHC is required for long-term survival of naive T cells (25, 26). However, to our knowledge, the data presented here are the first that directly implicate TCR signaling in T cell survival. Our results could reflect a unique requirement for CD3ϵ-mediated signals in T survival; however, they are also consistent with the idea that CD3ϵ contributes quantitatively to the TCR signaling response. A generalized defect in T cell survival was not observed in CD3$\epsilon^{\Delta/\Delta}$; ϵM-tg mice but instead was seen only in the H-Y TCR–transgenic background. Significantly, the self-ligand that positively selects and presumably maintains the survival of peripheral H-Y TCR$^+$ T cells is thought to bind with especially low affinity to the TCR (27, 28). Thus, the survival of H-Y TCR$^+$ CD8-SP T cells but not P14 TCR$^+$ CD8-SP T cells may critically depend upon the presence of the entire complement of TCR-ITAMs. Consistent with this idea, the generation of H-Y TCR$^+$ CD8-SP T cells but not P14 TCR$^+$ CD8-SP T cells by positive selection was found to be absolutely dependent upon TCR ζ chain ITAMs (9, 10). Moreover, P14 TCR$^+$ CD8-SP T cells but not H-Y TCR$^+$ CD8-SP T cells are triggered to undergo homeostatic proliferation when transferred to H-2D^b T cell–depleted hosts, again suggesting that H-Y TCR$^+$ CD8-SP T cells bind with lower affinity to self-ligands (28, 29). Another possibility that cannot presently be ruled out is that the mutant CD3ϵ chain expressed in CD3$\epsilon^{\Delta/\Delta}$; ϵM-tg mice is not inert but instead inhibits TCR signaling. An inhibitory role for ζ chains that contain partially (mono)phosphorylated ITAMs has been postulated based on data from in vitro studies (30). However, an inhibitory effect was not observed when a ζ chain containing mutated ITAMs capable of becoming mono- but not diphosphorylated was expressed in transgenic mice (9). Additional experiments will be required to determine the precise function of TCR signaling in general and CD3ϵ signals in particular in regulating T cell survival.

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