irradiation. Staining and analysis gates were as described in Fig. 1. The graph shows mean percentages of absolute cell numbers within the gates as compared with untreated animals.
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27. Four-week-old female C57BL/6 mice were injected intraperitoneally with dexamethasone (Sigma; 125 mg per kilogram of body weight) in PBS. Thymocytes from these and noninjected control mice were analyzed 48 hours later.
28. Analysis of HSA expression on thymocytes remaining 2 days after dexamethasone treatment revealed that mainly HSA^{lo} cells survived, indicating that only the most mature cells in each population are resistant to glucocorticoids.
29. Thymocytes from mice treated with dexamethasone 2 days previously were stained with anti-CD8–FITC and anti-CD4–PE (Becton Dickinson), and $CD4^{int}$-$CD8^{hi}$ cells were electronically sorted. Cells were incubated in complete medium at either 4° or 37°C overnight and then stained with anti-CD8–FITC, anti-CD4–PE, and anti-TCRβ–Red670 (Gibco-BRL) the next day. Seventy-five percent logarithmic contour plots of $TCR\beta^{hi}$ cells are shown. Using a forward and side scatter gate to exclude dead cells, we observed no cell loss (6.6×10^4 cells recovered in the experiment shown).
30. Mice deficient in surface MHC class I expression as a result of β_2M gene inactivation by homologous recombination were obtained from B. Koller and bred in our facilities. The original stock of mutant animals had been crossed to C57BL/6, and homozygous progeny were obtained by intercrossing heterozygous offspring. The line was maintained by intercrossing homozygous mutant offspring. Control mice were C57BL/6 mice bred in our facilities.
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34. We thank C. Eigsti and D. Stephany for analysis and sorting on the FACStar Plus, J.-C. Zuniga-Pflucker for helpful suggestions, B. J. Fowlkes for discussions and for critical review of the manuscript, and B. Koller for the $\beta_2M^{-/-}$ mice. J.P.M.v.M. is supported by a fellowship of the Fogarty International Center.

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Reconstitution of T Cell Receptor ζ–Mediated Calcium Mobilization in Nonlymphoid Cells

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T cell antigen receptor (TCR) activation involves interactions between receptor subunits and nonreceptor protein tyrosine kinases (PTKs). Early steps in signaling through the ζ chain of the TCR were examined in transfected COS-1 cells. Coexpression of the PTK $p59^{fynT}$, but not $p56^{lck}$, with ζ or with a homodimeric TCRβ-ζ fusion protein produced tyrosine phosphorylation of both ζ and phospholipase C (PLC)–γ1, as well as calcium ion mobilization in response to receptor cross-linking. CD45 coexpression enhanced these effects. No requirement for the PTK ZAP-70 was observed. Thus, $p59^{fynT}$ may link ζ directly to the PLC-γ1 activation pathway.

Lymphocyte activation through the TCR induces calcium ion mobilization, cell proliferation, and release of lymphokines and cytolytic factors. Early effects of TCR stimulation include tyrosine phosphorylation of the ζ chain (*1*) and CD3 subunits of the TCR (*2*); activation of cytoplasmic PTKs appears crucial in TCR-mediated activation (*3–6*). Unlike the tyrosine kinase growth factor receptors that activate similar signaling pathways (*7*), TCR subunits have no intrinsic enzymatic activities. However, two *src*-family PTKs, $p59^{fynT}$ (*8–10*) and $p56^{lck}$ (*11–15*), may link the TCR to signaling mechanisms. Cell lines and animals deficient in these kinases exhibit TCR signaling defects, as do T cell variants lacking the phosphotyrosine phosphatase CD45, a likely physiologic activator of the *lck* and *fyn* kinases (*16*).

The cytoplasmic domains of ζ and CD3ε can each transmit signals independently of other TCR subunits (*17, 18*). Three evenly spaced tyrosine pairs in the ζ tail and one pair in the CD3ε tail (*19*), lying within so-called tyrosine-based activation motifs (*20*), can become phosphorylated on T cell activation (*1, 2, 8, 12*). The role of these phosphorylations in TCR signaling awaits full elucidation.

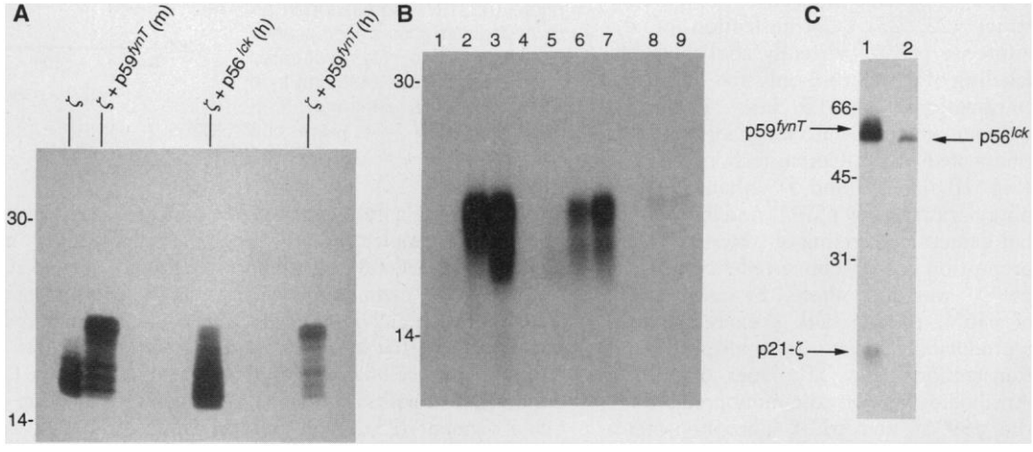

Fig. 1. Modification of ζ by $p59^{fynT}$ but not $p56^{lck}$ in COS-1 cells. Cells (1×10^6 to 2×10^6 per plate) were transfected by the DEAE-dextran method (*21*) and split 12 hours later. After 56 hours, transfectants (10^7 cells per sample) were lifted nonenzymatically (with 5 mM EDTA), washed, permeabilized on ice with L-α-lysolecithin (Sigma; 0.285 mg/ml) for 1 min, and then incubated with [γ-^{32}P]ATP (50 μCi per sample) for 15 min on ice (*12*). Cells were lysed with 1% NP-40 (Pierce) lysis buffer and centrifuged to remove nuclei. The supernatants were immunoprecipitated with 1D4.1 mAb (raised to a peptide comprising human ζ residues 153 to 164). Washed immunoprecipitates (10^7 cells per lane) were subjected to reducing SDS-PAGE (12.5% gels) and visualized by autoradiography. Molecular size standards (in kilodaltons) are at left. (**A**) COS-1 cells transfected with cDNAs encoding ζ only, ζ + (murine) $p59^{fynT}$(m), ζ + (human) $p56^{lck}$(h), and ζ + (human) $p59^{fynT}$(h). (**B**) The $p59^{fynT}$- and $p56^{lck}$-specific phosphorylations are enhanced by CD45. COS cells were cotransfected with cDNAs encoding ζ (lane 1), ζ + $p59^{fynT}$ (lane 2), ζ + $p59^{fynT}$ + CD45 (lane 3), ζ + $p56^{lck}$ (lane 4), ζ + $p56^{lck}$ + CD45 (lane 5), ζ + $p59^{fynT}$ + $p56^{lck}$ (lane 6), and ζ + $p59^{fynT}$ + $p56^{lck}$ + CD45 (lane 7). To examine $p56^{lck}$-specific effects, we cotransfected a 5′ antisense $p59^{fynT}$ cDNA segment (*22*) in samples 1, 4, and 5. The p21-ζ protein immunoprecipitated from activated peripheral blood T cells (lane 8) and from activated Jurkat leukemic T cells (lane 9) are shown for comparison. (**C**) Detection of a $p59^{fynT}$-p21ζ phosphoprotein complex. COS cells were triply transfected (ζ + $p59^{fynT}$ + $p56^{lck}$). Immunoprecipitates of $p59^{fynT}$ (lane 1) and $p56^{lck}$ (lane 2) were prepared with rabbit antisera [anti–human fyn and anti–human lck kinase (CT); UBI, Lake Placid, New York] from a divided lysate.

To dissect signaling pathways activated by TCR engagement, we have used transient gene expression in COS-1 monkey kidney epithelial cells. Cells were transfected with expression vectors CDM8 or πvx (*21*) carrying native or genetically modified complementary DNAs (cDNAs) encoding T cell proteins. After 48 to 72 hours, strong protein expression permitted analysis of protein associations, substrate phosphorylations, and metabolic effects of receptor stimulation.

We examined the relations between PTKs and TCR-ζ by transfecting cDNAs encoding ζ-only, ζ + p56^{lck}, or ζ + p59^{fynT} (*22*). Lysolecithin-permeabilized cells were labeled with [γ-^{32}P]adenosine triphosphate (ATP) (*12*), and immunoprecipitates from 1% NP-40 lysates were separated by SDS–polyacrylamide gel electrophoresis (PAGE) (Fig. 1). When coexpressed with human or murine p59^{fynT}, ζ underwent heavy ^{32}P-labeling with a nearly quantitative electrophoretic mobility–shift from 16 to 21 kD. An identical ζ mobility–shift resulting from tyrosine phosphorylation occurs in T cells on TCR stimulation (*11*). When coexpressed with p56^{lck}, ζ was modified mainly to intermediate (p16–p18) forms, whereas ζ transfected alone was only weakly labeled (Fig. 1). No reactivity of either kinase toward CD3γ, δ, or ε was detected in similar experiments (*23*).

A weak *fyn*-like kinase activity intrinsic to COS-1 cells was responsible for the ζ phosphorylation observed in ζ-only transfections. Immunoblotting (but not immunoprecipitation) of untransfected cells with antibodies to *fyn* detected a 60-kD protein that was absent from cells transfected with a p59^{fynT}-specific antisense cDNA construct (*22*, *23*). Cotransfection of the antisense p59^{fynT} virtually abolished ^{32}P-labeling of ζ in both ζ-only and ζ + p56^{lck} transfections (Fig. 1B, lanes 1 and 4), without affecting autophosphorylation of transfected p56^{lck}. Cotransfection of CD45 (Fig. 1B, lanes 3 and 5) enhanced phosphorylation of ζ by p59^{fynT} and led to weak but detectable labeling of ζ by p56^{lck}. The proportion of ζ converted to p21 by p59^{fynT} was little altered by coexpression of p56^{lck}, though bulk ζ expression was reproducibly lower in multiple chain transfections (Fig. 1B, lanes 6 and 7). Antibodies to *fyn* coimmunoprecipitated the p59^{fynT} and p21-ζ phosphoproteins from NP-40 lysates of ζ + p59^{fynT} or ζ + p59^{fynT} + p56^{lck} cotransfectants; antibodies to *lck* detected no such association with ζ (Fig. 1C). Thus, the p59^{fynT}-ζ interaction was specific, both in producing p21-ζ and in forming a detergent-resistant phosphoprotein complex.

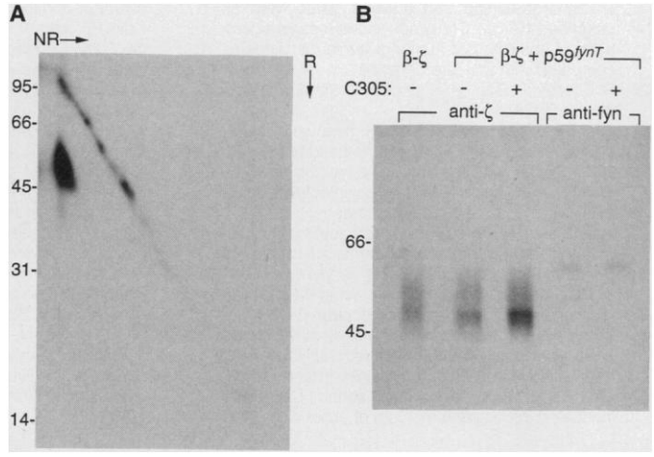

Fig. 2. (**A**) Cell surface expression of the 48-kD TCRβ-ζ fusion protein as a disulfide-linked homodimer. COS-1 (1 × 10^7 cells) transfected with the β-ζ fusion cDNA (*24*) were lifted nonenzymatically (5 mM EDTA), washed, radio-iodinated (Na^{125}I, Du Pont–NEN) by the lactoperoxidase method (*12*), lysed in 1% NP-40 lysis buffer, immunoprecipitated with 1D4.1 mAb, subjected to two-dimensional [nonreducing (NR)–reducing (R)] SDS-PAGE (12.5% gels), and autoradiographed. (**B**) Antibody cross-linking of β-ζ enhances its phosphorylation by p59^{fynT} in COS-1. COS-1 (2 × 10^7 cells per sample) transfected with cDNAs encoding β-ζ or β-ζ + p59^{fynT} were incubated with NMS (−) or C305 mAb (+) (1 μg per 10^6 cells) for 10 min at 37°C in buffered medium, followed by [γ-^{32}P]ATP labeling (*12*), lysis, immunoprecipitation with 1D4.1 mAb, reducing SDS-PAGE (10% gel), and autoradiography. This β-ζ transfection did not use the antisense p59^{fynT} cDNA construct. The β-ζ + p59^{fynT} cotransfectants were pooled and divided equally before the assay. Signals were quantified by a Phosphorimager with ImageQuant 2.0 software (Molecular Dynamics, Sunnyvale, California). Molecular size standards (in kilodaltons) are at left.

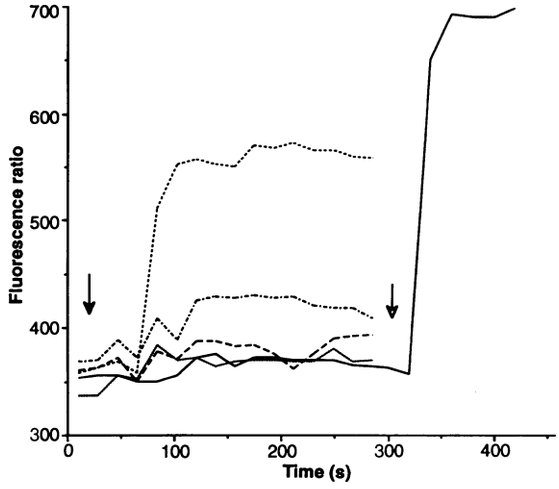

Fig. 3. Expression of p59^{fynT} required for antibody-induced calcium mobilization in β-ζ transfectants. COS-1 cells transfected with β-ζ only, β-ζ + p59^{fynT}, or β-ζ + p56^{lck} were harvested after 56 hours, loaded (45 min, 37°C) at 5 × 10^7 cells per milliliter with Indo 1-AM dye (5 μg/ml; Molecular Probes, Junction City, Oregon), and assayed in flow (FACScan, Becton Dickinson) for calcium-specific fluorescence (*12*) as a function of time after addition of antibody (1 μg per 10^6 cells) (closed arrow) or calcium ionophore (A23187, Sigma) (open arrow). Ordinate shows fluorescence intensity ratio of Ca^{2+}-saturated (405 nm)/Ca^{2+}-free (485 nm) Indo I-AM dye (units arbitrary). (···), TCRβ-ζ, C305; (– – –), β-ζ + p56^{lck}, C305; (- - -), β-ζ + p59^{fynT}, C305; (–·–·–·), β-ζ + p59^{fynT}, Mx21; and (——), β-ζ + p59^{fynT}, NMS.

We examined transmembrane signaling through the ζ cytoplasmic tail by using a TCRβ-ζ fusion cDNA (*24*) encoding a β-ζ fusion protein that lacks the targeting elements for intracellular retention and degradation that lie within the TCRβ transmembrane segment (*25*). High cell surface expression of β-ζ, principally as a disulfide-linked homodimer, was detected in transfected COS cells by ^{125}I-labeling (Fig. 2A), and β-ζ could be immunoprecipitated by antibodies to either β or ζ. Phosphorylation of β-ζ (Fig. 2B) was weak in β-ζ–only transfectants, three times heavier in β-ζ + p59^{fynT} cotransfectants, and was six times further enhanced by cross-linking of β-ζ on the latter cells with an immunoglobulin M (IgM) monoclonal antibody (mAb) to TCRβ (C305) before labeling. Phosphorylation of p59^{fynT} in cotransfected cells was unaltered by C305 (Fig. 2B). As with ζ, cotransfection with the p59^{fynT} antisense cDNA (*22*) virtually abolished background labeling of β-ζ (*23*).

To examine whether other signaling events might accompany β-ζ phosphorylation, we compared COS-1 transfectants in an antibody-stimulated fluorescence-activated cell sorter calcium mobilization assay (Fig. 3). Cells coexpressing β-ζ + p59^{fynT} exhibited a prominent calcium mobilization signal after a 1-min incubation with C305; a V_β8-specific IgG mAb (Mx21) produced a smaller positive response from the same

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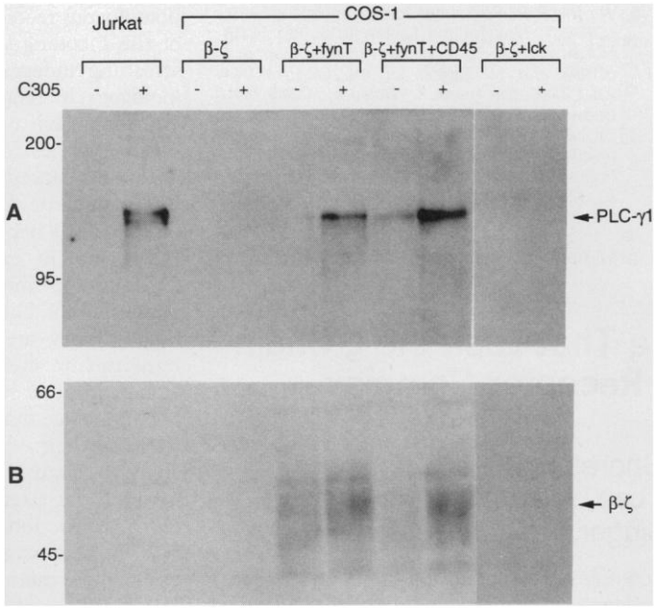

Fig. 4. Coexpression of $p59^{fynT}$ required for antibody-stimulated tyrosine phosphorylation of PLC-γ1 and β-ζ in COS-1. COS cells were transfected with cDNAs encoding β-ζ only, β-ζ + $p59^{fynT}$, β-ζ + $p59^{fynT}$ + CD45, or β-ζ + $p56^{lck}$. Jurkat T cells and transfectants were each pooled and split equally, then incubated in parallel with normal mouse serum (−) or with C305 antibody (+) at 1 μg per 10^6 cells (70 s, 37°C, in buffered medium), followed by rapid centrifugation and lysis in 1% NP-40 lysis buffer. Anti-phosphotyrosine immunoprecipitates (10^7 cells per lane) prepared with the 4G10 mAb (agarose-linked) were washed, subjected to reducing SDS-PAGE (10% gel), and transferred to nitrocellulose. Filters were blocked (5% dry milk in phosphate-buffered saline) and cut horizontally before immunoblotting. (**A**) Upper portion was blotted successively with anti–PLC-γ1–mixed mAbs (UBI, Lake Placid, New York), rabbit antibody to mouse IgG (Tago), and ^{125}I-labeled protein A (Amersham), followed by autoradiography (48 hours). Signals were quantified by a Phosphorimager with ImageQuant 2.0 software (Molecular Dynamics, Sunnyvale, California). (**B**) Lower portion was blotted with the anti-ζ mAb 1D4.1 and developed with alkaline phosphatase–conjugated antibody to mouse Ig (Promega, Madison, Wisconsin). Molecular size standards (in kilodaltons) are at left.

cells, whereas normal mouse serum (NMS) had no effect. Single (β-ζ only) and β-ζ + $p56^{lck}$ transfectants were unresponsive to both NMS and C305. Thus, $p59^{fynT}$ was both necessary and sufficient for calcium mobilization in COS-1 cells stimulated by antibody cross-linking of β-ζ. This response was kinetically similar to that produced by C305 in Jurkat T cells (*5*).

To test for involvement of PLC-γ1 in the observed calcium response, we assayed the effect of C305 on PLC-γ1 tyrosine phosphorylation in COS-1 transfectants. Anti-phosphotyrosine immunoprecipitates were prepared from NMS- or C305-treated cells, electrophoresed, and immunoblotted with antibodies to PLC-γ1 (Fig. 4A) and ζ (Fig. 4B). PLC-γ1 tyrosine phosphorylation was observed only in cells coexpressing β-ζ + $p59^{fynT}$ and was increased 10-fold by C305 treatment; CD45 coexpression enhanced this effect. No PLC-γ1 tyrosine phosphorylation was observed in either β-ζ–only or β-ζ + $p56^{lck}$ transfectants. Tyrosine phosphorylation of β-ζ also required $p59^{fynT}$ and resembled the [γ-^{32}P]ATP–labeling response (Fig. 2B) in its dependence on C305.

Thus, ζ-mediated signaling in a non–T cell, leading to ζ phosphorylation, PLC-γ1 activation, and calcium mobilization required coexpression of $p59^{fynT}$ but not $p56^{lck}$. The ability of $p59^{fynT}$ to associate with unmodified ζ, which is detectable only in very mild detergents (digitonin) (*8*, *26*), may facilitate ζ tyrosine phosphorylation (Fig. 1). The observed formation of a detergent-resistant $p59^{fynT}$-p21ζ complex (Fig. 2B), likely mediated by *fyn* SH2 domains, supports the view that TCR stimulation may lead to assembly of heteromultimeric signaling protein complexes (*27*, *28*).

Contact between ζ and $p56^{lck}$ in T cells may require stimulation by antigen–major histocompatibility complex or hybrid (CD3-CD4) antibodies (*12*, *13*). A recently cloned T cell–specific PTK, ZAP-70, binds phospho-ζ and strongly potentiates phosphorylation of a chimeric CD8-ζ substrate by $p56^{lck}$ or $p59^{fynT}$ (*28*). Calcium mobilization and PLC-γ1 activation in our system exhibited no requirement for ZAP-70, though possible expression by COS-1 cells of a *syk*-type PTK cannot yet be ruled out. The dependence on $p59^{fynT}$ of ζ signaling in COS cells resembles that of TCR signaling in mature thymocytes (*9*, *10*), whereas in peripheral (*10*) and Jurkat leukemic T cells (*14*, *27*, *28*) $p56^{lck}$ and ZAP-70 may play more dominant roles.

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22. Full-length cDNAs encoding human $p59^{fynT}$ and $p56^{lck}$ were amplified by Vent polymerase (New England Biolabs, Beverly, MA) from a first-strand cDNA library prepared with Jurkat T cell polyadenylate mRNA. Priming oligonucleotides were derived from published sequences [*fynT*: K. Semba *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 5459 (1986); *lck*: R. M. Perlmutter *et al.*, *J. Cell. Biochem.* **38**, 117 (1988)]. Primary structures were verified by dideoxy sequencing. Though only marginally active toward ζ and β-ζ in the assays reported here, the $p56^{lck}$ expressed in COS cells showed strong in vitro kinase activity, exceeding that of commercially purified $p56^{lck}$ enzyme (UBI, Lake Placid, NY), toward a natural substrate, the interleukin-2 receptor β chain (*29*). The antisense $p59^{fynT}$ construct was prepared by insertion of a blunted, inversely oriented 438–base pair 5′ restriction fragment (Sac II–Ppu MI, covering 5′-UT plus codons 1 to 72 of the open reading frame) from the murine $p59^{fynT}$ cDNA into the expression vector πvx.
23. C. G. Hall and C. Terhorst, unpublished data.
24. A 1.8-kb human ζ cDNA was isolated by probing of an HPB-ALL T cell cDNA library in the plasmid πvx (*21*) with the murine ζ cDNA [A. M. Weissman *et al.*, *Science* **239**, 1018 (1988)]. A 900-bp cDNA fragment encoding the extracellular domain (Met^1-Ile^{262}) of Jurkat TCRβ [Y. Yanagi *et al.*, *Nature* **308**, 145 (1984)] was generated by Vent polymerase chain amplification using the 3′ oligonucleotide 5′-GATATGATCAATGGTGGCAGACAG-3′, digested with Bcl I, and ligated to a Bam HI–digested 1.6-kb 3′ human ζ cDNA fragment to produce in-frame fusion to Asp^{28} of the predicted ζ sequence.

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30. We thank R. Perlmutter for the murine p59^{fynT} cDNA, T. Mak for the full-length Jurkat TCRβ cDNA (YT35), R. Klausner for the murine ζ cDNA, and M. Streuli for the CD45 cDNA (DL.LCA6). We thank A. Weiss for C305 antibody, H. Spits for Mx21 antibody, and B. Drucker for 4G.10 antibody and for helpful discussions and suggestions. Supported by PHS grant AI 15066 (C.T.) and CA 01486 (C.G.H.) and by the Leukemia Society of America (J.S.).

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T Cell Development in Mice That Lack the ζ Chain of the T Cell Antigen Receptor Complex

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The ζ subunit of the T cell antigen receptor complex is required for targeting nascent receptor complexes to the cell surface and for receptor-mediated signal transduction. To examine the significance of the ζ subunit in T cell development, mice deficient for ζ expression were generated by gene targeting. These $\zeta^{-/-}$ mice had few $CD4^+CD8^+$ thymocytes, and the generation of $CD4^+$ and $CD8^+$ single positive T cells was impaired but not completely abrogated. Peripheral T cells were present but were unusual in that they expressed small amounts of CD5 and few T cell receptors. Thus, ζ chain expression influences thymocyte differentiation but is not absolutely required for the generation of single positive T cells.

The T cell antigen receptor (TCR) is a complex multisubunit structure that consists of at least six different protein chains (TCRα and TCRβ; CD3γ, CD3δ, and CD3ε; and ζ) that are assembled in the endoplasmic reticulum and transported to the cell surface (*1*). The α and β chains are clonotypic and confer ligand specificity; the other subunits are invariant and function as signal-transducing molecules. The best characterized of the invariant subunits is ζ, which is assembled either as a disulfide-linked homodimer or as a disulfide-linked heterodimer with another member of the ζ family of proteins (η or the γ chain of the type I Fcε receptor) (*2*). The presence of ζ is required both for efficient transport of assembled TCR complexes to the cell surface and for efficient signal transduction (*1*, *3*). Signaling through the TCR antigen complex is thought to trigger intrathymic selection events during T cell development, particularly the negative and positive selection events that affect immature $CD4^+CD8^+$ thymocytes and regulate their differentiation into mature $CD4^+$ and $CD8^+$ T cells. Consequently, ζ expression should be critical for T cell development and for the generation of the mature T cell repertoire. We evaluated the importance of ζ in the maturation of T cells by assessing T cell differentiation in mice made deficient for ζ expression.

The ζ-deficient mice were derived from embryonic stem (ES) cells in which one of the ζ genes was specifically mutated by homologous recombination (*4*). Disruption of the ζ coding sequence was detected by screening independent ES clones for homologous integration of pζ-Δ/CT94 after electroporation of the targeting construct. Positive clones contained an inactivated ζ allele that lacked exon II (encoding the ζ transmembrane domain) and carried an insertion of the neomycin phosphotransferase (*neo*) gene in exon IV (Fig. 1A). This disruption of the ζ locus interferes with expression of both the ζ and η proteins because they are derived from the same transcript by alternative splicing (*5*). Two clones, J52 and J66, were used to generate chimeric mice that transmitted the mutated allele to their offspring. Southern (DNA) analysis of tail DNA from the progeny of heterozygote intermatings revealed the expected restriction pattern for the wild-type ($\zeta^{+/+}$), heterozygous mutant ($\zeta^{+/-}$), and homozygous mutant ($\zeta^{-/-}$) genotypes (Fig. 1B).

Expression of the endogenous and mutant ζ alleles was examined by Northern (RNA) blotting of total thymocyte and lymph node RNA. Thymocytes and lymph node cells from $\zeta^{+/-}$ heterozygous mice contained approximately 50% correctly sized (1.7 kb) transcripts when compared to $\zeta^{+/+}$ littermates and additionally contained small amounts of a mutant transcript of approximately 2.5 kb that may represent either an aberrantly or incompletely spliced mRNA species or a partial ζ transcript that contains the inserted *neo* gene (Fig. 1C). Thymocytes from $\zeta^{-/-}$ mice did not contain the wild-type ζ transcript and expressed only the mutant ζ transcript (Fig. 1C). Lymph node cells from $\zeta^{-/-}$ mice did not contain detectable ζ transcripts, either wild-type or mutant. Consistent with the absence of mature ζ transcripts in $\zeta^{-/-}$ mice, metabolic labeling studies of $\zeta^{-/-}$ thymocytes revealed that they did not synthesize ζ protein as determined by immunoprecipitation with a combination of polyclonal antibodies to ζ (anti-ζ 528 and anti-ζ 551), which are specific for the NH_2- and COOH-terminal sequences of the ζ cytoplasmic domain, respectively (Fig. 2) (*6*). Synthesis of η, which would additionally

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Table 1. Numbers of T cells in thymuses and lymph nodes from $\zeta^{+/-}$ and $\zeta^{-/-}$ mice. Numbers shown are means × 10^{-6} (for thymuses, $n = 4$ mice; for lymph nodes, $n = 3$ mice). Values in parentheses represent the percent of cells within quadrants as shown in Fig. 3. Thymocytes and lymph node cells (inguinal, axillary, and mesenteric) were obtained from 4- to 8-week-old $\zeta^{+/-}$ and $\zeta^{-/-}$ littermates.

Genotype	Source	Total T cells	$CD4^-CD8^-$	$CD4^+CD8^+$	$CD4^+CD8^-$	$CD4^-CD8^+$
$\zeta^{+/-}$	Thymus	194	7 (4)	168 (87)	13 (7)	7 (4)
$\zeta^{-/-}$	Thymus	28	8 (28)	19 (68)	<1 (2)	<1 (1)
$\zeta^{+/-}$	Lymph node	23	–	–	10 (43)	5 (22)
$\zeta^{-/-}$	Lymph node	17	–	–	2 (12)	1 (6)