Specific requirement for CD3ε in T cell development

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ABSTRACT T cell antigen receptor (TCR) and pre-TCR complexes are composed of clonotypic heterodimers in association with dimers of signal transducing invariant subunits (CD3γ, -δ, -ε, and ζ). The role of individual invariant subunits in T cell development has been investigated by generating gene-specific mutations in mice. Mutation of CD3γ, -δ, or ζ results in an incomplete block in development, characterized by reduced numbers of mature T cells that express low levels of TCR. In contrast, mature T cells are absent from CD3ε$^{-/-}$ mice, and thymocyte development is arrested at the early CD4$^-$CD8$^-$ stage. Although these results suggest that CD3ε is essential for pre-TCR and TCR expression/function, their interpretation is complicated by the fact that expression of the CD3γ and CD3δ genes also is reduced in CD3ε$^{-/-}$ mice. Thus, it is unclear whether the phenotype of CD3ε$^{-/-}$ mice reflects the collective effects of CD3γ, -δ, and -ε deficiency. By removing the selectable marker (PGK-NEO) from the targeted CD3ε gene via Cre/*lox*P-mediated recombination, we generated mice that lack CD3ε yet retain normal expression of the closely linked CD3γ and CD3δ genes. These (CD3ε$^{Δ/Δ}$) mice exhibited an early arrest in T cell development, similar to that of CD3ε$^{-/-}$ mice. Moreover, the developmental defect could be rescued by expression of a CD3ε transgene. These results identify an essential role for CD3ε in T cell development not shared by the CD3γ, CD3δ, or ζ-family proteins and provide further evidence that PGK-NEO can influence the expression of genes in its proximity.

Differentiation of thymocytes into mature, functional T cells requires the input of signals delivered through the T cell antigen receptor (TCR) and a precursor form of the TCR, the pre-TCR (1, 2). The TCR complexes expressed on mature T cells are composed of subunits originating from six different genes: TCRα and TCRβ (or TCRγ and TCRδ), CD3γ, CD3δ, CD3ε, and ζ (or a related family member) (3). The clonotypic (TCRα/β or TCRγ/δ) chains, which specify distinct lineages of T cells, are responsible for ligand recognition and are generated during development by programmed rearrangement of germline [V-(D)-J] gene segments. TCRα/β and TCRγ/δ heterodimers lack inherent signaling activity but associate noncovalently with dimers composed of the invariant signal transducing CD3 and ζ-family subunits. The generally accepted stoichiometry for the TCR complex is TCRαβ or TCRγδ/CD3γε/CD3δε/ζζ.

Rearrangement of genes encoding the lineage-specific αβ- and γδ-TCR chains is initiated in immature CD4$^-$ CD8$^-$ [or double negative (DN)] thymocytes that constitutively express the CD3γ, CD3δ, CD3ε, and ζ subunits (4). Productive rearrangement of both the TCRγ and TCRδ genes results in surface expression of γδTCR complexes and commitment of

DN thymocytes to the γδ-T cell lineage (5). On the other hand, productive rearrangement of the TCRβ locus results in surface expression of a "pre-TCR" complex composed of TCRβ chain paired with an invariant, pre-Tα chain in association with CD3 and ζ-chain dimers (2). Signals transduced by the pre-TCR induce commitment of DN thymocytes to the αβ-T cell lineage by triggering cell proliferation (resulting in "β-selection") and transition to the CD4$^+$CD8$^+$ [or double positive (DP)] stage, in which TCRα gene rearrangement is initiated (2, 6).

The role of each of the TCR subunits in T cell development has been examined by generating gene-specific mutations in mice. Mice rendered deficient in [V-(D)-J] recombination (Rag1$^{-/-}$ or Rag2$^{-/-}$) are devoid of T cells and contain only immature DN thymocytes (7, 8) whereas mutations that selectively block expression of the TCRα/β or TCRγ/δ chain heterodimers result in lineage-restricted defects (i.e., absence of α/β or γ/δ T cells, respectively) (6, 9, 10). Moreover, α/β-T cell development is arrested at the DN stage in TCRβ$^{-/-}$ mice and at the DP stage in TCRα$^{-/-}$ mice (6, 9), consistent with a requirement for TCRβ but not TCRα for pre-TCR assembly and function (2).

Mice lacking all members of the ζ-family (ζ, η, FcεRIγ) exhibit a partial block in T cell development at both the DN→DP and DP→CD4$^+$CD8$^-$ or CD4$^-$CD8$^+$ transitions (11). Small numbers of peripheral T cells are generated in ζ/η/FcεRIγ$^{-/-}$ mice despite the fact that they express extremely low levels of TCR (11). Thus, pre-TCR and TCR complexes can still be expressed and are capable of transducing developmental signals, albeit much less efficiently, in the absence of ζ-family dimers (11).

The phenotype of CD3ε$^{-/-}$ mice has shown that, unlike the ζ-family dimers, CD3 dimers are absolutely required for pre-TCR and TCR assembly and/or signal transduction (12). Both αβTCR$^+$ and γδTCR$^+$ T cells are absent from CD3ε$^{-/-}$ mice, and, although rearrangement of TCRβ occurs, thymocyte development is arrested at the immature DN stage (12). In contrast, mutation of either CD3γ or CD3δ results in a less severe developmental impairment (13, 14). As in ζ/η/FcεRIγ$^{-/-}$ mice, both CD3γ$^{-/-}$ and CD3δ$^{-/-}$ mice exhibit an incomplete block in development and contain low numbers of peripheral αβTCR^{lo} T cells (11, 13, 14). In addition, CD3δ$^{-/-}$ mice contain normal numbers of DP thymocytes, and γδ-T cell development appears unaffected (13). One interpretation of these results has been that CD3γ and CD3δ are functionally redundant such that CD3γε dimers or CD3δε dimers alone can, though with different efficiency, promote pre-TCR and TCR assembly. On the other hand, CD3ε is absolutely required because, in its absence, neither heterodimer can be formed.

This paper was submitted directly (Track II) to the *Proceedings* office.
Abbreviations: DN, double negative (CD4$^-$CD8$^-$); DP, double positive (CD4$^+$CD8$^+$); TCR, T cell antigen receptor; ES cell, embryonic stem cell.
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However, a potentially confounding factor in the interpretation of these data has been that thymocytes from CD3$\varepsilon^{-/-}$ mice were found to express abnormally low levels of CD3γ and CD3δ transcripts (12). Thus, it is has remained unclear whether the phenotype observed in CD3$\varepsilon^{-/-}$ mice reflects a specific requirement for CD3ε in early T cell development or the collective effects of CD3γ, CD3δ, and CD3ε deficiency. In this study, we generated mice that lack expression of CD3ε yet retain normal expression of the closely linked CD3γ and CD3δ genes. Significantly, the developmental defect in these mice was found to be identical to that of the previously reported CD3$\varepsilon^{-/-}$ mice and could be rescued by expression of a CD3ε transgene. Collectively, these results identify an absolute requirement for CD3ε in early T cell development not shared by the CD3γ, CD3δ, or ζ-family proteins.

MATERIALS AND METHODS

Generation of CD3$\varepsilon^{N/N}$, CD3$\varepsilon^{\Delta/\Delta}$, and CD3$\varepsilon^{tg}$ Mice. The CD3ε targeting vector pεNEO-*lox*P (Fig. 1*A*) was constructed by subcloning genomic DNA fragments that contained CD3ε exons 4 and 5 (5′ flank; 3.5 kilobases) and exons 7 and 8 (3′ flank; 3.0 kilobases) into plasmid pZINIMn, which contains both the *lox*P-PGK-NEO-*lox*P and PGK-HSV-TK expression cassettes. Embryonic stem (ES) cell electroporation and selection for homologous recombinants was performed essentially as described (15). CD3$\varepsilon^{\Delta/\Delta}$ mice (see allele designations below) were generated by mating CD3$\varepsilon^{N/N}$ mice with EIIa-*cre* transgenic mice (16) and then screening offspring for loss of the PGK-NEO cassette. The CD3ε transgene was generated by substituting the murine CD3ε coding sequences for the ζ cDNA sequences in construct ζ-CT108 (17). Four CD3ε transgenic founder lines were generated by zygote injection, and CD3ε protein expression was quantitated by Western blotting. Two founder lines [C7530 (2× expression relative to wild-type), and C7705 (0.25× expression relative to wild-type)] were used in the present experiments.

PCR Screening. Mice were genotyped initially by Southern blotting then subsequently by using a competitive PCR reaction that distinguishes between the CD3ε^+ (wild-type), CD3ε^N (mutant CD3ε with PGK-NEO insertion), and CD3ε^Δ(mutant CD3ε without PGK-NEO) alleles (Fig. 2). PCR was performed with a mixture of oligonucleotide 1 (CD3ε-exonV/5′-3′; TACAAAGTCTCCATCTCAGG), 2 (CD3ε-exonVII/3′-5′; TGGCCGCTCCTTGTTTTG), 3 (CD3ε-exonV/3′-5′; CTCGAGCTTTCAGGTACAA), and 4 (NEO; GGATTA-GATAAATG CCTGCT). PCR parameters were 95°C, 20″; 55°C, 20″; 72°C, 90″ × 35 cycles. Products were resolved on a 2% agarose gel and were visualized by staining with ethidium bromide.

RNA and Protein Analysis. For Northern blot analysis, RNA was purified from total thymocytes, was electrophoresed, was transferred to membranes, and was hybridized with CD3γ-, CD3δ-, CD3ε-, and ζ-specific probes as described (17). The ef-1α probe was generated by PCR of total embryo (fetal day 9.5) cDNA with oligos ef-1αA and ef-1αB (18). For Western blot analysis, thymocytes were enumerated, were lysed at a concentration of 1×10^8/ml in SDS-loading buffer plus β-mercaptoethanol, and were resolved by 15% SDS/PAGE. Separated proteins were transferred to poly(vinylidene difluoride) membranes, were blotted with a 1:100 dilution of polyclonal anti-CD3ε (A452; Dako), and were detected by chemiluminescence (ECL, Amersham).

Multicolor Flow Cytometry. Single-cell suspensions of thymocytes or lymph node cells were processed, stained, and analyzed as described (17). mAbs used for flow cytometric analysis (purchased from PharMingen unless noted otherwise) included unlabeled anti-CD16/CD32 (2.4G2; blocking antibody), and fluorescein isothiocyanate- or phycoerythrin conjugated anti-CD4 (RM4.5), anti-CD8α (53–6.7), anti-TCRβ (H57–597), anti-TCRδ (GL-3), anti-CD3ε (145–2C11), anti-CD25 (7D4), anti-B220 (RA3–6B2), and anti-CD44 (IM7). Quantum-red conjugated anti-CD4 and anti-CD8 were purchased from Sigma.

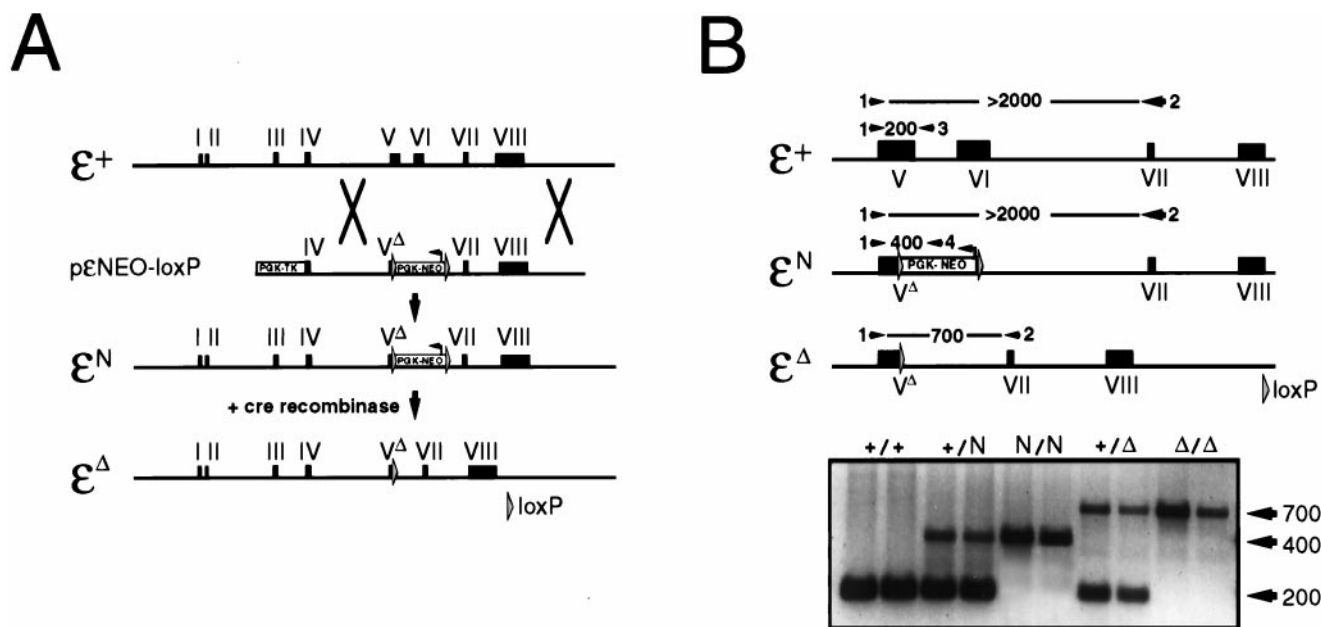FIG. 1.   Generation of CD3$\varepsilon^{N/N}$ and CD3$\varepsilon^{\Delta/\Delta}$ mice. (*A*) Representations of the CD3ε^+ (wild-type), CD3ε^N (PGK-NEO$^+$ mutant), and CD3ε^Δ (PGK-NEO$^-$ mutant) alleles. The CD3ε^N mutation was generated in ES cells by homologous recombination with the vector pεNEO-*lox*P as shown. The CD3ε^Δ allele subsequently was generated from the CD3ε^N allele by Cre/*lox*P-mediated recombination as described in *Materials and Methods*. (*B*) Competitive PCR strategy for distinguishing between the CD3ε^+, CD3ε^N, and CD3ε^Δ alleles. Mouse tail DNA was amplified with a mixture of oligonucleotides 1–4, and the products were visualized by gel electrophoresis. Allele-specific products were distinguished on the basis of size: CD3ε^+ (200-bp product of oligos 1+3); CD3ε^N (400-bp product of oligos 1+4); and CD3ε^Δ (700-bp product of oligos 1+2). Although oligos 1+2 also can amplify a product from both the CD3ε^+ and CD3ε^N alleles, the large size of the product (>2,000 bp) precludes its accumulation under the competitive PCR conditions. Results shown are from two independent DNA samples obtained from mice of the indicated genotypes.
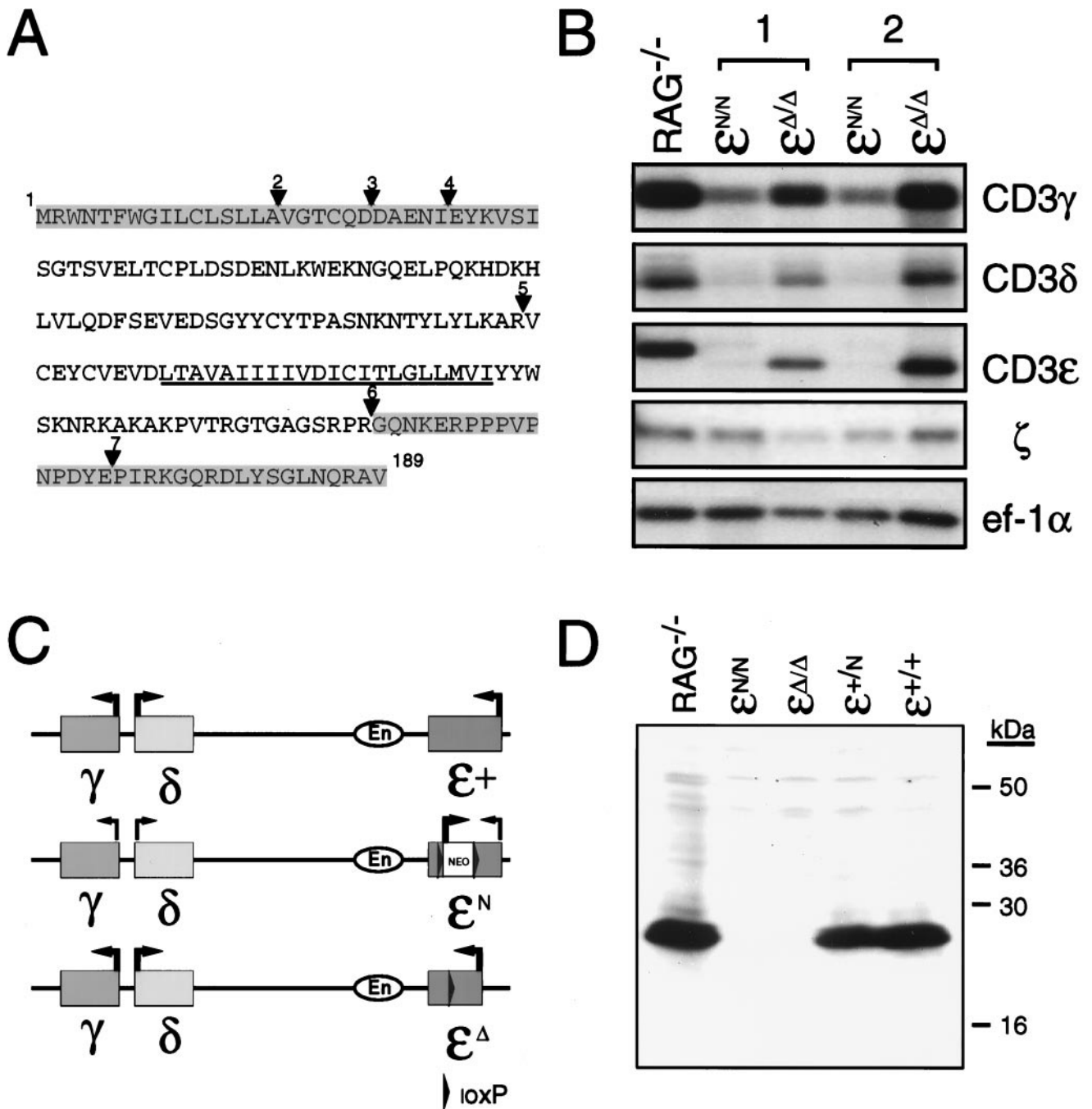
FIG. 2. (*A*) Predicted amino acid sequence of CD3ε$^\Delta$. Amino acid sequence of the fully processed CD3ε protein is shown. The transmembrane domain is underlined. Triangles indicate the location of introns (2–7) within the CD3ε gene. Shaded areas represent the protein that potentially could be expressed in CD3ε$^{\Delta/\Delta}$ mice based on the coding sequences that are retained within thymocyte transcripts from these mice. (*B*) Northern blot analysis of thymocyte total RNA from Rag$^{-/-}$ mice and from CD3ε$^{N/N}$ and CD3ε$^{\Delta/\Delta}$ mice that had been generated from two independently derived ES cell clones (1 and 2). Blots were hybridized with probes corresponding to CD3ε, CD3γ, CD3δ, and ζ and then were hybridized with a probe corresponding to the ubiquitously expressed ef-1α to assess the integrity and quantity of RNA in each lane. (*C*) Representation of the CD3-γ,-δ,-ε cluster on mouse chromosome 9. Location of the CD3 enhancer (En) element 3-prime of CD3ε is shown. Arrows indicate the direction of gene transcription. Thin lines indicate reduced gene expression. Depicted are the relative transcription levels observed from the (CD3)ε$^+$, (CD3)ε^N, and (CD3)ε$^\Delta$ alleles. (*D*) Western blot of thymocyte protein from Rag$^{-/-}$, (CD3)ε$^{N/N}$, (CD3)ε$^{\Delta/\Delta}$, (CD3)ε$^{+/N}$, and (CD3)ε$^{+/+}$ mice. Extracts from 7×10^6 thymocytes were run under reducing conditions on a 15% SDS-polyacrylamide gel, were transferred to poly(vinylidene difluoride) membrane, and were analyzed for CD3ε expression by using a rabbit antiserum raised against a peptide corresponding to amino acids within the C terminus of the CD3ε. Positions of the molecular mass standards are indicated in kilodaltons.

RESULTS AND DISCUSSION

Generation of CD3ε$^{N/N}$ and CD3ε$^{\Delta/\Delta}$ Mice. The vector pεNEO-*lox*P, which contains a PGK-NEO expression cassette flanked by *lox*P sites situated within a portion of the CD3ε gene, was used to generate targeted mutations of CD3ε in mouse ES cells (Fig. 1*A*). ES cell clones in which the targeting construct had integrated by homologous recombination contained a mutated CD3ε allele (ε^N) in which PGK-NEO was substituted for most of exon V and all of exon VI, which encode most of the extracellular domain and the entire transmembrane domain of CD3ε, respectively (Figs. 1*A* and 2*A*). Two positive ES clones were used to generate chimeric mice, which subsequently transmitted the mutated allele to their offspring.

Because it previously had been reported that mutation of CD3ε alters the expression of the CD3γ and CD3δ genes (12), thymocytes from mice homozygous for the CD3ε mutant allele (CD3ε$^{N/N}$) were screened for expression of CD3γ and CD3δ. Indeed, CD3ε$^{N/N}$ mice generated from both of the independently derived ES clones exhibited a phenotype identical to that previously described [i.e., a reduction in the level of CD3γ transcripts and a near absence of CD3δ transcripts (ref. 12 and Fig. 2B)]. CD3ε transcripts, which were of aberrant size, were also nearly undetectable in CD3ε$^{N/N}$ mice whereas expression of the unlinked ζ gene was unaffected (Fig. 2B).

It has been shown that the PGK-NEO cassette can negatively influence the expression of genes in its proximity, presumably by competing for regulatory sequences (19). Because the CD3γ, CD3δ, and CD3ε genes are linked closely, residing within 50 kilobases on mouse chromosome 9 (20), and are thought to be coregulated by an enhancer element located directly 3' of CD3ε (ref. 21 and Fig. 2C), it was conceivable that such a mechanism could account for the phenotype of CD3ε$^{N/N}$ mice. Consistent with this idea, multiple thymocyte-specific NEO transcripts were detectable in both CD3ε$^{+/N}$ and CD3ε$^{N/N}$ mice (data not shown). To ascertain whether the presence of PGK-NEO was in fact responsible for the decrease in CD3γ and CD3δ expression, the *lox*P-PGK-NEO-*lox*P cassette was removed from CD3ε$^{N/N}$ mice by Cre/*lox*P-mediated

recombination. CD3ε$^{N/N}$ mice were mated with EIIa-*cre* transgenic mice, which express Cre recombinase in all cells at the preimplantation stage of embryogenesis (16). Offspring of these matings initially were screened for loss of PGK-NEO (designated CD3ε^{Δ}) by Southern blotting and subsequently were tracked by a competitive PCR reaction that scores for the reduction in length of a product generated by primers corresponding to sequences within exon V and exon VII (Fig. 1B).

Analysis of thymocyte mRNA from CD3ε$^{Δ/Δ}$ mice by Northern blotting revealed a single abundant CD3ε transcript of reduced length compared with the wild-type CD3ε transcript (Fig. 2B). Sequencing of cDNAs generated from CD3^{Δ} transcripts by RT-PCR demonstrated that these mRNAs contained the intact coding sequences from exons I-IV and VII-VIII of CD3ε and thus represented a precise deletion of the exon V and exon VI coding sequences (Fig. 2A). Because these transcripts could conceivably direct the synthesis of a mutant CD3ε protein, thymocyte extracts from CD3$^{Δ/Δ}$ mice were screened by Western blotting with an antibody directed against a peptide corresponding to amino acids 156–168 of CD3ε, which should be retained within the intact CD3^{Δ} product (Fig. 2A). Immunoreactive protein was undetectable in CD3$^{Δ/Δ}$ mice, indicating that either the protein encoded by the CD3^{Δ} transcripts is highly unstable or that the epitope recognized by the antibody has been deleted (Fig. 2D). Sig-

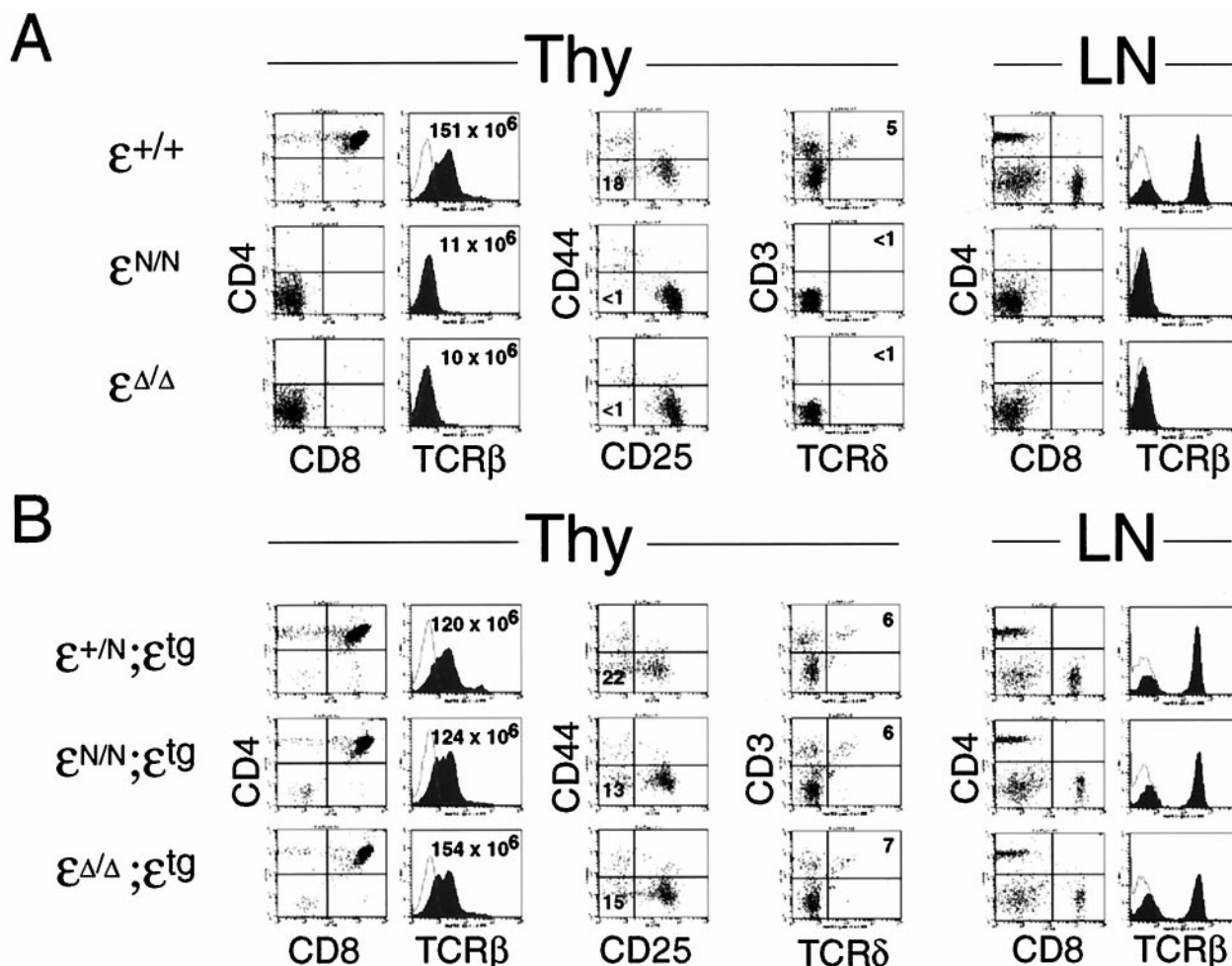FIG. 3. (A) Phenotype of CD3ε$^{N/N}$ and CD3ε$^{Δ/Δ}$mice. Thy, thymocytes; LN, lymph node. The left two panels show two-color (CD4 vs. CD8) and single-color (TCRβ) analysis of thymocytes from (CD3)ε$^{+/+}$, (CD3)ε$^{N/N}$, and (CD3)ε$^{Δ/Δ}$mice. Total thymocyte numbers from representative mice are provided in the two color plots. The central panels show two-color (CD44 vs. CD25) and two-color (CD3 vs. TCRδ) staining of gated (CD4$^-$CD8$^-$B220$^-$) thymocytes. Numbers in quadrants indicate percentage of gated cells within the quadrant. The right two panels show two-color (CD4 vs. CD8) and single-color (TCRβ) analysis of total lymph node cells. (B) Phenotype of CD3ε$^{N/N}$;ε^{tg} and CD3ε$^{Δ/Δ}$;ε^{tg} mice. The panels show representative data from (CD3)ε$^{+/N}$, (CD3)ε$^{N/N}$, and (CD3)ε$^{Δ/Δ}$mice that contain a CD3ε transgene under the control of the human CD2 promoter and enhancer sequences (founder line C7530). Analysis is identical to that described in A.

nificantly, in contrast to CD3ε$^{N/N}$ mice, thymocytes from CD3ε$^{Δ/Δ}$ mice contained normal levels of CD3γ and CD3δ transcripts, which were of the predicted size (Fig. 2*B*). Thus, the generation of CD3ε$^{Δ/Δ}$ mice enabled a specific examination of the requirement for CD3ε in development.

Phenotype of CD3ε$^{Δ/Δ}$ Mice. Analysis of T cell development in CD3ε$^{Δ/Δ}$ mice revealed a phenotype virtually indistinguishable from that of CD3ε$^{N/N}$ mice (ref. 12 and Fig. 3). Total thymocyte numbers in both CD3ε$^{Δ/Δ}$ and CD3ε$^{N/N}$ mice were <10% of control (CD3ε$^{+/+}$), and the thymocytes that were present consisted entirely of the DN cells (i.e., both DP and CD4$^+$CD8$^-$ or CD4$^-$CD8$^+$ thymocytes were absent) (Fig. 3*A*). DN thymocytes progress through four stages of maturation before their transition to the DP stage: CD44$^+$ CD25$^-$ → CD44$^+$ CD25$^+$ → CD44$^{-/lo}$ CD25$^+$ → CD44$^-$ CD25$^-$ (22). Rearrangement of the TCRβ locus is first detected in CD44$^{-/lo}$ CD25$^+$ thymocytes, and progression of these cells to the CD44$^-$ CD25$^-$ stage is thought to require expression of the pre-TCR (or TCR) complex (2, 22). Consistent with this idea, thymocyte development is arrested at the CD44$^{-/lo}$ CD25$^+$ stage in both Rag$^{-/-}$ and CD3ε$^{N/N}$ mice (refs. 7, 8, and 12 and Fig. 3*A*). Significantly, thymocyte development was arrested at the identical (CD44$^{-/lo}$ CD25$^+$) stage in CD3ε$^{Δ/Δ}$ mice (Fig. 3*A*). As observed previously in CD3ε$^{N/N}$ mice (12), TCRβ, TCRγ, and TCRδ gene rearrangements were readily detectable in thymocytes from CD3ε$^{Δ/Δ}$ mice (data not shown). Thus, these results demonstrate that CD3ε specifically is required for maturation of CD44$^{-/lo}$ CD25$^+$ thymocytes to the CD44$^-$ CD25$^-$ stage. In addition, the fact that T cell development is blocked at the identical stage in CD3ε$^{N/N}$ and CD3ε$^{Δ/Δ}$ mice indicates that CD3γ and CD3δ are incapable of compensating for the loss of CD3ε.

As predicted from the early block in thymocyte development, mature CD4$^+$CD8$^-$ or CD4$^-$CD8$^+$ T cells were absent from the periphery of CD3ε$^{Δ/Δ}$ mice (Fig. 3*A*). Moreover, surface αβTCR and γδTCR complexes were undetectable in both the thymus and peripheral lymphoid organs as assessed by staining for TCRβ and TCRδ chains (Fig. 3*A*). As previously reported for CD3ε$^{N/N}$ mice (12), the development of intestinal intraepithelial T cells also was arrested in CD3ε$^{Δ/Δ}$ mice whereas B and NK cell development appeared unaffected (data not shown).

Expression of a CD3ε Transgene Rescues T Cell Development in CD3ε$^{Δ/Δ}$ Mice. To confirm that the phenotype exhibited by CD3ε$^{Δ/Δ}$ mice could be attributed entirely to the absence of CD3ε, we reconstituted CD3ε expression in CD3ε$^{Δ/Δ}$ mice by the introduction of a CD3ε transgene. Our own investigations have shown that transgenes placed under the control of the T-lineage-specific human CD2 promoter and enhancer begin to be expressed in thymocytes at the DN, CD44$^{-/lo}$ CD25$^+$ stage and continue to be expressed at all subsequent stages of development (ref. 23 and data not shown). Thus, the CD3ε transgene construct should provide expression of CD3ε at the stage in development at which an arrest is first observed in CD3$^{Δ/Δ}$ mice (Fig. 3*A*). Because overexpression of CD3ε has been shown to block T cell development (24), we used transgenic founder lines that express relatively low levels of CD3ε for reconstitution experiments (0.25- and 2× wild-type). These transgenes did not inhibit thymocyte development when introduced into CD3$^{+/+}$ or CD3$^{+/N}$ mice (Fig. 3*B* and data not shown).

As depicted in Fig. 3*B*, reexpression of CD3ε in CD3ε$^{Δ/Δ}$ mice effectively reversed the developmental defects as assessed by the presence of DN, CD44$^-$ CD25$^-$ thymocytes, as well as DP and CD4$^+$CD8$^-$ and CD4$^-$CD8$^+$ thymocytes in CD3ε$^{Δ/Δ}$ CD3ε^{tg} mice. In addition, total thymocyte cellularity was increased markedly (to normal levels) in CD3ε$^{Δ/Δ}$; CD3ε^{tg} mice, and mature T cells were present in the periphery (Fig. 3). Finally, reexpression of CD3ε also restored surface expression of αβ- and γδ-TCR complexes on both immature and mature

T cells (Fig. 3 *A* and *B*). Thus, these results demonstrate that the defects in T cell development observed in CD3ε$^{Δ/Δ}$ mice are caused specifically by CD3ε deficiency. Moreover, because the CD3ε transgene would not be expected to restore expression of CD3ε at stages in development that precede the DN, CD44$^{-/lo}$ CD25$^+$ stage, these findings lend support to the idea that CD3ε first is required at the point at which expression of the pre-TCR begins (12).

Of interest, introduction of the CD3ε transgene also fully corrected the developmental defect in CD3ε$^{N/N}$ mice (Fig. 3*B*). This result was unexpected because DN thymocytes from CD3ε$^{N/N}$ mice contain abnormally low levels of CD3γ and CD3δ transcripts (ref. 12 and Fig. 2*B*), and thymocyte development is compromised in the absence of either CD3γ or CD3δ (13, 14). Analysis of CD3γ and CD3δ gene expression in total thymocytes from CD3ε$^{N/N}$;CD3ε^{tg} mice (which consisted mostly of DP cells) revealed normal levels of CD3γ or CD3δ transcripts (Fig. 4). Thus, the restoration of thymocyte development in CD3ε$^{N/N}$;CD3ε^{tg} mice could be attributed to normalization of CD3γ and CD3δ expression as well as reconstitution of CD3ε. There are several possible explanations for these results. For example, the inhibitory effect of PGK-NEO mice may be variable between individual cells, such that some DN thymocytes express relatively normal levels of CD3γ and CD3δ and these cells are expanded selectively when CD3ε is reexpressed. Alternatively, the transcriptional suppression of CD3γ and CD3δ by PGK-NEO may be cell cycle- or stage-dependent. Regardless of the mechanism, these results demonstrate that the inhibitory effect of PGK-NEO on CD3γ and CD3δ gene expression is not developmentally limiting if CD3ε expression is restored.

In summary, these results reveal a specific requirement for CD3ε in T cell development not exhibited by any of the other invariant subunits of the TCR complex (i.e., CD3γ, CD3δ, and ζ) (11, 13, 14). The present data are consistent with previous results obtained in T cell lines demonstrating that αβTCR complexes are still expressed, though at reduced levels, in the absence of CD3γ, CD3δ, or ζ but that CD3ε is absolutely required for surface expression of TCRα/β heterodimers (25–27). The absence of DP thymocytes and γδTCR$^+$ cells in
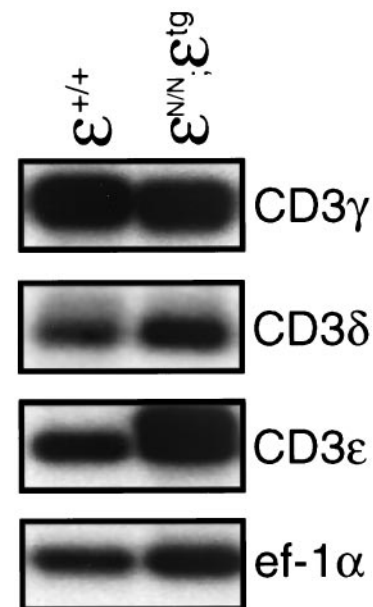

Fig. 4. Northern blot analysis of total thymocyte RNA from CD3ε$^{+/+}$ and CD3ε$^{N/N}$;ε^{tg} mice. Blots were hybridized with probes corresponding to CD3ε, CD3γ, CD3δ, and ζ and then were hybridized with the a probe corresponding to the ubiquitously expressed ef-1α to assess the integrity and quantity of RNA in each lane.

CD3$\varepsilon^{\Delta/\Delta}$ mice demonstrates that CD3ε is also required for the expression and/or function of the pre-TCR and $\gamma\delta$TCR complexes. That this need not necessarily have been the case is suggested by the discovery that the pre-TCR and $\gamma\delta$TCR differ from the $\alpha\beta$TCR in their requirement for CD3δ (13, 28). Taken together, current data indicate that a minimal requirement for pre-TCR/TCR expression and/or function is the expression of either a CD3$\gamma\varepsilon$ or CD3$\delta\varepsilon$ heterodimer, although the former are much more efficient at promoting the assembly and surface expression of pre-TCR and $\gamma\delta$TCR complexes. Finally, although these experiments demonstrate an obligate role for CD3ε in T cell development, it is important to note that the defect in CD3$\varepsilon^{\Delta/\Delta}$ mice results in loss of both the structural and signal transducing functions of CD3ε. Therefore, additional studies will be required to determine whether CD3ε-mediated signal transduction is required at specific stages of development.

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