Brief Definitive Report

Essential and Partially Overlapping Role of CD3γ and CD3δ for Development of αβ and γδ T Lymphocytes

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Summary

CD3γ and CD3δ are two highly related components of the T cell receptor (TCR)–CD3 complex which is essential for the assembly and signal transduction of the T cell receptor on mature T cells. In gene knockout mice deficient in either CD3δ or CD3γ, early thymic development mediated by pre-TCR was either undisturbed or severely blocked, respectively, and small numbers of TCR-$\alpha\beta^+$ T cells were detected in the periphery of both mice. γδ T cell development was either normal in CD3$\delta^{-/-}$ mice or partially blocked in CD3$\gamma^{-/-}$ mice. To examine the collective role of CD3γ and CD3δ in the assembly and function of pre-TCR and in the development of γδ T cells, we generated a mouse strain with a disruption in both CD3γ and CD3δ genes (CD3γδ$^{-/-}$). In contrast to mice deficient in either CD3γ or CD3δ chains, early thymic development mediated by pre-TCR is completely blocked, and TCR-$\alpha\beta^+$ or TCR-$\gamma\delta^+$ T cells were absent in the CD3γδ$^{-/-}$ mice. Taken together, these studies demonstrated that CD3γ and CD3δ play an essential, yet partially overlapping, role in the development of both αβ and γδ T cell lineages.

Key words: CD3γ • CD3δ • T cell receptor–CD3 complex • T cell development • knockout mouse

During thymocyte development, the genes coding for TCR-α and -β, pre-TCR-α (pTα), and the associated CD3 proteins (CD3γ, δ, ε, and ζ) are expressed in a temporal order (1). The pre-TCR–CD3 complex, consisting of pTα, TCR-β, and CD3 proteins, plays a major role in early thymocyte development and in the transition from $CD4^-CD8^-$ (double negative, DN) to $CD4^+CD8^+$ (double positive, DP) cells, as targeted mutations in pTα, TCR-β, RAG, and CD3 genes all result in an arrest of T cell development at the DN $CD44^-CD25^+$ check point (2, 3). Subsequently, TCR-α replaces pTα and the resulting TCR–CD3 complex mediates signal transduction cascades leading to further T cell development (2). Compared with αβ T cell development, γδ T cell development is less defined (4, 5). The majority of thymic γδ T cells do not express CD4 or CD8 antigens (6), and pTα and TCR-β are not involved the development of γδ T cells (7, 8). However, CD3 proteins are required for the development of this lineage (2).

Ample biochemical studies have shown that the CD3 proteins are important for assembly and efficient surface expression of TCR (9). In each TCR–CD3 complex, there are two copies of CD3ε and CD3ζ, yet only one copy of the highly homologous CD3γ and CD3δ (10–12). CD3ε forms heterodimers with CD3γ and CD3δ, and can also exist as a CD3εε homodimer, whereas CD3ζ exists as a CD3ζζ homodimer (11, 13, 14). TCRs lacking CD3γ, δ, ε, or ζ can reach the cell surface, albeit 10–100-fold less efficiently than wild-type receptors, because of a certain degree of redundancy in their assembly potential (15, 16). In immature thymocytes, the CD3 proteins are expressed (17–19), before the expression of pTα and TCR-β (1). Thus, CD3 proteins can be a part of the pre-TCR–CD3 complex or part of a clonotype-independent CD3 (CIC) complex (20). In these complexes, CD3γε dimers are consistently detected (20, 21), and some studies indicated the presence of a small quantity of CD3δε dimers (18–21). This led to the notion that CD3γ may be preferentially required over CD3δ in the assembly of pre-TCR complexes (22).

Recent studies on mutant mice deficient in either the CD3γ or CD3δ gene in part support this notion. Whereas transition from DN to DP αβ thymocytes appears to be normal in CD3$\delta^{-/-}$ mice (23), αβ T cell development in CD3$\gamma^{-/-}$ mice is blocked at the DN $CD44^-CD25^+$ check point (24). However, the blockade in T cell development in

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$CD3\gamma^{-/-}$ mice is incomplete, as small numbers of DP thymocytes were found and TCR-$\alpha\beta^{+}$ T cells were detected in the periphery (24). Moreover, in either mutant a considerable number of γδ T cells is present (23, 24). Therefore, it is likely that CD3γ and CD3δ play an essential, yet to some extent redundant, role in early development of T cells.

To examine the issue of partial overlap in function between CD3γ and CD3δ, a mouse strain with a disruption in both the CD3γ and CD3δ genes ($CD3\gamma\delta^{-/-}$) would be useful. A $CD3\gamma\delta^{-/-}$ mouse, however, could not be generated by breeding the $CD3\delta^{-/-}$ and $CD3\gamma^{-/-}$ mice, because the genes coding for CD3γ, δ, and ϵ are located in a single gene cluster and a mere 1.4-kb intergenic sequence separates the first exons of CD3γ and CD3δ genes (25). Therefore, we generated $CD3\gamma\delta^{-/-}$ mice by deleting the promoters and exons 1 of both genes.

Materials and Methods

Generation of $CD3\gamma\delta^{-/-}$ Mice. The targeting construct was generated by standard methods. In brief, a genomic DNA clone containing a 15.5-kb fragment of CD3γδ genes was isolated from a 129/sv mouse genomic DNA library (provided by Dr. Manley Huang, GenPharm Int., Mountain View, CA) and subcloned into pBluescriptSK+ (Stratagene, La Jolla, CA). A 2.8-kb SalI-XhoI DNA fragment containing the PGK-TK^{r} gene was isolated from pPGK-TK (provided by Dr. Manley Huang), and ligated to the XhoI site of pPGK-hygromycine^{r} (hyg^{r}) (a gift of Dr. Richard Mortensen). A 1.9-kb XbaI-XbaI intronic fragment between exon 1 and 2 of CD3δ was obtained by XbaI digestion of the 15.5-kb CD3γδ genomic DNA fragment. And a 3-kb intronic fragment between exon 1 and 2 of CD3γ was obtained by first subcloning a 5-kb EcoRI-XbaI fragment into SK+ followed by a HindIII cut, so that a HindIII site from the polylinker region of the plasmid was transferred to one end of the 3-kb fragment. The 1.9-kb XbaI-XbaI fragment and the 3-kb HindIII-HindIII fragment were inserted into the 5′ and 3′ sites of the PGK-Hyg^{r} gene. In the resulting construct, a 3.1-kb DNA fragment containing the 1.4-kb intergenic DNA fragment between the CD3γ and CD3δ genes and exons 1 of both genes were replaced by the 2.8-kb PGK-Hyg^{r} cassette. 10 μg of purified targeting molecules were electroporated into 10^{7} J-1 ES cells. ES cells were positively selected by hygromycin-B at 200 μg/ml and negatively selected by FIAU at 0.2 μM. 355 clones were selected and examined by Southern blots for homologous recombination using a 0.8-kb (StuI-XbaI) 5′ probe located outside of the construct. Eight clones were identified as targeted clones, which were confirmed by another Southern analysis with a hyg^{r} probe. Four of the targeted clones were injected into the blastocysts of either C57BL/6 or BALB/C origin, and 90–100% fur color chimerism was observed in 45 founder mice. Test breeding of the chimeras indicated that all of the males ($n = 28$ from 3 embryonic stem [ES] clones) transmitted the ES cell genome. Four males were mated to C57BL/6 females to generate heterozygous mice, and homozygous $CD3\gamma\delta^{-/-}$ lines were obtained by sibling breeding. Identical results were obtained from homozygous $CD3\gamma\delta^{-/-}$ lines of different ES clones.

Flow Cytometric Analysis. Single cell suspensions of thymocytes, LN cells, spleen cells, PBL, and small intestine intraepithelial lymphocytes (iIEL) were prepared as described (26, 27). Three-color staining of the cells was performed as previously reported elsewhere (28).

RNA Analysis. Northern blot analysis was performed as described (29).

Results

Generation of $CD3\gamma\delta^{-/-}$ Mice. To generate mice deficient in both CD3γ and CD3δ gene expression, a 3.1-kb DNA fragment containing the promoters (25) and exons 1 of the CD3γ and CD3δ genes was replaced by a PGK-Hyg^{r} cassette (Fig. 1 *A*). The PGK-hyg^{r} cassette was chosen here over the PGK-neo^{r} cassette to prevent a possible suppressive effect of the PGK-neo^{r} on neighboring gene expression (30, 31). Homozygous mice carrying this mutation in the CD3γ and δ genes were generated (Fig. 1 *B*). Northern blot analysis demonstrated that the expression of both CD3γ and CD3δ mRNA was absent in the $CD3\gamma\delta^{-/-}$ thymocytes (Fig. 2). Moreover, no aberrant expression of the truncated CD3γ or δ mRNAs were ever detected in Northern blotting of thymocytes from more than 20 $CD3\gamma\delta^{-/-}$ mice. However, the expression of the neighboring CD3ϵ gene and the nonlinked CD3ζ was normal (Fig. 2), and pTα expression was detected (data not shown).

αβ T Cell Development in the $CD3\gamma\delta^{-/-}$ Mice. Total cellularity of the thymi of $CD3\gamma\delta^{-/-}$ mice was 2–5% of that in wild-type or heterozygous littermates (Fig. 3 *A*). Flow cytometric analysis of the thymocytes showed that these cells are DN, with the majority of them being $CD44^{-}CD25^{+}$c-Kit^{-}Sca-1^{+}, identical to the thymocytes found in $RAG^{-/-}$ mice (Fig. 3 *B*). Northern blot analyses of the thymocytes of $CD3\gamma\delta^{-/-}$ mice did not detect the mRNA for rearranged TCR-α and TCR-β genes, whereas only the 1.0-kb germline C_{β} mRNA was detectable (Fig.

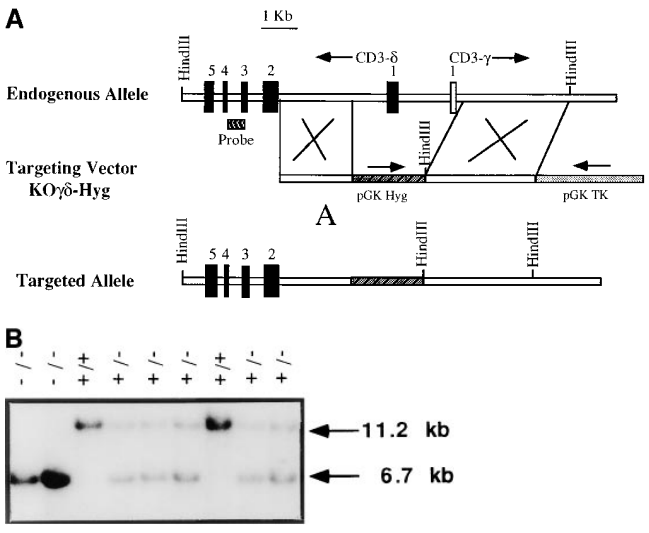

Figure 1. Disruption of CD3γδ genes. (*A*) Diagram of the CD3γδ targeting vector for homologous recombination. Exons 1–5 of the CD3δ gene and exon 1 of the CD3γ gene are numbered. Arrows indicate the transcriptional orientations of the CD3γδ genes. The 0.8-kb probe was used for screening the ES cell clones and for Southern analysis of tail DNA. (*B*) Southern blot analysis of tail DNA.

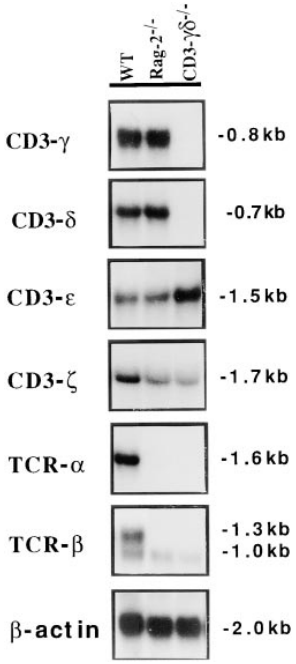

Figure 2. TCR–CD3 expression in the CD3γδ$^{-/-}$ mice. Northern blotting of thymocytes from wt, RAG$^{-/-}$ and CD3γδ$^{-/-}$ mice for the expression of CD3γ, δ, ϵ, ζ, and TCR-α and -β. The respective probes are indicated on the left, and the sizes on the right. Two mice of each type were analyzed in this blot.

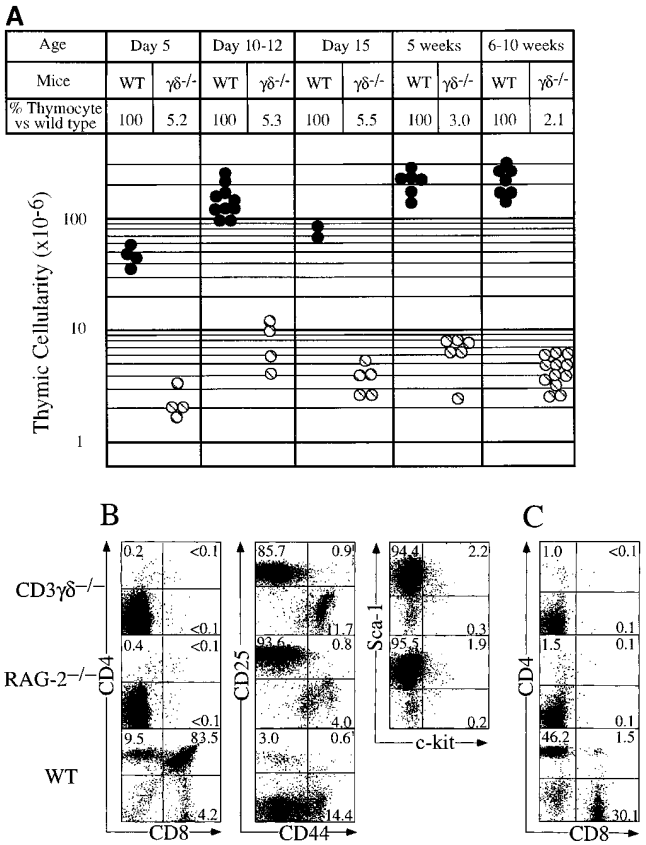

Figure 3. T cell deficiency in the CD3γδ$^{-/-}$ mice. (*A*) Thymic cellularity of the mutant mice. Each symbol represents the total number of thymocytes from a mouse. The ages of the mice are indicated. For each age group, the average number of thymocytes from mutant mice was compared with that of wild-type (including CD3γδ$^{+/-}$) littermates or age-matched, wild-type mice (28). (*B*) Flow cytometric analysis of thymocytes from CD3γδ$^{-/-}$, RAG-2$^{-/-}$ and wild-type mice for surface expression of CD4, CD8, CD44, CD25, Sca-1, and c-Kit. (*C*) Flow cytometric analysis of peripheral lymph node cells for surface expression of CD4 and CD8.

2). Consistent with these analyses, no mature αβ$^+$ T cells were detected in the LN, the spleen, or the gut of the CD3γδ$^{-/-}$ mice (Figs. 3 *C* and 4 *C*, Table 1). B cell development appeared unaffected (Table 1). Taken together, αβ T cell development in CD3γδ$^{-/-}$ mice is blocked at the same DN CD44$^-$CD25$^+$ check point as RAG$^{-/-}$ mice (32, 33).

γδ T Cell Development in CD3γδ$^{-/-}$ Mice. Next, γδ T cell development in CD3γδ$^{-/-}$ mice was examined. As shown in Fig. 4, *A* and *B*, γδ T cells were absent in the thymus and periphery of CD3γδ$^{-/-}$ mice. Since γδ T cells normally account for only a very small fraction of thymocytes and peripheral T cells, we assessed γδ T cell development in the small intestine, where γδ T cells represent a major population of the iIEL in wild-type mice. In CD3γδ$^{-/-}$ mice, γδ T cells were again nondetectable in the intestine (Fig. 4 *C*). However, normal number of CD8αα$^+$B220$^+$CD32$^+$NK1.1$^-$ cells, representing T cell progenitors in the gut (27) could be detected in the gut of CD3γδ$^{-/-}$ mice (Fig. 4, *C–E*, Table 1, and data not shown). Therefore, these analyses indicate that deficiency in CD3γ and δ completely blocked γδ T cell development beyond the CD8αα$^+$ stage.

Discussion

We report here that in the CD3γδ$^{-/-}$ double mutant mice, intrathymic development is completely arrested at the DN CD44$^-$CD25$^+$ prothymocyte stage, a central check point at which pre-TCR begins to mediate further thymocyte differentiation into the DP stage. This observation indicates that the function of pre-TCR is completely abrogated in CD3γδ$^{-/-}$ mice. In contrast, in recently reported CD3δ$^{-/-}$ mice, thymic development is undisturbed up to the DP stage (23), whereas the transition from DN to DP stages was severely but not completely blocked in CD3γ$^{-/-}$ mice (24). The phenotypes of CD3δ$^{-/-}$ and CD3γ$^{-/-}$ mice are consistent with the biochemical evidence that CD3γ is preferentially required over CD3δ in prothymocytes for the assembly of the pre-TCR–CD3 complex (22). However, the present data revealed that CD3δ also participated in vivo in the assembly and function of the pre-TCR–CD3 complex. Moreover, small numbers of TCR-αβ$^+$ T cells were detected in the periphery of CD3δ$^{-/-}$ and CD3γ$^{-/-}$ mice, but were absent in CD3γδ$^{-/-}$ mice. These observations are consistent with the biological evidence that in mature T cells, the TCR–CD3 complex lacking either CD3γ or δ could sometimes be detected on the cell surface at reduced levels. However, no surface expression of the TCR–CD3 complex could be detected in cells lacking both CD3γ and δ (15, 16). Taken together, CD3γ and CD3δ collectively play an essential, yet partially overlapping, role in the assembly and function of the pre-TCR. It is most likely that in the absence of

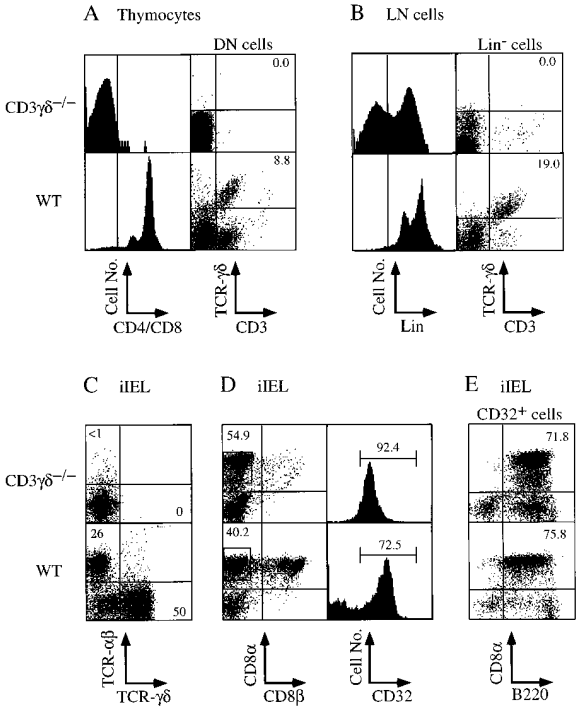

Figure 4. Flow cytometric analysis of the γδ T cell compartment in CD3γδ$^{-/-}$ mice. (*A*) Thymocytes were stained with anti-TCR-γδ-biotinylated (detected with RED-670), anti-CD3-PE, and anti-CD4/CD8-FITC. (*Left*) Profile of CD4/CD8 expression. (*Right*) Profile of TCR-γδ and CD3 expression in the analytically gated DN cells. (*B*) Lymph node cells were similarly analyzed as in *A*, except that a mixture of FITC-conjugated antibodies, i.e., anti-CD4, -CD8, –TCR-αβ, -B220, -Mac-3, and Gr-1 (collectively termed Lin), was used. (*C*) Expression of TCR-αβ and TCR-γδ in iIEL. (*D*) iIEL were stained with anti-CD8α, anti-CD8β, and anti-CD32. (*Left*) Profile of CD8α/CD8β expression. (*Right*) Profile of CD32 expression in the analytically gated CD8αα$^+$ cells. The CD8α$^+$ cells were all CD8αα$^+$ and were predominantly CD32$^+$. (*E*) iIEL were stained with anti-CD8α, anti-B220, and anti-CD32, and the profile of CD8α/B220 expression in the analytically gated CD32$^+$ cells is shown.

CD3γ and CD3δ, pre-TCR cannot be expressed on the surface of prothymocytes.

In addition to the structural requirement, CD3γ and CD3δ may regulate pre-TCR function through the signaling capacity of the immunoreceptor tyrosine-based activation motifs (ITAMs) presented in their cytoplasmic domains (34). It is known that not every ITAM plays a distinct role in pre-TCR function. For instance, pre-TCR function is competent in mutant mice deficient in the CD3ζ cytoplasmic domain (35). Moreover, the defect in pre-TCR function in CD3γ$^{-/-}$ (24), CD3ζ$^{-/-}$ (36), or RAG$^{-/-}$ (19, 27, 37) mice can be overcome by anti-CD3ε-mediated cross-linking. However, the same anti-CD3ε treatment in vivo in CD3γδ$^{-/-}$ mice failed to relieve the block at the DN check point (data not shown). Since the anti-CD3ε antibody used in all of these studies, namely 2C11 (or 500A2), binds CD3ε efficiently when either CD3γ or CD3δ is presented but poorly when both CD3γ and CD3δ are missing (38; data not shown), the lack

Table 1. *T Cell and B Cell Compositions in CD3γδ$^{-/-}$ Mice*

Tissue	Cell	CD3γδ$^{-/-}$	Wild type
Thymus	Cellularity (% of wt)	2.5 ± 1.1	100 ± 21
LN	TCR-αβ$^+$	0	73 ± 9
	B220$^+$CD19$^+$	84 ± 2	19 ± 4
Spleen	TCR-αβ$^+$	0	35 ± 3
	B220$^+$CD19$^+$	67 ± 6	47 ± 10
iIEL	TCR-αβ$^+$	0	41 ± 5
	TCR-γδ$^+$	0	50 ± 6
	CD8-α$^+$*	64 ± 5	66 ± 10

A total of 9–14 mice of each type were analyzed in five independent experiments, and data are pooled and shown as mean ± SD. Thymic cellularity was determined as shown in Fig. 3 *A*. Data for the peripheral lymphoid tissues and iIEL were obtained by cytometric analyses, and represent the percent of cells positive for the indicated marker in the whole lymphocyte population.

*CD8α$^+$ iIEL are CD8αα$^+$CD32$^+$B220$^+$NK1.1$^-$ T cell precursors in CD3γδ$^{-/-}$ mice (Fig. 4; 27). In wild-type mice, CD8α$^+$ iIEL consist of both CD8αα$^+$ and CD8αβ$^+$ cells, and are mostly TCR-αβ$^+$CD3$^+$ (27).

of thymocyte differentiation upon 2C11 treatment of CD3γδ$^{-/-}$ mice might be explained by the following nonexclusive possibilities: (*a*) pre-TCR could not be expressed on the surface of CD3γδ$^{-/-}$ prothymocytes; (*b*) the inefficient binding of 2C11 to CD3ε on the surface of CD3γδ$^{-/-}$ prothymocytes results in a weak signal that is below the threshold level for further thymic development; and (*c*) the cytoplasmic domains of CD3γ and CD3δ collectively play an essential role in pre-TCR function. The last possibility, nevertheless, is less likely because it has been shown that under artificial circumstances, either CD3ε or CD3ζ cytoplasmic domain alone can independently generate signals for thymocyte development to the DP stage (39). Thus, the ultimate assessment of the physiological role of the cytoplasmic domains of CD3γ and CD3δ awaits the generation of mutant mice in which the cytoplasmic domains of CD3γ and CD3δ are deleted.

An important observation of this study was that γδ T cell development was completely blocked in the CD3γδ$^{-/-}$ mice. In comparison, γδ T cell development was partially blocked in the CD3γ$^{-/-}$ mice and was undisturbed in CD3δ$^{-/-}$ mice (23, 24). Thus, this study demonstrated that CD3δ also plays a role in regulating the development of the γδ T cell lineage, and CD3γ and CD3δ collectively are essential for γδ T cell development. Like their regulation of αβ T cell development, CD3γ and CD3δ may regulate γδ T cell development by their structural contribution and/or signaling capacity. Nevertheless, the function of CD3γ or CD3δ for γδ T cells may not be a duplication of their respective roles for αβ T cells. For instance, although surface expression of TCR-αβ is severely reduced (8–10-fold) in CD3δ$^{-/-}$ mice, their TCR-γδ expression is only mildly

(less than twofold) reduced (23). On the other hand, severe reduction of both TCR-αβ and TCR-γδ expression in CD3$\gamma^{-/-}$ mice indicated a pivotal role of CD3γ in the assembly of TCR-αβ–CD3 and TCR-γδ–CD3 complexes (24). Taken together, it is likely that the complete block in γδ T cell development in CD3$\gamma\delta^{-/-}$ mice was a result of the incomplete TCR-γδ–CD3 complex not being expressed on cell surface in the absence of CD3γ and CD3δ. It remains to be investigated whether the cytoplasmic domains of CD3γ and CD3δ also have distinct functions in the development of γδ T cells.

In conclusion, in the CD3$\gamma\delta^{-/-}$ mice, early thymic development mediated by pre-TCR was completely blocked, and TCR-$\alpha\beta^{+}$ and TCR-$\gamma\delta^{+}$ T cells were absent. These observations are different from those made on either CD3$\delta^{-/-}$ or CD3$\gamma^{-/-}$ mice, in which pre-TCR function was either undisturbed or incompletely blocked, as TCR-$\alpha\beta^{+}$ and TCR-$\gamma\delta^{+}$ T cells were detected in the periphery. Taken together, these studies demonstrated that CD3γ and CD3δ play an essential, yet partially overlapping, role in the development of both αβ and γδ T cell lineages.

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References

1. Wilson, A., and H.R. MacDonald. 1995. Expression of genes encoding the pre-TCR and CD3 complex during thymus development. *Int. Immunol.* 7:1659–1664.
2. Tanaka, Y., L. Ardouin, A. Gillet, S.Y. Lin, A. Magnan, B. Malissen, and M. Malissen. 1995. Early T-cell development in CD3-deficient mice. *Immunol. Rev.* 148:171–199.
3. Wang, B., S.J. Simpson, G.A. Hollander, and C. Terhorst. 1997. Development of T lymphocyte and natural killer cell functions after bone marrow transplantation of severely immunodeficient mice. *Immunol. Rev.* 157:53–60.
4. Shortman, K., L. Wu, K.A. Kelly, and R. Scollay. 1991. The beginning and the end of the development of TCR gamma delta cells in the thymus. *Curr. Top. Microbiol. Immunol.* 173: 71–80.
5. Dudley, E.C., M. Girardi, M.J. Owen, and A.C. Hayday. 1995. α/β and γ/δ T cells can share a late common precursor. *Curr. Biol.* 5:659–669.
6. Zorbas, M., and R. Scollay. 1993. Development of γ/δ T cells in the adult murine thymus. *Eur. J. Immunol.* 23:1655–1660.
7. Mombaerts, P., A.R. Clarke, M.A. Rudnicki, J. Iacomini, S. Itohara, J.J. Lafaille, L. Wang, Y. Ichikawa, R. Jaenisch, M.L. Hooper, and S. Tonegawa. 1992. Mutations in T-cell antigen receptor genes α and β block thymocyte development at different stages. *Nature.* 360:225–231.
8. Fehling, H.J., A. Krotkova, C. Saint-Ruf, and H. von Boehmer. 1995. Crucial role of the pre-T-cell receptor α gene in development of α/β but not γ/δ T cells. *Nature.* 375:795–798 (see published erratum 378:419).
9. Exley, M., C. Terhorst, and T. Wileman. 1991. Structure, assembly and intracellular transport of the T cell receptor for antigen. *Semin. Immunol.* 3:283–297.
10. de la Hera, A., U. Muller, C. Olsson, S. Isaaz, and A. Tunnacliffe. 1991. Structure of the T cell antigen receptor (TCR): two CD3ε subunits in a functional TCR–CD3 complex. *J. Exp. Med.* 173:7–17.
11. Rutledge, T., P. Cosson, N. Manolios, J.S. Bonifacino, and R.D. Klausner. 1992. Transmembrane helical interactions: ζ chain dimerization and functional association with the T cell antigen receptor. *EMBO (Eur. Mol. Biol. Organ.) J.* 11:3245–3254.
12. Punt, J.A., J.L. Roberts, K.P. Kearse, and A. Singer. 1994. Stoichiometry of the T cell antigen receptor (TCR) complex: each TCR–CD3 complex contains one TCR-α, one TCR-β, and two CD3 epsilon chains. *J. Exp. Med.* 180: 587–593.
13. Alarcon, B., S.C. Ley, F. Sanchez-Madrid, R.S. Blumberg, S.T. Ju, M. Fresno, and C. Terhorst. 1991. The CD3-γ and CD3-δ subunits of the T cell antigen receptor can be expressed within distinct functional TCR/CD3 complexes. *EMBO (Eur. Mol. Biol. Organ.) J.* 10:903–912.
14. Manolios, N., F. Letourneur, J.S. Bonifacino, and R.D. Klausner. 1991. Pairwise, cooperative and inhibitory interactions describe the assembly and probable structure of the T-cell antigen receptor. *EMBO (Eur. Mol. Biol. Organ.) J.* 10: 1643–1651.
15. Hall, C., B. Berkhout, B. Alarcon, J. Sancho, T. Wileman, and C. Terhorst. 1991. Requirements for cell surface expression of the human TCR/CD3 complex in non-T cells. *Int. Immunol.* 3:359–368.
16. Kappes, D.J., and S. Tonegawa. 1991. Surface expression of alternative forms of the TCR/CD3 complex. *Proc. Natl. Acad. Sci. USA.* 88:10619–10623.
17. Punt, J.A., R.T. Kubo, T. Saito, T.H. Finkel, S. Kathiresan, K.J. Blank, and Y. Hashimoto. 1991. Surface expression of a T cell receptor β (TCR-β) chain in the absence of TCR-α, -δ, and -γ proteins. *J. Exp. Med.* 174:775–783.
18. Groettrup, M., A. Baron, G. Griffiths, R. Palacios, and H.

von Boehmer. 1992. T cell receptor (TCR) beta chain homodimers on the surface of immature but not mature α, γ, δ chain deficient T cell lines. *EMBO (Eur. Mol. Biol. Organ.) J.* 11:2735–2745.
19. Shinkai, Y., and F.W. Alt. 1994. CD3 epsilon-mediated signals rescue the development of $CD4^{+}CD8^{+}$ thymocytes in $RAG\text{-}2^{-/-}$ mice in the absence of TCR β chain expression. *Int. Immunol.* 6:995–1001.
20. Wiest, D.L., K.P. Kearse, E.W. Shores, and A. Singer. 1994. Developmentally regulated expression of CD3 components independent of clonotypic T cell antigen receptor complexes on immature thymocytes. *J. Exp. Med.* 180:1375–1382.
21. Berger, M.A., V. Dave, M.R. Rhodes, G.C. Bosma, M.J. Bosma, D.J. Kappes, and D.L. Wiest. 1997. Subunit composition of pre-T cell receptor complexes expressed by primary thymocytes: CD3δ is physically associated but not functionally required. *J. Exp. Med.* 186:1461–1467.
22. Borst, J., H. Jacobs, and G. Brouns. 1996. Composition and function of T-cell receptor and B-cell receptor complexes on precursor lymphocytes. *Curr. Opin. Immunol.* 8:181–190.
23. Dave, V.P., Z. Cao, C. Browne, B. Alarcon, G. Fernandez-Miguel, J. Lafaille, A. de la Hera, S. Tonegawa, and D.J. Kappes. 1997. CD3 δ deficiency arrests development of the α/β but not the γ/δ T cell lineage. *EMBO (Eur. Mol. Biol. Organ.) J.* 16:1360–1370.
24. Haks, M.C., P. Krimpenfort, J. Borst, and A.M. Kruisbeek. 1998. The CD3gamma chain is essential for development of both the TCR alpha beta and TCR gamma delta lineages. *EMBO (Eur. Mol. Biol. Organ.) J.* 17:1871–1882.
25. Saito, H., T. Koyama, K. Georgopoulos, H. Clevers, W.G. Haser, T. LeBien, S. Tonegawa, and C. Terhorst. 1987. Close linkage of the mouse and human CD3 gamma- and delta-chain genes suggests that their transcription is controlled by common regulatory elements. *Proc. Natl. Acad. Sci. USA.* 84:9131–9134.
26. Liu, C.P., R. Ueda, J. She, J. Sancho, B. Wang, G. Weddell, J. Loring, C. Kurahara, E.C. Dudley, A. Hayday, et al. 1993. Abnormal T cell development in $CD3\text{-}\zeta^{-/-}$ mutant mice and identification of a novel T cell population in the intestine. *EMBO (Eur. Mol. Biol. Organ.) J.* 12:4863–4875.
27. She, J., S.J. Simpson, A. Gupta, G. Hollander, C. Levelt, C.P. Liu, D. Allen, N. van Houten, B. Wang, and C. Terhorst. 1997. CD16-expressing CD8αα+ T lymphocytes in the intestinal epithelium: possible precursors of Fc gammaR-CD8αα+ T cells. *J. Immunol.* 158:4678–4687.
28. Wang, B., C.N. Levelt, M. Salio, D.-X. Zheng, J. Sancho, C.-P. Liu, J. She, M. Huang, K. Higgins, M.-J. Sunshine, et al. 1995. Over-expression of CD3ε transgenes blocks T lymphocyte development. *Int. Immunol.* 7:435–448.
29. Levelt, C.N., B. Wang, A. Ehrfeld, C. Terhorst, and K. Eichmann. 1995. Regulation of T cell receptor (TCR)-beta locus allelic exclusion and initiation of TCR-α locus rearrangement in immature thymocytes by signaling through the CD3 complex. *Eur. J. Immunol.* 25:1257–1261.
30. Malissen, M., A. Gillet, L. Ardouin, G. Bouvier, J. Trucy, P. Ferrier, E. Vivier, and B. Malissen. 1995. Altered T cell development in mice with a targeted mutation of the CD3-ε gene. *EMBO (Eur. Mol. Biol. Organ.) J.* 14:4641–4653.
31. Olson, E.N., H.H. Arnold, P.W. Rigby, and B.J. Wold. 1996. Know your neighbors: three phenotypes in null mutants of the myogenic bHLH gene MRF4. *Cell.* 85:1–4.
32. Mombaerts, P., J. Iacomini, R.S. Johnson, K. Herrup, S. Tonegawa, and V.E. Papaioannou. 1992. Rag-1-deficient mice have no mature B and T lymphocytes. *Cell.* 68:869–877.
33. Shinkai, Y., G. Rathbun, K.-P. Lam, E.M. Oltz, V. Stewart, M. Mendelsohn, J. Charron, M. Datta, F. Young, A.M. Stall, and F.W. Alt. 1992. Rag-2-deficient mice lack mature lymphocytes owing to inability to initiate V(D)J rearrangement. *Cell.* 68:855–867.
34. Cambier, J.C. 1995. Antigen and Fc receptor signaling. The awesome power of the immunoreceptor tyrosine-based activation motif (ITAM). *J. Immunol.* 155:3281–3285.
35. Shores, E.W., K. Huang, T. Tran, E. Lee, A. Grinberg, and P.E. Love. 1994. Role of TCR ζ chain in T cell development and selection. *Science.* 266:1047–1050.
36. Levelt, C.N., P. Mombaerts, B. Wang, H. Kohler, S. Tonegawa, K. Eichmann, and C. Terhorst. 1995. Regulation of thymocyte development through CD3: functional dissociation between p56lck and CD3 ζ in early thymic selection. *Immunity.* 3:215–222.
37. Levelt, C.N., P. Mombaerts, A. Iglesias, S. Tonegawa, and K. Eichmann. 1993. Restoration of early thymocyte differentiation in T-cell receptor β-chain-deficient mutant mice by transmembrane signaling through CD3 epsilon. *Proc. Natl. Acad. Sci. USA.* 90:11401–11405.
38. Bonifacino, J.S., C.K. Suzuki, J. Lippincott-Schwartz, A.M. Weissman, and R.D. Klausner. 1989. Pre-Golgi degradation of newly synthesized T cell antigen receptor chains: intrinsic sensitivity and the role of subunit assembly. *J. Cell Biol.* 109: 73–83.
39. Shinkai, Y., A. Ma, H.L. Cheng, and F.W. Alt. 1995. CD3 epsilon and CD3 ζ cytoplasmic domains can independently generate signals for T cell development and function. *Immunity.* 2:401–411.