The Common Cytokine Receptor γ Chain Controls Survival of γ/δ T Cells

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Summary

We have investigated the role of common γ chain (γ_c)-signaling pathways for the development of T cell receptor for antigen (TCR)-γ/δ T cells. TCR-γ/δ–bearing cells were absent from the adult thymus, spleen, and skin of γ_c-deficient (γ_c^-) mice, whereas small numbers of thymocytes expressing low levels of TCR-γ/δ were detected during fetal life. Recent reports have suggested that signaling via interleukin (IL)-7 plays a major role in facilitating TCR-γ/δ development through induction of V-J (variable-joining) rearrangements at the TCR-γ locus. In contrast, we detected clearly TCR-γ rearrangements in fetal thymi from γ_c^- mice (which fail to signal in response to IL-7) and reduced TCR-γ rearrangements in adult γ_c thymi. No gross defects in TCR-δ or TCR-β rearrangements were observed in γ_c^- mice of any age. Introduction of productively rearranged TCR Vγ1 or TCR Vγ1/Vδ6 transgenes onto mice bearing the γ_c mutation did not restore TCR-γ/δ development to normal levels suggesting that γ_c-dependent pathways provide additional signals to developing γ/δ T cells other than for the recombination process. Bcl-2 levels in transgenic thymocytes from γ_c^- mice were dramatically reduced compared to γ_c^+ transgenic littermates. We favor the concept that γ_c-dependent receptors are required for the maintenance of TCR-γ/δ cells and contribute to the completion of TCR-γ rearrangements primarily by promoting survival of cells committed to the TCR-γ/δ lineage.

T cells can be divided into two populations based on the structure of their TCRs. Most T cells express TCR heterodimers consisting of α and β chains, whereas a smaller population expresses an alternative TCR made of γ and δ chains. These two T cell populations share a number of features, including rearranging antigen receptor chains, the products of which associate with a set of invariant CD3 polypeptides responsible for signaling to the cell that the TCR heterodimer has been engaged (for review see reference 1). In contrast, γ/δ T cells differ from α/β T cells in their ontogeny, variable (V)[1] gene repertoires, and ultimate anatomical locations (for reviews see references 2 and 3). γ/δ T cells are the first TCR-expressing cells detected in the early fetal thymus, and persist in the adult thymus in small numbers. The first two waves of γ/δ T cells to appear during a fetal development express Vγ5-joining γ1 (Jγ1) and Vγ6-Jγ1 genes, both of which pair with δ chains composed of Vδ1-Dδ1-Jδ2 segment combinations. The most striking feature of these TCR-γ/δ genes is their lack of junctional diversity (2, 3). The Vγ5-expressing cells migrate to the skin to become the Thy1+ dendritic epidermal T cell (DETC) population, whereas Vγ6-expressing cells migrate to mucosal surfaces lining the reproductive tract and tongue (4). The production of Vγ5/Vδ1 and Vγ6/Vδ1 cells slows at the end of fetal life and another wave of TCR-γ/δ cells develops from this time onwards, which uses mainly Vγ4 and Vγ1 gene segments paired with a variety of Vδ genes and which displays extensive junctional diversity. In the adult, these γ/δ T cells constitute ~0.2% of the thymus and following export, seed the spleen and lymph nodes. The mechanisms that control the sequential appearance of γ/δ T cell subsets with distinct V gene segment usage, V(D)J junctional diversity, and unique homing properties are unknown (5, 6). Recent evidence suggests that the observed TCR-γ and TCR-δ gene rearrangements

[1]*Abbreviations used in this paper:* γc, common γ chain; DETC, dendritic epidermal T cell; DN, double negative; HSA, heat-stable antigen; J, joining; RAG, recombinase-activating gene; Tg, transgenic; TSLP, thymic stromal cell–derived lymphopoeitin; V, variable; wt, wild type.

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are temporally programmed and do not rely on selection of a particular subset of receptors among a diverse TCR-γ/δ combinatorial repertoire (7–9).

Deciphering the role played by cell–cell interactions and soluble cytokines provided by the thymic microenvironment constitutes a central question in the development of fetal versus adult γ/δ T cells. A variety of interleukins have been demonstrated to affect the growth and differentiation of TCR-γ/δ cells. For instance, freshly isolated γ/δ T cells from fetal thymus, skin, spleen, or peritoneal cavity can proliferate in vitro to IL-2, IL-7, or IL-15 (10–13), and in utero administration of antibodies to the IL-2Rβ chain block development of DETC (14). Gene ablation experiments in vivo have confirmed some of these findings. Mice deficient in IL-2Rβ (shared by IL-2 and IL-15), IL-7/IL-7Rα, or the common γ chain (γ_c; shared by IL-2, IL-4, IL-7, IL-9, and IL-15) each have defects in γ/δ T cell development (15–20). Still, the mechanism by which cytokine depletion affects the differentiation program of these cells is not completely understood. Cytokines could play a role in survival or proliferation of developing thymocytes, or alternatively might directly influence the TCR rearrangement process. Along these lines, experiments using mouse fetal liver cultures supplemented with IL-7 suggested that this cytokine could specifically induce the rearrangement of TCR-γ chain genes (21), and recently IL-7Rα–deficient mice were shown to have a selective block in TCR-γ gene recombination (22). In this report, we have analyzed mice deficient in γ_c to clarify the role of the γ_c in the development of fetal and adult γ/δ T cells. Our results demonstrate that γ_c-containing receptor complexes play a role for TCR-γ chain rearrangements in the adult, but not the fetal thymus, and more importantly, signaling through the γ_c provides essential survival signals for γ/δ T cells.

Materials and Methods

Mice. Mice harboring a null mutation of the γ_c have been described (19), γ_c-deficient mice, IL-7–deficient mice (kindly provided by R. Murray, DNAX Corp, Palo Alto, CA; reference 23), recombinase-activating gene 1–deficient mice ($RAG1^{-/-}$; ref. 24), and their littermate controls were maintained in specific pathogen-free conditions and used between 4 and 8 wk of age. Fetal tissues were obtained from timed-pregnant mice. Day 0 of embryonic development was considered to be the day a vaginal plug was observed.

Mice transgenic (Tg) for TCR Vγ1 and double Tg for TCR Vγ1 and TCR Vδ6 were constructed as follows. Genomic DNA fragments containing the rearranged TCR Vγ1 and Vδ6 genes were isolated from a cosmid library prepared in pWE15 using partially digested Sau 3A I DNA from the T3.13.1 TCR-γ/δ hybridoma (25). The Vγ1-Jγ4 clone (45 kb) contained 10 kb of upstream sequence and extended 26 kb downstream of the Cγ4 exon. The Vδ6-D-Jδ1 clone (34 kb) contained 5 kb of upstream DNA and 14 kb downstream of the Cδ exon. Tg constructs were mixed and the DNA microinjected into the pronuclei of fertilized embryos. Mice carrying the Tg TCRs were identified by PCR and backcrossed onto the C57Bl/6 background. Mice were screened using tail DNA and primers specific for the Tg Vγ1 TCR: forward, 5′-CCGGCAAAAAGCAAAAAAGTT-3′; and reverse, 5′-CCCATGATGTGCCTGACCAG-3′. PCR conditions were as follows: denaturation at 94°C for 20 s, annealing at 59°C for 25 s, and extension at 74°C for 25 s for 33 cycles.

Antibodies. The following Abs were used as conjugates to either FITC, PE, or biotin: anti–TCR-β (H57-597), anti–TCR-γ/δ (GL3), anti-TCR Vγ5 (536), anti-CD3 (2C11), anti-CD4, anti-CD8α, anti-CD24 (J11d), anti–Mac-1 (M1/70), and anti-CD32 (2.4G2). Streptavidin Tricolor (CALTAG Labs., South San Francisco, CA) was used to detect biotinylated antibodies. The antibody specific for TCR Vγ1 (2.11) has been described (25). A clonotypic antibody recognizing Vγ1/Vδ6 TCR heterodimer (1.9) was obtained in the same fusion and its specificity verified using a panel of γ/δ T cell hybridomas as described (25).

FACS® Analysis and Cell Sorting. Single cell suspensions obtained from thymus or spleen were lysed of red cells using hypotonic NH_4Cl solution. DETCs were isolated from ear skin using trypsinization and mechanical disaggregation as described (26). Nonspecific binding of mAbs to FcRs was reduced by preincubation with anti-CD32 mAb for 15 min. For surface staining, cells were incubated with saturating amounts of directly conjugated mAbs for 20 min, washed twice, and incubated with streptavidin Tricolor. For Bcl-2 staining, surface antigens were stained as above, and cells were washed in PBS and fixed in PBS containing 1% paraformaldehyde/0.01% Tween-20 for 90 min on ice. Cells were subsequently incubated with hamster anti–mouse Bcl-2 (clone 3F11; PharMingen, San Diego, CA) or purified hamster Ig. Cells were washed, incubated with biotinylated goat anti–hamster Ig, and finally with streptavidin Tricolor. Cells were analyzed on a FACScan® flow cytometer using CellQuest software (Becton Dickinson, Mountain View, CA). For isolation of early thymocyte precursors, cells were stained with CD4-FITC and CD8-PE, and double negative (DN) cells sorted using a FACStar Plus® cell sorter (Becton Dickinson).

PCR Analysis. DNA samples were extracted from total or fractionated populations of fetal or adult thymocytes using the salting-out technique (27). PCR reactions were done in a final volume of 20 μl and included a maximum of 100 ng of template DNA, 1 mM of each primer, 200 μM of each deoxynucleotide triphosphate, and 0.2 U of Taq DNA polymerase. Whole reaction mixtures were run on a 1.5% agarose gel, blotted to nylon membrane (Gene Screen Plus, New England Nuclear, Boston, MA), and hybridized with ^{32}P-labeled oligonucleotide probes.

The oligonucleotides and PCR conditions used for the analysis of TCR-β rearrangements were as described (28). For the TCR-γ and TCR-δ rearrangements, PCR were performed essentially as described (9) except that each cycle was shortened as follows: incubation at 94°C for 30 s, annealing at 50–60°C for 30 s, and extension at 72°C for 40 s. At least two sets of independent experiments were performed for each sample. To show that there is a linear relationship between product yield and the number of input target sequences, serial dilutions have been analyzed for each DNA sample. Hybridizing bands were quantitated using a phosphorimager (BAS1000; Raytest, Courbevoie, France). Before the analysis of the relative levels of TCR gene rearrangements, the quality and quantity of DNA present in each sample were checked by amplifying the nonrearranging trithorax gene (MTrx; reference 29) using primers MTrx1: 5′-AGGGTAAGCTGTGCTATGG-3′ and MTrx2: 5′-AGTAGTGTTTCCTCAGTCCCC-3′.

Results and Discussion

Absence of TCR-γ/δ Cells in γ_c-deficient Mice. In vitro data have suggested an important role for IL-2, IL-7, and IL-15

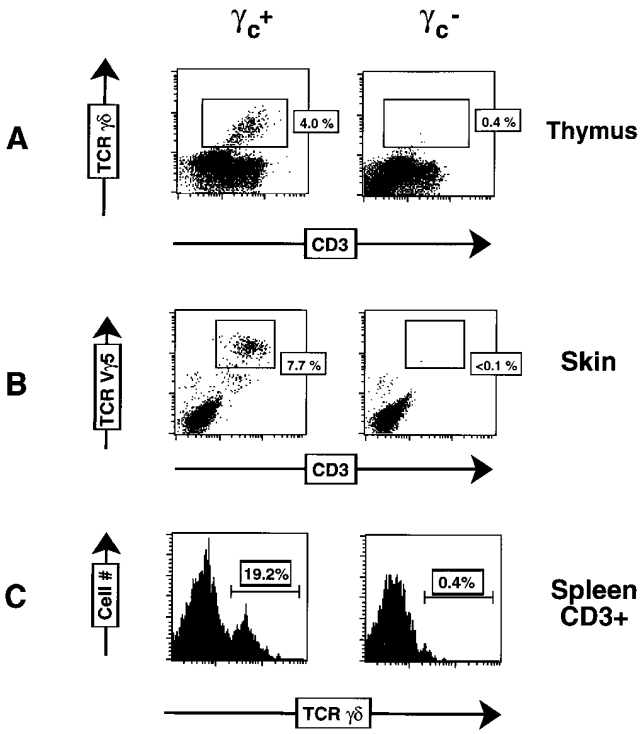

Figure 1. Flow cytometric analysis of γ/δ T cells from adult γ_c^+ and γ_c^- mice. (*A*) Thymocytes were stained with FITC–anti–pan-TCR-γ/δ (GL3), PE–anti-CD3, biotin-anti–CD4, and biotin–anti-CD8α. $CD4^-$ $CD8^-$ (DN) cells were electronically gated. (*B*) Skin-resident DETCs were isolated as indicated in Materials and Methods, and stained with biotin–anti-TCR Vγ5 and FITC–anti-CD3. (*C*) Splenocytes were monocyte depleted by adherence and stained with FITC–anti–pan-TCR-γ/δ, PE–anti-CD3, biotin–anti-CD4, and biotin–anti-CD8α. $CD3^+$ DN cells were electronically gated. Biotinylated antibodies were revealed with streptavidin-Tricolor.

in the survival, proliferation, and differentiation of cells belonging to the γ/δ T cell lineage (10–13). Because the γ_c receptor plays an integral role in the function of these cytokine receptors, we anticipated that γ_c^- mice would exhibit a defect in γ/δ T cell development. As most adult γ/δ T cells fail to express the CD4 and CD8 coreceptors and are found in the DN subset of T cells, we examined wild-type (wt) and γ_c^- DN thymocytes for the presence of cells bearing TCR-γ/δ receptors. Compared to control mice, adult thymi from γ_c^- mice showed a complete absence of TCR-γ/δ^+ cells (Fig. 1). Further analysis of the peripheral lymphoid organs, skin, and small intestine of adult γ_c-deficient mice failed to demonstrate any TCR-γ/δ^+ cells in the animal (Fig. 1 and data not shown). These results confirm and extend previous observations demonstrating the strict dependence on γ_c-containing receptors for the development of all types of γ/δ T cells present in adult mice (15–20).

Considering that γ/δ T cells constitute a minor cell population in the adult thymus, we next analyzed fetal thymi from γ_c^- mice, since they contain a higher frequency of γ/δ T cells (9). Total thymocyte cell numbers were clearly reduced in γ_c^- mice relative to control mice at all stages of

Table 1. *Cellularity of Fetal and Neonatal Thymi from γ_c^- Mice*

	Total cell number ($\times 10^5$)	
Age	γ_c^+	γ_c^-
FD15.5	2.46 ± 1.5 (6)*	0.3 ± 0.2 (6)
FD17.5	33.6 ± 9.1 (9)	3.8 ± 2.1 (10)
FD18.5	56.1 ± 20.3 (7)	7.0 ± 3.3 (3)
Neonate D1	96.8 ± 30 (6)	9.2 ± 2.7 (6)
Neonate D6	500 ± 15 (3)	22.6 ± 9.6 (3)

*Number of mice analyzed. Numbers are mean ± SEM.

fetal development examined (Table 1) although CD4 and CD8 expression was unaltered (data not shown). An ~10-fold reduction in cell number is apparent in γ_c^- fetal thymi, yet with age, thymocyte cell numbers increase in parallel with γ_c^+ mice. This suggests that alternative γ_c-independent signaling pathways (including that of the receptor tyrosine kinase c-kit; reference 30) support continuous thymic seeding and permit progressive thymocyte accumulation. Analysis of fetal (days 16–18) γ_c-deficient thymocytes revealed a reduced percentage of cells expressing TCR-γ/δ heterodimers (Fig. 2 and data not shown) and suggested that γ/δ T cells might have some capacity to develop in that context, but might subsequently be lost, perhaps due to poor survival or failure to mature. Consistent with this hypothesis, the few fetal TCR-γ/δ thymocytes found in γ_c^- mice have an immature phenotype characterized by low levels of TCR-γ/δ heterodimers and high levels of

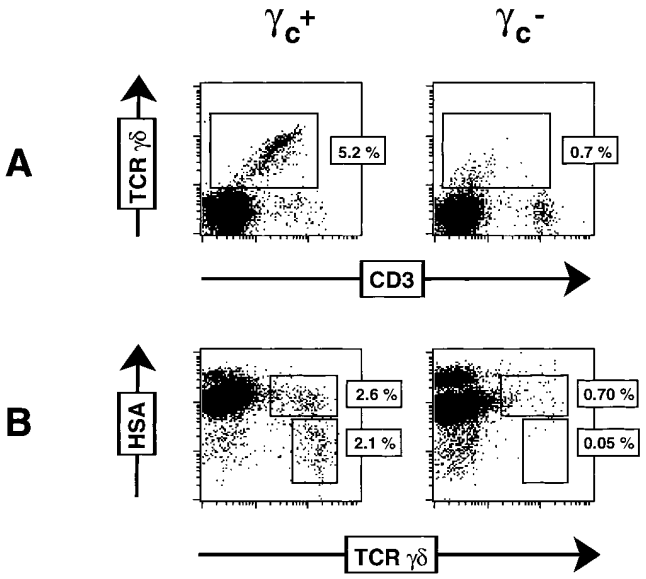

Figure 2. Flow cytometric analysis of fetal thymocytes from γ_c^+ and γ_c^- mice. (*A*) Thymocytes were stained with FITC–anti–pan-TCR-γ/δ and PE–anti-CD3. (*B*) Thymocytes were stained with FITC–anti-pan-TCR-γ/δ and PE–anti-HSA. In all experiments, nonspecific binding was blocked by the addition of anti-CD16/32.

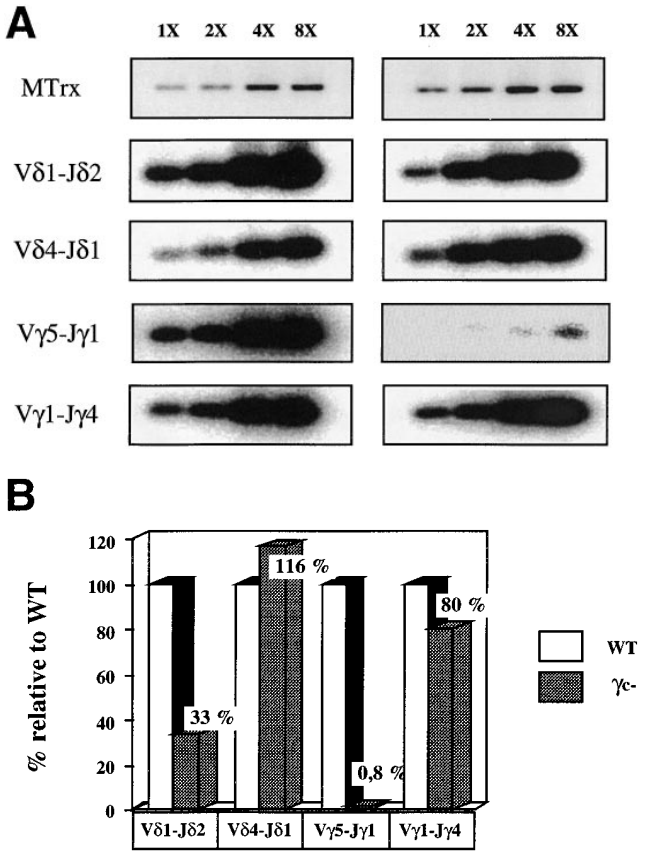

Figure 3. Quantitation of TCR-δ and TCR-γ rearrangements in fetal γ_c^+ and γ_c^- thymocytes. (*A*) Indicated rearrangements were amplified from DNA isolated from unfractionated D17 fetal γ_c^+ or γ_c^- thymi. Serial dilutions of DNA template were analyzed (*1*×, *2*×, *4*×, and *8*×). (*B*) Quantification of *A*. Hybridizing bands were scanned using a phosphorimager and the relative levels of rearrangements compared to wild-type (*WT*) γ_c^+ thymi after normalizing for input DNA using MTrx primers. See Materials and Methods for details.

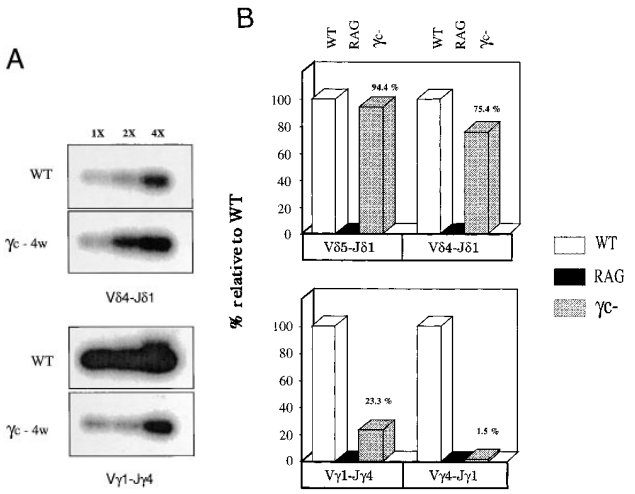

Figure 4. Quantitation of TCR-δ and TCR-γ rearrangements in adult γ_c^+ and γ_c^- thymocytes. (*A*) Rearrangements of Vδ4-Jδ1 and Vγ1-Jγ4 were amplified from DNA isolated from unfractionated γ_c^+ or γ_c^- thymi. Serial dilutions of DNA template were analyzed (*1*×, *2*×, and *4*×). (*B*) Quantification of *A*. Relative levels of rearrangements are compared to wild-type (*WT*) γ_c^+ thymi.

heat-stable antigen (HSA^{hi}; Fig. 2). Similar observations have been made in IL-7–deficient mice (16), pointing to IL-7 as the γ_c-dependent cytokine responsible for the defect observed in γ_c^- thymi.

Taken together, these observations confirm the major role played by the IL-7/γ_c–signaling pathway in γ/δ T cell development (16–20), but also suggest that factors independent of γ_c and IL-7 are present in the fetal, but not the adult, thymus and can support the appearance of the most immature γ/δ T cells. Recently, a novel cytokine, thymic stromal cell–derived lymphopoeitin (TSLP; reference 31), has been identified which shares biological activities with IL-7 and uses the IL-7Rα chain for signaling (32). We would hypothesize that TSLP could partially replace IL-7 during fetal development in γ_c^- mice. Two additional observations support this view. First, thymocytes from γ_c^- mice respond to TSLP (33) and second, mice with a deletion of the IL-7Rα chain have neither fetal nor adult TCR-γ/δ cells detectable by FACS® analysis (17). Therefore, the spectrum of cytokine receptors expressed by fetal and adult immature thymocytes may be identical, but fetal versus adult thymic stroma may differ in their abilities to produce cytokines, like TSLP. Along this line, it will be interesting to determine the relative expression of TSLP in fetal and adult thymi.

TCR Rearrangements in γ_c^- Thymocytes. We considered a number of nonexclusive hypotheses to explain the pronounced negative effect of γ_c deficiency on γ/δ T cell development: (*a*) γ_c may be required for committment to the TCR-γ/δ lineage (defect in α/β–γ/δ lineage branching), (*b*) γ_c may be required for initiation or completion of the site-specific DNA recombination process affecting the TCR-γ and/or TCR-δ loci (defect in TCR rearrangements), or (*c*) signals via γ_c may be essential for the survival of cells during the process of γ/δ T cell differentiation.

To explore these different possibilities, we analyzed the status of TCR gene rearrangements in fetal and adult thymocytes from wt and γ_c-deficient mice using a polymerase chain reaction technique that can specifically assess the presence of rearranged DNA from the various TCR gene loci (9, 28). The results shown in Fig. 3 were generated using fetal (day 17) thymocytes and PCR primer pairs specific for the Vγ5-Jγ1, Vγ1-Jγ4, Vδ1-Jδ2, and Vδ4-Jδ1 rearrangements. Although Vδ4-Jδ1 and Vγ1-Jγ4 rearrangements were nearly unaffected in γ_c^- fetal thymi, the canonical Vδ1-Jδ2 and Vγ5-Jγ1 rearrangements found in thymocytes destined to seed the skin epithelium (4) were clearly reduced (Fig. 3). As expected from the fact that the γ_c mutation does not block the development of α/β T cells (19, 20), DNA samples from γ_c thymi contained Dβ-Jβ and Vβ-Dβ-Jβ rearrangements that were as diverse as those found in wt samples (data not shown).

Similar studies were performed on adult γ_c^- thymi. As shown in Fig. 4, the Vγ1-Jγ4 rearrangements detected in 4-wk-old γ_c^- thymi represent 23% of those observed in wt

Table 2. *TCR-γ and TCR-δ Rearrangements in γ_c^- and IL-7$^{-/-}$ Thymi*

DNA source	Vδ4-Jδ1	Vγ4-Jγ1
γ_c^+	100.00*	100.00
RAG 1$^{-/-}$	2.1	0.4
γ_c^-	108.4	2.66
IL-7$^{-/-}$	114.5	3.79

*Rearrangements were quantitated and expressed as percentage of rearrangements found in DNA isolated from γ_c^+ total thymocyte preparations from 4-wk-old mice (see Materials and Methods). Results are representative of two independent experiments.

Table 3. *TCR-δ Rearrangements from γ_c^- Thymocyte Precursors*

DNA source	Vδ4-Jδ1	Vδ5-Jδ1
γ_c^+ total thymus	100.00*	100.00
RAG 1$^{-/-}$ thymus	1.61	1.56
γ_c^- total thymus	94.36	75.40
γ_c^- DN sort‡	111.92	55.58
CD3ε $^{\Delta 5/\Delta 5}$ thymus	73.24	101.43

*Indicated TCR-δ rearrangements were quantitated and expressed as percentage of rearrangements found in DNA isolated from γ_c^+ total thymocyte preparations (see Materials and Methods). Results are representative of two independent experiments.
‡Double negative thymocytes (CD4$^-$CD8$^-$) were sorted from γ_c^- thymi as described in Materials and Methods, and DNA was isolated for analysis.

thymi, respectively. Only limited Vγ4-Jγ1 rearrangements are observed in adult γ_c^- thymi, representing ~1% of the levels observed in adult wt thymi. In contrast, the extent of TCR-δ rearrangements found in adult thymi from γ_c-mutant mice resembled those present in wt adult thymi (Fig. 4 *B*, *top*). Thus, these results suggest that the γ_c mutation has little effect on adult TCR-δ rearrangements, but appears to selectively reduce adult TCR-γ rearrangements. Parallel studies using IL-7–deficient mice demonstrated a similar defect in TCR-γ rearrangements (Table 2).

Most developing α/β T cells contain TCR-δ gene segments that have rearranged during the DN stages of development (34, 35). As a result, TCR-α/β$^+$ thymocytes retain TCR-δ locus sequences (36–38), which could account for the split phenotype observed for the TCR-γ and TCR-δ loci in unfractionated adult γ_c^- thymocytes (Fig. 4). We therefore examined TCR-δ rearrangements in CD4$^-$CD8$^-$ precursors from γ_c mutant mice. As summarized in Table 3, the extent of TCR-δ rearrangement present in γ_c^- DN thymocytes was found to be similar to that observed in total γ_c^- thymocytes and in CD3ε$^{\Delta 5/\Delta 5}$ thymocytes, which are an enriched source of γ_c^+ DN cells with normal levels of TCR-δ gene rearrangements (34). Therefore, these findings demonstrate that TCR-δ genes do rearrange in the CD4$^-$CD8$^-$ precursors isolated from adult γ_c^- mice. TCR-γ rearrangements in γ_c^- DN thymocytes were also reduced (data not shown).

In conclusion, our analysis of TCR-γ and TCR-δ rearrangements in fetal and adult γ_c^- thymi distinguishes TCR-γ/δ cell development during these two stages. Although noncanonical TCR-γ and TCR-δ rearrangements were both present during the fetal period, TCR-γ, but not TCR-δ, rearrangements were severely reduced in the adult γ_c^- thymus. The implications of these observations are the following. First, during fetal life, the absence of γ_c signaling pathways does not impair the ability to rearrange the TCR-γ or TCR-δ loci. This suggests that γ_c-independent factors may compensate for the lack of IL-7/γ_c–mediated signals. Second, the fact that fetal and adult thymi from γ_c^- mice do contain TCR-γ and TCR-δ rearrangements, but fail to give rise to mature γ/δ T cells strongly suggests that other defects (e.g., survival of already committed or successfully rearranged γ/δ T cells) likely accounts for the defective γ/δ T cell development observed in γ_c thymi.

Lack of TCR-γ/δ Cell Survival in γ_c-deficient Mice Tg for Rearranged TCR Vγ1 or TCR Vγ1/Vδ6. To further address potential defects in α/β–γ/δ lineage branching and/or γ/δ T cell survival, we crossed γ_c-deficient mice with mice Tg for a functionally rearranged TCR Vγ1 gene or with double Tg mice harboring the same TCR Vγ1 and a productively rearranged TCR Vδ6 gene. The T3.13.3 hybridoma from which these rearranged TCR chains were isolated corresponds to the subset of adult TCR-γ/δ cells and the TCR Vγ1/Vδ6 heterodimer demonstrates extensive junctional diversity (25). In addition, the Vγ1 Tg construct contains the necessary flanking DNA sequences to ensure proper expression in TCR-γ/δ precursors, as well as the silencer element required to prevent its adventitious expression in TCR-α/β lineage cells (39). Founder mice expressing the Vγ1 or Vγ1/Vδ6 constructs were identified and crossed onto the γ_c-deficient background.

Expression of the Vγ1 Tg alone in mice with or without the γ_c mutation did not alter absolute thymocyte cell numbers or the expression of mature CD4 or CD8 single positive thymocytes (Table 4; Fig. 5). Total thymocyte preparations from nontransgenic littermates contained only a very small percentage of cells marked with the anti-TCR Vγ1 antibody, whereas γ_c^+ Vγ1 Tg animals demonstrate an increase in both the frequency (Fig. 5) and absolute numbers of Vγ1 γ/δ T cells (Table 4). Importantly, the Vγ1$^+$ cells were negative for TCR-β chains, demonstrating that the Tg was correctly expressed in TCR-γ/δ cells and that the flanking silencer element was operative in the α/β T cells found in these Tg mice (Fig. 5). γ_c^- TCR-γ Tg animals showed a population of Vγ1$^+$ cells in total thymus preparations; these Tg$^+$ thymocytes were more clearly demonstrated in the DN compartment (Fig. 5). Despite expression of the Tg Vγ1 chain, γ/δ T cells in γ_c^- mice were still severely reduced. Compared with γ_c^+ Tg animals, there was a 120-fold reduction in absolute numbers of Vγ1$^+$ cells (Table 4). Considering that the γ_c chain plays a role in the

Table 4. *Transgenic γ/δ T Cell Development in γ_c^- Mice*

Mouse	*n**	Total thymocyte cell No. ($\times 10^6$)	Thymic TCR-γ/δ cell No. ($\times 10^5$)	Splenic lymphoid cell No. ($\times 10^6$)	Splenic TCR-γ/δ cell No. ($\times 10^5$)
γ_c^+ Non-Tg	>10	247 ± 34	2.5± 0.3 (0.1%‡)	66.5 ± 10.9	3.3 ± 0.6 (0.6%‡)
γ_c^- Non-Tg	>10	11.7 ± 6.6	ND	8.2 ± 3.2	<0.05 (<0.05%)
γ_c^+ TCR Vγ1 Tg	5	198 ± 23.1	29 ± 3.4 (1.4%)	45.2 ± 4.6	20 ± 6.5 (4.0%)
γ_c^- TCR Vγ1 Tg	7	8.22 ± 4.6	0.24 ± 0.1 (0.3%)	4.7 ± 1.1	0.14 ± 0.07 (0.4%)

*Number of mice analyzed.
‡Percentage of cells positive for TCR Vγ1 determined by FACS® analysis.
ND: see Fig. 1.

generation of the earliest noncommitted thymic precursors ($CD44^+CD25^-$ cells; reference 40) which are 15-fold reduced in γ_c^- mice (data not shown), part of the dramatic reduction in $V\gamma1^+$ thymocytes in γ_c^- Tg mice stems from the limited number of thymocyte precursors available to express the Tg Vγ1 receptor. Taking this into account, γ/δ T cells are still eightfold reduced (120-fold/15-fold) in γ_c^- Tg mice relative to γ_c^+ Tg controls, suggesting that additional mechanisms are responsible for the defective γ/δ T cell development. Similar results were obtained using mice expressing the same TCR Vγ1 chain and a rearranged TCR Vδ6 (Fig. 6 and data not shown). We conclude that a rearranged TCR-γ/δ Tg does not restore normal γ/δ T cell development in the absence of γ_c. Moreover, since γ_c^- mice can express the TCR-γ/δ transgenes, a defect in TCR-α/β–γ/δ lineage branching can be effectively ruled out.

Considering that thymically derived γ/δ T cells seed the spleen and lymph nodes of postnatal mice, we further examined the peripheral lymphoid compartments for transgenic γ/δ T cells. As shown in Fig. 6, a large population of cells expressing the transgenic TCR-γ/δ receptor are present in the spleen of γ_c^+ Tg animals; these cells coexpress CD3, but are TCR-β negative (data not shown) and accumulate to levels which are approximately six-fold higher in absolute numbers than γ_c^+ nontransgenic animals (Table 4). In contrast, the periphery of γ_c^- Tgs contain only a few cells expressing the Tg TCR-γ/δ receptor, and these cells fail to accumulate in the spleen. In terms of absolute numbers, γ_c^- Tg γ/δ cells are reduced 142-fold compared with γ_c^+ Tg littermates (Table 4). Taken together, our results suggest a major role for γ_c-dependent signals in the survival of γ/δ T cells.

To investigate whether γ_c^- Tg γ/δ T cells had a defect in survival, we examined Tg thymocytes for the expression of the antiapoptotic factor, Bcl-2. Engagement of γ_c-dependent receptors has been shown to maintain high levels of Bcl-2, which appear to protect lymphoid cells from cell death (41–43). Although $V\gamma1^+$ thymocytes from γ_c^+ mice expressed Bcl-2, Tg^+ γ/δ T cells from γ_c^- mice were essentially negative for Bcl-2 (Fig. 7). These results are consistent with a defect in γ/δ T cell survival in the absence of γ_c, although the relative contribution of Bcl-2 in supporting γ/δ T cell development remains to be determined.

Concluding Remarks. Most γ/δ T cells start their development within the thymus where they rearrange their TCR-γ and TCR-δ genes via site-specific DNA recombination reactions triggered by the specialized stromal microenvironment found in the thymus. IL-2, IL-7, and IL-15 bind to specific receptors that share the γ_c and these cyto-

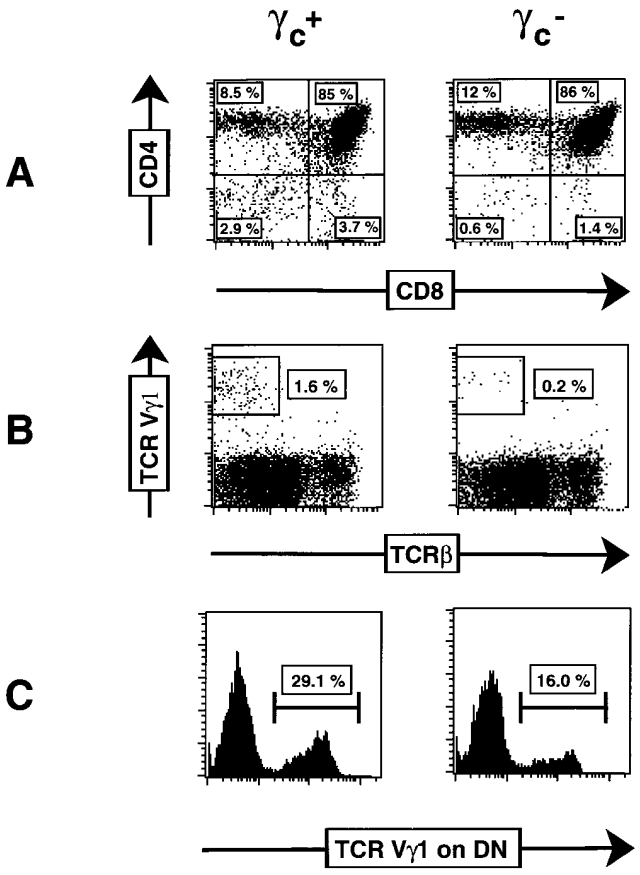

Figure 5. Flow cytometric analysis of thymocytes from γ_c^+ or γ_c^- mice transgenic for a rearranged TCR Vγ1 receptor. (*A*) Cells were stained with FITC–anti-CD8α and PE–anti-CD4. (*B*) Cells were stained with FITC–anti–TCR Vγ1 and biotin–anti–TCR-β. (*C*) Cells were stained with FITC–anti–TCR Vγ1, PE–anti-CD4, and PE–anti-CD8α. $CD4^-CD8^-$ (DN) cells were electronically gated.

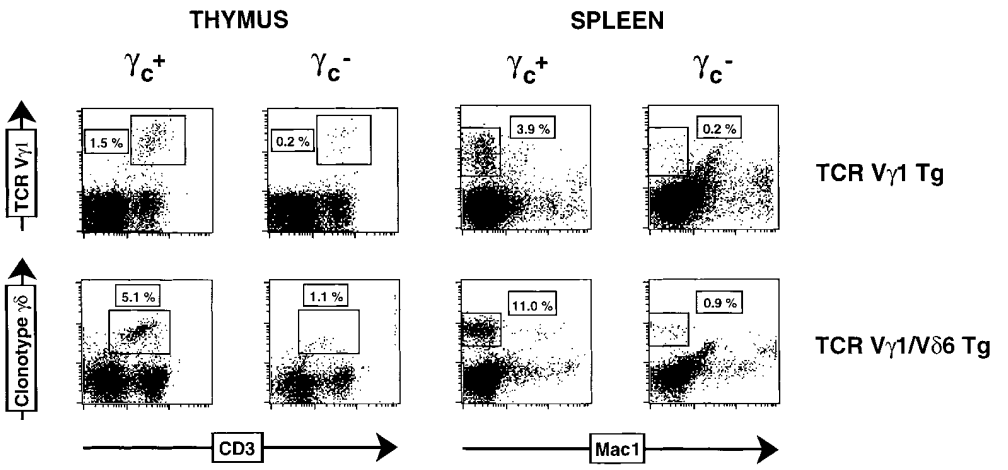

Figure 6. Flow cytometric analysis of thymocytes and splenocytes of γ_c^+ and γ_c^- mice transgenic for TCR Vγ1 or double transgenic for TCR Vγ1/Vδ6. Cells were stained with combinations of FITC–anti–TCR Vγ1, FITC–anti–TCRγ/δ clonotype, PE–anti-CD3, and PE–anti-Mac1.

kines have been postulated to play an important role in the survival, growth, and differentiation of γ/δ cells (10–13). In a recent study using mice deficient in IL-7Rα chains, TCR-γ gene rearrangements were found to be selectively abolished, and as a consequence, these mice lacked both fetal and adult TCR-γ/δ cells (22). The authors concluded that ligands binding to the IL-7Rα chain (IL-7 and TSLP) are likely to be mandatory for the process of TCR-γ rearrangements within intrathymic TCR-γ/δ cell precursors (22). Although we do not refute this conclusion, our data reveal an additional function of γ_c-containing receptors (likely due to IL-7), that is independent of the TCR-γ rearrangement process. Thymocytes from adult γ_c-deficient mice do not contain detectable TCR-γ/δ cells and showed only low levels of TCR-γ rearrangements, thereby limiting the potential synthesis of TCR-γ polypeptides. Complementation of such γ_c-deficient mice with TCR-γ and TCR-γ/δ transgenes only partially rescued thymic γ/δ T cell development and did not permit accumulation of peripheral γ/δ T cells. Therefore, the developmental blockade affecting the adult γ/δ T cell lineage in γ_c-deficient mice results not only from the limited amounts of rearranged TCR-γ genes but also from the fact that IL-7 promotes the survival of γ/δ T cell precursors containing TCR-γ and TCR-γ/δ polypeptides. The survival role played by IL-7 in γ/δ T cell development is supported by our observation that fetal thymocytes from γ_c-deficient mice do contain TCR-γ and TCR-δ rearrangements, but fail to generate appreciable numbers of γ/δ T cells intrathymically or to export them to the periphery. Further evidence is provided by the fact that γ_c^- γ/δ T cells contain dramatically reduced levels of the antiapoptotic factor Bcl-2. Previous reports have shown that engagement of γ_c-containing receptors maintains cellular Bcl-2 protein levels (41–43), thereby promoting lymphoid cell survival. Whether the near-absent Bcl-2 levels are directly responsible for the survival defect in γ_c^- γ/δ T cells or simply an epiphenomenon related to decreased cell survival remains to be determined.

Thus, we would like to propose that for γ/δ T cells, the primary function of γ_c-containing receptors is to promote survival. The ability of the γ_c^- fetal thymus to support the survival of γ/δ T cells (possibly via TSLP) would explain the presence of TCR-γ rearrangements in IL-7–deficient mice and γ_c-deficient mice, and their mere absence in IL-7Rα–deficient mice. After successful expression of a functional TCR-γ/δ complex, γ/δ T cells would still require signals via γ_c to survive, mature, and seed the periphery. This idea gains support from the analysis of IL-7–deficient mice, where $V\gamma5^{lo}HSA^{hi}$ fetal thymocytes are readily detectable, but fail to mature into $V\gamma5^{hi}HSA^{lo}$ cells (16). As a result, skin DETCs are not detectable in IL-7–deficient mice (data not shown).

Based on these results, one is led to ask why development of α/β T cells is less severely impaired in γ_c-deficient mice than that of γ/δ T cells. In the α/β lineage, it has been recently documented that TCR-β rearrangements are accompanied by a selective process allowing only those cells displaying a productively rearranged Vβ gene to reach the next stage of differentiation (β selection). At a later time point, a second phase of selection, denoted TCR-α/β selection, occurs to ensure MHC restriction and self tolerance. For γ/δ T cells, TCR-γ and TCR-δ rearrangements are probably achieved concurrently and not subjected to pre-TCR–based epigenetic control mechanisms operating during α/β T cell development (34). In this model of γ/δ T development, γ/δ T cell precursors might not receive any pre-TCR or TCR signals, and engagement of γ_c-containing cytokine receptors may constitute the only means to support the survival of these cells. In marked contrast, in

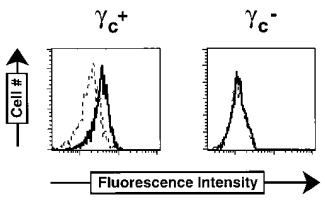

Figure 7. Intracellular levels of Bcl-2 are diminished in γ_c^- γ/δ T cells. Cells were surface stained with FITC–anti–TCR Vγ1, fixed, and permeabilized. Bcl-2 staining was performed as described in Materials and Methods. Dotted lines indicate staining with hamster Ig and solid lines with hamster anti–mouse Bcl-2–specific Ig.

α/β T cell precursors, the γ_c-dependent survival signals and the pre-TCR dependent survival signals may partially overlap, explaining how, in the absence of survival signals dependent on γ_c-containing receptors, signals emanating at a latter time point from the pre-TCR may rescue the development of a few α/β T cell precursors.

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