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) 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 fetal life, and skin of γ−/− 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−/− mice (which fail to signal in response to IL-7) and reduced TCR-γ rearrangements in adult γ−/− thymi. No gross defects in TCR-α or TCR-β rearrangements were observed in γ−/− mice of any age. Introduction of productively rearranged TCR Vγ1 or TCR Vγ1/Vδ1 transgenes onto mice bearing the γ− mutation did not restore TCR-γ/δ development to normal levels suggesting that γ−/δ-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−/− mice were dramatically reduced compared to γ+ transgenic littermates. We favor the concept that γ−/δ-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.

*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 lymphopoietin; V, variable; wt, wild type.
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, 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-2Rα chain block development of DETC (14). Gene ablation experiments in vivo have confirmed some of these findings. Mice deficient in IL-2R β (shared by IL-2 and IL-15), IL-7/IL-7Rα, or the common γ chain (γc; shared by 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 γc to clarify the role of the γc in the development of fetal and adult γ/δ T cells. Our results demonstrate that γc-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 γc provides essential survival signals for γ/δ T cells.

Materials and Methods

Mice. Mice harboring a null mutation of the γc have been described (19), γc-deficient mice, IL-7-deficient mice (kindly provided by R. Murray, DNA X 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 cosm id 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-DJδ1 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′-CCGGCAAAAAAGCAGAAAAGTT-3′; and reverse, 5′-CCCATGATGCTGCTGACAG-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 35 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-CD8a, anti-CD24 (11D), 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 Vγ1Vδ6 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 NH4Cl solution. DETCs were isolated from ear skin using trypsinization and mechanical disaggregation as described (26). Nonspecific binding of mAbs to Fcr 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 withhamster anti-mouse Bcl-2 (clone 3F11; Pharmingen, San Diego, CA) or purified hamster Ig. Cells were washed, incubated with biotinylated goat antihamster Ig, and finally with streptavidin Tricolor. Cells were analyzed on a FACSScan 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 32P-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′-AGTAGTGGTTTTCTCAGTCCC-3′.

Results and Discussion

A subset of TCR-γ/δ cells in γc-deficient mice. In vitro data have suggested an important role for IL-2, IL-7, and IL-15...
in the survival, proliferation, and differentiation of cells belonging to the γδ T cell lineage (10–13). Because the γc receptor plays an integral role in the function of these cytokine receptors, we anticipated that γc-deficient mice would exhibit a defect in γδ T cell development. As most adult γδ 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 γc-deficient DN thymocytes for the presence of cells bearing TCR-γδ receptors. Compared to control mice, adult thymus from γc-deficient mice showed a complete absence of TCR-γδ1 cells (Fig. 1). Further analysis of the peripheral lymphoid organs, skin, and small intestine of adult γc-deficient mice failed to demonstrate any TCR-γδ1 cells in the animal (Fig. 1 and data not shown). These results confirm and extend previous observations demonstrating the strict dependence on γc-containing receptors for the development of all types of γδ T cells present in adult mice (15–20).

Considering that γδ T cells constitute a minor cell population in the adult thymus, we next analyzed fetal thymus from γc-deficient mice. Total thymocyte cell numbers were clearly reduced in γc-deficient fetal thymus, yet with age, thymocyte cell numbers increase in parallel with γc+ mice. This suggests that alternative γc-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) γc-deficient thymocytes revealed a reduced percentage of cells expressing TCR-γδ heterodimers (Fig. 2 and data not shown) and suggested that γδ 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-γδ thymocytes found in γc-deficient mice have an immature phenotype characterized by low levels of TCR-γδ heterodimers and high levels of...
role played by the IL-7/

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has been identified which shares biological activities with
stromal cell–derived lymphopoietin (TSLP; reference 31), immature
adult, thymus and can support the appearance of the most
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dent of the IL-7R

mice respond to TSLP (33) and second, mice with a dele-
tion of the IL-7Rα chain for signaling (32). We

heat-stable antigen (HSA; Fig. 2). Similar observations
have been made in IL-7-deficient mice (16), pointing to
IL-7 as the γc-dependent cytokine responsible for the de-
fect observed in γc− thymi.

Taken together, these observations confirm the major
role played by the IL-7/γc-signaling pathway in γδ T cell
development (16–20), but also suggest that factors indepen-
dent of γc and IL-7 are present in the fetal, but not the
adult, thymus and can support the appearance of the most
immature γδ T cells. Recently, a novel cytokine, thymic
stromal cell–derived lymphopoietin (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 γc− mice. Two additional ob-
servations support this view. First, thymocytes from γc−
mice respond to TSLP (33) and second, mice with a dele-
tion of the IL-7Rα chain have neither fetal nor adult
TCR-γδ cells detectable by FACS® analysis (17). There-
fore, the spectrum of cytokine receptors expressed by fetal
and adult immature thymocytes may be identical, but fetal

Figure 3. Quantitation of TCR-δ and TCR-γ rearrangements in fetal
γc+ and γc− thymocytes. (A) Indicated rearrangements were amplified
from DNA isolated from unfractionated D17 fetal γc+ or γc− thymi. Se-
nal dilutions of DNA template were analyzed (1×, 2×, 4×, and 8×). (B) Q
uantification of A. Hybridizing bands were scanned using a phosphor-
imager and the relative levels of rearrangements compared to wild-type
(WT) γc+ thymi after normalizing for input DNA using MTtrx primers.
See Materials and Methods for details.

Quantification of TCR-δ and TCR-γ rearrangements detected in
4-wk-old γc− thymi. As shown in Fig. 4, the Vγ1-Jγ4 rearrangements detected in
wt thymi contained Dγb-Jγ1 and Vγb-Dβ-Jβ rearrangements that were as diverse as
those found in wt samples (data not shown).

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

Figure 4. Quantitation of TCR-δ and TCR-γ rearrangements in adult
γc+ and γc− thymocytes. (A) R earrangements of Vδ4-Jδ1 and Vγ1-Jγ4 were amplified from DNA isolated from unfractionated γc+ or γc− thymi. Se-
nal dilutions of DNA template were analyzed (1×, 2×, 4×, and 8×). (B) Q
uantification of A. Relative levels of rearrangements are compared to
wild-type (WT) γc+ thymi.
thymi, respectively. Only limited Vγ4-Jγ1 rearrangements are observed in adult γc− thymi, representing ~1% of the levels observed in adult wt thymi. In contrast, the extent of TCR-δ rearrangements found in adult thymi from γc−-mutant mice resembled those present in wt adult thymi (Fig. 4 B, top). Thus, these results suggest that the γc− 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 γc− thymocytes (Fig. 4). We therefore examined TCR-δ rearrangements in CD4−CD8− thymocytes from wt mutant mice. As summarized in Table 3, the extent of TCR-δ rearrangement present in γc− DN thymocytes was found to be similar to that observed in total γc− thymocytes and in CD3eδ/δ− thymocytes, which are an enriched source of γc− 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 γc− mice. TCR-γ rearrangements in γc− DN thymocytes were also reduced (data not shown).

In conclusion, our analysis of TCR-γ and TCR-δ rearrangements in fetal and adult γc− 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 γc− thymus. The implications of these observations are the following. First, during fetal life, the absence of γc− signaling pathways does not impair the ability to rearrange the TCR-γ or TCR-δ loci. This suggests that γc− independent factors may compensate for the lack of IL-7/γc−-mediated signals. Second, the fact that fetal and adult thymi from γc− 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 γc− thymi.

Lack of TCR-γ/δ Cell Survival in γc− mice Tg for R 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 γc− 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 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-thymocytes, as well as the slencer 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 γc− deficient background.

Expression of the Vγ1 Tg alone in mice with or without the γc− 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 γc− Vγ1 Tg animals demonstrated 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 slencer element was operative in the α/β T cells found in these Tg mice (Fig. 5). γc− 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 γc− mice were still severely reduced. Compared with γc− Tg animals, there was a 120-fold reduction in absolute numbers of Vγ1+ cells (Table 4). Considering that the γc− chain plays a role in the

### Table 2. TCR-γ and TCR-δ R rearrangements in γc− and IL-7−/− Thymi

| DNA source   | V64-Jδ1 | Vγ4-Jγ1 |
|--------------|---------|---------|
| γc−          | 100.00† | 100.00  |
| RAG 1−/−     | 2.1     | 0.4     |
| γb           | 108.4   | 2.66    |
| IL-7−/−      | 114.5   | 3.79    |

†R rearrangements were quantitated and expressed as percentage of rearrangements found in DNA isolated from γc− total thymocyte preparations from 4-wk-old mice (see Materials and Methods). Results are representative of two independent experiments. **Table 3. TCR-δ R rearrangements from γc− T thymocyte Precursors**

| DNA source   | V64-Jδ1 | Vδ5-Jδ6 |
|--------------|---------|---------|
| γc+ total thymus | 100.00† | 100.00† |
| RAG 1−/− thymus | 1.61    | 1.56    |
| γc− total thymus | 94.36   | 75.40   |
| γc− DN sort† | 111.92  | 55.58   |
| CD3eδ5/δ5 thymus | 73.24   | 101.43  |

†Indicated TCR-δ rearrangements were quantitated and expressed as percentage of rearrangements found in DNA isolated from γc− total thymocyte preparations (see Materials and Methods). Results are representative of two independent experiments. †Double negative thymocytes (CD4−CD8−) were sorted from γc− thymi as described in Materials and Methods, and DNA was isolated for analysis.
Table 4. Transgenic γδ T Cell Development in γc- Mice

| Mouse          | n* | T total thymocyte cell N.o. (× 10⁶) | Thymic TCR-γδ cell N.o. (× 10⁵) | Splenic lymphoid cell N.o. (× 10⁶) | Splenic TCR-γδ cell N.o. (× 10⁵) |
|----------------|----|-----------------------------------|---------------------------------|-----------------------------------|----------------------------------|
| γc+ Non-Tg     | >10| 247 ± 34                          | 2.5 ± 0.3 (0.1%)                | 66.5 ± 10.9                       | 3.3 ± 0.6 (0.6%)                |
| γc- Non-Tg     | >10| 11.7 ± 6.6                        | ND                              | 8.2 ± 3.2                         | <0.05 (<0.05%)                  |
| γc+ TCR Vγ1 Tg | 5  | 198 ± 23.1                        | 29 ± 3.4 (1.4%)                 | 45.2 ± 4.6                        | 20 ± 6.5 (4.0%)                 |
| γc- 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 γc- mice (data not shown), part of the dramatic reduction in Vγ1+ thymocytes in γc- Tg mice stems from the limited number of thymocyte precursors available to express the Tg Vγ1 receptor. Taking this into account, γδ T cells are still eightfold reduced (120-fold/15-fold) in γc- Tg mice relative to γc+ Tg controls, suggesting that additional mechanisms are responsible for the defective γδ 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-γδ Tg does not restore normal γδ T cell development in the absence of γc. Moreover, since γc- mice can express the TCR-γδ transgenes, a defect in TCR-α/β-γδ lineage branching can be effectively ruled out.

Considering that thymically derived γδ T cells seed the spleen and lymph nodes of postnatal mice, we further examined the peripheral lymphoid compartments for transgenic γδ T cells. As shown in Fig. 6, a large population of cells expressing the transgenic TCR-γδ receptor are present in the spleen of γc+ 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 γc+ nontransgenic animals (Table 4). In contrast, the periphery of γc- Tgs contain only a few cells expressing the Tg TCR-γδ receptor, and these cells fail to accumulate in the spleen. In terms of absolute numbers, γc- Tg γδ T cells are reduced 142-fold compared with γc+ Tg littermates (Table 4). Taken together, our results suggest a major role for γc-dependent signals in the survival of γδ T cells.

To investigate whether γc- Tg γδ T cells had a defect in survival, we examined Tg thymocytes for the expression of the antiapoptotic factor, Bcl-2. Engagement of γc-dependent receptors has been shown to maintain high levels of Bcl-2, which appear to protect lymphoid cells from cell death (41-43). Although Vγ1+ thymocytes from γc+ mice expressed Bcl-2, Tg γδ T cells from γc- mice were essentially negative for Bcl-2 (Fig. 7). These results are consistent with a defect in γδ T cell survival in the absence of γc, although the relative contribution of Bcl-2 in supporting γδ T cell development remains to be determined.

Figure 5. Flow cytometric analysis of thymocytes from γc+ or γc- mice transgenic for a rearranged TCR Vγ1 receptor. (A) Cells were stained with FITC-anti-CD8 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.

Concluding Remarks. Most γδ 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 γc and these cyto-

1282 γc Signaling Controls γδ T Cell Survival
kines have been postulated to play an important role in the survival, growth, and differentiation of γ/δ T cells (10–13). In a recent study using mice deficient in IL-7R α 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-7R α 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 γc-containing receptors (likely due to IL-7), that is independent of the TCR-γ rearrangement process. Thymocytes from adult γc-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-γα polypeptides. Complementation of such γc-deficient mice with TCR-γ and TCR-γ/δ transgenes only partially rescued thymic γδ T cell development and did not permit accumulation of peripheral γδ T cells. Therefore, the developmental blockade affecting the adult γδ T cell lineage in γc-deficient mice results not only from the limited amounts of rearranged TCR-γ/δ genes but also from the fact that IL-7 promotes the survival of γδ T cell precursors containing TCR-γ and TCR-γ/δ polypeptides. The survival role played by IL-7 in γδ T cell development is supported by our observation that fetal thymocytes from γc-deficient mice do contain TCR-γ and TCR-δ rearrangements, but fail to generate appreciable numbers of γδ T cells intrathymically or to export them to the periphery. Further evidence is provided by the fact that γc-γδ T cells contain dramatically reduced levels of the antiapoptotic factor Bcl-2. Previous reports have shown that engagement of γc-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 γc-γδ T cells or simply an epiphenomenon related to decreased cell survival remains to be determined.

Thus, we would like to propose that for γδ T cells, the primary function of γc-containing receptors is to promote survival. The ability of the γc- fetal thymus to support the survival of γδ T cells (possibly via TSLP) would explain the presence of TCR-γ rearrangements in IL-7-deficient mice and γc-deficient mice, and their mere absence in IL-7R α-deficient mice. After successful expression of a functional TCR-γδ complex, γδ T cells would still require signals via γc to survive, mature, and seed the periphery. This idea gains support from the analysis of IL-7-deficient mice, where Vγ5HSA16 fetal thymocytes are readily detectable, but fail to mature into Vγ5HSA16 T 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 αβ T cells is less severely impaired in γc-deficient mice than that of γδ T cells. In the αβ lineage, it has been recently documented that TCR-β rearrangements are accompanied by a selective process allowing only those cells displaying a productively rearranged Vb 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 γδ T cells, TCR-γ and TCR-δ rearrangements are probably achieved concurrently and not subjected to pre-TCR–based epigenetic control mechanisms operating during αβ T cell development (34). In this model of γδ T development, γδ T cell precursors might not receive any pre-TCR or TCR signals, and engagement of γc-containing cytokine receptors may constitute the only means to support the survival of these cells. In marked contrast, in...
α/β T cell precursors, the γδ-dependent survival signals and the pre-TCR dependent survival signals may partially overlap, explaining how, in the absence of survival signals dependent on γδ-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.

This work was supported by grants from the Centre National de la Recherche Scientifique, the Institut National de la Sante et de la Recherche Medicale, the Association pour le Recherche sur le Cancer, Ligue Nationale Contre le Cancer, and the Commision of the European Communities.

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Received for publication 22 April 1997 and in revised form 4 August 1997.

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