

Interleukin-7 Receptor is Indispensable for Proliferation and Survival in Thymic $\gamma\delta$ T Cell Development

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ABSTRACT

Background: Interleukin-7 receptor (IL-7R) α -deficient mice have small numbers of B cells and $\alpha\beta$ T cells in periphery, they totally lack $\gamma\delta$ T cells. In addition, the V-J recombination and transcription of TCR γ genes is also severely impaired in IL-7R α -deficient mice. Stat5, a signaling molecule of the IL-7R, induces germline transcription in the TCR γ locus, and promotes V-J recombination and $\gamma\delta$ T cell development. However, the roles for IL-7R signaling pathway in thymic or extrathymic $\gamma\delta$ T cell development are largely unknown. **Methods:** To clarify the role of the IL-7 receptor in proliferation and survival of $\gamma\delta$ T cells, we introduced the TCR $\gamma\delta$ transgene, V γ 2/V δ 5, into IL-7R α -deficient mice, and investigated the development of $\gamma\delta$ T cells. **Results:** We found that V γ 2/V δ 5 transgene restored $\gamma\delta$ T cells in the epithelium of the small intestine (IEL) but not in the thymus and the spleen. Further addition of a *bcl-2* transgene resulted in partial recovery of $\gamma\delta$ T cells in the thymus and the spleen of these mice. **Conclusion:** Taken together, this study revealed that the IL-7R α is indispensable for proliferation and survival mainly in thymic $\gamma\delta$ T cell development. (*Immune Network* 2005;5(1):23-29)

Key Words: IL-7R, TCR γ gene, V γ 2/V δ 5 transgenic mouse, thymus

Introduction

A small number of bone marrow-derived cells give rise to the NK, intrathymic dendritic cell, and $\alpha\beta$ and $\gamma\delta$ T cells. $\gamma\delta$ T cell development has unique features in contrast to $\alpha\beta$ T cell development (1). IL-7 is an essential cytokine for $\gamma\delta$ T cell development. IL-7 exerts its effect through interaction with the IL-7R, which is composed of a unique α chain (IL-7R α) and the common cytokine receptor γ chain (γ c) (2). Recently, thymic stromal lymphopoietin (TSLP) has shown to transmit signals through IL-7R α and TSLPR heterodimer (3). Injection of neutralizing antibodies to IL-7 or IL-7R α , or genetic ablation of IL-7, IL-7R α or γ c, leads to a block of lym-

phocyte development (4,5). While IL-7R α -deficient mice have small numbers of B cells and $\alpha\beta$ T cells in periphery, they totally lack $\gamma\delta$ T cells (6,7). The IL-7R transmits at least two types of signal in lymphocyte progenitors (8). One signal is for survival and proliferation. For instance, the IL-7R induces the expression of Bcl-2 in T cell precursors (9), and introduction of *bcl-2* transgene restores $\alpha\beta$ T cell development in IL-7R α -deficient mice (10). The IL-7R supports the proliferation of lymphocyte precursors through the activation of phosphatidylinositol-3 (PI3) kinase (11). Peripheral $\alpha\beta$ T cells in IL-7R α -deficient mice are also defective in survival and proliferation (12). The second signal from the IL-7R is to promote recombination and transcription in the IgH and TCR γ loci. For example, IL-7R signaling induces germline transcription and DNA rearrangement in D-distal V segments in pro-B cells (13). The V-J recombination and transcription of TCR γ gene is also severely impaired in IL-7R α -deficient mice (14). Stat5, a signaling molecule of the IL-7R, induces germline transcription in

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the TCR γ locus, and promotes V γ -J γ recombination and $\gamma\delta$ T cell development (15).

In addition to IL-7, other cytokines play substantial roles in $\gamma\delta$ T cell development. IL-15 achieves its effects through interaction with the IL-15R, which is composed of a unique α chain (IL-15R α), the common β chain (IL-2R β), and the γ_c (16). Deletion of IL-15, IL-15R α , or IL-2R β results in a block of NK cell development and impaired $\gamma\delta$ T cell development in the epithelium. IL-2R β -deficient mice have decreased numbers of $\gamma\delta$ IEL (17). These results suggest that IL-15 plays important roles in the development of $\gamma\delta$ IEL and DETC. Besides IL-15, IL-2 is also implicated to be involved in thymic and extrathymic T cell development (18).

The roles for each signaling pathway in $\gamma\delta$ T cell development are largely unknown. Introduction of a *bcl-2* transgene restored $\alpha\beta$ but not $\gamma\delta$ T cell development in IL-7R α -deficient mice. Similarly, a *bcl-2* transgene alone did not rescue B cells, $\gamma\delta$ T cells and NK cells in γ_c -deficient mice (19). However, these experiments did not clarify the role of Bcl-2 in $\gamma\delta$ T cell development, because the IL-7R signaling is necessary for the recombination and transcription of TCR γ genes. Introduction of a V γ 1.1/V δ 6 TCR $\gamma\delta$ transgene failed to restore $\gamma\delta$ T cells in IL-7R α -deficient mice, suggesting that the IL-7R plays some role in proliferation and/or survival of $\gamma\delta$ T cells (20).

To clarify the roles of the cytokine receptors in proliferation and survival of $\gamma\delta$ T cells, we introduced a TCR $\gamma\delta$ transgenes, V γ 2/V δ 5 into IL-7R α -deficient mice and found that they restored $\gamma\delta$ T cells in IEL and the skin but not in the thymus and the spleen. Further addition of a *bcl-2* transgene resulted in partial recovery of $\gamma\delta$ T cells in the thymus and the spleen of these mice. In contrast, the same V γ 3/V δ 1 TCR transgene failed to rescue $\gamma\delta$ T cells in the skin of IL-2R β -deficient mice (21). Thus, this study reveals that the IL-7R is indispensable for the rearrangement of TCR γ genes in all $\gamma\delta$ T precursors and promotes both proliferation and survival in thymic $\gamma\delta$ T cell development.

Materials and Methods

Antibodies. The following monoclonal antibodies were used. FITC-conjugated antibodies; 53-6.7 (anti-CD8 α), H57-597 (anti- $\alpha\beta$ TCR), 8D6 (anti-V γ 2/V δ 5), 53-2.1 (anti-Thy-1.2), KH95 (anti-H-2D^b). Biotin-conjugated antibodies; H57-597 (anti- $\alpha\beta$ TCR). Phycoerythrin (PE)-conjugated antibodies; GK1.5 (anti-CD4), GL3 (anti- $\gamma\delta$ TCR), 34-2-12 (anti-H-2D^d). Other monoclonal antibodies were purchased from PharMingen (San Diego, CA).

Mice. IL-7R α -deficient (14) and H2K-bcl-2 transgenic

mice (22) were reported previously and were bred on the (129/Ola \times C57BL/6) hybrid background. The *bcl-2* transgene is driven by a H-2K promoter and expressed on virtually all blood cells. Transgenic mice containing productively rearranged V γ 2/V δ 5 TCR genes were described before (23). The V γ 2/V δ 5 TCR is derived from a $\gamma\delta$ T cell line specific for a product of a gene in the TL region in the TLb haplotype (24). All mice used in V γ 2/V δ 5 TCR transgenic experiments were of the H-2^{d/d} haplotype confirmed by staining spleen cells with anti-H-2D^b and anti-H-2D^d antibodies. All mice were maintained in animal facilities at Seoul National University College of Medicine.

Cell preparations and flow cytometric analysis. Thymocytes and spleen cells were harvested in PBS supplemented with 2% fetal bovine serum and 0.02% sodium azide (FACS solution). Red blood cells were lysed, and cells were washed in the FACS solution. IEL were isolated from small intestine as described previously (25). Flow cytometric analysis was performed as described (21). Debris, erythrocytes, and dead cells

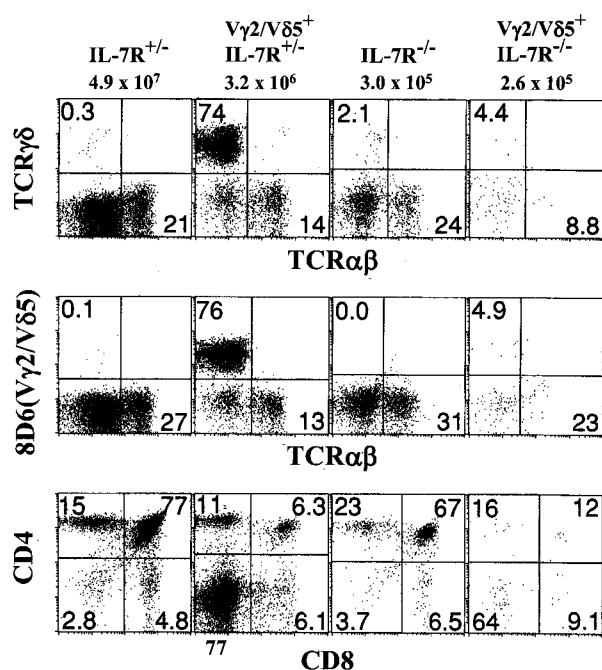


Figure 1. Introduction of a V γ 2/V δ 5 TCR transgene fails to restore $\gamma\delta$ T cells in the thymus of IL-7R α -deficient mice. Thymocytes were isolated from IL-7R α ^{+/+}, TCR Tg⁺ IL-7R α ^{+/+}, IL-7R α ^{-/-}, and TCR Tg⁺ IL-7R α ^{-/-} mice. Cells were stained with either FITC-anti-TCR $\alpha\beta$ and PE-anti-TCR $\gamma\delta$, FITC-anti-V γ 2/V δ 5 TCR and biotin-anti-TCR $\alpha\beta$ followed by PE-streptavidin, or FITC-anti-CD8 and PE-anti-CD4 antibodies. The percentages of cells for a given phenotype are shown. Representative data from 8-week-old littermates are shown. The cell numbers recovered from each mouse are shown above each panel.

were excluded from the analysis by forward and side scatters and propidium iodide gatings. Viable cells were analyzed by a FACS Calibur with CellQuest software version 3.1 (Becton Dickinson, San Jose, CA).

Results

Introduction of a $V_{\gamma 2}/V_{\delta 5}$ TCR transgene fails to restore $\gamma\delta$ T cells in the thymus and the spleen of IL-7R α -deficient mice. To test the role of the IL-7R on proliferation and/or survival of $\gamma\delta$ T cells, we introduced a $V_{\gamma 2}/V_{\delta 5}$ TCR transgene into the IL-7R α -deficient mice to bypass the defective V_{γ} -J γ recombination. We backcrossed the $V_{\gamma 2}/V_{\delta 5}$ TCR transgenic mice to IL-7R $\alpha^{-/-}$ mice and chose four types of mice, namely, IL-7R $\alpha^{+/-}$, $V_{\gamma 2}/V_{\delta 5}$ TCR Tg⁺ IL-7R $\alpha^{+/-}$, IL-7R $\alpha^{-/-}$, and $V_{\gamma 2}/V_{\delta 5}$ TCR Tg⁺ IL-7R $\alpha^{-/-}$. Thymocytes and spleen cells were isolated from the mice, and analyzed by flow cytometry (Fig. 1). While only 0.3% of thymocytes expressed $\gamma\delta$ TCR in IL-7R $\alpha^{+/-}$ mice, 74% of thymocytes were $\gamma\delta$ T cells expressing the $V_{\gamma 2}/V_{\delta 5}$ TCR transgene in TCR Tg⁺ IL-7R $\alpha^{+/-}$ mice. In TCR Tg⁺ IL-7R $\alpha^{-/-}$ mice 77% of thymocytes were CD4⁺8⁻, suggesting that most of the transgenic $\gamma\delta$ T cells were CD4⁺8⁻. The number of thymocytes was reduced in Tg⁺ IL-7R $\alpha^{-/-}$ transgenic mice compared with IL-7R $\alpha^{+/-}$ mice (Fig. 2A), suggesting an adverse effect of TCR $\gamma\delta$ transgenes on $\alpha\beta$ T cell development as pro-

posed previously (26). In contrast, we did not detect any distinct TCR $\gamma\delta^{+}$ cells in IL-7R $\alpha^{-/-}$ mice as we and others reported previously (6,7). Although we sometimes observed TCR $\gamma\delta^{+}$ signals, we concluded that they were caused by nonspecific staining as judged by CD3 and TCR $\gamma\delta$ staining (data not shown). In TCR Tg⁺ IL-7R $\alpha^{-/-}$ mice, however, we found few, if any, $\gamma\delta$ T cells. Spleen cells gave similar results to thymocytes: in TCR Tg⁺ IL-7R $\alpha^{+/-}$ mice a prominent $\gamma\delta$ T cell population developed in spleen, while no distinct $\gamma\delta$ T cells were detected in IL-7R $\alpha^{-/-}$ and TCR Tg⁺ IL-7R $\alpha^{-/-}$ mice (data not shown).

Next we compared the overall numbers of total and $\gamma\delta$ T cells (Fig. 2A, B, D and E). The numbers of $\gamma\delta$ T cells dramatically increased in the thymus and the spleen of TCR Tg⁺ IL-7R $\alpha^{+/-}$ mice compared with IL-7R $\alpha^{+/-}$ mice. The introduction of the TCR $\gamma\delta$ transgene, however, resulted in a slight increase of $\gamma\delta$ T cells in the thymus and no increase in the spleen of IL-7R $\alpha^{-/-}$ mice. These results showed that the introduction of the $V_{\gamma 2}/V_{\delta 5}$ TCR transgene alone failed to rescue $\gamma\delta$ T cell development in the thymus and the spleen of IL-7R $\alpha^{-/-}$ mice and that the IL-7R may play an important role in proliferation and/or survival of $\gamma\delta$ T cells in these organs.

The $V_{\gamma 2}/V_{\delta 5}$ TCR transgene partially recovers $\gamma\delta$ IEL

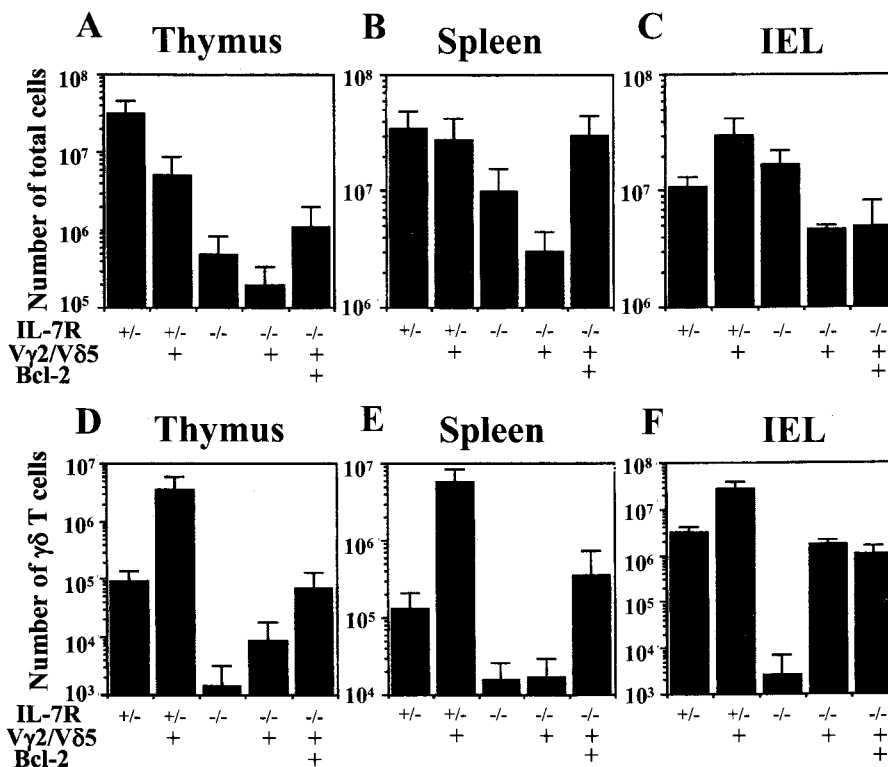


Figure 2. Numbers of total and $\gamma\delta$ T cells of IL-7R α -deficient mice with $V_{\gamma 2}/V_{\delta 5}$ TCR and *bcl-2* transgenes. Numbers of total (A, B, and C) and $\gamma\delta$ T (D, E, and F) cells were counted in the thymus (A and D), the spleen (B and E), and IEL (C and F) from IL-7R $\alpha^{+/-}$, TCR Tg⁺ IL-7R $\alpha^{+/-}$, IL-7R $\alpha^{-/-}$, and TCR Tg⁺ IL-7R $\alpha^{-/-}$, and *bcl-2* Tg⁺ TCR Tg⁺ IL-7R $\alpha^{+/-}$ mice of 8-week-old. The mean \pm S.E. is calculated from four mice. The numbers of $\gamma\delta$ T cells were calculated from the total cell numbers and the percentage of $\gamma\delta$ T cells in each mice.

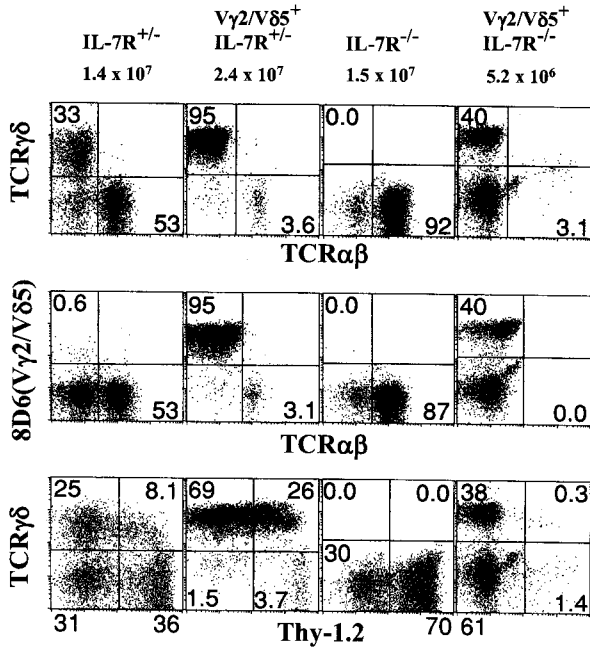


Figure 3. The V γ 2/V δ 5 TCR transgene partially restores $\gamma\delta$ IEL in IL-7R α -deficient mice. IEL were isolated from IL-7R α ^{+/+}, TCR Tg⁺ IL-7R α ^{+/+}, IL-7R α ^{-/-}, and TCR Tg⁺ IL-7R α ^{-/-} mice. Cells were stained with either FITC-anti-TCR $\alpha\beta$ and PE-anti-TCR $\gamma\delta$, FITC-anti-V γ 2/V δ 5 TCR and biotin-anti-TCR $\alpha\beta$ followed by PE-streptavidin, or FITC-anti-Thy-1.2 and PE-anti-TCR $\gamma\delta$ antibodies. The percentages of cells for a given phenotype are shown. Representative data from 8-week-old littermates are shown. The cell numbers recovered from each mouse are shown above each panel.

in IL-7R α -deficient Mice. To assess the role of the IL-7R in extrathymic $\gamma\delta$ T cell development, we next analyzed IEL of the IL-7R α -deficient mice with the V γ 2/V δ 5 TCR transgene (Fig. 3). IL-7R α ^{+/+} mice showed a clear $\gamma\delta$ T cell population in IEL. The majority of IEL in TCR Tg⁺ IL-7R α ^{+/+} mice were $\gamma\delta$ T cells expressing the V γ 2/V δ 5 TCR transgene. As reported before (14), IL-7R α ^{-/-} mice completely lacked $\gamma\delta$ IEL. In contrast to the thymus and the spleen, TCR Tg⁺ IL-7R α ^{-/-} mice contained distinct transgenic $\gamma\delta$ T cells in IEL. $\alpha\beta$ IEL development in these mice was blocked probably because of the adverse effect of the transgene (26). The numbers of $\gamma\delta$ IEL in TCR Tg⁺ IL-7R α ^{-/-} mice were recovered almost to the levels of IL-7R α ^{+/+} mice, but never reached to the levels of TCR Tg⁺ IL-7R α ^{+/+} mice (Fig. 2F). These results demonstrated that introduction of the V γ 2/V δ 5 TCR transgene partially restored $\gamma\delta$ IEL in the IL-7R α -deficient mice. They also suggested that, although IL-7 plays a substantial role in expansion and survival of IEL, other cytokine(s) like IL-15 probably supports this process in concert with IL-7. In IL-7R α ^{+/+} and

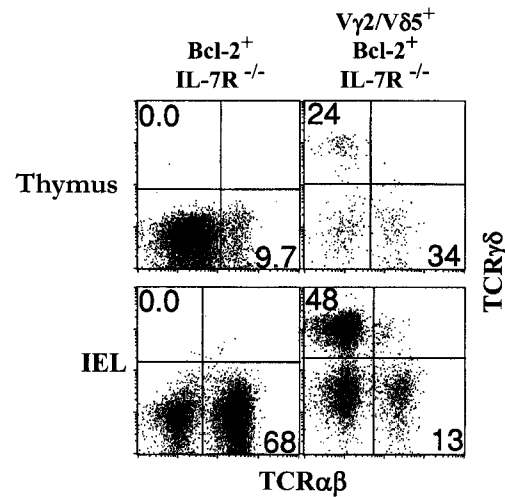


Figure 4. Introduction of a *bcl-2* transgene partially restores transgenic $\gamma\delta$ T cells in the thymus of IL-7R α -deficient mice. Thymocytes and IEL were isolated from *bcl-2* Tg⁺ IL-7R α ^{-/-} and V γ 2/V δ 5 TCR Tg⁺ *bcl-2* Tg⁺ IL-7R α ^{-/-} mice. Cells were stained with FITC-anti-TCR $\alpha\beta$ and PE-anti-TCR $\gamma\delta$ antibodies. The percentages of cells for a given phenotype are shown.

TCR Tg⁺ IL-7R α ^{+/+} mice, we observed both Thy-1⁺ and Thy-1⁻ $\gamma\delta$ IEL populations (Fig. 3). In TCR Tg⁺ IL-7R α ^{-/-} mice, however, Thy-1⁺ $\gamma\delta$ IEL were very few. This result suggested that the expression of Thy-1 antigen on $\gamma\delta$ IEL is induced by IL-7R signaling.

Introduction of a bcl-2 transgene partially rescues transgenic $\gamma\delta$ T cells in the thymus and the spleen of IL-7R α -deficient mice. *Bcl-2* transgenes significantly restored $\alpha\beta$ T cells in the thymus of IL-7R α -deficient mice (9,10). This result suggested that the IL-7R mainly transmits a survival signal in $\alpha\beta$ T cell development. In contrast, B cells and $\gamma\delta$ T cells were not restored in *bcl-2* Tg⁺ IL-7R α ^{-/-} mice (15). This was partly because of impaired V-J recombination in the TCR γ locus. To test the role of the IL-7R on cell survival of $\gamma\delta$ T cells, we introduced an H-2K-*bcl-2* transgene into the V γ 2/V δ 5 TCR Tg⁺ IL-7R α ^{-/-} mice. Thymocytes and IEL were isolated from *bcl-2* Tg⁺ IL-7R α ^{-/-} and *bcl-2* Tg⁺ TCR Tg⁺ IL-7R α ^{-/-} mice, and analyzed by flow cytometry (Fig. 4). No distinct $\gamma\delta$ T cells were detected in the thymus and IEL of *bcl-2* Tg⁺ IL-7R α ^{-/-} mice as reported before. In *bcl-2* Tg⁺ TCR Tg⁺ IL-7R α ^{-/-} thymus, however, we observed more obvious transgenic $\gamma\delta$ T cells than in TCR Tg⁺ IL-7R α ^{-/-} thymus (Fig. 1A). The levels of the transgene expression in TCR Tg⁺ IL-7R α ^{-/-} mice were as high as those in TCR Tg⁺ IL-7R α ^{+/+} mice, suggesting that the defective development of $\gamma\delta$ T cells in TCR Tg⁺ IL-7R α ^{-/-} mice was not necessarily due to impaired transcription of the transgene

(14). As an effect of the *bcl-2* transgene, $\alpha\beta$ T cell development was also partially recovered. We obtained similar results with spleen cells (data not shown). In contrast, after the introduction of the *bcl-2* transgene $\gamma\delta$ IEL did not change much in TCR Tg⁺ IL-7R $\alpha^{-/-}$ mice, except that $\alpha\beta$ IEL were partially restored.

Next we compared the overall numbers of total and $\gamma\delta$ T cells (Fig. 2). The numbers of $\gamma\delta$ T cells in the thymus and the spleen of *bcl-2* Tg⁺ TCR Tg⁺ IL-7R $\alpha^{-/-}$ mice were rescued almost to the levels of IL-7R $\alpha^{+/-}$ mice, but never reached to the levels of TCR Tg⁺ IL-7R $\alpha^{+/-}$ mice. In contrast, the *bcl-2* transgene did not affect the numbers of $\gamma\delta$ IEL in TCR Tg⁺ IL-7R $\alpha^{-/-}$ mice. These results showed that the introduction of the *bcl-2* transgene partially restored $\gamma\delta$ T cell development in the thymus and the spleen of IL-7R α -deficient mice. These results supported the idea that the IL-7R plays a substantial role in survival of $\gamma\delta$ T cells in the thymus and the spleen by inducing Bcl-2. However, they also implied that the IL-7R transmits a proliferation signal in $\gamma\delta$ thymocyte development. In contrast, the IL-7R is dispensable for Bcl-2 induction in $\gamma\delta$ IEL development, probably because the IL-2R β can compensate for the IL-7R.

Discussion

In this study, we first showed that introduction of the V γ 2/V δ 5 TCR transgene alone failed to rescue $\gamma\delta$ T cell development in the thymus and the spleen of IL-7R $\alpha^{-/-}$ mice, suggesting that the IL-7R may play an important role in proliferation and/or survival of $\gamma\delta$ T cells in these organs (Fig. 1 and 2). The same transgene, however, partially restored $\gamma\delta$ IEL in IL-7R $\alpha^{-/-}$ mice, showing that both IL-7 and other cytokine(s) such as IL-15 play a substantial role in expansion of $\gamma\delta$ IEL (Fig. 2 and 3). Further introduction of the *bcl-2* transgene in these mice partially restored $\gamma\delta$ T cells in the thymus and the spleen (Fig. 2 and 4), demonstrating that the IL-7R transmits both proliferation and survival signals for $\gamma\delta$ T cells in these organs. The results also showed that the defective development of $\gamma\delta$ T cells in these mice was not due to impaired expression of the transgene (14). In contrast to V γ 2/V δ 5, the introduction of a V γ 3/V δ 1 TCR transgene into IL-7R $\alpha^{-/-}$ mice completely restored V γ 3⁺ T cells in the fetal thymus and DETC in the adult skin (21). On the other hand, the same V γ 3/V δ 1 transgene alone, or together with the *bcl-2* transgene, failed to rescue DETC in IL-2R $\beta^{-/-}$ mice (21). These results demonstrated that the IL-2R β , rather than the IL-7R, plays an essential role in proliferation and survival of dendritic epidermal T cells (DETC) in the fetal thymus and the

skin. Thus, this study proved that IL-7R α and IL-2R β serve differential functions in proliferation and survival of $\gamma\delta$ T cells.

The IL-7R transmits at least two signals during $\gamma\delta$ T cell development. One is for proliferation and survival, and the other is for recombination and transcription of the TCR γ locus. As we and others previously showed, the V-J recombination of TCR γ genes was severely impaired in IL-7R α -deficient mice (14). In addition, Stat5, a signaling molecule of the IL-7R, induced germline transcription in the TCR γ locus, and promoted V γ -J γ recombination and $\gamma\delta$ T cell development (15). In this study two TCR γ δ transgenes were shown to restore $\gamma\delta$ T cells in IL-7R α -deficient mice: the V γ 2/V δ 5 transgene partially recovered $\gamma\delta$ IEL. On the other hand, the V γ 3/V δ 1 transgene completely rescued DETC in the fetal thymus and the skin (21). These results suggested that IL-7R signaling is indispensable for rearrangement of TCR γ genes even in the tissues where IL-15 is available. This is either because the IL-15R α or the IL-2R β is not expressed at very early stages where the rearrangement of TCR γ genes take place, or there is an IL-7R-specific signal for inducing recombination.

Thymic $\gamma\delta$ T cell development depends on IL-7R signaling for both proliferation and survival. In previous reports, the IL-7R induced the expression of *Bcl-2* in T cell precursors (9), and introduction of a *bcl-2* transgene alone restored $\alpha\beta$ T cell development in IL-7R α -deficient mice (10). This is probably because $\alpha\beta$ T cell precursors receive proliferation and survival signals from pre TCR after they manage to express TCR β chain (27). In contrast, $\gamma\delta$ T cell precursors seem to depend entirely on the IL-7R for their proliferation and survival in the thymus and the spleen. Bcl-2 is probably induced in $\gamma\delta$ T cell precursors by IL-7R signaling and plays an essential role in their survival, as suggested by the result that the *bcl-2* transgene partially rescued $\gamma\delta$ T cells in the thymus and the spleen of V γ 2/V δ 5 TCR Tg⁺ IL-7R $\alpha^{-/-}$ mice. The same result also implied that besides the survival signal $\gamma\delta$ T cell precursors receive a proliferation signal from the IL-7R. This is mediated probably by the MAP kinase cascade and PI3 kinase. It is also conceivable that the IL-7R supports the survival of $\gamma\delta$ T cells by keeping the transcription of the TCR γ genes (15). Because the *bcl-2* transgene partially rescued $\gamma\delta$ T cells in TCR Tg⁺ IL-7R $\alpha^{-/-}$ mice and the transgene expression on recovered $\gamma\delta$ T cells was not lowered, our results, however, suggested that the defective development of $\gamma\delta$ T cells in these mice was not due to impaired expression of the transgene.

Extrathymic $\gamma\delta$ T cell development depends on

not only the IL-7R but also the IL-2/IL-15R. While IL-7R α -deficient mice completely lack $\gamma\delta$ IEL (14), IL-15-, IL-15R-, and IL-2R β -deficient mice show only decreased numbers of $\gamma\delta$ IEL (17). Because the $V_{\gamma 2}/V_{\delta 5}$ TCR transgene did not completely rescue $\gamma\delta$ IEL in IL-7R α -deficient mice, it is implied that the IL-7R induces proliferation and/or survival of $\gamma\delta$ IEL besides rearrangement of TCR γ genes. Furthermore, introduction of the *bcl-2* transgene did not recover $\gamma\delta$ IEL further in $V_{\gamma 2}/V_{\delta 5}$ TCR Tg⁺ IL-7R $\alpha^{-/-}$ mice, suggesting that the IL-7R transmits at least a proliferation signal in $\gamma\delta$ IEL. In addition, $\gamma\delta$ IEL probably receive proliferation and survival signals from the IL-2/IL-15R in response to IL-15 produced by intestinal epithelial cells. Thus, the IL-7R and the IL-2/IL-15R play roles at early and late stages of $\gamma\delta$ IEL development, respectively. Thy-1⁺ $\gamma\delta$ IEL were very few in $V_{\gamma 2}/V_{\delta 5}$ TCR Tg⁺ IL-7R $\alpha^{-/-}$ mice (Fig. 3). In contrast, IL-15-, IL-15R α -, and IL-2R β -deficient mice have mostly Thy-1⁺ $\gamma\delta$ IEL (17). These results suggested that only IL-7R signaling can induce Thy-1 expression on $\gamma\delta$ IEL although the IL-2/IL-15R mobilizes a similar set of signaling molecules to the IL-7R. Thus, the Thy-1⁺ and Thy-1⁻ IEL populations in normal mice may correspond to the early and late stages of their development, respectively.

In conclusion, these data suggest that the IL-7R α is indispensable for proliferation and survival mainly in thymic $\gamma\delta$ T cell development.

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