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Immunology

Interleukin 7 receptor-deficient mice lack γδ T cells

(homologous recombination/T-cell differentiation/cytokine/thymus/intraepithelial lymphocyte)

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ABSTRACT **The interleukin 7 receptor (IL-7R) plays a crucial role in early B- and T-cell development. It consists of a unique α chain and a common γ chain [IL-2 receptor γ chain (IL-2Rγ)]. Gene inactivation of IL-7, IL-7R, and IL-2Rγ resulted in severe impairment of B and T lymphopoiesis in mice. In addition, IL-2Rγ-deficient mice lack γδ T cells in the skin and have the impaired development of natural killer (NK) cells and intraepithelial lymphocytes. To explore the role of IL-7/IL-7R system in γδ T- and NK-cell development, we have generated and analyzed IL-7R-deficient mice. γδ T cells were absent from skin, gut, liver, and spleen in the deficient mice. In contrast, αβ T and B cells were detected in reduced, but certain, numbers, and NK cells developed normally. The γδ T-cell development in fetal and adult thymus was also completely blocked. These results clearly demonstrate that the signal from IL-7R is indispensable for γδ T-cell development in both thymic and extrathymic pathways. On the contrary, it is suggested that NK-cell development requires cytokine(s) other than IL-7.**

Interleukin 7 (IL-7) is a growth factor for early B- and T-cell precursors. It was first characterized by its ability to support the growth of B-cell precursors. Subsequently, it has been shown to support survival and growth of early thymocytes and promote rearrangement of T-cell receptor (TCR) β and γ chains in fetal thymus and fetal liver cultures (1, 2). *In vivo* administration of neutralizing antibodies to IL-7 and IL-7 receptor (IL-7R) resulted in the inhibition of both B and T lymphopoiesis (3, 4). IL-7R consists of two polypeptides: a unique α chain (IL-7Rα) and a common γ chain (IL-2R γ chain, IL-2Rγ) (5, 6). IL-7R shares the IL-2Rγ with the receptors for IL-2, IL-4, IL-9, and IL-15 (7).

γδ T cells have unique features in contrast to αβ T cells (8, 9). γδ T cells expressing specific Vγ chains appear as several successive waves in the developing thymus and each of them shows specific tissue distribution in the adult mouse. For instance, Vγ3 T cells reside as Thy-1$^+$ dendritic epidermal T cells (DETCs) in the skin, while Vγ4 T cells exist in the epithelium of the lung, tongue, and vagina. In contrast, Vγ5 T cells develop in the gut as intraepithelial lymphocytes (IELs). γδ T cells recognize nonpeptide antigens, such as pyrophosphate derivatives and polypeptide antigens like nonclassical class I molecules and heat shock proteins, and are involved in the resistance against leukemic cells (10). Little is known, however, about the mechanism of γδ T-cell development. In fetal thymic organ culture, addition of IL-7 promotes expansion of mature γδ T cells but prevents generation of mature αβ T cells (11, 12). The epithelial cells in the skin and the gut produce IL-7 (13, 14), and DETCs proliferate in respond to IL-7 (13). Additionally, IL-7 induced rearrangement of Vγ2 and Vγ4 but not of Vγ3 or Vγ5 genes and sustained expression of RAG-1 and RAG-2 genes (1, 2). Collectively, these results strongly suggest that IL-7 may be also involved in the development and the maintenance of γδ T cells in the thymus and the periphery.

Gene inactivation studies have been carried out to elucidate the *in vivo* function of various cytokines and their receptors. In IL-2Rγ-deficient mice, numbers of T, B, and natural killer (NK) cells are reduced (15, 16). In addition, DETCs are absent from the skin and the development of IELs is severely diminished. In contrast, mice deficient for IL-2, IL-4, or both cytokines have normal numbers of mature T and B cells (17, 18). On the other hand, in both IL-7- and IL-7R-deficient mice, the numbers of lymphocytes are reduced (19, 20), suggesting that the impairment of T and B lymphopoiesis in IL-2Rγ-deficient mice is caused by depletion of the signal by IL-7. However, studies have not been reported on the development of γδ T and NK cells in IL-7R-deficient mice.

To elucidate the role of IL-7/IL-7R system in γδ T-cell development, we have generated and analyzed IL-7R-deficient mice. γδ T cells were absent from these mice, while decreased, but certain, numbers of mature αβ T cells were detected. On the contrary, NK cells normally developed in the mice. These results suggested that αβ and γδ T-cell lineages have a different manner of dependency on the signal from IL-7R for their development.

MATERIALS AND METHODS

Gene Targeting. Mouse IL-7R genomic clones were isolated from a 129/SVJ mouse genomic library (Stratagene). The targeting construct was assembled in pBluescript II KS (+) plasmid. The phosphoglycerate kinase promoter-driven neomycin-resistance (PGK-*neo*) cassette and the MC1 promoter-driven herpes simplex virus-thymidine kinase (MC1-*tk*) cassette were cloned into the *Sal*I–*Spe*I and the *Apa*I sites, respectively, by blunt-end ligation. A 4.1-kb *Xba*I–*Cla*I fragment just 5′ to the exon 2 and a 5.7-kb *Hin*dIII–*Stu*I fragment within the second intron were blunt-end cloned into the *Xho*I and the *Xba*I sites. As a result, the targeting vector substituted the *neo* cassette for the 1.0-kb *Cla*I–*Hin*dIII fragment containing the exon 2 and was designated as pNTIL-7RA (Fig. 1*A*).

E14–1 embryonic stem (ES) cells (a gift from D. Kitamura, Science University of Tokyo) were cultured on primary embryonic fibroblasts in culture medium supplemented with leukemia inhibitory factor (Amrad, Melbourne, Australia;

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Abbreviations: IL, interleukin; IL-*n*R, IL-*n* receptor; IL-2Rγ, IL-2R γ chain; TCR, T-cell receptor; DETC, dendritic epidermal T cell; IEL, intestinal intraepithelial lymphocyte; NK, natural killer; ES, embryonic stem; IHL, intrahepatic lymphocyte; FITC, fluorescein isothiocyanate; PE, phycoerythrin.

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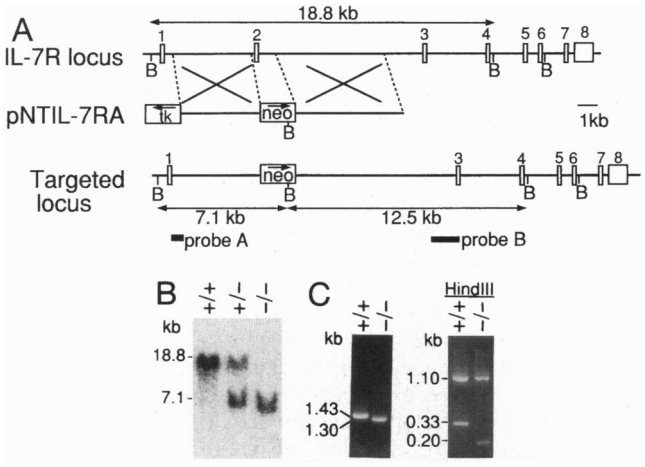

FIG. 1. Strategy for the disruption of the IL-7R gene. (*A*) Genomic structure of the wild-type IL-7R locus, the IL-7R targeting vector (pNTIL-7RA), and the IL-7R targeted locus. Boxes represent exons. The size of diagnostic restriction fragment and the location of probes used for Southern blot analysis are shown. B, *Bam*HI. (*B*) Southern blot analysis of offspring from intercrosses of heterozygous mice. Genomic DNA from the tails was digested with *Bam*HI and hybridized with probe A. Wild-type (+/+), heterozygous (+/−) and homozygous (−/−) mice are shown. (*C*) Expression of IL-7R mRNA in IL-7R$^-$ mice. Reverse transcriptase-coupled PCR was done with spleen RNA and IL-7R primers. PCR products before (*Left*) and after (*Right*) *Hin*dIII digestion are shown.

1000 units/ml) as described (21). The targeting vector, pNTIL-7RA, was linearized with *Not*I and electroporated into ES cells in PBS (20 μg of DNA per 1×10^7 cells) by a Bio-Rad Gene Pulser (210 V, 500 μFD). Selection was begun with G418 at 225 μg/ml (active weight) 24 hr after plating. Selection with 1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-5-iodouracil (FIAU) at 0.2 μM started 36 hr after plating and continued for 84 hr. Doubly drug-resistant colonies were picked on the days 8, 10, and 11. Genomic DNA was digested with *Bam*HI, and a Southern blot was probed with a 0.4-kb *Dra*I–*Xba*I fragment (probe A) or a 1.2-kb *Kpn*I–*Hin*dIII fragment (probe B) (Fig. 1*B*). Of 145 clones, two were targeted for IL-7R gene. These ES cells were injected into blastocysts of C57BL/6N mice. Chimeric animals were bred with C57BL/6J mice. One ES-cell clone (D10) transmitted the mutation into the germ line. All mice were maintained under the specific pathogen-free conditions.

RNA Analysis. Total RNA was isolated from spleen with guanidinium isothiocyanate and was analyzed by reverse transcriptase-coupled PCR. Oligo(dT)-primed cDNA was prepared by Molony murine leukemia virus RNase H$^-$ reverse transcriptase (GIBCO/BRL) at 37°C for 1 hr. PCR was carried out for 30 cycles consisting of denaturation (for 30 sec at 94°C), annealing (for 1 min at 64°C), and extension (for 2 min at 72°C). The primer pair for PCR was as follows: IL-7R5′-1, 5′-CGTCTAGAATGATGGCTCTGGGTA-3′; IL-7R3′-1, 5′-GGAGATCTCAGTGATCATTTGGT-3′. The underlined sequences represent the restriction sites of *Xba*I and *Bgl*II, respectively.

Cell Preparations. Bone marrow cells, spleen cells, thymocytes, and peritoneal cells were harvested in Hanks' balanced salt solution (without phenol red) supplemented with 2% fetal bovine serum, 10 mM Hepes (pH 7.4), and 0.02% NaN_3 (HBSS) as described (22). Red blood cells were lysed, and cells were washed once in HBSS. IELs were isolated from small intestine as described (23). Intrahepatic lymphocytes (IHLs) were isolated as described with minor modification (24). Isolation of epidermal cells and immunofluorescence staining of epidermal sheets were done as described (25).

Antibodies and Flow Cytometric Analysis. The following monoclonal antibodies were used (22, 26). Fluorescein isothiocyanate (FITC)-conjugated antibodies: RA3–6B2, anti-CD45R/B220; 145–2C11, anti-CD3; 53–6.7, anti-CD8α; 53–2.1, anti-Thy-1.2. Phycoerythrin (PE)-conjugated antibodies: GK 1.5, anti-CD4. Biotin-conjugated antibodies: M41, anti-μ chain; A7R34, anti-IL-7Rα chain (4); H57–597, anti-$\alpha\beta$ TCR; GL3, anti-$\gamma\delta$ TCR; 53–7.3, anti-CD5; PK136, anti-NK 1.1; M181.1, anti-Vγ3; TM-β1, anti-IL-2Rβ (27); 040–10, anti-I-A^{b,k}. Modified anti-IL-2Rβ, anti- IL-7Rα, and anti-Vγ3 antibodies were gifts from T. Sudo (Basic Research Laboratories, Toray Industries, Inc., Kamakura), M. Miyasaka (Osaka University), and I. MacNeil (Ariad Pharmaceuticals, Cambridge), respectively. Modified anti-μ chain and anti-NK 1.1 antibodies were provided by K. Takatsu (University of Tokyo). Biotin-anti-I-A^{b,k} antibody was purchased from Meiji Milk Products Co. Ltd. (Tokyo, Japan). Other monoclonal antibodies were purchased from PharMingen. PE-streptavidin was from Biomeda Corp.

Flow cytometric analysis was done as described (22). Viable cells were analyzed by FACScan with LYSIS II software (Becton-Dickinson). Debris, erythrocytes, and dead cells were excluded from the analysis by forward and side scatter and propidium iodide gatings.

RESULTS

Generation of IL-7R-Deficient Mice. To produce a targeted disruption of the IL-7R gene, we constructed an IL-7R disruption vector (pNTIL-7RA) containing a 9.8-kb homologous DNA fragment, the PGK-*neo* cassette replacing the exon 2, and the MC1-*tk* cassette at the 5′ end of the targeting vector (Fig. 1*A*). ES cells were transfected with linearized pNTIL-7RA by electroporation, and clones resistant to both G418 and 1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-5-iodouracil were isolated. Homologous recombinant clones were detected by Southern blot analysis with probe A, a flanking sequence on the 5′ side. Two out of 145 double-drug-resistant clones showed the expected pattern of homologous recombination. Furthermore, homologous recombination and single integration in these clones were confirmed by hybridization with the probe B, a flanking sequence on the 3′ side, and the *neo* probe (data not shown). These targeted clones were injected into C57BL/6 blastocysts. One clone gave rise to chimera mice with a considerable contribution from ES cells. The chimera mice were intercrossed with C57BL/6 mice, and mice heterozygous for the mutation in the IL-7R locus were identified among offspring with agouti coat color by Southern blot analysis (Fig. 1*B*). Heterozygotic siblings were intercrossed to generate homozygotes. Animals homozygous for the IL-7R mutation were obtained at the expected Mendelian frequency. The mice were on the (129/Ola × C57BL/6) F^2 hybrid background. They were phenotypically indistinguishable at a gross level from their heterozygous and wild-type littermates.

To verify the mutation in the IL-7R gene, expression of mRNA was investigated by reverse transcriptase-coupled PCR analysis in spleen cells from wild-type and homozygous mice (Fig. 1*C*). Our gene targeting vector should have deleted the exon 2 (139 bp) of the IL-7R gene, including conserved cysteine motif. A pair of primers, encompassing the whole coding region of IL-7R, was used to amplify cDNA from spleen cells. While we observed a PCR product of approximately 1.43 kb in wild-type mice, the product in homozygous mice was smaller. Because a *Hin*dIII site is located in the exon 3 of the IL-7R gene, we digested the products with *Hin*dIII. The PCR product in wild-type mouse yielded 0.33-kb and 1.1-kb fragments, corresponding to exons 1 to 3 and exons 3 to 8, respectively. On the other hand, the product in homozygous mice gave rise to 0.20-kb and 1.1-kb fragments. The difference in size of the smaller fragment (0.13 kb) was approximately the

same as the length of exon 2. This suggests that the IL-7R mRNA detected in homozygous mice lacks the exon 2. The deletion of exon 2 theoretically causes a frame-shift mutation in IL-7R gene, and homozygous mice should produce a truncated and nonfunctional, if any, IL-7R. To check the expression of IL-7R on cell surface, we analyzed bone marrow cells by flow cytometry with an anti-IL-7R antibody (Fig. 2*A*). We detected only 0.1% of the cells reactive with the antibody in homozygous mice, while 10% of the cells expressed the receptor in wild-type mice. In addition, spleen cells were also analyzed with the same antibody. They did not express IL-7R on cell surface in homozygous mice (data not shown). These results confirmed that the targeted mutation resulted in a null allele.

Lymphocyte and NK-Cell Development in IL-7R$^-$ Mice. The number of white blood cells in peripheral blood was slightly decreased in IL-7R$^-$ mice. Differential counting showed that lymphoid cells were 2-fold reduced (data not shown). Bone marrow of IL-7R$^-$ mice was also slightly reduced in cellularity. Flow cytometric analysis showed that B-cell differentiation is severely impaired in IL-7R$^-$ mice (Fig. 2 *A* and *B*). The percentages of B220$^+$IgM$^+$ (immature B) and B220^{high}IgM$^+$ (mature B) cells were 40-fold reduced relative to in wild-type mice. On the other hand, B220$^+$IgM$^-$ (pro-B to pre-B) cells were only 10-fold reduced in IL-7R$^-$ mice. We further dissected the B220$^+$IgM$^-$ fraction with the markers of BP-1, heat stable antigen, CD43, and c-kit and found that B-cell precursors tend to be blocked at HSA^{lo} BP-1$^-$ fraction (fraction A, ref. 28) (data not shown). These results are consistent with the previous observation that B-cell development from fractions A to B is severely impaired in IL-7R-deficient mice (20).

Thymus of IL-7R$^-$ mice was greatly reduced in size and cellularity (20- to 70-fold reduction). Although the percentage of CD4$^+$8$^+$ cells was slightly decreased in IL-7R$^-$ mice, thymocyte differentiation did not appear to be arrested (Fig. 2*C*). However, dissection of CD4$^-$8$^-$ thymocytes with CD44 and CD25 markers revealed that the percentage of cells at the CD44$^+$CD25$^-$ stage was higher than that in control mice (data not shown). This result is also consistent with the previous report (20). Spleen was 3- to 12-fold reduced in cellularity. The number of mature IgM$^+$B220$^+$ B cells was 12- to 45-fold reduced (data not shown). Mature T cells bearing $\alpha\beta$ TCR were also detected in the spleen of IL-7R$^-$ mice, although they were 5- to 33-fold reduced in number relative to in wild-type mice (data not shown). The ratio of CD4$^+$ to CD8$^+$ T cells was slightly increased in IL-7R$^-$ mice (Fig. 2*D*). These results also suggested that lymphocyte production in the thymus and the bone marrow is not completely blocked and small numbers of lymphocytes can develop and accumulate in the periphery. To evaluate the role of IL-7R in lymphocyte function, we checked the response of spleen cells to Con A or lipopolysaccharide by [^{3}H]thymidine uptake. As percentages of T and B cells were reduced in the spleen of IL-7R$^-$ mice, we compensated proliferative response to the mitogens. In these proliferation assays, T and B cells from IL-7R$^-$ mice and controls responded similarly (data not shown).

Because NK cells are absent in IL-2Rγ- and Jak3-deficient mice (15, 16, 29), we checked whether IL-7R is essential for NK-cell development. On the (129/Ola × C57BL/6) F^2 hybrid background, we expect to get NK1.1$^+$ mice in three out of four. Spleen cells were analyzed with anti-CD3 and anti-NK1.1 antibodies. Percentage of NK1.1$^+$CD3$^-$ cells was 2- to 5-fold increased in IL-7R$^-$ mice (Fig. 2*E*). Because the spleen is reduced in cellularity, total number of NK1.1$^+$CD3$^-$ cells was approximately the same as in wild-type mice. To examine whether NK cells were functional, we tested spleen cells by using a ^{51}Cr release cytotoxicity assay with NK-sensitive

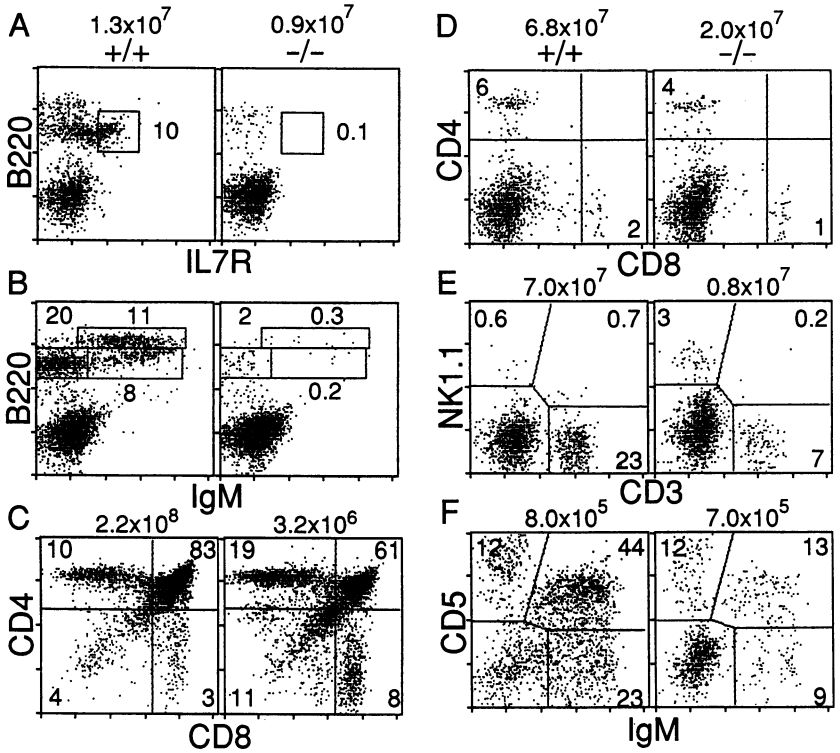

FIG. 2. Flow cytometric analyses of bone marrow, thymus, spleen, and peritoneal cells from wild-type (+/+) and IL-7R$^-$(−/−) mice. (*A* and *B*) Bone marrow cells stained with FITC-anti-B220 and either biotin-anti-IL-7Rα in *A* or biotin-anti-IgM in *B*. (*C*) Thymocytes stained with FITC-anti-CD8α and PE-anti-CD4. (*D* and *E*) Spleen cells stained with FITC-anti-CD8α and PE-anti-CD4 in *D* or FITC-anti-CD3 and biotin-anti-NK1.1 in *E*. (*F*) Peritoneal cells stained with FITC-anti-IgM and biotin-anti-CD5. Biotinylated antibodies were visualized with PE-streptavidin. The percentages of cells for a given phenotype are shown. Representative data from a 5-week-old mouse in *A–D* and *F* and an 8-week-old mouse in *E* are shown. Cell number recovered from each mouse is shown above each panel.

RLmale1 cells. After compensation, similar cytotoxic activity was observed with IL-7R$^-$ cells as control cells (data not shown). These data indicate that the signal from IL-7R is not essential for development and function of NK cells. Staining of peritoneal cells with anti-μ and anti-CD5 antibodies showed that both IgM$^+$CD5$^+$ (B-1) and IgM$^+$CD5$^-$ (conventional) B cells were reduced in percentage in IL-7R$^-$ mice (Fig. 2*F*). This result suggests that B-1 as well as conventional B cells depend on the IL-7/IL-7R system for their development and/or survival.

Absence of $\gamma\delta$ T Cells from IL-7R$^-$ Mice. Because $\gamma\delta$ T cells in the skin and the gut were severely reduced in number in IL-2Rγ- and Jak3-deficient mice (15, 16, 29), we next checked $\gamma\delta$ T-cell development in IL-7R$^-$ mice. The cell number of IELs recovered was 2-fold reduced in IL-7R$^-$ mice. Staining with CD3, $\gamma\delta$ TCR and $\alpha\beta$ TCR markers revealed that $\gamma\delta$ T cells were absent in IELs of IL-7R$^-$ mice, while they were readily detected in wild-type mice. On the other hand, $\alpha\beta$ T cells were present in both wild-type and IL-7R$^-$ mice (Fig. 3*A*). Because $\gamma\delta$ IELs are of extrathymic origin (30), this result suggests that the extrathymic pathway of $\gamma\delta$ T-cell development depends on the signal from IL-7R.

We next analyzed IHLs with anti-$\gamma\delta$ TCR, anti-CD3, anti-IL-2Rβ antibodies (Fig. 3*B*). No distinct population of $\gamma\delta$ T cells was detected in IL-7R$^-$ mice, while they were apparently present in wild-type mice (5%). On the other hand, $\alpha\beta$ T cells were reduced in percentage but still present in IL-7R$^-$ mice. As in the spleen, the percentage of IL-2Rβ^+ CD3$^-$ NK cells was 6-fold increased in IHLs of IL-7R$^-$ mice. This result also supports the conclusion above that NK-cell development does not depend on the signal from IL-7R. IL-2Rβ^+ CD3^{int} cells were reduced in percentage as well as IL-2Rβ^- CD3$^+$ cells. IL-2Rβ^+ CD3^{int} T cells are supposed to be of extrathymic origin (31). This result suggests that both the thymic and the extrathymic pathways of $\alpha\beta$ T-cell development depend on IL-7R.

To analyze DETCs in the skin, epidermal sheets were stained with anti-Thy-1 antibody. Thy-1$^+$ DETCs were completely absent in the epidermis of IL-7R$^-$ mice (Fig. 4*A*). This result was further confirmed by flow cytometric analysis (Fig. 4*B*). Thy-1$^+$ I-A$^-$ DETCs were greatly reduced in percentage in IL-7R$^-$ mice, while Thy-1$^-$ I-A$^+$ Langerhans cells were unchanged. In addition, no distinct population of $\gamma\delta$ T cells was detected in the spleen of IL-7R$^-$ mice (Fig. 4*C*). These results suggested that all kinds of mature $\gamma\delta$ T cells are absent in IL-7R$^-$ mice.

To determine whether the development in the thymus is blocked or the survival in the periphery is severely impaired, Vγ3 T cells, the precursors of DETCs, were analyzed in fetal thymus. Fetal thymocytes at day 17 of gestation were stained with anti-Vγ3 and anti-CD3 antibodies. Although V$\gamma3^+$ CD3$^+$ cells were detected in wild-type fetuses (4%), no distinct population of V$\gamma3^+$ CD3$^+$ cells was observed in IL-7R$^-$ fetuses (Fig. 5*A*). Furthermore, $\gamma\delta$ TCR$^+$ CD3$^+$ cells, the precursors of the majority of $\gamma\delta$ T cells in the spleen and the lymph node, were also absent in adult thymus of IL-7R$^-$ mice (Fig. 5*B*). These results suggested that the development of $\gamma\delta$ T cells is severely blocked in fetal and adult thymus of IL-7R$^-$ mice.

DISCUSSION

In the present study, we demonstrated that $\gamma\delta$ T cells are absent from IL-7R-deficient mice. In contrast, $\alpha\beta$ T cells and B cells are reduced but still exist in certain numbers, and NK cells develop normally. In light of these observations, we propose a hierarchy of dependency on the IL-7/IL-7R system among lymphoid cells. (*i*) $\gamma\delta$ T cells completely depend on the IL-7/IL-7R system for their generation and/or survival. (*ii*) $\alpha\beta$ T and B cells partially depend on IL-7R for their generation. Lastly, NK cells seem not to require a signal from IL-7R. $\gamma\delta$ T cells were not detected in various peripheral tissues, suggesting that the thymic and extrathymic pathways and the epithelial- and non-epithelial-associated pathways are severely impaired in IL-7R-deficient mice. These results indicate that IL-7 is involved in the mechanism common to the development of all kinds of $\gamma\delta$ T cells. IL-7 produced by the keratinocytes and the gut epithelial cells may play a role in the survival of epithelial-associated $\gamma\delta$ T cells in the periphery (13, 14). Furthermore, IL-7 has been shown to promote rearrangement of TCR γ genes in fetal liver culture system (2). On the other hand, IL-7R is not essential for V-D-J recombination of TCR α- and β-chain genes or induction of RAG genes in $\alpha\beta$ T-cell precursors, because certain numbers of mature $\alpha\beta$ T cells developed in IL-7- and IL-7R-deficient mice. Collectively, two possibilities can be proposed to explain the lack of $\gamma\delta$ T cells. (*i*) IL-7R may deliver an essential signal for rearrangement of TCR γ genes, either by making the TCR γ locus accessible to

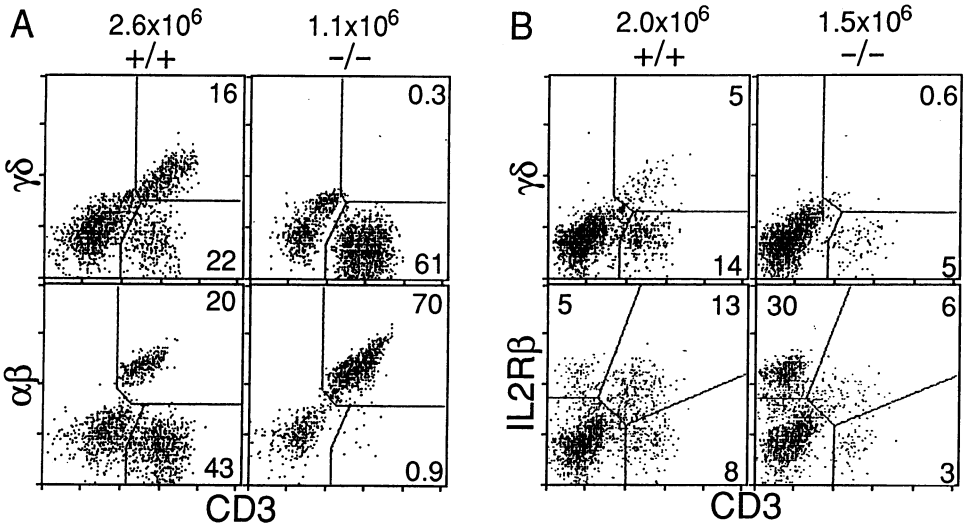

FIG. 3. Absence of $\gamma\delta$ T cells in IELs and IHLs of IL-7R$^-$ mice. (*A*) IELs stained with FITC-anti-CD3 and either biotin-anti-$\gamma\delta$ TCR (*Upper*) or biotin-anti-$\alpha\beta$ TCR (*Lower*). (*B*) IHLs stained with FITC-anti-CD3 and either biotin-anti-$\gamma\delta$ TCR (*Upper*) or biotin-anti-IL2Rβ (*Lower*). Biotinylated antibodies were visualized with PE-streptavidin. The percentages of cells for a given phenotype are shown. Representative data from 8-week-old wild-type (+/+) and IL-7R$^-$ (−/−) mice are shown. Cell number recovered from each mouse is shown above each panel.

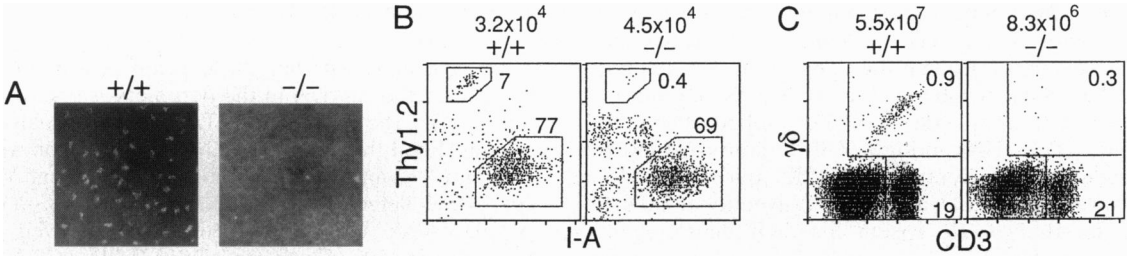

FIG. 4. Absence of γδ T cells in the skin and the spleen of IL-7R$^-$ mice. (*A*) Immunofluorescence analysis of the epidermis. Epidermal sheets of 5-week-old mice were stained with FITC-anti-Thy1.2 and examined under a fluorescence microscope. (*B*) Flow cytometric analysis of epidermal cells. Epidermal cells of an 8-week-old mouse were stained with FITC-anti-Thy1.2 and biotin-anti-I-A, followed by PE-streptavidin. (*C*) Flow cytometric analysis of adult spleen. Spleen cells of a 7-week-old mouse were stained with biotin-anti-γδ TCR and FITC-anti-CD3 antibodies, followed by PE-streptavidin. The percentages of cells for a given phenotype are indicated. Wild-type (+/+) and IL-7R$^-$ (−/−) mice are shown. Cell number recovered from each mouse is shown above each panel.

recombinases or by inducing locus-specific transacting factors. In this respect, analysis of transgenic γδ T cells in IL-7R-deficient mice may help to test this possibility. (*ii*) IL-7 may be an indispensable factor for the survival of γδ, but not αβ, T-cell precursors. It is also to be noted that at least one additional cytokine, thymic-stromal-derived lymphopoietin (TSLP), binds to IL-7Rα (20). Thus, it is yet to be elucidated whether IL-7 and TSLP have any differential role on the generation of γδ T cells. IL-7R transduces its signal via several molecules: Jak1, Jak3, Stat5, fyn, and phosphatidylinositol 3-kinase (7, 32). From a study of breeding *lck*-deficient mice with TCR γδ transgenic mice, it has been shown that *lck* is crucial for the thymic, but not intestinal, maturation of γδ T cells (33). Because Jak3-deficient mice lack γδ T cells in the skin and the gut (29), most of the signal from IL-7R for γδ T-cell development is probably delivered by Jak3 kinase. It is yet to be clarified whether *lck* also plays a role in the signal transduction of IL-7R.

The pre-TCR, composed of pTα and TCR β chains, is expressed on early thymocytes and important for their differentiation into CD4$^+$8$^+$ stage (34). pTα-deficient mice have severely impaired development of αβ, but not γδ, T cells (35). In this respect, it is possible to explain the difference in the mechanism of αβ and γδ T-cell development as follows. IL-7R plays an important role in both αβ and γδ T-cell lineages during the early stages of development. In αβ T-cell precursors, however, the signal from pre-TCR takes over, driving them into the further steps of development. On the contrary, the γδ T-cell development continues to depend on the signal from IL-7R, and its depletion causes a complete loss of mature cells. Like pre-TCR in αβ T-cell precursors, a similar pre-B-cell receptor, composed of surrogate light and μ heavy chains, drives B-cell precursors to mature (36). Therefore, it may be reasonable that we observed decreased, but certain, numbers of mature B cells in periphery in IL-7R-deficient mice. Differentiation of CD4$^-$8$^-$3$^-$ triple negative thymocytes takes place in four successive stages: CD44$^+$25$^-$, CD44$^+$25$^+$, CD44$^-$25$^+$, and CD44$^-$25$^-$ stages (34). As described above, differentiation of CD4$^-$8$^-$3$^-$ thymocytes was most profoundly hampered at the CD44$^+$25$^-$ stage in IL-7R-deficient mice. In contrast, they tend to be blocked at the CD44$^-$25$^+$ stage in pTα-deficient mice (35). Because thymocyte differentiation is not completely arrested, it might be possible that small numbers of αβ T-cell precursors can proceed into the CD44$^+$25$^+$ and the CD44$^-$25$^+$ stages and be rescued by the signal from pre-TCR in IL-7R-deficient mice. However, since it has been reported (37) that αβ and γδ lineages do not diverge until late in development, there remains a possibility that γδ precursors might utilize the signal from pre-TCR at some developmental stages but that they might continue to depend on the signal from IL-7R even after the commitment into γδ lineage.

In addition to IL-7, the c-kit ligand is also a major factor driving expansion of very immature thymocytes. *W/W* mice have 40-fold reduced numbers of Thy-1^{lo} CD2$^{-/lo}$ CD4$^-$8$^-$ cells compared with wild-type littermates (38). Thus, the signal from c-kit may also drive thymocyte differentiation at early stages in IL-7R-deficient mice. Furthermore, the number of γδ IELs decreased in older *W/W^v* and *Sl/Sl^d* mutant mice, while γδ T cells were normal in thymus, spleen, and skin (39). Because this result may have been affected by residual activity of the incomplete mutations in the *W^v* and the *Sl^d* alleles, it is still to be clarified whether c-kit, like IL-7R, plays a differential role in αβ and γδ T-cell development.

Little is known about the stages of extrathymic αβ T-cell development. We observed reduced, but certain, numbers of IL-2Rβ$^+$ CD3^{int} αβ T cells in the liver. This result implies that the pre-TCR probably plays an important role in extrathymic as well as thymic αβ T-cell development. On the other hand, NK cells do not depend on the signal from IL-7R for their development, although IL-2Rγ-deficient mice have severely reduced numbers of NK cells (15, 16). The signal responsible for NK-cell generation is not known. Because IL-2-deficient mice have a reduced, but still substantial, level of NK-cell activity (40), it is possible that other cytokines binding to

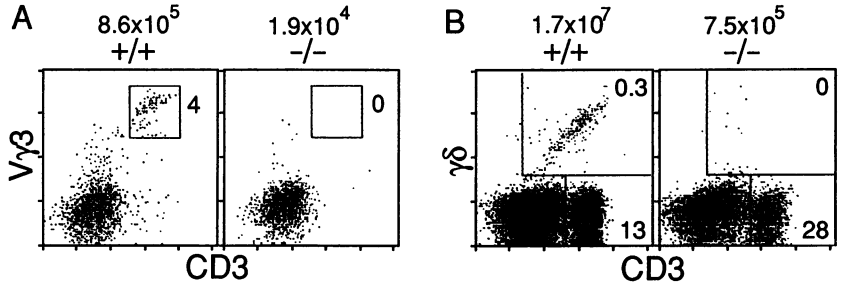

FIG. 5. γδ T-cell development in fetal and adult thymus of IL-7R$^-$ mice. Fetal thymocytes at day 17 of gestation (*A*) and thymocytes of 7-week-old mice (*B*) were stained with FITC-anti-CD3 and biotin-anti-Vγ3, followed by PE-streptavidin. The percentages of cells for a given phenotype are shown. Wild-type (+/+) and IL-7R$^-$ (−/−) mice are shown. Cell number recovered from each mouse is shown above each panel.

IL-2Rβ, such as IL-15, play an important role in NK-cell development.

IL-7R-deficient mice showed an unequivocal phenotype that all kinds of γδ T cells are absent. Thus, they will provide a unique system to analyze the mechanism of αβ and γδ T-cell development. They can be used to dissect the machinery for the proliferation and the differentiation of T-cell precursors, by intercrossing with various kinds of transgenic and knockout mice.

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