Brief Definitive Report

Interleukin-7 Receptor α Is Essential for the Development of γδ$^+$ T Cells, but Not Natural Killer Cells

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Summary

Mice that lack a functional γc subunit of the receptors for interleukin (IL)-2, IL-4, IL-7, IL-9, and IL-15 display profound defects in lymphoid development. The IL-7/IL-7R system represents a critical interaction for conventional T and B cell development. In this report, the role of IL-7Rα in the development of lymphoid lineages other than conventional T and B cells was examined. We demonstrate that γδ$^+$ T cells were absent in IL-7Rα–deficient mice, whereas the development and function of natural killer cells were normal. Thus, IL-7Rα function is required for the development of γδ$^+$ T cells but not natural killer cells.

Lymphocyte development is critically dependent on cytokines for the expansion and differentiation of multipotential progenitors. Mutations in the γc subunit of the receptors for IL-2, IL-4, IL-7, IL-9, and IL-15 (1–8) in humans result in X-linked severe combined immunodeficiency (SCID) (9). This disease is characterized by the absence or greatly reduced numbers of T cells and NK cells with the presence of nonfunctional B cells (10). Mice lacking γc exhibit defects in lymphoid development that appear more severe than human X-linked SCID. These mice are deficient in the development of T cells, B cells, NK cells, Thy-1$^+$ γδ dendritic epidermal T cells (DETC), and intraepithelial lymphocytes (IEL) (11, 12). Because γc is shared by at least five cytokine receptors, this phenotype likely results from the disruption of the function of several cytokines. Recently, IL-7 has been identified as one key cytokine for lymphocyte development. For mice treated with mAbs to IL-7 or IL-7Rα (13–15), or deficient in expression of the IL-7 or the IL-7Rα genes (16, 17), T and B cell development was blocked at very early stages. It is not known, however, whether signaling through IL-7Rα is also critical for supporting the development of other lymphoid lineages, such as NK and γδ$^+$ DETC cells. In this study, this issue was examined by phenotypic and functional analysis of IL-7Rα–deficient mice. We show here that γδ$^+$ T cells are absent, while the development and function of NK cells are normal in IL-7Rα–deficient mice.

Materials and Methods

Animals. IL-7Rα deficient mice, back-crossed for five generations to C57BL/6 mice, were kindly provided by Immunex Research and Development Corp. (Seattle, WA) (17). C57BL/6 mice were purchased from The Jackson Laboratory (Bar Harbor, ME) or obtained from the Specialized Animal Facility at the University of Miami. 5–7-wk-old age-matched C57BL/6 mice were used as controls throughout the study.

Flow Cytometric Analysis. Cells from the thymus, spleen, and skin were subjected to multicolor staining and analyzed on a FACScan® (Becton Dickinson & Co., Mountain View, CA) flow cytometer using LYSIS II software. Before staining, cells were incubated with an mAb to the FcR (2.4G2) to prevent nonspecific binding, and then sequentially stained with biotinylated antibodies, PE-avidin, FITC-mAbs, and Cy-Chrome-mAbs. Data were collected from 10^4 viable cells, as determined either by a combination of forward and side scatter or by staining with propidium iodide. The following mAbs were purchased from PharMingen (San Diego, CA): biotin-GL3 (anti-γδ TCR), biotin-H57-597 (anti-αβ TCR), biotin-PK136 (anti-NK1.1), Cy-Chrome–RM-4-5 (anti-CD4), and Cy-Chrome–53-6.7 (anti-CD8α). The following antibodies were purified and labeled in our laboratory: FITC-145-2C11(anti-CD3ε) and FITC-HO13.4 (anti-Thy1.2). The anti–IL-2Rβ mAb 5H4 was produced in our laboratory and described elsewhere (18).

NK Assay. To assay spontaneous NK activity, splenocytes were incubated with ^{51}Cr-labeled YAC-1 target cells (10^4) in 0.2 ml of RPMI 1640 medium containing 5% FCS in round-bottom plates for 6 h, and an aliquot (0.1 ml) of the culture fluid was counted in a gamma scintillation counter as described (19). IL-2–activated NK (LAK) activity was examined by culturing splenocytes (2×10^6/ml) with 500 U/ml mouse rIL-2 for 72 h at 37°C in a humidified atmosphere containing 7% CO_2 and then harvesting the adherent cells as effectors. To test the enhancement of NK activity by Poly(I):(C) (Sigma Immunochemicals, St. Louis, MO), mice were injected intraperitoneally with 100 μg Poly(I):(C) 48 and 72 h before harvesting the spleen cells. Data are calculated as previously described (19) and reported as the means of triplicate determinations with SD that varied by <10%.

Epidermal Cell Preparation. Epidermal cells were prepared by using the method described by Sullivan et al. (20). Shaved mouse torso skins were removed and trimmed of subcutaneous fat and blood vessels. Each pelt was cut into 0.5-cm strips and placed dermal side up in 0.3% trypsin (Boehringer Mannheim, Indianapolis,

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IN) PBS solution for 1–2 h at 37°C. Epidermal cells were carefully scraped from the dermis and placed in fresh 0.3% trypsin PBS containing 20 μg/ml of DNAase I (Sigma) at 37°C for 10 min, followed by addition of FCS to inactivate trypsin. Cells were further disaggregated mechanically by drawing and expressing the cell suspension with a 5-ml syringe. Disaggregated cells were then centrifuged over Ficoll-Paque (Pharmacia Biotech, Uppsala, Sweden) at 25°C for 20 min. The cell band at the medium–Ficoll interface was harvested and washed three times with RPMI 1640 medium before FACS® analysis.

Results

The Development and Function of NK Cells in IL-7Rα–deficient Mice. To examine whether NK cell development is normal in the IL-7Rα–mutant mice, splenocytes were stained with mAbs to NK1.1 and CD3. NK-1.1$^+$ CD3$^-$ cells were easily detected in the IL-7Rα–deficient mice (Fig. 1). Although the percentage of NK-1.1$^+$ CD3$^-$ cells in the spleen from IL-7Rα–deficient mice is ~10-fold higher than that in spleen cells from age-matched C57BL/6 mice, the actual numbers of these cells are similar for both normal and mutant mice (IL-7Rα +/+: $3.3 \pm 0.3 \times 10^6$, $n = 6$; IL-7Rα −/−: $3.5 \pm 0.2 \times 10^6$, $n = 6$). Since NK cells express relatively high levels of the IL-2Rβ chain (21), splenocytes were further analyzed with the 5H4 mAb to the IL-2Rβ chain. As expected, almost all the NK-1.1$^+$ cells reacted with the anti–IL-2Rβ mAb (Fig. 1). These results indicate that NK cell development is not obviously affected by the inactivation of the IL-7Rα gene.

To determine whether NK cells in the IL-7Rα mutant mice are functional, unfractionated splenocytes from untreated or Poly(I):(C)–treated mice were tested for their lytic activity on the NK-sensitive target, YAC-1 cells. Poly(I):(C) has been shown to induce IFN production from macrophages, which in turn enhance NK activity (22). Splenocytes from IL-7Rα–deficient mice readily lysed YAC-1 targets at levels that were higher than detected for normal C57BL/6 mouse spleen cells. (Fig. 2 *A*). The stronger lytic activity by IL-7Rα–deficient splenocytes is most likely caused by a higher number of NK-1.1$^+$ cells added in the assay. The lysis of YAC-1 targets by splenocytes from Poly(I):(C)–treated IL-7Rα–deficient and normal mice was greatly increased, reaching a maximal level at E/T of 100:1 for cells from the IL-7Rα–mutant mice (Fig. 2 *A*). These findings indicate that the basal and Poly(I):(C)–induced NK activities are not affected by the inactivation of IL-7Rα gene.

High concentration of IL-2 induces NK cells to differentiate into LAK cells (23). To determine whether NK cells differentiate into LAK cells from the spleens of IL-7Rα–mutant mice, splenocytes from normal and IL-7Rα–mutant mice were cultured with a high concentration of IL-2 for 72 h, and the IL-2–activated NK activity was assessed for the adherent cells. The IL-2–activated cells from the IL-7Rα–mutant mice efficiently lysed YAC-1 targets (Fig. 2 *B*). Thus, IL-7Rα is not required for IL-2–induced NK activation. Collectively, these results demonstrate that the development and function of NK cells are not dependent on IL-7Rα.

γδ$^+$ T Cells Are Absent in IL-7Rα–deficient Mice. Thy-1$^+$ DETC in the skin consist of a large population of cells that almost exclusively express γδ TCR (24). To examine whether γδ$^+$ DETC were present in the skin of IL-7Rα–mutant mice, epidermal cells were prepared and directly stained with mAbs specific for T cells. Epidermal cells from the Ficoll interface from control C57BL/6 mice contained readily detectable γδ$^+$ DETC that varied from 2 to 10% in different experiments. Similar percentages of CD3$^+$ and

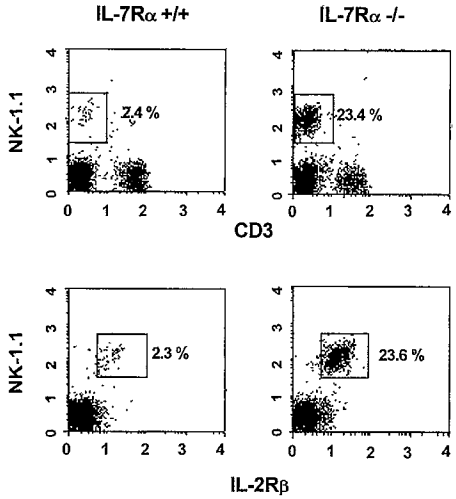

Figure 1. The presence of NK cells in the spleens of IL-7Rα mutant mice. Splenocytes from 6-wk-old control C57BL/6 (*IL-7Rα* +/+) and IL-7Rα–deficient mice (*IL-7Rα* −/−) were stained with biotin–NK-1.1, followed by PE-streptavidin and either FITC–anti-CD3 (*A*) or FITC–anti-IL-2Rβc (*B*), and were analyzed by flow cytometry. Boxed areas are the populations referred to in the text.

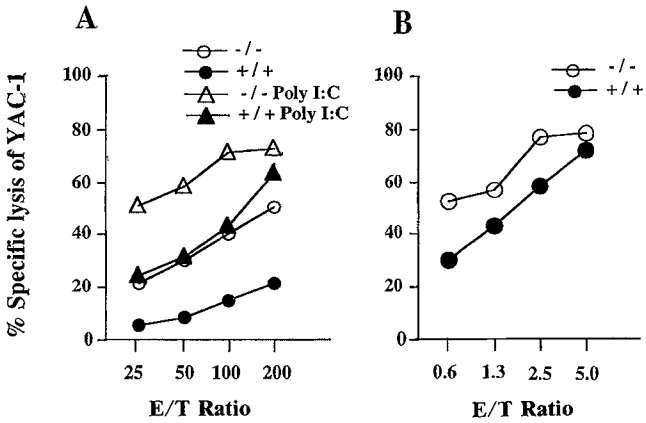

Figure 2. NK activity from IL-7Rα–deficient mice. (*A*) NK activity of fresh splenocytes from untreated and Poly(I):(C)–treated 5–6-wk-old control C57BL/6 (+/+) and IL-7Rα–deficient mice (−/−). (*B*) NK activity of the adherent fraction of spleen cells from 5–6-wk-old control C57BL/6 (+/+) and IL-7Rα–deficient mice (−/−) cultured with mIL-2 (500 U/ml) for 72 h. Data represent the mean value of triplicates from one of three similar experiments.

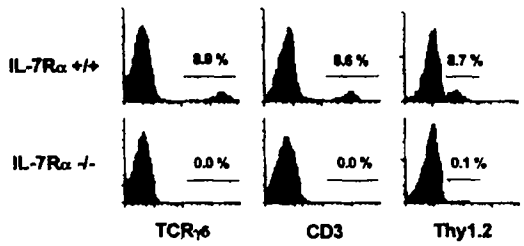

Figure 3. Absence of γδ⁺ DETC in the skin of IL-7Rα–mutant mice. Flow cytometric analyses were performed on epidermal cells from 6-wk-old control C57BL/6 (*IL-7Rα* +/+) and IL-7Rα–deficient mice (*IL-7Rα* −/−). Interface epidermal cells were preincubated with an mAb to FcR, followed by staining with either biotin–anti-TCRγδ, PE-streptavidin, FITC–anti-CD3, or FITC–anti-Thy1.2. Percentages of positive cells are indicated.

Thy1.2⁺ cells were also detected in the control skin. A representative FACS® profile is shown in Fig. 3. By contrast, γδ⁺ DETC were not detected in the skin from IL-7Rα–mutant mice (Fig. 3). These epidermal cells also lacked cells that express CD3 and Thy1.2 (Fig. 3). These results were further confirmed by direct examination of epidermal sheets under fluorescence microscopy (data not shown).

TCR γδ⁺ cells account for a very minor subset of cells in thymus and spleen (25, 26). To detect γδ⁺ T cells in the thymus, cells were analyzed by three-color staining. CD4⁻CD8⁻ thymocytes were gated and examined for expression of γδ or αβ TCR. The "double-negative" thymocytes from control mice contained 20–25% CD3⁺ cells, of which 30–40% expressed γδ TCR and 60–70% expressed αβ TCR (Fig. 4 *A*). By contrast, very few CD4 CD8⁻ TCR γδ⁺ thymocytes were detected in the IL-7Rα–deficient mice, while a correspondingly higher percentage of CD4⁻CD8⁻ TCR αβ⁺ thymocytes were found (Fig. 4 *A*).

Since the majority of CD4⁺ or CD8⁺ T cells in the spleen express αβ TCR (26), CD3⁺CD4⁻CD8⁻ splenocytes were gated and analyzed for the expression of γδ TCR. This subset accounted for ~0.7% and 0.1% of unfractionated splenocytes from the control and IL-7Rα–deficient mice, respectively. Within this population, 36% of the control cells expressed γδ TCR, and an equal percentage of the cells expressed αβ TCR (Fig. 4 *B*). In the IL-7Rα–mutant mice, very few CD3⁺CD4⁻CD8⁻ splenocytes expressed γδ TCR, whereas a majority of these cells expressed αβ TCR (Fig. 4 *B*).

Discussion

Three important points emerge from this study. First, the absence of γδ⁺ T cells and normal development and function of NK cells in IL-7Rα–deficient mice indicate that inactivation of IL-7 function in γc-mutant mice accounts for part, but not all, of the defects in the development of multiple lymphoid lineages. Second, both IL-7– and thymic stromal cell–derived lymphopoeitin (TSLP) (17) are not critically involved in the development and function of NK cells. Third, signaling through IL-7Rα appears more critical for the development of γδ T cells than for αβ T cells.

As IL-7 and IL-7Rα–mutant mice exhibit severe defects in conventional T and B cell development, it is clear that signaling through the IL-7Rα is critical for both T and B cell development, and that the phenotype of γc–mutant mice and human X-linked SCID is as least partially explained by a deficiency in the IL-7/IL-7R system. Furthermore, IL-7Rα is a subunit for not only IL-7, but also TSLP, which shares many biological properties with IL-7 (17, 27). It is likely that the more severe phenotype in IL-7Rα–mutant mice reflects the consequences of inactivation of both IL-7 and TSLP function. However, the presence of a normal number of functional NK cells in IL-7Rα–deficient mice demonstrates that the IL-7R system does not solely explain the phenotype of γc–mutant mice and indicate that the development of NK is dependent on a cytokine whose receptor uses γc, but is independent of IL-7 and IL-7Rα. Since the development of NK cells is normal in IL-2– and IL-4–deficient mice (28, 29), it is highly likely that these cytokines do not critically control NK development.

Given the tremendous heterogeneity of γδ⁺ T cells with respect to their generation, anatomical localization, and V gene segment usages (for review see reference 24), we were surprised that no γδ⁺ T cells were detected in the skin, thymi, and spleens of IL-7Rα–mutant mice. Furthermore, in studies that have investigated the role of γc in IEL development, γδ⁺ IELs were not detected in the small intestines of IL-7Rα–deficient mice (He, Y.-W., and T.R.

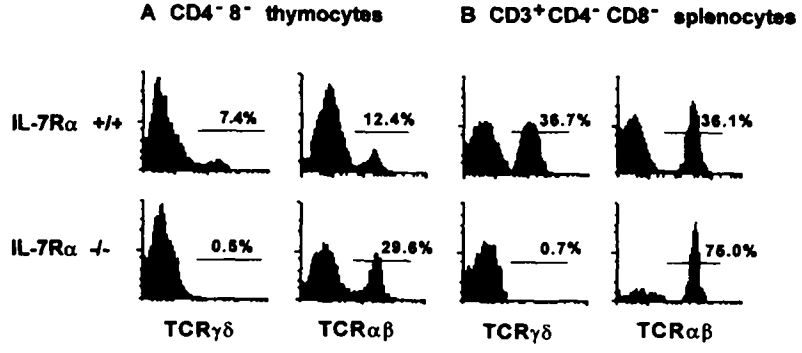

Figure 4. Absence of γδ⁺ T cells in the spleens and thymi of IL-7Rα mutant mice. (*A*) Thymocytes were stained with either biotin–anti-TCRγδ or biotin–anti-TCRαβ, followed by PE-streptavidin, FITC–anti-CD4, and Cy-Chrome–anti-CD8; CD4⁻CD8⁻ "double-negative" cells were gated and analyzed for αβ and γδ TCR expression by collecting 5 × 10³ viable cells. (*B*) Splenocytes were stained with either biotin–anti-TCRγδ or biotin–anti-TCRαβ, followed by PE-streptavidin, FITC–anti-CD3, Cy-Chrome–anti-CD4, and Cy-Chrome–anti-CD8; the CD3⁺CD4⁻CD8⁻ cells were gated and analyzed for αβ and γδ TCR expression by collecting 5 × 10³ viable cells. Percentages of positive cells are indicated in both panels.

Malek, unpublished data). Thus, IL-7Rα signaling appears to be required for the development of all γδ$^+$ T cells. Our findings are consistent with several in vitro studies that demonstrated a role for IL-7 in inducing expression of γδ TCR by CD3$^-$CD4$^-$CD8$^-$ thymocytes (30) and in supporting the growth of DETC (31). However, the precise role of IL-7Rα for γδ T cell development is unknown. Signaling via this receptor may be critical for commitment to the γδ T cell lineage, or after commitment, it may be essential for γδ TCR rearrangement.

There is an obvious dichotomy in the requirement for IL-7Rα function for development of γδ$^+$ and αβ$^+$ T cells. Although the overall number of thymocytes and splenic T cells was reduced ~10-fold in the IL-7Rα–mutant mice (17), essentially all the CD4$^+$ or CD8$^+$ mature T cells in these organs expressed αβ TCR (He, Y.-W., and T.R. Malek, unpublished observation). Furthermore, correspondingly higher than normal percentages of CD4$^-$CD8$^-$ thymocytes and splenic T cells also expressed αβ TCR. These data indicate that IL-7Rα signaling is not essential for the rearrangement of the αβ TCR. This conclusion is in contrast to the observation that IL-7 induced V(D)J rearrangement of the TCR β gene and sustained the expression of *RAG*-1 and *RAG*-2 genes in vitro in thymocyte suspensions from mouse embryos (32). When mice are treated with mAbs to IL-7, the earliest T cell precursors, i.e., CD44$^+$CD25$^-$CD4$^-$CD8$^-$ thymocytes, inefficiently make the transition to the next stage of thymocyte maturation, i.e., CD44$^+$CD25$^+$CD4$^-$CD8$^-$ thymocytes (14). It is only after this time that TCR β gene rearrangement occurs (33). Thus, we favor a predominant role for IL-7Rα signaling for the expansion and/or survival of the earliest T cell precursors in the thymus without an essential role in the induction of αβ TCR rearrangement.

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