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IL-7 Produced by Thymic Epithelial Cells Plays a Major Role in the Development of Thymocytes and TCRγδ+ Intraepithelial Lymphocytes

Soichiro Shitara,* Takahiro Hara,*,1 Bingfei Liang,*, Keisuke Wagatsuma,*, Saulius Zuklys,‡ Georg A. Holländer,‡,§ Hiroshi Nakase,‡ Tsutomu Chiba,‡ Shizue Tani-ichi,* and Koichi Ikuta*

IL-7 is a cytokine essential for T cell development and survival. However, the local function of IL-7 produced by thymic epithelial cells (TECs) is poorly understood. To address this question, we generated IL-7−/− mice and crossed them with FoxN1 promoter-driven Cre (FoxN1-Cre) mice to establish knockout mice conditionally deficient for the expression of IL-7 by TECs. We found that αβ and γδ T cells were significantly reduced in the thymus of IL-7−/− FoxN1-Cre mice. Proportion of mature single-positive thymocytes was increased. In lymph nodes and the spleen, the numbers of T cells were partially restored in IL-7−/− FoxN1-Cre mice. In addition, γδ T cells were absent from the fetal thymus and epidermis of IL-7+/− FoxN1-Cre mice. Furthermore, TCRγδ+ intraepithelial lymphocytes (IELs) were significantly decreased in the small intestines of IL-7+/− FoxN1-Cre mice. To evaluate the function of IL-7 produced in the intestine, we crossed the IL-7−/− mice with villin promoter-driven Cre (Vil-Cre) mice to obtain the mice deficient in IL-7 production from intestinal epithelial cells. We observed that αβ and γδ IELs of IL-7−/− vil-Cre mice were comparable to control mice. Collectively, our results suggest that TEC-derived IL-7 plays a major role in proliferation, survival, and maturation of thymocytes and is indispensable for γδ T cell development. This study also demonstrates that IL-7 produced in the thymus is essential for the development of γδ IELs and indicates the thymic origin of γδ IELs. The Journal of Immunology, 2013, 190: 000–000.

Interleukin-7 is a cytokine essential for lymphocyte development and survival. The IL-7R consists of the common cytokine receptor γ chain and the unique IL-7R α-chain (IL-7Rα). Mice deficient in IL-7 or IL-7Rα show marked reductions in T and B cells (1, 2). Early lymphocytes depend on IL-7 for their proliferation and survival in the thymus and bone marrow. In addition, IL-7 plays an important role in the differentiation of positively selected CD8 T cells in the thymus (3, 4). Furthermore, IL-7R and STAT5 control V(D)J recombination in the IgH and TCRγ loci (5–7). In the periphery, IL-7 regulates T cell homeostasis by enhancing survival and proliferation of naive and memory T cells (8). Furthermore, IL-7−/− mice showed marked reductions in TCRγδ+ intraepithelial lymphocytes (IELs) in the intestine (9).

IL-7 is produced in the thymus, lymph nodes (LNs), skin, intestines, and liver. In the thymus, IL-7 mRNA is detected in thymic epithelial cells (TECs) and mesenchymal stromal cells (10, 11). In the bone marrow, IL-7 expression is detected in mesenchymal stromal cells (12). In the periphery, IL-7 mRNA is detected in fibroblastic reticular cells at T cell zone of LNs (13). Furthermore, IL-7 is expressed in epidermal keratinocytes and IECs (14, 15). Recently, IL-7 reporter mice confirmed IL-7−/− expressing cells in thymus, bone marrow, and peripheral lymphoid organs (16–19). However, little is known about the local function of IL-7 produced by each cell type.

Development of IELs depends on common cytokine receptor γ chain cytokines. IELs are composed of TCRαβ+ IELs (αβ IELs) and TCRγδ+ IELs (γδ IELs). Although IL-7−/− mice totally lack γδ T cells in the thymus and intestines, many αβ IELs develop without IL-7 (20), suggesting that IL-7 is essential for γδ IEL development. In contrast, IL-15−/− mice show reduced numbers of γδ IELs and CD8α+ αβ IELs (21), indicating that IL-15 plays some role in IEL development. A fate-mapping experiment showed that αβ IELs originate from the thymus (22). Because γδ IELs develop in reduced but substantial numbers in nude mice (23), the issue on thymic versus extrathymic generation of γδ IELs is still a matter of debate (24, 25).

To address these questions, we established IL-7−/− mice and crossed them with FoxN1 promoter-driven Cre (FoxN1-Cre) and villin promoter-driven Cre (Vil-Cre) transgenic (Tg) mice to...
obtain conditional knockout mice deficient in IL-7 production from TECs and IECs, respectively. We found that the numbers of thymocytes were severely reduced and the populations of CD4+8+ or CD4+8- single-positive (SP) thymocytes were changed in FoxN1-Cre IL-7f/f mice, suggesting that IL-7 produced by TECs plays a major role in T cell development within the thymus. In addition, the numbers of γδ IELs were severely decreased in the small intestine of FoxN1-Cre IL-7f/f mice but not Vil-Cre IL-7f/f mice, indicating that IL-7 produced by TECs plays an important role in γδ IEL differentiation. Therefore, this study demonstrates that IL-7 produced by TECs is essential for development of thymocytes and has novel functions in maturation of SP thymocytes. Moreover, it indicates the thymic origin of γδ IELs.

Materials and Methods

Mice

IL-7flox/x and FoxN1-Cre Tg mice were reported previously (26, 27). Vil-Cre Tg mice were obtained from The Jackson Laboratory (28). These Tg mice were bred on a C57BL/6 background. Mice were maintained under specific pathogen-free conditions in the Experimental Research Center for Infectious Diseases in the Institute for Virus Research, Kyoto University (Kyoto, Japan). All mouse protocols were approved by Kyoto University.

Abs and flow cytometry

The following fluorescent dye- or biotin-conjugated Abs were used: CD3 (145-2C11), CD4 (GK1.5), CD8α (53-67.2), TCRβ (H57-597), TCRγδ (GL2), CD11b (M1/70), CD19 (17-0191), B220 (6B2), CD25 (PC61), CD27 (LG3A10), CD44 (IM7), L-selectin (CD62L) (MEL-14), CD69 (H1.2F3), c-Ki (2B8), GR-1 (RB6-8C5), TER119, MHC class II (MHC II) (M5/114,15.2), Qu-2 (6H9/1-9), NK1.1 (PK136), HSA (M1/69), IL-7Rα (A7R34), and rat IgG2a isotype control (eB2Rα). The modified Abs were obtained from eBioscience (San Diego, CA), Biologend (San Diego, CA), Vector Laboratories (Burlingame, CA), and R&D Systems (Minneapolis, MN). PE–streptavidin was purchased from eBioscience. Cells were analyzed by FACS Calibur and FACS Canto II flow cytometers (BD Biosciences, Franklin Lakes, NJ) and analyzed with FlowJo software (Tree Star, Ashland, OR).

Cell isolation

IELs were isolated from small intestine as described previously (29). Isolation of epidermal cells and immunofluorescence staining of epidermal sheets were performed as described previously (30). IECs were isolated as reported previously (31).

Real-time RT-PCR

Total RNA was extracted with TRizol reagent (Invitrogen, Carlsbad, CA), and reverse transcribed using ReverTra Ace (Toyobo, Osaka, Japan). All mouse protocols were approved by Kyoto University. In addition, some medullary TECs expressed Aire in IL-7f/f FoxN1-Cre mice (Supplemental Fig. 1B). These results suggest that the IL-7 gene was successfully inactivated in TECs of IL-7f/f FoxN1-Cre mice.

It was previously reported that thymocytes were reduced by 90–95% in IL-7−/− mice (1). However, little is known about the function of IL-7 produced by TECs. To address this question, we analyzed the thymocytes of IL-7f/f FoxN1-Cre mice by flow cytometry. Although the proportion of CD4+CD8+ double-positive (DP) thymocytes was decreased in IL-7f/f FoxN1-Cre mice, those of CD4+CD8− double-negative (DN), CD4−CD8− SP (CD4 SP), CD4−CD8+ SP (CD8 SP), CD3−TCRβ+ (αβ T), CD3−NK1.1+ (NK), and CD3−NK1.1+ (NKT) cells were unchanged or slightly increased (Fig. 2A). In contrast, the proportion of CD3−TCRγδ+ (γδ T) cells was severely reduced. The absolute numbers of total, DN, DP, CD4 SP, CD8 SP, αβ T, NK, and NKT cells were decreased by 36–40%, 23–24%, 24–27%, and 11-fold, respectively (Fig. 2B). The numbers of γδ T cells were severely decreased by 180-fold. These results suggest that IL-7 produced by TECs plays a major role in T cell development and is essential for γδ T cells in the thymus.

T cell development begins from early thymic progenitors (ETPs), which progress to DN2, DN3, and DN4 thymocytes (33). CD4+ and CD8+ T cells are divided into DN2a, DN2b, DN3a, and DN3b subsets (34, 35). Therefore, we next analyzed early thymocyte subsets in IL-7f/f FoxN1-Cre mice by flow cytometry. We found severe reduction in ETP (1/1700) subsets, slight recovery in DN2b (1/39) and DN3a (1/94), and examined by a confocal laser scanning microscope (TSC-SP2; Leica Microsystems, Tokyo, Japan).

Statistics

An unpaired two-tailed Student t test was used for all of the statistical analysis.

Results

Thymocytes are severely reduced in IL-7f/f FoxN1-Cre mice

To investigate the role of IL-7 produced by TECs, we crossed IL-7f/f mice with FoxN1-Cre Tg mice to obtain conditional knockout mice deficient in TEC-derived IL-7. We analyzed IL-7 transcripts by real-time RT-PCR. The levels of IL-7 mRNA were drastically reduced in IL-7f/f FoxN1-Cre thymus, suggesting that TECs produce most IL-7 in the thymus (Fig. 1). The levels of IL-7 were comparable in other organs except for LNs and the liver. The observed differences in these organs may be explained by a decrease in the number of fibroblastic reticular cells expressing IL-7 that correlates with a reduction of T cell zones in LNs of IL-7f/f FoxN1-Cre mice (32). Histologically, IL-7f/f FoxN1-Cre mice showed a smaller thymus size, but the structure with cortex and medulla was maintained (Supplemental Fig. 1A). In addition, some medullary TECs expressed Aire in IL-7f/f FoxN1-Cre mice (Supplemental Fig. 1B). These results suggest that the IL-7 gene was successfully inactivated in TECs of IL-7f/f FoxN1-Cre mice.

Infectious Diseases in the Institute for Virus Research, Kyoto University, Tg lines were on a C57BL/6 background. Mice were maintained under specific pathogen-free conditions in the Experimental Research Center for Infectious Diseases in the Institute for Virus Research, Kyoto University. FoxN1-Cre mice (32). Histologically, IL-7 f/f FoxN1-Cre mice were decreased in IL-7f/f FoxN1-Cre thymus, suggesting that TECs produce most IL-7 in the thymus (Fig. 1). The levels of IL-7 were comparable in other organs except for LNs and the liver. The observed differences in these organs may be explained by a decrease in the number of fibroblastic reticular cells expressing IL-7 that correlates with a reduction of T cell zones in LNs of IL-7f/f FoxN1-Cre mice (32). Histologically, IL-7f/f FoxN1-Cre mice showed a smaller thymus size, but the structure with cortex and medulla was maintained (Supplemental Fig. 1A). In addition, some medullary TECs expressed Aire in IL-7f/f FoxN1-Cre mice (Supplemental Fig. 1B). These results suggest that the IL-7 gene was successfully inactivated in TECs of IL-7f/f FoxN1-Cre mice.

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subsets, and again severe reduction in DN3b (1/540) and DN4 (1/440) subsets (Fig. 3A, 3B). An earlier report showed that ETPs were IL-7Rα2/lo (36), whereas another group reported that ETPs expressed IL-7Rα (37). By flow cytometry, we demonstrated that ETPs expressed the IL-7Rα at intermediate levels (Fig. 3C).

Levels of IL-7Rα expression on ETPs were lower than those of CD4 SP and CD8 SP thymocytes but higher than those of regulatory T cells. Thus, these results demonstrate that IL-7 produced by TECs is required for early thymocyte development.

Proportion of mature SP thymocytes is increased in IL-7f/f FoxN1-Cre mice

The elevated frequency of CD4 SP and CD8 SP cells led us to explore the possibility that thymocyte maturation might be affected in IL-7f/f FoxN1-Cre mice. After positive selection, SP thymocytes first differentiate into the semimature stage with CD69highHSAhigh Qa-2lowCD62Llow phenotype and then into the mature stage with CD69lowHSAlowQa-2highCD62Lhigh phenotype (38). Thus, we analyzed the CD4 SP (CD3highCD4+ except CD8low) cells of IL-7f/f FoxN1-Cre mice with the above maturation markers by flow cytometry. The proportion of HSAlow and Qa-2high CD4 SP cells was elevated in IL-7f/f FoxN1-Cre mice (Fig. 4). In addition, CD8 SP (CD3highCD8+) cells showed similar results. Altogether, these results suggest that the proportion of mature SP thymocytes is increased in IL-7f/f FoxN1-Cre mice.

T cell development is impaired in fetal thymus of IL-7f/f FoxN1-Cre mice

It was reported that γδ T cell development is severely blocked in fetal thymus of IL-7R−/− mice (2). To determine the roles of IL-7 produced by TECs in fetal thymus, we analyzed fetal thymocytes of IL-7f/f FoxN1-Cre mice at embryonic day 17.5 by flow cytometry. The proportion of DP thymocytes was decreased by 2-fold in fetal thymus of IL-7f/f FoxN1-Cre mice, whereas that of DN thymocytes were increased by 2-fold (Fig. 5A). The numbers of total, DN, and DP cells were decreased by 24-, 11-, and 41-fold, respectively (Fig. 5B). Furthermore, αβ and γδ T cells were absent in fetal thymus of IL-7f/f FoxN1-Cre mice. These results suggest that IL-7 produced by TECs plays a vital role in T cell development at fetal stages.

T cells partially recover in the periphery of IL-7f/f FoxN1-Cre mice

Because IL-7 regulates survival of peripheral T cells as well as thymocyte development (8), it remained unclear whether a defect in IL-7 production from TECs has any effects in T cell survival in the periphery. To address this question, we analyzed LN cells of IL-7f/f FoxN1-Cre mice by flow cytometry. The proportion of CD3+ T cells was decreased by 2-fold in IL-7f/f FoxN1-Cre mice compared with IL-7f/f mice (Fig. 6A). The numbers of total LN, CD4 T, CD8 T, αβ T, γδ T, and NKT cells were decreased by 1.6-, 5.6-, 3.1-, 4.2-, 200-, and 2.4-fold, respectively (Fig. 6B), suggesting that T cells have partially recovered in LNs. The cell
numbers of B and NK cells in LNs were slightly but not significantly increased. Next, we analyzed the phenotype of T cells with CD44 and CD62L Abs and divided them into naive (CD44^low^CD62L^+^), memory (CD44^+^CD62L^+^), and activated (CD44^+^CD62L^low^) phenotypes. In contrast to control mice, IL-7^f/f^ FoxN1-Cre mice showed increased proportions of memory and activated phenotypes of CD4 T cells and memory phenotype of CD8 T cells in LNs (Fig. 6C, 6D). Spleen cells showed similar results in IL-7^f/f^ FoxN1-Cre mice (Supplemental Fig. 2A). The numbers of CD4 SP, CD8 SP, CD8αβ T, γδ T, and NKT cells were decreased by 3.6-, 4.8-, 4.2-, 16-, and 3-fold, respectively, in IL-7^f/f^ FoxN1-Cre spleen (Supplemental Fig. 2B). Although B cells were not changed, NK cells were increased by 1.6-fold in the spleen. In IL-7^f/f^ FoxN1-Cre mice, the proportions

**FIGURE 4.** Proportion of mature SP thymocytes is increased in adult IL-7^f/f^ FoxN1-Cre mice. (A) Flow cytometric analysis of CD3^high^CD4^+, except CD8^low^ (CD4 SP) and CD3^high^CD8^+^ (CD8 SP) thymocytes from 4-wk-old IL-7^f/f^ and IL-7^f/f^ FoxN1-Cre littermate mice. Numbers in histograms indicate the percentages of each fraction. The data represent six independent experiments with similar results. (B) Percentages of indicated cells. Values are the mean ± SE of six mice. **p < 0.01.

**FIGURE 5.** T cell development is severely impaired in fetal thymus of IL-7^f/f^ FoxN1-Cre mice. (A) Flow cytometric analysis of embryonic day 17.5 fetal thymus. Data represent three IL-7^f/f^ and five IL-7^f/f^ FoxN1-Cre mice. (B) Absolute numbers of total thymocytes and indicated cells. Values are the mean ± SE of three to five mice. The data represent two independent experiments with similar results. **p < 0.01, ***p < 0.001.

**FIGURE 6.** T cells are partially recovered in LNs of IL-7^f/f^ FoxN1-Cre mice. (A) Flow cytometric analysis of inguinal LN cells from 4-wk-old IL-7^f/f^ and IL-7^f/f^ FoxN1-Cre littermate mice. The data represent four to seven independent experiments with similar results. (B) Absolute numbers of total LN cells and indicated cells. Values are the mean ± SE of four mice. *p < 0.05, **p < 0.01, ***p < 0.001.
of memory and activated phenotypes of splenic CD4 and CD8 T cells were also increased (Supplemental Fig. 2C, 2D). Thus, the numbers of T cells were partially restored in peripheral lymphoid organs compared with the thymus of IL-7f/f FoxN1-Cre mice.

γδ T cells are absent from the epidermis of IL-7f/f FoxN1-Cre mice

Previous studies reported that γδ T cells were absent from the skin of IL-7Rα−/− mice (2). Therefore, we analyzed dendritic epidermal T cells (DETCs) in epidermal sheets by flow cytometry. The proportion and cell numbers of TCRγδ+ DETCs were greatly reduced in the epidermis of IL-7f/f FoxN1-Cre mice, whereas MHC class II+ Langerhans cells were unchanged (Fig. 7A, 7B). These results were further confirmed by immunohistochemistry. TCRγδ+ DETCs were completely absent in the epidermis of IL-7f/f FoxN1-Cre mice (Fig. 7C). Because the precursors of DETCs develop in fetal thymus, these results were consistent with the observation that γδ T cell development is severely impaired in fetal thymus of IL-7f/f FoxN1-Cre mice (Fig. 5).

Development of γδ IELs is severely impaired in IL-7f/f FoxN1-Cre mice but not in IL-7f/f Vil-Cre mice

To address the question of the origin of γδ IELs, we analyzed IELs of IL-7f/f FoxN1-Cre mice by flow cytometry. The proportion of γδ IELs was significantly decreased in IL-7f/f FoxN1-Cre mice compared with control mice (Fig. 8A). The cell numbers of γδ IELs were reduced by 120-fold, whereas αβ IELs were decreased by 9-fold at 4 wk (Fig. 8B). Kinetic analyses showed that the numbers of αβ IELs gradually increased and reached to the levels of control mice at 12 wk, whereas those of γδ IELs remained at low levels. These results suggest that IL-7 produced in the thymus plays an essential role in development of γδ IELs.

To evaluate the function of IL-7 produced in the intestine, we next crossed the IL-7f/f mice with Vil-Cre Tg mice to obtain conditional knockout mice deficient in IEC-derived IL-7. We first measured IL-7 transcripts of IECs isolated from IL-7f/f and IL-7f/f Vil-Cre littermate mice. The levels of IL-7 transcripts were significantly reduced in IECs of IL-7f/f Vil-Cre mice, suggesting that the IL-7 gene was successfully deleted in IECs (Fig. 9A). We analyzed IELs of IL-7f/f Vil-Cre mice by flow cytometry. The proportion and numbers of αβ and γδ IELs were comparable to control mice (Fig. 9B, 9C). These results demonstrate that IL-7

![FIGURE 7](http://www.jimmunol.org/)

![FIGURE 8](http://www.jimmunol.org/)

![FIGURE 9](http://www.jimmunol.org/)
produced by IECs is dispensable for γδ IEL development. Collectively, these results suggest that the majority of γδ IELs differentiate in the thymus and migrate into the intestine. Thus, these results indicate the thymic origin of γδ IELs.

Discussion
In this study, we clarified the precise function of IL-7 produced from TECs by analyzing IL-7f/f FoxN1-Cre mice. We showed that thymocyte numbers were decreased in these mice (Fig. 2 and 3). Remarkably, γδ T cells were almost absent in the thymus. In addition, T cell maturation was increased (Fig. 4). In the fetal thymus, thymocytes were also drastically reduced (Fig. 5). In addition, we found that the numbers of T cells were partially restored in LNs and spleens (Fig. 6, Supplemental Fig. 2). Furthermore, γδ T cells were abrogated in the epidermis and diminished in the small intestine (Figs. 7, 8). This study demonstrates that IL-7 produced by TECs is essential for the development of thymocytes and TCRgd IELs.

Thymocytes were significantly decreased in IL-7f/f FoxN1-Cre mice. However, this reduction was less severe than in IL-7−/− mice (1). In addition to TECs, mesenchymal stromal cells also express IL-7 in the thymus (11, 19). With this in mind, although the disruption of the IL-7 gene in TECs resulted in a severe reduction of IL-7 transcripts in the IL-7f/f FoxN1-Cre thymus (Fig. 1), IL-7 produced from mesenchymal stromal cells might have slightly compensated for this loss. Thus, IL-7 produced by TECs and mesenchymal stromal cells might play major and minor roles, respectively, in the thymus.

TEC-derived IL-7 is essential for early thymocyte development. We found severe reduction in ETP, DN2a, DN3b, and DN4 subsets but slight recovery in DN2b and DN3a subsets (Fig. 3A, 3B). In addition, we demonstrated that ETPs express the IL-7Rα at intermediate levels (Fig. 3C). Therefore, it is possible that ETP and DN2a thymocytes greatly depend on IL-7. In addition, a decrease of IL-7 signal in transition from DN2a to DN2b stage reportedly induces Bcl11b expression, which is essential for T lineage commitment (39). Thus, in FoxN1-Cre IL-7−/−KO mice, the DN2a/DN2b transition may be accelerated, resulting in a partial recovery of DN2b and DN3a thymocytes. After productive rearrangements in the TCRβ locus, DN3a cells differentiate into DN3b stage (40). Last, IL-7 signal is required for β-selection-induced proliferation of thymocytes in transition from DN3a to DN3b stage and thereafter (41). Therefore, in FoxN1-Cre IL-7−/−KO mice, the DN3a/DN3b transition may be impaired, resulting in a reduction of DN3b and DN4 thymocytes.

IL-7 is essential for γδ T cell development in fetal thymus. γδ T cells are severely reduced in fetal thymus of IL-7R−/− mice (2). γδ T cells expressing invariant Vγ3Vβ61 TCR develop specifically in fetal thymus and distribute in the epidermis as DETCs in adult (42). We did not detect γδ T cells in the fetal thymus of IL-7f/f FoxN1-Cre mice (Fig. 5). In addition, DETCs were absent from the epidermis of IL-7f/f FoxN1-Cre mice (Fig. 7). Considering strong expansion capacity of DETCs in the skin (43), these results suggest that the development of γδ T cells is almost completely blocked in fetal thymus of IL-7f/f FoxN1-Cre mice. Therefore, our study demonstrates that IL-7 produced by TECs is essential for γδ T cell development in fetal thymus.

The issue of thymic versus extrathymic generation of γδ IELs is still a matter of debate. IL-7−/− mice completely lack γδ IELs (20), suggesting that IL-7 is essential for the development of γδ IELs. In our study, γδ IELs were more severely reduced than αβ IELs at 4 wk in IL-7f/f FoxN1-Cre mice (Fig. 8). αβ IELs gradually recovered during 8 and 12 wk, whereas γδ IELs remained low. In the thymus, although most γδ T cells were absent, a certain extent of αβ T cells remained in IL-7f/f FoxN1-Cre mice. Therefore, it is probable that these αβ T cells first occupy IEL niche in young mice, competing against homing or extrathymic development of γδ T cells in the intestines. We think that this is one reason for a large difference in γδ IELs between IL-7f/f FoxN1-Cre and nude mice. Furthermore, αβ and γδ IELs were unchanged in IL-7f/f Vil-Cre mice, suggesting that IL-7 produced in intestinal epithelial cells is dispensable for the development of γδ IELs (Fig. 9). This result was consistent with the report that the majority of γδ IELs do not express IL-7Rα (44). Collectively, our study clearly supports the idea on the thymic origin of γδ IELs.

We found that there is no difference in IL-7 transcript levels in skin between IL-7f/f FoxN1-Cre and IL-7f/f mice (Fig. 1). It is expected that the FoxN1-Cre transgene is expressed in the hair follicle, the hair cortex and outer root sheath and in the follicular matrix as well as in epidermal keratinocytes. Because IL-7 is highly expressed in lymphatic vessels of the skin (higher than in keratinocytes) (19), it might be that the difference of IL-7 expression between IL-7f/f FoxN1-Cre and IL-7f/f mice may become unclear.

In conclusion, we defined the in vivo roles of IL-7 produced by TECs for thymocyte development. TEC IL-7 is important for the development of thymocytes and γδ IELs. Our study thus provides strong evidence for the thymic origin of γδ IELs. These findings will accelerate the elucidation of IL-7 functions produced by different stromal cells, and IL-7–floxed mice should serve as a powerful tool to dissect the local function of IL-7 in lymphoid organs.

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Disclosures
The authors have no financial conflicts of interest.

References


