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Ontogeny and Regulation of IL-7-Expressing Thymic Epithelial Cells1

Monica Zamisch,* Billie Moore-Scott,† Dong-ming Su,‡ Philip J. Lucas,‡ Nancy Manley,† and Ellen R. Richie2*§

Epithelial cells in the thymus produce IL-7, an essential cytokine that promotes the survival, differentiation, and proliferation of thymocytes. We identified IL-7-expressing thymic epithelial cells (TECs) throughout ontogeny and in the adult mouse thymus by in situ hybridization analysis. IL-7 expression is initiated in the thymic fated domain of the early primordium by embryonic day 11.5 and is expressed in a Foxn1-independent pathway. Marked changes occur in the localization and regulation of IL-7-expressing TECs during development. IL-7-expressing TECs are present throughout the early thymic rudiment. In contrast, a major population of IL-7-expressing TECs is localized to the medulla in the adult thymus. Using mouse strains in which thymocyte development is arrested at various stages, we show that fetal and postnatal thymi differ in the frequency and localization of IL-7-expressing TECs. Whereas IL-7 expression is initiated independently of hemopoietic-derived signals during thymic organogenesis, thymocyte-derived signals play an essential role in regulating IL-7 expression in the adult TEC compartment. Moreover, different thymocyte subsets regulate the expression of IL-7 and keratin 5 in adult cortical epithelium, suggesting that despite phenotypic similarities, the cortical TEC compartments of wild-type and RAG-1−/− mice are developmentally and functionally distinct. The Journal of Immunology, 2005, 174: 60–67.

Interleukin-7 is a pleiotropic cytokine that is produced by thymic and bone marrow stromal cells and is essential for T and B cell lymphopoiesis (reviewed in Refs. 1 and 2). The IL-7R consists of an α-chain that is also a component of the thymic stromal lymphopoietin receptor, and the common cytokine receptor γ-chain (γc)3 that is present in IL-2, -4, -9, -15, and -21 receptors (3). Signaling through the IL-7R initiates multiple signaling cascades, including activation of protein tyrosine kinases Jak1 and Jak3 that associate with the intracellular domains of the IL-7Rα and γc chains, respectively. Activated Jak1 and Jak3 phosphorylate STAT1 and STAT5, resulting in altered gene expression patterns (4). IL-7-mediated signals modulate the expression of genes that affect thymocyte survival, proliferation, and differentiation. Disruption of IL-7 signaling in IL-7−/−, IL-7Rα−/−, and γc−/− mice severely reduces thymic cellularity and impairs thymocyte development (5–7). This phenotype is due, in part, to reduced survival of CD4−CD8− double-negative (DN) thymocyte progenitors because introduction of a bcl-2 transgene or deletion of the death effector Bax rescues T cell development in IL-7Rα−/− and γc−/− mice (8–10). IL-7 signaling also promotes the survival of DN thymocytes undergoing transition through the β-selection checkpoint to the CD4+CD8+ double-positive (DP) stage (11) and proliferation of positively selected CD4+CD8− and CD4+CD8+ single-positive (SP) thymocytes (12). In addition to enhancing the survival and proliferation of αβ-lineage thymocytes, IL-7 regulates TCRγ gene rearrangement by controlling locus accessibility, and is, therefore, essential for the development of γδ T cells (13–15).

Although recent studies suggest a less stringent requirement for IL-7 in promoting survival of fetal compared with adult thymocytes (13, 16), IL-7 expression has been reported in the early fetal thymus (17, 18). Intrathymic production of IL-7 is primarily a function of thymic epithelial cells (TECs) (19, 20). The TEC compartment is heterogeneous, consisting of subcapsular, cortical, and medullary subsets defined by morphological properties, antigenic profiles, and keratin expression patterns (reviewed in Refs. 21 and 22). However, because prior studies analyzed IL-7 expression by RT-PCR, the relative localization of TEC subsets that express IL-7– in the fetal and adult thymic microenvironment was not determined. Furthermore, the earliest developmental stage at which IL-7 is expressed in the thymic primordium has not been established. Given the functional significance of IL-7 signaling and the compartmentalization of thymocyte and epithelial subsets, we performed in situ hybridization (ISH) analyses to explore the emergence, distribution, and regulation of IL-7-expressing TECs during ontogeny and in postnatal mice. The data show striking differences in the localization and frequency of IL-7 expression in fetal and adult TECs. In addition, examination of IL-7 expression in the third pharyngeal pouch endoderm of wild-type and nude mice reveals IL-7 to be an early marker of TEC fate. Finally, differences in the thymocyte subsets that regulate IL-7 expression patterns demonstrate that cortical TECs in RAG-1−/− mice are developmentally and functionally immature compared with the wild-type cortical TEC compartment.

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3 Abbreviations used in this paper: γc, common γ-chain; CLP, common lymphoid progenitor; CMJ, corticomedullary junction; DN, double negative; DP, double positive; E, embryonic day; ECM, extracellular matrix; ISH, in situ hybridization; K5, keratin 5; SP, single positive; TEC, thymic epithelial cell.
**Materials and Methods**

**Mice and tissue preparation**

C57BL/6d, RAG-1⁻/⁻, and Tg mice were purchased from The Jackson Laboratory. RAG-2⁻/⁻/γc⁻/⁻ mice were purchased from Taconic Farms. Ikaros-null mice were the generous gift of K. Georgopoulos (Harvard Medical School, Charleston, MA). Embryos were obtained by setting up timed matings for 16 h and considering the morning of finding the vaginal plugs as embryonic day 0.5 (E0.5). Embryos were fixed in Bouin’s solution for 3–5 h; washed overnight in 70% ethanol; dehydrated in series of 30, 50, 70, and 100% ethanol washes; and paraffin embedded. Thymi from postnatal mice were obtained by dissection and fixed as above, except that the dehydration protocol used 70, 90, and 100% ethanol. For whole mount in situ hybridization, embryos were fixed in 4% paraformaldehyde/PBST overnight, washed twice in PBST, and dehydrated in series of 30, 50, 70, 90, and 100% methanol.

**In situ hybridization**

Paraffin section ISH was performed, as previously described (24), with the exception that the proteinase K digestion was omitted. The IL-7 riboprobe was obtained by RT-PCR on RNA from the cell line mouse plasmacytoma line J558 obtained from American Type Culture Collection (Manassas, VA). The PCR used the following oligos: 5' oligo, 5'-GCGACTGAGTACCGACTACACCCACCTCCCGCACACC-3'; 3' oligo, 5'-CGATCCGGATCCACAGATTCCTGAGGTGTTACCTGAC-3'. The 5' oligo ends right before the ATG site, and the 3' oligo starts 30 bp downstream from the stop codon. After adding BstHI sites to each end, the 546-bp fragment was cloned into the blunt EcoRI site of BSII SK⁺ (Strategene). The 534-bp BamHI fragment was sequenced using T3 and T7 primers of BSII.

The Gem2 probe was generated by PCR amplification, as described (25). The Foxn1 probe was PCR amplified from E11.5 mouse cDNA using primers encompassing the eighth and ninth exons and 3' UTR (26). The IL-7, Foxn1, and Gem2 riboprobes (sense and antisense) were labeled with digoxigenin during in vitro transcription. BM purple (Roche) was used as a chromagen for detection of the hybridized probe, and the sections were counterstained with nuclear fast red. Whole mount ISH was performed, as described (27).

**Semiquantitative RT-PCR**

Wild-type and nude E12 fetal thymic lobes were separately collected in TRizol (Invitrogen Life Technologies) and homogenized using Micro Pellet Pestles (Nalge Nunc International). RNA was isolated, the genomic DNA was depleted with DNase I (Invitrogen Life Technologies), and reverse transcription of total RNA to cDNA was performed using SuperScript II (Invitrogen Life Technologies), following manufacturer’s protocol. Equal amounts of cDNA from each strain were added to a final 20 μl PCR mix using the Qiagen TaqPCR kit. PCR conditions for IL-7 and Foxn1 were: initial denaturation of 94°C 3 min; 25–35 cycles of 94°C 0.5 s, 55–65°C 0.5 s, 72°C 1 min; and final extension of 72°C 10 min. PCR conditions for IL-7Rα were initial denaturation of 95°C 1 min; 40 cycles of 95°C 30 s, 55°C 30 s, 72°C 45 s; and final extension of 72°C 10 min. PCR products were visualized by 5% acrylamide gel electrophoresis and ethidium bromide staining. Band densities were measured and analyzed using the Molecular Analyst software (BioRad, version 1.4.1). Primer sequences were: IL-7 (5'), 5'-GAC ACA CCC ACC TCC CGC A-3'; IL-7 (3'), 5'-TCT CAG TAG TCT CTT TAG G-3'; GADPH (5'), 5'-GTC TAC ATG TTC CAG TAT GAC TCC ACT CAC-3'; GADPH (3'), 5'-GAA ACG TTA GAT CTA GAT TGT GAG ATT TCT CGT-3'; IL-7Rα (5'), 5'-GAC ATG AAG ATT CCT ACT GAT TGG-3'; IL-7Rα (3'), 5'-GCC GAG CTT GCC TTC GGC AAT GAA ACT CAC AT-3'; actin (5'), 5'-GTT GGA CAC TCT CTA CAC C-3'; actin (3'), 5'-GTT GCC ACC TCT CCT CGA AGT C-3'.

**Immunohistochemistry**

Paraffin-embedded tissue sections were deparaffinized and rehydrated in 100–75% ethanol. OCT-embedded frozen tissue sections were air dried 30 min before acetone fixation. Sections were incubated overnight at 4°C with optimal dilutions of polyclonal anti-mouse keratin 5 (K5; Covance Research) and/or monoclonal anti-mouse c-kit (BD Pharmingen). Immunoreactivity as seen by c-kit was enhanced by tyramide amplification (PerkinElmer Life Sciences). Controls included slides incubated with nonimmune rabbit-matched Ig or isotype-matched mouse Ig. For costaining, sections were incubated simultaneously with primary Abs from different species. Microscopic analysis was performed with an Olympus ProVis AX70 microscope (Olympus).

**Thymic suspensions and FACs sorting**

Thymocytes and TECs were released from E12.5 thymi by trypsin digestion, as previously described (28). Single cell suspensions were stained with allophtocyanin-conjugated anti-CD45 Ab (clone 30-F11) (BD Biosciences). Hemopoietic and stromal cells were isolated by sorting the Ab-stained cells for CD45⁻ (>98% purity) and CD45⁺ (>94% purity) subsets, respectively, using a Corixa Elite flow cytometer.

**Results**

IL-7 expression is restricted to the thymus domain of the early primordium

IL-7 is a secreted cytokine that binds to MHC class II-positive TECs and fibroblasts in a heparin sulfate-dependent manner (29). To avoid detecting cells that bind, but do not synthesize IL-7, we evaluated IL-7 mRNA expression by ISH analysis. Although IL-7 expression was not detected in the parathyrengal region at E10.5 (data not shown), IL-7 message is expressed in the third parathyrengal pouch endoderm by E11.5. Fig. 1 shows that IL-7 expression is restricted to the epithelium of the third pouch and is not detected in other parathyrengal compartments. At E11.5, the parathyroid- and thymus-specific domains of the third pouch are contiguous within a common primordium that has not yet separated from the pharynx (30). A higher magnification view (Fig. 1B) shows that IL-7 expression is restricted to the ventral aspect of the third pouch, the domain that is fated to develop into thymus as opposed to parathyroid (31). Although IL-7 plays an essential role in thymocyte development, the early appearance of IL-7-expressing TECs during ontogeny suggested that it may function to stimulate TEC progenitors via an autocrine pathway. To explore this possibility, we assessed IL-7Rα expression on stromal as well as hemopoietic cells obtained from fetal thymi. Single cell suspensions of E12 thymi were stained with anti-CD45 and sorted to isolate CD45⁺ stromal cells and CD45⁻ hemopoietic cells. Consistent with earlier reports showing that IL-7Rα is expressed on fetal thymocyte progenitors (32–34), RT-PCR analysis of CD45⁻ cells revealed a

**FIGURE 1.** IL-7 expression in the third pharyngeal pouch of E11.5 C57BL/6d/embryo. In situ hybridization analysis of IL-7 expression in a sagittal section of an E11.5 embryo. A, IL-7-expressing cells are localized in the third pharyngeal pouch. Numbers designate each pharyngeal pouch. Dorsal is left; anterior is up. B, A high magnification image of the third pouch shows that IL-7-expressing cells are present in the ventral aspect of the third pouch in the common thymus/parathyrengal primordium. C, RT-PCR analysis demonstrates that IL-7Rα expression is detected in FACs-sorted CD45⁻ hemopoietic cells, but not in CD45⁺ stromal cells from E12 fetal thymi. Two dilutions of each cDNA were used for PCR.
obtained with CD45

Foxn1 previously described for sors, but does not function as an autocrine factor for TEC thymic primordium promotes development of thymocyte precursors, but does not function as an autocrine factor for TEC progenitors.

The IL-7 expression pattern at E11.5 was similar to that previously described for Foxn1 (31). Foxn1 is a forkhead class transcription factor that is expressed in the thymic primordium by E11.25 and is required for TEC development. Nude mice are homozygous for a null mutation in Foxn1, resulting in defective hair and thymus development due to impaired epithelial differentiation in skin and thymus (31, 35, 36). TECs in the nude thymus are arrested at a stage corresponding to an early progenitor cell type, suggesting that TECs are specified to a thymus fate, but fail to differentiate (35, 37, 38). Comparative whole mount ISH analysis shows that Foxn1 and IL-7 are expressed in overlapping domains at E11.5 (Fig. 2, A and B).

In this comparison, the IL-7 domain appears slightly smaller than the Foxn1 domain, although development at this stage is rapid, and this could simply represent slight differences in the developmental stages of the embryos, or in the exact plane of the section. In contrast, Gcm2 is a transcription factor that is expressed specifically in the parathyroid-specific domain of the third pouch endoderm (31). Comparison of the IL-7 and Gcm2 expression patterns at E11.5 confirmed that IL-7 expression is restricted to the ventral and distal thymus-specific domain of the developing primordium (Fig. 2, B and C).

Foxn1 does not regulate IL-7 expression

Because Foxn1 is required for TEC differentiation and is expressed within a similar developmental time frame as IL-7, we considered that IL-7 might be a downstream target of Foxn1-mediated transcriptional regulation. To explore this possibility, we examined IL-7 expression in the rudimentary thymic remnant of early embryos from nude mice. Fig. 3, A and B, shows that IL-7 expression is readily detected by ISH analysis in the thymic primordium of E12 nude mice. Semiquantitative RT-PCR analysis confirmed the presence of IL-7 message in the E12 nude thymus anlage, although a stronger signal was observed in wild-type compared with nude fetal thymus (Fig. 3C). These results demonstrate that induction of IL-7 expression is independent of Foxn1-mediated regulation during thymic organogenesis. Furthermore, the presence of IL-7 message in the absence of functional Foxn1 establishes IL-7 as an early developmental marker of endodermal commitment to a thymic epithelial cell fate.

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**FIGURE 2.** IL-7 expression is restricted to the thymus-specific portion of the shared parathyroid-thymus primordium. Images shown are the inside surface of E11.5 embryos hemisected parasagittally and stained in whole mount by in situ hybridization. The developing third pouch primordium is outlined in each panel. Dorsal is left; anterior is up. A, Foxn1 is expressed in the ventral primordium. B, IL-7 expression is similar to Foxn1. C, Gcm2 expression marks the dorsal, parathyroid-specific domain.

**FIGURE 3.** IL-7 is expressed in the third pouch endoderm of nude mice. A, ISH analysis of IL-7 expression in a transverse section of an E12 nude embryo shows that IL-7 expression is independent of Foxn1 expression. B, A higher magnification image of IL-7-expressing cells in the third pharyngeal pouch. C, RT-PCR analysis confirms that IL-7 is expressed in wild-type and nude thymus from E12 embryos.

**FIGURE 4.** Changes in the frequency and localization of IL-7-expressing TECs during ontogeny. Top row. Shows low magnification images, and bottom row shows high magnification images of ISH analyses of IL-7-expressing cells in transverse sections of thymi from embryos at the indicated ages. There is a notable decrease in the frequency of IL-7-expressing cells that are localized near the subcapsular region by E16.5.
found in the perimedullary region at the CMJ (Fig. 5D). Hemopoietic progenitors gain entry from the bloodstream into the postnatal thymus by extravasation at these sites (40, 41). The perivascular localization of IL-7-expressing cells suggests that as thymocyte progenitors enter the postnatal thymus, they encounter high concentrations of IL-7.

Initiation of IL-7 expression in the fetal thymus is independent of thymocyte-derived signals

We previously reported that hemopoietic-derived inductive signals are not required to establish initial patterning of fetal TEC subsets as determined by expression of distinct keratin species and antigenic profiles (42). To determine whether hemopoietic-derived signals are required to initiate IL-7 expression in the thymic rudiment, we examined IL-7-expressing TECs in two experimental models that sustain early blocks in T cell development. The Ikaros transcription factor plays an essential role in commitment of hemopoietic progenitors to the lymphoid lineage. Targeted deletion of the C-terminal region of the Ikaros gene results in a null phenotype characterized by failure of B and NK development throughout life and an absence of T cell precursors during the early fetal period (23). In contrast, thymocyte development is arrested at the c-kit+CD25+ DN2 differentiation stage in mice that are deficient for both RAG-2 and the γc receptor polypeptide chain (RAG2/γc−/−) (43). As shown in Fig. 6A, fetal thymi from E13.5 RAG2/γc−/− mice contain few c-kit-positive thymocyte progenitors, and such cells are undetectable in E13.5 Ikaros−/− thymi. Nevertheless, Fig. 6B shows that IL-7 is abundantly expressed in E13.5 fetal thymi from both RAG2/γc−/− and Ikaros−/− mice. Furthermore, the localization pattern of IL-7-expressing TECs was comparable to that observed in wild-type controls. Therefore, the induction of IL-7 expression in fetal TECs occurs independently of thymocyte-derived signals.

Thymocyte-derived signals are required to regulate IL-7 expression in the postnatal thymus

Although thymocyte/TEC interactions are not required to specify phenotypic and functional characteristics of the thymic epithelial compartment during early ontogeny, thymocyte-derived signals are essential for proper organization and development of cortical and medullary TEC subsets during late fetal development and in the adult (42, 44–46). To determine whether thymocytes are also required to regulate IL-7 expression in the postnatal thymic epithelial compartment, we performed a comparative ISH analysis of IL-7 expression in adult thymi from wild-type, RAG-2/γc−/−, and RAG-1−/− mice. In contrast to the developmental block at the DN3 stage in RAG-2/γc−/− mice, thymocyte development is arrested at the DN3 stage in RAG-1−/− mice (43, 47).

Fig. 7 shows that, as noted above, IL-7-expressing TECs are predominantly localized in the medullary region of the wild-type adult thymus, whereas the majority of cortical TECs do not express detectable levels of IL-7 message. K5 is expressed by TECs in the medulla and at the corticomedullary junction, but not by cortical TECs (45). An analysis of K5 expression in a serial section confirmed the overlap in localization of IL-7 and K5 expression in the

FIGURE 5. The majority of IL-7-expressing cells are localized at the CMJ and in the medulla of postnatal thymi. A, ISH analysis of IL-7 expression in the newborn thymus shows positive cells in the subcapsular region and outlining the medullary regions. B, A consecutive H&E-stained section of the same newborn thymus delineates cortical and medullary regions. C, By 1 wk of age, IL-7-expressing cells are found throughout the medullary region and surround large vessels at the CMJ. D, A higher magnification image of the boxed area in C shows IL-7-expressing cells lining large vessels. E, The majority of IL-7-expressing cells are found in the medulla and corticomedullary junction of a 4-wk-old thymus hybridized with an antisense IL-7 probe. F, No positive cells are present in medulla or cortex of a consecutive section hybridized with a sense IL-7 probe.

FIGURE 6. Thymocyte-derived signals are not necessary for induction of IL-7 expression in the early fetal thymus. A, Cryostat sections of E13.5 wild-type C57BL6/J, RAG2/γc-deficient, and Ikaros null embryos were stained with anti-K5 and anti-c-kit. Reactivity with anti-K5 was detected with Texas Red-conjugated anti-Ig, and a tyramide amplification system was used to detect c-kit FITC staining. B, The top row shows low magnification images, and the bottom row shows high magnification images of IL-7-expressing cells in transverse sections of thymi from E13.5 wild-type, RAG-2/γc−/−, and Ikaros null embryos.

FIGURE 7. IL-7 and K5 are differentially regulated in thymi of adult wild-type, RAG-1−/−, and RAG-2/γc−/− mice. Top row, Shows ISH analyses of IL-7-expressing cells. Bottom row, Consecutive sections are stained with anti-K5 and developed with Texas Red-conjugated anti-Ig. IL-7 and K5 expression are found throughout the RAG-2/γc−/− thymus. K5 expression, but not IL-7 expression, is down-regulated in the RAG-1−/− thymus.
wild-type thymus. In striking contrast, IL-7-expressing TECs are present throughout the RAG-1−/− and RAG-2/γc−/− thymi. The widespread IL-7 expression pattern in the adult RAG-2/γc−/− thymus is consistent with the fact that the epithelial compartment in the severely hypoplastic RAG-2/γc−/− thymus has an abnormal two-dimensional organization and consists of immature TECs that coexpress K8 and K5 (42). K5 immunostaining of a serial section verifies that the majority of TECs in the RAG-2/γc−/− thymus are K5 positive. In contrast, expression of IL-7 throughout the adult RAG-1−/− thymus was unexpected because we previously demonstrated that thymocytes blocked at the DN3 stage could signal cortical TEC progenitors to down-regulate K5 expression and organize into a three-dimensional structure (45). These findings suggest that distinct thymocyte-derived signals control IL-7 expression and K5 down-regulation in the RAG-1−/− cortex and reveal that TECs in the cortex of RAG-1−/− mice are developmentally and functionally distinct from wild-type cortical TECs.

IL-7 expression pattern in RAG-2−/− TCRβ transgenic thymus

To further examine the possibility that thymocyte-derived signals regulate the IL-7 expression pattern in the adult thymic cortex, we performed ISH analysis for IL-7 on thymi obtained from RAG-2−/− mice that express a TCRβ transgene. Introduction of a productively rearranged TCRβ transgene into RAG-2-deficient mice restores the ability of DN3 thymocytes to undergo β-selection and differentiate to the DP stage (48). Although RAG-2−/− TCRβ+ DN thymocytes traverse the β-selection checkpoint, the absence of TCRαβ on DP thymocytes precludes positive selection and maturation to the SP stage. As expected, an increase in thymic cellularity and the appearance of DP thymocytes were observed in RAG-2−/− TCRβ+ mice (Fig. 8). In contrast to the consistent pattern of IL-7 expression found in the RAG-1−/− thymus, IL-7-expressing TECs are primarily concentrated in the subcapsular region of RAG-2−/− TCRβ+ mice. Furthermore, there is a notable decrease in the frequency of IL-7-expressing TECs in the expanded cortex, a phenotype that is similar to the paucity of IL-7-expressing TECs observed in the wild-type cortex. Taken together, these data support the notion that progression through the β-selection checkpoint renders thymocytes competent to induce cortical expansion and alter the pattern of IL-7 expression in the cortical TEC compartment.

Discussion

This work presents four major findings concerning the appearance and regulation of IL-7-expressing TECs. First, IL-7 expression is an early marker of thymic epithelial fate. Second, IL-7-expressing TECs are present throughout the early thymic rudiment, but are organized into discrete zones in the adult thymus. Third, although hematopoietic cells are not required to initiate IL-7 expression during thymic organogenesis, thymocyte-derived signals are necessary to maintain a normal pattern of IL-7-expressing TECs in the adult thymus. Finally, the early arrest in thymocyte differentiation in RAG-1−/− mice results in a developmentally immature cortical TEC compartment as reflected by the widespread distribution of IL-7-expressing TECs.

IL-7 expression is initiated between E10.5 and E11.5 in the pharyngeal pouch endoderm of the common thymus/parathyroid primordium, specifically in the domain that is fated to develop into the thymic epithelial compartment. Thus, IL-7 expression can be considered as a functional marker of early TECs, and is likely to be expressed in the recently described K8+K5+MTS24+ progenitors that give rise to cortical and medullary epithelial compartments (37, 38). Despite its early appearance, IL-7 expression was not detected by ISH in all epithelial cells within the thymic fated domain. This may reflect maturation-dependent constraints on the level of IL-7 expression or the existence of distinct epithelial lineages. Further studies of precursor-progeny relationships and lineage analysis are necessary to distinguish these alternative explanations.

Although there are notable similarities in the temporal and spatial expression of IL-7 and Foxn1 during fetal thymic development, the presence of IL-7 mRNA in the nude thymic rudiment demonstrates that Foxn1 transcriptional activity is not required for IL-7 gene expression. The reduced IL-7 signal intensity in RT-PCR analysis of nude compared with wild-type fetal thymi suggests that Foxn1 may modulate the level of IL-7 expression. However, this interpretation is subject to the caveat that wild-type and nude thymi are not strictly comparable because thymic epithelial differentiation is arrested in nude mice and, therefore, the lower signal may be an indirect consequence of impaired TEC differentiation. Regardless, the Foxn1-independent expression of IL-7 in thymic fated epithelial cells of the common primordium identifies IL-7 as the earliest known marker of TEC fate in thymic ontogeny.

The early appearance of IL-7 message suggested that cross talk between epithelial and hematopoietic progenitors might not be required to initiate IL-7 expression in the fetal thymus. This premise was supported by ISH data showing that high levels of IL-7 are expressed throughout RAG2/γc−/− and Ikaros−/− fetal thymi in which there is a partial or complete block, respectively, in early...
appropriate intrathymic milieu for promoting early fetal thymus is an important factor in establishing an appropriate microenvironment. Indeed, IL-7Rs are expressed on bone marrow progenitors encounter high concentrations of IL-7 as they migrate across the condensing mesenchymal capsule (50). Because IL-7Rs are expressed on lymphoid progenitors that emerge in the fetal liver at E11 to E12 (32–34), it is likely that T cell precursors are signaled by IL-7/IL-7R interactions upon encountering the thymic microenvironment. The first wave of hematopoietic precursors that migrate into the fetal thymus gives rise to thymocytes that express an invariant TCR αβ characterized by a lack of functional diversity (51, 52). IL-7R-mediated signals are required to initiate TCRγ gene rearrangement (13–15). Therefore, it seems likely that the prevalence of IL-7-expressing TECs in the early fetal thymus is an important factor in establishing an appropriate intrathymic milieu for promoting γδ T cell development. In contrast, IL-7 may play a redundant role in promoting in αβ T cell development during fetal life because there is a relatively normal distribution, albeit reduced number, of each major thymocyte subset in fetal IL-7−/− mice, whereas thymocyte development is arrested before the DN3 stage in the adult (16).

In contrast to their widespread distribution in early ontogeny, a striking dichotomy exists in the localization of IL-7-expressing cells in cortical vs medullary compartments as the thymic microenvironment becomes specialized into functionally distinct zones of the late fetal and postnatal thymus. Earlier reports using RT-PCR showed that IL-7 is expressed by MHC class II-positive TECs, but the intrathymic localization of IL-7-expressing TECs was not determined (12, 20, 53). Most notably, the medulla contains the highest concentration of IL-7-expressing cells in the adult thymus, whereas IL-7-expressing TECs are sparsely distributed in the cortical and subcapsular regions. The high frequency of IL-7-expressing cells in the medulla is not surprising given that IL-7Rs are up-regulated on positively selected CD4+CD8− and CD4−CD8+ thymocytes and that IL-7 drives expansion of SP medullary thymocytes (12). Furthermore, IL-7-mediated signals promote the differentiation and maintain the viability of CD4−CD8+ thymocytes (39). The paucity of IL-7-expressing TECs in the cortex corresponds to the lack of IL-7R expression on DP cortical thymocytes (54). In the absence of IL-7 signaling, nonselected DP thymocytes down-regulate bcl-2 expression and undergo apoptosis.

Although IL-7-expressing cells are relatively rare in the cortex, IL-7 expression is pronounced in perivascular cells of the large vessels at the CMJ, where circulating hematopoietic precursors enter the postnatal thymus (40, 41). Thus, it is likely that blood-borne bone marrow progenitors encounter high concentrations of IL-7 as they migrate out of the vascular system into the postnatal thymic microenvironment. Indeed, IL-7Rs are expressed on bone marrow-derived common lymphoid progenitors (CLPs), which are clonogenic multipotent lymphoid-restricted precursors. Whereas CLPs generate B lineage-committed progeny in the bone marrow, CLPs give rise to T cells in the thymic microenvironment (55, 56). However, distinct IL-7R-negative T lineage progenitors were recently described in the adult murine thymus (57, 58). Therefore, further studies are needed to clarify the significance of IL-7 signaling for intrathymic hematopoietic progenitors.

It is well established that lineage-committed DN thymocytes express IL-7Rs that transduce survival and differentiation signals as a result of IL-7 binding (11, 59). Differentiating DN precursors migrate from the CMJ through the deep cortex to the subcapsular region, where the DN3 to DN4 developmental transition occurs in thymocytes that traverse the β-selection checkpoint (60). The impact of IL-7 has been shown to be highly dose dependent with low doses promoting and high doses inhibiting thymocyte development (61). Although IL-7-expressing TECs are sparsely distributed throughout the cortex and subcapsular region of the adult thymus, effective IL-7 signaling may nevertheless occur via several mechanisms. Trigueros et al. (11) demonstrated that IL-7 signals are necessary for efficient progression of proliferating DN4 cells to the DP stage. They further suggested that pre-TCR-mediated signaling in DN4 thymocytes reduces the activation threshold, permitting an effective response to low levels of IL-7 (11). A similar mechanism was found to operate during B cell development in that pre-BCR-induced MAPK signals permit B cells to respond to suboptimal concentrations of IL-7 (62). Furthermore, IL-7 binds to extracellular matrix (ECM) components such as fibronectin and heparan sulfate on TECs, resulting in activation of the surface integrin VLA-4, thereby enhancing thymocyte/epithelial interactions (29, 63, 64). Interestingly, DN thymocyte migration is directed in part by interactions between surface integrins on thymocytes and ECM components on TECs (65). Therefore, migrating DN thymocytes that bind to ECM components on cortical TECs are likely to encounter low levels of IL-7 in the thymic cortex.

Development of the thymic epithelial microenvironment is considered to occur in a stepwise manner dependent on signals from thymocytes at specific stages of maturation (66). The present study demonstrates that distinct thymocyte subsets are responsible for changes in the expression patterns of K5 and IL-7 in cortical TECs of the postnatal thymus. We previously demonstrated that down-regulation of K5 in the thymic cortex depends on cross talk between K8+K5− TEC precursors and T lineage-committed thymocytes (45, 67). Thus, K5 is down-regulated in the well-organized cortex of RAG-1-deficient mice, whereas human CD3ε transgenic and RAG2/γc−/− thymi that sustain earlier blocks in thymopoiesis retain a primitive, poorly organized TEC population consisting of K8+K5− TEC progenitors. We now show that in contrast to K5, IL-7 continues to be expressed throughout the thymic epithelium of RAG-1-deficient mice. Based on these data, we suggested that signals emanating from thymocytes that have advanced beyond the DN3 stage of thymocyte development are required to induce IL-7 down-regulation, or alternatively are required to stimulate selective expansion of IL-7-negative precursors in the thymic cortex. This interpretation is consistent with the IL-7 expression pattern observed in RAG-2−/−/TCRβ+ mice. Although IL-7-expressing cells were concentrated in the subcapsular region, the scarcity of IL-7-expressing cells in thymic cortex was similar to the results obtained from ISH analysis of wild-type thymus.

Finally, the presence of IL-7-expressing TECs throughout the RAG-1−/− thymus, despite generation of a three-dimensional epithelial meshwork (66) consisting primarily of K8+K5− cells, indicates that RAG-1−/− cortical TECs are not developmentally equivalent to the wild-type cortical epithelial compartment. We
speculate that RAG-1−/− cortical TECs are arrested at an immatu-
rate stage of development due to the absence of appropriate in-
ductive signals generated by thymocytes that have traversed
the D3N to DN4 checkpoint.

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