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CUTTING EDGE

Cutting Edge: Thymocyte-Independent and Thymocyte-Dependent Phases of Epithelial Patterning in the Fetal Thymus[1]

David B. Klug,[2,3] Carla Carter,[3] Irma B. Gimenez-Conti, and Ellen R. Richie[4]

Thymic epithelial cells (TECs) in adult mice have been classified into distinct subsets based on keratin expression profiles. To explore the emergence of TEC subsets during ontogeny, we analyzed keratin 8 and keratin 5 expression at several stages of fetal development in normal C57BL/6J mice. In addition, thymic epithelial development and compartmentalization were explored in recombination-activating gene 2/common cytokine receptor γ-chain-deficient and Ikaros-null mice that sustain early and profound blocks in thymocyte differentiation. The results demonstrate that initial patterning of the thymic epithelial compartment as defined by differential keratin expression does not depend on inductive signals from hematopoietic cells. However, thymocyte-derived signals are required during late fetal stages for continued development and maintenance of TEC subsets in the neonate and adult. ***The Journal of Immunology,*** **2002, 169: 2842–2845.**

Thymic organogenesis is a precisely regulated process during which inductive interactions among epithelial, mesenchymal, and hematopoietic cells are indispensable for organ development (reviewed in Refs. 1 and 2). Formation of the early thymic primordium is initiated at approximately embryonic day (E)[5]11, as the third pharyngeal pouch endoderm forms an epithelial bud that becomes encompassed by neural crest mesenchyme of the third and fourth pharyngeal arches (3, 4). Signaling between cells of epithelial and mesenchymal origin is a general principle that governs the development of many organ systems. However, thymic organogenesis is unique in that signals derived from cells of hematopoietic as well as mesenchymal origin are required to induce proper differentiation of the epithelial compartment (5–9).

Programmed differentiation of epithelial cells in skin and other tissues is accompanied by changes in keratin expression pattern. The keratin superfamily of intermediate filament proteins contains >20 members, which are expressed as heterodimers of acidic and basic keratin species. Keratins are considered biochemical markers of epithelial differentiation because they are expressed in a developmentally regulated and tissue-specific manner (10). K8 and its partner K18 are uniformly expressed in simple epithelia. In contrast, immature basal cells of stratified epithelia express K5/K14 heterodimers, which are down-regulated during terminal differentiation as other keratin species are up-regulated.

The epithelial compartment in the thymus is unique in that it cannot be classified strictly as simple or stratified epithelium. Thymic epithelial cells (TECs) are organized into a three-dimensional network rather than forming epithelial sheets arranged on a basement membrane as is characteristic of epithelial organization in other organs (11). The mesh-like arrangement of TECs facilitates thymocyte migration among and interaction with thymocyte subsets located in the subcapsular, cortex, and medullary regions (11, 12). TECs have been characterized according to location, morphology, and function (1, 13). As in other epithelial tissues, keratins serve as differentiation markers that distinguish thymic epithelial subsets. We have demonstrated that the thymic cortex contains a predominant subset of $K8^+K18^+K5^-K14^-$ cells and a minor subset of $K8^+K18^+K5^+K14^-$ cells (7, 14). TECs in the latter population (hereafter referred to as $K8^+K5^+$) are concentrated at the corticomedullary junction and scattered throughout the cortical and subcapsular regions. The medulla contains a major $K8^-K18^-K5^+K14^+$ subset and a minor $K8^+K18^+K5^-K14^-$ population that is distinguished from the cortical subset by globular morphology and *Ulex europaeus* agglutinin lectin binding properties. Previous studies of the adult murine thymus revealed that $K8^+K5^+$ precursors generate the major cortical $K8^+K5^-$ TEC subset in a process dependent on signals from T lineage-committed thymocytes (7, 15). Two recent reports have shown that progenitor activity is restricted to a subset of $K8^+K5^+$ TECs that expresses MTS24 cell surface glycoprotein (16, 17). Ectopic grafts of isolated and reaggregated $MTS24^+$ TECs can differentiate into cortical and medullary TEC subsets that support thymocyte development.

Although it is well established that proper differentiation of the thymic epithelial compartment requires signals from mesenchymal

Department of Carcinogenesis, University of Texas, M. D. Anderson Cancer Center, Smithville, TX 78957

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[2] Current address: Experimental Immunology Branch, National Cancer Institute, Bethesda, MD 20892.

[3] D.B.K. and C.C. contributed equally to this work.

[4] Address correspondence and reprint requests to Dr. Ellen R. Richie, Department of Carcinogenesis, University of Texas, M. D. Anderson Cancer Center, Science Park-Research Division, Smithville, TX 78957. E-mail address: erichie@odin.mdacc.tmc.edu

[5] Abbreviations used in this paper: E, embryonic day; K, keratin; TEC, thymic epithelial cell; RAG, recombination-activating gene; γ_c, common cytokine receptor γ-chain; Fgf, fibroblast growth factor; h, human.

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cells and thymocytes, it was not known whether thymocyte-derived signals were necessary to establish initial patterning of TEC subsets defined by keratin expression in the thymic anlage. Therefore, we compared TEC development and compartmentalization in wild-type mice to that which occurs in the recombination-activating gene (RAG)2/common γ-chain (γ_c)-deficient and Ikaros-null mice that sustain early and profound blocks in thymocyte differentiation. Our results demonstrate that there is an early developmental window within which thymic epithelial subsets defined by keratin expression patterns develop independently of thymocyte-mediated signals during thymic organogenesis. However, thymocyte-derived signals are required during late fetal development to generate and sustain a normal thymic epithelial compartment in the neonate and adult.

Materials and Methods

Mice

C57BL/6J and human (h)CD3ε-transgenic mice were purchased from The Jackson Laboratory (Bar Harbor, ME). RAG2/γ_c mice were purchased from Taconic Farms (Germantown, NY). Ikaros-null mice were the generous gift of Dr. K. Georgopoulos (Harvard Medical School, Charlestown, MA) (18).

Antibodies

Polyclonal anti-mouse K5 was obtained from Covance Research (Richmond, CA). Troma-1 mAb (anti-K8) was purchased from the Developmental Studies Hybridoma Bank (Iowa City, IA) (19). MTS10, c-*kit*, and CD25 mAbs were purchased from BD PharMingen (San Diego, CA). Immunoreactivity detected with fluorochrome-conjugated anti-Ig (Jackson ImmunoResearch Laboratories, West Grove, PA) was enhanced as indicated by tyramide amplification (PerkinElmer Life Sciences, Boston, MA).

Immunohistology

Serial sections (5 μm) from OCT-embedded frozen tissue were fixed in acetone and incubated overnight at 4°C with optimal dilutions of anti-K8 and/or anti-K5 Abs before washing and incubation with fluorochrome-conjugated secondary reagents. Control slides were incubated with nonimmune serum or isotype-matched Ig. Analysis was performed with an Olympus ProVis AX70 microscope (Olympus, Melville, NY).

Results and Discussion

To investigate the emergence of TEC subsets during thymic organogenesis, we analyzed keratin expression patterns in murine fetal thymi obtained at various gestational stages. Fig. 1 shows that the epithelium of the early thymic anlage at E11.5 is arranged in a two-dimensional bilayer consisting of cells that express K8 but not K5. By E12.5, the epithelial compartment assumes a clustered organization, and a prominent $K8^+K5^+$ subset emerges that is centrally located and surrounded by $K8^+K5^-$ TECs. The centralized clusters of $K8^+K5^+$ TECs persist in E13.5 fetal thymi. By E15.5 smaller clusters of $K8^+5^+$ TECs are observed emanating toward the periphery of the thymus interspersed among the $K8^+K5^-$ subset. K5 expression is generally highest in the innermost cells of the $K8^+K5^+$ clusters, whereas cells at the boundary express less K5, suggesting a transitional population. Some TECs in the central clusters were $K8^{low}K5^+$, consistent with later development of a $K8^-K5^+$ phenotype characteristic of the predominant medullary subset (7). There is a notable change by E17.5, when the cortex becomes well organized and consists predominantly of $K8^+K5^-$ TEC similar to the adult thymus. Although an abundant subset of TECs that coexpress K8 and K5 is still apparent, they are no longer organized into central cores. We also observed incipient medullary regions that contain $K8^-K5^+$ TECs, similar to the predominant medullary TEC subset in the adult (7).

Development of the thymic epithelial compartment depends on inductive signals from hematopoietic as well as mesenchymal cells. The migration of hematopoietic progenitors into the thymic

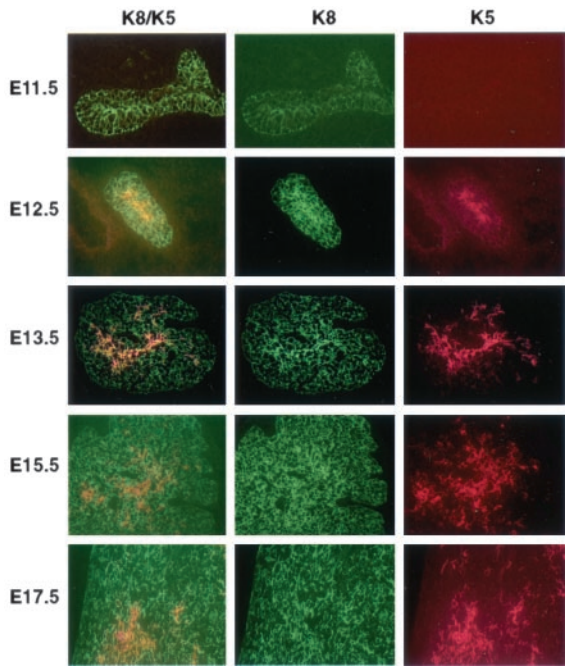

FIGURE 1. K8 and K5 expression in TECs during ontogeny in C57BL/6J mice. Cryostat sections of embryos (E11.5–E13.5) or dissected thymic lobes (E15.5 and E17.5) were stained with Abs to K8 and K5 followed by incubation with FITC- or Texas Red-conjugated anti-Ig secondary reagents. The original magnification was ×200.

primordium is initiated between E11 and E12, which corresponds temporally to the appearance of $K8^+K5^+$ TEC clusters (20, 21). This correlation suggests that interactions with differentiating thymocytes might induce K5 up-regulation in fetal TECs. Alternatively, the appearance of $K8^+K5^+$ TEC clusters by E12.5 might occur independently of thymocyte-derived signals. To explore these possibilities, we examined keratin expression patterns in fetal thymi from RAG2/γ_c-deficient and Ikaros mutant mice. The absence of a functional γ_c gene in RAG2/γ_c-deficient mice precludes IL-7/IL-7R interactions, and the lack of RAG2 prevents TCR gene rearrangement. Consequently, thymocyte cellularity is drastically reduced ($<10^5$ cells) and there is a severe block in T, B, and NK cell development in RAG2/γ_c-deficient mice (22). The Ikaros transcription factor is indispensable for commitment of hematopoietic stem cells to the lymphoid lineage. Targeted deletion of the carboxyl-terminal region of the Ikaros gene results in a null phenotype characterized by failure of B cell development and an absence of T cell precursors during the fetal period (18). Fig. 2 shows that, despite these early T cell developmental blocks, the epithelial compartment in E13.5 and E15.5 RAG2/γ_c-deficient and Ikaros thymi organizes into a three-dimensional structure containing a predominant $K8^+K5^-$ TEC subset and centralized $K8^+K5^+$ TEC clusters. Thus, thymocyte-derived signals are not required to generate the $K8^+K5^+$ clusters in early fetal thymic development.

It has been reported that IL-7 is not as essential for thymopoiesis in fetal compared with adult thymi (23). This may account for the small but detectable population of $CD25^+$ double negative thymocytes in E13.5 RAG2/γ_c-deficient thymi. However, it is unlikely that these cells are responsible for up-regulating K5 expression because $CD25^+$ thymocytes are not detectable in E13.5 Ikaros-null thymi that contain $K8^+K5^+$ central clusters (Fig. 3*A*). Moreover, although a few c-*kit*$^+$ thymocytes are observed in Ikaros-null thymi at E15.5, these early progenitors are not found at

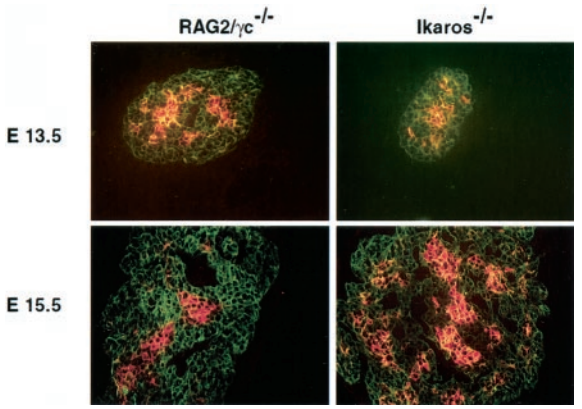

FIGURE 2. Organization of TEC subsets defined by keratin expression patterns at E13.5 and E15.5 in fetal thymi from RAG2/γ_c-deficient and Ikaros-null mice. Cryostat sections were stained with Abs to K8 and K5 as described for Fig. 1.

E13.5 (Fig. 3*B*). These data support the premise that initial patterning of the thymic epithelium into $K8^+K5^-$ and $K8^+K5^+$ TEC subsets occurs independently of thymocyte-derived signals.

Interestingly, the $K8^+K5^+$ clusters in E13.5 thymi from normal, RAG2/γ_c, and Ikaros-null mice express the medullary marker MTS10 (Fig. 3*C*). The brightest $MTS10^+$ cells are found within the central region of $K8^+K5^+$ clusters, whereas the surrounding $K8^+K5^-$ fetal TECs are MTS10 negative. Mature T cells, which are required for medullary region organization (5, 24), are not yet present in the E13.5 normal thymus and the developmental blocks in RAG2/γ_c-deficient and Ikaros mice preclude or delay their appearance. Thus, the MTS10 expression pattern is consistent with the notion that the $K8^+K5^+$ subset contains progenitors of medullary as well as cortical epithelium.

Although hematopoietic precursors do not determine initial patterning of the fetal thymic rudiment, thymocyte/TEC interactions are indispensable for maintaining TEC differentiation and organization in the adult thymus (5, 7, 25). This is apparent in adult mice that sustain a T cell developmental arrest at the $CD4^-CD8^-CD44^+CD25^-$ precursor stage due to expression of a hCD3ϵ transgene. The severely hypoplastic thymi in hCD3ϵ mice have a disorganized TEC compartment that reverts to a two-dimensional organization and consists almost entirely of $K8^+K5^+$ TECs (6, 7, 11). Not surprisingly, we found a similar TEC phenotype in adult RAG2/γ_c-deficient thymi, which also have a profound block in early T cell development (data not shown). Given that thymocyte-derived signals are required to maintain compartmentalization and architecture of the adult thymic epithelium but are not involved in establishing the fetal thymic epithelial network, we examined the duration of the developmental window within which TEC differentiation proceeds independently of thymocyte/TEC interactions. As shown in Fig. 4, the newborn C57BL/6J thymus has a well-developed cortex with $K8^+K5^-$ TECs that are oriented perpendicular to the capsule. Small medullary regions are forming that contain $K8^-K5^+$ TECs surrounded by $K8^+K5^+$ TECs at the corticomedullary junction. Although epithelial organization is similar in newborn and E17.5 thymi, the $K8^+K5^+$ subset is more prominent at E17.5. In striking contrast, newborn RAG2/γ_c-deficient and hCD3ϵ-transgenic thymi are notably hypoplastic, with a keratin expression pattern similar to that observed at E13.5–E15.5 (i.e., prominent centralized clusters of $K8^+K5^+$ TECs). Well-organized mature medullary regions containing $K8^-K5^+$ TECs are absent. The early fetal-like keratin expression pattern persists until ~1 wk of age, after which the majority of TECs assume the aberrant $K8^+K5^+$ phenotype characteristic of adult RAG2/γ_c-deficient and hCD3ϵ-transgenic thymi (data not shown and Ref. 7). Thus, thymocyte-derived signals impinge upon TEC development by E15.5, a time frame that is coincident with the appearance of $CD25^+$ immature thymocytes.

In conclusion, this study demonstrates that regional differences in keratin expression patterns are established early in the genesis of the thymic rudiment. We find that the epithelial cells of the E11.5 thymic primordium express K8 before up-regulating K5 expression. At this developmental stage, K5 is expressed in pharyngeal ectoderm but not in pharyngeal endoderm (data not shown). The $K8^+K5^-$ phenotype of early TECs is consistent with other studies that maintain the thymus is derived exclusively from endoderm (3, 4). Similarly, Gill et al. (17) reported that K8 is widely expressed in the E11.5 thymic primordium, whereas only rare $K5^+$ cells are present. In contrast, Bennett et al. (16) found K5 expression to be more generalized throughout the E11.5 anlage. Discrepancies in the appearance of $K5^+$ TECs during the earliest stage of thymic organogenesis may be due to differences in mouse strains and/or staging criteria.

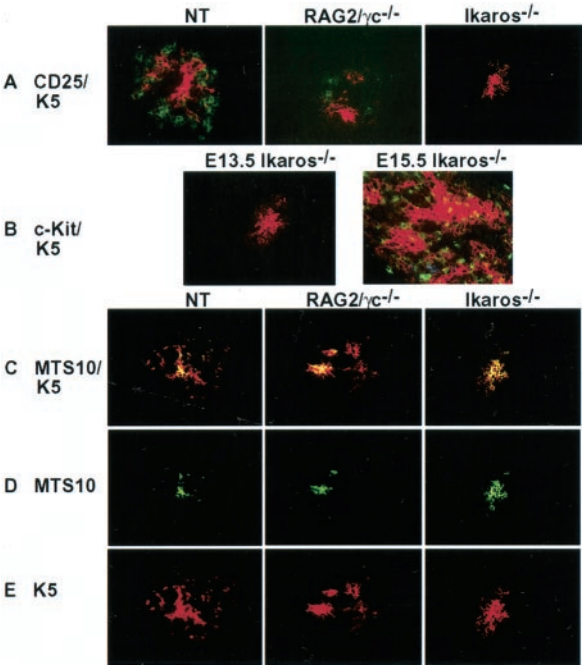

FIGURE 3. Thymocyte-derived signals are not necessary for early patterning of the E13.5 fetal TEC subsets defined by K8, K5, and MTS10 expression. Cryostat sections of E13.5 normal C57BL/6J, RAG2/γ_c-deficient, and Ikaros-null embryos were stained with anti-K5 and anti-CD25 (*A*), anti-c-*kit* (*B*), or anti-MTS10 (*C*). Anti-K5 reactivity was detected with Texas Red-conjugated anti-Ig. The tyramide amplification system was used to detect MTS10, c-*kit*, and CD25 staining.

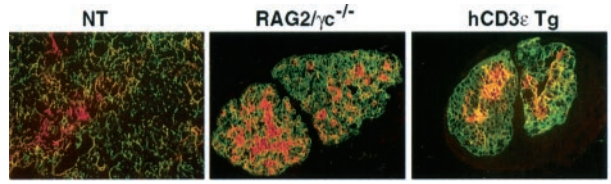

FIGURE 4. Thymocyte-derived signals are required to sustain normal TEC differentiation in the neonate. Cryostat sections of newborn thymic lobes from C57BL/6J, RAG2/γ_c-deficient, and Ikaros-null mice were stained with Abs to K8 and K5 as described for Fig. 1.

It is not yet clear whether the $K8^+K5^-$ epithelial cells in the developing thymic rudiment are equivalent to $K8^+K5^-$ cortical TECs in the adult. The $K8^+K5^-$ epithelial cells in the early thymic primordium may be lost during fetal development, similar to the developmental fate of the $K8^+K5^-$ periderm, the transient outermost layer of embryonic epidermis that disappears before birth (26). Regardless, by approximately E12.5, K5 is up-regulated in a discrete subset of TECs that are localized toward the central region of the developing thymus. Thus, heterogeneity within the epithelial compartment is established early during thymic organogenesis.

Itoi et al. (20) reported that the thymic epithelium converts from a stratified bilayered epithelium at E11 to a clustered organization by E12 and a meshwork structure by E13. Our findings are consistent with this report and further show that K5 is up-regulated in a subset of TECs concomitant with or shortly after the thymic epithelium assumes a three-dimensional structure. Moreover, we have shown that $K8^+K5^+$ TEC clusters are produced in the absence of thymocyte precursors. $K8^+K5^+$ TECs contain $MTS24^+$ progenitors of the cortical and medullary TEC compartments (16, 17). Therefore, we conclude that initial development of functional TEC progenitors is independent of hematopoietic-derived signals. Mesenchyme-derived inductive signals may be responsible for early patterning of the thymic epithelial compartment. Byrne et al. (27) found that K5 expression in the developing epidermis does not correlate with morphogenesis per se, but rather with changes in the embryonic origin of underlying mesenchyme. Earlier studies demonstrated that neural crest-derived mesenchymal cells play a crucial role in thymic development (8, 9, 28). Fibroblast growth factor (Fgf)7 and Fgf10 produced by mesenchymal cells surrounding the thymic primordium activate proliferation of FgFR2-IIIb-expressing TECs (29). Mesenchymal cells may also impart cues that induce differentiation and initial patterning of the thymic rudiment. In any case, we demonstrate that there is a discrete developmental window beyond which thymocyte-derived signals are required to sustain TEC organization and differentiation as defined by keratin expression patterns. Further studies are needed to define the various signaling pathways that induce thymocyte-independent and -dependent phases of TEC differentiation.

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References

1. Boyd, R. L., C. L. Tucek, D. I. Godfrey, D. J. Izon, T. J. Wilson, N. J. Davidson, A. G. D. Bean, H. M. Ladyman, M. A. Ritter, and P. Hugo. 1993. The thymic microenvironment. *Immunol. Today 14:445.*
2. Manley, N. R. 2000. Thymus organogenesis and molecular mechanisms of thymic epithelial cell differentiation. *Semin. Immunol. 12:421.*
3. Cordier, A. C., and S. M. Haumont. 1980. Development of thymus, parathyroids, and ultimo-branchial bodies in NMRI and nude mice. *Am. J. Anat. 157:227.*
4. Gordon, J., A. R. Bennett, C. C. Blackburn, and N. R. Manley. 2001. Gcm2 and Foxn1 mark early parathyroid- and thymus-specific domains in the developing third pharyngeal pouch. *Mech. Dev. 103:141.*
5. Shores, E. W., W. Van Ewijk, and A. Singer. 1994. Maturation of medullary thymic epithelium requires thymocytes expressing fully assembled CD3-TCR complexes. *Int. Immunol. 6:1393.*
6. Hollander, G. A., B. Wang, A. Nichogiannopoulou, P. P. Platenburg, W. van Ewijk, S. J. Burakoff, J.-C. Gutierrez-Ramos, and C. Terhorst. 1995. Developmental control point in induction of thymic cortex regulated by a subpopulation of prothymocytes. *Nature 373:350.*
7. Klug, D. B., C. Carter, E. Crouch, D. Roop, C. J. Conti, and E. R. Richie. 1998. Interdependence of cortical thymic epithelial cell differentiation and T-lineage commitment. *Proc. Natl. Acad. Sci. USA 95:11822.*
8. Bockman, D. E., and M. L. Kirby. 1984. Dependence of thymic development on derivatives of the neural crest. *Science 223:498.*
9. Shinohara, T., and T. Honjo. 1996. Epidermal growth factor can replace thymic mesenchyme in induction of embryonic thymus morphogenesis in vitro. *Eur. J. Immunol. 26:747.*
10. Franke, W. W., D. L. Schiller, R. Moll, S. Winger, E. Schmid, I. Engelbrecht, H. Denk, R. Krepler, and B. Platzer. 1991. Diversity of cytokeratins: differentiation specific expression of cytokeratin polypeptides in epithelial cells and tissues. *J. Mol. Biol. 153:933.*
11. van Ewijk, W., B. Wang, G. Hollander, H. Kawamoto, E. Spanopoulou, M. Itoi, T. Amagai, Y. F. Jiang, W. T. Germeraad, W. F. Chen, and Y. Katsura. 1999. Thymic microenvironments, 3-D versus 2-D? *Semin. Immunol. 11:57.*
12. Lind, E. F., S. E. Prockop, H. E. Porritt, and H. T. Petrie. 2001. Mapping precursor movement through the postnatal thymus reveals specific microenvironments supporting defined stages of early lymphoid development. *J. Exp. Med. 194:127.*
13. Anderson, G., and E. J. Jenkinson. 2001. Lymphostromal interactions in thymic development and function. *Nat. Rev. Immunol. 1:31.*
14. Klug, D. B., E. Crouch, C. Carter, L. Coghlan, C. J. Conti, and E. R. Richie. 2000. Transgenic expression of cyclin D1 in thymic epithelial precursors promotes epithelial and T cell development. *J. Immunol. 164:1881.*
15. Sano, S., Y. Takahama, T. Sugawara, H. Kosaka, S. Itami, K. Yoshikawa, J. Miyazaki, W. van Ewijk, and J. Takeda. 2001. Stat3 in thymic epithelial cells is essential for postnatal maintenance of thymic architecture and thymocyte survival. *Immunity 15:261.*
16. Bennett, A. R., A. Farley, N. F. Blair, J. Gordon, L. Sharp, and C. C. Blackburn. 2002. Identification and characterization of thymic epithelial progenitor cells. *Immunity 16:803.*
17. Gill, J., M. Malin, G. A. Hollander, and R. Boyd. 2002. Generation of a complete thymic microenvironment by $MTS24^+$ thymic epithelial cells. *Nat. Immunol. 3:635.*
18. Wang, J.-H., A. Nichogiannopoulou, L. Wu, L. Sun, A.-H. Sharpe, M. Bigby, and K. Georgopoulos. 1996. Selective defect in the development of the fetal and adult lymphoid system in mice with an Ikaros null mutation. *Immunity 5:537.*
19. Kemler, R., P. Brulet, M.-T. Schnebelen, J. Gaillard, and F. Jacob. 1981. Reactivity of monoclonal antibodies against intermediate filament proteins during embryonic development. *J. Embryol. Exp. Morphol. 64:45.*
20. Itoi, M., H. Kawamoto, Y. Katsura, and T. Amagai. 2001. Two distinct steps of immigration of hematopoietic progenitors into the early thymus anlage. *Int. Immunol. 13:1203.*
21. Suniara, R. K., E. J. Jenkinson, and J. J. Owen. 2000. An essential role for thymic mesenchyme in early T cell development. *J. Exp. Med. 191:1051.*
22. Colucci, F., C. Soudais, E. Rosmaraki, L. Vanes, V. L. Tybulewicz, and J. P. Di Santo. 1999. Dissecting NK cell development using a novel alymphoid mouse model: investigating the role of the c-*abl* proto-oncogene in murine NK cell differentiation. *J. Immunol. 162:2761.*
23. Crompton, T., S. V. Outram, J. Buckland, and M. J. Owen. 1998. Distinct roles of the interleukin-7 receptor α chain in fetal and adult thymocyte development revealed by analysis of interleukin-7 receptor α-deficient mice. *Eur. J. Immunol. 28:1859.*
24. Surh, C. D., B. Ernst, and J. Sprent. 1992. Growth of epithelial cells in the thymic medulla is under the control of mature T cells. *J. Exp. Med. 176:611.*
25. van Ewijk, W., E. W. Shores, and A. Singer. 1994. Crosstalk in the mouse thymus. *Immunol. Today 15:214.*
26. Takaishi, M., Y. Takata, T. Kuroki, and N. Huh. 1998. Isolation and characterization of a putative keratin-associated protein gene expressed in embryonic skin of mice. *J. Invest. Dermatol. 111:128.*
27. Byrne, C., M. Tainsky, and E. Fuchs. 1994. Programming gene expression in developing epidermis. *Development 120:2369.*
28. Manley, N. R., and M. R. Capecchi. 1995. The role of *Hoxa-3* in mouse thymus and thyroid development. *Development 121:1989.*
29. Revest, J. M., R. K. Suniara, K. Kerr, J. J. Owen, and C. Dickson. 2001. Development of the thymus requires signaling through the fibroblast growth factor receptor R2-IIIb. *J. Immunol. 167:1954.*