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The Early Postnatal Life: A Dynamic Period in Thymic Epithelial Cell Differentiation

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The microenvironments formed by cortical (c) and medullary (m) thymic epithelial cells (TECs) play a non-redundant role in the generation of functionally diverse and self-tolerant T cells. The role of TECs during the first weeks of the murine postnatal life is particularly challenging due to the significant augment in T cell production. Here, we critically review recent studies centered on the timely coordination between the expansion and maturation of TECs during this period and their specialized role in T cell development and selection. We further discuss how aging impacts on the pool of TEC progenitors and maintenance of functionally thymic epithelial microenvironments, and the implications of these chances in the capacity of the thymus to sustain regular thymopoiesis throughout life.

Keywords: thymus, thymic epithelial cells, tolerance, early postnatal life, aging

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INTRODUCTION

The current pandemic caused by the SARS-CoV-2 virus underscores the importance of maintaining a pool of immunologically competent T cells, which are capable of responding to virtually any new foreign threats while tolerant to the host own tissues. The establishment of a diverse T cell receptor (TCR) repertoire arises from the random recombination of V(D)J gene segments during T cell development in the thymus. Yet, the arbitrariness underlying this process can also produce autoreactive T lymphocytes. The thymus has developed several control mechanisms to simultaneously establish T cell immunity against non-self elements and impose self-tolerance. Particularly important in the choreography of T cell selection are thymic epithelial cells (TECs), which represent a key component of the thymic stromal microenvironment. TECs are typically subdivided into functionally distinct cortical (cTEC) and medullary (mTEC) lineages (1). While cTECs primarily mediate T cell lineage commitment and positive selection, mTECs fine-tune the negative selection of autoreactive thymocytes or promote their deviation into the T regulatory cell lineage (2). It is conceptually accepted that cTECs and mTECs differentiate from thymic epithelial progenitors (TEPs) present within the embryonic and postnatal thymus (2). Deficits in the function of TECs arise with aging, cytoablative regimens and infection, leading to a lower naïve T cell output. These thymic failures are pertinent in the elderly and patients undergoing bone marrow transplantations (BMT), contributing to their poor T-cell responses to new pathogens or predisposing to autoimmunity (3). Thus, the preservation of a regular thymic function also depends on the maintenance and differentiation potential of bipotent or lineage restricted TEPs.

In this review, we focus on critical changes in the molecular traits of TECs that occur during the first weeks of the murine postnatal life, and integrate how these alterations might precede events coupled with thymic involution.

THE BUILD-UP OF TEC MICROENVIRONMENTS

The initiation of TEC development coincides with the onset of thymus organogenesis, which starts around day 9-10 of the murine embryonic gestation (E9-10) (4). The expression of Forkhead box protein N1 (Foxn1) in the ventral area of the common thymus and parathyroid primordium marks a critical step in TEC specification (5). Still, Foxn1 expression needs to be continuously maintained during the differentiation of c/mTEC, wherein it imposes a complex genetic program that confers them the capacity to support distinct stages of thymopoiesis (6). TEPs formed during early thymus ontogeny constitute the primordial building blocks for the establishment and maintenance of c/mTEC microenvironments (7–9). Our comprehension about the mechanisms underlying TEC differentiation has considerably advanced with the identification of distinct populations containing bipotent or lineage-restricted progenitor activity (10–21) [further detailed below and reviewed in (22, 23)]. These studies led to the proposal of different refined models of TEC differentiation, whereby TEPs traverse through transitional stages that share a closer or distinct relationship with cTEC- or mTEC-unipotent precursors, prior to the commitment in mature c/mTEC subsets [reviewed in (2, 24, 25)]. Yet, it remains unclear the trajectories and molecular elements governing the differentiation of TEC progenitors into mature c/mTEC lineages.

The expansion and functionalization of c/mTEC compartment during early postnatal stages generates a supportive microenvironment that increases thymopoiesis, reaching its peak during young adulthood. Thereafter, T cell production progressively declines with aging, becoming residual in the aged thymus (26). During these periods, TECs undergo concomitant alterations in their composition and differentiation program. Although the density of TECs based on flow cytometry analysis might be underestimated (27), the number of TECs vigorously expands during postnatal life and early adulthood, followed by a progressive decline with age (28, 29). Changes in the size of TEC microenvironment appears to relate with the function of the thymus. While a reduction in the TEC compartment below a certain threshold restrains thymopoiesis (30, 31), the expansion of the thymic epithelial niche, for example *via* transgenic expression of Foxn1 or Cyclin D1, increases T cell generation (32, 33). Along this line, the frequency of cycling TECs is elevated during fetal life, progressively declines during the postnatal life and become a rare fraction in the aged mouse thymus (28). Transcriptomic analysis revealed that the expression of cell-cycle regulators is downregulated in TECs as early as 1 month (34). Moreover, the enforced expression of cMyc in TECs promotes the expansion of the TEC compartment, *via* the engagement of a genetic program akin to the one found in embryonic TECs (35). These results suggest that the loss in the proliferative rate of TECs, together with other alterations such as changes in cell survival and rate of differentiation, may contribute to a reduction in the size of TEC compartments with age. In the next sections, we outline specific cellular and molecular alterations that take place in c/mTEC during early postnatal life, and conjecture how those changes may anticipate subsequent functional losses in the capacity of TECs to sustain regular thymopoiesis in the long-term.

THE ASSEMBLY OF FUNCTIONALLY DEDICATED CTEC AND MTEC COMPARTMENTS

The first weeks of the postnatal life marks a period of intense turnover and functional diversification in the TEC niche, wherein key mature subsets in tolerance induction are generated or expanded (23). During this period, the changes in the cellularity and functionality of cTECs appear to unfold concomitant with the expansion and diversification of mTECs (11, 12, 36–38). This leads to a conspicuous inversion in the cTEC/mTEC ratio within the first 2 weeks after birth, which correlates with the intensification of thymopoiesis (11, 12, 28). In this regard, the consequent rise in the number of positive and negative selection events, will impose an increase demand on TEC compartments. Given that mature cTECs and mTECs have a limited life-span, the maintenance and specialization of their microenvironment seem to depend on the continual differentiation of their progenitors. These functional requirements are in part met by a symbiotic relationship with thymocytes (discussed further below) that stimulate specific proliferative and differentiation programs in TECs (39).

It remains surprising how little we know about the molecular program that underlies the differentiation of cTECs. Despite these gaps, several studies highlight that cTECs undergo molecular and functional changes during neonatal and puberty periods. In particular, cTECs downregulate the expression of key thymopoietic factors, such as Dll4 and IL-7, during the first weeks of postnatal life, which result from continual lymphoepithelial interactions (37, 38, 40, 41). These quantitative and qualitative disruptions in cTECs appear to anticipate the bona fide hallmarks that characterize TECs in the involuted thymus. In contrast to cTECs, our understanding of the cartography of mTEC differentiation is more complete (22). This process depends on reciprocal signals provided by several types of hematopoietic cells (1). These lymphoepithelial interactions, commonly referred as thymic crosstalk, engage specific members of the tumor necrosis factor receptor superfamily (TNFRSF), including receptor activator of NF-κB (RANK), CD40 and lymphotoxin β receptor (LTβR), in mTECs and their progenitors, leading to the activation of a nuclear factor kappa B (NF-κB)-dependent maturation program [reviewed in (1, 22)]. The cooperative action of TNFRSF members is not only important for the expansion of mTEC niches but also for their functional diversification. Upon the initial subdivision in

mTEC^{low} and mTEC^{high} (42), the discovery of Autoimmune regulator (Aire)-, Ccl21- and forebrain embryonic zinc finger-like protein 2 (Fezf2)-expressing cells revealed that mTECs harbors a variety of functionally distinct mature subsets (1, 22). Although Aire$^+$ and Fezf2$^+$ cells emerge during embryonic life (1, 22), their abundance significantly increases in the first weeks of life. In this regard, RANK-mediated signaling is essential to the expansion of Aire$^+$ mTECs, whereas CD40 also contributes to this process (43, 44). Although LTβR signaling was initially coupled to the development of Aire$^+$ (45) and Fezf2$^+$ lineages (46), subsequent studies indicated its involvement in the architecture of postnatal medullary compartment (47). Aire and Fezf2 regulate the capacity of mTECs to express large sets of non-overlapping tissue restricted antigens (TRAs), which are randomly organized in patterns of gene expression at the single cell level (48–50) and are reported to decrease their levels with age (51–53). In this regard, an earlier study underscore the importance of Aire expression in mTECs during neonatal period (54), which corelates with their capacity to control the generation of a unique population of T regulatory cells (55). It remains to be determined whether Aire expression during this temporal window particularly impacts on the quantity or quality of TRAs expression by mTECs.

The role of mTECs in tolerance induction extends beyond their promiscuous gene expression capacity. CCL21-producing cells represent a prototypical example of alternative roles of mTECs. CCL21-expressing mTEC represent a subset of mTEC^{lo} and control the migration of positively selected thymocytes towards the medulla (56, 57). CCl21$^+$ cells emerge during embryogenesis and their numbers also undergo a marked increase during the first weeks of life (57). Recent single cell RNA sequencing analysis suggests that Aire- and Ccl21a-expressing mTEC subsets do not share a direct lineage relationship (58). Moreover, the discoveries that Aire$^+$mTECs differentiate into Post-Aire cells (59, 60) further extended our view on the heterogeneity within thymus medulla. Post-Aire mTECs shutdown the expression of Aire, certain TRAs, CD80 and MHCII, while acquiring traits of terminally differentiated keratinocytes (61, 62). Two reports identified a highly differentiated mTECs that share molecular traits with tuft cells found at mucosal barriers. Fate-mapping analysis suggests that this subset can develop *via* an AIRE-dependent and AIRE-independent pathway (63, 64). Although their complete functional relevance remains elusive, tuft-like mTECs appear to regulate the development of invariant NKT cells and ILCs (63, 64). Future studies may uncover new specialized mTEC subsets and their role in imposing the limits of tolerance, or alternative processes in thymus biology.

THE THYMIC EPITHELIAL CELL PROGENITOR RESERVOIR

The diversification of TECs during the first weeks of life is dictated by the intricate balance between the rate of proliferation and differentiation of mature subsets. The rapid turnover of TEC microenvironments, with an estimated replacement time of one to two weeks to mTECs (28, 59), implicates the requirement for a regular generation of mature TECs from their upstream progenitors. One possibility is that bipotent TEPs continually produce lineage-committed precursors lacking long-term self-renewal capacity. Alternatively, and not mutually exclusive, the abundance of bipotent TEPs might decrease with age, being the maintenance of cortical and medullary epithelial niches assured by downstream compartment-restricted precursors. In the last years, several studies provide evidence for the existence of an arsenal of subsets enriched in purported bipotent TEC progenitors in the postnatal thymus (10, 13–15). One approach has employed *in vitro* 2D-clonogenic (10) or spheroids (13) assays to respectively isolate TEC progenitors that reside within EpCAM$^+$Ly51$^+$cTECs or EpCAM$^-$ cells, which were expanded *in vitro* and revealed the capacity to give rise to c/mTEC. Nonetheless, a more recent study indicate that cells isolated from EpCAM$^-$derived spheroids represent mesenchymal progenitors (65). Other methodologies resolved bipotent progenitor activity within defined subsets of UEA-1$^-$MHII^{lo} Sca-1$^+$ TECs (14) and MHCII^{hi} Ly51$^+$Plet1$^+$ cTECs (15). Both strategies employ reaggregate organ cultures (RTOCS) to determine the precursor-product lineage relationship to mature cells. Despite the advances, it remains to be determined the physiological contribution of these cells to the TEC microenvironment in the adult thymus. Thus, we still lack experimental evidence that demonstrates the existence of bona-fide bipotent TEC progenitors in the postnatal thymus, and their identification at the single cell level.

Downstream of TEC progenitors, complementary studies documented how mTEC compartments evolved from bipotent TEP and mTEC-restricted precursors (mTEPs), including mTEC-restricted SSEA-1+ and podoplanin+ (PDPN) mTEPs (16, 18). Fate-mapping studies show that the adult mTEC network arise from fetal- and newborn-derived TEPs expressing beta5t (β5t), a prototypical cTEC marker. Yet, the contribution of β5t+ TEPs to the adult mTEC niche decreases with age (19, 20), suggesting that the maintenance of the adult medullary epithelium is assured by mTEPs. Although bipotent TEPs might lose the expression of some traits found in the embryo (e.g. β5t), it is also possible that the abundance and/or the self-renewal properties of bipotent TEPs and/or lineage-restricted progenitors decline with time. Supporting this view, the clonogenic activity of purported bipotent TEPs that reside within the cortex decrease with age (10) and Cld3,4$^+$SSEA1$^+$ mTEC-restricted cells become rare in the adult thymus (16). Given that the numbers of embryonic TEPs dictates the size of functional TEC microenvironments (30), we infer that the loss in the TEC network that takes place with age may result from the decrease in the bioavailability and self-renewal capacity of TEPs early in life.

The advent of single cell RNA sequencing (scRNAseq) analysis have also contributed to our understanding of the heterogeneity and dynamic of TEC progenitors. This approach has emerged as a new unbiased method to identify novel subsets, providing a valuable platform to analyze their developmental

trajectories and determine their relationships with progenitor subsets identified by conventional methodologies. In this regard, new clusters termed "pre-Aire mTEC 1 and 2" (66) appear to present molecular traits similar to the ones found in podoplanin+ (PDPN) mTEPs (18). A subsequent study identified a novel cluster of "intertypical TECs" (51) that harbors traits akin to the ones found in podoplanin+ (PDPN) mTEPs (18), UEA-1^{-}MHII^{lo}Sca-1^{+} (14) and MHCII^{hi} Ly51^{+}Plet1^{+} (15) TECs. Since "intertypical TECs" are further segmented in distinct 4 subclusters, it would be interesting to determine if they associate to a particular bipotent or unipotent subset. Moreover, scRNAseq analysis reveal the existence of a previously unrecognized cluster of "perinatal cTECs". Interestingly, this subset harbors cells with a highly proliferative status and their abundance declines with age (51). Moreover, the combination of scRNAseq and fate mapping analysis revealed that $\beta5t^{+}$ TEPs acquire senescent-like properties with age, potentially explaining their failure to contribute to mTEC lineage beyond the neonatal stage (19, 20). Together, these findings indicate that the integration of multiple experimental approaches provides a more complete strategy to resolve the intricacies of the TEC compartment. Future studies should attempt to identify specific markers to resolve the newly characterized populations at a single level.

CONCLUDING REMARKS

The aforementioned studies underscore that the period between birth and early adulthood is a time of intense alterations in TEC microenvironments, which prepares them to the highly demand role of choreographing the selection of growing number of T cell precursors. In this sense, it is remarkable to appreciate the synchronous coordination between TEC differentiation and the requisites imposed by T cell development. Yet, the erosion of the pool of TEC progenitors seem to accompany the generation of specialized subsets with key roles in tolerance induction. We reason that an in-depth molecular analysis of TEC differentiation during early postnatal may provide insights on how TEC niches are maintained, and can be repaired in the aged thymus. Despite recent advances, it remains unclear how changes in the bioavailability of TEPs impact on the maintenance of TEC microenvironment across life, and ultimately on thymic output. Another unexplored area pertains to the physiological causes underlying the presumed age-dependent decrease and/or senescence of TEPs. Knowledge in these areas will not only permit to comprehend the basic principles that governs thymic function, but also target pathways for the treatment of disorders coupled to dysfunctional thymic/T cell responses.

AUTHOR CONTRIBUTIONS

NA and RP wrote the manuscript. All authors contributed to the article and approved the submitted version.

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REFERENCES

1. Anderson G, Takahama Y. Thymic Epithelial Cells: Working Class Heroes for T Cell Development and Repertoire Selection. *Trends Immunol* (2012) 33:256–63. doi: 10.1016/j.it.2012.03.005
2. Alves NL, Takahama Y, Ohigashi I, Ribeiro AR, Baik S, Anderson G, et al. Serial Progression of Cortical and Medullary Thymic Epithelial Microenvironments. *Eur J Immunol* (2014) 44:16–22. doi: 10.1002/eji.201344110
3. Hollander GA, Krenger W, Blazar BR. Emerging Strategies to Boost Thymic Function. *Curr Opin Pharmacol* (2010) 10:443–53. doi: 10.1016/j.coph.2010.04.008
4. Rodewald HR. Thymus Organogenesis. *Annu Rev Immunol* (2008) 26:355–88. doi: 10.1146/annurev.immunol.26.021607.090408
5. Gordon J, Bennett AR, Blackburn CC, Manley NR. Gcm2 and Foxn1 Mark Early Parathyroid- and Thymus-Specific Domains in the Developing Third Pharyngeal Pouch. *Mech Dev* (2001) 103:141–3. doi: 10.1016/S0925-4773(01)00333-1
6. Zuklys S, Handel A, Zhanybekova S, Govani F, Keller M, Maio S, et al. Foxn1 Regulates Key Target Genes Essential for T Cell Development in Postnatal Thymic Epithelial Cells. *Nat Immunol* (2016) 17:1206–15. doi: 10.1038/ni.3537
7. Gill J, Malin M, Hollander GA, Boyd R. Generation of a Complete Thymic Microenvironment by MTS24(+) Thymic Epithelial Cells. *Nat Immunol* (2002) 3:635–42. doi: 10.1038/ni812
8. Bennett AR, Farley A, Blair NF, Gordon J, Sharp L, Blackburn CC. Identification and Characterization of Thymic Epithelial Progenitor Cells. *Immunity* (2002) 16:803–14. doi: 10.1016/S1074-7613(02)00321-7
9. Rossi SW, Jenkinson WE, Anderson G, Jenkinson EJ. Clonal Analysis Reveals a Common Progenitor for Thymic Cortical and Medullary Epithelium. *Nature* (2006) 441:988–91. doi: 10.1038/nature04813
10. Meireles C, Ribeiro AR, Pinto RD, Leitao C, Rodrigues PM, Alves NL. Thymic Crosstalk Restrains the Pool of Cortical Thymic Epithelial Cells With Progenitor Properties. *Eur J Immunol* (2017) 47:958–69. doi: 10.1002/eji.201746922

11. Ribeiro AR, Meireles C, Rodrigues PM, Alves NL. Intermediate Expression of CCRL1 Reveals Novel Subpopulations of Medullary Thymic Epithelial Cells That Emerge in the Postnatal Thymus. *Eur J Immunol* (2014) 44:2918–24. doi: 10.1002/eji.201444585
12. Ribeiro AR, Rodrigues PM, Meireles C, Di Santo JP, Alves NL. Thymocyte Selection Regulates the Homeostasis of IL-7-Expressing Thymic Cortical Epithelial Cells In Vivo. *J Immunol* (2013) 191:1200–9. doi: 10.4049/jimmunol.1203042
13. Ucar A, Ucar O, Klug P, Matt S, Brunk F, Hofmann TG, et al. Adult Thymus Contains FoxN1(-) Epithelial Stem Cells That Are Bipotent for Medullary and Cortical Thymic Epithelial Lineages. *Immunity* (2014) 41:257–69. doi: 10.1016/j.immuni.2014.07.005
14. Wong K, Lister NL, Barsanti M, Lim JM, Hammett MV, Khong DM, et al. Multilineage Potential and Self-Renewal Define an Epithelial Progenitor Cell Population in the Adult Thymus. *Cell Rep* (2014) 8:1198–209. doi: 10.1016/j.celrep.2014.07.029
15. Ulyanchenko S, O'Neill KE, Medley T, Farley AM, Vaidya HJ, Cook AM, et al. Identification of a Bipotent Epithelial Progenitor Population in the Adult Thymus. *Cell Rep* (2016) 12:2819–32. doi: 10.1016/j.celrep.2016.02.080
16. Sekai M, Hamazaki Y, Minato N. Medullary Thymic Epithelial Stem Cells Maintain a Functional Thymus to Ensure Lifelong Central T Cell Tolerance. *Immunity* (2014) 41:753–61. doi: 10.1016/j.immuni.2014.10.011
17. Hamazaki Y, Fujita H, Kobayashi T, Choi Y, Scott HS, Matsumoto M, et al. Medullary Thymic Epithelial Cells Expressing Aire Represent a Unique Lineage Derived From Cells Expressing Claudin. *Nat Immunol* (2007) 8:304–11. doi: 10.1038/ni1438
18. Onder L, Nindl V, Scandella E, Chai Q, Cheng HW, Caviezel-Firner S, et al. Alternative NF-kappaB Signaling Regulates mTEC Differentiation From Podoplanin-Expressing Presursors in the Cortico-Medullary Junction. *Eur J Immunol* (2015) 45:2218–31. doi: 10.1002/eji.201545677
19. Ohigashi I, Zuklys S, Sakata M, Mayer CE, Hamazaki Y, Minato N, et al. Adult Thymic Medullary Epithelium Is Maintained and Regenerated by Lineage-Restricted Cells Rather Than Bipotent Progenitors. *Cell Rep* (2015) 13:1432–43. doi: 10.1016/j.celrep.2015.10.012
20. Mayer CE, Zuklys S, Zhanybekova S, Ohigashi I, Teh HY, Sansom SN, et al. Dynamic Spatio-Temporal Contribution of Single Beta5t+ Cortical Epithelial Precursors to the Thymus Medulla. *Eur J Immunol* (2015) 4:846–56. doi: 10.1002/eji.201545995
21. Akiyama N, Takizawa N, Miyauchi M, Yanai H, Tateishi R, Shinzawa M, et al. Identification of Embryonic Precursor Cells That Differentiate Into Thymic Epithelial Cells Expressing Autoimmune Regulator. *J Exp Med* (2016) 213:1441–58. doi: 10.1084/jem.20151780
22. Alves NL, Ribeiro AR. Thymus Medulla Under Construction: Time and Space Oddities. *Eur J Immunol* (2016) 46:829–33. doi: 10.1002/eji.201646329
23. Abramson J, Anderson G. Thymic Epithelial Cells. *Annu Rev Immunol* (2017) 35:85–118. doi: 10.1146/annurev-immunol-051116-052320
24. Hamazaki Y. Adult Thymic Epithelial Cell (TEC) Progenitors and TEC Stem Cells: Models and Mechanisms for TEC Development and Maintenance. *Eur J Immunol* (2015) 45:2985–93. doi: 10.1002/eji.201545844
25. Ishikawa T, Akiyama N, Akiyama T. In Pursuit of Adult Progenitors of Thymic Epithelial Cells. *Front Immunol* (2021) 12:621824. doi: 10.3389/fimmu.2021.621824
26. Lynch HE, Goldberg GL, Chidgey A, Van den Brink MR, Boyd R, Sempowski GD. Thymic Involution and Immune Reconstitution. *Trends Immunol* (2009) 30:366–73. doi: 10.1016/j.it.2009.04.003
27. Dumont-Lagacé M, Brochu S, St-Pierre C, Perreault C. Adult Thymic Epithelium Contains Nonsenescent Label-Retaining Cells. *J Immunol* (2014) 192:2219–26. doi: 10.4049/jimmunol.1302961
28. Gray DH, Seach N, Ueno T, Milton MK, Liston A, Lew AM, et al. Developmental Kinetics, Turnover, and Stimulatory Capacity of Thymic Epithelial Cells. *Blood* (2006) 108:3777–85. doi: 10.1182/blood-2006-02-004531
29. Alves NL, Richard-Le Goff O, Huntington ND, Sousa AP, Ribeiro VS, Bordack A, et al. Characterization of the Thymic IL-7 Niche In Vivo. *Proc Natl Acad Sci USA* (2009) 106:1512–7. doi: 10.1073/pnas.0809559106
30. Jenkinson WE, Bacon A, White AJ, Anderson G, Jenkinson EJ. An Epithelial Progenitor Pool Regulates Thymus Growth. *J Immunol* (2008) 181:6101–8. doi: 10.4049/jimmunol.181.9.6101
31. Chen L, Xiao S, Manley NR. Foxn1 is Required to Maintain the Postnatal Thymic Microenvironment in a Dosage-Sensitive Manner. *Blood* (2009) 113:567–74. doi: 10.1182/blood-2008-05-156265
32. Robles AI, Larcher F, Whalin RB, Murillas R, Richie E, Gimenez-Conti IB, et al. Expression of Cyclin D1 in Epithelial Tissues of Transgenic Mice Results in Epidermal Hyperproliferation and Severe Thymic Hyperplasia. *Proc Natl Acad Sci USA* (1996) 93:7634–8. doi: 10.1073/pnas.93.15.7634
33. Zook EC, Krishack PA, Zhang S, Zeleznik-Le NJ, Firulli AB, Witte PL, et al. Overexpression of Foxn1 Attenuates Age-Associated Thymic Involution and Prevents the Expansion of Peripheral Cd4 Memory T Cells. *Blood* (2011) 118:5723–31. doi: 10.1182/blood-2011-03-342097
34. Ki S, Park D, Selden HJ, Seita J, Chung H, Kim J, et al. Global Transcriptional Profiling Reveals Distinct Functions of Thymic Stromal Subsets and Age-Related Changes During Thymic Involution. *Cell Rep* (2014) 9:402–15. doi: 10.1016/j.celrep.2014.08.070
35. Cowan JE, Malin J, Zhao Y, Seedhom MO, Harly C, Ohigashi I, et al. Myc Controls a Distinct Transcriptional Program in Fetal Thymic Epithelial Cells That Determines Thymus Growth. *Nat Commun* (2019) 10:5498. doi: 10.1038/s41467-019-13465-y
36. Rode I, Boehm T. Regenerative Capacity of Adult Cortical Thymic Epithelial Cells. *Proc Natl Acad Sci USA* (2012) 109:3463–8. doi: 10.1073/pnas.1118823109
37. Alves NL, Huntington ND, Mention JJ, Richard-Le Goff O, Di Santo JP. Cutting Edge: A Thymocyte-Thymic Epithelial Cell Cross-Talk Dynamically Regulates Intrathymic IL-7 Expression In Vivo. *J Immunol* (2010) 184:5949–53. doi: 10.4049/jimmunol.1000601
38. Fiorini E, Ferrero I, Merck E, Favre S, Pierres M, Luther SA, et al. Cutting Edge: Thymic Crosstalk Regulates Delta-Like 4 Expression on Cortical Epithelial Cells. *J Immunol* (2008) 181:8199–203. doi: 10.4049/jimmunol.181.12.8199
39. Alves NL, Huntington ND, Rodewald HR, Di Santo JP. Thymic Epithelial Cells: The Multi-Tasking Framework of the T Cell "Cradle". *Trends Immunol* (2009) 30:468–74. doi: 10.1016/j.it.2009.07.010
40. Koch U, Fiorini E, Benedito R, Besseyrias V, Schuster-Gossler K, Pierres M, et al. Delta-Like 4 Is the Essential, Nonredundant Ligand for Notch1 During Thymic T Cell Lineage Commitment. *J Exp Med* (2008) 205:2515–23. doi: 10.1084/jem.20080829
41. Rodrigues PM, Ribeiro AR, Serafini N, Meireles C, Di Santo JP, Alves NL. Intrathymic Deletion of IL-7 Reveals a Contribution of the Bone Marrow to Thymic Rebound Induced by Androgen Blockade. *J Immunol* (2018) 200:1389–98. doi: 10.4049/jimmunol.1701112
42. Gray D, Abramson J, Benoist C, Mathis D. Proliferative Arrest and Rapid Turnover of Thymic Epithelial Cells Expressing Aire. *J Exp Med* (2007) 204:2521–8. doi: 10.1084/jem.20070795
43. Akiyama T, Shimo Y, Yanai H, Qin J, Ohshima D, Maruyama Y, et al. The Tumor Necrosis Factor Family Receptors RANK and CD40 Cooperatively Establish the Thymic Medullary Microenvironment and Self-Tolerance. *Immunity* (2008) 29:423–37. doi: 10.1016/j.immuni.2008.06.015
44. Hikosaka Y, Nitta T, Ohigashi I, Yano K, Ishimaru N, Hayashi Y, et al. The Cytokine Rankl Produced by Positively Selected Thymocytes Fosters Medullary Thymic Epithelial Cells That Express Autoimmune Regulator. *Immunity* (2008) 29:438–50. doi: 10.1016/j.immuni.2008.06.018
45. Boehm T, Scheu S, Pfeffer K, Bleul CC. Thymic Medullary Epithelial Cell Differentiation, Thymocyte Emigration, and the Control of Autoimmunity Require Lympho-Epithelial Cross Talk *Via* Ltbetar. *J Exp Med* (2003) 198:757–69. doi: 10.1084/jem.20030794
46. Takaba H, Morishita Y, Tomofuji Y, Danks L, Nitta T, Komatsu N, et al. Fezf2 Orchestrates a Thymic Program of Self-Antigen Expression for Immune Tolerance. *Cell* (2015) 163:975–87. doi: 10.1016/j.cell.2015.10.013
47. Cosway EJ, Lucas B, James KD, Parnell SM, Carvalho-Gaspar M, White AJ, et al. Redefining Thymus Medulla Specialization for Central Tolerance. *J Exp Med* (2017) 214:3183–95. doi: 10.1084/jem.20171000
48. Sansom SN, Shikama-Dorn N, Zhanybekova S, Nusspaumer G, Macaulay IC, Deadman ME, et al. Population and Single-Cell Genomics Reveal the Aire Dependency, Relief From Polycomb Silencing, and Distribution of Self-Antigen Expression in Thymic Epithelia. *Genome Res* (2014) 24:1918–31. doi: 10.1101/gr.171645.113
49. Meredith M, Zemmour D, Mathis D, Benoist C. Aire Controls Gene Expression in the Thymic Epithelium With Ordered Stochasticity. *Nat Immunol* (2015) 16:942–9. doi: 10.1038/ni.3247

50. Brennecke P, Reyes A, Pinto S, Rattay K, Nguyen M, Kuchler R, et al. Single-Cell Transcriptome Analysis Reveals Coordinated Ectopic Gene-Expression Patterns in Medullary Thymic Epithelial Cells. *Nat Immunol* (2015) 16:933–41. doi: 10.1038/ni.3246
51. Baran-Gale J, Morgan MD, Maio S, Dhalla F, Calvo-Asensio I, Deadman ME, et al. Ageing Compromises Mouse Thymus Function and Remodels Epithelial Cell Differentiation. *Elife* (2020) 9:e56221. doi: 10.7554/eLife.56221
52. Bredenkamp N, Nowell CS, Blackburn CC. Regeneration of the Aged Thymus by a Single Transcription Factor. *Development* (2014) 141:1627–37. doi: 10.1242/dev.103614
53. Griffith AV, Fallahi M, Venables T, Petrie HT. Persistent Degenerative Changes in Thymic Organ Function Revealed by an Inducible Model of Organ Regrowth. *Aging Cell* (2012) 11:169–77. doi: 10.1111/j.1474-9726.2011.00773.x
54. Guerau-de-Arellano M, Martinic M, Benoist C, Mathis D. Neonatal Tolerance Revisited: A Perinatal Window for Aire Control of Autoimmunity. *J Exp Med* (2009) 206:1245–52. doi: 10.1084/jem.20090300
55. Yang S, Fujikado N, Kolodin D, Benoist C, Mathis D. Immune Tolerance. Regulatory T Cells Generated Early in Life Play a Distinct Role in Maintaining Self-Tolerance. *Science* (2015) 348:589–94. doi: 10.1126/science.aaa7017
56. Kozai M, Kubo Y, Katakai T, Kondo H, Kiyonari H, Schaeuble K, et al. Essential Role of CCL21 in Establishment of Central Self-Tolerance in T Cells. *J Exp Med* (2017) 214:1925–35. doi: 10.1084/jem.20161864
57. Lkhagvasuren E, Sakata M, Ohigashi I, Takahama Y. Lymphotoxin Beta Receptor Regulates the Development of CCL21-Expressing Subset of Postnatal Medullary Thymic Epithelial Cells. *J Immunol* (2013) 190:5110–7. doi: 10.4049/jimmunol.1203203
58. Wells KL, Miller CN, Gschwind AR, Wei W, Phipps JD, Andersonz MS, et al. Combined Transient Ablation and Single-Cell RNA-Sequencing Reveals the Development of Medullary Thymic Epithelial Cells. *Elife* (2020) 9:e60188. doi: 10.7554/eLife.60188
59. Nishikawa Y, Nishijima H, Matsumoto M, Morimoto J, Hirota F, Takahashi S, et al. Temporal Lineage Tracing of Aire-Expressing Cells Reveals a Requirement for Aire in Their Maturation Program. *J Immunol* (2014) 192:2585–92. doi: 10.4049/jimmunol.1302786
60. Nishikawa Y, Hirota F, Yano M, Kitajima H, Miyazaki J, Kawamoto H, et al. Biphasic Aire Expression in Early Embryos and in Medullary Thymic Epithelial Cells Before End-Stage Terminal Differentiation. *J Exp Med* (2010) 207:963–71. doi: 10.1084/jem.20092144
61. Metzger TC, Khan IS, Gardner JM, Mouchess ML, Johannes KP, Krawisz AK, et al. Lineage Tracing and Cell Ablation Identify a Post-Aire-Expressing Thymic Epithelial Cell Population. *Cell Rep* (2013) 5:166–79. doi: 10.1016/j.celrep.2013.08.038
62. Wang X, Laan M, Bichele R, Kisand K, Scott HS, Peterson P. Post-Aire Maturation of Thymic Medullary Epithelial Cells Involves Selective Expression of Keratinocyte-Specific Autoantigens. *Front Immunol* (2012) 3:19. doi: 10.3389/fimmu.2012.00019
63. Miller CN, Proekt I, von Moltke J, Wells KL, Rajpurkar AR, Wang H, et al. Thymic Tuft Cells Promote an IL-4-Enriched Medulla and Shape Thymocyte Development. *Nature* (2018) 559:627–31. doi: 10.1038/s41586-018-0345-2
64. Bornstein C, Nevo S, Giladi A, Kadouri N, Pouzolles M, Gerbe F, et al. Single-Cell Mapping of the Thymic Stroma Identifies IL-25-Producing Tuft Epithelial Cells. *Nature* (2018) 559:622–6. doi: 10.1038/s41586-018-0346-1
65. Sheridan JM, Keown A, Policheni A, Roesley SNA, Rivlin N, Kadouri N, et al. Thymospheres Are Formed by Mesenchymal Cells With the Potential to Generate Adipocytes, But Not Epithelial Cells. *Cell Rep* (2017) 21:934–42. doi: 10.1016/j.celrep.2017.09.090
66. Dhalla F, Baran-Gale J, Maio S, Chappell L, Hollander GA, Ponting CP. Biologically Indeterminate Yet Ordered Promiscuous Gene Expression in Single Medullary Thymic Epithelial Cells. *EMBO J* (2020) 39:e101828. doi: 10.1101/554899

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