REVIEW

The role of thymic epithelium in thymus development and age-related thymic involution

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Abstract : The establishment of an adaptive immune system is critical for protecting our bodies from neoplastic cancers and invading pathogens such as viruses and bacteria. As a primary lymphoid organ, the thymus generates lymphoid T cells that play a major role in the adaptive immune system. T cell generation in the thymus is controlled by interactions between thymocytes and other thymic cells, primarily thymic epithelial cells. Thus, the normal development and function of thymic epithelial cells are important for the generation of immunocompetent and self-tolerant T cells. On the other hand, the degeneration of the thymic epithelium due to thymic aging causes thymic involution, which is associated with the decline of adaptive immune function. Herein we summarize basic and current knowledge of the development and function of thymic epithelial cells and the mechanism of thymic involution. J. Med. Invest. 71 : 29-39, February, 2024

Keywords **:** *thymus, T cell, thymic epithelial cell, thymic involution*

INTRODUCTION

Adaptive immunity is a sophisticated defense system in the human body. Lymphoid T cells, one of the major cell populations in the adaptive immune system, are capable of discriminating between self and non-self antigens. T cells respond to invading pathogens with antigen specificities but tolerate components in the body. Immunocompetent and self-tolerant T cells are generated in the thymus. The thymus is composed of hematopoietic cells, such as T cells ; thymic epithelial cells (TECs) ; and non-TEC stromal cells. These cells form a three-dimensional network by interacting with each other, constructing a thymic microenvironment consisting of the cortex and the medulla. The cortical microenvironment is the site for early T cell development and positive selection of functionally competent T cells, whereas the medullary microenvironment is the site for negative selection of self-reactive T cells and the development of regulatory T cells to establish self-tolerance. The functional characteristics of these two microenvironments are typified by the functions of cortical thymic epithelial cells (cTECs) and medullary thymic epithelial cells (mTECs), respectively (1). Abnormalities in the function and development of TECs lead to immune diseases such as immunodeficiencies and autoimmune diseases. Therefore, the functional competence of TECs for generating immunocompetent and self-tolerant T cells is important for the proper operation of the adaptive immune system.

T cell generation in the thymus decreases in an age-dependent manner. The thymus undergoes the earliest age-related chronic involution among the organs of the body (2). Thymic involution is also induced transiently by such factors as infection, chemotherapy, and stress (3). The reduction of T cell production due to age-related thymic involution is linked to the decline of adaptive immune function, which, in turn, may be associated with a reduced response to vaccination and an increased tumor incidence in the elderly (4). It has been reported that thymic involution increases the generation of senescent T cells that may be associated with age-related diseases (5, 6). Accumulated evidence suggests that the age-related alteration of TEC properties is involved in thymic involution. In this regard, the regeneration of TECs to restore T cell production in the thymus is a promising treatment strategy.

Herein, we discuss the development and function of the thymus, focusing on the developmental and functional mechanisms of TECs. We also discuss the mechanisms of thymic involution, centering on the degeneration of TECs in the process of age-related thymic involution.

DEVELOPMENT OF THYMUS

Development of thymic epithelium

Thymus development is initiated in mid-gestation. In mice, TECs, marked by the expression of the transcription factor forkhead box protein N1 (Foxn1), develop in a part of the endoderm-derived epithelium of the third pharyngeal pouch around day 11 of gestation. Cells with characteristics of cTECs and mTECs are detectable around day 12 of gestation. The differentiation of cTECs and mTECs from a common thymic epithelial progenitor cell is regulated by Foxn1 (7-10). During embryonic thymus organogenesis, the majority of TECs express cTEC-associated molecules such as CD205 and the thymoproteasome component β5t, suggesting that embryonic TECs expressing cTEC-associated molecules possess progenitor activity. Cell lineage analysis using re-aggregated thymic organ culture or fate mapping revealed that embryonic TECs expressing cTEC molecules contain progenitor cells capable of giving rise to mTECs (11, 12). Therefore, it is believed that cTECs and mTECs are derived from common progenitors that progress from a lineage-uncommitted progenitor stage to a transitional TEC progenitor stage that expresses cTEC-associated molecules.

How TECs are supplied to the adult thymus following organogenesis completion is controversial. Bipotent TEC progenitors in the adult thymus have been identified by two independent studies (13, 14). The bipotent progenitor activities of TECs isolat-

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ed from the adult thymus were demonstrated experimentally by re-aggregating these cells with embryonic thymic cells, although the progenitor activity of these cells in the physiological condition has not yet been identified. On the other hand, we have reported that mTECs in the postnatal thymus are maintained by mTEC lineage cells derived from progenitor cells that express β5t from the embryonic period to the neonatal period (15). Once the thymic medulla is fully formed, the contribution of $\beta5t^+$ TEC precursors to the supply of mTECs is severely limited, if not eliminated (15, 16). Indeed, mTEC-specific embryonic stem cells that are capable of generating functional mTECs responsible for the establishment of self-tolerance in T cells have been identified and these stem cells are derived from β5t-expressing TEC progenitors (15, 17). Notably, although the development of cTECs and mTECs is severely impaired in the Foxn1-deficient thymus, mTEC stem cells are detected even in the absence of Foxn1 expression, suggesting that mTEC stem cells emerge independently of Foxn1 (18). In addition to mTEC-specific stem cells, mTEC progenitors have been identified by tracing the differentiation potential or performing the trajectory analysis of single cell-based transcriptome profiles. Several mTEC progenitors have been reported by different groups (Fig. 1) (19-25), including *Sox9*$^+$ TECs, $RANK^+$ TECs, keratin 19-expressing TECs, and $CCL21^+$ TECs in the embryonic thymus (19, 20, 23, 24). The reported progenitors also include podoplanin$^+$ TECs that localize to the cortico-medullary junction where TEC progenitors are suggested to be located, as well as transient amplifying cells termed TAC-TECs (22, 25). However, the relationships among these mTEC progenitor cells have remained unknown. Recent studies have reported that Notch signaling is required for the production and maintenance of mTEC-specific progenitor cells and almost all mTECs have a history of receiving Notch signals, suggesting the involvement of Notch signaling in mTEC fate determination (26, 27).

The molecular mechanisms that regulate mTEC development and medullary organization are known to involve tumor necrosis factor (TNF) superfamily cytokine signaling and NFκB signaling (18, 28, 29). Epigenetic regulators, including histone deacetylase 3 (HDAC3) and polycomb repressive complex 2 (PRC2), are also involved in the regulation of mTEC development. HDAC3 controls mTEC development independently of NFκB signaling and by repressing Notch signaling (30). Indeed, the forced expression of Notch1 in TECs results in the blockage of mTEC development (26, 27). It has also been reported that mice with TEC-specific inhibition of PRC2 function have a hypoplastic thymus and show a marked decrease in the number of mature mTECs after birth (31). The transcriptional profile in TECs, including molecules related to protein processing and antigen presentation, is altered, and the diversity of T cell receptor (TCR) repertoire is reduced by the functional deficiency of PRC2, indicating that the differentiation and function of postnatal TECs are affected by the epigenetic regulation of gene expression (31).

In contrast to mTECs, little is known about the molecular mechanisms that regulate cTEC development, although it has long been understood that interactions with immature T cells are important for the differentiation and maintenance of cTECs (32). The Wnt/β-catenin signaling pathway is known to be involved in early thymus development, as shown in mouse studies that manipulated core components, including the T cell factor (TCF)/lymphoid enhancing factor (LEF) family transcription factors, Wnt, or β-catenin, either systemically or specifically in keratin 5^+ epithelial cells (33-37). However, the role of the TEC-specific Wnt/β-catenin signaling pathway was not addressed in those studies owing to extrathymic abnormalities or insufficient gene manipulation. By using the Cre-mediated targeting to Foxn1- or β5t-expressing cells, we and others showed that the deletion of β-catenin, a major mediator of the Wnt/β-catenin signaling pathway, in TECs resulted in a decrease in the number of cTECs but not mTECs after the neonatal period, but did not affect the cortical and medullary thymic structures (38, 39). Furthermore, the increase in β-catenin function specifically in TECs leads to the impaired differentiation of cTECs and mTECs (38-40). These results suggest that Wnt/β-catenin signaling is not essential for cTEC and mTEC differentiation, although the signal intensity defines the characteristics of TECs and the number of cTECs.

ROLE OF THYMIC EPITHELIUM IN T CELL GENERATION

Role of cortical thymic epithelium

Bone marrow-derived T-lymphoid progenitor cells enter the thymus through the blood vessels at the cortico-medullary junction and move to the cortex. Chemokines, including CXCR4 ligand CXCL12 and CCR9 ligand CCL25 produced by cTECs and CCR7 ligands CCL19 and CCL21 produced by mTECs, contribute to the thymic entry of T progenitor cells (41-43). cTECs also express Notch ligand delta-like 4 and cytokine IL-7, which are important for the lineage specification and development of T cells (44, 45). In addition to the regulation of T cell development, cTECs play a crucial role in the positive selection of newly generated T cells. cTECs have unique machinery that produces positive selection-inducing self-peptides by expressing enzymes, including thymus-specific serine protease (TSSP), cathepsin L (CTSL), and thymoproteasomes. TSSP and CTSL are involved in the generation of MHC class II-associated self-peptides for inducing the positive selection of CD4-lineage cells, whereas thymoproteasomes generate MHC class I-associated self-peptides for inducing the positive selection of CD8-lineage cells (46-49). TSSP and CTSL are expressed in other cells including dendritic cells (DCs). In contrast, thymoproteasomes are expressed only in cTECs because β5t, a component unique to thymoproteasomes, is specifically expressed in cTECs (48, 50). The transcription of β5t-encoding *Psmb11* is directly regulated by Foxn1 (51). However, Foxn1 is expressed in cTECs, mTECs, and skin keratinocytes. Therefore, other mechanisms, in addition to Foxn1, may contribute to the regulation of the cTEC-specific transcription of *Psmb11*. The cTEC-specific expression of thymoproteasomes is important for the generation of self-peptides that are optimal for the positive selection of CD8-lineage cells. It has been theorized that differential self-peptide display between the cortex and the medulla optimizes T cell generation, allowing positively selected T cells to escape from negative selection (52). In contrast to thymoproteasomes in the cortex, MHC class I-associated peptides are generated by immunoproteasomes or proteasomes in antigen-presenting cells in the thymic medulla. Furthermore, two independent studies have reported that the negative selection of CD8-lineage thymocytes is enhanced without affecting the positive selection when both positive and negative selection-inducing peptides are generated by the same proteasomes (53, 54). On the other hand, we have reported that the generation of CD8T cells is impaired in thymoproteasome-deficient mice, even in the absence of the thymic medulla or medullary antigen-presenting cells, including mTECs, DCs, and B cells (55). We have also reported that thymoproteasome deficiency reduces positively selected CD8-lineage thymocytes even when thymocyte apoptosis by negative selection is inhibited by the transgenic expression of the anti-apoptotic molecule Bcl-2 (55). Therefore, thymoproteasomes regulate the positive selection of CD8T cells independent of the thymic medulla, medullary antigen-presenting cells, and thymocyte negative selection. A comparative analysis of MHC class I-associated peptides displayed by mouse embryonic fibroblasts ectopically expressing thymoproteasomes

or immunoproteasomes has revealed that thymoproteasomes generate self-peptides with optimal binding affinity to TCRs to induce positive selection (49). However, thymoproteasome-dependent self-peptides displayed by cTECs have not yet been identified. The identification of those self-peptides would enhance our understanding of the mechanism of thymoproteasome-dependent positive selection.

It is also interesting to note that cTECs envelop developing thymocytes and such cTECs are known as thymic nurse cells (56). Each thymic nurse cell engulfs approximately 100 to 150 thymocytes and provides a microenvironment that optimizes T cell selection (56, 57).

Role of medullary thymic epithelium

Positively selected T cells in the cortex migrate to the thymic medulla to undergo further selection. To establish self-tolerance in the medulla, T cells expressing TCRs that bind with high affinity to self-peptide-MHC complexes are eliminated by negative selection or differentiate into regulatory T cells that suppress the immune response. Positively selected T cells increase the expression of CCR7, a chemokine receptor (58). On the other hand, mTECs express CCR7 ligands CCL19 and CCL21. There are two types of CCL21 proteins in mice, CCL21Ser and CCL21Leu, which contain serine and leucine at the 65th amino acid, respectively (59). CCL21Ser is encoded by *Ccl21a*, whereas CCL21Leu is encoded by several genes including *Ccl21b* and *Ccl21d*. Among these CCR7 ligands, CCL21Ser plays an essential role in the thymus ; in its absence, the migration of T cells from the cortex to the medulla is impaired (60). As a consequence, the negative selection of self-reactive T cells in the thymic medulla is impaired, and autoimmune disease develops in mice as a result of CCL21Ser deficiency, even though the expression of other CCR7 ligands is intact (60). On the other hand, the impairment of T cell migration into the medulla and the development of autoimmune diseases are undetectable in mice deficient in CCL19 (60). Therefore, CCL21Ser has a nonredundant role in the establishment of self-tolerance in T cells in the thymic medulla.

mTECs are unique in terms of the ectopic expression of tissue-specific antigens (TSAs) to establish self-tolerance in T cells. The expression of TSAs is regulated by Aire, which is responsible for the autoimmune polyendocrinopathy-candidiasis-ectodermal syndrome (61-63). Although not a conventional transcription

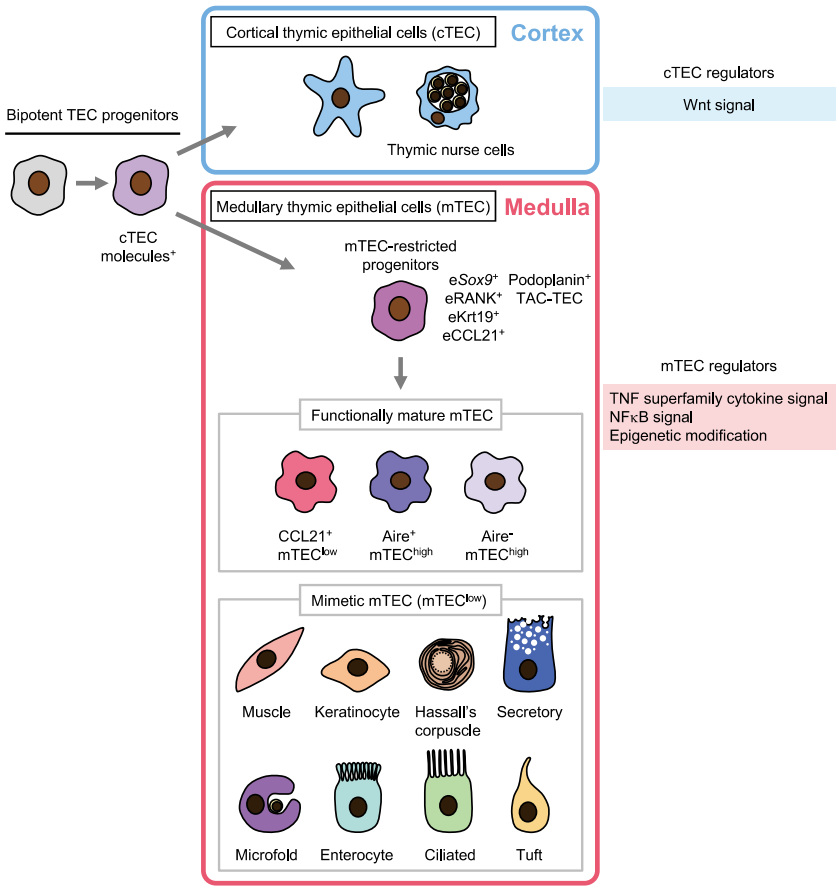

Figure 1. Thymic epithelial cell heterogeneity.
cTECs and mTECs differentiate from common thymic epithelial cell (TEC) progenitors through intermediate progenitor cells expressing cTEC-associated molecules. mTECs are divided into two populations, $mTEC^{low}$ and $mTEC^{high}$, which are $CD80^{low}$MHC class II^{low} and $CD80^{high}$MHC class II^{high}, respectively. The $mTEC^{high}$ subset contains Aire-expressing mTECs. The $mTEC^{low}$ subset contains progenitors that give rise to the $mTEC^{high}$ subpopulation. Several mTEC-restricted progenitors have been identified, including $Sox9^+$ embryonic TECs ($eSox9^+$), $RANK^+$ embryonic TECs ($eRANK^+$), $keratin19^+$ embryonic TECs ($eKrt19^+$), and $CCL21^+$ embryonic TECs ($eCCL21^+$). mTEC progenitors also include $podoplanin^+$ TECs and TAC-TECs. Functionally mature $CCL21^+$ mTECs, as well as post-Aire mTECs differentiated from the $mTEC^{high}$ subpopulation, also belong to the $mTEC^{low}$ subset. Recent single cell-based comprehensive analysis of TECs has revealed heterogeneous TEC subpopulations including post-Aire mimetic mTECs, which transcriptomically mirror extrathymic cells. The mimetic TECs are named according to their counterparts (muscle, keratinocyte, Hassall's corpuscle, secretory/neuroendocrine, microfold, enterocyte/hepatocyte, ciliated, and tuft mTECs).

factor, Aire accounts for the expression of thousands of genes in mTECs. Furthermore, it has been reported that the transcription factor Fezf2 regulates the expression of Aire-independent TSAs (64). On the other hand, it has also been reported that Fezf2 deficiency has little impact on the expression of Aire-independent TSAs, but affects late-mTEC development (65, 66). The significance of Fezf2 function in the establishment of self-tolerance is still controversial. mTECs are roughly divided into two populations, $mTEC^{low}$ and $mTEC^{high}$, which are $CD80^{low}$MHC class II^{low} and $CD80^{high}$MHC class II^{high}, respectively (Fig 1). The $mTEC^{high}$ subset contains Aire-expressing mTECs and is essential for the presentation of self-antigens to developing thymocytes. On the other hand, the $mTEC^{low}$ subset contains progenitor/immature mTECs that are capable of giving rise to $mTEC^{high}$, as well as mTECs that are further differentiated from $mTEC^{high}$, termed post-Aire mTECs (19, 67). mTEC heterogeneity was clarified on the basis of morphological differences, such as Hassall's corpuscles, ciliated columnar epithelial cells, and neurosecretory epithelial cells (68). Recent comprehensive analyses of TECs at single cell-level have revealed that mTECs are a more heterogeneous population than previously recognized (21, 69-71). Regarding post-Aire mTECs, single-cell assays for chromatin accessibility have revealed several distinct clusters, each of which is characterized by lineage-defining transcription factors for skin, lung, liver, and intestinal cells (71). Furthermore, these lineage-defining transcription factors detected in each mTEC cluster are associated with chromatin accessibility patterns that correspond to each cell type, including keratinocytes, microfold cells, endocrine cells, and tuft cells (69, 71, 72). These post-Aire mTECs transcriptomically mirror the extrathymic cells but retain the mTEC signature and are therefore termed mimetic mTECs (Fig. 1). Importantly, the expression of a model antigen in mimetic mTECs is sufficient to induce T cell tolerance by inducing the negative selection of self-reactive T cells (71). In addition, mimetic mTECs have roles other than the establishment of self-tolerance in T cells. Studies have shown that thymic tuft cells play a role in the development and function of iNKT2 cells (69, 73). Endocrine mimetic mTECs are involved in the regulation of thymic cellularity, whereas microfold mimetic mTECs contribute to the generation of IgA^+ plasma cells in the thymus (74).

The $mTEC^{low}$ subpopulation also includes CCL21-expressing mTECs. Single cell-based transcriptome profiling of TECs has shown that a cluster with the *Ccl21a* transcript has the transcriptional signature of progenitor cells (21). However, whether $CCL21^+$ mTECs are immature mTECs or post-Aire mature mTECs has not been directly investigated. Interestingly, our recent study revealed that CCL21-producing embryonic mTECs have the potential to give rise to $Aire^+$ mTECs, and almost all mTECs are derived from cells that have transcribed *Ccl21a*, indicating that embryonic CCL21-expressing mTECs have progenitor activity (24). On the other hand, the differentiation potential of postnatal $CCL21^+$ mTECs into $Aire^+$ mTECs was not detected (24). Therefore, $CCL21^+$ mTECs detected in the postnatal thymus may be terminally differentiated cells that lack progenitor activity. The conversion of thymocyte-attracting mTECs into antigen-presenting mTECs, such as $Aire^+$ mTECs, may contribute to the diversity in the medullary thymic epithelium. More importantly, the establishment of self-tolerance in T cells is regulated by mTEC subpopulations including thymocyte-attracting CCL21-expressing mTECs and antigen-presenting mTECs such as Aire-expressing mTECs and post-Aire mimetic mTECs.

THYMIC INVOLUTION

Acute thymic involution

The thymus involutes transiently or chronically. Acute thymic involution is caused by several factors including radiation, chemotherapy, infection, and hormones ; thus, upon the removal of these factors, thymus size is restored. It is well known that sex hormones affect thymus size. An increase in androgen production at puberty has been implicated in thymic involution, whereas androgen deprivation results in the thymus size recovery (75). T cell-specific disruption of the androgen receptor (AR) has little effect on thymus size, whereas TEC-specific disruption of AR results in thymic enlargement, suggesting that TECs are targets of androgen-driven thymic involution (76-78). It is also well known that the thymus undergoes involution during pregnancy, which may contribute to feto-maternal tolerance. Thymic involution during pregnancy is triggered by progesterone-induced alteration of TEC function. Progesterone receptor (Pgr) expression is increased and the transcriptome profiles of cTECs are altered during pregnancy, including the expression of such chemokines as *Ccl25*, *Ccl21*, *Ccl19*, and *Cxcl12*, which are involved in the thymus seeding of T progenitors (79-81). Importantly, thymic involution during pregnancy contributes to normal fertility, as the prevention of progesterone-mediated thymic involution by TEC-specific Pgr deficiency results in a reduced litter size in mice (81).

Age-related chronic thymic involution

Unlike acute thymic involution, age-related thymic involution is a chronic process (Fig. 2). It is generally accepted that thymus volume is largest at puberty and decreases with age. In humans, however, the loss of thymus tissue begins as early as one year of age, decreasing at a rate of 3% yearly until middle age and 1% yearly thereafter (82, 83). Along with the loss of thymus tissue, adipose tissue replaces the thymus tissue with age (83, 84). In contrast to the human thymus, the increase of adipose tissue in the mouse thymus is not remarkable. However, thymus mass peaks at approximately 4 weeks of age and gradually decreases thereafter. Epithelial-mesenchymal transition (EMT) is involved in adipogenesis in the thymus, which is dependent on the increased expression of PPARγ, a master regulator of adipogenesis, in TECs (85, 86). It has been suggested that the age-dependent decline of ghrelin receptor-mediated signaling induces adipogenesis programming, including an increased PPARγ expression, in the thymus, thus promoting EMT (87). Furthermore, a recent study has indicated that the interaction of CD147 on T cells and annexin A2 on TECs, triggered by TGF-β signaling, promotes the EMT process, and T cell-specific deficiency of CD147 delays thymic involution (88). Thus, the inhibition of PPARγ expression in TECs or CD147–annexin A2 interaction is promising as a means of inhibiting EMT-mediated thymic involution.

Other studies have shown that age-related thymic inflammation is also associated with thymic involution, which may be triggered by the activation of inflammasomes through age-related intra-thymic accumulation of lipotoxic danger signals (89, 90). Mice deficient in the inflammasome component Nlrp3 or Asc are protected from thymic involution and immune senescence during aging (89).

It is also important to note that caloric restriction (CR), which is widely recognized to contribute to lifespan increase, prevents the increase of intrathymic adipogenesis and thymic involution in aged mice (91). CR decreases the expression of pro-EMT and proadipogenic regulators, such as fibroblast-specific protein-1, FoxC2, PPARγ, and FABP4, in the thymus of aged mice (91). The increase in the expression of molecules related to the prevention of thymic involution, including ghrelin, leptin, and Igf1, in the

thymus of short-term calorie-restricted mice has also been reported (91, 92). A recent study on healthy humans has reported that a 14% reduction in calorie intake enhances thymic mass and function (93). It also induces transcriptional remodeling in adipose tissue, resulting in the upregulation of mitochondrial biogenesis, anti-inflammatory response, and pro-longevity effects (93). Interestingly, aged mice deficient in Pla2g7, a pro-inflammatory enzyme that is decreased in adipose tissue-resident macrophages in calorie-restricted humans, showed improved adipose tissue metabolism, decreased ceramide-mediated inflammasome activation, and protection from thymic involution (93). Therefore, CR-dependent metabolic regulation is another factor that controls age-related thymic involution. However, severe CR, such as a 40% reduction, can increase susceptibility to infections despite its protective effect against age-related thymic involution (91, 94, 95).

Age-related thymic involution is associated with various immunological consequences. Thymic involution decreases the *de novo* generation of naïve T cells, which has been suggested to be involved in the decline of adaptive immune function. Elderly people are susceptible to microbial invasions they have not previously encountered, but maintain defenses against previously experienced infections ; however, the decline in immune function is associated with increased susceptibility to infection, increased cancer incidence, and poor response to vaccines in the elderly. Mathematical modeling revealed a strong link between the age-dependent decline of T cell production in the thymus and infectious diseases and cancers (4).

The reduction of T cell production due to thymic involution is related to the production of senescent T cells that show defective antigen response, biased secretion of proinflammatory cytokines, and resistance to apoptosis (96-98). Senescent T cells are also increased in chronic inflammatory environments, and the association of senescent T cells with inflammatory disorders, such as systemic lupus erythematosus, obesity-induced adiposity, insulin resistance, cardiovascular diseases, and cancers has been reported (96, 99-101), although the direct relationship between thymic involution and these diseases remains to be elucidated.

Aging of the thymus changes not only the production of T cells but also the functionality of cells in the thymus. Aged mouse models display dysregulated CD3 expression in thymic T cells, resulting in impaired TCR response, including failure of activation marker CD69 upregulation and reduced proliferation (102). Thymic DCs and macrophages show increased expression of proinflammatory genes with age, which are associated with age-related thymic inflammation (89, 90, 103). Moreover, the expression of Aire and Aire-dependent TSAs is diminished in aged thymic B cells, suggesting a link between aging and age-related autoimmune diseases (104). Importantly, it has been proposed that changes in thymic stromal cells contribute to age-related thymic involution. A previous study has demonstrated that the transplantation of bone marrow cells from young mice failed to increase *de novo* T cell generation in the thymus of aged recipient

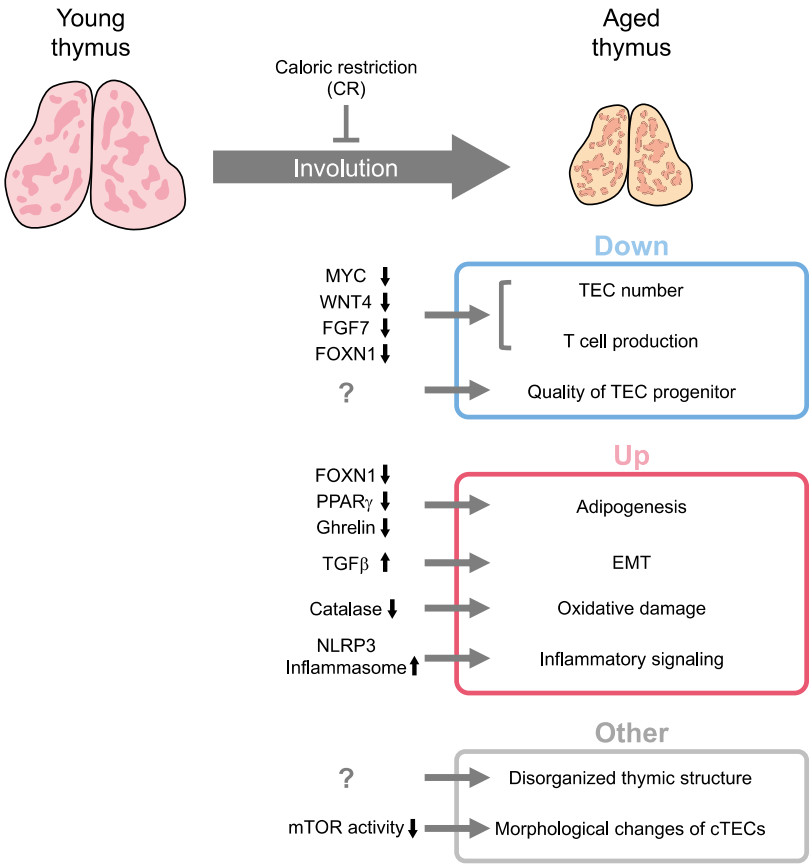

Figure 2. Age-related thymic involution.
Age-related thymic involution is accompanied by quantitative and qualitative changes in the thymus. Thymus volume gradually decreases with age after puberty. During thymic involution, the number of TECs, the quality of TEC progenitors, and the *de novo* generation of naïve T cells are decreased, whereas adipose tissue is increased in the thymus. Representative factors involved in thymic involution are also shown.

mice (105). On the other hand, another study has revealed that hematopoietic cells from aged mice were able to generate T cells equivalent to those from young mice when fetal thymus was transplanted into aged or young recipient mice to create a thymic environment in which thymic stromal cells were derived from the fetus whereas hematopoietic cells were derived from the recipient mice (106). These findings suggest that the aging of thymic stromal cells rather than hematopoietic cells causes an age-related decline of T cell generation in the thymus. Moreover, accumulated evidence suggests that the degeneration of TECs drives thymic involution. In the following section, we summarize the age-dependent degeneration of TECs.

TEC DEGENERATION DURING AGE-RELATED THYMIC INVOLUTION

Age-related decline in TEC numbers

Age-related thymic involution is accompanied by a decrease in the number of TECs (107). This may be mediated by age-dependent changes in the transcriptional profile of molecules associated with cell proliferation. It has been reported that the activity of E2F3, a transcription factor critical for cell proliferation, is decreased in cTECs and the mTEC^{low} subset, and the decreased E2F3 activity and the downregulation of cell cycle regulators may result in reduced cell cycle progression in these cells, thus contributing to the reduction in TEC cellularity during early thymic involution (103). Transcription factor Myc is a key regulator of TECs, supporting rapid thymic development and TEC proliferation during embryonic stages. Myc activity in TECs is highest on day 13.5 of gestation and declines thereafter (108). The forced expression of Myc in TECs results in the long-term maintenance of an embryonic-specific transcriptional program and increases in thymus size and thymocyte number, suggesting that the maintenance of Myc activity may attenuate the reduction in thymus size and thymic function during thymic involution (108). The number of TECs is also regulated by growth factors secreted either by TECs or by surrounding cells such as mesenchymal cells. It was reported that the pharmacological supplementation of Fgfr2b ligand Fgf7 increased the number of TECs and the ectopic expression of Fgf7 in TECs resulted in a sustained increase in the number of TECs in the aged thymus (109-111). These findings suggest that growth factors also contribute to quantitative changes in TECs.

Age-dependent alteration of gene expression in TECs

It has been reported that Foxn1 is involved in postnatal thymus development by controlling both proliferation and differentiation of TECs in a dose-dependent manner and that the decrease in the number of Foxn1-expressing TECs is initiated at an early stage of thymic involution (112-115). The overexpression of Foxn1 in TECs under the control of keratin-14 promoter or Foxn1-Cre attenuates age-related thymic involution, whereas the overexpression of Foxn1 under the control of keratin-5 promoter does not attenuate thymic involution, although it prevents the decrease in TEC differentiation, particularly for mTECs (114, 116, 117). These studies suggest that the increased expression of Foxn1 contributes to the prevention of thymic involution even though the effect varies between TEC subpopulations, and that Foxn1-independent pathways also contribute to thymic involution.

Transcriptional profiling of TECs has revealed age-related alterations in various molecules, including cell cycle regulators, growth factors, and inflammatory cytokines, during thymic involution (103, 108). Furthermore, alterations of the gene expression of Wnt family members and Wnt signaling molecules have been shown in early thymic involution, suggesting the involvement of the Wnt pathway in TEC degeneration (113, 116, 118). The reduction of Wnt4 expression in cTECs coincides with the reduction of Foxn1 expression during early thymic involution. An *in vitro* experiment has revealed that Wnt4 induces Foxn1 expression through the Wnt/β-catenin pathway (119) ; thus, the reduction of Wnt4 expression may lead to a reduction of Foxn1 expression in TECs during thymic involution. However, the direct link between Wn4 and Foxn1 expression during thymic involution is uncertain, as the disruption of the Wnt/β-catenin pathway in TECs has no effect on Foxn1 expression *in vivo* (38, 39).

Recent single-cell transcriptome profiling has revealed previously unrecognized gene expression signatures in TECs during the aging process with alterations of both subset composition and transcriptional states of TECs during thymic involution (21). It has also been reported that the primary targets of aging are thymic epithelial progenitor cells rather than mature TECs, with changes in progenitor quality (21, 111). Current evidence suggests that age-related quiescence or reduced differentiation potential of TEC progenitors leads to a reduction in TEC maintenance, resulting in reduced thymic function.

Age-related alterations in TEC properties

In addition to the age-related alterations in the number and transcriptome profiles of TECs, alterations in TEC properties, including morphology, may also be involved in thymic involution. Morphological analysis of individual TECs has shown that the morphology of cTECs changed dramatically, their projections contracting with age ; this phenomenon was not found in mTECs (57). The change in cTEC morphology may be regulated by mTOR signaling, a cell and tissue size regulator, as pathway analysis of transcriptome profiles has shown that the mTOR signaling pathway decreased with age and increased dramatically in the early stages of TEC regeneration (57). The dramatic change in cTEC structure may contribute to the reduction in thymic size during thymic involution in addition to increased cell death or decreased cell proliferation in aged TECs.

In cTECs undergoing intense metabolic activity due to lymphocyte proliferation, a deficiency of antioxidant enzyme catalase increases susceptibility to oxidative damage, resulting in accelerated thymic involution (120). The genetic complementation of catalase in thymic stromal cells attenuates age-related rapid thymic atrophy, suggesting that the levels of oxidative damage contribute in part to thymic involution (120). On the other hand, a recent study has indicated that the overexpression of catalase reduced autophagy in thymic stromal cells and impaired thymocyte clonal deletion, resulting in the increased infiltration of autoreactive T cells in the lung and liver (121). These findings suggest that low levels of catalase promote high levels of autophagic activity in thymic stromal cells, which are required for self-antigen presentation and T cell selection to regulate central T cell tolerance. However, autophagic activity decreases with age and is associated with senescence in some cells and tissues (122). Moreover, autophagy is induced by the inhibition of mTOR signaling (123), which may be involved in the regulation of cTEC morphology (57). Future studies should elucidate how oxidative damage, autophagic activity, and mTOR-mediated metabolic regulation are linked to age-related alterations in TEC properties during thymic involution.

CONCLUSION

Despite playing a critical role in immune system establishment, the function of the thymus remained unknown until the

1960s. For a long time, the thymus was considered a vestigial organ that lost its original function because of size diminishment in adults. Consequently, the thymus was one of the last organs in our body for which its function was definitively established. Furthermore, research on TECs lagged behind the study of T cells, likely owing to the challenges in isolating TECs for detailed molecular analyses. Today, it is widely accepted that the thymic epithelium mainly regulates the generation of T cells in the thymus and that the optimal function of the thymic epithelium is important for thymus function. Thus, it is essential to understand the mechanism responsible for the establishment of thymus function by the epithelium. However, much remains unknown as to why the thymus undergoes the earliest age-related chronic involution in the body. In the human body, an enormous number of T cells are generated daily in the thymus, but most of them are eliminated by apoptosis due to thymic selection, and that process probably consumes a large amount of energy (124). In this regard, thymic involution may reduce energy consumption in the thymus, allowing energy to be used for other biological processes, thereby benefitting other organs. Alternatively, it has been speculated that thymic involution reduces the risk of T cell leukemia. As the generation of hematopoietic cells in the bone marrow decreases with age, thymic involution may prevent T cells from residing in inappropriate intrathymic niches for long periods, thus preventing the creation of pro-leukemic environments in the thymus (125). Age-related thymic involution is involved in immunosenescence and is considered to be detrimental to the body. The development of ways to promote thymic rejuvenation in the elderly is anticipated ; however, it is necessary to elucidate the significance of age-related thymic involution to understand aging and treat age-dependent changes in biological function.

COI STATEMENT

The authors declare no conflicts of interest associated with this manuscript.

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