

# We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900

Open access books available

185,000

International authors and editors

200M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index  
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?  
Contact [book.department@intechopen.com](mailto:book.department@intechopen.com)

Numbers displayed above are based on latest data collected.  
For more information visit [www.intechopen.com](http://www.intechopen.com)



# Age-Related Thymic Atrophy: Mechanisms and Outcomes

*Rachel Thomas and Dong-Ming Su*

## Abstract

Age-related thymic atrophy or involution, a hallmark of thymic aging, takes place both in humans and animals. In this chapter, we will discuss age-related thymic atrophy, outlining the underlying cellular and molecular mechanisms of its occurrence. We will also address the downstream influences on the aged T cell immune system, not only regarding insufficiency against pathogens, but also hyper-reactivity to self. Particularly, we will focus on how thymic atrophy disrupts efficient establishment of central T cell immune tolerance primarily via impairment of thymocyte negative selection, resulting in an increased number of self-reactive conventional T cells, and on thymic-derived regulatory T cell generation. Finally, we will provide a framework for understanding the significant role that the atrophied thymus plays in shaping inflammaging: a chronic, low-grade, systemic inflammatory phenotype observed in aged individuals in the absence of acute infection. The involvement of T cell adaptive immunity in mediating inflammaging plays a crucial role in the progression of many age-related neurological and cardiovascular diseases.

**Keywords:** thymic atrophy, aging, inflammaging, central tolerance, regulatory T (Treg) cells

## 1. Introduction

The thymus gland is the primary central lymphoid organ involved in development and selection of T lymphocytes (T cells) [1]. It is also responsible for the establishment of central T cell immune tolerance, which includes two mechanisms: thymocyte negative selection, through which most self (auto)-reactive T cells are depleted [2], and the generation of CD4 single positive (CD4<sup>SP</sup>) FoxP3<sup>+</sup> regulatory T (Treg) cells [3], which act to suppress self-reactive T cell-mediated reactions in the periphery [4]. It is thought that Treg cells provide some level of compensation for imperfections in negative selection that allow some self-reactive T cells to escape this protective process [5]. As part of the aging process, the thymus undergoes progressive involution or atrophy in most vertebrates, exhibiting not only morphological changes, but also a functional decline resulting in [6, 7] significantly lowered thymic output [8].

The theoretical causes of this age-related diminishment of thymopoiesis are two-fold. First, is the notion of a hematopoietic defect. This stems from the observations that there are reduced numbers of hematopoietic stem cell (HSC) progenitors produced by the bone marrow with age, [9] that could cause a reduction in early T-cell progenitors (ETP) entering the thymus [10]. Second, is the notion of a

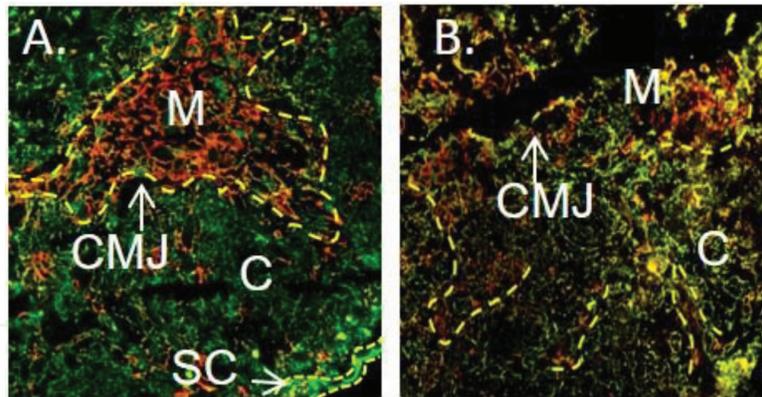
non-hematopoietic defect, which suggests that the primary age-related atrophy of the thymus is derived from HSC niche cells [11, 12] and thymic stromal cells, or ETP niches [13, 14]. The myriad of changes that characterize thymic atrophy first occur within the thymic niche and then extend to the ETPs as a result of age. We believe that these substantial age-related alterations in thymic microstructure and micro-environment, which provide important thymic factors, contribute more heavily to the diminished thymopoiesis observed in the elderly [7, 13]. The primary thymic stromal cells are thymic epithelial cells (TECs), including two subpopulations distinct in their localization, function, and molecular expression patterns, namely medullary TECs (mTECs) and cortical TECs (cTECs) [15]. Compelling evidence show that age-related thymic atrophy is tightly associated with postnatal TEC homeostasis, which is regulated by TEC autonomous transcription factors (TFs), such as Forkhead box N1 (FoxN1) [16].

Age-related changes to immune system function, often referred to as immunosenescence [17–20], are generally thought of as immune insufficiency, such as reduced anti-infection and vaccine immunity [21] and reduced tumor surveillance [22, 23]. However, self-reactive immune responses are elevated in the elderly, which is a result of inflammaging, a chronic, low-grade, systemic inflammatory phenotype in the absence of acute infection observed in aged individuals [24–31]. Immunosenescence and inflammaging are antagonistic phenotypes, but they actually comprise two sides of the same coin in terms of age-related immune dysregulation [19, 20, 32, 33]. It has been proposed that the basal inflammatory state defined by inflammaging greatly contributes to many age-related degenerative diseases, including neurodegenerative diseases, such as Alzheimer's disease, metabolic diseases, and cardiovascular diseases, among others [30, 34, 35].

Here, we will outline the cellular and molecular mechanisms underlying the occurrence of age-related thymic atrophy including some of the aforementioned hallmarks, and its effects on general T cell output. We will also describe its effects on the establishment of central T cell immune tolerance via a combination of both mechanistic arms of central tolerance: thymocyte negative selection and thymic-derived CD4<sup>SP</sup>FoxP3<sup>+</sup> T regulatory (tTreg) cell generation. We will discuss why we believe many aspects of the adaptive immune system's role in the development of inflammaging can be attributed to these thymic manifestations. Finally, in light of new trends in T cell immune system aging, we will expand on some future research goals in the field of thymic atrophy interventions and therapeutics as a potential conduit for normalizing aged T cell-mediated immunity. This is of clinical significance for combating age-related neurological and cardiovascular diseases.

## 2. Hallmarks of age-related thymic atrophy

During aging, the thymus undergoes progressive atrophy [36]. In addition to a reduction in thymic mass (size and thymocyte numbers), there is substantial remodeling of the thymic microstructure. The thymus is characterized by two primary compartments, namely the cortex and the medulla. In between the cortex and medulla, there is a zone termed the corticomedullary junction (CMJ) (**Figure 1a**). These two compartments contain specialized thymic epithelial cells (TECs), cortical (cTECs) or medullary (mTECs), and these cellular compartments are responsible for different stages of thymocyte development and selection [37, 38]. Regarding thymic microstructure, the aged, involuted thymus, in addition to an overall decline in TEC-associated markers, such as keratin and major histocompatibility complex class-II (MHC-II), also manifests altered ratios of cTECs to mTECs, and an overt change in microstructure due to disrupted



**Figure 1.**  
*Thymic microstructural changes characterized by K8 and K5 fluorescent staining. In the aged thymus, the CMJ is not clear, because the medulla is disorganized and medullary TECs are dispersed and do not form a distinct compartmental region. Normally, K8<sup>+</sup> TECs (green) are primarily localized in the cortical region, while K5<sup>+</sup> TECs (red) are primarily localized in the medullary region. A) Young (~2 months old) murine thymus; B) aged (>18 months old) murine thymus. C = cortex, M = medulla, CMJ = corticomedullary junction, SC = subcapsule.*

CMJ, resulting in a disorganized medullary region (**Figure 1b**). A decline in MHC-II<sup>hi</sup> expressing TECs is a sign of the reduction of mature mTECs [39, 40]. Additionally, increased numbers of fibroblasts [39] and accumulation of adipose tissue in the thymus is also observed [40].

Increased senescent cells ( $\beta$ -Gal<sup>+</sup>, p21<sup>+</sup>, and TAP63<sup>+</sup>) [41] in the aged thymus are also present, and it has been demonstrated that TECs contribute to the senescence observed in the aged thymus [39, 41, 42]. This possibly contributes to an increased inflammatory environment (increased levels of IL-6, IL-1 $\beta$ , etc.) within the involuted thymus [30, 43]. Additionally, there is augmented apoptosis in TECs of the atrophied thymus, contributing to diminished stromal cellularity [39].

### 3. Mechanisms of age-related thymic atrophy

#### 3.1 Mechanisms of diminished thymic input and output associated with aged thymus

Perhaps the most noted outcome of age-related thymic atrophy is diminished thymic output and thymopoiesis. This attracts attention and has led many groups to examine whether the bone marrow (BM) derived hematopoietic stem cell (HSC) lymphoid progenitors are sufficiently able to seed the thymus during aging. This is because HSCs are reduced [9] with a myeloid biased development in advanced age [44]. There have been many studies investigating this aspect of thymopoiesis and it is suggested that age-related HSCs contain defects [9] that could contribute to insufficient entry of early T-cell progenitors (ETPs) into the aged thymus [10]. Thus, this result could explain decreased thymic output with age [45].

Mechanisms of diminished thymic input resulting in thymic involution and declined thymic output are mainly based on bone marrow transplantation (BMT) experiments using mouse models. In these models, transferring aged HSCs into young mice could not rejuvenate the thymic involution induced by irradiation prior to bone marrow transplantation [46]. Additionally, the HSC progenitors have been shown to exhibit an age-related skewed proportion within the HSC pool towards myeloid lineage versus lymphoid lineage [44, 47–49]. It has also been observed that early stage thymocytes, defined as the ETPs in the triple negative-1 (TN1) thymocyte population, from aged mice demonstrated decreased differentiation

after *in vitro* fetal thymic organ culture [10]. This group also reported declined proliferation and enhanced apoptosis of these early thymocytes taken from aged animals compared to young controls. The overall assertion was that the deficiency in thymocyte differentiation and development past this early stage was attributed to the production of the HSCs in the aged bone marrow [10]. Therefore, aged HSCs and ETPs were regarded as having an intrinsic defect [50].

Given the comprehensive microenvironments in young and aged animals, and the vulnerability of HSCs or ETPs during *in vitro* preparation, these experiments using BMT and ETP culture may not provide the necessary rigor for the conclusions drawn from them, and certainly do not adequately reflect physiological conditions. Therefore, we designed an age-mismatched experimental system with less *in vitro* preparation to reexamine these biological events [13, 51]. One design was to utilize young or aged IL-7R knockout mice as recipients [13, 52, 53], in which their BM niche is relatively open and available to accept exogenous BM cells without irradiation [52, 54]. After grafting young BM cells into young and aged IL-7R knockout mice, the young BM cells produced a young profile in young recipients, but the same young BM cells produced an old profile in aged recipients [13], which implies that the microenvironment directs BM cell aging, rather than the HSCs themselves [14]. The other design was to utilize mouse fetal thymus transplantation into young or aged mice, in which BM progenitors from young or aged recipients seed the grafted young thymus *in vivo* [51]. After grafting fetal thymic lobes into young and aged wild-type recipient mice, BM progenitors from young and old mice were able to grow equally well in the engrafted thymus (with young thymic microenvironment) [51]. In addition, aged HSCs seeding the engrafted thymus did not demonstrate any intrinsic defects [13, 55]. These comprehensive experiments provide solid evidence that the non-hematopoietic microenvironment, rather than HSCs, direct hematopoietic progenitor aging [14], thereby mediating the kinetics of thymic involution [7].

An important fact linking these potential mechanisms is the unique cross-talk or interaction that occurs between the developing hematopoietic progenitors (such as thymocytes) and the stromal microenvironment (such as TECs) in the thymus [15]. For example, there are reports that several key thymic factors involved in this cross-talk are adversely impacted by age-related thymic atrophy. One such factor is IL-7, secreted by TECs, which is important for thymopoiesis and has been shown to be reduced in the aged thymus [56]. Interestingly, direct exogenous supplementation of IL-7 helped to improve aged thymopoiesis [57]. On the other hand, thymocytes provide signals to promote TEC development, at least during thymic organogenesis [58, 59], but the dynamics of this phenomenon during thymic aging remain unknown.

In general, adult organ size is governed by the tissue-specific stem cell pool [60, 61]. It is known that there are two types of tissue-specific stem pools: infinite pools, such as in the liver, and restricted pools, such as in the pancreas. For example, if the liver is injured, its infinite stem pool can expand at a high capacity; whereas, if the pancreas is injured, the expansion of its tissue-specific stem cell pool is very limited due to its restricted and finite epithelial progenitor pool. The thymic epithelial progenitor pool has characteristics of the restricted, finite epithelial progenitor pool [61]. Therefore, it is conceivable that aging TECs exhibit limited turnover compared to mobile thymocytes, which are periodically entering from the BM [62, 63].

Taken together, deficiencies in thymocyte-TEC interactions in the thymus [15] promote thymic atrophy during aging. However, given the fact that thymocytes are mobile with a relatively short period of thymic residency, while TECs have permanent residency in the thymus, experimental evidence [13, 51] and the “seed and soil” theory describing how the soil (stem niche) directs seed (HSC) fate [64–66],

lead us to conclude that age-related thymic involution begins with defects in the TEC compartment.

### 3.2 Mechanisms of thymic stromal cell-mediated structural thymic atrophy

In light of the aforementioned evidence of age-related TEC defects and the decline in total TEC numbers in the aged, atrophied thymus, we now move to discuss the underlying mechanisms of these alterations. Many studies have been conducted to identify factors involved in the cellular and molecular aspects of TEC aging (cytokines, transcription factors, microRNAs, sex steroids, etc.). The single most predominant factor currently accepted as significantly contributing to this phenomenon is the TEC autonomous transcription factor FoxN1. This idea was based on the athymic nude mouse phenotype [67, 68]. FoxN1 is expressed mainly in epithelial cells of the thymus and skin to regulate epithelial cell differentiation in these organs [67]. It is thereby responsible for thymic organogenesis and subsequent T cell development in the thymus [16], as well as hair follicle development in the skin [69, 70]. Many past and current studies utilize nude mice, which exhibit a null mutation in FoxN1 resulting in the lack of hair and the thymus, which explains the lack of T cells in these mice [71, 72].

FoxN1 is noted to be reduced in expression in the age-related atrophied thymus and has even been described as one of the first markers of the onset of thymic involution [73, 74]. The question is whether this reduced FoxN1 expression is due to TEC aging, which results in a decline in many TEC-associated genes, or if primary FoxN1 decline with aging induces a TEC defect that then results in age-related thymic involution. This cause-and-effect relationship had been substantially debated prior to the generation of a conditional knock-out (cKO) FoxN1 mouse model [75]. In this model, the murine FoxN1 gene is *loxP*-floxed and the *uCreER<sup>T</sup>* is introduced through crossbreeding [76]. In this model, the tamoxifen (TM)-inducible ubiquitous Cre-recombinase (*uCreER<sup>T</sup>*) transgene has a low level of spontaneous activation, even without TM induction [77, 78], causing gradual excision of the FoxN1<sup>*lox/lox*</sup> gene over time. This results in progressive loss of FoxN1 with age and thymic involution that is positively correlated with reduced FoxN1 levels [79]. Supplying exogenous FoxN1, such as via plasmid [79] or transgene [80, 81], into the aged thymus greatly reduces thymic atrophy and improves function. Additionally, the use of FoxN1 reporter mice has enabled further elucidation of the timeline and kinetics of thymic atrophy with age [82]. For example, one group recently published a study demonstrating that the reduction in FoxN1 initiates the onset of thymic involution, beginning predominantly in the cTEC compartment [82]. Therefore, a decline in FoxN1 expression with aging causally induces flaws in TEC homeostasis, thereby resulting in age-related thymic atrophy, as opposed to the notion that age-induced thymic atrophy causes FoxN1 decline in the thymus.

## 4. Outcomes of age-related thymic atrophy

Overt outcomes of age-related thymic atrophy include reduction of functional naïve T cells, which is related to a decline in T cell receptor (TCR) repertoire diversity [8, 55, 83, 84]. However, the atrophied thymus is still functioning, albeit with limitations, in the elderly, continuing to select T cells for the lifetime of the individual. This causes a potential for the atrophied thymus to generate harmful T cells that could increase autoimmune predisposition the elderly [26]. Therefore, we will review recent research progress regarding this area of concern.

#### 4.1 Decreased naïve conventional T cell output

As stated previously, the most readily observed outcome of age-related thymic involution is the decline in thymic output, which includes reduced naïve conventional T (Tcon) cell output over time [85] and fewer recent thymic emigrants (RTEs) [8]. However, peripheral T cell numbers are not decreased in aged individuals [36, 86, 87]. The actual effect is an overall diminished TCR repertoire diversity observed in the aged peripheral T cell pool [8, 55, 83, 84], which is due to oligoclonal expansion of memory T cells along with insufficient RTE output. This has been suggested to contribute to the decreased capacity for new immune responses to infection and poor vaccination efficacy, which are typical phenotypes of immunosenescence [17–20], observed in the elderly [35].

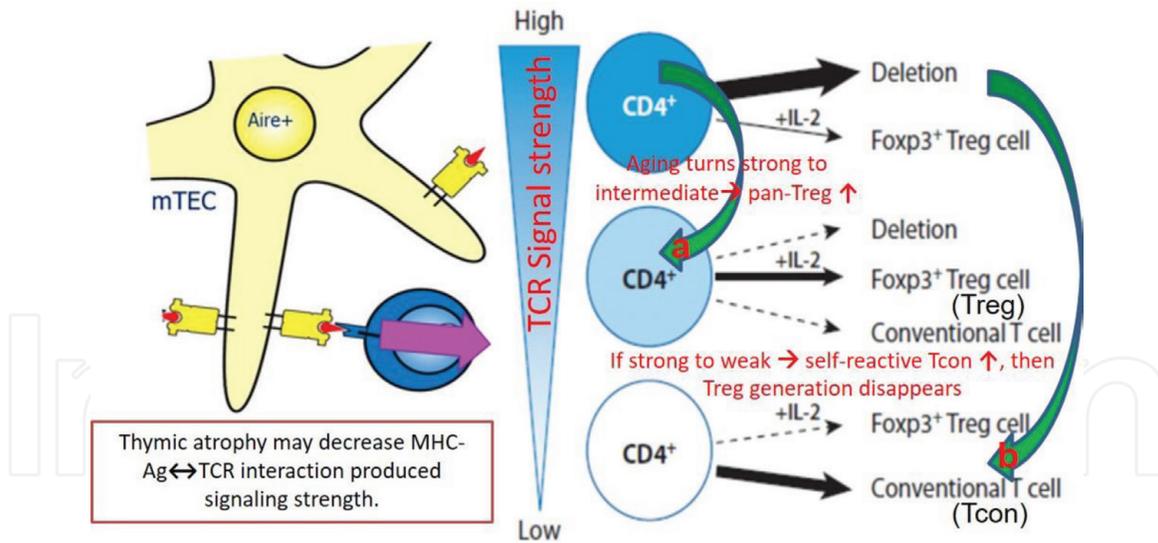
This phenotype has been recapitulated in FoxN1 cKO mice, which have accelerated aging in the thymus, but maintain a young periphery, as they exhibit impaired peripheral T cell responses in infection with influenza virus [88]. This study also demonstrated a direct role for thymic atrophy in the impairment of T cell function during aging.

#### 4.2 Increased self-reactive conventional T cells due to perturbed negative selection

In light of the alterations in thymocyte number and diminished naïve T cell output with age-related thymic atrophy, it is of paramount importance to understand the effects of the altered thymic micro-environment on central tolerance establishment of the thymocytes that are still being developed in the atrophied thymus.

Under the current paradigm, negative selection is the process by which thymocytes with high affinity for self-peptides presented by MHC are deleted from the developing thymocyte repertoire via apoptosis [2, 38, 89]. Studies also show that when these high affinity TCRs receive strong signaling, negative selection takes place [90, 91]. However, the TCR signaling strength is not based solely on TCR affinity, but is also influenced by avidity, or the quantity of interactions between self-peptide/MHC (self-pMHC) complexes and the TCR (**Figure 2**). Therefore, if the thymocyte-intrinsic factors (i.e., TCR affinity and number), of self-reactive thymocytes are unchanged, the TCR signaling strength varies based on the ability of effective self-pMHC-II expression. In other words, if self-antigen can be normally presented in the MHC-II groove, the reciprocal TCR signaling should be produced through a strong interaction. We know that MHC-II is expressed on mTECs, however, aging induces mTEC defects (**Figure 1b**), resulting in reduced capacity for self-pMHC-II ligand expression. Therefore, we suggest that a strong signaling strength shifts either to an intermediate strength, which favors CD4<sup>SP</sup>FoxP3<sup>+</sup> tTreg cell generation (**Figure 2**, arrow-a), or to a low strength, which results in the generation of self-reactive thymocytes (**Figure 2**, arrow-b). The self-reactive thymocytes via this pathway are neither depleted nor shifted to Treg cells, but become Tcon cells that are released to the periphery. If they encounter specific self-tissues, they may become effector T (Teff) cells that can attack self-tissues and induce pathological inflammation.

The FoxN1 cKO mouse model is a useful model for studying the capacity for efficient self-pMHC-II ligand expression, because it exhibits a defect in the non-hematopoietic TECs, but maintains intrinsically normal hematopoietic lineage cells and a young periphery. We demonstrated that thymic involution perturbs negative selection, as revealed by the enhanced release of autoreactive interphotoreceptor retinoid-binding protein (IRBP)-specific Tcon cells from the atrophied thymus of FoxN1 cKO mice compared to the thymus from young normal controls [25]. This



**Figure 2.** Signaling strength decides self-reactive  $CD4^{SP}$  T clone fates. Interaction between self-pMHC on mTEC and self-reactive TCR on  $CD4^{SP}$  thymocyte produces three types of signaling strength: a strong signal leads to negative selection, resulting in depletion, an intermediate signal leads to tTreg generation, and a weak signal results in thymocyte survival to differentiate into Tcon. Thymic aging (green arrow—*a*) shifts signaling strength from strong to intermediate and relatively enhances polyclonal tTreg generation; while some antigen-specific interactions exhibit an extremely weak signal, resulting in diminished antigen-specific tTreg cells and increased antigen-specific Tcon cells (green arrow—*b*).

result is presumably due to decreased self-pMHC-II expression, confirmed via assessment of a mock self-antigen in normal versus atrophied thymus [92].

### 4.3 Changes in thymic-derived regulatory T cell generation

As mentioned earlier, central tolerance establishment encompasses two mechanisms. The first mechanism, negative selection, is not entirely perfect [5] resulting in some self-reactive T clones being released into the periphery as Tcon cells. The second defense against self-reactivity is  $CD4^{SP}FoxP3^{+}$  peripheral Treg (pTreg) cell-mediated autoimmune suppression. It is believed that 80–95% of pTreg cells are generated within the thymus, as thymic-derived T regulatory (tTreg) cells [93–95]. Under the current paradigm, the processes of both negative selection and tTreg generation in the thymus utilize the same set of agonist self-peptides [93, 96]. Whether self-reactive thymocytes developing in the thymus are negatively selected or develop into tTreg cells depends on TCR signaling strength, or the sum of TCR affinity and avidity, (or the number of TCR interactions with self-peptide/MHC) when all other variables, such as IL-2, etc., are fixed. Put simply, strong signaling induces the apoptosis of self-reactive thymocytes, intermediate signaling leads to tTreg generation, and weak signaling results in the survival of thymocytes that differentiate into Tcon cells (**Figure 2**). This paradigm implies that depletion or survival for thymocytes is dependent on overall TCR signaling strength [38, 93].

Although there are cell extrinsic factors that can impact thymocyte development, such as the thymic cytokine milieu (IL-2 [97, 98], TGF- $\beta$  [98, 99], etc.), we propose that there are two cell types that directly regulate TCR signaling strength. One is intrinsic to thymocytes and the other is intrinsic to TECs. When the TCR binds to self-peptide/MHC on an antigen presenting cell, the immunoreceptor tyrosine-based activation motifs (ITAMs) are activated and the Zap70 kinase is subsequently phosphorylated. A mouse model with a knock-in allele of TCR zeta ( $\zeta$ ) chain gene with tyrosine-to-phenylalanine mutations in 6 out of 10 ITAMs led to a 60% decrease in TCR signaling potential [100]. This mouse model exhibited a

defect in negative selection, but an increase in tTreg generation [100]. The second variable is the relative expression level of self-peptide/MHC on TECs. Transgenic expression of a microRNA targeting the MHC class-II transactivator (CIITA) resulted in reduced MHC-II on mTECs [37], leading to insufficient mTEC presentation of self-peptides thus reducing the overall avidity of the TCR interaction with self-peptide/MHC. This also resulted in the enhancement of tTreg generation at the expense of negative selection [37].

In the aged thymus, as we mentioned earlier, mTECs are flawed and self-antigen cannot be normally presented in the MHC-II groove, which results in a diminished interaction with TCRs on developing thymocytes. This is similar to the second scenario described above, in which a defect exists in the TEC compartment causing reduced TCR signaling strength. We observed a relatively enhanced tTreg generation in the atrophied thymus, exhibiting no change in overall tTreg numbers, but an increased ratio of tTreg to tTcon cells in the aged, atrophied thymus compared to young controls [92]. This is probably a demonstration of the atrophied thymus attempting to compensate for defective negative selection [25] in order to maintain central T cell tolerance in the elderly.

If self-reactive TCR signaling strength is too low these thymocytes may neither be depleted nor form tTreg cells, but rather may directly differentiate into self-reactive Tcon cells. As an artifact of impaired promiscuous self-antigen expression in mTECs through an autoimmune regulator (*Aire*) knock-out model, the *Aire*-dependent TCAF3 epitope of prostate antigen cannot be promiscuously expressed on mTECs [101]. This resulted in prostate-specific thymocytes, which should be negatively selected, but in contrast were redirected into prostate-reactive Tcon cells. The authors observed loss of prostate-specific tTreg cells for this same epitope, and heightened prostate-reactive Tcon cells that infiltrated the prostate of these mice causing auto-inflammatory lesions [102, 103]. Defects in self-peptide expression on mTECs due to protein knock-out [104], are beginning to suggest that some of the same impairments exhibited by the atrophied thymus, may impact antigen-specific (monoclonal) tTreg generation, meanwhile increasing this same self-antigen specific Tcon generation, despite an unchanged or increased total (polyclonal) tTreg population [105]. It will be interesting to see what further subtle implications the aging thymus has on central tolerance establishment via potentially altering certain self-tissue specific tTreg populations and altering the overall aged Treg TCR repertoire, in spite of a relatively increased aged polyclonal Treg population [92].

#### 4.4 Overall contribution to inflammaging

Inflammaging or the age-related, persistent increase in basal pro-inflammatory phenotype, has long been thought to be primarily a result of senescent somatic cells exhibiting senescence-associated secretory phenotype (SASP) [30, 31, 34, 106]. However, it has come to be appreciated that chronic immune activation in the elderly contributes to a pro-inflammatory secretory milieu. This activation in the elderly includes chronic innate immune activation, which may result from immunosenescence related to accumulation of memory T cells. Chronic innate immune activation is also attributed to long-term virus, such as cytomegalovirus (CMV) [29, 107, 108], infection; or a degeneration-associated autotoxic reaction [109]. However, age-related autoimmune predisposition (an adaptive immune activation), induced by adaptive immune reaction to self-tissues by self-reactive T cells, has recently been recognized as a potential factor and/or synergistic cause of chronic inflammation in the elderly [25, 34]. Therefore, the role of the adaptive immune system in mediating inflammaging, as a result of self-reactive T cell immune

responses to self-tissue that increases with age, is directly related to the atrophied thymus [25, 34].

Since it has recently been confirmed that the involuted thymus releases self-reactive Tcon cells as a result of perturbed negative selection, the direct implications of age-related thymic atrophy on the risks of inflammaging and the associated subclinical increase in the pro-inflammatory milieu has become more clear [25].

Subsequent alterations in tTreg development may also play an unappreciated role in the increased self-reactivity associated with aging, as changes in the tTreg repertoire may in fact impair sufficient suppression of appropriate self-reactivity in the periphery, however, this still remains largely uninvestigated.

## 5. Trends in rejuvenation of age-related thymic atrophy

Rejuvenation of aged thymic function is one of the strategies to reduce inflammaging because it can reduce self-reactive Tcon cell release and potentially readjust tTreg cell function so that the adaptive immune aspects of inflammaging may be ameliorated. Several strategies to rejuvenate the atrophied thymus have been reported, including: (1) TEC stem cell-based strategies, including utilization of human embryonic/pluripotent stem cells [110–112], FoxN1<sup>eGFP/+</sup> knock-in epithelial cells [113], young TEC-based [114] or inducible TEC-based [115] strategies; (2) cytokine-to-TEC based therapy, such as keratinocyte growth factor (KGF) [116, 117] and IL-22 [118–120]; (3) genetically-based methods (enhancement of exogenous FoxN1 expression with FoxN1 cDNA plasmid and FoxN1 transgene) [79–81], and (4) epigenetically-based methods (via exosomes extracted from young healthy serum) [121].

As to the genetic rejuvenation strategy via exogenous FoxN1, intrathymic injection of plasmid vectors carrying FoxN1-cDNA into middle-aged and aged mice was able to partially rescue thymic atrophy and function. The investigators observed increased thymic size and thymocyte number in the treated group compared to mice receiving empty vector [79]. Another group utilized an inducible FoxN1 overexpression reporter gene system, and it was demonstrated that *in vivo* upregulation of FoxN1 expression in middle-aged and aged mice resulted in increased thymic size and thymocyte numbers as well as increased numbers of early thymic progenitor cells [81]. Additionally, the ratio of mTECs to cTECs, which is normally declined, was restored to normal levels [81].

As to cell-based therapy, this has also been investigated as a potential source of thymic rejuvenation via the use of exogenous TECs from newborn thymi. The investigators, after observing that circulating factors alone (via a heterochronic parabiosis model, in which young and aged mice are surgically joined resulting in mutual influence of blood-borne factors [122–130]) did not rejuvenate the aged thymus, utilized a model of direct transplantation of TECs from newborn mice intrathymically into middle-aged recipients [114]. This group observed renewed growth of the thymus as well as enhanced T cell generation [114].

Other groups are investigating the use of reprogrammed mouse embryonic fibroblasts (MEF), as sources of exogenous FoxN1, as a means of generating *de novo* ectopic thymus. One such group generated induced TECs (iTECs) from MEF cells by initiating FoxN1 expression that converted MEF cells into epithelial-like cells *in vitro* [115]. Then, these iTECs, after some testing, were re-aggregated and grafted under the kidney capsule of syngenic adult mice to evaluate the ability of these iTECs to develop into a functional thymus-like organ. Interestingly, the grafts were seeded by host T cell progenitors and reflected thymocyte distributions associated

with the normal thymus at endpoint (4 weeks after engraftment). Additionally, typical thymus microstructure was observed in these grafts [115].

The overarching conclusions taken from these cytokine, cellular, genetic, or epigenetically-based rejuvenation strategies are that FoxN1 expression is a key target for rejuvenating TECs, resulting in a more functional thymus able to produce normal T cells. However, we need to recognize that any rejuvenation therapy has its pitfalls. For example, intrathymic injection of newborn TECs can rejuvenate middle-aged thymus [114], but the source of newborn TECs is limited and may not be ideal as a translational therapy. Additionally, generation of an ectopic *de novo* thymus under the kidney capsule [115] can generate naïve T cells, but this does not remedy the increased self-reactive T cells released by the original atrophied thymus remaining in the host. Also, the use of cytokines may help revitalize the thymus, but as a systemic therapy could present various detrimental side-effects. Therefore, further studies to develop practical and effective therapies are necessary.

## 6. Conclusion

In conclusion, age-related thymic atrophy is a dynamic process beginning early in life that shapes T cell development and the establishment of central T cell tolerance. There is substantial clinical significance in further exploring the underlying mechanisms of its effects on the various subsets of T cells developed in the atrophied thymus, namely Treg and Tcon cells. Also, continued investigation into potential avenues of thymic rejuvenation are striving to reverse the adverse effects of age-related thymic atrophy on the aged T cell immune system, since increased self-reactive T cells are observed with age, contributing to inflammaging. Moreover, there are numerous areas still to explore in this field with far-reaching applications.

## Acknowledgements

We would like to thank Dr. Rance Berg (Department of Microbiology, Immunology and Genetics, UNTHSC) for critical reading of this manuscript.

## Conflict of interest

The authors have no conflict of interest.

## Funding

Supported by NIH/NIAID grant R01AI121147 to D-M. S. The funder had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

IntechOpen

### **Author details**

Rachel Thomas<sup>1</sup> and Dong-Ming Su<sup>2\*</sup>

1 Cell Biology, Immunology, and Microbiology Graduate Program, Graduate School of Biomedical Sciences, University of North Texas Health Science Center, Fort Worth, Texas, USA

2 Department of Microbiology, Immunology, and Genetics, University of North Texas Health Science Center, Fort Worth, Texas, USA

\*Address all correspondence to: [dongming.su@unthsc.edu](mailto:dongming.su@unthsc.edu)

### **IntechOpen**

© 2019 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/3.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. 

## References

- [1] Kurd N, Robey EA. T-cell selection in the thymus: A spatial and temporal perspective. *Immunological Reviews*. 2016;**271**(1):114-126
- [2] Palmer E. Negative selection—Clearing out the bad apples from the T-cell repertoire. *Nature Reviews Immunology*. 2003;**3**(5):383-391
- [3] Hsieh CS, Lee HM, Lio CW. Selection of regulatory T cells in the thymus. *Nature Reviews Immunology*. 2012;**12**(3):157-167
- [4] Cebula A, Seweryn M, Rempala GA, Pabla SS, McIndoe RA, Denning TL, et al. Thymus-derived regulatory T cells contribute to tolerance to commensal microbiota. *Nature*. 2013;**497**(7448):258-262
- [5] Gallegos AM, Bevan MJ. Central tolerance: Good but imperfect. *Immunological Reviews*. 2006;**209**:290-296
- [6] Rezzani R, Nardo L, Favero G, Peroni M, Rodella LF. Thymus and aging: Morphological, radiological, and functional overview. *Age (Dordrecht, Netherlands)*. 2014;**36**(1):313-351
- [7] Palmer DB. The effect of age on thymic function. *Frontiers in Immunology*. 2013;**4**:316
- [8] Hale JS, Boursalian TE, Turk GL, Fink PJ. Thymic output in aged mice. *Proceedings of the National Academy of Sciences of the United States of America*. 2006;**103**(22):8447-8452
- [9] Sudo K, Ema H, Morita Y, Nakauchi H. Age-associated characteristics of murine hematopoietic stem cells. *The Journal of Experimental Medicine*. 2000;**192**(9):1273-1280
- [10] Min H, Montecino-Rodriguez E, Dorshkind K. Reduction in the developmental potential of intrathymic T cell progenitors with age. *Journal of Immunology (Baltimore, Md. : 1950)*. 2004;**173**(1):245-250
- [11] Kovtonyuk LV, Fritsch K, Feng X, Manz MG, Takizawa H. Inflamm-aging of Hematopoiesis, hematopoietic stem cells, and the bone marrow microenvironment. *Frontiers in Immunology*. 2016;**7**:502
- [12] Latchney SE, Calvi LM. The aging hematopoietic stem cell niche: Phenotypic and functional changes and mechanisms that contribute to hematopoietic aging. *Seminars in Hematology*. 2017;**54**(1):25-32
- [13] Sun L, Brown R, Chen S, Zhuge Q, Su DM. Aging induced decline in T-lymphopoiesis is primarily dependent on status of progenitor niches in the bone marrow and thymus. *Aging*. 2012;**4**(9):606-619
- [14] Su DM, Aw D, Palmer DB. Immunosenescence: A product of the environment? *Current Opinion in Immunology*. 2013;**25**(4):498-503
- [15] Anderson G, Jenkinson EJ. Lymphostromal interactions in thymic development and function. *Nature Reviews Immunology*. 2001;**1**(1):31-40
- [16] Vaidya HJ, Briones Leon A, Blackburn CC. FOXP1 in thymus organogenesis and development. *European Journal of Immunology*. 2016;**46**(8):1826-1837
- [17] Pawelec G. Age and immunity: What is “immunosenescence”? *Experimental Gerontology*. 2018;**105**:4-9
- [18] McElhaney JE, Effros RB. Immunosenescence: What does it mean to health outcomes in older adults?

Current Opinion in Immunology.  
2009;**21**(4):418-424

[19] Pawelec G. Immunosenescence comes of age. Symposium on aging research in immunology: The impact of genomics. EMBO Reports. 2007;**8**(3):220-223

[20] Pawelec G, Wagner W, Adibzadeh M, Engel A. T cell immunosenescence in vitro and in vivo. Experimental Gerontology. 1999;**34**(3):419-429

[21] Nikolich-Zugich J. The twilight of immunity: Emerging concepts in aging of the immune system. Nature Immunology. 2018;**19**(1):10-19

[22] Zinger A, Cho WC, Ben-Yehuda A. Cancer and aging-the inflammatory connection. Aging and Disease. 2017;**8**(5):611-627

[23] Machiela MJ. Mosaicism, aging and cancer. Current Opinion in Oncology. 2019;**31**(2):108-113

[24] Xia J, Wang H, Guo J, Zhang Z, Coder B, Su DM. Age-related disruption of steady-state thymic medulla provokes autoimmune phenotype via perturbing negative selection. Aging and Disease. 2012;**3**(3):248-259

[25] Coder BD, Wang H, Ruan L, Su DM. Thymic involution perturbs negative selection leading to autoreactive T cells that induce chronic inflammation. Journal of Immunology (Baltimore, MD: 1950). 2015;**194**(12):5825-5837

[26] Coder B, Su DM. Thymic involution beyond T-cell insufficiency. Oncotarget. 2015;**6**(26):21777-21778

[27] De Martinis M, Franceschi C, Monti D, Ginaldi L. Inflammaging and lifelong antigenic load as major determinants of ageing rate and longevity. FEBS Letters. 2005;**579**(10):2035-2039

[28] Franceschi C, Bonafe M, Valensin S, Olivieri F, De Luca M, Ottaviani E, et al. Inflammaging. An evolutionary perspective on immunosenescence. Annals of the New York Academy of Sciences. 2000;**908**:244-254

[29] Brunner S, Herndler-Brandstetter D, Weinberger B, Grubeck-Loebenstern B. Persistent viral infections and immune aging. Ageing Research Reviews. 2011;**10**(3):362-369

[30] Freund A, Orjalo AV, Desprez PY, Campisi J. Inflammatory networks during cellular senescence: Causes and consequences. Trends in Molecular Medicine. 2010;**16**(5):238-246

[31] Franceschi C, Capri M, Monti D, Giunta S, Olivieri F, Sevini F, et al. Inflammaging and anti-inflammaging: A systemic perspective on aging and longevity emerged from studies in humans. Mechanisms of Ageing and Development. 2007;**128**(1):92-105

[32] Fulop T, Larbi A, Dupuis G, Le Page A, Frost EH, Cohen AA, et al. Immunosenescence and Inflamm-aging As two sides of the same coin: Friends or foes? Frontiers in Immunology. 2017;**8**:1960

[33] Gui J, Mustachio LM, Su DM, Craig RW. Thymus size and age-related thymic involution: Early programming, sexual dimorphism, progenitors and stroma. Aging and Disease. 2012;**3**(3):280-290

[34] Fulop T, Witkowski JM, Olivieri F, Larbi A. The integration of inflammaging in age-related diseases. Seminars in Immunology. 2018;**40**:17-35

[35] Palmer S, Albergante L, Blackburn CC, Newman TJ. Thymic involution and rising disease incidence with age. Proceedings of the National Academy of Sciences of the United States of America. 2018;**115**(8):1883-1888

- [36] Aspinall R, Andrew D. Thymic involution in aging. *Journal of Clinical Immunology*. 2000;**20**(4):250-256
- [37] Hinterberger M, Aichinger M, Prazeres da Costa O, Voehringer D, Hoffmann R, Klein L. Autonomous role of medullary thymic epithelial cells in central CD4(+) T cell tolerance. *Nature Immunology*. 2010;**11**(6):512-519
- [38] Klein L, Kyewski B, Allen PM, Hogquist KA. Positive and negative selection of the T cell repertoire: What thymocytes see (and don't see). *Nature Reviews Immunology*. 2014;**14**(6):377-391
- [39] Aw D, Silva AB, Maddick M, von Zglinicki T, Palmer DB. Architectural changes in the thymus of aging mice. *Aging Cell*. 2008;**7**(2):158-167
- [40] Chinn IK, Blackburn CC, Manley NR, Sempowski GD. Changes in primary lymphoid organs with aging. *Seminars in Immunology*. 2012;**24**(5):309-320
- [41] Burnley P, Rahman M, Wang H, Zhang Z, Sun X, Zhuge Q, et al. Role of the p63-FoxN1 regulatory axis in thymic epithelial cell homeostasis during aging. *Cell Death & Disease*. 2013;**4**:e932
- [42] Masters AR, Haynes L, Su DM, Palmer DB. Immune senescence: Significance of the stromal microenvironment. *Clinical and Experimental Immunology*. 2017;**187**(1):6-15
- [43] Sempowski GD, Hale LP, Sundy JS, Massey JM, Koup RA, Douek DC, et al. Leukemia inhibitory factor, oncostatin M, IL-6, and stem cell factor mRNA expression in human thymus increases with age and is associated with thymic atrophy. *Journal of Immunology*. 2000;**164**(4):2180-2187
- [44] Pang WW, Price EA, Sahoo D, Beerman I, Maloney WJ, Rossi DJ, et al. Human bone marrow hematopoietic stem cells are increased in frequency and myeloid-biased with age. *Proceedings of the National Academy of Sciences of the United States of America*. 2011;**108**(50):20012-20017
- [45] Linton PJ, Dorshkind K. Age-related changes in lymphocyte development and function. *Nature Immunology*. 2004;**5**(2):133-139
- [46] Tyan ML. Age-related decrease in mouse T cell progenitors. *Journal of Immunology (Baltimore, MD: 1950)*. 1977;**118**(3):846-851
- [47] Morrison SJ, Wandycz AM, Akashi K, Globerson A, Weissman IL. The aging of hematopoietic stem cells. *Nature Medicine*. 1996;**2**(9):1011-1016
- [48] Waterstrat A, Van Zant G. Effects of aging on hematopoietic stem and progenitor cells. *Current Opinion in Immunology*. 2009;**21**(4):408-413
- [49] Van Zant G, Liang Y. Concise review: Hematopoietic stem cell aging, life span, and transplantation. *Stem Cells Translational Medicine*. 2012;**1**(9):651-657
- [50] Min H, Montecino-Rodriguez E, Dorshkind K. Effects of aging on early B- and T-cell development. *Immunological Reviews*. 2005;**205**:7-17
- [51] Zhu X, Gui J, Dohkan J, Cheng L, Barnes PF, Su DM. Lymphohematopoietic progenitors do not have a synchronized defect with age-related thymic involution. *Aging Cell*. 2007;**6**(5):663-672
- [52] Peschon JJ, Morrissey PJ, Grabstein KH, Ramsdell FJ, Maraskovsky E, Gliniak BC, et al. Early lymphocyte expansion is severely impaired in interleukin 7 receptor-deficient mice. *The Journal of Experimental Medicine*. 1994;**180**(5):1955-1960

- [53] Sun L, Hurez VJ, Thibodeaux SR, Kioussis MJ, Liu A, Lin P, et al. Aged regulatory T cells protect from autoimmune inflammation despite reduced STAT3 activation and decreased constraint of IL-17 producing T cells. *Aging Cell*. 2012;**11**(3):509-519
- [54] Porritt HE, Rumfelt LL, Tabrizifard S, Schmitt TM, Zuniga-Pflucker JC, Petrie HT. Heterogeneity among DN1 prothymocytes reveals multiple progenitors with different capacities to generate T cell and non-T cell lineages. *Immunity*. 2004;**20**(6):735-745
- [55] Gui J, Zhu X, Dohkan J, Cheng L, Barnes PF, Su DM. The aged thymus shows normal recruitment of lymphohematopoietic progenitors but has defects in thymic epithelial cells. *International Immunology*. 2007;**19**(10):1201-1211
- [56] Aspinall R, Andrew D. Thymic atrophy in the mouse is a soluble problem of the thymic environment. *Vaccine*. 2000;**18**(16):1629-1637
- [57] Henson SM, Snelgrove R, Hussell T, Wells DJ, Aspinall R. An IL-7 fusion protein that shows increased thymopoietic ability. *Journal of Immunology (Baltimore, MD: 1950)*. 2005;**175**(6):4112-4118
- [58] van Ewijk W, Hollander G, Terhorst C, Wang B. Stepwise development of thymic microenvironments in vivo is regulated by thymocyte subsets. *Development*. 2000;**127**(8):1583-1591
- [59] Shores EW, Van Ewijk W, Singer A. Disorganization and restoration of thymic medullary epithelial cells in T cell receptor-negative scid mice: Evidence that receptor-bearing lymphocytes influence maturation of the thymic microenvironment. *European Journal of Immunology*. 1991;**21**(7):1657-1661
- [60] Stanger BZ, Tanaka AJ, Melton DA. Organ size is limited by the number of embryonic progenitor cells in the pancreas but not the liver. *Nature*. 2007;**445**(7130):886-891
- [61] Jenkinson WE, Bacon A, White AJ, Anderson G, Jenkinson EJ. An epithelial progenitor pool regulates thymus growth. *Journal of Immunology*. 2008;**181**(9):6101-6108
- [62] Foss DL, Donskoy E, Goldschneider I. The importation of hematogenous precursors by the thymus is a gated phenomenon in normal adult mice. *The Journal of Experimental Medicine*. 2001;**193**(3):365-374
- [63] Donskoy E, Foss D, Goldschneider I. Gated importation of prothymocytes by adult mouse thymus is coordinated with their periodic mobilization from bone marrow. *Journal of Immunology*. 2003;**171**(7):3568-3575
- [64] Moore KA, Lemischka IR. Stem cells and their niches. *Science*. 2006;**311**(5769):1880-1885
- [65] Wilson A, Trumpp A. Bone-marrow haematopoietic-stem-cell niches. *Nature Reviews. Immunology*. 2006;**6**(2):93-106
- [66] Prockop SE, Petrie HT. Regulation of thymus size by competition for stromal niches among early T cell progenitors. *Journal of Immunology*. 2004;**173**(3):1604-1611
- [67] Nehls M, Pfeifer D, Schorpp M, Hedrich H, Boehm T. New member of the winged-helix protein family disrupted in mouse and rat nude mutations. *Nature*. 1994;**372**(6501):103-107
- [68] Nehls M, Kyewski B, Messerle M, Waldschutz R, Schuddekopf K, Smith AJ, et al. Two genetically separable steps in the differentiation

of thymic epithelium. *Science*. 1996;**272**(5263):886-889

[69] Schlake T. The nude gene and the skin. *Experimental Dermatology*. 2001;**10**(5):293-304

[70] Brissette JL, Li J, Kamimura J, Lee D, Dotto GP. The product of the mouse nude locus, Whn, regulates the balance between epithelial cell growth and differentiation. *Genes & Development*. 1996;**10**(17):2212-2221

[71] Flanagan SP. 'Nude', a new hairless gene with pleiotropic effects in the mouse. *Genetical Research*. 1966;**8**(3):295-309

[72] Pantelouris EM. Absence of thymus in a mouse mutant. *Nature*. 1968;**217**(5126):370-371

[73] Ortman CL, Dittmar KA, Witte PL, Le PT. Molecular characterization of the mouse involuted thymus: Aberrations in expression of transcription regulators in thymocyte and epithelial compartments. *International Immunology*. 2002;**14**(7):813-822

[74] Rode I, Martins VC, Kublbeck G, Maltry N, Tessmer C, Rodewald HR. FoxN1 protein expression in the developing, aging, and regenerating Thymus. *Journal of Immunology*. 2015;**195**(12):5678-5687

[75] Cheng L, Guo J, Sun L, Fu J, Barnes PF, Metzger D, et al. Postnatal tissue-specific disruption of transcription factor FoxN1 triggers acute thymic atrophy. *The Journal of Biological Chemistry*. 2010;**285**(8):5836-5847

[76] Zhang Z, Burnley P, Coder B, Su DM. Insights on FoxN1 biological significance and usages of the "nude" mouse in studies of T-lymphopoiesis. *International Journal of Biological Sciences*. 2012;**8**(8):1156-1167

[77] Matsuda T, Cepko CL. Controlled expression of transgenes introduced by in vivo electroporation. *Proceedings of the National Academy of Sciences of the United States of America*. 2007;**104**(3):1027-1032

[78] Bleul CC, Corbeaux T, Reuter A, Fisch P, Monting JS, Boehm T. Formation of a functional thymus initiated by a postnatal epithelial progenitor cell. *Nature*. 2006;**441**(7096):992-996

[79] Sun L, Guo J, Brown R, Amagai T, Zhao Y, Su DM. Declining expression of a single epithelial cell-autonomous gene accelerates age-related thymic involution. *Aging Cell*. 2010;**9**(3):347-357

[80] 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**(22):5723-5731

[81] Bredenkamp N, Nowell CS, Blackburn CC. Regeneration of the aged thymus by a single transcription factor. *Development (Cambridge, England)*. 2014;**141**(8):1627-1637

[82] O'Neill KE, Bredenkamp N, Tischner C, Vaidya HJ, Stenhouse FH, Peddie CD, et al. FoxN1 is dynamically regulated in thymic epithelial cells during embryogenesis and at the onset of thymic involution. *PLoS One*. 2016;**11**(3):e0151666

[83] Swain S, Clise-Dwyer K, Haynes L. Homeostasis and the age-associated defect of CD4 T cells. *Seminars in Immunology*. 2005;**17**(5):370-377

[84] Goronzy JJ, Lee WW, Weyand CM. Aging and T-cell diversity. *Experimental Gerontology*. 2007;**42**(5):400-406

- [85] Petrie HT. Role of thymic organ structure and stromal composition in steady-state postnatal T-cell production. *Immunological Reviews*. 2002;**189**:8-19
- [86] Hodes RJ. Telomere length, aging, and somatic cell turnover. *The Journal of Experimental Medicine*. 1999;**190**(2):153-156
- [87] Franceschi C, Valensin S, Fagnoni F, Barbi C, Bonafe M. Biomarkers of immunosenescence within an evolutionary perspective: The challenge of heterogeneity and the role of antigenic load. *Experimental Gerontology*. 1999;**34**(8):911-921
- [88] Guo J, Feng Y, Barnes P, Huang FF, Idell S, Su DM, et al. Deletion of FoxN1 in the thymic medullary epithelium reduces peripheral T cell responses to infection and mimics changes of aging. *PLoS One*. 2012;**7**(4):e34681
- [89] Stritesky GL, Jameson SC, Hogquist KA. Selection of self-reactive T cells in the thymus. *Annual Review of Immunology*. 2012;**30**:95-114
- [90] Josefowicz SZ, Lu LF, Rudensky AY. Regulatory T cells: Mechanisms of differentiation and function. *Annual Review of Immunology*. 2012;**30**:531-564
- [91] Li MO, Rudensky AY. T cell receptor signalling in the control of regulatory T cell differentiation and function. *Nature Reviews Immunology*. 2016;**16**(4):220-233
- [92] Oh J, Wang W, Thomas R, Su DM. Capacity of tTreg generation is not impaired in the atrophied thymus. *PLoS Biology*. 2017;**15**(11):e2003352
- [93] Klein L, Robey EA, Hsieh CS. Central CD4(+) T cell tolerance: Deletion versus regulatory T cell differentiation. *Nature Reviews Immunology*. 2019;**19**(1):7-18
- [94] Pohar J, Simon Q, Fillatreau S. Antigen-specificity in the thymic development and peripheral activity of CD4(+)FOXP3(+) T regulatory cells. *Frontiers in Immunology*. 2018;**9**:1701
- [95] Lathrop SK, Santacruz NA, Pham D, Luo J, Hsieh CS. Antigen-specific peripheral shaping of the natural regulatory T cell population. *The Journal of Experimental Medicine*. 2008;**205**(13):3105-3117
- [96] Wirnsberger G, Hinterberger M, Klein L. Regulatory T-cell differentiation versus clonal deletion of autoreactive thymocytes. *Immunology and Cell Biology*. 2011;**89**(1):45-53
- [97] Malek TR, Bayer AL. Tolerance, not immunity, crucially depends on IL-2. *Nature Reviews Immunology*. 2004;**4**(9):665-674
- [98] Liu Y, Zhang P, Li J, Kulkarni AB, Perruche S, Chen W. A critical function for TGF-beta signaling in the development of natural CD4+CD25+Foxp3+ regulatory T cells. *Nature Immunology*. 2008;**9**(6):632-640
- [99] Konkel JE, Jin W, Abbatiello B, Grainger JR, Chen W. Thymocyte apoptosis drives the intrathymic generation of regulatory T cells. *Proceedings of the National Academy of Sciences of the United States of America*. 2014;**111**(4):E465-E473
- [100] Hwang S, Song KD, Lesourne R, Lee J, Pinkhasov J, Li L, et al. Reduced TCR signaling potential impairs negative selection but does not result in autoimmune disease. *The Journal of Experimental Medicine*. 2012;**209**(10):1781-1795
- [101] Leonard JD, Gilmore DC, Dileepan T, Nawrocka WI, Chao JL, Schoenbach MH, et al. Identification of natural regulatory T cell epitopes reveals convergence on a dominant autoantigen. *Immunity*. 2017;**47**(1):107.e8-117.e8

- [102] Malchow S, Leventhal DS, Savage PA. Organ-specific regulatory T cells of thymic origin are expanded in murine prostate tumors. *Oncoimmunology*. 2013;**2**(7):e24898
- [103] Malchow S, Leventhal DS, Lee V, Nishi S, Socci ND, Savage PA. Aire enforces immune tolerance by directing autoreactive T cells into the regulatory T cell lineage. *Immunity*. 2016;**44**(5):1102-1113
- [104] Kieback E, Hilgenberg E, Stervbo U, Lampropoulou V, Shen P, Bunse M, et al. Thymus-derived regulatory T cells are positively selected on natural self-antigen through cognate interactions of High functional avidity. *Immunity*. 2016;**44**(5):1114-1126
- [105] Lages CS, Suffia I, Velilla PA, Huang B, Warshaw G, Hildeman DA, et al. Functional regulatory T cells accumulate in aged hosts and promote chronic infectious disease reactivation. *Journal of Immunology (Baltimore, MD: 1950)*. 2008;**181**(3):1835-1848
- [106] Campisi J. Cellular senescence: Putting the paradoxes in perspective. *Current Opinion in Genetics & Development*. 2011;**21**(1):107-112
- [107] Nikolich-Zugich J. Ageing and life-long maintenance of T-cell subsets in the face of latent persistent infections. *Nature Reviews. Immunology*. 2008;**8**(7):512-522
- [108] High KP, Akbar AN, Nikolich-Zugich J. Translational research in immune senescence: Assessing the relevance of current models. *Seminars in Immunology*. 2012;**24**(5):373-382
- [109] McGeer PL, McGeer EG. Inflammation and the degenerative diseases of aging. *Annals of the New York Academy of Sciences*. 2004;**1035**:104-116
- [110] Parent AV, Russ HA, Khan IS, LaFlam TN, Metzger TC, Anderson MS, et al. Generation of functional thymic epithelium from human embryonic stem cells that supports host T cell development. *Cell Stem Cell*. 2013;**13**(2):219-229
- [111] Sun X, Xu J, Lu H, Liu W, Miao Z, Sui X, et al. Directed differentiation of human embryonic stem cells into thymic epithelial progenitor-like cells reconstitutes the thymic microenvironment in vivo. *Cell Stem Cell*. 2013;**13**(2):230-236
- [112] Soh CL, Giudice A, Jenny RA, Elliott DA, Hatzistavrou T, Micallef SJ, et al. FoxN1 (GFP/w) reporter hESCs enable identification of integrin-beta4, HLA-DR, and EpCAM as markers of human PSC-derived FoxN1(+) thymic epithelial progenitors. *Stem Cell Reports*. 2014;**2**(6):925-937
- [113] Barsanti M, Lim JM, Hun ML, Lister N, Wong K, Hammett MV, et al. A novel FoxN1(eGFP/+) mouse model identifies Bmp4-induced maintenance of FoxN1 expression and thymic epithelial progenitor populations. *European Journal of Immunology*. 2017;**47**(2):291-304
- [114] Kim MJ, Miller CM, Shadrach JL, Wagers AJ, Serwold T. Young, proliferative thymic epithelial cells engraft and function in aging thymuses. *Journal of Immunology (Baltimore, MD: 1950)*. 2015;**194**(10):4784-4795
- [115] Bredenkamp N, Ulyanchenko S, O'Neill KE, Manley NR, Vaidya HJ, Blackburn CC. An organized and functional thymus generated from FoxN1-reprogrammed fibroblasts. *Nature Cell Biology*. 2014;**16**(9):902-908
- [116] Erickson M, Morkowski S, Lehar S, Gillard G, Beers C, Dooley J, et al. Regulation of thymic epithelium by keratinocyte growth factor. *Blood*. 2002;**100**(9):3269-3278

- [117] Alpdogan O, Hubbard VM, Smith OM, Patel N, Lu S, Goldberg GL, et al. Keratinocyte growth factor (KGF) is required for postnatal thymic regeneration. *Blood*. 2006;**107**(6):2453-2460
- [118] Bhandoola A, Artis D. Immunology. Rebuilding the thymus. *Science*. 2012;**336**(6077):40-41
- [119] Dudakov JA, Hanash AM, Jenq RR, Young LF, Ghosh A, Singer NV, et al. Interleukin-22 drives endogenous thymic regeneration in mice. *Science*. 2012;**336**(6077):91-95
- [120] Chaudhry MS, Velardi E, Dudakov JA, van den Brink MR. Thymus: The next (re)generation. *Immunological Reviews*. 2016;**271**(1):56-71
- [121] Wang W, Wang L, Ruan L, Oh J, Dong X, Zhuge Q, et al. Extracellular vesicles extracted from young donor serum attenuate inflammaging via partially rejuvenating aged T-cell immunotolerance. *FASEB Journal: Official Publication of the Federation of American Societies for Experimental Biology*. 2018;**32**(11):5899-5912. DOI: 10.1096/fj201800059R
- [122] Conboy IM, Conboy MJ, Wagers AJ, Girma ER, Weissman IL, Rando TA. Rejuvenation of aged progenitor cells by exposure to a young systemic environment. *Nature*. 2005;**433**(7027):760-764
- [123] Pishel I, Shytikov D, Orlova T, Peregudov A, Artyuhov I, Butenko G. Accelerated aging versus rejuvenation of the immune system in heterochronic parabiosis. *Rejuvenation Research*. 2012;**15**(2):239-248
- [124] Ruckh JM, Zhao JW, Shadrach JL, van Wijngaarden P, Rao TN, Wagers AJ, et al. Rejuvenation of regeneration in the aging central nervous system. *Cell Stem Cell*. 2012;**10**(1):96-103
- [125] Brack AS, Conboy MJ, Roy S, Lee M, Kuo CJ, Keller C, et al. Increased Wnt signaling during aging alters muscle stem cell fate and increases fibrosis. *Science*. 2007;**317**(5839):807-810
- [126] Loffredo FS, Steinhauser ML, Jay SM, Gannon J, Pancoast JR, Yalamanchi P, et al. Growth differentiation factor 11 is a circulating factor that reverses age-related cardiac hypertrophy. *Cell*. 2013;**153**(4):828-839
- [127] Katsimpardi L, Litterman NK, Schein PA, Miller CM, Loffredo FS, Wojtkiewicz GR, et al. Vascular and neurogenic rejuvenation of the aging mouse brain by young systemic factors. *Science*. 2014;**344**(6184):630-634
- [128] Villeda SA, Plambeck KE, Middeldorp J, Castellano JM, Mosher KI, Luo J, et al. Young blood reverses age-related impairments in cognitive function and synaptic plasticity in mice. *Nature Medicine*. Jun 2014;**20**(6):659-663
- [129] Villeda SA, Luo J, Mosher KI, Zou B, Britschgi M, Bieri G, et al. The ageing systemic milieu negatively regulates neurogenesis and cognitive function. *Nature*. 2011;**477**(7362):90-94
- [130] Villeda SA, Wyss-Coray T. The circulatory systemic environment as a modulator of neurogenesis and brain aging. *Autoimmunity Reviews*. 2013;**12**(6):674-677