

Review

# The Origin and Implication of Thymic Involution

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**ABSTRACT:** Age-related regression of the thymus is associated with a decline in naïve T cell output which is thought to contribute to the reduction in T cell diversity in older individuals that is partially responsible for an increase in susceptibility and severity of infections, cancers and autoimmune diseases. Thymic involution is one of the most dramatic and ubiquitous changes in the ageing immune system, but the precise regulators remain anonymous. However, a picture is emerging, implicating extrinsic and intrinsic factors that may contribute towards age-associated thymic involution. In this review we assess the role of the thymic microenvironment as a possible target of thymic involution, question whether thymocyte development in the aged thymus is functional and explore why the thymus involutes.

**Key words:** Thymus; Thymocytes; Thymic microenvironment; Thymic involution

The thymus is a primary lymphoid organ responsible for the production of a diverse repertoire of immunocompetent T cells [1]. Principally, pluripotent stem cells enter into the thymus where they will differentiate into T cells by receiving instructions from the specialised thymic microenvironment that regulate phases of maturation, proliferation, gene rearrangement and selection [2, 3].

It is now recognised that the function of the immune system deteriorates with age and this is believed to be primarily responsible for the increased susceptibility and higher incidences of infectious diseases, poor response to vaccination, neoplastic and autoimmune conditions in older people [4-6]. Both the innate and the adaptive immune responses are affected by age [7, 8]; however, the exact mechanisms involved in immunosenescence are not fully understood.

One of the most characteristic changes of the ageing immune system is the regression, or involution, of the thymus [9-11]. Moreover, age-associated thymic involution seems to occur in all vertebrates that possess a thymus, indicating that this is an evolutionary ancient and conserved event [12]. Age-associated thymic involution is associated with a reduction in tissue mass and thymic cellularity, loss of tissue structure and

abnormal architecture; leading to a decline in naïve T cell output [9-11]. The regression of the thymus is intimately linked to constriction of peripheral T cell diversity, alterations in their phenotype and function, and corrosion of telomeres due to replicative senescence [13-15]. These changes in the peripheral T cell compartment are believed to be, in part, responsible for the clinical signs of immunosenescence [4-6]. Thus, thymic involution can be regarded as one of the leading regulators of ageing [16]. Yet despite age-associated thymic involution being recognized for over 70 years, little is known about the precise mechanisms that contribute to this event. However, evidence suggest that both extrinsic and intrinsic factors may contribute towards age-associated thymic involution [6, 7, 10, 17], which we will explore together with discussing why thymic atrophy might occur at all.

## Are thymic epithelial cells regulators of thymic involution?

Many theories and mechanisms have been proposed to explain the process of age-associated thymic atrophy. Among these are intrinsic and extrinsic factors, however recent studies have begun to elucidate the primary

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culprits and most are the result of alteration within the thymic microenvironment or act via transforming this unique site of T cell development.

Given the dramatic loss in thymic cellularity with age, people proposed progenitors that migrate from the bone marrow [BM] to the thymus were in some way defective [5-7]. Indeed, initial studies by Tyan, transferring BM from older donors to young mice suggested haematopoietic stem cells [HSC] were less efficient at repopulating the thymus following irradiation than BM from young animals [18]. Additionally, transplantation of Lin<sup>-</sup>c-kit<sup>+</sup> haematopoietic progenitors from older mice into younger mice displayed reduced differentiation ability to T and Natural Killer lineages compared with the same population from younger mice [19]. Similarly, purified HSC from older mice also exhibit decreased differentiation potential towards lymphoid lineage *in vitro* [20]. This age-related alteration in differentiation potential is even more pronounced in early thymic progenitors [ETP] when seeded into fetal thymic organ cultures [21]. ETP derived from young mice were capable of reconstituting all stages of T cell development, whereas those from older mice, although able to produce fully mature thymocytes, generated significantly lower number of thymocytes. Nevertheless, a number of studies transferring young BM into aged lethally irradiated hosts have shown that thymic and splenic repopulation and mitogenic responses are consistently lower, implying these deficiencies are independent of the age of the thymocyte progenitors [22, 23]. Furthermore, young BM injected into aged mice failed to restore thymic histological abnormalities [23] and BM from aged and young mice donors is able to repopulate the thymus and spleen in equal numbers irrespective of the recipient's age [22]. These conflicting results may be the consequence of employing different experimental conditions, including using whole BM or purified progenitors in different strains of mice. An elegant study conducted by Zhu and colleagues sought to overcome these inconsistencies by examining the capacity of progenitors *in situ* by grafting a fetal thymus to the kidney capsule in young and old mice [24]. Despite the native thymus from older animals still having significantly lower actual total and subset numbers, the thymi grafts in both young and older hosts had similar total thymic cellularity. In addition, the ability of wildtype thymocyte progenitors to pattern the cortical and medullary regions of transplanted *Rag*<sup>-/-</sup> lobes is not affected by age [24]. Therefore, these results imply that the functional capacity of ETP from older mice is not drastically disparate from ETP in younger mice. Further investigation suggests that the decline in cellularity is, in part, due to the inability of the aged

thymic microenvironment to support and maintain ETP [25]. Intravenously injected lineage negative-enriched BM from young mice into sublethally irradiated one month old and 18 month old mice showed absolute numbers of donor cells was similar in young and older hosts after three days. However, seven to 10 days after injection, the number of donor cells in older thymi was severely reduced compared to those identified in younger thymi, suggesting a decline in their proliferative capacity. Additionally, in nine month-old mice there is a significant increase the proportion of Annexin V<sup>+</sup> ETP, suggesting an increase in apoptosis of ETP with age [26]. Moreover, the frequency of ETP expressing Ki67 [nuclear antigen found in proliferating cells] decreases progressively with age and is significantly reduced in cells from 20 month old mice [21]. Therefore, although intrinsic age-related alteration thymocyte progenitors may play a role in thymic involution [27], it appears the thymic microenvironment itself is responsible for influencing many of the defects exhibited by these precursors.

Studies observing the detrimental effects of sex hormones on thymocytes [6] and the renowned observations of transient thymic involution during pregnancy that is reversed postpartum or after lactation; giving rise to the notion that involution possibly begins at puberty [28]. For instance, 8 month-old mice following castration demonstrate amplification in ETP number [26]. Concomitantly, the distorted thymic architecture, including the disruption of the cortico-medullary junction and decline in MHC class II expression is restored to resemble young thymi [29]. Analysis of the stromal compartment presents an increase in TEC number and a rise in Ki67<sup>+</sup> TEC, implying TEC regeneration is at least partially due to enhance proliferation following castration [30]. These alterations have a positive effect on thymopoiesis, as the proportion of BrdU<sup>+</sup> thymocytes improves after castration whilst the number of Annexin V<sup>+</sup> thymocytes decreases and the quantity of recent thymic emigrants [RTE] exported to the periphery is augmented [29]. Additionally, the thymic stroma has been identified as the target of androgen-induced regression [31]. Given what we know about the role of the thymic stroma in maintenance and sustainability of ETP and that castration reorganises the thymic microenvironment to resemble young thymi, this further supports the theory that the thymus itself is the major regulator of age-associated thymic atrophy. However, the effects of castration appear to affect the thymic microenvironment transiently with positive effects lasting only 20 week post sex hormone removal [32]. Furthermore, the often held view that age-associated thymic involution is

initiated, in part, with the onset of puberty is now being questioned as there is increasing evidence to suggest that this process is initiated significantly earlier in life [32-34].

It is now well established that the thymic microenvironment undergoes a series of morphological, phenotypical and architectural alteration with age. These include a downregulation of thymic epithelial markers, including general markers such as keratin and MHC class II [33, 35] and characteristic markers that are restricted to distinct subpopulations of TEC, including cortex and medulla [23, 36, 37, 38, 39]. This appears to be due to both a qualitative and quantitative loss of TEC. Using flow cytometric analysis Gray and colleagues noted the expansion of a population of MHC class II<sup>lo</sup> TEC with age in the mouse thymus, which were also smaller than MHC class II<sup>hi</sup> TEC [30]. MHC class II TEC are responsible for production of IL-7 [40], which has also been implicated to be involved in thymic atrophy [17]. There appears to be an age-dependent decline in production of IL-7 [39] which is a critical cytokine for several stages of thymopoiesis, promoting survival, proliferation and development. Administration of older mice with exogenous IL-7 increases thymic weight and cellularity [41] and is accompanied by an increase in thymic egression [42]. This therapy scaled-up to treat rhesus macaques, demonstrates an increase in TREC levels and they have improved immune responses to influenza vaccination [43]. Therefore, the decline in IL-7 described may be due to a loss in MHC class II<sup>+</sup> TEC, which further exacerbates the symptoms of thymic involution and may be treated, in part, with additional IL-7. Further evidence to suggest the essential role of TEC in thymic involution comes from some very elegant experiments that demonstrate Foxn1 is required for the maintenance in the postnatal thymus [44]. Creating a mouse, where Foxn1 is expressed throughout fetal stages but progressively declines postnatally, it was observed that the thymus undergoes alterations that mimic age-associated thymic involution. This includes cell autonomous changes in thymic architecture and a significant decline in thymic cellularity. Other studies have shown that there is an age-related decline in FOXN1 expression [39] therefore, although it does not specifically prove downregulation of Foxn1 is the causal mechanism of thymic involution, it does suggest the possibility that it contributes to age-related regression and thus again reiterates a pivotal role of TEC in thymic atrophy. Indeed, treatments actually focused on TEC have been shown to have therapeutic effects in rejuvenation of the thymus. Administration of keratin growth factor [KGF], required for TEC proliferation, enhances thymic cellularity considerably in 15 month old

mice, to the extent of equivalent thymic cellularity in untreated six week old mice, with a single course of KGF treatment eliciting effects up to two months after supplementation [45]. In conjunction, the immune function of KGF-treated mice, judged by antigen-specific antibody production induced by keyhole limpet haemocyanin, is improved in older mice. Concurrently, there is a restoration of the TEC organisation in older mice treated with KGF, which is accompanied by an increase in intrathymic expression of IL-7 and EVA.

Undoubtedly, the process of thymic involution is a complex one, involving the interplay of many mechanisms, yet there is always a common factor and it is increasingly clear that the thymic stroma, and in particular TEC, are an essential regulator of age-related thymic atrophy. The series of morphological, phenotypical and architectural changes in TEC may have detrimental affect; including reducing available niches for thymocyte colonisation and consequently thymic cellularity and altering T cell development. Considering various investigations reporting regeneration of the thymus involve either supplementing factors provided by TEC, such as IL-7 [17, 42] or the therapies themselves are capable of restoring the thymic microenvironment to thymic architecture observed in younger mice [33, 34, 45], it suggests that TEC are regulators of thymic atrophy. Therefore, any potential therapies to rejuvenate the thymus in the elderly should focus on regenerating the thymic microenvironment.

#### **Does the quality of recent thymic emigrants change with age?**

Despite preconceived notions that the thymus became redundant with age, various groups have shown that the thymus is still active late in life, generating new T cells albeit at a lower frequency [46-49]. Nevertheless, this has led to some presumptions of its own, including that the recent thymic emigrants [RTE] exported to the periphery in older individuals are phenotypically and functionally akin to those produced in younger subjects. However, investigations are now beginning to question this and provide evidence to the contrary, with several studies exposing age-associated deficiencies in RTE with age. Specifically, RTE in aged mice undergo phenotypic maturation with delayed kinetics compared to younger mice, accompanied by lower production of IL-2, decrease in proliferative capacity and weak expression of early activation markers [47]. Additionally, CD4<sup>+</sup> RTE have been showed to be defective in their ability to increase intracellular calcium concentration following TCR crosslinking with age, secreting less IL-2 and proliferative less compared to CD4<sup>+</sup> RTE populations

from younger mice [47]; whilst such cells from aged mice exhibit reduced helper and memory activity [50, 51].

Arguably, these changes could be the consequence of the exposure of RTE to the ageing peripheral environment, however we and others have observed that although crude measures of thymocyte development is not drastically altered in the elderly, there are age-associated changes in thymopoiesis [52]. For instance, there is a reduction in the percentage of CD3<sup>+</sup> thymocytes in ageing mice [53, 54] and human [55], which appears to be associated with a decline in the amount of CD3 complexes per cell (mean fluorescence index) [54]. Given the important role of CD3 in transducing TCR signalling, a decrease in the level of CD3 molecules is likely to affect the ability of T cells to respond to TCR-mediated stimulation and potentially impair thymopoiesis [56]. Indeed, stimulation through the TCR using Concanavalin A and IL-2 revealed a decline in the ability of thymocytes from older mice to proliferate [54, 57, 58], which may be a failure of such cells to enter the G2/M phase of the cell cycle [54]. These studies imply that the aged RTE may have acquired these defects due to age-associated changes in thymocyte development. Indeed, aged peripheral T cells from either human or mice have been showed that exhibit increase in apoptosis [59, 60] and we and others have observed that thymocytes from older animals are resistant to apoptosis [54, 58, 61].

Thus it could be argued that thymocyte development in the aged may not be functional and that the age-associated defects observed in RTE could arise from intrinsic defects imprinted on the developing T cells in the thymus. Moreover, given the importance of the thymic microenvironment in thymopoiesis, such alterations in thymocyte development is likely to occur due to the age-associated deterioration of the thymus environment. Although maintenance of the peripheral T cell pool is predominately via homeostatic proliferation and the number of newly generated T cells entering this population is small, there is still the potential for these cells to contribute to the age-related changes observed in T cells from the elderly. RTE are excluded from the niche-based regulation of peripheral T cell numbers [62] and are preferentially selected for survival in the periphery over existing resident T cells [63], therefore the thymus is able to influence the T cell pool throughout adult life with considerable control over the composition of the peripheral T cell pool repertoire.

### Why does the thymus involute?

Examination of the thymus in many vertebrates including avian, amphibian and teleost reveals that it

undergoes age-associated involution, indicating that this process is an evolutionary conserved event which appears to occur early in life [64]. Due to improvements in sanitation, food availability and medical care the average lifespan has been extended drastically over the last couple of centuries. Yet healthy life expectancy has not risen in conjunction as in the absence of any long-term evolutionary pressure the immune system that was designed to function for approximately 40 years has to continue for an additional four decades. Thus the consequence of thymic involution together with inflamm-ageing [65] which manifest as immunosenescence is highly apparent in the elderly [64].

Of all the evolutionary hypotheses of ageing, the disposable soma theory, based on the allocation of resources by an organism between maintenance and repair and other functions to maximise Darwinian fitness appears to be the most compatible with regression of the thymus [66]. Energy is a scarce commodity required for somatic maintenance and repair, which continuously competes against various other functions including thermogenesis, locomotion, growth and reproduction for this resource. The theory postulates that the primary cause of ageing is the accumulation of cellular and molecular damage, because of limitations in somatic maintenance and repair function [67]. Regression of the thymus occurs much earlier than most acknowledged features of ageing [28] and this is thought to be, in part, because it is an energy expensive organ [9]. The investment to produce a single functional, self-MHC restricted T cell is great especially considering the extensive proliferation entailed and that the majority of developing thymocytes are eliminated as they fail to conform to the criteria of selection. Furthermore, the contribution of the thymus to the overall survival of the individual is not necessary once a T cell repertoire is established, highlighted by the fact that thymectomising adults does not compromise their lifespan, whereas those thymectomised at birth die prematurely [1].

Therefore, it may be of benefit to the individual to forgo somatic maintenance of the thymus and downregulate thymopoiesis in order to devote energy to other activities. Indeed, there are a number of scenarios where energy is at a premium that the thymus does undergo atrophy, known as acute thymic involution, where the phenomenon is reversible. During pregnancy the thymus shrinks in weight and cellularity whilst these changes may be related to the need to prevent fetal immune rejection it may also be in concordance to energy conservation [68, 69]. Moreover, the thymus has been designated the barometer of malnutrition as the thymus displays significant reduction (acute involution) following starvation [70]. Interestingly administration of

exogenous leptin, which plays a key role in energy expenditure, is able to prevent starvation-induced thymic alterations [70]. Thus, it would appear that in situation where resources are limited the thymus is highly susceptible to modulation. Indeed, there is evidence to suggest that the immune system, in particularly the thymus is susceptible to developmental programming [71]. Such that a reduction of thymic mass, is observed, in offspring feed suboptimal nutrition during fetal and early postnatal life [72, 73]; which may result in immune dysfunction in later life.

Ageing and cancer have been described as different sides of the same coin, with senescence being described as a method to prevent tumour formation; acting as a stress-induced barrier that limits the proliferative potential of damaged cells [74]. Thus, given the high turnover of cells within the thymus, it is tempting to speculate an advantage of thymic involution could be to reduce the prevalence of thymic tumors. In support of such hypothesis, recent study deleting p16ink4a in the T lineage ameliorated several ageing phenotypes, including a significant slowing in the rate of thymic involution [75].

## Conclusion

Of all the changes of the ageing immune system, regression of the thymus involution is the most of the most dramatic, ubiquitous and recognisable; which appears to be an evolutionary conserved process. Yet despite age-associated thymic involution being recognised for over 70 years, little is known about the mechanisms that contribute to this event. While, the defects of developing thymocytes have been explored, an often overlook candidate is the TEC. Indeed, a picture is now emerging that the changes in the thymic microenvironment contributes towards thymic involution and that thymocytes leaving from the aged thymus may be defective; thereby questioning whether the thymus is able to produce functional T cells even in the older individual. Moreover, data is now emerging that TEC offers a potential target for rejuvenation.

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