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Changes in Primary Lymphoid Organs With Aging

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Abstract

Aging is associated with decreased immune function that leads to increased morbidity and mortality in the elderly. Immune senescence is accompanied by age-related changes in two primary lymphoid organs, bone marrow and thymus, that result in decreased production and function of B and T lymphocytes. In bone marrow, hematopoietic stem cells exhibit reduced self-renewal potential, increased skewing toward myelopoiesis, and decreased production of lymphocytes with aging. These functional sequelae of aging are caused in part by increased oxidative stress, inflammation, adipocyte differentiation, and disruption of hypoxic osteoblastic niches. In thymus, aging is associated with tissue involution, exhibited by a disorganization of the thymic epithelial cell architecture and increased adiposity. This dysregulation correlates with a loss of stroma-thymocyte 'cross-talk', resulting in decreased export of naïve T cells. Mounting evidence argues that with aging, thymic inflammation, systemic stress, local Foxn1 and keratinocyte growth factor expression, and sex steroid levels play critical roles in actively driving thymic involution and overall adaptive immune senescence across the lifespan. With a better understanding of the complex mechanisms and pathways that mediate bone marrow and thymus involution with aging, potential increases for the development of safe and effective interventions to prevent or restore loss of immune function with aging.

Keywords

Aging; Bone Marrow; Lymphopoiesis; Stroma; Thymopoiesis; Thymus

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1. Introduction

Aging-associated immune deficiency represents a key component of the overall pathophysiological effects of aging and has multiple well-documented impacts on human health and quality of life. Hallmarks of aging with respect to immune function include enhanced susceptibility to infection, poor responses to vaccination, and increased autoimmunity, all of which increase morbidity and mortality in the elderly. Even in middle-aged individuals, attenuated immune function contributes to reduced ability to combat infectious diseases such as influenza. Immune deficiency is exacerbated by conditions such as cancer and chronic viral infections. Decreased immune function commonly occurs after chemotherapy, radiation, or bone marrow/stem cell transplantation, and is compounded by age-related immune senescence.

Multiple physiological changes at the cell, organ, and system level are associated with aging. Increasingly, individual genes are being identified with age-related changes in expression or function that influence the whole organism by impacting general cellular physiology (*e.g.,* mitochondrial function, oxidative stress, telomere length) or individual organs or tissues. Aging, like any other complex system, represents an integration of multiple inputs with downstream influences on a variety of levels; interplay between tissue-specific compartments and residents, systemic mediators, and cell-intrinsic regulators. Careful investigation into these mechanisms may provide an opportunity to identify novel avenues for therapeutic approaches to delay or reduce the effects of aging.

Bone marrow and thymus represent the two critical primary lymphoid organs negatively impacted by aging. Bone marrow serves as a reservoir for hematopoietic stem cells (HSCs) and is a critical site for sustained production of common lymphoid progenitor cells (CLPs). Whereas the thymus provides the main venue for development and education of T cell progenitors, bone marrow serves as the primary site for development of B cells. These complex tissues play a crucial role in providing cellular components of the immune system across the lifespan; however, they are highly susceptible to the combined impacts of aging. While the effect of aging on thymocyte precursors has already been addressed in this issue [1], here we present a discussion of the changes observed in bone marrow and thymus associated with aging and more specifically detail a number of the key pathways and processes dysregulated in these primary lymphoid organs that lead to immune deficiencies with aging.

2. Bone Marrow

Bone marrow can be divided generally into two compartments: the HSC compartment, which includes HSCs and the products of their differentiation; and the stromal compartment, which includes mesenchymal stem cells and the stromal products of their differentiation, such as osteoblasts and adipocytes. Changes in each of these compartments are known to contribute to senescence of lymphocyte production with aging (Figure 1).

2.1 Effects of aging on the bone marrow HSC compartment

Aging has been demonstrated to have several important effects on the reservoir of HSCs needed to sustain lymphopoiesis in bone marrow. In mice, quantities of HSCs within bone marrow seem to paradoxically increase with aging [2–6]. The degree of this increase differs depending upon the strain of mouse studied, placing an important role for genetics in the effect of aging on long-term HSC activity [7, 8]. As an example, de Haan and colleagues observed that the frequency and numbers of HSCs, which were defined as day 35 “cobblestone area forming cells,” in bone marrow increase in C3H/He, CBA/J, DBA/2, BALB/c, and C57Bl/6 mice with aging. Aged DBA/2 mice possess a distinctly higher

frequency of HSCs in bone marrow compared to the other four strains [2]. In humans, genetic differences are even further accentuated, with studies reporting either decreased numbers [9], increased numbers [10], or no change in numbers [11] of HSCs in bone marrow with aging. Increased quantities of HSCs in mice have been attributed to greater HSC proliferative activity with aging: in 23 month-old mice, 12.5% of HSCs were observed to be in $S/G_2/M$ phases of the cell cycle, whereas younger mice, up to 15 months of age, had only 2.6 – 3.9% of HSCs with the same proliferative markers [12]. Increased proliferative activity may be detrimental to the ability of HSCs to sustain functions such as lymphopoiesis and self-renewal despite their greater numbers. For instance, although the number of HSCs increases in bone marrow of mice with aging [2–5, 12], they also show decreased regenerative capacity in terms of efficiency for repopulating bone marrow of lethally irradiated recipients [4, 6, 12]. This decline in regenerative function for HSCs with aging despite increased quantities does not appear to be mouse strain-dependent [13]. Importantly, reduced self-renewal potential has also been demonstrated. For example, bone marrow harvested from recipients of 20 – 24 month-old HSCs provides impaired lymphopoietic and erythropoietic reconstitution of secondary irradiated recipients compared to bone marrow taken from recipients of 2 – 3 month old HSCs [14]. Thus, HSCs exhibit diminished functional quality with aging, which may correlate with their increased tendency to proliferate.

Such changes in bone marrow HSC quality contribute to decreased lymphopoiesis with aging. It was initially noted that in C57Bl/6 mice, HSCs from aged bone marrow outcompete HSCs from young bone marrow when both types of HSCs are co-transplanted into lethally irradiated recipients [14, 15]. Interestingly, this phenomenon is not observed in CBA, DBA/2, or BALB/c mice [14, 15]. The unexpected competitive advantage of aged HSCs over young HSCs led to further studies, which showed that aged bone marrow produces significantly greater numbers of myeloid cells than young bone marrow in competitive engraftment assays [12]. In a key study, the ability of aged HSCs to outcompete young HSCs was found to reflect increased engraftment of self-renewing myeloid-skewed, “lymphoid defective” HSCs [5]. A six-fold increase in such lymphoid defective HSCs was also observed between 2 and 18 months of age in non-transplanted mice, accounting for over 40% of the paradoxical increase in HSC quantity over time [5]. These findings indicate that expanded populations of aged HSCs consist of a large number of HSCs that are in fact intrinsically skewed toward myelopoiesis and thus not truly uncommitted progenitors.

This concept of myeloid skewing of bone marrow HSCs plays a significant role in lymphoid senescence with aging. Myeloid skewing has been validated using microarray analyses, which show upregulation of myeloid genes and downregulation of lymphoid genes in bone marrow HSCs taken from 22 – 24 month-old mice compared to genes expressed in younger bone marrow HSCs at 2 – 3 months of age [3]. In mice, myeloid skewing of HSCs with aging results in decreased bone marrow production of CLPs, pre-pro-B cells, pro-B cells, pre-B cells, and total B cells [3, 16–19]. The question of whether the production of IgM^{hi} immature B cells and B-1 cells changes with aging remains controversial [16, 20–22]. Decreased production of pre-pro- and pro-B cells in bone marrow with aging has been attributed to reduced CLP proliferation [19], whereas the decline in the numbers of pre-B cells has been associated with loss of recombination activating gene 2 (RAG2) expression and V(D)J recombinase activity [18]. In humans, aging has also been associated with decreased production of B cell-committed progenitors, accompanied by decreased overall lymphocytic cellularity, increased lymphocyte apoptosis, and percentages of B and T cells peaking in bone marrow at 50 to 59 years of age [10, 23]. Thus, myeloid skewing of bone marrow HSCs provides a key initial step toward a series of changes that result in diminished lymphocyte production with aging.

2.2 Oxidative stress is a key mechanism for intrinsic HSC dysfunction with aging

Loss of genomic longevity likely plays an important role in the failure of HSCs within bone marrow to maintain lymphopoiesis with aging. Decreased genomic longevity occurs because telomeres shorten in HSCs after every cell division, whether for differentiation or self-renewal. In telomerase-deficient mice, this loss of telomeric DNA over time with aging correlates with decreased HSC function [24, 25]. However, shortening of telomeres cannot be the only factor contributing to declining HSC function in bone marrow because overexpression of telomerase does not extend HSC function [26].

Oxidative stress, resulting in production of reactive oxygen species (ROS), must be considered as an additional cause of bone marrow HSC dysfunction with aging. ROS-mediated DNA damage is associated with loss of genomic integrity and is exacerbated by the fact that DNA damage repair gene expression decreases in bone marrow HSCs with aging [6]. In mice, increased ROS levels in HSCs have been associated with decreased HSC survival and function [27–29], which can be rescued [28, 29] or delayed [30] by antioxidant treatment. In a key series of experiments, Ito and colleagues used $Atm^{-/-}$ mice predisposed toward accumulating DNA damage in bone marrow HSCs over time to examine the effect of ROS on HSC function. Aged HSCs from these animals demonstrated a markedly impaired ability to sustain hematopoiesis through 16 weeks post-transplantation into lethally irradiated recipients. The HSCs also exhibited decreased capacity for self-renewal. Overexpression of telomerase in these cells did not rescue their function. When intracellular levels of ROS were measured, they were significantly higher in $Atm^{-/-}$ HSCs than in wild-type HSCs. Treatment with N-acetylcysteine or catalase decreased intracellular ROS levels in HSCs and restored hematopoietic reconstitutive capacity of the cells [28], thus emphasizing the additional impact of ROS on HSC functional decline with aging.

Age-related changes in ROS activity within bone marrow have been evaluated extensively. Bone marrow HSCs with low ROS activity exhibit normal lymphoid production, ability to maintain quiescence, and self-renewal potential [27]. Meanwhile, bone marrow HSCs with high ROS activity increase in percentage with age and show myeloid-skewed differentiation and decreased self-renewal [27]. Aging is associated with a 25% decrease in HSCs within bone marrow that have low ROS activity [27]. Levels of ROS likely increase in bone marrow in part because production of antioxidants, such as glutathione [31] and peroxiredoxin 2 [32], by bone marrow stromal cells and their precursors declines with aging. In humans, ROS-induced DNA damage also accumulates in bone marrow HSCs with aging, associated with loss of HSC regenerative capacity and self-renewal, HSC dysfunction, and cell cycle arrest [33]. It is important to note that although antioxidant supplementation does not appear to decrease mortality in clinical trials [34], specific effects of antioxidants on human bone marrow HSC ROS activity and senescence have not been assessed. Reversal of aging-related increases in ROS activity within bone marrow HSCs using exogenous glutathione in human subjects has likewise remained difficult to test because the peptide is not imported into cells in a simple manner [35, 36], and indiscriminate elevations of glutathione levels may favor resistance of malignant cells to apoptosis [37]. Thus, the available data together suggest that increased oxidative stress contributes significantly toward senescence of lymphoid production from bone marrow HSCs with aging.

2.3 Decreased support for lymphopoiesis by the bone marrow stromal compartment with aging

Aging produces changes in bone marrow stroma that result in loss of support for lymphopoiesis. One study, in which 2 – 3 month-old bone marrow HSCs were transplanted into 2 – 3 month-old and 22 – 24 month-old lethally irradiated recipients, suggested that aging of the recipient bone marrow microenvironment does not produce long-term adverse

effects in B cell lymphopoiesis [3]. This assertion, however, was based upon measurements of circulating mature B cells over time, which could simply reflect homeostatic proliferation, and were not based upon assessments of B cell precursors within bone marrow itself. When bone marrow B cell precursor populations are examined, reduced B cell lymphopoiesis in response to aging of bone marrow stroma becomes evident. For example, it has been shown that the presence of older bone marrow stroma correlates with decreased production of pre-B cells from pro-B cells, decreased expression of RAG2 in pro-B cells, and decreased V(D)J recombinase activity in pro-B cells [18]. These changes occur independent of HSC age. In fact, aging results in decreased stromal support for overall HSC regenerative capacity, as observed by poor peripheral leukocyte reconstitution in lethally irradiated recipients of age-matched bone marrow HSCs [13]. It is well-accepted that changes in bone marrow stroma with aging negatively impact sustained lymphopoiesis. For example, in mice a 52% decrease in ability of bone marrow stromal cells to support B cell lymphopoiesis with aging is associated with reduced stromal IL-7 production [38]. The identical mechanism may not apply to humans, as IL-7 appears to be dispensable for B cell development in patients with defects in IL-7 signaling [39].

2.4 Aging-induced failure of bone marrow stromal cells to maintain HSC niches favorable for sustained lymphopoiesis

In bone marrow, mesenchymal stem cells (MSCs) have the ability to differentiate into osteoblasts or adipocytes [40]. Osteoblasts are stromal cells that play a central role in maintaining bone marrow lymphopoeisis. HSCs have been demonstrated to be enriched within the osteoblastic niches of bone marrow [41]. In a central study, Visnjic and colleagues reported the results of conditional ablation of osteoblasts using a transgenic mouse model. They found that loss of osteoblasts produces increased myelopoiesis, severely decreased lymphopoiesis with greater than 30% reduction in bone marrow B cells, and decreased numbers of HSCs in bone marrow [42]. In addition, increased osteoblastic activity correlates with increased numbers of bone marrow HSCs in a Notch-dependent manner [43]. On the other hand, adipocytes are stromal cells that inhibit the preservation and activity of bone marrow HSCs. Naveiras and colleagues observed a 2 – 3-fold reduction in HSCs within adipocyte-rich bone marrow compared to non-adipocytic rich bone marrow. Using both lipoatrophic A-ZIP/F1 mice and inhibition of adipocyte formation in wild-type mice, they further demonstrated significantly enhanced HSC hematopoietic reconstitution in lethally irradiated recipients that lack bone marrow adipocytes [44].

Changes in the bone marrow stromal compartment play a critical role in declining HSC function with aging. Aging is generally associated with increased production of poorly characterized factors in bone marrow that promote growth and not loss of stroma [45]. Thus, abnormal, not deficient, differentiation of MSCs in producing osteoblast-rich niches needed to maintain HSC function may be central to age-related loss of stromal support for lymphopoeisis [46]. Aging produces an altered balance of osteoblast and adipocyte differentiation from MSCs, resulting in increased bone marrow adipogenesis [9] and diminished osteoblastic proliferative capacity, function, and number [47, 48]. As a result of these changes, HSCs are removed from osteoblastic niches and show decreased adhesion to stroma [49]. Thus, failure to preserve and maintain the niches necessary for preserving HSCs and their capacity for lymphopoiesis contributes significantly toward senescence of bone marrow lymphoid function with aging.

Mechanisms for altered bone marrow osteoblast/adipocyte balance with aging have not been fully elucidated, but some critical pathways have been defined. Peroxisome proliferator-activated receptor gamma 2 (PPARγ2) serves as the “master regulator” of osteoblast/adipocyte balance in stromal cell differentiation from MSCs: activation of PPARγ2 results in upregulation of adipogenesis-associated genes and downregulation of osteoblastic genes

[50]. In a key study, 20 – 26 month-old and 6 – 8 month-old bone marrow cells were treated with a PPARγ agonist. Aged bone marrow produced a significantly greater number of adipocytes than younger marrow, demonstrating increased sensitivity of older bone marrow cells to the PPARγ agonist. Quantitative real-time RT-PCR analyses further revealed a 10-fold increase in PPARγ2 mRNA expression in older bone marrow compared to younger bone marrow cells [51]. It remains unclear why PPARγ2 expression rises with aging, although age-related elevations in PPARγ activity have been associated with increased oxidative stress that produces decreased binding of steroid receptor coactivator-1 (SRC-1) to PPARγ [52]. Results of recent microarray analyses comparing gene expression in aged versus young bone marrow MSCs [31] and adipocytes [53] will hopefully shed light on additional factors that contribute to dysregulation of the bone marrow osteoblast/adipocyte balance with aging. Finally, although most bone marrow adipocytes arise from MSCs, bone marrow myeloid cells have also demonstrated the capacity to undergo adipogenesis with aging [54]. This observation implies that myeloid skewing in aged bone marrow could augment shifting of the osteoblast/adipocyte balance in favor of adipocytes. However, the degree to which this mechanism contributes to adipocyte formation *in vivo* has not been fully established.

2.5 Hypoxia: a critical factor for HSC longevity preserved by healthy bone marrow stromal niches

Bone marrow HSCs are optimized for survival in hypoxic conditions with increased expression of oxidative repair or protective proteins and decreased expression of motility proteins [55]. When cultured under hypoxic conditions (1% oxygen), they demonstrate a 7.6-fold increase in efficiency for repopulating bone marrow of lethally irradiated recipients compared to HSCs cultured in normoxia [56]. Hypoxia preserves HSC longevity by promoting their existence in the G_0 state [57].

Important mechanisms encourage the subsistence of bone marrow HSCs in hypoxia. Most obviously, hypoxia benefits HSCs by protecting them from ROS-mediated DNA damage, which allows them to maintain normal lymphoid production and self-renewal potential [27]. Proteomic analysis of bone marrow HSCs reveals that they express upregulated glycolytic pathway components, allowing them to produce energy for survival without using the ROS-generating process of oxidative phosphorylation [55]. More importantly however, hypoxia preserves long-term bone marrow HSC function and lymphopoiesis through its impact on hypoxia inducible factor-1 alpha (HIF-1α), which binds to hypoxia response elements in promoter regions of DNA to direct transcription [58]. In a fundamental series of experiments, Takubo and colleagues demonstrated the critical role of hypoxia-induced HIF-1α activity in HSCs. They first compared bone marrow HSCs cultured in 1% oxygen and 20% oxygen and demonstrated increased expression and stability of HIF-1α in hypoxic HSCs. Conditional deletion of HIF-1α was then used to demonstrate that it is required in bone marrow HSCs to maintain the cells in G_0, upregulate glucose transporter 1 and pyruvate dehydrogenase for glycolysis, and attenuate ROS production. Aged HIF-1α deficient animals exhibit accelerated loss of bone marrow HSCs. Exhaustion of bone marrow HSCs occurs as a result of loss of HIF-1α mediated suppression of $p16^{Ink4a}$ and $p19^{Arf}$ [59]. HIF-1α also upregulates stem cell telomerase expression and activity [60] and interacts with Notch, resulting in activation of Notch-responsive genes [61]. Finally, hypoxia plays an important role in maintaining HSC function through its ability to induce vascular endothelial growth factor alpha (VEGFα in a manner that does not seem to require HIF-1α [59]. VEGFα appears to be important for maintaining the regenerative capacity, but not necessarily quantity, of bone marrow HSCs [62].

In addition to its effects on bone marrow HSCs, hypoxia enhances the ability of stromal cells to support long-term bone marrow HSC function. Protection against ROS activity may

play an important role: increased ROS and lipoxygenase levels in stromal progenitors with aging are known to result in increased lipid oxidation, leading to decreased *wnt* suppression of PPARγ, decreased binding of SRC-1 to PPARγ, and reduced numbers of osteoblasts [52, 63]. Rat bone marrow MSCs cultured in low oxygen conditions (5% oxygen) demonstrate increased osteoblastic activity [64]. Hypoxia-induced VEGFα expression supports osteoblastic function [62]. It has been suggested that human bone marrow MSCs shift away from adipogenesis toward osteoblast differentiation under hypoxic conditions [65], although this finding is not consistently observed [66]. As in bone marrow HSCs, hypoxia results in upregulation of HIF-1α in bone marrow MSCs, associated with increased expression of telomerase [66, 67].

Not surprisingly, healthy bone marrow provides hypoxic microenvironments favorable for HSC longevity [59]. While room air at sea level contains oxygen at a concentration of approximating 21%, it has long been known that bone marrow is desaturated and contains oxygen concentrations ranging from 1% – 7% [66, 68, 69]. In pioneering work, Parmar and colleagues injected mice with pimonidazole, which localizes to hypoxia. They then isolated bone marrow cells and used intracellular anti-pimonidazole antibody staining to demonstrate that HSCs are distributed within the most hypoxic areas of bone marrow. They supported this observation using tirapazamine, which is toxic to cells under hypoxic conditions but which is neutralized by oxygen. Treatment of mice with tirapamazine resulted in 90% – 95% loss of bone marrow HSCs [70]. Subsequent work has confirmed that these near-anoxic regions of bone marrow in which HSCs are sustained and enriched correspond to the same endosteal niches in which osteoblasts reside, localized away from vascular flow [71]. These findings suggest that a key role for osteoblasts in supporting lymphopoiesis involves maintaining hypoxic niches within the bone marrow that are favorable for preventing senescence of HSC lymphoid production. Loss of osteoblastic niches with aging thus has a direct impact on bone marrow HSCs through disruption of these favorable hypoxic microenvironments (Figure 1).

2.6 Role of inflammation in reduced bone marrow lymphopoiesis with aging

Increased inflammation or repetitive inflammatory insults with aging may contribute to decreased bone marrow production of lymphoid cells, although definitive studies remain lacking. Nakamura, Maeda, and colleagues demonstrated using Src homology-2-containing inositol 5′-phosphatase (SHIP)-deficient mice that myeloid skewing of HSCs with aging requires signaling through SHIP that can occur in response to IL-6. IL-6, in turn, is produced by the myeloid products of HSC differentiation themselves [72, 73]. This positive feedback cycle could amplify myeloid skewing over time. Elevated ROS activity in the bone marrow with aging has been demonstrated to have the ability to induce local IL-6 production [74]. Finally, systemic IL-6 levels increase with aging [75], which may further enhance myeloid skewing. Increased IL-6 levels with aging may also indirectly decrease bone marrow HSC function by somehow affecting bone marrow osteoblast/adipocyte balance. Significantly higher levels of IL-6 have been found in bone marrow of osteoporotic patients, correlating with reduced osteoblastic activity, compared to non-osteoporotic patients [76]. This observation requires further evaluation, as a later report did not find an association between increased bone marrow adipogenesis and IL-6 levels with aging. In this report, however, bone marrow IL-6 concentrations were not assessed [9].

2.7 Bone marrow lymphoid senescence and future research considerations

Despite an increased understanding of changes in bone marrow with aging that contribute to its senescence as a lymphoid organ, many questions remain unanswered. For example, it is not completely known why the hypoxic bone marrow niches favorable for sustained lymphopoiesis change or why aged HSCs appear to lose adhesion to or localize away from

these niches. Recent studies have reported that P-selectin expression increases in HSCs with aging [6, 77]. Because P-selectin is highly involved in cellular adhesion and migration, this finding may shed some light on mechanisms behind the changes in HSC distribution with aging. Further definition of HIF-1α-mediated and non-HIF-1α-mediated signaling pathways needed for maintaining long-term HSC survival and function in hypoxic conditions would help identify additional molecular processes that contribute to senescence with aging and potential therapeutic intervention. Finally, genetic, epigenetic [6], and environmental [19] variables are known to modulate age-induced HSC senescence. However, the specific factors and mechanisms by which they together and individually affect the bone marrow have not yet been fully characterized. Infections, diet, and hormones could all be hypothesized to influence bone marrow senescence with aging. The role of growth hormone, in particular, remains poorly defined. Growth hormone reverses age-associated adipogenesis but increases myelopoiesis in the bone marrow of rats [78]. In mice, sustained overexpression of growth hormone (GH) and insulin-like growth factor-1 (IGF-1) is associated with exhaustion of bone marrow HSCs [79]. Thus, additional research is required to better understand the conditions that alter the progression of the bone marrow toward lymphoid senescence with aging.

3. Thymus

The thymus is the major source of self-restricted, self-tolerant naïve T cells required for robust adaptive immunity, demonstrated by the profoundly immune compromised status of athymic individuals. Lymphoid progenitor cells from bone marrow commit to the T cell lineage, differentiate into functional T cells within the thymus, and are then exported to the periphery, where they act to effect and control immune responses. The capacity of the thymus to produce T cells is determined by the structure and function of the thymic stroma, composed of thymic epithelial cells (TECs) and other non-lymphoid cells that provide a series of microenvironmental niches to promote distinct stages of T cell development and repertoire selection. The thymus develops during fetal life, reaches its maximal output during early postnatal life, and declines in size and output during young adulthood and throughout adult life through the process of age-related involution.

3.1 Changes in thymic stromal structure and function during the lifespan

The thymus originates from endodermal cells in the ventral third pharyngeal pouches during mid-gestation in mouse embryos; thus all TECs have a common endodermal origin [80]. Once organogenesis is initiated, a complex set of cellular interactions between TECs, surrounding mesenchyme, and immigrating lymphoid and endothelial progenitor cells results in the development of an intricate cellular environment [81]. The postnatal thymus consists of developing T cells, known as thymocytes, and the non-lymphoid thymic stromal elements that in aggregate comprise the microenvironments that promote the different stages of thymocyte differentiation. These elements include TECs, mesenchyme, endothelium, and non-lymphoid hematopoietic cells (*e.g.*, dendritic cells and macrophages). TECs represent the primary functional stromal cell type and are required for promoting most stages of thymocyte differentiation.

After birth, the thymus continues to develop and organize its compartmental structure while being periodically seeded with hematopoietic progenitors from bone marrow [82]. During the early postnatal period, it expands in size and increases the rate of output of naïve T cells, filling the ‘empty’ peripheral environment [83–85]. The postnatal thymus is organized into regions or compartments that contain different populations of TECs and thymocytes. The outer and inner compartments are termed the cortex and medulla, respectively, and the zone where they meet is known as the corticomedullary junction (CMJ) (Figure 2). These compartments are characterized by the presence of two main subsets of TECs that exhibit a

striking functional dichotomy. Cortical TECs (cTECs) are responsible for T lineage commitment, early thymocyte expansion and differentiation, and positive selection, while medullary TECs (mTECs) are required for proper induction of central tolerance and the final stages of thymocyte maturation prior to thymic egress. While initial development of the cTEC and mTEC lineages appears to occur autonomously, elaboration of properly expanded and organized cortical and medullary compartments depends upon the presence of developing thymocytes [86, 87]. This well-established, mutually inductive process is termed "cross-talk" [88–90] and contributes to the regulation of thymus organogenesis and homeostasis.

3.2 The role of thymic involution in immunosenescence

Loss of T cell production from thymus comprises a significant component of immune senescence. At some point, although the exact timing of onset remains controversial and may be species-specific, the thymus enters a period of decline, resulting in stereotypic degeneration of the organ, termed "thymic involution" [91, 92]. Aging is associated with gradual disorganization of thymic compartments, changes in TEC subset ratios, and reduced T cell production. While the mechanisms underlying these processes remain poorly characterized, the final result is clearly deteriorated thymus structure with severely reduced output of naïve T cells.

Thymic involution results in age-related T cell abnormalities that contribute to reduced immune system function. The primary effect on peripheral T cells is a decline in frequency and function of naïve T cells, leading to a restricted T cell repertoire and decreased immune responses to novel challenges. Increased representation of memory T cells occurs as a consequence of aging. Diminished capacity of T cells to proliferate and produce cytokines in older individuals is thought to result from defects accumulated after prolonged T cell residence in the periphery [93]. The reduced number and repertoire of naïve T cells in the peripheral pool occurs largely due to diminished output from aged thymus [94].

3.3 Changes in thymic stromal structure and function associated with thymic senescence

During thymic involution, the TEC component of the thymic stroma is not maintained. Age-related defects in the thymus epithelial compartment include a reduction in the number of TECs with age, skewing of the cTEC:mTEC ratio, and a decline in the most differentiated MHC class II^{high} TEC subsets [95, 96]. Changes in TEC subsets are associated with the appearance of a less complex medullary architecture [97] and progressive disorganization of the stromal architecture, as observed by blurring of the CMJ (Figure 2). These age-related changes are accompanied by replacement of thymocytes in perivascular spaces with adipose tissue and the appearance of areas devoid of epithelial cells [94, 98].

One of the more dramatic changes appears in the form of increased adipose tissue within the involuted thymus and is especially pronounced in humans. Recent data support a possible intrathymic origin for adipose cells [99, 100]. Since thymocyte numbers begin to decline prior to obvious increases in thymic fat, it seems unlikely that increased adiposity in the thymus alone causes aging-related thymic involution. However, evidence is mounting that adipose cells can produce an array of cytokines and other signaling molecules that directly affect thymopoiesis [101, 102]. Data also support a correlation between reduced adiposity and increased thymic function, such as during caloric restriction [103]. Obesity and increased intrathymic fat have also been associated with reduced thymic function [99, 104]. Thus, while increases in thymic adipocytes may not necessarily initiate involution, their increasing presence with age may accelerate or directly aggravate the impact of age on thymic function. Further research in this area may yield beneficial information relevant to both mechanisms of thymic involution and strategies to ameliorate it.

3.4 Loss of thymocyte-stromal cross-talk as a key mechanism for thymic senescence

'Cross-talk' between TEC and developing thymocytes is required for development of normal thymic architecture. Several key inductive interactions have been identified, including a requirement for receptor activator of nuclear factor kappa-B (NF-κb) ligand and CD40L signaling from thymocytes for the differentiation of specific mTEC subtypes and a need for NF-κb pathway signaling for medullary compartment formation and expansion [105]. It remains unclear whether the same mechanisms required for initial formation of a functional thymus play a role in its degeneration.

Thymic involution likely occurs as a consequence of both intrinsic defects in thymocyte progenitors and failure to maintain a functional TEC compartment. However, the interdependence of these cellular components significantly complicates analysis of their relative contributions to involution. Age-related decreases in the function of bone marrow HSCs and their ability to generate high-quality lymphoid progenitors have already been discussed. Recent studies have shown that within thymus the number of early T lineage precursors (ETPs) in the double-negative 1 subset declines with age [91, 106] and that purified ETPs from old donors have reduced activity in fetal thymus reconstitution assays [91], suggesting that intrinsic defects in thymocyte progenitors contribute to reduced thymopoiesis in old age [92, 107]. However, whether these defects exist intrinsic to thymocyte precursors or secondary to influences from their exposure to the aged thymic microenvironment before isolation remains undetermined. Reconstitution of aged thymuses with bone marrow precursors from young mice do not restore thymic compartmental structure or fully rescue deficient thymopoiesis, and reconstitution of young mice using aged bone marrow cells [108] or intrathymic injection of young ETPs into an aged thymus [109] do not impair thymopoiesis. Similarly, when fetal thymi are transplanted into aged mice, they are efficiently colonized by the aged bone marrow-derived cells and do not involute [109]. These data suggest that reduced ability of aged thymi to support thymopoiesis may come from direct or indirect age-related defects in thymic stroma.

Decreased availability of stromal epithelial niches for thymocyte progenitors in the involuted thymus or a deterioration in the quality of these niches could account in part for reduced naïve T cell output with aging. Evidence supporting this notion comes from several studies that demonstrate that increased TEC number produces a proportional increase in thymus size. Expression of cyclin D1 under a keratin 5 promoter in TECs results in a dramatic increase in thymus size and is sufficient to delay or prevent involution [110]. Similarly, expression of simian vacuolating virus 40 T antigen under control of the *Foxn1* promoter leads to markedly increased thymus size with normal stromal organization [111]. Genetic inactivation of the cell cycle inhibitor p27 (*p27^{KIP1}, Cdkn1b*) also results in excessive thymus size and increased output with normal stromal organization [112–114]. Conversely, limiting TEC number by either restricting the number of epithelial progenitors from which the thymus develops [115] or by suppressing the proliferative potential of the epithelial progenitor/stem compartment [116] results in thymic hypoplasia. Collectively, these studies suggest that niche availability serves as a key determinant for thymus output and that restriction in the number and/or function of thymic micronvironmental niches is a critical cellular and structural component of thymic involution. Nonetheless, while thymocyte numbers and phenotypes in these models of delayed or blocked involution have been studied, the absence of data regarding the effects of involution on thymic output and the functionality of the peripheral T cell pool remains problematic.

3.5 The roles of inflammation, stress, and cytokines in thymic involution and senescence

Age-related changes in thymic cytokine production are known to play a role in thymic senescence and involution. These changes may result from inflammation or stress. In a

critical investigation, human thymuses were studied from normal human subjects ranging from 3 days to 78 years of age [102]. mRNA analyses showed that aging is associated with increased expression of leukemia inhibitory factor (LIF), oncostatin M (OSM), stem cell factor (SCF), IL-6, and macrophage colony stimulating factor (M-CSF) by thymic adipocytes and TECs. Increased expression of LIF, SCF, IL-6, and M-CSF further correlates with decreased production of RTEs. In mice, administration of LIF, OSM, SCF, or IL-6 results in thymic involution [102]. The identification of three IL-6 family cytokines, LIF, OSM, and IL-6, associated with thymic involution suggests an active role for inflammation in thymic senescence with aging. Subsequent investigations showed that stress, applied by endotoxin administration, can also produce thymic involution mediated by LIF and multiple other pro-inflammatory cytokines through mechanisms that depend in part upon steroid activity [117, 118].

3.6 Stromal cell factors associated with thymic senescence and rebound

Evidence is building that the involuted thymus can be rejuvenated to some degree, even in very old individuals. Despite dramatic changes in appearance and function, even the very aged thymus retains some function: although the total number of recent thymic emigrants (RTEs) declines with age, output of RTEs remains relatively stable as a function of overall thymus size [102, 119], although it is not clear whether the generated T cells have equivalent function to those produced from a younger thymus. It has long been known that the thymus can be induced to regenerate by a variety of experimental interventions, a process termed “thymic rebound”. The persistent, albeit low level of thymic function and the ability to induce thymic rebound in aged animals further indicates that the involuted thymus retains the cellular capacity for regeneration. The ability to reverse thymic involution in experimental animal models has yielded valuable information about potential mechanisms of age-related thymic senescence.

Few genes have been definitively and specifically linked with directly regulating the development, maintenance and rebound of the thymus. One protein that appears to play a key role is the transcription factor Foxn1, the gene mutated in the classic functionally athymic mouse mutant, ‘nude’. Foxn1 is a forkhead class transcription factor that is required for TEC differentiation [111, 120–124]. Foxn1 has also been implicated in promoting proliferation [96, 125], raising the possibility that it functions to balance or coordinate differentiation and proliferation. Because of its central role in the fetal thymus, Foxn1 may be a key regulator of TEC maintenance and function in the postnatal thymus. The *Foxn1* gene is extensively expressed in both cTECs and mTECs in the postnatal thymus [96, 111, 121, 122], although its expression and protein levels are differentially regulated in various TEC subsets [96, 111, 126]. Reducing but not eliminating *Foxn1* gene expression soon after birth causes rapid premature thymic involution that is phenotypically similar to age-related involution [96]. Consistent with these results, downregulation of *Foxn1* occurs early in thymic involution [127], while transgenic overexpression of Foxn1 delays involution [128]. Foxn1 has been proposed to regulate fibroblast growth factor receptor 2 isoform IIIb (FGFR2IIIb) transcription [129], which is needed for proliferation and differentiation of TECs [130–133]. Mechanisms underlying changes in *Foxn1* expression in the postnatal thymus and how this process relates to other proposed agents of involution have not been determined. However, Foxn1 expression/function must clearly be considered as a prime downstream mediator of agents or pathways capable of inducing involution or rebound.

Keratinocyte growth factor (KGF) has been identified as another key factor in thymic senescence and rebound. KGF signaling through FGFR2IIIb is critical for fetal thymus organogenesis [132] and is linked to proliferation and differentiation in both fetal and postnatal TECs [130–133]. Administration of KGF increases thymus size, TEC differentiation, and thymocyte production in aged animals [131, 133–137]. Treatment with

KGF also protects the postnatal thymus from damage after irradiation [133, 136, 138, 139]. KGF has multiple targets outside the thymus, and the mechanisms by which it acts in the postnatal thymus and the functional quality of the thymus after KGF-induced rebound have not been fully investigated.

3.7 Sex steroids and regulation of thymic involution and rebound

Puberty presents one of the most commonly proposed physiological causes for thymic involution. This concept is based upon the idea that increased levels of steroid hormones associated with sexual maturity have negative effects on the thymus. Support comes from the observation that chemical or surgical castration, resulting in sex-steroid ablation (SSA) of male mice or rats, produces thymic rebound [140, 141]. The effect appears to be reversed by administration of testosterone or dihydrotestosterone [142], although the literature is not consistent about this effect [143]. Thymic rebound after SSA correlates with increased thymus size and thymocyte number, increased production of naïve T cells, and evidence of recovered CMJ distinction [92, 106]. Androgens and estrogens administered in high doses can also cause dramatic degeneration of the thymus, although it has not been demonstrated that this degeneration occurs by the same cellular or molecular basis as age-related involution.

Androgens work primarily by binding to the androgen receptor (AR), a member of the steroid hormone receptor superfamily. AR is an intracellular receptor that is sequestered in the cytoplasm by binding to heat shock protein 90. It translocates to the nucleus upon hormone binding to modulate transcription of genes through binding to well-characterized androgen response elements. The molecular complexes that AR recruits to target genes, including mediator components and chromatin remodeling complexes, and the mechanisms by which they influence gene expression have been extensively studied. Recent evidence has identified two additional pathways of androgen action: through intracellular conversion of testosterone to estrogen by aromatase and binding to promoters using the estrogen receptor (ER), or through a poorly characterized second messenger pathway independent of transcriptional regulation [144]. In support of the ER-based pathway, aromatase inhibitors have been reported to produce thymic rebound, although perhaps not as effectively as castration [145]. AR signaling affects many downstream targets and cellular functions. It has been shown to influence proliferation by interacting directly with both Rb and cyclin D1 [146, 147], and it regulates differentiation by direct transcriptional regulation of effector targets in multiple tissues.

The ability of androgen depletion or blockade to promote thymic rebound and the enlarged thymic phenotypes associated with mutation of the androgen or estrogen receptor supports the possibility that systemic hormonal changes associated with aging underlie at least some of the changes seen during thymic involution. AR has been shown to be expressed in TECs by both expression and functional analysis [142]. Consistent with SSA-induced rebound, deletion of the AR results in an enlarged thymus in adult mice and resistance to androgen-induced thymic atrophy [142]. Adoptive transfers with *Ar* null mutant bone marrow have shown that this function appears to reside at least partially in the thymic stroma [142], although the possibility that some effects of AR deletion may depend on AR activity in hematopoietic cells cannot be excluded. Furthermore, castration and other global changes in sex steroid production or receptor deletion have global and systemic physiological effects, including complex effects on other hormones such as estrogens, IGF-1, and GH, which have themselves been implicated in affecting thymus function [107, 148]. Thus, the mechanism of thymic rebound in this model may involve a complex interaction between multiple cell types and may have both direct and indirect effects on the thymus.

Challenges exist in translating results from the SSA model of hormone-induced involution to human age-related immunosenescence. First, a histologic and morphometric study of human thymic structure from different ages concluded that the thymus reaches its peak size and productivity soon after birth rather than at puberty, begins to decrease immediately afterwards with a gradual decrease in output through middle age, and continues to degenerate throughout life [149–151]. These results differ superficially from measures of thymus function in mice, which show that thymic function, as measured by thymocyte numbers, at least broadly corresponds to the age of sexual maturity [95, 96]. However, it should be noted that in rodents, weaning and sexual maturity occur very close together, while humans have a prolonged postnatal growth phase prior to puberty. Thus, the apparent difference between mice and humans in the timing of onset for involution may have more to do with life history differences than with differences in the mechanisms promoting involution. Second, the fact that serum testosterone levels drop significantly with aging in both rodents and humans [152–155] questions the effect of androgens on involution during natural aging. If increased androgens during puberty and adulthood play a role in suppressing thymus size and function, as castration-induced rebound suggests, then the natural decrease in androgen levels that occurs with aging might be expected to relieve that suppression to some degree. Yet thymus involution continues progressively, or at least does not reverse, throughout natural aging. Third and finally, a recent report has raised further issues concerning the degree that SSA-induced thymic rebound represents true “rejuvenation” of the thymus [97]. Griffith and colleagues performed a bioinformatics-based comparison of the stromal cell transcriptional profiles of the cortical and medullary regions of wild-type thymi across time before, during, and after castration. This analysis produced two key findings. First, castration-induced rebound exists transiently, and the rebounded thymus re-involutes within a few weeks after castration. Second, and more importantly, the gene expression profile of the maximally rebounded thymus seems indistinguishable from the profile of aged, rather than young, thymus. Most critically, expression of tissue-restricted antigens for negative selection and of Foxn1 did not recover to a ‘young’ profile. These results suggest that SSA may induce expansion of the thymus but does not significantly rejuvenate it, arguing that SSA-induced rebound does not generate a functionally ‘young’ microenvironment. Although SSA-induced rebound was first reported over 100 years ago [156], it remains unclear what relationship sex steroids have to the normal life cycle of the thymus or which cells in the thymus provide the most critical target for these effects during involution or induction of rebound.

3.8 Missing mechanisms in understanding involution and rebound

Eventual thymic involution with sustained Foxn1 expression, re-involution after castration, and failure of castration-induced rebound to generate sustained thymic rebound or to recapitulate the molecular characteristics of a young microenvironment all point to unidentified mechanisms of thymic senescence. Indeed, compared to other organs, very little is known about the molecular and cellular mechanisms controlling generation and maintenance of the thymic microenvironment or about the cellular composition of the microenvironment itself and the lineage relationships between different TEC subsets. For example, the field has relied almost exclusively on the role of a single TEC-specific transcription factor, Foxn1, to explain thymus development, homeostasis, and involution. Its pivotal role in fetal thymus development and in maintaining postnatal thymus homeostasis identifies Foxn1 as a linchpin of thymus biology, tying together development and maintenance of the thymic microenvironment across the lifespan, and it remains essential to understand the mechanisms by which it regulates the balance between proliferation and differentiation in TECs. However, while Foxn1 undeniably contributes to all of these processes, it is clearly not sufficient to drive all the important processes in the thymus. Two recent studies have shown that TEC-null mutants for Foxn1 demonstrate differential

expression of some early cTEC and mTEC markers, suggesting that some TEC differentiation occurs independently from Foxn1 [111, 157]. Another recent study has identified additional transcription factors that may be candidates for future analysis of both Foxn1-dependent and -independent processes in TECs across the lifespan [158].

4. Conclusions

High demand and clinical need exist for therapies to ameliorate immune deficiency caused by aging. Substantial preclinical and clinical approaches are being explored to enhance lymphopoiesis for the elderly in primary lymphoid tissues (*i.e.*, bone marrow and thymus). However, inadequate basic knowledge of cellular targets and molecular pathways that cause age-associated involution in these critical primary lymphoid organs creates a significant barrier to developing and implementing such strategies.

Upon reflection of specific changes observed in bone marrow and thymus with aging, common themes come to the fore. The deliberate and highly regulated subcompartments of primary lymphoid organs appear to fail with aging from both cell-intrinsic and extrinsic pressures. Increased tissue inflammation and obvious colonization of stromal tissue with adipocytes is noted with aging. With loss of protected and highly specialized functional niches for nurturing and providing essential cross-talk to developing lymphocytes, there is a breakdown in lymphopoiesis and subsequent profound immune deficiency with aging.

Better understanding of the biology underlying these changes should lead to the development of more specific and effective strategies for reversing or even preventing age-related primary lymphoid tissue involution, thus improving overall immune function in the elderly and aging populations. For example, determining the origin of adipocytes and the factors that control their development could be important for preventing or reversing the effects of involution. Therapies aimed at sustaining or restoring stromal architecture and function may be able to prevent or restore loss of lymphoid tissue function after involution, regardless of the primary cause. A desirable outcome would include the identification of specific cellular or molecular targets that can be exploited for tissue restoration with few or no side effects. Thus, regeneration of aged bone marrow or thymus or prevention of its deterioration remains a potentially productive avenue of research to induce significant improvements in immune status in aged individuals.

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Abbreviations

AR	androgen receptor
CLPs	common lymphoid progenitor cells
CMJ	corticomedullary junction
cTECs	cortical TECs
ER	estrogen receptor
ETPs	early T lineage precursors

FGFR2IIIb	fibroblast growth factor receptor 2 isoform IIIb
GH	growth hormone
HIF-1α	hypoxia inducible factor-1 alpha
HSCs	hematopoietic stem cells
IGF-1	insulin-like growth factor-1
KGF	keratinocyte growth factor
LIF	leukemia inhibitory factor
M-CSF	macrophage colony stimulating factor
mTECs	medullary TECs
MSCs	mesenchymal stem cells
NF-κb	nuclear factor kappa-B
OSM	oncostatin M
PPARγ2	peroxisome proliferator-activated receptor gamma 2
RAG2	recombination activating gene 2
ROS	reactive oxygen species
RTEs	recent thymic emigrants
SCF	stem cell factor
SHIP	Src homology-2-containing inositol 5′-phosphatase
SSA	sex-steroid ablation
SRC -1	steroid receptor coactivator-1
TECs	thymic epithelial cells
VEGFα	vascular endothelial growth factor alpha

References

1. Dorshkind K. Genetic Regulation of Thymocyte Progenitor Aging. Seminars in Immunology. 2012
2. de Haan G, Nijhof W, Van Zant G. Mouse Strain-Dependent Changes in Frequency and Proliferation of Hematopoietic Stem Cells During Aging: Correlation Between Lifespan and Cycling Activity. Blood. 1997; 89:1543–1550. [PubMed: 9057635]
3. Rossi DJ, Bryder D, Zahn JM, Ahlenius H, Sonu R, Wagers AJ, et al. Cell intrinsic alterations underlie hematopoietic stem cell aging. Proceedings of the National Academy of Sciences of the United States of America. 2005; 102:9194–9199. [PubMed: 15967997]
4. Hotta T, Hirabayashi N, Utsumi M, Murate T, Yamada H. Age-related changes in the function of hemopoietic stroma in mice. Experimental Hematology. 1980; 8:933–936. [PubMed: 16398026]
5. Sudo K, Ema H, Morita Y, Nakauchi H. Age-Associated Characteristics of Murine Hematopoietic Stem Cells. Journal of Experimental Medicine. 2000; 192:1273–1280. [PubMed: 11067876]
6. Chambers SM, Shaw CA, Gatza C, Fisk CJ, Donehower LA, Goodell MA. Aging Hematopoietic Stem Cells Decline in Function and Exhibit Epigenetic Dysregulation. PLoS Biol. 2007; 5:e201. [PubMed: 17676974]
7. Müller-Sieburg CE, Riblet R. Genetic control of the frequency of hematopoietic stem cells in mice: mapping of a candidate locus to chromosome 1. Journal of Experimental Medicine. 1996; 183:1141–1150. [PubMed: 8642256]

8. Phillips RL, Reinhart AJ, Van Zant G. Genetic control of murine hematopoietic stem cell pool sizes and cycling kinetics. Proceedings of the National Academy of Sciences. 1992; 89:11607–11611.
9. Tuljapurkar SR, McGuire TR, Brusnahan SK, Jackson JD, Garvin KL, Kessinger MA, et al. Changes in human bone marrow fat content associated with changes in hematopoietic stem cell numbers and cytokine levels with aging. Journal of Anatomy. 2011; 219:574–581. [PubMed: 21923862]
10. Kuranda K, Vargaftig J, de la Rochere P, Dosquet C, Charron D, Bardin F, et al. Age-related changes in human hematopoietic stem/progenitor cells. Aging Cell. 2011; 10:542–546. [PubMed: 21418508]
11. Povsic TJ, Zhou J, Adams SD, Bolognesi MP, Attarian DE, Peterson ED. Aging Is Not Associated With Bone Marrow–Resident Progenitor Cell Depletion. The Journals of Gerontology Series A: Biological Sciences and Medical Sciences. 2010; 65A:1042–1050.
12. Morrison SJ, Wandycz AM, Akashi K, Globerson A, Weissman IL. The aging of hematopoietic stem cells. Nat Med. 1996; 2:1011–1016. [PubMed: 8782459]
13. Sakuma A, Tsuboi I, Morimoto K, Horie T, Sugimoto K, Mori KJ. Age-related decreases in the reconstituting ability of hemopoietic cells and the ability of hemopoietic microenvironment to support hemopoietic reconstitution in senescence accelerated (SAM-P) mice. Mechanisms of Ageing and Development. 1994; 73:127–135. [PubMed: 8051954]
14. Chen J, Astle CM, Harrison DE. Genetic regulation of primitive hematopoietic stem cell senescence. Experimental Hematology. 2000; 28:442–450. [PubMed: 10781902]
15. Harrison DE. Long-term erythropoietic repopulating ability of old, young, and fetal stem cells. Journal of Experimental Medicine. 1983; 157:1496–1504. [PubMed: 6854204]
16. Johnson KM, Owen K, Witte PL. Aging and developmental transitions in the B cell lineage. International Immunology. 2002; 14:1313–1323. [PubMed: 12407022]
17. Miller JP, Allman D. The Decline in B Lymphopoiesis in Aged Mice Reflects Loss of Very Early B-Lineage Precursors. Journal of Immunology. 2003; 171:2326–2330.
18. Labrie JE, Sah AP, Allman DM, Cancro MP, Gerstein RM. Bone Marrow Microenvironmental Changes Underlie Reduced RAG-mediated Recombination and B Cell Generation in Aged Mice. Journal of Experimental Medicine. 2004; 200:411–423. [PubMed: 15314072]
19. Min H, Montecino-Rodriguez E, Dorshkind K. Effects of Aging on the Common Lymphoid Progenitor to Pro-B Cell Transition. Journal of Immunology. 2006; 176:1007–1012.
20. Kline GH, Hayden TA, Klinman NR. B Cell Maintenance in Aged Mice Reflects Both Increased B Cell Longevity and Decreased B Cell Generation. Journal of Immunology. 1999; 162:3342–3349.
21. Alter-Wolf S, Blomberg BB, Riley RL. Old mice retain bone marrow B1 progenitors, but lose B2 precursors, and exhibit altered immature B cell phenotype and light chain usage. Mechanisms of Ageing and Development. 2009; 130:401–408. [PubMed: 19428460]
22. Barber CL, Montecino-Rodriguez E, Dorshkind K. Reduced production of B-1–specified common lymphoid progenitors results in diminished potential of adult marrow to generate B-1 cells. Proceedings of the National Academy of Sciences. 2011; 108:13700–13704.
23. Ogawa T, Kitagawa M, Hirokawa K. Age-related changes of human bone marrow: a histometric estimation of proliferative cells, apoptotic cells, T cells, B cells and macrophages. Mechanisms of Ageing and Development. 2000; 117:57–68. [PubMed: 10958923]
24. Rossi DJ, Bryder D, Seita J, Nussenzweig A, Hoeijmakers J, Weissman IL. Deficiencies in DNA damage repair limit the function of haematopoietic stem cells with age. Nature. 2007; 447:725–729. [PubMed: 17554309]
25. Vaziri H, Dragowska W, Allsopp RC, Thomas TE, Harley CB, Lansdorp PM. Evidence for a mitotic clock in human hematopoietic stem cells: loss of telomeric DNA with age. Proceedings of the National Academy of Sciences. 1994; 91:9857–9860.
26. Allsopp RC, Morin GB, Horner JW, DePinho R, Harley CB, Weissman IL. Effect of TERT over-expression on the long-term transplantation capacity of hematopoietic stem cells. Nat Med. 2003; 9:369–371. [PubMed: 12669037]
27. Jang Y-Y, Sharkis SJ. A low level of reactive oxygen species selects for primitive hematopoietic stem cells that may reside in the low-oxygenic niche. Blood. 2007; 110:3056–3063. [PubMed: 17595331]

28. Ito K, Hirao A, Arai F, Matsuoka S, Takubo K, Hamaguchi I, et al. Regulation of oxidative stress by ATM is required for self-renewal of haematopoietic stem cells. Nature. 2004; 431:997–1002. [PubMed: 15496926]
29. Tothova Z, Kollipara R, Huntly BJ, Lee BH, Castrillon DH, Cullen DE, et al. FoxOs Are Critical Mediators of Hematopoietic Stem Cell Resistance to Physiologic Oxidative Stress. Cell. 2007; 128:325–339. [PubMed: 17254970]
30. Shipounova IN, Svinareva DA, Petrova TV, Lyamzaev KG, Chernyak BV, Drize NI, et al. Reactive oxygen species produced in mitochondria are involved in age-dependent changes of hematopoietic and mesenchymal progenitor cells in mice. A study with the novel mitochondria-targeted antioxidant SkQ1. Mechanisms of Ageing and Development. 2010; 131:415–421. [PubMed: 20600239]
31. Wilson A, Shehadeh L, Yu H, Webster K. Age-related molecular genetic changes of murine bone marrow mesenchymal stem cells. BMC Genomics. 2010; 11:229. [PubMed: 20374652]
32. Wang W, Gou L, Xie G, Tong A, He F, Lu Z, et al. Proteomic Analysis of Interstitial Fluid in Bone Marrow Identified That Peroxiredoxin 2 Regulates H_2O_2 Level of Bone Marrow during Aging. Journal of Proteome Research. 2010; 9:3812–3819. [PubMed: 20568815]
33. Yahata T, Takanashi T, Muguruma Y, Ibrahim AA, Matsuzawa H, Uno T, et al. Accumulation of oxidative DNA damage restricts the self-renewal capacity of human hematopoietic stem cells. Blood. 2011; 118:2941–2950. [PubMed: 21734240]
34. Bjelakovic G, Nikolova D, Gluud LL, Simonetti RG, Gluud C. Mortality in Randomized Trials of Antioxidant Supplements for Primary and Secondary Prevention. Journal of the American Medical Association. 2007; 297:842–857. [PubMed: 17327526]
35. Jensen GL, Meister A. Radioprotection of human lymphoid cells by exogenously supplied glutathione is mediated by gamma-glutamyl transpeptidase. Proceedings of the National Academy of Sciences. 1983; 80:4714–4717.
36. Puri RN, Meister A. Transport of glutathione, as gamma-glutamylcysteinylglycyl ester, into liver and kidney. Proceedings of the National Academy of Sciences. 1983; 80:5258–5260.
37. Ballatori N, Krance SM, Notenboom S, Shujie S, Kim T, Hammond CL. Glutathione dysregulation and the etiology and progression of human diseases. Biological Chemistry. 2009; 390:191–214. [PubMed: 19166318]
38. Stephan RP, Reilly CR, Witte PL. Impaired Ability of Bone Marrow Stromal Cells to Support B-Lymphopoiesis With Age. Blood. 1998; 91:75–88. [PubMed: 9414271]
39. Puel A, Ziegler SF, Buckley RH, Leonard WJ. Defective IL7R expression in $T^{-}B^{+}NK^{+}$ severe combined immunodeficiency. Nature Genetics. 1998; 20:394. [PubMed: 9843216]
40. Beresford JN, Bennett JH, Devlin C, Leboy PS, Owen ME. Evidence for an inverse relationship between the differentiation of adipocytic and osteogenic cells in rat marrow stromal cell cultures. Journal of Cell Science. 1992; 102:341–351. [PubMed: 1400636]
41. Zhang J, Niu C, Ye L, Huang H, He X, Tong W-G, et al. Identification of the haematopoietic stem cell niche and control of the niche size. Nature. 2003; 425:836–841. [PubMed: 14574412]
42. Visnjic D, Kalajzic Z, Rowe DW, Katavic V, Lorenzo J, Aguila HL. Hematopoiesis is severely altered in mice with an induced osteoblast deficiency. Blood. 2004; 103:3258–3264. [PubMed: 14726388]
43. Calvi LM, Adams GB, Weibrecht KW, Weber JM, Olson DP, Knight MC, et al. Osteoblastic cells regulate the haematopoietic stem cell niche. Nature. 2003; 425:841–846. [PubMed: 14574413]
44. Naveiras O, Nardi V, Wenzel PL, Hauschka PV, Fahey F, Daley GQ. Bone-marrow adipocytes as negative regulators of the haematopoietic microenvironment. Nature. 2009; 460:259–263. [PubMed: 19516257]
45. Chertkov JL, Gurevitch OA. Age-related changes in hemopoietic microenvironment. Enhanced growth of hemopoietic stroma and weakened genetic resistance to hemopoietic cells in old mice. Experimental Gerontology. 1981; 16:195–198. [PubMed: 7026269]
46. Muguruma Y, Yahata T, Miyatake H, Sato T, Uno T, Itoh J, et al. Reconstitution of the functional human hematopoietic microenvironment derived from human mesenchymal stem cells in the murine bone marrow compartment. Blood. 2006; 107:1878–1887. [PubMed: 16282345]

47. Kassem M, Marie PJ. Senescence-associated intrinsic mechanisms of osteoblast dysfunctions. Aging Cell. 2011; 10:191–197. [PubMed: 21210937]
48. Jiang SY, Shu R, Xie YF, Zhang SY. Age-related changes in biological characteristics of human alveolar osteoblasts. Cell Proliferation. 2010; 43:464–470. [PubMed: 20735438]
49. Köhler A, Schmithorst V, Filippi M-D, Ryan MA, Daria D, Gunzer M, et al. Altered cellular dynamics and endosteal location of aged early hematopoietic progenitor cells revealed by time-lapse intravital imaging in long bones. Blood. 2009; 114:290–298. [PubMed: 19357397]
50. Shockley KR, Lazarenko OP, Czernik PJ, Rosen CJ, Churchill GA, Lecka-Czernik B. PPARγ2 nuclear receptor controls multiple regulatory pathways of osteoblast differentiation from marrow mesenchymal stem cells. Journal of Cellular Biochemistry. 2009; 106:232–246. [PubMed: 19115254]
51. Moerman EJ, Teng K, Lipschitz DA, Lecka-Czernik B. Aging activates adipogenic and suppresses osteogenic programs in mesenchymal marrow stroma/stem cells: the role of PPAR-γ2 transcription factor and TGF-β/BMP signaling pathways. Aging Cell. 2004; 3:379–389. [PubMed: 15569355]
52. Miard S, Dombrowski L, Carter S, Boivin L, Picard F. Aging alters PPARγ in rodent and human adipose tissue by modulating the balance in steroid receptor coactivator-1. Aging Cell. 2009; 8:449–459. [PubMed: 19485965]
53. Liu L-F, Shen W-J, Ueno M, Patel S, Kraemer F. Characterization of age-related gene expression profiling in bone marrow and epididymal adipocytes. BMC Genomics. 2011; 12:212. [PubMed: 21545734]
54. Majka SM, Fox KE, Psilas JC, Helm KM, Childs CR, Acosta AS, et al. De novo generation of white adipocytes from the myeloid lineage via mesenchymal intermediates is age, adipose depot, and gender specific. Proceedings of the National Academy of Sciences. 2010; 107:14781–14786.
55. Unwin RD, Smith DL, Blinco D, Wilson CL, Miller CJ, Evans CA, et al. Quantitative proteomics reveals posttranslational control as a regulatory factor in primary hematopoietic stem cells. Blood. 2006; 107:4687–4694. [PubMed: 16507774]
56. Cipolleschi MG, Dello Sbarba P, Olivotto M. The role of hypoxia in the maintenance of hematopoietic stem cells. Blood. 1993; 82:2031–2037. [PubMed: 8104535]
57. Hermitte F, Brunet de la Grange P, Belloc F, Praloran V, Ivanovic Z. Very Low O_2 Concentration (0.1%) Favors G0 Return of Dividing $CD34^+$ Cells. Stem Cells. 2006; 24:65–73. [PubMed: 16123391]
58. Simsek T, Kocabas F, Zheng J, DeBerardinis RJ, Mahmoud AI, Olson EN, et al. The Distinct Metabolic Profile of Hematopoietic Stem Cells Reflects Their Location in a Hypoxic Niche. Cell Stem Cell. 2010; 7:380–390. [PubMed: 20804973]
59. Takubo K, Goda N, Yamada W, Iriuchishima H, Ikeda E, Kubota Y, et al. Regulation of the HIF-1α Level Is Essential for Hematopoietic Stem Cells. Cell Stem Cell. 2010; 7:391–402. [PubMed: 20804974]
60. Coussens M, Davy P, Brown L, Foster C, Andrews WH, Nagata M, et al. RNAi screen for telomerase reverse transcriptase transcriptional regulators identifies HIF1α as critical for telomerase function in murine embryonic stem cells. Proceedings of the National Academy of Sciences. 2010; 107:13842–13847.
61. Gustafsson MV, Zheng X, Pereira T, Gradin K, Jin S, Lundkvist J, et al. Hypoxia Requires Notch Signaling to Maintain the Undifferentiated Cell State. Developmental Cell. 2005; 9:617–628. [PubMed: 16256737]
62. Rehn M, Olsson A, Reckzeh K, Diffner E, Carmeliet P, Landberg G, et al. Hypoxic induction of vascular endothelial growth factor regulates murine hematopoietic stem cell function in the low-oxygenic niche. Blood. 2011; 118:1534–1543. [PubMed: 21670467]
63. Almeida M, Ambrogini E, Han L, Manolagas SC, Jilka RL. Increased Lipid Oxidation Causes Oxidative Stress, Increased Peroxisome Proliferator-activated Receptor-γ Expression, and Diminished Pro-osteogenic Wnt Signaling in the Skeleton. Journal of Biological Chemistry. 2009; 284:27438–27448. [PubMed: 19657144]

64. Lennon DP, Edmison JM, Caplan AI. Cultivation of rat marrow-derived mesenchymal stem cells in reduced oxygen tension: Effects on in vitro and in vivo osteochondrogenesis. Journal of Cellular Physiology. 2001; 187:345–355. [PubMed: 11319758]
65. Wagegg M, Gaber T, Lohanatha F, Jakstadt M, Kasper G, Duda G, et al. Adipogenic and Osteogenic Switch in Differentiation of Human Mesenchymal Stem Cells (hMSCs) Is Triggered by Hypoxia in a HIF-1 Dependent Manner. [abstract]. Arthritis & Rheumatism. 2010; 62:630.
66. D'Ippolito G, Diabira S, Howard GA, Roos BA, Schiller PC. Low oxygen tension inhibits osteogenic differentiation and enhances stemness of human MIAMI cells. Bone. 2006; 39:513–522. [PubMed: 16616713]
67. Holzwarth C, Vaegler M, Gieseke F, Pfister S, Handgretinger R, Kerst G, et al. Low physiologic oxygen tensions reduce proliferation and differentiation of human multipotent mesenchymal stromal cells. BMC Cell Biology. 2010; 11:11. [PubMed: 20109207]
68. Grant JL, Smith B. Bone Marrow Gas Tensions, Bone Marrow Blood Flow, and Erythropoiesis in Man. Annals of Internal Medicine. 1963; 58:801–809. [PubMed: 13949863]
69. Harrison JS, Rameshwar P, Chang V, Bandari P. Oxygen saturation in the bone marrow of healthy volunteers. Blood. 2002; 99:394–394. [PubMed: 11783438]
70. Parmar K, Mauch P, Vergilio J-A, Sackstein R, Down JD. Distribution of hematopoietic stem cells in the bone marrow according to regional hypoxia. Proceedings of the National Academy of Sciences. 2007; 104:5431–5436.
71. Winkler IG, Barbier V, Wadley R, Zannettino ACW, Williams S, Lévesque J-P. Positioning of bone marrow hematopoietic and stromal cells relative to blood flow in vivo: serially reconstituting hematopoietic stem cells reside in distinct nonperfused niches. Blood. 2010; 116:375–385. [PubMed: 20393133]
72. Nakamura K, Kouro T, Kincade PW, Malykhin A, Maeda K, Coggeshall KM. Src Homology 2–containing 5-Inositol Phosphatase (SHIP) Suppresses an Early Stage of Lymphoid Cell Development through Elevated Interleukin-6 Production by Myeloid Cells in Bone Marrow. Journal of Experimental Medicine. 2004; 199:243–254. [PubMed: 14718513]
73. Maeda K, Mehta H, Drevets DA, Coggeshall KM. IL-6 increases B-cell IgG production in a feed-forward proinflammatory mechanism to skew hematopoiesis and elevate myeloid production. Blood. 2010; 115:4699–4706. [PubMed: 20351305]
74. Zhang J, Dai J, Lu Y, Yao Z, O'Brien CA, Murtha JM, et al. In vivo visualization of aging-associated gene transcription: evidence for free radical theory of aging. Experimental Gerontology. 2004; 39:239–247. [PubMed: 15036418]
75. Wei J, Xu H, Davies JL, Hemmings GP. Increase of plasma IL-6 concentration with age in healthy subjects. Life Sciences. 1992; 51:1953–1956. [PubMed: 1453878]
76. Pino AM, Ríos S, Astudillo P, Fernández M, Figueroa P, Seitz G, et al. Concentration of adipogenic and proinflammatory cytokines in the bone marrow supernatant fluid of osteoporotic women. Journal of Bone and Mineral Research. 2010; 25:492–498. [PubMed: 19653807]
77. Sullivan C, Chen Y, Shan Y, Hu Y, Peng C, Zhang H, et al. Functional Ramifications for the Loss of P-Selectin Expression on Hematopoietic and Leukemic Stem Cells. PLoS ONE. 2011; 6:e26246. [PubMed: 22039451]
78. French RA, Broussard SR, Meier WA, Minshall C, Arkins S, Zachary JF, et al. Age-Associated Loss of Bone Marrow Hematopoietic Cells Is Reversed by GH and Accompanies Thymic Reconstitution. Endocrinology. 2002; 143:690–699. [PubMed: 11796526]
79. Kucia M, Shin DM, Liu R, Ratajczak J, Bryndza E, Masternak MM, et al. Reduced number of VSELs in the bone marrow of growth hormone transgenic mice indicates that chronically elevated Igf1 level accelerates age-dependent exhaustion of pluripotent stem cell pool: a novel view on aging. Leukemia. 2011; 25:1370–1374. [PubMed: 21566652]
80. Gordon J, Wilson VA, Blair NF, Sheridan J, Farley A, Wilson L, et al. Functional evidence for a single endodermal origin for the thymic epithelium. Nat Immunol. 2004; 5:546–553. [PubMed: 15098031]
81. Gordon J, Manley NR. Mechanisms of thymus organogenesis and morphogenesis. Development. 2011; 138:3865–3878. [PubMed: 21862553]

82. Goldschneider I. Cyclical mobilization and gated importation of thymocyte progenitors in the adult mouse: evidence for a thymus-bone marrow feedback loop. Immunol Rev. 2006; 209:58–75. [PubMed: 16448534]

83. Adkins B. T-cell function in newborn mice and humans. Immunol Today. 1999; 20:330–335. [PubMed: 10379052]

84. Adkins B. Peripheral $CD4^+$ lymphocytes derived from fetal versus adult thymic precursors differ phenotypically and functionally. J Immunol. 2003; 171:5157–5164. [PubMed: 14607915]

85. Min B, McHugh R, Sempowski GD, Mackall C, Foucras G, Paul WE. Neonates support lymphopenia-induced proliferation. Immunity. 2003; 18:131–140. [PubMed: 12530982]

86. Anderson G, Jenkinson WE, Jones T, Parnell SM, Kinsella FA, White AJ, et al. Establishment and functioning of intrathymic microenvironments. Immunol Rev. 2006; 209:10–27. [PubMed: 16448531]

87. Gill J, Malin M, Sutherland J, Gray D, Hollander G, Boyd R. Thymic generation and regeneration. Immunol Rev. 2003; 195:28–50. [PubMed: 12969308]

88. Ritter MA, Boyd RL. Development in the thymus: it takes two to tango. Immunol Today. 1993; 14:462–469. [PubMed: 8216725]

89. van Ewijk W, Shores EW, Singer A. Crosstalk in the mouse thymus. Immunol Today. 1994; 15:214–217. [PubMed: 8024681]

90. Anderson G, Jenkinson EJ. Lymphostromal interactions in thymic development and function. Nat Rev Immunol. 2001; 1:31–40. [PubMed: 11905812]

91. Min H, Montecino-Rodriguez E, Dorshkind K. Reduction in the developmental potential of intrathymic T cell progenitors with age. J Immunol. 2004; 173:245–250. [PubMed: 15210781]

92. Min H, Montecino-Rodriguez E, Dorshkind K. Reassessing the role of growth hormone and sex steroids in thymic involution. Clin Immunol. 2006; 118:117–123. [PubMed: 16188505]

93. Haynes L, Swain SL. Why aging T cells fail: implications for vaccination. Immunity. 2006; 24:663–666. [PubMed: 16782020]

94. Taub DD, Longo DL. Insights into thymic aging and regeneration. Immunol Rev. 2005; 205:72–93. [PubMed: 15882346]

95. Gray DH, Seach N, Ueno T, Milton MK, Liston A, Lew AM, et al. Developmental kinetics, turnover, and stimulatory capacity of thymic epithelial cells. Blood. 2006; 108:3777–3785. [PubMed: 16896157]

96. Chen L, Xiao S, Manley NR. Foxn1 is required to maintain the postnatal thymic microenvironment in a dosage-sensitive manner. Blood. 2009; 113:567–574. [PubMed: 18978204]

97. Griffith AV, Fallahi M, Venables T, Petrie HT. Persistent degenerative changes in thymic organ function revealed by an inducible model of organ regrowth. Aging Cell. 2012; 11:169–177. [PubMed: 22103718]

98. Takeoka Y, Chen SY, Yago H, Boyd R, Suehiro S, Shultz LD, et al. The murine thymic microenvironment: changes with age. Int Arch Allergy Immunol. 1996; 111:5–12. [PubMed: 8753837]

99. Youm YH, Yang H, Amin R, Smith SR, Leff T, Dixit VD. Thiazolidinedione treatment and constitutive-PPARgamma activation induces ectopic adipogenesis and promotes age-related thymic involution. Aging Cell. 2010; 9:478–489. [PubMed: 20374200]

100. Youm YH, Yang H, Sun Y, Smith RG, Manley NR, Vandanmagsar B, et al. Deficient ghrelin receptor-mediated signaling compromises thymic stromal cell microenvironment by accelerating thymic adiposity. J Biol Chem. 2009; 284:7068–7077. [PubMed: 19054770]

101. Dixit VD. Thymic fatness and approaches to enhance thymopoietic fitness in aging. Curr Opin Immunol. 2010; 22:521–528. [PubMed: 20650623]

102. 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. J Immunol. 2000; 164:2180–2187. [PubMed: 10657672]

103. Yang H, Youm YH, Dixit VD. Inhibition of thymic adipogenesis by caloric restriction is coupled with reduction in age-related thymic involution. J Immunol. 2009; 183:3040–3052. [PubMed: 19648267]

104. Yang H, Youm YH, Vandanmagsar B, Rood J, Kumar KG, Butler AA, et al. Obesity accelerates thymic aging. Blood. 2009; 114:3803–3812. [PubMed: 19721009]
105. Zhu M, Fu YX. Coordinating development of medullary thymic epithelial cells. Immunity. 2008; 29:386–388. [PubMed: 18799147]
106. Heng TS, Goldberg GL, Gray DH, Sutherland JS, Chidgey AP, Boyd RL. Effects of castration on thymocyte development in two different models of thymic involution. J Immunol. 2005; 175:2982–2993. [PubMed: 16116185]
107. Montecino-Rodriquez E, Min H, Dorshkind K. Reevaluating current models of thymic involution. Semin Immunol. 2005; 17:356–361. [PubMed: 15967677]
108. Mackall CL, Punt JA, Morgan P, Farr AG, Gress RE. Thymic function in young/old chimeras: substantial thymic T cell regenerative capacity despite irreversible age-associated thymic involution. Eur J Immunol. 1998; 28:1886–1893. [PubMed: 9645370]
109. 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; 6:663–672. [PubMed: 17681038]
110. Klug DB, Crouch E, Carter C, Coghlan L, Conti CJ, Richie ER. Transgenic expression of cyclin D1 in thymic epithelial precursors promotes epithelial and T cell development. J Immunol. 2000; 164:1881–1888. [PubMed: 10657637]
111. Nowell CS, Bredenkamp N, Tetelin S, Jin X, Tischner C, Vaidya H, et al. Foxn1 regulates lineage progression in cortical and medullary thymic epithelial cells but is dispensable for medullary sublineage divergence. PLoS Genet. 2011; 7:e1002348. [PubMed: 22072979]
112. Nakayama K, Ishida N, Shirane M, Inomata A, Inoue T, Shishido N, et al. Mice lacking p27(Kip1) display increased body size, multiple organ hyperplasia, retinal dysplasia, and pituitary tumors. Cell. 1996; 85:707–720. [PubMed: 8646779]
113. Kiyokawa H, Kineman RD, Manova-Todorova KO, Soares VC, Hoffman ES, Ono M, et al. Enhanced growth of mice lacking the cyclin-dependent kinase inhibitor function of p27(Kip1). Cell. 1996; 85:721–732. [PubMed: 8646780]
114. Fero ML, Rivkin M, Tasch M, Porter P, Carow CE, Firpo E, et al. A syndrome of multiorgan hyperplasia with features of gigantism, tumorigenesis, and female sterility in p27(Kip1)-deficient mice. Cell. 1996; 85:733–744. [PubMed: 8646781]
115. Jenkinson WE, Bacon A, White AJ, Anderson G, Jenkinson EJ. An epithelial progenitor pool regulates thymus growth. J Immunol. 2008; 181:6101–6108. [PubMed: 18941199]
116. Senoo M, Pinto F, Crum CP, McKeon F. p63 Is essential for the proliferative potential of stem cells in stratified epithelia. Cell. 2007; 129:523–536. [PubMed: 17482546]
117. Sempowski GD, Rhein ME, Scearce RM, Haynes BF. Leukemia inhibitory factor is a mediator of Escherichia coli lipopolysaccharide-induced acute thymic atrophy. European Journal of Immunology. 2002; 32:3066–3070. [PubMed: 12385026]
118. Billard MJ, Gruver AL, Sempowski GD. Acute Endotoxin-Induced Thymic Atrophy Is Characterized By Intrathymic Inflammatory and Wound Healing Responses. PLoS One. 2011; 6:e17940. [PubMed: 21437240]
119. Hale JS, Boursalian TE, Turk GL, Fink PJ. Thymic output in aged mice. Proc Natl Acad Sci U S A. 2006; 103:8447–8452. [PubMed: 16717190]
120. Kaestner KH, Knochel W, Martinez DE. Unified nomenclature for the winged helix/forkhead transcription factors. Genes Dev. 2000; 14:142–146. [PubMed: 10702024]
121. 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; 372:103–107. [PubMed: 7969402]
122. Nehls M, Kyewski B, Messerle M, Waldschütz R, Schüddekopf K, Smith AJ, et al. Two genetically separable steps in the differentiation of thymic epithelium. Science. 1996; 272:886–889. [PubMed: 8629026]
123. Blackburn CC, Augustine CL, Li R, Harvey RP, Malin MA, Boyd RL, et al. The *nu* gene acts cell-autonomously and is required for differentiation of thymic epithelial progenitors. Proc Natl Acad Sci USA. 1996; 93:5742–5746. [PubMed: 8650163]

124. Su DM, Navarre S, Oh WJ, Condie BG, Manley NR. A domain of Foxn1 required for crosstalk-dependent thymic epithelial cell differentiation. Nat Immunol. 2003; 4:1128–1135. [PubMed: 14528302]

125. Itoi M, Kawamoto H, Katsura Y, Amagai T. Two distinct steps of immigration of hematopoietic progenitors into the early thymus anlage. Int Immunol. 2001; 13:1203–1211. [PubMed: 11526101]

126. Itoi M, Tsukamoto N, Amagai T. Expression of Dll4 and CCL25 in Foxn1-negative epithelial cells in the post-natal thymus. Int Immunol. 2007; 19:127–132. [PubMed: 17158094]

127. 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. Int Immunol. 2002; 14:813–822. [PubMed: 12096041]

128. 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

129. Tsai PT, Lee RA, Wu H. BMP4 acts upstream of FGF in modulating thymic stroma and regulating thymopoiesis. Blood. 2003; 102:3947–3953. [PubMed: 12920023]

130. Suniara RK, Jenkinson EJ, Owen JJ. An essential role for thymic mesenchyme in early T cell development. J Exp Med. 2000; 191:1051–1056. [PubMed: 10727466]

131. Erickson M, Morkowski S, Lehar S, Gillard G, Beers C, Dooley J, et al. Regulation of thymic epithelium by keratinocyte growth factor. Blood. 2002; 100:3269–3278. [PubMed: 12384427]

132. Revest JM, Suniara RK, Kerr K, Owen JJ, Dickson C. Development of the thymus requires signaling through the fibroblast growth factor receptor r2-iiib. J Immunol. 2001; 167:1954–1961. [PubMed: 11489975]

133. Rossi SW, Jeker LT, Ueno T, Kuse S, Keller MP, Zuklys S, et al. Keratinocyte growth factor (KGF) enhances postnatal T-cell development via enhancements in proliferation and function of thymic epithelial cells. Blood. 2007; 109:3803–3811. [PubMed: 17213286]

134. Rossi S, Blazar BR, Farrell CL, Danilenko DM, Lacey DL, Weinberg KI, et al. Keratinocyte growth factor preserves normal thymopoiesis and thymic microenvironment during experimental graft-versus-host disease. Blood. 2002; 100:682–691. [PubMed: 12091365]

135. Dooley J, Erickson M, Larochelle WJ, Gillard GO, Farr AG. FGFR2IIIb signaling regulates thymic epithelial differentiation. Dev Dyn. 2007; 236:3459–3471. [PubMed: 17969154]

136. 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; 107:2453–2460. [PubMed: 16304055]

137. Min D, Panoskaltsis-Mortari A, Kuro-o M, Holländer GA, Blazar BR, Weinberg KI. Sustained thymopoiesis and improvement in functional immunity induced by exogenous KGF administration in murine models of aging. Blood. 2007; 109:2529–2537. [PubMed: 17138819]

138. Kelly RM, Highfill SL, Panoskaltsis-Mortari A, Taylor PA, Boyd RL, Hollander GA, et al. Keratinocyte growth factor and androgen blockade work in concert to protect against conditioning regimen-induced thymic epithelial damage and enhance T-cell reconstitution after murine bone marrow transplantation. Blood. 2008; 111:5734–5744. [PubMed: 18334670]

139. Min D, Taylor PA, Panoskaltsis-Mortari A, Chung B, Danilenko DM, Farrell C, et al. Protection from thymic epithelial cell injury by keratinocyte growth factor: a new approach to improve thymic and peripheral T-cell reconstitution after bone marrow transplantation. Blood. 2002; 99:4592–4600. [PubMed: 12036893]

140. Fitzpatrick FT, Kendall MD, Wheeler MJ, Adcock IM, Greenstein BD. Reappearance of thymus of ageing rats after orchidectomy. J Endocrinol. 1985; 106:R17–19. [PubMed: 4045335]

141. Hince M, Sakkal S, Vlahos K, Dudakov J, Boyd R, Chidgey A. The role of sex steroids and gonadectomy in the control of thymic involution. Cell Immunol. 2008; 252:122–138. [PubMed: 18294626]

142. Olsen NJ, Olson G, Viselli SM, Gu X, Kovacs WJ. Androgen receptors in thymic epithelium modulate thymus size and thymocyte development. Endocrinology. 2001; 142:1278–1283. [PubMed: 11181545]

143. Greenstein BD, Fitzpatrick FT, Adcock IM, Kendall MD, Wheeler MJ. Reappearance of the thymus in old rats after orchidectomy: inhibition of regeneration by testosterone. J Endocrinol. 1986; 110:417–422. [PubMed: 3760740]

144. Liu PY, Death AK, Handelsman DJ. Androgens and cardiovascular disease. Endocr Rev. 2003; 24:313–340. [PubMed: 12788802]

145. Greenstein BD, de Bridges EF, Fitzpatrick FT. Aromatase inhibitors regenerate the thymus in aging male rats. Int J Immunopharmacol. 1992; 14:541–553. [PubMed: 1521922]

146. Balk SP, Knudsen KE. AR, the cell cycle, and prostate cancer. Nucl Recept Signal. 2008; 6:e001. [PubMed: 18301781]

147. Lu J, Danielsen M. Differential regulation of androgen and glucocorticoid receptors by retinoblastoma protein. J Biol Chem. 1998; 273:31528–31533. [PubMed: 9813067]

148. van den Brink MR, Alpdogan O, Boyd RL. Strategies to enhance T-cell reconstitution in immunocompromised patients. Nat Rev Immunol. 2004; 4:856–867. [PubMed: 15516965]

149. Steinmann GG. Changes in the human thymus during aging. Curr Top Pathol. 1986; 75:43–88. [PubMed: 3514161]

150. Steinmann GG, Klaus B, Muller-Hermelink HK. The involution of the ageing human thymic epithelium is independent of puberty. A morphometric study. Scand J Immunol. 1985; 22:563–575. [PubMed: 4081647]

151. George AJ, Ritter MA. Thymic involution with ageing: obsolescence or good housekeeping? Immunol Today. 1996; 17:267–272. [PubMed: 8962629]

152. Wang C, Leung A, Sinha-Hikim AP. Reproductive aging in the male brown-Norway rat: a model for the human. Endocrinology. 1993; 133:2773–2781. [PubMed: 8243304]

153. Lacombe A, Lelievre V, Roselli CE, Salameh W, Lue YH, Lawson G, et al. Delayed testicular aging in pituitary adenylate cyclase-activating peptide (PACAP) null mice. Proc Natl Acad Sci U S A. 2006; 103:3793–3798. [PubMed: 16505386]

154. Gooren LJ. Late-onset hypogonadism. Front Horm Res. 2009; 37:62–73. [PubMed: 19011289]

155. Hijazi RA, Cunningham GR. Andropause: is androgen replacement therapy indicated for the aging male? Annu Rev Med. 2005; 56:117–137. [PubMed: 15660505]

156. Henderson J. On the relationship of the thymus to the sexual organs. Journal of Physiology. 1904; 31:222–229. [PubMed: 16992746]

157. Zamisch M, Moore-Scott B, Su DM, Lucas PJ, Manley N, Richie ER. Ontogeny and regulation of IL-7-expressing thymic epithelial cells. J Immunol. 2005; 174:60–67. [PubMed: 15611228]

158. Wei Q, Condie BG. A focused in situ hybridization screen identifies candidate transcriptional regulators of thymic epithelial cell development and function. PLoS One. 2011; 6:e26795. [PubMed: 22087235]

Highlights

- Hypoxic niches are critical for sustaining bone marrow stem cell lymphopoiesis
- ROS and IL-6 accelerate bone marrow lymphoid senescence with aging
- HIF-1α and PPARγ2 modulate bone marrow lymphopoiesis with aging
- Loss of stromal-thymocyte cross-talk contributes to thymic involution with aging
- Intrathymic changes in Foxn1, KGF, and sex steroids drive thymic senescence

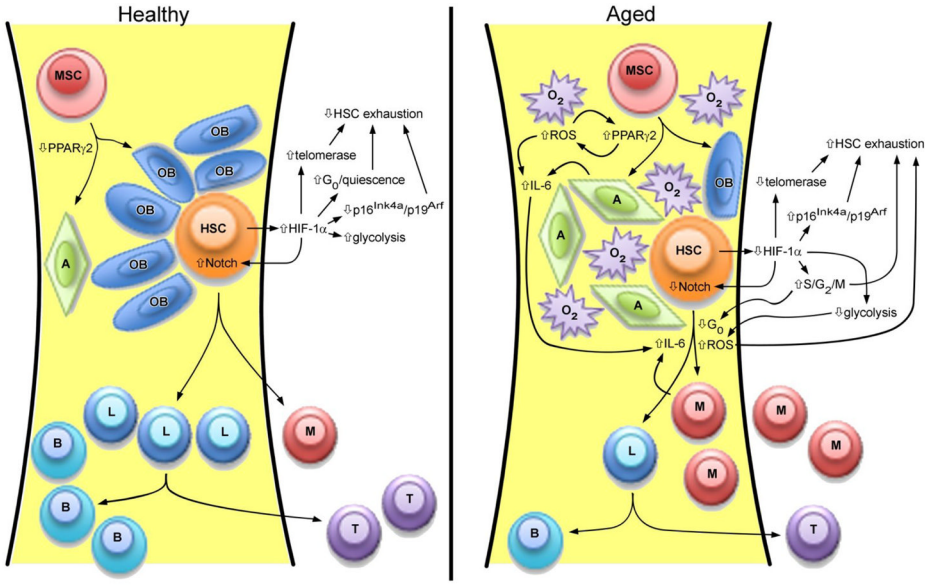

Figure 1. Mechanisms of bone marrow lymphoid senescence with aging

Aging produces changes within the bone marrow (yellow shaded area), including increased production of adipocytes (A) from mesenchymal stem cells (MSC), loss of hypoxic osteoblast (OB)-enriched niches, exhaustion of hematopoietic stem cells (HSC), skewing of HSCs toward myeloid cell (M) formation, and defective production of lymphocyte progenitors (L) for B cell (B) and T cell (T) differentiation.

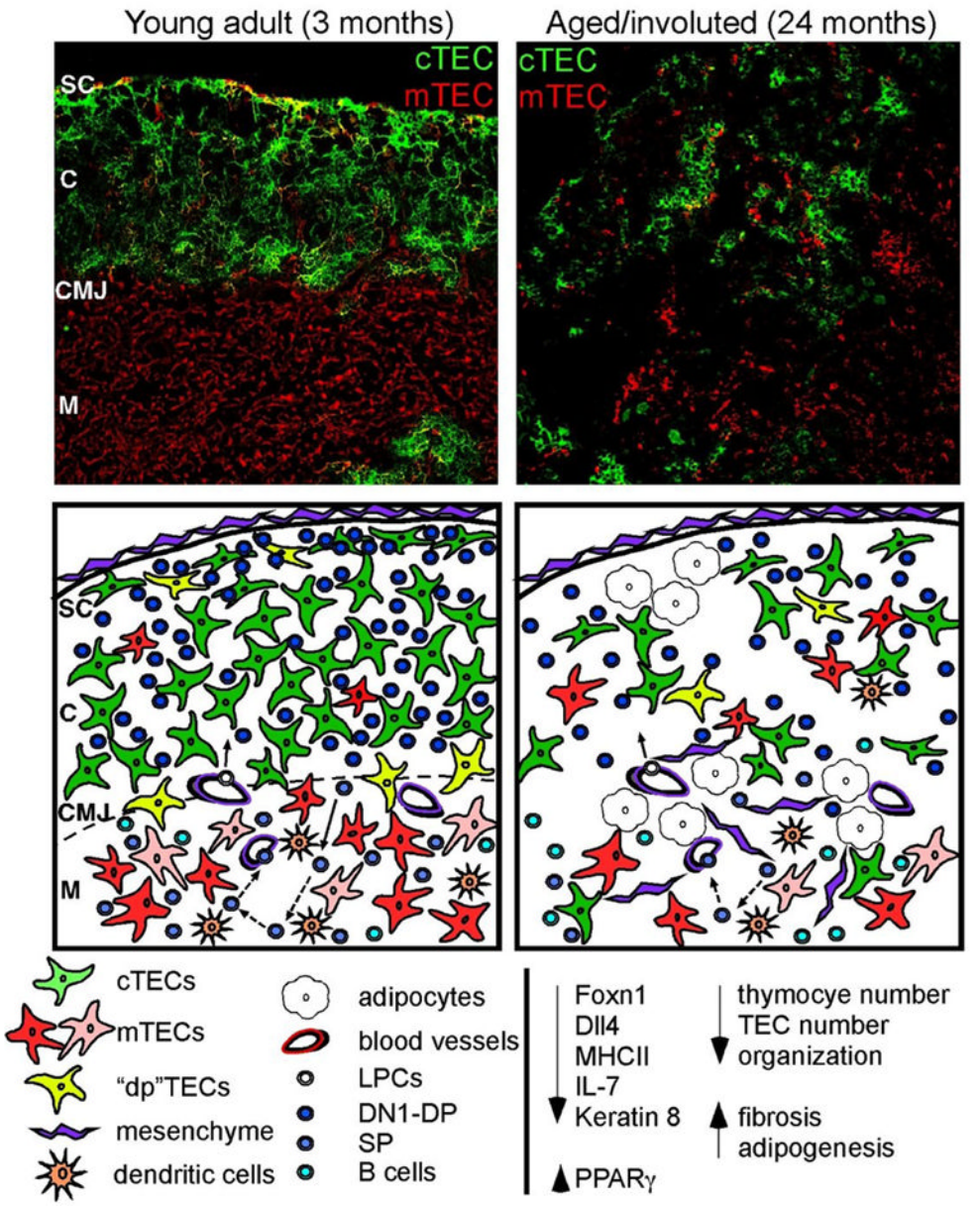

Figure 2. Organization and composition of young and aged thymus

Top panels show sections of 3 (left) and 24 (right) month-old thymus tissues stained with immunofluorescent antibodies to Ly51 for cTECs (green) and keratins 5 or 14 for mTECs (red). The young adult thymus is characterized by a well-organized cortical-medullary structure with clearly defined subcapsular (SC), cortical (C), medullary (M), and corticomedullary junction (CMJ) zones. The involuted aged thymus demonstrates intermingled cTECs and mTECs, loss of TECs expressing both cTEC and mTEC markers (in yellow, double positive or "dp" TECs), and TEC-free regions. Illustrated diagrams depict the general cellular composition and organization of the young and aged thymus. A key for cell types is provided at the bottom left; the bottom right panel shows molecular and cellular changes associated with the aged thymus. Other abbreviations: Dll4, delta-like 4; DN1-DP, double negative 1-double positive cells; LPCs, lymphocyte progenitor cells; SP, single positive cells