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Thymic involution and immune reconstitution

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Abstract

Chronic thymus involution associated with aging results in less efficient T-cell development and decreased emigration of naïve T cells to the periphery. Thymic decline in the aged is linked to increased morbidity and mortality in a wide range of clinical settings. Negative consequences of these effects on global health make it of paramount importance to understand the mechanisms driving thymic involution and homeostatic processes across the lifespan. There is growing evidence that thymus tissue is plastic and that the involution process might be therapeutically halted or reversed. We present here progress on the exploitation of thymosuppressive and thymostimulatory pathways using factors such as keratinocyte growth factor, interleukin 7 or sex steroid ablation for therapeutic thymus restoration and peripheral immune reconstitution in adults.

Introduction

The thymus is a vital organ for homeostatic maintenance of the peripheral immune system. It is in this mediastinal tissue that T cells develop and are extensively educated for export to the periphery and establishment of a functional and effective immune system (Box 1). One of the striking paradoxical features of the thymus is that it undergoes profound age-associated atrophy. Loss of thymic epithelial space (TES), i.e. thymus involution or atrophy, results in less efficient T-cell development, or thymopoiesis, and decreased emigration of naïve T cells. Under most circumstances, thymic decline is of minimal consequence to a healthy individual, but the reduced efficacy of the immune system with age has direct etiological linkages with an increase in diseases including opportunistic infections, autoimmunity and the incidence of cancer (see articles by Chen *et al.* and Weksler *et al.* also in this issue). Furthermore the inability of adults to restore immune function after insult induced by chemotherapy, ionizing radiation exposure and infections (e.g. HIV-1) leads to increased morbidity and often mortality in the aged. For these reasons, it is important to understand what drives normal age-related thymic atrophy and loss of thymic output. Elucidation of these crucial mechanisms and systems will allow us to better prevent or treat immunosenescence-associated problems in an aging world.

Important advances have begun to shed light on the intricate processes that mediate chronic age-associated thymic involution. In this review, we will present the emerging paradigm that thymus tissue homeostasis is regulated by a balance of thymosuppressive and

thymostimulatory factors and the potential for these pathways to be exploited for therapeutic thymus restoration and peripheral immune reconstitution.

Thymic involution associated with aging

Age-related thymic atrophy (human and mouse)

In 1985, Steinman defined the morphology of the human thymus and elegantly demonstrated that thymic function gradually starts to decrease from the first year of life [1]. Crucial to this observation was the appreciation of dual components of the human thymus, the true thymic epithelial space (TES) in which thymopoiesis occurs and the nonepithelial, nonthymopoietic perivascular space (PVS) [1]. The TES (cortex and medulla) contains keratin-positive thymic epithelium that nurtures developing thymocytes, but the PVS stroma is keratin negative and does not harbor thymopoiesis (Figure 1a) [2].

Steinman and colleagues also showed that the lymphoid component within the TES begins to involute shortly after birth and decreases ~3%/yr through middle age (35-45 years of age) and continues to decrease ~1%/yr throughout the rest of life. The expansion of the PVS (adipocytes, peripheral lymphocytes, stroma) with age results in a shift in the ratio of true TES to PVS, with the TES shrinking to <10% of the total thymus tissue by 70 years of age. These changes are exemplified in Figure 1a, which shows stained thymus tissue sections from young (~3 days) and aged (60 years) healthy donors [2]. Also shown in Figure 1a are CD4 and CD8 flow cytometric analyses of freshly isolated thymocytes from young (3 days) and aged (demonstrates ~78 years) healthy donors. This the well-described loss of maturing DP thymocytes with advancing age, and is further supported by an *in situ* loss of TCR gene rearrangement [3]. When extrapolated, these observations suggest the thymus would completely cease to produce new T cells at ~105 years of age [4].

Overall, the progressive loss of TES and functional thymopoiesis with human aging results in a decreased output of naïve T cells to the periphery and the detrimental creation of a restricted peripheral T-cell repertoire [5]. Using sjTREC (single joint T-cell receptor excision circle) analysis (see Box 2 for details), we have reported a steady three log decline in circulating naïve T cells across an 80-year lifespan (Figure 1b) [3]. Each new T cell that is produced in the aged thymus is healthy; however, there are far fewer thymocytes produced in the aged thymus than in the young thymus. Therefore, biological pathways and factors in youth that drive robust thymopoiesis must gradually diminish and coincide with constriction of TES (i.e. involution), which results in a diminished quantity and quality of thymocytes exported over time (i.e. thymopoiesis). The question, however, of whether epithelial tissue degeneration is causative for or a response to reduction in developing thymocytes is still an open question and active area of investigation.

Box 1. The thymus and thymocyte development

The thymus is made up of two main histologic components: the perivascular space (PVS) and the thymic epithelial space (TES) [69]. The latter is the site of active T-cell development, or thymopoiesis, and is further subdivided into the cortical and medullary functional compartments. Thymic progenitor cells migrate to the thymus from the bone marrow and begin their differentiation and education process as CD3⁻CD4⁻CD8⁻ triple negative (TN) thymocytes [70]. Subsequent maturation and education of these cells involves an orchestrated interplay between developing thymocytes and the complex soluble and cellular components of the thymic microenvironment [71]. TN cells acquire CD3 and progress to CD4⁻CD8⁻ double-negative (DN) thymocytes. These early T-cell progenitors then migrate through distinct subsets: DN I (CD44⁺CD25⁻) to DN II (CD44⁺CD25⁺) to DN III (CD44⁻CD25⁺) to DN IV (CD44⁻CD25⁻) and then to CD4⁺CD8⁺ double-positive (DP)

thymocytes. Rearrangement of T-cell antigen receptor (TCR) genes occurs and is followed by positive and negative thymocyte selection. During this education process, cells downregulate one of their co-receptors to become CD4 or CD8 single-positive (SP) naïve T cells and are exported to the periphery as functional T helper or cytotoxic T cells, respectively.

Crucial to our understanding of human thymic involution and the potential development of therapeutics is the use of mouse aging models (see Box 2 and article by Maue *et al.* also in this issue). To this end, it has been important that we fully understand the nature of age-associated thymic involution in wild-type mice. Several years ago, this question was addressed in a comprehensive analysis of 6- to 90-week-old BALB/c mice [6]. We demonstrated that both thymus weight and number of thymocytes significantly decreased with age. This decrease was detectable by as early as 12 weeks of age, compared with thymus at age 6 weeks. After 35 weeks of age, thymus weight bottomed out at ~45% of the original 6-week size, whereas the number of thymocytes decreased from 6 to 90 weeks of age. This retained thymic weight was most probably caused by the residual thymocyte-depleted stroma. mTRECs (murine T cell receptor excision circles) per milligram of thymus tissue significantly decreased with increasing age during the 90 weeks studied, thereby demonstrating a gradual decrease in thymopoiesis with age.

Box 2. Investigating thymic function

Mouse model systems offer substantial advantages when studying the intricacies of age-associated thymic involution. Hallmarks of thymic health such as tissue weight, cellularity, histology and thymocyte phenotype can be readily measured and quantified in the mouse. Moreover, comparative gene expression profiling, molecular interaction analyses and mechanistic studies can be easily performed with the availability of a wide range of induced recombinant mutant mouse strains.

Noninvasive molecular analysis of signal-joint T-cell receptor (TCR) excision circles (sjTRECs) in thymus tissue, thymocytes and peripheral T-cell subsets by quantitative real-time PCR has been illuminating with regard to human thymic involution and peripheral naïve T-cell homeostasis in a variety of clinical settings [3,72]. sjTRECs are nonreplicated episomal circles of excised genomic DNA exclusively found in T cells [10]. sjTRECs are carried by T cells until they are diluted by peripheral expansion into T-cell clonal progeny. A similar quantitative assay has been developed for assessing mouse thymopoiesis called mouse sjTREC (mTREC) [6]. This assay has expanded our ability to quantify thymic function and TCR gene rearrangement across the lifespan in mouse models. A comprehensive review of techniques for monitoring thymopoiesis and TCR diversity can be found in Hudson *et al.* [73].

Splenocytes from aged BALB/c mice were also examined to determine the impact of thymic atrophy associated with aging on peripheral naïve T-cell levels and mTREC levels [6]. At 90 weeks of age, the number of mTRECs in CD4⁺ splenocytes significantly dropped compared with 6-week-old mice. By contrast, mTRECs per 100 000 CD8⁺ splenocytes began decreasing at 12 weeks of age and significantly dropped below the limit of detection of the assay at 90 weeks of age. These data suggested that, despite decreasing thymic output, the CD4⁺ compartment maintained a constant level of TREC⁺ cells by at least 61 weeks of age. Despite a steady decline in thymopoiesis with age, we demonstrated detectable thymic function in adult mice. The most persuasive data for this observation are the findings of continued mTREC production within the aged thymus as expressed as mTRECs per 100 000 thymocytes. This is

similar to what we and others have reported for thymic involution associated with aging in humans [3,7].

Mechanisms of thymic involution

Several possible mechanisms for age-related thymic involution have been suggested. These include blockage of T-cell receptor gene rearrangement, decreased self-peptide MHC molecules, and depletion of T-cell progenitors [8]. An emerging area of investigation to define intrathymic mechanisms of age-induced thymic atrophy is focused on the loss or disruption of key cross-talk events between developing thymocytes and the supportive thymic stroma (both cortex and medulla).

Thymopoiesis is also regulated by crucial cytokines produced by the thymic epithelial cells of the stroma. These cytokines work in concert to tightly regulate the maturation and education of lymphocytes. However, as the body ages, expression patterns of these cytokines change. Thymic epithelial cells can produce several hematopoietic cytokines such as G-CSF, GM-CSF, interleukin 1 (IL-1), IL-3, IL-6, IL-7, macrophage-colony stimulating factor (M-CSF), stem cell factor (SCF), transforming growth factor β (TGF- β), oncostatin M (OSM), and leukemia inhibitory factor (LIF) [3,8]. Intrathymic and systemic production of these potent cytokines regulates the complex process of thymopoiesis and thymic involution.

To specifically address the role of cytokines in regulating thymic involution, we profiled cytokine mRNA steady-state levels in 45 normal human thymus tissues (aged 3 days to 78 years, Duke Human Vaccine Institute Thymus Tissue Bank) during aging and correlated cytokine mRNA levels with thymic sjTREC levels [3]. We found that LIF, OSM, SCF, IL-6 and M-CSF steady-state mRNAs were expressed significantly higher in aged human thymus [3]. The increase in steady-state mRNA expression of LIF, SCF, IL-6 and M-CSF correlated with age and also correlated with decreasing thymic sjTRECs. We also observed that mRNA levels of IL-7, a potent tropic cytokine for thymopoiesis, remained constant in the aged human thymus. This suggested that loss of thymostimulatory factor (e.g. IL-7) expression alone is not mechanistically sufficient for age-induced thymic involution. When exogenously administered to young mice, these IL-6 family cytokines (i.e. LIF, OSM and IL-6) induced rapid and acute thymus gland involution and decreased thymic export to the periphery [3]. LIF has also been specifically implicated as a crucial mediator of thymic atrophy induced by bacterial endotoxin [9], an acute phenomenon similar to age-related thymic atrophy. In these studies, we also defined the essential role of both systemic- and intrathymic-derived corticosteroids as key final effectors in thymic atrophy [9]. Last, RNA expression studies on human thymus tissue and adipose tissue have demonstrated that IL-7 as well as LIF, OSM, IL-6 and M-CSF are all produced by adipocytes and thymic epithelial cells [3].

Overall, these studies promote the concept that intrathymic cytokines, whether their loss or their increase, play a crucial role in actively driving thymic involution. These studies, however, are just a shot in the dark into the workings of a complex postnatal tissue. To fully elucidate the factors that drive thymopoiesis in youth and thymic atrophy associated with aging, a more broad systems biology approach needs to be used to identify key proteins, their location in the thymus and the pathways they modulate.

Thymopoiesis persists in the elderly

Despite extensive documentation that the thymus (human and mouse) becomes atrophic with age and that this process begins early in life, there is growing evidence that thymus tissue is plastic, thymopoiesis continues in TES stromal islands and the involution process has the potential to be therapeutically halted or reversed. Histological and molecular studies of human thymus tissue support this speculation and suggest that thymopoiesis persists into the eighth

decade of life [2,3,7]. Figure 1c shows stained thymus tissue from a 78-year-old woman in which islands of cortical and medullary thymic epithelial tissue can be seen as dark blue patches in a sea of adipocyte-infiltrated PVS. Immunohistochemical staining of these TES islands with antibodies for cytokeratin, CD1a and Ki67 (a cell proliferation marker) provide evidence of healthy thymic epithelium and actively developing thymocytes (Figure 1d). Molecular analysis of adult thymus tissue corroborates the histological analyses and demonstrates thymopoiesis continuing into at least the fifth decade of life [3,7,10,11]. The functional capacity of TES islands in the elderly might be exploited with thymic reconstitution therapies to augment thymopoiesis and promote output of naïve T cells.

Enhancement of immune reconstitution by cytokine modulation

Cytotoxic therapies such as chemotherapy and radiotherapy are common treatments for malignant disease and are often coupled with hematopoietic stem cell transplant (HSCT). Delayed immune reconstitution in adults has been correlated with increased morbidity and mortality caused by infection [12-17] and tumor recurrence [18-20]. One of the most implicated predictive factors for this poor recovery is patient age. Crucial to immune recovery, therefore, is a functional thymus. Thus, it is of paramount importance to develop strategies that enhance thymic output and promote immune reconstitution, particularly in aged individuals.

Some cytokines and other signaling factors have been found to have a stimulating effect on the aged mouse thymus. For instance thymopoiesis can be increased in mice after administration of ghrelin, an anti-inflammatory peptide linked to metabolism and adipogenesis [21]. Growth hormone has also been shown to be thymostimulatory in murine models of thymic involution induced by aging and ablative bone marrow transplantation [22]. We will focus here on promising studies involving the use of keratinocyte growth factor (KGF) and IL-7.

Keratinocyte Growth Factor

KGF (Box 3) is approved by the US Food and Drug Administration for oral mucositis prophylaxis in patients receiving myeloablative therapies before HSCT [23]. The stimulatory effects of KGF on the thymus have also been studied in mouse models of thymic involution and suggest it to be a viable therapeutic candidate for accelerated immune recovery.

Box 3. Keratinocyte growth factor and interleukin 7 in the thymus

Keratinocyte growth factor (KGF), also known as fibroblast growth factor 7 (FGF7), is produced primarily by cells of mesenchymal origin and is an epithelial cell mitogen. Within the thymus, thymocyte production of KGF increases during differentiation, with the highest levels being produced by single-positive (SP) thymocytes [74]. A population of thymic fibroblasts has been identified as the predominant producer of KGF in the thymic stromal compartment [74]. The KGF receptor, FGF2IIIb, is expressed predominantly on, but not exclusively by, epithelial cells [75]. In the thymus, FGFR2IIIb is expressed on thymic epithelial cells but not on developing T cells [76].

Interleukin 7 (IL-7), a nonredundant cytokine for both T- and B-cell development in mice, is a 25-kDa glycoprotein produced predominantly by bone marrow and thymic stromal cells [77], as well as keratinocytes and enterocytes [78]. IL-7 receptor (IL-7R) is made up of an α -chain (CD127) and the common cytokine receptor γ -chain (γ c) [79]. IL-7R is expressed on common lymphoid precursors, developing B cells, triple-negative (TN) and SP thymocytes, thymic dendritic cells (DCs), CD4⁺, CD8⁺ and $\gamma\delta$ T cells and monocytes, as well as nonhematopoietic cells such as keratinocytes and intestinal epithelial cells [78]. IL-7 augments survival of TN and SP thymocytes [80,81] and is also essential for $\gamma\delta$ T cells [31] and thymic DC [82] development.

In young and old mice receiving immune ablative cyclophosphamide, dexamethasone or irradiation, KGF administration results in a significant increase in thymic cellularity compared with untreated control mice [24]. Age-related thymic microenvironment disruption in old mice was also reversed with KGF administration. In aged mice, these changes translated into an increase in peripheral T-cell numbers and function [24,25]. Continued KGF treatment led to an increase in thymic cellularity [25]. In young mice treated with KGF, there was a transient expansion of the thymic epithelial cell (TEC) compartment and differentiation of immature TECs. This led to enhanced thymocyte development and increased T-cell export [26]. KGF pretreatment of murine recipients of allogeneic [24,27] and syngeneic [27] bone marrow transplants (BMTs) led to enhanced thymopoiesis and peripheral T-cell reconstitution [24, 27]. Similarly, KGF treatment of rhesus macaques before CD34⁺ peripheral blood progenitor transplant restored thymic architecture and improved thymic-dependent T-cell reconstitution. Specifically, naïve T-cell and sjTREC numbers were increased in the KGF-treated group as was peripheral T-cell function [28].

Interleukin-7

IL-7 is crucial for both T- and B-cell development in mice (Box 3). Lymphocyte development is severely impaired in mice lacking IL-7 or IL7 receptor (IL-7R) or treated with anti-IL-7 antibodies. Thymic size and cellularity is dramatically decreased in IL-7 and IL7-R knockout mice [29,30]. This results in a decreased number of $\alpha\beta$ T cells and an absence of $\gamma\delta$ T cells [31]. In humans, severe combined immunodeficiency syndrome (SCID) and a complete lack of T cells results from a defect in IL-7R [32]. Unlike mice, humans do not require IL-7 for normal B-cell development [32].

IL-7 has been administered to mice as a means of reversing age-related thymic involution, with varying degrees of success. After IL-7 administration, an increase can be observed in triple-negative (TN) thymocytes [33], whereas another study found an increase in early TN thymocytes, but not in the transition to TN4 cells, thymic size or thymic output when aged murine recipients were intrathymically transplanted with an IL-7-producing stromal cell line [34]. However, several other reports did not observe specific augmentation of thymopoiesis or thymic export after IL-7 administration to aged mice [6,35-38].

Although there is still controversy as to whether IL-7 levels are altered with age, what is clear is that the administration of IL-7, after periods of immunodeficiency, enhances T-cell reconstitution. In both syngeneic and allogeneic (young and 9 months old) BMT mouse models, administration of IL-7 leads to enhanced T-cell reconstitution through increased thymic T-cell development and homeostatic proliferation, as well as decreased T-cell apoptosis but increased T-cell function [39-45].

Enhancement of immune reconstitution by modulation of sex steroids

Acceleration of thymic decline at puberty is intriguing and suggests a link with increasing levels of physiologic sex steroids. Exogenous administration of sex steroids, however, in the adult causes almost total collapse of thymopoiesis through apoptosis, primarily of the immature cortical thymocytes [46]. Thymic expression of androgen receptors increases with age, and this might contribute to alterations in the downstream signaling pathways with development, which leads to sex steroid-induced cell death and production of immunosuppressive cytokines such as TGF- β [47]. Hence, one of the key questions is how sex steroids can mediate their inhibitory effects on adult thymus. Because the receptors are expressed on both thymocytes [48] and stromal cells [49], age-dependent atrophy could be caused by direct loss of both cell types. However, given the well-recognized symbiotic developmental relationship between the multiple subsets of thymocytes and stromal cells [50-52], the effects on either compartment could be caused by loss or modulation of the other.

Because sex steroids are clearly linked to thymic degeneration, intervening in this pathway represents a potential means of reversing thymic atrophy. As early as 1896, castration was shown to increase the size of what we now know as the thymus. More recently, several groups have showed that castration does induce thymic rejuvenation in rodents, the effects of which could be achieved either by surgical removal of the gonads or chemical blockage of sex steroid production. The latter is accomplished by interfering with the normally cyclic hypothalamic-derived luteinizing hormone-releasing hormone (LHRH; gonadotrophin-releasing hormone or GnRH) signaling to the pituitary gland to induce gonadotrophins and is termed sex steroid ablation (SSA) [53]. This is most commonly achieved by supersaturating pituitary signaling using potent agonist variants of LHRH. Such drugs are standard care for many millions of patients with sex steroid-exacerbated disease, for example, prostate cancer, endometriosis and some forms of breast cancer [54-56]. Although intervention in gonadotrophin signaling can also be achieved using LHRH antagonists, the agonists have been successful clinically.

LHRH receptor is expressed in thymus, potentially enabling LHRH agonists (LHRH-A) to have direct stimulatory effects that might contribute to improved T-cell function. Indeed, it was found that preincubating rat thymocytes with LHRH-A or LHRH peptide induced a significant dose-dependent increase in proliferative response to the mitogen concanavalin-A. This response was abolished with simultaneous addition of a LHRH antagonist [57].

What are the initial events in thymic rejuvenation after SSA?

Given the relatively long duration (months in mice, years in humans) for thymic decline, it was remarkable to observe the rapidity of regeneration and synchronous expansion of thymocyte subsets in aged mice undergoing SSA [58]. In addition to decreased levels of thymocyte apoptosis, which is elevated with age, within 3 days there was already a significant increase in the proportion of all four TN subsets is observed. This was reflected numerically by day 5, by which time they had returned to levels indistinguishable from young controls [59]. The increase in TN cells was accompanied by enhanced proliferation in all thymocyte subsets and increased levels of the IL-7R α . Interestingly, early T-lineage progenitors (ETPs), the most immature intrathymic progenitors, were also significantly elevated by day 5, but not because of enhanced proliferation. Because we have also found that SSA improved bone marrow function, with numerical and functional increases in hematopoietic stem cells [60], the effect on ETPs might reflect more robust influx of thymic progenitors. CD4⁺CD8⁺ (double positive, DP) thymocytes were also expanded by day 5, presumably by *de novo* differentiation from in the increased TN population, but there were no major changes in the mature CD4⁺ or CD8⁺ single-positive (SP) cells until day 10, when CD4⁺ cells were detected at increased levels. Total thymocyte numbers were restored to those of young controls by day 7 and further elevated by 2-4 weeks.

Importantly, there was no evidence of any pathological T-cell development (i.e. autoreactivity), one explanation for which was the normalization of the thymic stromal microenvironment with complete restoration of the well-defined cortical and medullary epithelium occurring soon after, and perhaps induced by, thymocyte recovery. There was an increase in the proportion of the major histocompatibility complex class II high expressing (MHCII-high) TECs by day 14, with a return to normal proportions of MHCII-high and -low expressing TECs by day 28 [61]. The origins of the epithelial regeneration were at least in part caused by increased levels of proliferation of the individual subsets, restoring a normal young cortical TEC:medullary TEC ratio. Although medullary epithelial cells expressing low levels of MHCII (mTEC-low) are probably progenitors of mTEC-high cells, a key question is the contribution of putative thymic epithelial stem cells, equivalent to those enriched by the expression of MTS 24, a lymphocyte progenitor marker, in the early embryo [62]. MTS 24⁺ cells do proliferate during SSA regeneration, but formal proof of their progenitor status in adults is still lacking. There

was also a recovery of normal numbers and distribution of dendritic cells after SSA in mice [58].

In light of the fact that a major concern in the clinic is failure of adults to restore immune capacity after chemotherapy or ionizing radiation therapy, the impact of SSA on recovery of mice from the cytostatic agent cyclophosphamide was also examined. The effect of SSA was that it both reduced initial thymocyte loss and importantly, even in young mice, increased thymocyte number as quickly as 3 days. This reconstitution resulted in a more rapid total recovery of all T-cell subsets within 7 days compared with untreated controls, which were only half their pretreatment levels. Again, there was a major initial increase at the level of TN thymocytes with SSA. This was caused at least in part by enhanced proliferation but not by increased levels of ETPs serving as the pool for downstream T-cell development [59].

As expected, the dramatic increase in thymus function after SSA was translated to the periphery where naïve T cell levels, particularly CD4⁺, increased to normal levels, as did the CD4:CD8 ratio, which typically declines with age [58]. There was also increased peripheral T-cell function as manifested by increased responsiveness to CD3-CD28 receptor cross-linking and mitogens *in vitro* and challenge with human herpes simplex virus, transplantable tumors and vaccines [58,63]. Given the history of LHRH-A from a clinical perspective, it is important to note that the androgen ablation as standard of care for prostate cancer patients enhances naïve thymic-derived T-cell numbers [58], T-cell responsiveness to prostate antigens, perhaps by breaking self-tolerance and also their ability to home to and infiltrate the prostate gland, adding an important additional mechanism to the reduction in tumor burden [64,65].

Because thymopoiesis is reliant on a constant source of blood-borne progenitors, we examined in detail the impact of SSA on bone marrow function in the aged and after BMT or HSCT. There was a numerical increase in lineage⁻, Sca1⁺, c-Kit⁺ hematopoietic stem cells (LSK) of both donor and host-type in allogeneic and congenic HSCT after highdose ionizing irradiation [58,60,64]. This increased engraftment with SSA should have major applications in improving the efficacy of HSCT, including facilitating the use of umbilical cord blood hematopoietic stem cells (HSCs) for adult recipients by reducing the number of required cells. SSA again augmented thymus and peripheral T-cell recovery, retained the graft versus leukemia (GVL) response but did not exacerbate graft versus host disease (GVHD) in the allotransplant setting [64].

We have evaluated the effectiveness of LHRH-A for improving HSCT and restoring thymus function and immune competence in a clinical setting of high-dose myeloablative chemotherapy for hematological malignancies in adults [66]. The LHRH-A-treated patients had quicker recovery of neutrophils and platelets, increased levels of circulating T cells over 12 months, and most importantly, marked improvement in thymus function manifest as rejuvenated production of phenotypically naïve and sjTREC⁺ T cells from ~6 months. This is a similar time frame to that of reconstitution in children [66]. New T cells also displayed a broad T-cell receptor repertoire and increased responsiveness to *in vitro* stimulation.

Concluding remarks

The thymus and human immune system are generally believed to have evolved to last 40-50 years. With modern advances in medicine, the average lifespan is now twice that at ~80 years. We are therefore expecting an immune system to work beyond its 'designed' lifespan. As a result, with age, one becomes more susceptible to infection, chronic disease, cancer and autoimmune disorders and less able to generate protective immune responses to vaccination. These consequences can have a significant impact on global public health. Currently ~18% of the US population is over 65, and the proportion of this age group will steadily continue to

increase in years to come. The worldwide cost of medical treatment for problems associated with age-related immunosenescence is staggering in light of the number of people at risk.

Aging causes drastic architectural changes in the thymus, but residual functional areas of tissue remain late in life. Therapies are being developed that exploit the thymopoietic potential of these areas to promote reconstitution of the organ and increase output of appropriately educated naïve T cells (Figure 2). It is important to appreciate that these approaches might negatively impact thymic selection and peripheral tolerance with potentially long-term ramifications.

KGF is currently in phase II and III clinical trials as a treatment for mucositis in allogeneic BMT patients after high-dose chemotherapy [67]. Recombinant nonglycosylated human IL-7 (CYT 99 007; Cytheris, Inc.) has been used in preliminary human studies and shown to increase thymic export and expand both CD4 and CD8 T cells of naïve and memory phenotypes [68]. SSA also seems to provide a global boost to thymus and bone marrow function, with increased naïve T-cell production and functional improvement of the peripheral immune system. Possible mechanisms for thymic rejuvenation include restoration of the thymic epithelial microenvironment, an increase in thymus seeding by early T-lineage progenitors or increased proliferation and/or differentiation of TN, DP or SP thymocytes. Overall, investment in understanding the mechanisms and crucial pathways that drive thymic involution associated with aging is essential for development of innovative and safe therapies to combat immunosenescence that naturally occurs with age or as a result of immunoablative treatments.

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Figure 1.

Thymic function decreases with age. **(a)** Top panel shows cytokерatin-immunostained and hematoxylin & eosin (H&E)-counterstained thymus sections from healthy young and aged human donors. In the aged thymus, the perivascular space (P) is increased in size and the thymopoietic cortex (C) and medulla (M) are significantly constricted. Lower panel shows flow cytometric analysis of CD4 and CD8 on thymocytes isolated from healthy young and aged human thymus donors; a clear reduction in double positive (DP) CD4⁺CD8⁺ T cells be seen in aged individuals. **(b)** sjTREC (single joint T-cell receptor excision circle) measurement per microgram of circulating peripheral blood mononuclear cell (PBMC) DNA from healthy human donors ranging from <1 to ~80 years of age demonstrates a steady decline of naïve T-cell output with age. **(c)** Human thymus tissue from a 78-year-old female. Tissue was immunostained with anti-cytokeratin and H&E counterstained to show remaining islands of cortical and medullary tissue (blue). **(d)** Islands of cortical and medullary tissue in aged thymus show signs of active thymopoiesis by CD1a and Ki67 immunostain.

Figure 2.

Factors involved in thymic involution and reconstitution. Young thymus produces self-tolerant T cells expressing a broad T-cell receptor (TCR) repertoire, and this is supported in a well-delineated cortex and medulla by functionally distinct stromal cell populations. With age, there is a gradual reduction in total thymic cellularity, an increase in perivascular space (PVS), disruption of the thymic architecture, a reduced production of naïve T cells and a restriction in the peripheral TCR repertoire. Involution of the thymus occurs in parallel with increased production of sex steroids in humans. Treatment with thymostimulatory cytokines, such as keratinocyte growth factor (KGF), interleukin 7 (IL-7), growth hormone (GH), leptin and ghrelin, or sex steroid ablation therapy (SSA), can promote regeneration of an atrophic thymus—increasing total cellularity, restoring thymic architecture, increasing output of naïve T cells and rejuvenating the diversity of the peripheral TCR repertoire. This might occur by several mechanisms including an increase in thymus seeding by early T-lineage progenitors (ETPs) or increased proliferation and/or differentiation of triple negative (TN), double positive (DP) or single positive (SP) thymocytes.