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Thymic Rejuvenation and Aging

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Abstract

The thymus is a vital organ for homeostatic maintenance of the peripheral immune system. It is within this mediastinal tissue that T cells develop and are extensively educated and exported to the periphery for establishment of a functional and effective immune system. A striking paradoxical feature of this critical lymphoid tissue is that it undergoes profound age-associated involution. Thymic decline is of minimal consequence to healthy individuals, but the reduced efficacy of the immune system with age has direct aetiological linkages with an increase in diseases including opportunistic infections, autoimmunity, and incidence / burden of cancer. Furthermore the inability of adults to restore immune function following insult induced by chemotherapy, ionizing radiation exposure or therapy, and infections (e.g. HIV-1) leads to increased morbidity and often mortality in the elderly. For these reasons, it is important that investigators strive to translate their understanding of mechanisms that drive thymic involution, and develop safe and effective strategies to rejuvenate the thymus in settings of clinical need. In this review, we present a discussion of the current status of thymic rejuvenation efforts associated with: sex steroid ablation, cytokines, growth factors, and hormones.

Keywords

Aging; Thymopoiesis; Immune Reconstitution; Cytokines; Steroids and Hormones

INTRODUCTION

The thymus is made up of two compartments, the true thymic epithelial space (TES) and the perivascular space (PVS). The thymic epithelial space consists of the cortex and the medulla, which is where T cell development, maturation and induction of self-tolerance occur in a process known as thymopoiesis [1]. The cortex is the location of early thymocyte development including T cell receptor gene rearrangement and positive selection [2]. The medulla is the location of subsequent thymocyte development. Thymocytes that reach the medulla have successfully undergone T cell receptor gene rearrangement and positive selection. In the medulla, thymocytes undergo negative selection to remove auto-reactive T cells from the mature repertoire [2,3]. The perivascular space, composed of adipocytes, peripheral lymphocytes and stroma, is a non-epithelial and non-thymopoietic component of the thymus.

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Thymic function gradually starts to decrease from the first year of life [4]. With age there is an expansion of the perivascular space and a simultaneous shrinkage of the thymic epithelial space [5]. The lymphoid component within the TES (Cortex and Medulla) begins to involute shortly after birth and decreases ~3%/year through middle age (35–45 years of age) and ~1%/year throughout the rest of life. Involution of the TES and expansion of the PVS (adipocytes, peripheral lymphocytes, stroma) with age alters the TES to PVS ratio and leads to the TES becoming <10% of the total thymus tissue by age 70 years [4]. Reduction of TES and functional thymopoiesis, with aging, subsequently results in loss of TCR gene rearrangement and decreased output of naïve T cells to the periphery [6]. Using single joint T cell receptor excision circle analysis (sjTREC), a molecular marker for active thymopoiesis, we and others have reported a steady three-log decline in circulating naïve T cells across an 80 year lifespan [6]. Thus thymic involution with age results in diminished thymopoiesis, i.e. decreased quantity and quality of T cells exported across the lifespan (Figure 1) [7].

Age-associated thymic involution occurs in most vertebrates that possess a thymus but currently no evolutionary explanation has been confirmed. Shanley, *et al.* have speculated that robust thymocyte development and T cell production occur early in life to create a diverse innate repertoire and ensure survival of the individual. Since the majority of thymocytes do not make it to the peripheral pool, creating a diverse T cell repertoire is considered energy consuming. As the organism enters its reproductive stage (and has an established T cell repertoire) it has been suggested that the host benefits from down-regulation of thymopoiesis and rechanneling of energy to other systems, particularly reproduction [8]. Dowling and colleagues have suggested that there is a direct link between peripheral selection and the need for thymic involution with age; they purport that the thymus involutes to allow peripheral selection of a well-adapted T cell repertoire [9]. They suggest that thymic involution encourages peripheral homeostatic proliferation and survival of a limited repertoire of clones.

Mouse aging models have contributed to our understanding of thymic involution and aging. In a comprehensive study of BALB/c mice, aged 6 to 90 weeks old [10], we demonstrated that thymus weight and thymus cellularity significantly decreased with age. The decrease in thymus weight and thymocyte number was detected as early as 12 weeks of age. After 35 weeks of age, thymus weight decreased to no less than ~45% of the original 6week size, while the number of thymocytes steadily decreased across the mouse life span of 6 to 90 weeks. This retained thymic weight is attributed to the residual thymocyte-depleted stroma. As was done with human thymus, we used sjTREC to measure active thymopoiesis and found a significant decrease, with increasing age, of sjTREC per milligram of mouse thymus tissue. These findings thus demonstrated a gradual decrease in murine thymopoiesis with age, similar to that seen in humans [7].

More recent investigation has focused on cross-talk mechanisms between the supporting thymic stromal microenvironment and the developing thymocytes [11,12]. The thymus consists of cells of stromal and hematopoietic origin, and includes thymic epithelial cells (TECs), mesenchymal cells, endothelial cells and dendritic cells. TECs in the cortex and medulla form a complex network that supports T cell differentiation [13]. Differentiating thymocytes undergo migration through cortex and medulla and encounter functionally distinct microenvironments which regulate positive and negative selection [13]. Recent work by Petrie and colleagues [14,15] has shown that during thymocyte development lymphoid progenitors enter the thymus at the cortico–medullary junction, then migrate to the outer cortex, and finally return to the medulla.

To further support the idea that T cell development requires many unique interactions that can only be provided in the thymus, work by Anderson and colleagues [16] has shown that the thymus must function as a whole and in a three-dimensional structure for T cell differentiation to occur [17]. Taken together these studies demonstrate that in addition to T cell progenitors and developing thymocytes, the thymic stromal microenvironment and overall thymic tissue structure play vital roles in productive thymopoiesis and are disrupted with aging. Thus when developing thymic rejuvenation strategies, one needs to consider all cellular and non-cellular components of the thymus and the unique microenvironments and niches that nurture and educate T cells (Figure 1).

Sex Steroid Ablation

Although thymic involution is initiated in the first year of life, it is more readily observed/ documented around the time of in puberty. This is also a developmental timeframe when there is a dramatic increase in circulating sex steroids. Due to this temporal connection between puberty, sex steroids and loss of thymic function, sex steroid ablation (SSA) has been a long-standing area of investigation for potentially rejuvenating the thymus.

Various studies have examined the role of sex steroids on the thymus and have found a connection between sex steroids and the rejuvenation of the thymus. Mice castrated before puberty showed a delay in thymic atrophy [18–22], removal of sex steroids after puberty restores thymic architecture, increases in thymic cellularity and enhancement of thymopoiesis in aged mice and humans [23], and lastly, Boyd and colleagues have noted that the number of early thymic progenitors, which are decreased with age, are normalized after surgical castration [20,23].

Sex steroid ablation can be accomplished surgically or with administration of luteinizing hormone releasing hormone (LHRH) analogues, has been shown to have a positive effect on lymphoid progenitors which promote immune recovery following bone marrow transplant and cytoablative therapy [24–28]. While castration would not be considered in humans as a means for routine treatment of thymic involution, the use of LHRH could be considered as human studies with LHRH analogues have resulted in increased thymic weight and reversal of age-related thymic structural defects [19,29–31].

In addition to castration and the use of LHRH to reverse thymic involution, other studies that are taking advantage of sex steroid ablation involve intracellular androgen receptors (iARs). Expression of iARs has been shown to be present in both male and female thymus; in thymic stromal cells and in all thymocyte subsets [32–36]. Olsen and colleagues have studied androgen receptor signaling in thymus and suggest that the significant enlargement of the thymus, seen in castrated rodents, is mediated by the androgen receptor (AR) [37]. Additional studies support this rejuvenation approach by their report that thymic regeneration following castration is reversed in a dose-dependent manner by administration of testosterone or estrogen [22,32–34,38].

Although surgical sex steroid ablation is promising, a pharmaceutical-mediated approach to thymus rejuvenation is more likely tolerated in clinical settings. Lupron (Leuprolide) is one such therapeutic candidate. Lupron acts on the pituitary gland and controls the release of luteinizing hormone and follicle-stimulating hormone by desensitizing LHRH receptors resulting in a decrease of luteinizing hormone and follicle-stimulating hormone leading to a decrease in both estrogen and testosterone sex steroid production [39]. A currently recruiting clinical trial sponsored by the NCI/NIH is underway to assess the ability of Lupron to stimulate immune system function (i.e. thymopoiesis) in subjects receiving allogeneic hematopoietic stem cell transplant therapy (NCT01338987). Lupron/leuprolide was recently evaluated in a now closed study sponsored by M.D. Anderson Cancer Center

(NCT00254397; closed 10/2012). In this study the investigators tested the hypothesis that leuprolide augments immune responses to a melanoma-specific peptide vaccine (no results posted).

Cytokines and Growth Factors: IL-7, KGF and IL-22

Similar to bone marrow and peripheral sites, cytokines within the thymus are crucial for thymopoiesis. Moreover, biological pathways that control the thymic cytokine microenvironment represent candidate targets for therapies to reverse age-induced thymic involution. Thymic epithelial cells produce a number of colony stimulating factors and hematopoietic cytokines such as IL-1, IL-3, IL-6, IL-7, transforming growth factor TGF- β , oncostatin M (OSM), and leukemia inhibitory factor (LIF) [6,40–43]. Intrathymic and systemic production of these potent cytokines/growth factors regulate the complex process of thymopoiesis and are responsible for stromal cross-talk. Thus cytokines and growth factors are viable candidates for therapies aimed at rejuvenating thymopoiesis. IL-7, KGF and IL-22 are the most currently promising and will be discussed below.

Interleukin 7 is a 25 kDa glycoprotein produced by stromal cells in thymus and bone marrow. IL-7 promotes both survival and differentiation of immature triple negative, and mature single positive thymocytes. In the periphery IL-7 regulates T cell survival and function [44,45]. IL-7 binds the IL-7 receptor (IL-7R) which is expressed on many immune system cells, including lymphoid precursors CD3–CD4–CD8– (TN), CD4+CD8+ (DP) thymocytes and single positive CD4 or CD8 T cells [46].

Schluns et al. demonstrated that administration of IL-7 to normal non-lymphopenic mice resulted in expansion of both naïve and memory CD4 and CD8 peripheral T cells [47]. In addition, treatment with IL-7 restored thymopoiesis in IL-7 deficient mice. From various studies we know that the IL-7 receptor is a target for therapeutic intervention, particularly for restoring T cells in subjects undergoing T cell depleting regimens for medical treatments [47]. A recombinant form of human IL-7, CYT 99 007, has been given to patients in varying doses to promote thymic rejuvenation. It is reported that CYT 99 007 appears to have low toxicity and a dose-dependent sustained expansion of naïve and memory CD4 and CD8 T cells, as predicted by mouse pre-clinical studies [48]. Although promising, there are no current open IL-7 trials focusing on thymus rejuvenation and/or immune reconstitution in adults (clinicaltrials.gov; May 2013).

Keratinocyte growth factor (KGF, FGF7) has been identified as another key factor in thymic senescence and potential rebound. KGF signaling through FGFR2IIIb is critical for fetal thymus organogenesis [49] and is linked to proliferation and differentiation in both fetal and postnatal TECs [49–53]. Exploitation of KGF as a means to promote thymic microenvironment regeneration has been explored in a variety of pre-clinical settings and has moved into human clinical trials.

Administration of KGF has been well-documented to increase thymus size, TEC differentiation, and thymocyte production in aged animals [51–56]. Early studies in this area by Min and others demonstrated that KGF treatment promotes function of TECs and improves thymic function/output in aged mice [57]. Following one course of KGF treatment, Min et al. demonstrated that aged mice had an increase in thymic cellularity which was maintained for two months. KGF-treated aged mice in this study had restoration of defined cortical and medullary compartments, increased output of naïve phenotype CD4 T cells, and increased humoral responses to a T-dependent antigen [57]. This initial two-month improvement in thymic cellularity was sustained with monthly KGF follow-up administration. 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, are areas of active investigation.

A review of recently verified clinical trials (clinicaltrials.gov; May 2013) revealed a number of trials specifically investigating the regenerative potential of KGF (Palifermin or Kepivance) on thymic function in adults (ages 18–50 years old). Cambridge University Hospitals (NCT01712945) has a currently recruiting study investigating the hypothesis that giving KGF will prevent secondary autoimmunity problems associated with Campath-1H treatment for multiple sclerosis, due to its reported ability to promote thymic T cell regeneration in adults. An open phase II study sponsored by Indiana University is investigating the impact KGF administration on thymic rejuvenation in the setting of haplotype-mismatched hematopoietic stem cell (CD34+) transplant (NCT00593554). In addition to looking at improvement in treatment-related mortality, the investigators are also focusing on thymus-mediated immune recovery. Lastly, a study completed in July 2012 sponsored by the Fred Hutchinson Cancer Research Center and the University of Washington (NCT01233921) specifically investigated the pharmacodynamics of palifermin on thymic function in adult patients undergoing stem cell transplant for hematologic cancer. The primary hypothesis of this study was that KGF-induced thymic regeneration would prevent chronic graft versus host disease (no results posted). Together these active and recently closed studies demonstrate the active enthusiasm for the use of recombinant KGF as a supportive therapy to rejuvenate an age-damaged thymus and encourage robust immune recovery.

Interleukin 22 (IL-22), an IL-10 gene family member, is a mediator of cellular inflammatory responses [58] and has recently become a cytokine of interest to the field of thymus tissue rejuvenation, based on the ground-breaking work from the van den Brink laboratory. IL-22 is produced by T helper 17 cells and innate lymphoid cell subsets [59]. Although IL-22 primarily is known for its role in mucosal surface barrier maintenance by promoting innate antimicrobial molecules, IL-22 is also reported to mediate epithelial regeneration after tissue injury. Dudakov et al. demonstrated that IL-22 signals through the IL-22 receptor on the surface of thymic epithelial cells and promotes the proliferation and survival of thymocytes [59]. This is of particular interest because thymopoiesis is a process that involves many cell types, including thymic epithelial cells, and the supporting thymic stromal microenvironment [60]. Dudakov and colleagues went on to show that administration of IL-22 to wild-type mice after total body irradiation resulted in a significant increase in thymic cellularity, both developing thymocytes and TEC subsets [59]. Interestingly, immune recovery in irradiated IL-22 deficient mice was impaired, compared to controls [59]. These pre-clinical proof-of-concept studies demonstrating rejuvenation of damaged thymus tissue suggest that IL-22 therapy is worth further investigation and potential translation to clinical studies aimed at rejuvenating aged/involved thymus. There are currently no active studies in clinicaltrials.gov focused on IL-22 therapy and aged thymus rejuvenation.

Hormones: Growth Hormone and Ghrelin

Two hormones of long-standing interest to the thymic rejuvenation field are growth hormone and ghrelin. Growth hormone (GH) is a peptide hormone that impacts the immune system via its stimulatory effects on insulin-like growth factor 1 (IGF-1) [61]. Taub et al. have shown that hematopoietic cells can produce GH that indirectly promote immune reconstitution via IGF-1 production. IGF-1 provides anti-apoptotic signals and promotes immune cell survival [61]. IGF-1 may also act directly on thymic stromal cells as a component of the thymus microenvironment cross-talk network and stimulate IL-7 production. Intrathymic IL-7 in turn promotes and regulates T cell survival and function [62].

Multiple studies have demonstrated that exogenous administration of recombinant GH allows for regeneration of an aged or damaged thymus in a variety of settings (reviewed in [11,63]). Specifically, mouse studies have demonstrated that following syngeneic bone marrow transplantation, GH administration leads to overall enhanced hematopoiesis [64], and that in adult mice GH treatment results in increased spleen and bone marrow hematopoietic progenitors [65].

The NIA/NIH is currently recruiting for a hallmark study (NCT00663611) to investigate the impact of subcutaneous pulsatile GH (Somatropin) on overall age-induced immune senescence in adults (25–50 years old). The multipart study is first confirming a pulsatile pattern and dose delivered of GH via a pump. They will then investigate the impact of GH on immune function and metabolic profile in two expanded arms (+/- GH). This critical study will be highly informative to the field.

The HIV/AIDS research community has understandably embraced investigation into therapeutic approaches to rejuvenate an aged or damaged thymus. With the advent of anti-retrovirals and subsequent viral control in AIDS patients, support care to encourage robust thymus-mediated immune recovery is needed [66]. Three studies have recently closed (NCT0071240, NCT00287677, NCT00119769) all focusing on using recombinant GH as a therapeutic immune stimulant to promote thymus regrowth and recapitulation of a broad naïve T cell repertoire in adults being actively treated for HIV/AIDS. Although no outcome results are currently posted, these studies will shed light on the potential use of GH to promote thymic rejuvenation in adults with enhanced underlying immunodeficiency.

Another hormone of interest for thymus regeneration is Ghrelin. This metabolic hormone is believed to be a major regulator of food intake in humans by its effects on feeding centers in the hypothalamus [61]. In addition to inducing hunger, ghrelin has been shown to bind to various immune cell subsets with ghrelin-specific receptors [61]. Ghrelin also appears to localize with lipid rafts of activated T cells, site of T cell signaling [61], thus suggesting that ghrelin may be a potential target for T cell stimulation. Dixit et al. have reported that ghrelin reduces age-associated inflammatory responses in mice, and that in an aged thymus, ghrelin and GH levels decrease and correlate with functional thymic involution and generalized elevated inflammation [67]. In addition to suppression of inflammation, ghrelin has been shown to have a stimulatory effect on thymus cellularity. Administration of ghrelin to aged mice revealed an increase of early thymic progenitors in the thymus and an increase in the number of recent thymic emigrants exported to the periphery, as assessed by sjTREC [61].

Additional studies conducted by Taub and colleagues show that ghrelin might also play a role in improving thymic architecture [61]. Ghrelin infusions resulted in increased cortical and medullary epithelial cells along with an increase in early thymic progenitors in the thymus. Overall these studies support the potential use of ghrelin as a therapeutic for thymus rejuvenation. There are currently no open studies in clinicaltrials.gov focused on ghrelin and thymic rejuvenation.

CONCLUSION

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. As a result, with age we become more susceptible to infection, chronic disease, cancer and autoimmune disorders, and are less able to generate protective immune responses to vaccination. These consequences can have a significant impact on global public health.

By not fully appreciated mechanisms, aging causes drastic architectural changes in thymus that result in profound tissue involution, dramatic reduction in T cell export and reduced

overall peripheral immunity. There is however, residual thymic function in small islands of epithelium late in life. Thus, therapies are needed to exploit this thymopoietic potential and promote regeneration of the organ to increase output of naïve T cells when clinically needed. Promising basic research in the development of sex steroid ablation, cytokine treatment (KGF, IL-7 and IL-22) and hormone therapy (GH, Ghrelin) has been presented. These thymus rejuvenation approaches are at different stages of development along the translational pipeline. To augment and enhance our ability to develop safe and effective thymic/immune rejuvenation in adults, a substantial commitment needs to be made to understanding the mechanisms that mediate this age-associated process.

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HIGHLIGHTS

- Thymus is a vital organ for homeostatic maintenance of a functional immune system.
- Reduced efficacy of the immune system leads to increased morbidity in the elderly.
- Currently being explored: sex steroid ablation, cytokine, growth factor and hormone therapy.

Figure 1.

Young thymus produces self-tolerant T cells expressing a broad T cell receptor (TCR) repertoire 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), a disruption of the thymic architecture, a reduced production of naïve T cells, and a restriction in the peripheral TCR repertoire. The goal of therapeutic thymus rejuvenation is restoration of thymic architecture, increased output of naïve T cells and regeneration of a diverse the peripheral TCR repertoire.