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Thymic stromal cells: roles in atrophy and age-associated dysfunction of the thymus

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Abstract

Atrophy of the thymus, the primary site of T lymphocyte generation, is a hallmark of the aging immune system. Age-associated thymic atrophy results in diminished output of new, naïve T cells, with immune sequelae that include diminished responses to novel pathogenic challenge and vaccines, as well as diminished tumor surveillance. Although a variety of stimuli are known to regulate transient thymic atrophy, mechanisms governing progressive age-associated atrophy have been difficult to resolve. This has been due in part to the fact that one of the primary targets of age-associated thymic atrophy is a relatively rare population, thymic stromal cells. This review focuses on changes in thymic stromal cells during aging and on the contributions of periodic, stochastic, and progressive causes of thymic atrophy.

1. Introduction

The thymus is the primary site of generation of new, naïve T lymphocytes, which are generated in the thymus by periodic recruitment of circulating multi-potent thymus seeding progenitors (Bhandoola et al., 2007; Foss et al., 2001; Goldschneider et al., 1986; Luc et al., 2012; Luis et al., 2016). Mutually inductive signaling in the unique stromal microenvironment of the thymus, comprised of a heterogeneous population of thymic epithelial cells (TECs), as well as mesenchymal, neural, vascular, and hematopoietic populations (including dendritic cells, macrophages, and B cells), directs these multi-potent progenitors along a well-characterized program of differentiation, proliferation, and selection to generate a self-tolerant, self-restricted T cell population (Abramson and Anderson, 2017; Klug et al., 1998; Petrie and Zuniga-Pflucker, 2007). However, the thymus begins to atrophy relatively early in life, with concomitant reduction in production of new, naïve T cells (Aspinall et al., 2010; Hale et al., 2006; Hartwig and Steinmann, 1994; Steinmann et al., 1985). This results in shift toward an oligoclonal memory T cell population with diminished T cell receptor diversity (Ernst et al., 1990; Hale et al., 2006; Haynes et al., 2000b; Nikolich-Zugich and Rudd, 2010; Utsuyama et al., 1992). The resulting immunodeficiencies include diminished vaccine responsiveness and tumor surveillance, as

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well as decreased response to new infections, especially viral infection (including a well characterized loss of immunity against influenza A epitopes) ((Yager et al., 2008), reviewed in (Nikolich-Zugich and Rudd, 2010) (Dicarlo et al., 2009; Montecino-Rodriguez et al., 2013)).

Both the thymic stromal population and the hematopoietic stem cells (HSC) that ultimately give rise to T cells undergo extensive changes with age. Changes in HSCs include altered homing and increased bias toward myeloid, rather than lymphoid development (reviewed in (Chinn et al., 2012; Montecino-Rodriguez et al., 2013; Palmer, 2013)). However, thymic atrophy, which begins at approximately 7 weeks of age in mice (Manley et al., 2011), precedes the initiation of these changes in the hematopoietic population, which begin later in life, at around 7 months of age in mice (Montecino-Rodriguez et al., 2013). A large body of evidence supports the notion that the stromal population in the thymus is a primary target of age-associated thymic dysfunction (recently reviewed in (Lepletier et al., 2015; Masters et al., 2017)). For instance, transplant experiments employing age mismatched donors and hosts support the notion that age-associated defects in thymus size and architecture are primarily a function of the age of the thymic microenvironment rather than the age of the transplanted bone marrow cells (Doria et al., 1997; Mackall et al., 1998; Zhu et al., 2007). Functional assessments of recent thymic emigrants (RTE) in age mismatched bone marrow chimeras showed that the aged thymic microenvironment and lymphoid-intrinsic factors both contribute to age-associated declines in RTE function (Clise-Dwyer et al., 2007), and recent work has shown that transplant of young TECs into aged thymi can increase T cell production (Kim et al., 2015). Consistent with these studies, we, and others (Sutherland et al., 2005), have found that the lymphoid phenotype in the thymus is essentially unchanged at 12 months of age in mice, and that age-associated transcriptional changes predominately occur in stromal, rather than lymphoid populations (Griffith et al., 2012).

2. Histological and transcriptional characteristics of aging thymic stroma

Histological changes in thymus architecture have been observed as early as the first few years of life in humans, including the expansion of perivascular space and adipose tissue relative to functional cortical and medullary tissue (Steinmann et al., 1985), and loss of cortico-medullary organization (Henry and Anderson, 1987). In the mouse, histological changes are obvious by 3 months of age, and also include increases in adipose tissue (Yang et al., 2009), as well as loss of cortico-medullary junction integrity and cortical thinning (Manley et al., 2011), and decreased complexity of medullary islet morphology (Griffith et al., 2012) (and reviewed in (Manley et al., 2011; Montecino-Rodriguez et al., 2013; Taub and Longo, 2005)). In humans, total thymus size is considered to remain relatively constant during aging, though functional lymphopoietic tissue is replaced by perivascular space and adipose tissue over time, whereas in murine studies, declines in total thymus size (weight) are well characterized (Haynes et al., 2000a). Nonetheless, in both mice and humans, as well as many other vertebrate species as discussed below (Kendall, 1981), the volume of lymphopoietic tissue within the thymus and thymic generation of new T cells decline beginning early in life and are progressive during aging.

Transcriptome-based approaches have revealed extensive changes in stromal cells. Murine studies based on microdissected whole thymus tissue showed that the stromal compartment, especially cortical stroma, undergoes broad transcriptional changes within the first year of life, while relatively few changes are apparent in major thymic T cell subsets (Griffith et al., 2012). In these studies, transcriptional changes may reflect altered relative frequencies of stromal subpopulations and/or alterations in gene expression within individual stromal populations. Stromal transcriptional changes include changes in Wnt pathway signaling associated with stem cell expansion and epithelial cell division (Ferrando-Martinez et al., 2015; Kvell et al., 2010; Wei et al., 2015). In particular, studies have found decreased expression of *Wnt3a* and *Wnt4*, and increases in *Wnt10a* and *Wnt5b* in the aged thymus (Bredenkamp et al., 2014; Griffith et al., 2012; Ki et al., 2014; Kvell et al., 2010; Wei et al., 2015). These results are consistent with known roles for *Wnt3a* and *Wnt4* in cell proliferation (Liu et al., 2010) and *Foxn1* expression (Balciunaite et al., 2002; Kvell et al., 2010; Wei et al., 2015), respectively. Increased expression of *Wnt5b* is consistent with known roles for *Wnt5b* in promotion of adipogenesis (van Tienen et al., 2009).

Expression of tissue restricted self-antigens, critical for tolerizing developing T cells (Derbinski et al., 2005; Klein et al., 2014), declines with age in the stromal population as a whole (Griffith et al., 2012), and within the epithelial compartment (Bredenkamp et al., 2014), revealing a potential mechanistic link between aging and age-associated increases in autoimmunity (Muller and Pawelec, 2015). There is also evidence to support the notion that thymus atrophy is associated with declines in negative selection of autoreactive T cells in a mouse model of thymus atrophy induced by *Foxn1* deficiency (Coder et al., 2015). In addition, diminished T cell output after thymus atrophy may promote autoimmunity by allowing homeostatic expansion of autoreactive T cells in the periphery (Hakim and Gress, 2007; King et al., 2004).

Studies based on purified stromal populations, including cortical and medullary thymic epithelial cells (cTECs, mTECs), dendritic cells, and fibroblasts, revealed transcriptional changes associated with atrophy as early as 3 months of age in mice (Ki et al., 2014). Among these cell types, most age-associated changes were found in dendritic cells and MHCII^{lo} mTECs. Within the MHCII^{lo} mTEC (mTEC^{lo}) population, age-associated changes included diminished expression of cell cycle regulators (*i.e.* *Cdc20*, *Cdc6*). In both cTECs and the mTEC^{lo} population, significant decreases were found in transcription of targets of the transcription factor E2F3, an important cell cycle regulator. These declines in expression of genes important for proliferation are consistent with evidence that TEC proliferation declines during aging (Gray et al., 2006), and that enforced TEC proliferation increases thymus size in aged mice (Garfin et al., 2013; Robles et al., 1996). In dendritic cells, aging was associated with increased expression of proinflammatory regulators such as *Il1a*, *Il1b*, *Il6* and *Tnf* (Ki et al., 2014).

Another critical change that has been identified in the aging thymus is diminished expression of *Foxn1* in epithelial cells. *Foxn1* is a transcriptional regulator necessary for TEC development and function (Nehls et al., 1994; Vaidya et al., 2016). Expression of *Foxn1* diminishes with age in the thymus of mice (Chen et al., 2009; Ortman et al., 2002) and humans (Reis et al., 2015), and overexpression is sufficient to restore not only thymus

size (Zook et al., 2011), but also medullary islet complexity and TRA expression, even in 12 month-old mice, highlighting its potential as a therapeutic target for long-term reversal of thymus size and function (Bredenkamp et al., 2014; Gallo et al., 2017; Rode et al., 2015).

3. Mechanisms governing thymic atrophy

Although broad changes in thymus structure and function during aging have been described, the mechanisms governing age-associated thymic atrophy have been difficult to resolve. This is in part due to the diversity of periodic, episodic, and cumulative events that influence thymus size and function over the lifespan (Figure 1). Several key contributors have emerged, as described below.

Significant reciprocal feedback occurs between the thymus and endocrine system (reviewed in (Montecino-Rodriguez et al., 2005)). The thymus has role in regulating reproductive functions (Besedovsky and Sorkin, 1974; Pierpaoli and Sorkin, 1972; Weinstein, 1978), and there is a clear inverse relationship between androgens and thymus size (Olsen and Kovacs, 1996; Olsen et al., 1998). The effects of sex steroids on thymus size are remarkable, including atrophy of the thymus during pregnancy (Tibbetts et al., 1999) and rebound after castration (Heng et al., 2005; Leposavic et al., 1996; Sutherland et al., 2005; Utsuyama and Hirokawa, 1989). However, the transient nature of thymic rebound after castration ((Griffith et al., 2012; Leposavic et al., 1996; Utsuyama and Hirokawa, 1989)), and diminished rebound in older rodents relative to young, support the notion that other age-associated factors are the predominant factors in determining steady state thymus size (Montecino-Rodriguez et al., 2005). This notion is also supported by studies reviewing thymus size in species from diverse vertebrate classes exhibiting seasonal breeding patterns (Osteichthyes, Amphibia, Reptilia, Aves, and Mammalia), indicating that during each breeding season, when sex steroids are present at relatively high levels, the thymus diminishes in size temporarily, regenerating each year, but to a diminished degree, as the organisms age (Kendall, 1981). Growth hormone (GH) and insulin-like growth factor-I (IGF-I) have also been characterized as regulators of age-associated thymic atrophy, as both hormones promote thymus growth (Bar-Dayana and Small, 1994; Kelley et al., 1986; Montecino-Rodriguez et al., 1998), and production of GH declines with age (Lamberts et al., 1997). As with androgens, while the effects of hormone treatment on thymus size are large in relatively young mice, effects in older mice are more limited, suggesting other age-associated factors prevent sustained reversal of thymic atrophy (Montecino-Rodriguez et al., 2005).

The hormonal stimuli discussed above may be periodic, that is, occurring at regular intervals (e.g. breeding season), or sporadic (episodic) (e.g. pregnancy), and while their effects on thymus size can be large in magnitude, they are generally reversible. Sporadic events such as infection-induced inflammation and glucocorticoid release in response to a variety of physiological stressors also negatively affect thymus size (reviewed in (Majumdar and Nandi, 2017; Taub and Longo, 2005)). Recent work has shown that miR-205 expression in thymic epithelial cells (TECs) promotes recovery from episodic stressors such of this type through enhanced expression of *Foxn1*, as well as critical chemokine transcriptional targets (Hoover et al., 2016).

Over the last several years, immune sensing of metabolic dysregulation has emerged as an important factor in age-associated thymic atrophy, but the mechanisms regulating this association are not yet completely understood. Calorie restriction has been shown to partially mitigate age-associated thymic atrophy (Yang et al., 2009). Conversely, obesity has been associated with decreases in thymus output (Howard et al., 1999). Adipogenesis within the thymus and thymic atrophy are promoted by age-associated decreases in expression of ghrelin, an orexigenic and anti-inflammatory peptide, and ghrelin receptor (growth hormone secretagogue receptor) in thymic stromal cells (Dixit et al., 2007; Youm et al., 2009). Mechanistic links between aging, thymic adiposity, and thymic atrophy have been revealed by recent work showing that lipotoxic “danger-associated molecular patterns” (DAMPs), such as ceramide and free cholesterol, increase during aging (de Mello-Coelho et al., 2017; Youm et al., 2012), and can initiate NLRP3 inflammasome signaling and IL-1 β production in thymic myeloid cells (Youm et al., 2012). *Il1r* expression was found mainly in the TEC compartment, and since IL-1 β causes thymic dysfunction (Morrissey et al., 1988a; Morrissey et al., 1988b) these studies suggest that lipotoxic DAMPs inhibit thymus function via IL-1 β signaling in TEC (Youm et al., 2012). In agreement with these studies, Ki *et al* found high levels of *Il1r* expression in TECs, and also found that the antagonists *Il2rn* and *Il1r2* were preferentially expressed by mTECs (Ki et al., 2014). The authors noted that IL-1 therefore likely has differential effects on the cortex and medulla. Together, these studies support the notion that DAMP-initiated NLRP3 signaling may preferentially trigger IL-1 β signaling in the thymic cortex, perhaps contributing to the noted decline in cortical:medullary ratio with age (Brelinska et al., 2008; Griffith et al., 2012; Li et al., 2003; Steinmann et al., 1985; Sutherland et al., 2005). In addition, the longevity factor and ketogenic hormone FGF21 has also been shown to decrease thymic adiposity and mitigate thymic atrophy, presumably by diminishing lipotoxic DAMP signals in the thymus during aging (Youm et al., 2016). This is consistent with the observation that mutation of the FGF21 obligate co-receptor, β Klotho, promotes thymic atrophy (Kuro-o et al., 1997).

Thymic stromal cells may be especially sensitive to damage induced by inflammation and DAMPs, such as reactive oxygen species (ROS), for several reasons. First, the early stages of lymphopoiesis that occur in the thymus involve exceptionally high levels of proliferation (Lind et al., 2001; Penit, 1988). While the lymphocytes exit cell cycle, and eventually emigrate from the thymus, stromal cells, especially in the cortex, are constantly juxtaposed to the periodically recruited lymphoid progenitors exhibiting high rates of proliferation (Petrie and Zuniga-Pflucker, 2007), and will be continually exposed to the cell-permeable products thereof, including ROS, such as hydrogen peroxide. We have recently identified a deficiency in the peroxide-quenching enzyme catalase in thymic stromal cells. This deficiency renders thymic stromal cells particularly sensitive to ROS-induced DNA damage, which is found at much higher levels in thymic epithelial cells than in thymic lymphocytes. Accumulated metabolic damage promotes atrophy of the thymus, which can be mitigated by dietary or genetic complementation of antioxidant activity (Griffith et al., 2015). The cellular damage induced by ROS, which may be generated as byproducts of metabolism and/or DAMP-induced (Youm et al., 2012) or dendritic cell-mediated inflammation (Ki et al., 2014), represents an example of a continual, cumulative, and relatively irreversible age-associated change. Because ROS may be generated as a result of inflammation (reviewed in

(Laskin et al., 2011)), and may also serve as NLRP3-activating DAMPs (reviewed in (Tschopp and Schroder, 2010)), a feed-forward loop of cellular damage may be established once inflammation and/or ROS emerge.

In addition to the factors discussed above, careful examination of differences in thymic atrophy among 16 recombinant inbred mouse strains have revealed evidence of genetic determinants that may regulate maximal thymus size and pace of atrophy on chromosomes 3 and 9, respectively (Hsu et al., 2003). Recent work has also identified genetic differences in thymic epithelial cell number and lymphocyte:epithelial ratio among CBA/J and PWK/PhJ mouse strains (Nagakubo et al., 2017). These studies highlight the fact that there are genetic factors regulating thymus atrophy during aging that remain to be identified.

4. Conclusions

As illustrated in Figure 1, both transient and cumulative events contribute to declining thymus output during aging. To the best of our knowledge, direct measurements of total lymphocyte cellularity in the human thymus during aging have not been reported. However, careful histological analysis of human thymus during aging (Steinmann et al., 1985) agrees with estimates of thymic output in humans based on T cell receptor excision circle (TREC) analysis (Douek et al., 1998; Jamieson et al., 1999; Thome et al., 2016) and mathematical modeling (Bains et al., 2009), indicating that peak thymus output occurs in the first few years of life, and yet continues to occur through the 9th decade of life (Mitchell et al., 2010). These data informed our estimates corresponding to thymus output in human and experimental models illustrated in Figure 1. Although the thymus retains a remarkable capacity to regenerate after removal of a negative stimulus (Chaudhry et al., 2016; Dudakov et al., 2012; Majumdar and Nandi, 2017; Rode and Boehm, 2012; Sutherland et al., 2005), age progressively limits the size reached at return to steady state. Persistent regeneration of thymus size and function holds great promise for extending the healthspan, but remains a challenge, and approaches aimed at preventing atrophy are relatively unexplored. A more complete understanding of the causes of thymic atrophy will inform studies that aim to reverse or prevent it.

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Highlights

- This article reviews the role of thymic stromal cells in age-associated atrophy of the thymus.
- Age-associated changes in stromal phenotype, transcriptome, and function are reviewed.
- Periodic, stochastic, and progressive causes of atrophy are discussed.

Figure 1.