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Transcriptome analysis of age-, gender- and diet-associated changes in murine thymus ☆

Ana Lustig [a], Ashani T. Weeraratna [a], William W. Wood III [b], Diane Teichberg [b], Dorothy Bertak [a], Arnell Carter [a], Suresh Poosala [b], Jeffrey Firman [b], Kevin G. Becker [b], Alan B. Zonderman [b], Dan L. Longo [a], Dennis D. Taub [a,*]

[a] *Laboratory of Immunology, National Institute on Aging-Intramural Research Program, National Institutes of Health, 5600 Nathan Shock Drive, Baltimore, MD 21224, USA*
[b] *The Research Resources Branch, National Institute on Aging-Intramural Research Program, National Institutes of Health, Baltimore, MD 21224, USA*

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Abstract

The loss of thymic function with age may be due to diminished numbers of T-cell progenitors and the loss of critical mediators within the thymic microenvironment. To assess the molecular changes associated with this loss, we examined transcriptomes of progressively aging mouse thymi, of different sexes and on caloric-restricted (CR) vs. *ad libitum* (AL) diets. Genes involved in various biological and molecular processes including transcriptional regulators, stress response, inflammation and immune function significantly changed during thymic aging. These differences depended on variables such as sex and diet. Interestingly, many changes associated with thymic aging are either muted or almost completely reversed in mice on caloric-restricted diets. These studies provide valuable insight into the molecular mechanisms associated with thymic aging and emphasize the need to account for biological variables such as sex and diet when elucidating the genomic correlates that influence the molecular pathways responsible for thymic involution.
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1. Introduction

Alterations in immune function with age in both humans and animals are important to the health of aging individuals. Older humans are much more susceptible to microbial infections of the soft tissues, skin, the abdomen and both the urinary and respiratory tracts [1,2]. These older subjects also demonstrate an increased incidence of infectious endocarditis, tuberculosis, meningitis and herpes zoster, and the mortality rates for these diseases in older patients are often two to three times higher than in younger people with the same disease [3–6]. In addition, an increased prevalence of specific cancers and certain autoimmune diseases have been observed with advancing age [6–9]. The increased prevalence of these conditions and the higher morbidity and mortality from infections strongly suggest functional defects in a deteriorating immune system with advancing age [10–16]. Many studies have centered on possible loss or alterations in the number of circulating T lymphocytes and T-cell subsets [17,18]. This focus on T lymphocytes seems reasonable given that the thymus is known to atrophy with progressive aging and correlates with a significant loss in its capability to generate de novo T cells for export into the peripheral T-cell pool [19]. Interestingly, this loss in thymic output with age does not result in any significant change in the total number of peripheral T cells as the maintenance of peripheral T-cell numbers appears to be regulated via a thymus-independent

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* Corresponding author. Fax: +1 410 558 8284.
E-mail address: taubd@grc.nia.nih.gov (D.D. Taub).

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homeostatic process involving expansion of mature peripheral T cells. This peripheral homeostatic expansion of mature T cells results in a much more limited T-cell receptor (TCR) repertoire with age [19,20] and is believed to contribute to the diminished capacity of older T cells to proliferate upon mitogenic and other stimulation when compared with their younger counterparts [20–23]. However, given that the loss in thymic function is one of the earliest and most consistent steps in the progression to immune dysfunction, the involuting thymus seems to be a promising target to which to direct therapy with the specific goal of reversing thymic atrophy and restoring thymopoiesis and T-cell export [19,24–26].

For a number of years in gerontological research, efforts have been made to identify new and unique biomarkers that will define the biological, physiological and chronological changes that occur with age [27]. Numerous studies have focused on the comparison of calorically restricted (CR) primates and rodents to their *ad libitum* (AL)-fed animal controls, where significant differences in a variety of physiological processes have been observed as well as a prolongation in the animals' lifespan [28,29]. This delay of aging by CR appears to depend upon a delay, or sometimes ablation, of a broad spectrum of age-associated pathophysiological changes and a 30–50% increase in maximum life span. These studies are quite complicated as CR induces a plethora of biological changes including decreases in oxidative stress [30], glycation or glycoxydation [31,32], body temperature and circulating thyroid hormone levels associated with a hypometabolic state [33,34] as well as alterations in gene expression and protein degradation [29,32] and a number of neuroendocrine and inflammatory changes [35–37]. Despite the identification of many notable physiological differences between such dietary-restricted animals, no specific biomarkers have ever been reproducibly identified that could accurately identify the age of the organism or predict lifespan.

Microarray analysis has been an efficient way to profile the changes associated with disease onset, especially in the arena of both cancer and infectious disease [27]. By identifying the global gene expression profiles between disease and non-disease states, researchers are often able to isolate individual genes that may be used either as markers for disease, or as molecular targets as well as whole pathways that may be involved in the studied disease. Similar studies have now been initiated to examine age-related changes in a number of tissues and cellular subsets by microarray analysis in an effort to identify genes or patterns of gene expression associated with physiological aging, lifespan, and age-related disease states [27,38–42]. Only a few studies to date have focused on immunologic aging with the majority of studies focusing on an examination of bulk T-cell populations [43]. Limited replication of analysis, small sample sizes and poor array validation have restricted the interpretation of such data with regard to age-related immunological changes. To date, no studies have focused on gene expression changes within the aging thymus using microarray analysis with an additional focus on additional biological variables such as gender and dietary restrictions.

In an effort to understand the potential forces driving age-associated thymic involution and the cellular pathways involved in these phenomena, we have performed microarray analyses on thymi derived from young and old mice to identify differences in gene expression patterns which may be attributed to aging. We have included mice of both sexes in this study to determine sex-specific differences in aging, as well as mice that have been placed on various dietary regimens. Our results demonstrate that many of the putative thymic aging-responsive genes are in fact dependent upon variables like sex and diet, some of which are independent of age. Only a small fraction of the total gene products examined demonstrated thymic aging-dependent gene expression changes that were also independent of sex or diet. Complex biological interactions are therefore responsible for the breadth of alterations in gene expression generally observed in the expression analyses of progressively aging thymi.

2. Materials and methods

2.1. Mice

Specific pathogen-free C57BL/6 mice were purchased through the Office of Biological Resources and Resource Development of the National Institute on Aging (Bethesda, MD). All mice were maintained in an AAALAC-certified barrier facility and were acclimated for 2 weeks before use. All mice were fed autoclaved food. Water was ingested *ad libitum*. All mice with evidence of disease (e.g., enlarged spleen, gross tumors) were excluded from these studies. The distribution and characteristics of the animals utilized in this study are described in Table 1. In this study, mice were fed either diets consisting of 100% regular feed (*ad libitum*) or caloric-restricted (CR) mice as follows: Up to 13 weeks of age, 100% regular feed, followed by 90% fortified feed for 1 week, 75% for 1 week, then 60% fortified feed after that until the age at which the mice were sacrificed. For the verification studies, flash-frozen thymi for RNA and protein analyses from both C57BL/6 and Balb/C mice were obtained from the NIA aged rodent tissue bank (Bethesda, MD).

2.2. RNA extraction and microarray analysis

All data from microarray experiments have been deposited into the GEO Accession Database (number in progress). For each array sample, the RNA was prepared using the entire thymus. The tissue was processed using a Bead Beater (Bio-Spec, Bartlesville, OK) followed by RNA purification using the RNEasy Mini Kit (Qiagen, Valencia, CA). The RNA was examined for quantity and quality using a Bioanalyzer (Agilent

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