BIOGERONTOLOGY

Functional Unity of the Thymus and Pineal Gland and Study of the Mechanisms of Aging

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The data on the morphology and functions of the thymus and pineal gland in individuals of different age are analyzed and common mechanisms of involution of these organs during aging and the consequencies of this process are discussed. Based on the data on the molecular changes in the thymus and pineal gland during aging, the authors hypothesize the functional unity of these organs and their mutual complementarity in the maintenance of normal immune and endocrine status during aging.

Key Words: *thymus; pineal gland; aging; neuroimmunoendocrine interrelationships*

An appreciable part of diseases associated with aging is linked with reduction of the nervous, immune, and endocrine system functioning [2]. Reduction of humoral and cellular immunity in elderly people leads to an increase in the incidence of autoimmune, endocrine, infectious, and tumor diseases. Neuroimmunoendocrine age-associated changes, primarily the age-associated impairment of the thymic and pineal (epiphyseal) functions, are now believed to play an important role in the mechanisms of aging and development of age-associated diseases [12,15,21,22].

Ample data on changes linked with involution of the thymus and pineal gland (PG) during aging suggest a close functional relationship between these organs; changes in the nature of this relationship can play the key role in some mechanisms of aging [4,9,19,23]. The unity of the thymus and PG in the regulation of various biological processes is confirmed by the data of a study [14] indicating that 20% PG area is occupied by the lymphoid tissue presented by T cells secreting cytokines identical to those expressed in the thymus [11].

In order to detect the common features of involution of the thymus and PG and the mechanisms of probable interactions of these organs during aging, let us discuss in detail their age-associated changes.

The thymus is a lymphoepithelial organ. Its main function is the development of T cells, including maturing, differentiation, and selection of T-cell clones capable of recognizing foreign antigens. Lymphocyte maturation is realized by their migration over different zones of the thymus and interactions with the microenvironment cells. Septa originating from the connective tissue capsule divide the organ into lobules. The outer (cortical) layer of the thymus is presented by thymic epithelial cells (TEC) and dividing lymphoblasts (early prothymocytes). The TEC are subdivided into 3 groups: secretory TEC, synthesizing thymosins, thymopoietin, thymic serum factor, and class II main histocompatibility complex molecules; nursing cells isolating the thymocytes from the adjacent cells; and perivascular cells protecting thymocytes from circulating blood antigens. With migration towards the medullary layer of the thymus, prothymocytes lose CD1

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antigen and acquire capacity to express CD3, CD4, and CD8 antigens. In the medullary layer the thymocytes which cannot recognize class II histocompatibility molecules start apoptosis, while the remaining cells differentiate into T-helpers ($CD3^+CD4^+CD8^-$) and T-killers ($CD3^+CD4^-CD8^+$).

Morphological involution of the thymus starts from the first year of life and consists in a steady reduction of its weight throughout the life span mainly at the expense of the cortical layer replacement by connective tissue [16]. The process of thymus involution reaches the peak by 50-60 years and later virtually does not progress. The greater part of the thymic lobes is replaced by fatty and connective tissue in elderly, senile, and long lived subjects, this precluding the differentiation between the cortical and medullary layers. Hypoplasia of thymocytes, forming groups of 5-6 cells, is seen in the remaining lobes. The cellular microenvironment of the thymus reduces by 3-5% by middle age, and then by less than 1% annually.

Functional activity of the thymus also decreases with age, but does not disappear completely even in senile age. The thymic serum factor disappears from the blood by 50-60 years, the counts of T-killers reduce significantly, and less so the counts of T-helpers. Studies of vascular endothelial growth factor (VEGF) in the thymus throughout its entire ontogenesis showed that this factor was expressed in the subcapsular zone, cortical and medullary matter in all age groups. In elderly subjects, the expression of VEGF in thymic tissues is lower than in young people, this presumably reflecting the structural involution of the gland. In senile and in long lived subjects the expression of VEGF increases, presumably under the effect of hypoxia. In elderly subjects the expression of serotonin by T cells and microenvironment cells is reduced, but the expression of this hormone does not cease even in long lived subjects, this indicating the reversible nature of involutive changes in the thymus [13,14]. This hypothesis is confirmed by the results of studies demonstrating an increase of endothelin-1 in the aging thymus, which can serve as a compensatory mechanism maintaining functional activity of this organ during aging [10].

Involution of the thymus is not irreversible and is effectively corrected by intrathymic implantation of the PG and by therapy with melatonin and epithalone, a synthetic peptide created on the base of epithalamin (a substance isolated from extract of the pineal gland) [1,19]. These data leave no doubts that the aging of the thymus is the key process responsible for reduction of the functional activity of the immune system. Reduction of humoral and cellular immunity in subjects aged over 60 years increases significantly the probability of infections and carcinogenesis, often leading to lethal outcomes.

The pineal gland is a structure of the epithalamic region of the brain regulating biorhythms and ontogenesis by producing the main pineal gland hormone melatonin. The pineal gland is enveloped in a connective tissue capsule with blood vessels, from which cords, separating it into lobules, originate. The gland parenchyma consists of pinealocytes, type 1 clear cells, type 2 dark cells, and some neurons. It is assumed that serotonin is produced from tryptophan in clear pinealocytes, while dark cells synthesize melatonin from serotonin. Type 1 cells are round or irregularly shaped, have a large nucleus of irregular shape with diffuse chromatin and clear karyoplasm. Type 2 pinealocytes are elongated, have processes, and are diffusely scattered among type 1 cells. The nuclei of type 2 cells contain heterochromatin and have numerous secretory granules with melatonin in their cytoplasm. The main function of the PG is secretion of melatonin, a hormone regulating circadian rhythms of organs and the organism, characterized by an antioxidant effect, and stimulating the T-cellular immunity.

During aging, morphological and functional changes develop in the PG.

Autopsy studies of the pineal gland in humans aged 10-50 years have shown that the gland increases during this period from 80-100 to 150-160 mg at the expense of increase in the fibrous tissue volume without appreciable changes in the pinealocyte counts [5,6]. Life-time studies of the PG by magnetic imaging have confirmed the results of autopsy material studies and have indicated that the volume of the gland increases from birth till the age of 13 years, while at 13-16 years the organ starts to shrink, which is related to sexual maturing; at 17-21 years the PG volume increases again, and then slightly reduces and stabilizes in elderly age [5]. The reduction of the gland volume in pubertal age is characterized by gender-specific differences: in girls the process is paralleled by reduction of melatonin level, while in boys no relationship of this kind has been detected.

Morphological changes in the PG during aging include accumulation of psammoma bodies (colloid with calcificate incorporations) in the pinealocytes. According to some authors [3,5,24], psammoma bodies are present in the pineal gland in subjects aged from 19 to 78 years. A trend to an increase of this parameter with aging has been noted, but appreciable levels of colloid calcificates in the pineal gland are often detected in young age. The absence of a clear-cut relationship between the level of psammoma bodies and human age indicates functional intactness of PG even in elderly and senile age.

Functional changes in aging PG are more pronounced in comparison with its morphological changes. According to electron microscopy findings, the

age-specific changes in type 2 pinealocytes include modification of the nucleus shape, reduction of functional activity of mitochondria, and accumulation of lipofuchsin granules and autophagosomes [20]. The count of type 2 pinealocytes immunopositive to melatonin reduces progressively with aging, this indicating a reduction of this hormone production in the PG. A pronounced reduction of melatonin concentration during the night hours has been found in elderly subjects, while in the daytime its level is the same as in young people.

However, nocturnal secretion of melatonin is reduced in just 71% elderly subjects, while in 29% representatives of this age group the PG melatonin-producing function is retained [6]. Subjects aged over 60 years with retained PG function exhibit better physical and mental performance, normal circadian rhythms of cardiovascular work and autonomic regulation, and normal titer of the thymic serum factor, this fact confirming the relationship between the pineal gland and the thymus. Reduced melatonin expression by pinealocytes in elderly age leads to total systems disorders in homeostasis, manifesting by shifts in biological rhythms, emergence of disorders in the work of the nervous, immune, and endocrine systems [4-9]. For example, age-specific changes in melatonin secretion cause disorders in the sleeping/consciousness cycle and stimulates the LPO, promoting an increase of cancer risk. In addition, reduction of melatonin production is assumed to contribute to the development of age-associated neurodegenerative diseases and high risk of myocardial infarction [2]. Physical and mental performance of elderly people with functional insufficiency of the PG are 20-40% lower than normally.

One of the leading functions of PG realized through melatonin effects is its regulation of humoral and cellular immunity, immune cell proliferation, and immune mediators production. Melatonin stimulated humoral immune response in rats by increasing the levels of IgG1 and IgM and promoted thymocyte proliferation during age-associated involution of the thymus [2]. Recovery of circadian dynamics of absolute counts of $CD4^+CD8^+$ cells under the effect of pineal melatonin indicates its possible involvement in T-cell maturing and differentiation [4]. However, we cannot rule out the possibility of normal differentiation of T cells in elderly people with reduced function of the pineal gland, because melatonin production in the thymus of elderly subjects has been described [11].

These data indicate that PG is one of the central objects of gerontological studies, as the morphofunctional changes associated with its involution trigger a cascade of molecular reactions linked with age-specific dysfunctions of the nervous, immune, and endocrine systems. In addition, age-specific involution of the immune and endocrine systems largely linked with aging of the thymus and PG confirms close functional relationships between these organs.

The thymus and PG are endocrine organs producing a series of similar bioactive molecules, the most important of which are melatonin and serotonin. In the thymus serotonin-containing cells are T-lymphocyte precursors ($CD4^-CD8^-$), immature cortical cells ($CD4^+CD8^+$), mature medullary cells ($CD4^+CD8^-$ and $CD4^-CD8^+$), and TEC; in addition, melatonin expression by thymic cells has been described [11]. Serotonin is secreted by type 1 cells and melatonin by type 2 pinealocytes in PG.

The thymus is the central organ of the immune system, but PG also contains lymphoid tissue; in addition, melatonin secreted by pinealocytes regulates the proliferation and differentiation of thymic T-lymphocytes.

During aging, functional activities of the thymus and PG decrease though do not cease completely. The weights of both organs decrease in elderly people, the connective tissue replaces the cortical zone of the thymus (in which the initial stages of prothymocyte differentiation take place), and the pineal gland parenchyma (in which melatonin-producing pinealocytes are located) degenerates. Inhibition of pineal melatonin synthesis during aging leads to disorders in T cell proliferation and differentiation in the thymus, which together with reduced functional activity of this organ augments immune system dysfunction.

The data on the close functional relationship between the thymus and pineal gland during aging suggest that retained functional activity of one of these organs under conditions of significant involution of the other one can have a positive impact for the maintenance of the thymus—PG system in general. It is known that in the majority of cases aging is a heterogeneous process, and involution of the thymus and PG is uneven [15,18]. For example, functional activity of the pineal gland does not decrease in 29% elderly people and high level of melatonin release by this organ can promote recovery of the thymic function even under conditions of its significant involution [17]. Presumably, retention of thymic functions during aging inhibits involution processes in the PG.

By the present time the data on mutual effects of functional activities of the thymus and PG during aging are scanty, but experimental studies of the effects of epithalamin (synthetic epiphyseal peptide) and melatonin hormone on functional activity of the thymus have shown that these substances inhibit significantly involution of the thymus. Presumably, further studies of the functional relationship between the thymus and PG during aging will open new vistas in our understanding of the age-specific involution of the immune and endocrine systems as well.

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