Review paper

The thymus in neuro-endocrine-immune network

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Abstract

On the grounds of concise review of literature comprising the most important findings achieved by different investigators during over fifty years of intensive research, authors describe the present state-of-art knowledge on the immunoregulatory role of thymus. Being responsible for creation of immune competence of T lymphocytes, the thymus is able to control immune functions also by regulation of immunity indirectly by neurohormonal mechanisms. They comprise both synergistic (e.g. growth hormone, prolactin, enkephalins, thyroid hormones) and antagonistic (e.g. adrenal and sex hormones) connections between the thymus and neurohormonal system. This way, remaining active for the whole life of the organism, providing both cellular and hormonal influences, the thymus integrates and maintains the homeostatic tasks of neuro-endocrine-immune network related to its metabolic, procreative, regenerative, tolerogenic and defensive functions.

Key words: *the thymus and neurohormonal regulation of immunity.*

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Introduction

The thymus is the first completely developed organ in our individual life. Its activity, the most vigorous in fetal and neonatal period, is continued with gradually decreased intensities till the end of our life. The thymus is the center of development of immunocompetence of immigrant bone marrow-derived lymphocytes which progressively differentiate into separate T-lymphocyte subclasses. Intrathymic environment composed of the network of thymic epithelial cells (TEC) presents to incoming cells the template of self histocompatibility antigens of the HLA-first and the HLA-second class. They are ligands for recombinantly developing T cell receptors (TCR) which together with co-recognizing structures (CD4 or CD8) create the mechanisms of immune recognition. The T lymphocytes with the shape of TCR able to recognize the self histocompatibility antigens (HLA-restriction), thus able to discriminate between the correctness of self or incorrectness of self deformed by influence of foreign elements (including infective antigens), are positively verified and selected by the supportive influence of thymic epithelial cells. TEC are producers of some cytokines, growth factors (e.g. IL-1, IL-7, oxytocine, enkephalins) and specific polypeptides collectively described as a "thymic hormones". These substances are highly sensitive for enzymatic proteolysis and their life-span in circulation is very short. Their repeated impulses delivered by the active thymus are indispensable for completion and finalization of the process of maturation of T lymphocytes which takes about two weeks in human [1]. Leaving the thymus, newly matured T lymphocytes representing the all known classes of the population (T-inducer-helper, T-cytotoxic, T-regulatory cells), reach the peripheral lymphoid system as a recent thymic emigrants (RTE). This is the physiologic way or replenishment of cellular resources of immune system exploited by its numerous defensive, regenerative, tolerant and regulatory functions. The thymic supplementation of immune system with the new cellular cohorts to replace the exploited resources is a whole-life process, the most intensive at the perinatal period. Nevertheless, even in the adult and elderly individuals the thymic lymphopoietic and endocrine functions are maintained, as indicated by the appearance of the new RTE cells in the circulation [2-4]. In consequence, the proper activity of the thymus leads to the functional efficiency of immune system in the full scope of its activities and the

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abrogation of thymic activity results in progressing immune deficiencies of different kinds.

The functional connections between the thymus and neuro-endocrine system

The thymus is a composition of the three cellular systems originating from embrional, ectodermal, endodermal and mesenchymal elements, thus representing the main constructive elements of developing organism. During ontogeny the endodermal TEC which developed from pharyngeal pouch and ectodermal TEC which developed from brachial cleft, all connected with mesenchymal elements, make the two-lobe organ located primarily in the cervical region and finally descending to the mediastinum. The non-lymphoid portion of the embryonic thymus elaborates chemotactic factors attracting immigration of fetal liver and bone marrow stem cells which create thymic lymphoid cell populations (thymocytes, macrophages).

The organ receives its innervation form several nerves: the vagus, the phrenic nerve, the recurrent laryngeal nerve and the descendens cervicales (ansa hypoglossi). Both adrenergic and cholinergic terminals regulate the thymic development and function by influence of respective neurotransmitters [5]. For example, cortisone injection into mice activate cholinergic nerves increasing acetylocholine (AChE) activity within the areas of thymocyte disintegration [6]. On the other hand, the propranolol, an adrenergic blocker, given to newborn mice evoked an arrest of the thymic development and consequent impairment of thymic dependent immune functions [7]. The high susceptibility of the thymus to the stress is well known feature of the organ. The stressing signals of the physical (temperature, radioactivty, strong electromagnetic fields), chemical (toxins) or even psychological nature (dramatic events, accidents, lost of spouse) may lead to the thymic atrophy with the immunodeficient consequences remembering to the some degree those observed in animals after neonatal thymectomy (wasting disease). The symptoms of wasting disease observable after neonatal thymectomy in mice [8] demonstrate the negative reflection of the full range of beneficial influences of the thymus and T lymphocytes in the organism. They show what was lost. The lack of T cells is observed in subcortical regions of lymph nodes, in periarteriolal sheets in the spleen, in the lymph, blood and tissues. In contrast to the presence of B, NK , K cells, monocytes and macrophages and their products, the symptoms of immunodeficiency develop including severe infections of the skin, mucous membranes, respiratory, digestive and urogenital tracts. The growth of animal is inhibited and general atrophy destructs different tissues and organs, including bone marrow. The immune autoagressive reactions and spontaneous tumors will frequently develop before the animals die.

The thymus is strongly subdued in its functions to the influences of central nervous system and endocrine system. From its own side, the thymus directly by hormonal influences or indirectly by immune functions, affects the neuro-endocrine system.

Neuro-hormonal influences on the thymus

Numerous products of hypothalamo-pituitary axis (HP) regulate thymic development, involution, endocrine activity and generation of T lymphocyte repertoire. Under the supervision of HP, also hormones of peripheral endocrine organs (thyroxine, corticosteroids and sex hormones) exert the regulatory influences on the thymus.

Kelley *et al*. [9] has demonstrated for the first time complete regeneration of the thymus in old rats after implantation of pituitary derived epithelial cell line GH3 secreting growth hormone and prolactin. Further investigations detected that growth hormone (GH) appeared to stimulate several functions of the thymus including secretion of thymic hormones, bone marrow cell immigration, production of extracellular matrix proteins regulatory for intrathymic traffic of maturing thymocytes, their proliferation and export [10-14]. The treatment with somatotropin of growth hormone deficient patients resulted in several fold increase of the concentration of thymic hormone thymosin $\alpha 1$ in the serum [15].

Prolactin (PRL), the other hormone of anterior part of pituitary, highly active *post partum* efficiently contributes to the regeneration and activity of the thymus temporarily inhibited during pregnancy by influence of progesterone [16]. Similar effects are exerted by pituitary oxytocin which is also produced by thymic neural-crest derived neuropeptide secreting cells. The local oxytocin is potent to replace the lacking IL-2 for costimulation of thymocyte proliferation [17]. The prolactin combining with specific PRL receptors present on TEC stimulates them to synthetise the thymic hormone thymulin [18]. The hypothalamo-pituitary-adrenal axis (HPA) exerts complex modulatory influences on the whole immune system with the thymus at the top. Direct influence of ACTH on the cultures of TEC appeared to stimulate their endocrine activity. In contrast to that, ACTH increasing adrenal production of corticosteroids initiates their immunosuppressive effects by abrogation of thymic hormonal activity and reducing the number of thymocytes and peripheral T cells [1, 19-21]. The only exception with no direct immunosuppressive influences in the whole steroid family is dihydroepiandrostendiol (DHEA), the precursor of corticosteroids and sex hormones which, in turn, appear to exert strong immunosuppressive influences with the leading role of progesterone in this respect [16]. The other hormonal agent representing strong stimulatory influence on thymic lymphopoiesis is product of thyroid - triiodotyronine (fT3). The positive correlation exists between fT3 concentration in the serum and endocrine activity of TEC [22-24]. Triiodotyronine activates the expression of receptors and ligands in extracellular matrix (fibronectin, laminine, VLA-5, VLA-6), accelerating the

intercellular cooperation between TEC and maturing thymocytes and prompting on this way the process of replenishment of peripheral immune system with the new resources of multifunctional T cell population [25-29]. Our own investigations of several immune parameters characterizing T lymphocyte kind and functions in hypothyroid and hyperthyroid patients revealed less pronounced T cell deficit in the later group, thus suggesting the stimulatory effect of endocrine thyroid on thymic dependent functions of immune system [30].

The influence of the thymus on neuro-hormonal functions of the organism

The tasks of the thymus are not limited to the maturation, diversification and selection of T lymphocyte population. The endocrine influence of the thymus relates also to the function of hypothalamo-pituitary axis. Due to this influence, the thymus participate indirectly in regulation of tissue metabolism which is directly controlled by peripheral endocrine organs: thyroid, pancreas and adrenals. Moreover, the proper functioning of hypothalamo-pituitary-gonadal axis is dependent on the thymic endocrine influence on the hypothalamic production of respective releasing hormones. The first observations suggesting possibility of existence of other than immunologic activity of the thymus result from appearance of the symptoms of wasting disease in neonatally thymectomised mice. The animals, even in the germ-free circumstances, demonstrated inhibition of the growth and dystrophic changes of numerous tissues, including bone marrow, connective tissue, bones, muscles, subdermal adipose tissue, skin, salivary glands, mucous membranes and gonads.

The consecutive investigations undertaken next by numerous authors (Pierpaoli and Sorkin, Fabris *et al*., Comsa, Deschaux *et al*. among others), univocally confirmed the existence of endocrine functions of the thymus. In pituitaries of thymectomised mice the degranulation of cells producing growth hormone and prolactin has been observed in parallel to the stable decrease of the concentrations of these hormones in the serum. Poly-hormonal disorders comprising thyroid, adrenal, pancreatic and gonadal hormones and related to them pituitary tropic hormones were observed in different species of thymectomised animals (rats, hamsters, guinea pigs, mouse). These deficits were restored after the animals were implanted with the thymus. The insufficiency of salivary glands and dessication of oral and conjunctival mucous membranes are observed in man suffering from Sjögren syndrome and in nude, thymectomised and aging mice. All these abnormalities are due to the deficit of β-adrenergic receptors which are indispensable to absorb the water [31]. Transplantation of the thymus or treatment with thymic hormones results in redistribution of the receptors and in amelioration of the pathological symptoms.

Participation of the endocrine thymus in development and regulation of hormonal mechanisms determining procreative abilities of the organism is presently the best known fragment of the thymic influence on hypothalamo-pituitary

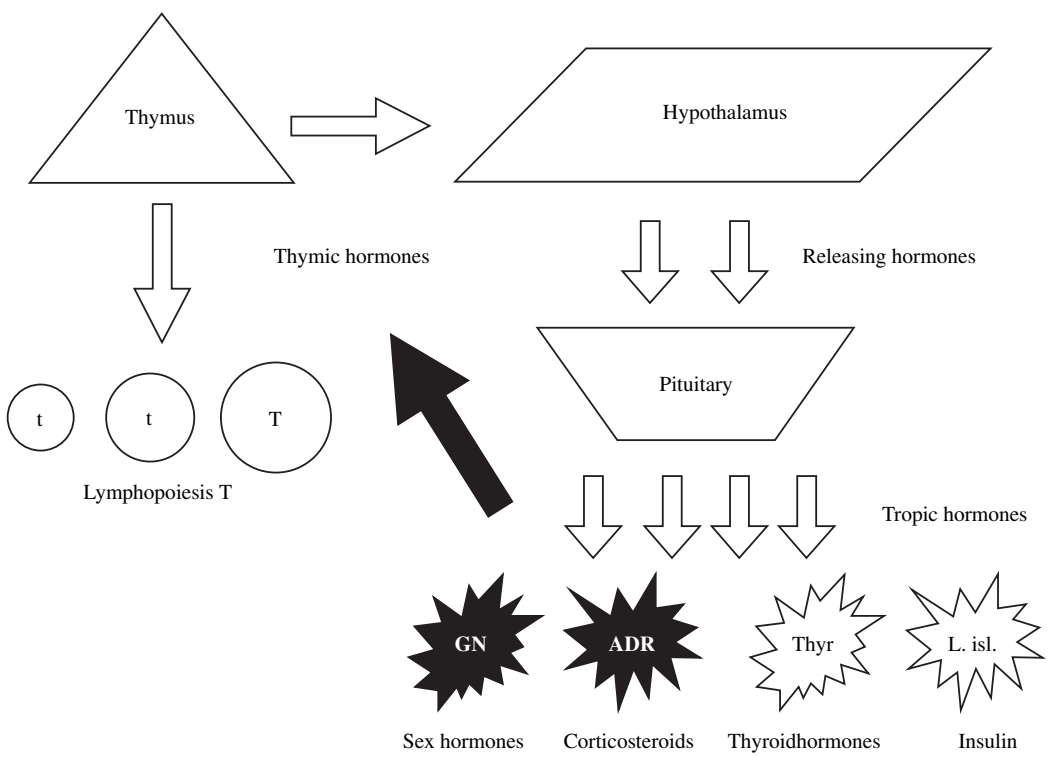

Fig. 1. The thymus and neurohormonal system

axis. The oogenesis and spermatogenesis may develop in prepubertal period and to be continued after puberty under influence of pituitary gonadotropins (FSH and LH). Their absence in nude mice results in infertility of the animals. Over 30 years of investigations clarified the mechanisms of the influence of the thymus on hypothalamo-pituitary-gonadal axis. In late 70. Lintern-Moore and Pantelouris [32] have observed that administration of gonadotropins to athymic mice normalizes ovarial development. In 80. Rebar and coworkers [33, 34] investigating the hormonal sequence in young and adult athymic mice have determined that the deficits comprised consequent steps of hormonal hierarchy. They were observed at the level of hypothalamus (deficient production of releasing hormone for luteinizing hormone LH-RH), through the level of pituitary (gonadotropin deficits), descending to the level of ovary and serum (decreased concentrations of estrogens and progesterone). The sequenced hormonal deficits observed in athymic nude mice could suggest that the lacking thymic hormonal influence was the reason for the appearance of the deficits. To answer the questions if the endocrine thymic influence can stimulate hypothalamo-pituitary-gonadal hormonal cascade, and if so, which of the thymic products can do it, and at which level, the Rebar's team in cooperation with A.L. Goldstein and his coworkers performed next series of experiments [33]. The hypothalamic and pituitary tissues were cultured in the environment containing thymosin β4 or thymosin α1, both well known and sequenced thymic hormonal products. The experiments have shown that only in the system comprising both hypothalamic and pituitary tissues increased production of LH-RH and LH took place under influence of thymosin β4. No thymosin α1 was active in this respect, nor thymosin β4 was able to stimulate the hormonal production if pituitary tissues were cultured in the absence of hypothalamic tissues [33, 34]. These observations, valid for animals, have its respective reflection also in the humans. *Post-mortem* investigations in girls who died from inherited syndromes of thymic atrophy (DiGeorge, ataxia-teleangiectasia) revealed the total absence of oocytes in atrophied ovaries.

The influence of thymic hormones on the hypothalamo-pituitary system is not limited to the gonadal axis. Healy *et al*. [35] discovered in 1983 the similar effect of thymic products (thymosin fr.5 and tymosin α1) on the hypothalamo-pituitary-adrenal axis. The experiments were performed on thymectomised monkeys (Macaca fascicularis) in which the concentrations of ACTH, β-endorphin and cortisol were estimated by radioimmunoassay. The animals were equipped with special vests to provide location of the catheter inside the vein vessel for collection of blood specimens and for administration of thymic hormones (thymosin fr. 5, thymosin α1, thymosin β4). These precautions have made the animals safe from possible stressing direct contacts with the scientific staff. In thymectomised monkeys the levels of ACTH, β-endorphins and cortisol were significantly lower 6 weeks after thymectomy as compared to the levels estimated 6 weeks before thymectomy. The administration of thymosin fr. 5 but not thymosin α1 or thymosin β4 increased the concentrations of ACTH, β-endorphin and cortisol in tested blood specimens. To find out at which level (hypothalamus?, pituitary?, adrenals?) the administered thymic hormones were active, Hall and coworkers [36] continued the investigations on rats in which two weeks before the experiment the catheter was introduced directly to the hypothalamus. Thymosin fr. 5 and thymosin α1administered this way increased the production of adrenal steroids. None of thymic hormones was active in this respect if was injected intravenously or intra-peritoneally.

Concluding remarks

The cited historical experiments performed by Rebar, Healy and Hall and next confirmed by more recent investigations [20-22, 25, 26, 28-30, 37] indicate that the hypothalamus is a common central point of action for different thymic hormones and that thymic influence on hypothalamus finally results in respective changes of activity of peripheral endocrine organs. Thus, the thymus occupies the central position in the neuro-endocrine-immune network integrating its homeostatic tasks in the range of metabolic, procreative, regenerative, tolerogenic and defensive functions of the organism. The time of thymic activity is not limited to the childhood but the organ remains or should remain active, albeit with changing intensities adjusted to different periods of life (e.g. pregnancy, illness stress, recovery from illnesses), for the whole life-span. Being responsible for creation of immune competence represented by multifunctional population of T lymphocytes, the thymus is able to control immune functions not only by supplementation of immune system with T cells but also by regulation of immunity indirectly by neurohormonal mechanisms. They comprise both synergistic (e.g. growth hormone, prolactin, enkephalins, thyroid hormones) and antagonistic (e.g. adrenal and sex hormones) connections between the thymus and neurohormonal system. In this respect, whereas immunoinhibitory influences of hypothalamo-pituitary-adrenal and –gonadal axis create important feedback control system in physiological circumstances, the excessive clinical use of corticosteroids or sex hormones, what is inhibitory for thymic functions, may bring the harmful effects for balanced activity of neuro-endocrine-immune network.

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