EXPERIMENTAL BIOLOGY

Effect of Orchiectomy on Structures of the Thymus Expressing Major Histocompatibility Complex Class II Molecules

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Morphological and immunohistochemical study was performed to evaluate the effect of orchiectomy on structures of the thymus expressing major histocompatibility complex class II (MHC-II) proteins. Male sex hormone deficiency in the organism led to an increase in the number of MHC-II$^+$ cells in the medulla and cortex of the thymic lobule. At the same time, the count of these cells in the corticomedullary zone decreased after orchiectomy. These changes modify homeostasis and activate immune processes.

Key Words: *major histocompatibility complex class II proteins; antigen-presenting cells; orchiectomy; thymus*

The presence of hormone receptors on the surface of immunocompetent cells and ability of immune cells to synthesize some hormones reflect the reciprocal regulation of 2 major systems in the organism (immune system and endocrine system) [2,4,7]. The major histocompatibility complex (MHC) is an important component of the immune system. This complex determines the T-cell immune response and provides the interaction between lymphocytes and macrophages in the immune response. The majority of T cells do not recognize free native antigens. This antigen passes through other cells and is presented on the cell membrane with MHC class I protein or MHC class II protein. This process triggers the cascade of T cell-mediated immune reactions [6].

Here we studied the effect of endocrine deficiency of male sex hormones on structures of the thymus expressing MHC-II.

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MATERIALS AND METHODS

Experiments were performed on adult male Wistar rats (n=30) weighing 200 g, maintained in a vivarium under standard conditions, and feeding a balanced diet. The animals were divided into 2 groups: intact (n=20, group 1) and orchiectomized rats (n=10, group 2).

All experimental manipulations were performed according to the rules of studies on laboratory animals. The thymus was removed under deep ether anesthesia on day 30 after orchiectomy (OEC) and fixed in 4% formalin. Cryostat sections (20 μ) were prepared on a Frigocut E cryostat (Reichert-Jung) after postfixation in 30% sucrose. The sites of antigen binding to MHC-II were identified by the standard immunohistochemical method. Endogenous peroxidase activity was inhibited by incubation of sections in 3% H_2O_2 for 30 min followed by washing with 0.1 M phosphate buffer. Nonspecific binding was suppressed by incubation of sections in 10% goat serum for 1 h and addition of primary anti-

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bodies against MHC-II proteins (1:4, rat anti-rat RT1Bu, Class II polymorphic, Serotec). Biotinylated antibodies (goat anti-rat IgG, Biocarta) and ABC complex (Vector Laboratories) served as secondary antibodies. Incubation with 3.3'-diaminobenzidine tetrachloride (Sigma) produced a specific brown color of MHC-II-containing structures.

Photographing, analysis, and morphometry of immunohistochemical preparations were performed using a MIKMED-5 light microscope with a MOV-1 device (μ). The quantitative distribution of MHC-II$^+$ cells was estimated by microscopic counting in 10 fields of view (3 sections from each animal, ×100).

The arithmetic means were subjected to statistical analysis by Student's *t* test.

RESULTS

Our previous studies showed that OEC in rats is followed by hypertrophy of the thymus due to increase of the medulla and cortex [5].

The present study showed that OEC causes hypertrophy of the thymic lobule (as differentiated from the control group, Fig. 1). MHC-II$^+$ cells were arranged in several rows along the corticomedullary zone of the lobule in animals of both groups. The size of angular cells with light nucleus in the central area of the cytoplasm was 7.3-9.2 μ. MHC-II-producing cells were diffusely localized and sometimes formed small aggregates in the cortex and medulla of the thymic lobule (Fig. 1). Morphological characteristics and localization of MHC-II$^+$ cells in the thymus were similar to those of luminescent granular cells, which belong to the population of dendrite macrophages [1].

MHC-II is expressed only in immune cells. They received the name of professional antigen-presenting cells (APC) and are presented by dendrite cells, macrophages, and B lymphocytes. The complex of antigen-peptide and MHC-II molecules on the surface of these cells is recognized only by T helper cells (CD4$^+$). If these changes are accompanied by necessary and sufficient coreceptor interactions with APC, the T cell receives an activation signal for proliferation and differentiation. The T cell expresses sufficient amount of membrane molecules and cytokines, which is necessary for the interaction with B lymphocytes and leukocytes. These processes lead to activation of immune function. APC create the microenvironment of thymocytes and are involved in the selection of T-lymphocyte clones responding to MHC-II with a foreign antigen and inducing apoptosis in defective autoantigen-reacting T cells [3,6].

After OEC, the number of MHC-II-expressing cells in the medulla and cortex of thymic lobules increased by 1.5 and 1.1 times, respectively, compared to the control (Table 1). Mature thymocytes migrate from the corticomedullary zone of the central immune organ into T-zones of peripheral lymphoid organs. The number of APC in the cor-

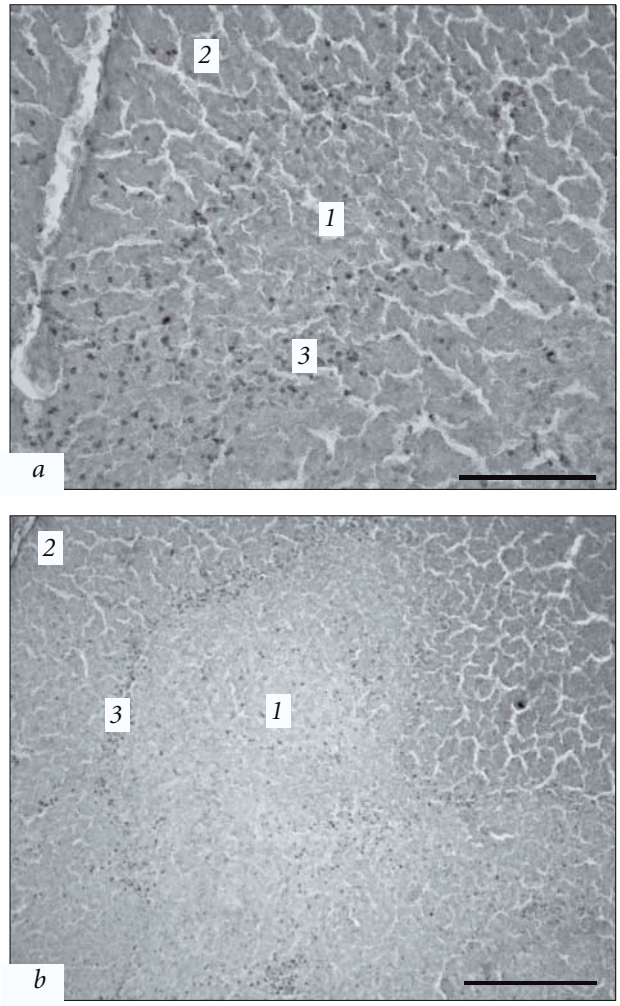

Fig. 1. MHC-II$^+$ cells in the medulla (*1*), cortex (*2*), and corticomedullary zone (*3*) of the thymic lobule under normal conditions (*a*) and after OEC (*b*, ×100).

TABLE 1. Quantitative Distribution of MHC-II-Expressing Cells in the Thymus under Normal Conditions and after OEC (*M*±*m*)

Thymic structure	Group	
	1	2
Cortex	7.00±0.96	8.0±0.9
Corticomedullary zone	13.00±1.12	11.0±0.5*
Medulla	6.00±0.81	9.00±1.31

Note. *p<0.05 compared to group 1.

ticomedullary zone decreases by 1.18 times against the background of male sex hormone deficiency (Table 1).

Thus, experimental deficiency of male sex hormones due to OEC illustrates the interrelation of the immune and endocrine systems. OEC is followed by an increase in the number of MHC-II$^+$ cells in the microenvironment of thymocytes in the thymic cortex and medulla. Maturation of T cells occurs in deep layers of the thymic cortex and is completed in the medulla. These changes can be interpreted as activation of immune processes under conditions of male sex hormone deficiency in the organism.

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