Antigen-Induced Changes in the Endocrine Function of the Thymus in CBA Mice during Aging: Role of Peptide Factors Released by the Pineal Gland

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The titer of thymic serum factor was measured in adult and old CBA females 15, 30, and 60 min, 24, 48, 72, and 96 h after injection of 3% suspension of sheep erythrocytes and changes of this parameter under the effect of epithalamin were studied in old immunized mice. The titer of thymic serum factor increased appreciably in adult mice virtually at all periods of the study after immunization (a drop was observed only 72 h after immunization). In old mice the titer of thymic serum factor virtually did not change after injection of sheep erythrocytes, while immunization of old mice preinjected with epithalamin significantly increased this parameter. Not only the values, but their dynamics in old mice injected with epithalamin corresponded to those in immunized adult animals.

Key Words: *thymic serum factor; T-dependent antigen; age; mice; epithalamin*

Drastic decrease in the immune response to foreign antigens during aging can be determined by changes in the count of T cells and their regulatory subpopulations, spectrum of cytokines produced by them, and specific features of cell-cell interactions [3]. These disorders are largely determined by age-associated weakening of the endocrine function of the thymus [3,6]. Thymectomy decreases blood concentrations of antibodies, while injection of bioactive thymic factors to old mice increases this parameter [7]. The function of thymic epithelial cells can be activated by antigenic stimuli [5].

The pineal gland regulates the rhythmic processes and is sensitive to stress factors, to which T-dependent antigens can be referred. The pineal gland is closely related to the thymic-lymphatic system [2,6,12] and produces bioactive substances of not only indole (melatonin), but also peptide nature [9]. Epithalamin (bioactive peptide) administered to old mice stimulates the immune response, increased the count of T cells, and restored their subpopulation composition [4,6,9].

We studied age-specific changes in the thymic endocrine function of CBA mice treated with T-dependent antigen and evaluated the possibility of its restoration in old animals by epithalamin (peptide factor of the pineal gland).

MATERIALS AND METHODS

The study was carried out on adult (4-5 months) and old (22-24 months) CBA/Ca female mice from Breeding Center of Institute of Gerontology.

For studies of age-specific antigen-induced changes, adult and old mice were intraperitoneally immunized with T-dependent antigen (3% sheep erythrocyte suspension, SE) in a single dose of 0.2 ml in the morning hours (9.00-10.00). Since injection can change blood hormone level [5], controls received a single injection of the same volume of 0.9% NaCl. Blood for analysis was collected in the morning after decapitation. The animals were decapitated under ether narcosis 15, 30, 60 min, 24, 48, 72, and 96 h after injection of SE or NaCl.

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Epithalamin was injected to old mice in the morning hours in accordance with our protocol developed with consideration for age-specific sensitivity of the immune system to this peptide [4]. The course consisted of 5 subcutaneous injections with 2-3-day intervals (single dose 0.5 mg/100 g). Old mice of the control group received 5 subcutaneous injections of 0.9% NaCl. On day 3 after the last injection old mice were immunized intraperitoneally with a single dose of 3% SE suspension (0.2 ml). Blood from immunized old mice was collected in the morning (9.00-10.00).

Intact adult and old mice were also used in the study.

The endocrine function of the thymus was evaluated by the titer of thymic serum factor (TSF), one of the true hormones of the gland [10,11]. The results were expressed in $\log_2$ of the titer.

The data were statistically processed using Student's *t* test.

RESULTS

The titer of TSF in intact adult mice was higher (5.7±0.4, n=21) than in old mice (4.1±0.5, n=9; p<0.05). In adult mice TSF titer decreased to 4.3±0.3 (n=5) 15 min after injection of 0.9% NaCl solution compared to intact animals (p<0.05). During other periods of the study TSF titer in adult animals after injection of 0.9% NaCl did not differ from its level in intact animals; TSF titer in old animals injected with NaCl did not differ from its level in intact animals during the entire period of the study (p>0.05).

In adult animals TSF titer significantly increased 15 min after immunization with 3% SE suspension in comparison with adult mice injected with 0.9% NaCl (Table 1). Significant difference from intact adult animals was observed 30 and 60 min and 24, 48, and 96 h after injection of 3% SE suspension. On the other hand, TSF titer in immunized old mice did not differ from that in old intact animals and was appreciably lower than in immunized adult mice (Table 1).

In the majority old mice pretreated with epithalamin immunization significantly increased TSF titer in comparison with immunized old controls (Table 2). It is noteworthy that in 3 of 4 experimental mice the titer was lower 72 h after immunization than during other periods of the study (3.3±0.4, p<0.05). After injection of epithalamin TSF titer in immunized old mice did not differ from that in immunized adult animals (p>0.05; Table 1), except the 30th minute postinjection.

Hence, the thymus of adult CBA mice reacts to injection of T-dependent antigen (SE) by modification of the endocrine function; phasic nature of the thymic function manifests in alternation of intensification and normalization periods. In old mice the function of the thymus is weak and does not react to antigenic stimulation. Epithalamin not only stimulated the endocrine function of the thymus in old mice in response to the antigen, but approximated it to that in adult animals.

The number and spectrum of mediators produced by activated immune cells (macrophages, lymphocytes) change during the first minutes and hours after antigenic stimulation [5,12]. The mediators serve as an afferent signal for the hypothalamic-pituitary-adrenal system. Elevated blood glucocorticoid level ensures adequate (by intensity) immune reaction and is a factor of immunospecificity [5,12]. TSF, whose level sharply increases during the inductive phase of the immune response, can also serve as a link between the immune and neuroendocrine systems. Injection of fraction 5 of thymosin into the cerebral lateral ventricles of adult mice sharply increases blood concentrations of ACTH and glucocorticoids in adult mice as early as after 2 h, while in nude mice the reaction of

TABLE 1. TSF Titer ($\log_2$) in Adult and Old CBA Mice at Different Periods after Injection of 3% SE Suspension ($M\pm m$)

Period of study	Adult mice	Old mice
15 min	6.6±0.9 (5)	4.1±0.6* (5)
30 min	9.2±0.5^{+} (7)	3.7±0.5* (7)
60 min	9.3±0.3^{+} (6)	2.5±0.6* (3)
24 h	7.6±0.5^{o} (8)	3.0±0.6* (5)
48 h	8.5±0.9 (6)	3.2±0.2* (5)
72 h	4.9±0.5^{ox} (5)	3.0±0.6* (4)
96 h	7.3±0.7^{o} (8)	3.5±0.8* (5)

Note. p<0.05 compared to *adult animals, $^{+}$with the corresponding parameter 15 min postinjection, ^{o}with the corresponding parameter after 60 min, ^{x}with the corresponding parameter 24, 48, and 96 h postinjection.

TABLE 2. TSF Titer ($\log_2$) in Old Mice in Different Periods after Immunization and Its Changes under the Effect of Epithalamin ($M\pm m$)

Period of study	0.9% NaCl	Epithalamin
15 min	4.1±0.7 (8)	6.4±0.6* (5)
30 min	4.5±0.4^{o} (6)	7.5±0.4* (6)
60 min	5.3±0.7 (6)	8.0±0.9* (6)
24 h	5.7±0.5 (6)	8.1±0.9* (6)
48 h	—	8.8±0.9* (4)
72 h	—	4.5±1.4^{+} (4)
96 h	5.6±0.3 (10)	7.2±0.5* (10)

Note. p<0.05 compared to *animals injected with 0.9% NaCl, $^{+}$the corresponding parameter 48 h postinjection, ^{o}the corresponding parameter 96 h postinjection.

the adrenal cortex to stress is much weaker [8,12]. The increase in blood level of thymic hormones during the productive phase of the immune response is most likely directed towards activation of T-helpers [10]. Hormonal shifts under the effects of antigens, in turn, modulate the function of the thymus [5,7,12].

Disorders in the thymic reaction to antigenic stimulus in old mice are determined by structural changes in the gland and specific features in its interactions with the neuroendocrine system [3,6,8]. Epithalamin decelerated age-associated structural changes in the thymus, increased blood concentration of melatonin (adaptive hormone), restored the balance between neurotransmitters in the hypothalamus and its sensitivity to peripheral hormonal signals in old mice [1,2,6,9], as a result the time course of TSF titer fluctuations during immune response was restored in old mice.

Hence, our findings confirm the role of the pineal gland in the mechanisms of age-associated changes in adaptive reactions of the thymus (the central organ of the immune system). The endocrine function of the thymus in immunized adult mice undergoes phasewise changes in the course of the immune response and is characterized by alternation of periods of hyperactivity and normal activity. The reaction of the thymus to the antigen is leveled with aging. Epithalamin (peptide factor of the pineal gland) not only stimulated the endocrine function of the thymus in immunized old mice, but approximated the parameters of its changes to those in adult mice.

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