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EFFECT OF ENDOGENOUS GLUCOCORTICOIDS ON MORPHOLOGY OF THE MOUSE THYMUS AND SPLEEN

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Dependence of T cell reactivity to transplantation H-Y antigens on the physiological level of endogenous glucocorticoids (corticosterone) in inbred mouse lines was demonstrated previously [6, 7]. A decrease in the level of endogenous glucocorticoids in adrenalectomized mice is accompanied by widening of the cortex of the thymus and the appearance of an immune response to H-Y antigens [4]. The thymus affects the abundance of the T-dependent zones in the white pulp of the spleen [9] and controls differentiation of stem cells in the spleen toward myelopoiesis or erythropoiesis [5]. The cortex of the thymus contains cortisone-sensitive precursors of T lymphocytes, whereas the medulla of the thymus contains cortisone-resistant precursors of T lymphocytes. Precursors of T lymphocytes migrate from the thymus into the spleen, where they are converted into mature immunocompetent cells [10].

This paper describes a study of the possible dependence of the ratio between the cortex and medulla of the thymus and the white and red pulp of the spleen on the endogenous corticosterone level in inbred lines of mice of haplotypes $H\text{-}2^k$ and $H\text{-}2^b$.

EXPERIMENTAL METHOD

Female CBA, C57BL/6, and AKR mice aged 3-4 months, which differed in their reactivity to H-Y antigens of skin grafts [7, 11], were used. Mice were obtained from the Inbred Animals Nursery, Academy of Medical Sciences of the USSR (Stolbovaya). The plasma corticosterone level was determined by the method in [8]. Blood was taken from the retro-orbital plexus with a Pasteur pipet at the same time of day and all mice were kept under identical conditions. The thymic and splenic indices (weight of organ in g/body weight in g) × 100, was determined for the animals. Morphometric studies of histological sections of the thymus and spleen, stained with hematoxylin and eosin or azure-eosin, were carried out by means of an ocular grid [1]. The number of points on the ocular grid which coincided with the corresponding morphological structures of the thymus (cortex and medulla) and spleen (white and red pulp) was counted in sections. In sections of each organ 40 measurements were made, and the distribution of 1000 points of the ocular grid determined. In the experiments of series I intact mice were used, adrenalectomized mice in series II. The adrenalectomized mice were given NaCl solution. The thymus and spleen were obtained on the 5th and 10th days after bilateral adrenalectomy. In one experiment hydrocortisone was injected subcutaneously into intact CBA and C57BL/6 mice in a dose of 0.025 mg (1.25 mg/kg body weight) daily for 10 days, after which the thymic and splenic indices were determined; control animals were given phys-

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TABLE 1. Ratio between Cortex and Medulla in Thymus and White and Red Pulp in Spleen of Inbred Lines of Mice (intact), Differing in Their Endogenous Corticosterone Level

Group of animals	Line of mice	Corticosterone level, μg% (M ± m)	Thymus			Spleen		
			index (M ± m)	cortex (g_1 ± m)	medulla (g_1 ± m)	index (M ± m)	pulp	
							white (g_2 ± m)	red (g_2 ± m)
1	CBA	79,4±2,6 (*n*=25)	0,14±0,01 (*n*=30)	60,3±2,9 (*n*=6)	39,2±3,4 (*n*=6)	0,6±0,05 (*n*=30)	67,7±3,0 (*n*=6)	31,7±3,0 (*n*=6)
2	C57BL/6	32,6±3,0 (*n*=28)	0,24±0,02 (*n*=30)	72,5±2,8 (*n*=6)	25,8±2,8 (*n*=6)	0,8±0,09 (*n*=30)	35,5±3,0 (*n*=6)	64, 3±3,0 (*n*=6)
3	AKR	24±3,5 (*n*=33)	—	75,2±3,3 (*n*=5)	24,4±3,9 (*n*=5)	—	29±3,6 (*n*=5)	70±3,6 (*n*=5)

Legend. Here and in Table 2: g_1) mean index of size of cortex and medulla of thymus (in %); g_2) mean index of volume of white and red pulp of spleen (in %); 40 measurements made on histological sections of each organ, with determination of distribution of 1000 points of ocular grid; n) number of animals.

TABLE 2. Ratio of Cortex and Medulla in Thymus and White and Red Pulp in Spleen of Adrenalectomized CBA and C57BL/6 Mice

Group of animals	Line of mice	Corticosterone level, μg% (M ± m)	Thymus			Spleen		
			index (M ± m)	cortex (g_1 ± m)	medulla (g_1 ± m)	index (M ± m)	pulp	
							white (g_2 ± m)	red (g_2 ± m)
1 (adrenalectomy)	CBA	41±3,5 (*n*=16)	0,21±0,02 (*n*=20)	70±3,2 (*n*=6)	0,57±0,05 (*n*=6)	34,2±3,2 (*n*=20)	65,4±3,0 (*n*=6)	30,1±3,2 (*n*=6)
2 (mock operation)	CBA	83,4±4,2 (*n*=16)	0,12±0,015 (*n*=20)	59±3,1 (*n*=5)	38,3±3,4 (*n*=5)	0,56±0,06 (*n*=20)	65,6±2,8 (*n*=5)	32,7±2,9 (*n*=5)
3 (intact mice)	CBA	—	0,012±0,003 (*n*=15)	—	—	0,016±0,01 (*n*=15)	—	—
4 (adrenalectomy)	C57BL/6	17,2±2,3 (*n*=12)	0,28±0,01 (*n*=20)	76,1±2,5 (*n*=7)	23,6±2,5 (*n*=7)	0,73±0,06 (*n*=20)	22,3±2,7 (*n*=6)	77,4±2,7 (*n*=6)
5 (mock operation)	C57BL/6	33,5±2,8 (*n*=12)	0,25±0,02 (*n*=20)	67,8±3,2 (*n*=7)	36,3±2,9 (*n*=7)	0,8±0,07 (*n*=20)	34,9±3,3 (*n*=5)	65±3,0 (*n*=5)
6 (intact mice)	C57BL/6	—	0,11±0,025 (*n*=15)	—	—	0,059±0,06 (*n*=15)	—	—

Legend. Intact mice received hydrocortisone.

iological saline. Student's t test was used for statistical analysis of the experimental results [2].

EXPERIMENTAL RESULTS

The thymus of CBA mice, with a high endogenous corticosterone level, had a narrower cortex and wider medulla than C57BL/6 and AKR mice, with a low corticosterone level (Table 1, groups 1-3). The ratio between cortex and medulla in the thymus of CBA was 1:1.5, in C57BL/6 mice 1:2.8, and in AKR mice 1:3.1; the thymic index in CBA mice, moreover, was less than in C57BL/6 mice ($P < 0.01$). The white pulp in the spleen of CBA mice was larger than the red (Fig. 1), whereas in C57BL/6 and AKR mice the opposite relations were found between them (Fig. 2). In the adrenalectomized CBA mice the thymic index was increased, the cortex of the thymus widened, its medulla reduced, and in the spleen the red pulp was larger than the white (Table 2, groups 1 and 2, $P < 0.01$), i.e., the morphology of the thymus and spleen of adrenalectomized CBA mice and of intact C57BL/6 and AKR mice, with a low corticosterone level, became similar. In adrenalectomized C57BL/6 mice the thymic index was unchanged, but in this line also, under conditions of endogenous hypocorticism the cortex of the thymus widened whereas the medulla was reduced, and the volume of red pulp in the spleen increased (Table 2, groups 4 and 5). The width of the cortex of the thymus and the volume of white pulp in the spleen of intact and adrenalectomized CBA, C57BL/6, and AKR mice exhibited negative correlation with each other ($r = -0.89$). The thymus and spleen of CBA mice were found to be more sensitive to hydrocortisone than the thymus and spleen of C57BL/6 mice, as could be demonstrated by the values of the thymic and splenic indices after injection of hydrocortisone into the mice (Table 2, groups 3 and 6).

The higher level of endogenous glucocorticoids in CBA mice may be one cause of the increase in size of the white pulp in the spleen, for in hypercorticism the number of eryth-

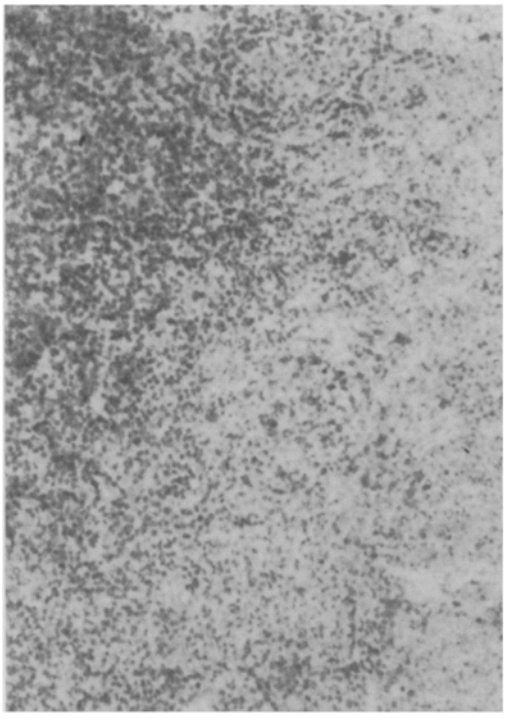

Fig. 1

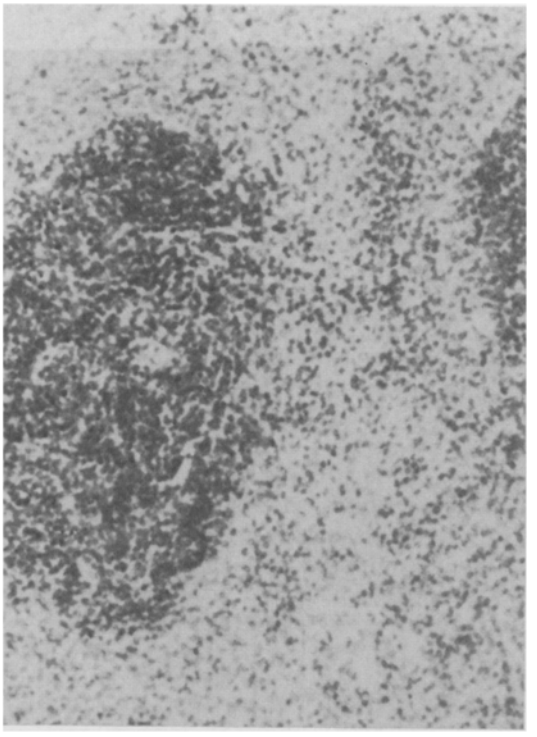

Fig. 2

Fig. 1. Large lymphatic follicle in spleen of CBA mouse. Here and in Fig. 2, magnification 90. Azure-eosin.

Fig. 2. Lymphatic follicles in spleen of C57BL/6 mouse.

roid colonies in the spleen is reduced and the number of granulocytic colonies increased [3]. Glucocorticoids may perhaps influence spleen morphology indirectly through the thymus, for widening of the cortex of the thymus and a reduction in size of its medulla may be accompanied by a compensatory decrease in size of the white pulp in the spleen, although a direct action of glucocorticoids on the spleen is not ruled out. The higher level of endogenous corticosterone and the greater sensitivity of tissues of the thymus and spleen to glucocorticoids in CBA mice compared with these same parameters in C57BL/6 mice may also affect reactivity to H-Y antigens. Preservation of a definite level of endogenous corticosterone in the adrenalectomized animals was due to the presence of accessory adrenal tissue in mice.

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