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Cellular Composition of the Thymus in Wistar Rats in Experimental Intracerebral Hemorrhage

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The time course of changes in cellular composition of the thymus after experimental intracerebral hemorrhage was studied in rats with various prognostic resistance to emotional stress. Increased migration of T lymphocyte precursors to the subcapsular zone, activation of T lymphocyte differentiation in the thymus, reduced number of mitotic cells, destruction and intensive migration of thymocytes from the thymus were observed. The severity of changes in cell composition in all layers of the thymus after experimental intracerebral hemorrhage was different in rats resistant and predisposed to emotional stress. Predisposition to emotional stress and stress before surgical modeling of intracerebral hemorrhage affected the severity of changes in the thymus. It is shown that autoimmune and adaptation mechanisms, which are closely interrelated, play an important role in the pathogenesis of the intracerebral hemorrhage.

Key Words: *thymus; intracerebral hemorrhage; stress; Wistar rats*

Immune mechanisms have been proven to play an important role in the pathogenesis of acute cerebrovascular failure. However, there is no consensus about their impact on its course and outcome [8]. Nonspecific adaptive responses developing due to activation of the hypothalamic–pituitary–adrenal complex, *i.e.* adaptation syndrome, also play a role in the pathogenesis of acute cerebrovascular events [4]. In particular, they are expressed in the immunosuppression due to weakening of T-cell immunity [7]. The thymus is the central organ of immunogenesis. The protective responses of the organism and adaptation to the environment are largely determined by functional state and activity of the thymus [3]. Animals with different prognostic resistance to emotional stress (ES) demonstrate different changes in stress-marker organs, including thymus, in response to ES [5]. The study was aimed at investigation of the cellular composition of the thymus in rats with different prognostic resistance to ES under conditions of experimental intracerebral hemorrhage (ICH).

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MATERIALS AND METHODS

Experiments were carried out on male Wistar rats. The animals were divided into groups by their open-field behavior and prognostic evaluation of their resistance to stress: rats prognostically resistant and liable to ES and ambivalent animals [2]; prognostically stable and liable to ES rats were used in further experiments (108 animals). The animals in the first two groups were randomized into 3 subgroups: control (subgroup 1), rats with ICH (subgroup 2), and rats exposed to ES

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(aggressive conflict behavior with hanging by the tail for 24 h) before surgical modeling of ICH (subgroup 3). ICH was modeled by injection of autoblood (60 μl) according to stereotactic coordinates corresponding to the left caudate nucleus. One, 3, and 7 days after surgery, the animals were sacrificed by intraperitoneal injection of hexachloranum (400 mg/kg) and the thymuses were removed. Histological sections of the thymus were stained with hematoxylin and eosin, picrofuchsin, azure II-eosin, and toluidine blue by standard techniques.

For studying of the cellular structure of the thymus, the cells were counted under a microscope with a ×10 eyepiece and a ×90 oil-immersion objective using built-in 25-knot ocular morphometric grid with 10-μ spacing. Cell composition was determined in the subcapsular zone of the cortex, central zone of the cortex, and medulla. The following cell types were counted: epithelial reticular cells, lymphoblasts, large lymphocytes, medium lymphocytes, small lymphocytes, immature and mature plasma cells, neutrophils, eosinophils, macrophages, cells with mitotic figures, and destructively altered cells. The absolute content of all cell elements per unit area (880 μ^2) was determined and the percents of cells per unit area (880 μ^2) were calculated.

The data were processed by parametric and nonparametric methods. For all tests, 5% significance level was chosen.

RESULTS

After experimental ICH, changes in the total number of cells in the thymus cortex were observed. On day 1, it was most pronounced in rats prognostically liable to ES not stressed before ICH modeling: 41.53±1.11 cells per unit area of 880 μ^2, which was lower than in the control subgroup (59.00±2.39) by 29.7%. In the subgroup of non-stressed animals resistant to ES, the total number of cells decreased by 26.5% (to 40.40±1.52). On day 3, in this zone and in the subcapsular zone, the most marked changes were observed in two subgroups; in non-stressed animals prognostically resistant to ES, the total number of cells was by 40% lower than in controls (67.00±3.67); in stressed rats predisposed to ES it was by 42.1% lower than in controls. On day 7, the minimum total number of cells was observed in the subgroup of resistant non-stressed animals (39.10±1.64, by 41.6% below the control level); areas of connective tissue growth and fibrosis were seen. In other experimental subgroups, this parameter was also significantly below the control level ($p<0.01$). These changes were largely due to a decrease in the number of small lymphocytes, which were the most abundant cells in the cortex (Table 1). The number of these cells was significantly decreased relative to these of the corresponding control subgroups. On day 1, the most pronounced changes were observed in animals predisposed to ES. In rats not exposed to stress before

TABLE 1. Number of Small Lymphocytes and Destructively Altered Cells per Unit Area of 880 μ2 in the Thymus Cortex of Rats Prognostically Liable and Resistant to ES on days 1, 3, 7 after ICH and in the Control (*M±m*)

Group, experimental conditions			Small lymphocytes	Destructively-changed cells
ES-resistant	Control		45.00±1.63	0.80±0.23
	ICH	day 1	38.00±2.05**	1.50±0.38
		day 3	27.70±1.22**	2.3±0.2**
		day 7	23.80±1.15**	2.40±0.29**
	ICH+stress	day 1	39.47±2.03**	0.73±0.15
		day 3	30.10±1.29**	1.80±0.39*
		day 7	29.30±3.22**	1.10±0.17
ES-liable	Control		47.50±2.37	1.40±0.25
	ICH	day 1	29.73±1.42**	1.67±0.15
		day 3	29.40±1.21**	1.20±0.19
		day 7	24.60±1.56**	1.47±0.3
	ICH+stress	day 1	28.07±1.35**	5.33±1.47**
		day 3	17.60±0.62**	1.27±0.11
		day 7	30.67±1.36**	1.2±0.1

Note. Here and in Table. 2: *$p<0.05$, **$p<0.01$ compared to the control.

TABLE 2. Number of Large Lymphocytes, Blasts, and Cells with Mitotic Figures per Unit Area of 880 μ² in the Subcapsular Zone of the Thymus of Rats Prognostically Liable and Resistant to ES on days 1, 3, 7 after ICH and in the Control (*M*±*m*)

Group, experimental conditions			Large lymphocytes	Blasts	Mitosis
ES-resistant	Control		1.30±0.34	0.50±0.16	1.40±0.26
	ICH	day 1	2.60±0.38**	1.60±0.15**	0.60±0.25**
		day 3	3.00±0.32**	2.10±0.48**	0.60±0.15**
		day 7	3.30±0.28**	2.0±0.2**	0.50±0.21**
	ICH+stress	day 1	2.47±0.34**	2.87±0.23**	0.73±0.20**
		day 3	3.40±0.51**	3.40±0.21**	0.40±0.21**
		day 7	3.60±0.47**	2.60±0.21**	0.90±0.26
ES-liable	Control		1.01±0.31	0.60±0.13	1.00±0.27
	ICH	day 1	1.33±0.15*	2.00±0.21**	0.33±0.12**
		day 3	1.60±0.21**	2.07±0.26**	0.27±0.11**
		day 7	1.20±0.17	2.07±0.31**	0.33±0.12**
	ICH+stress	day 1	1.53±0.21*	2.47±0.16**	0.33±0.12**
		day 3	1.87±0.26**	2.27±0.11**	0.13±0.09**
		day 7	1.8±0.3**	2.60±0.13**	0.2±0.1**

ICH modeling, the count of small lymphocytes was 29.73±1.42 per unit area, which is 37.4% below the control level. In stressed rats, this parameter was below the control by 41%. On day 3, the number of small lymphocytes in the cortex of rats predisposed to ES and stressed before ICH was minimum (17.60±0.62 per unit area of 880 μ^2), which was by 63% below the control. By day 7, the least count of small lymphocytes was found in non-stressed animals.

The dynamics of destructively altered cells in the cortex of the thymus in rats with different prognostic resistance to ES after ICH was ambiguous and did not correspond to that of the number of small and medium-sized lymphocytes (Table 1). On day 1, in the group of stressed animals predisposed to ES, a significant increase of destructively altered cells by 381% compared to the control value of 1.40±0.25 was detected. The number of destructions in non-stressed animals prognostically resistant to stress increased by 187.5% from the corresponding control subgroups (to 1.50±0.38). On the day 3, this parameter significantly surpassed the control in ES-resistant animals and on day 7 only in non-stressed resistant animals. These results attest to activation of migration processes and possible completion of phagocytosis of destructively altered cells.

The number of blasts and large lymphocytes in the subcapsular zone and in the whole cortex increased against the background of reduced count of mitotic cells (Table 2). In control subgroups, the counts of blast forms and large lymphocytes in the subcapsular zone of ES-resistant rats were 0.50±0.16 and 1.30±0.34, respectively; in ES-liable rats, the corresponding counts were 0.60±0.13 and 1.01±0.31. On day 1, the greatest increase in the number of blasts was observed in animals exposed to ES before ICH modeling. In resistant and liable animals, this parameter increased by 5.7 and 4.1 times, respectively. The number of large lymphocytes was maximum in animals resistant to ES: in non-stressed and stressed rats it surpassed the control values by 2 and 1.9 times, respectively. On day 3, no significant changes in the number of blasts were noted, but in the subgroup of resistant stressed animals it increased by 6.8 times compared to the control (to 3.40±0.21 blasts per unit area of 880 μ^2). The number of large lymphocytes increased in rats resistant to ES: by 2.3 and 2.6 times in non-stressed and stressed animals compared to the control. In animals prognostically liable to ES, the number of large lymphocytes also increased: by 1.6 and 1.8 times in non-stressed and stressed rats, respectively (to 1.60±0.21 and 1.87±0.26 cells per unit area, respectively). On day 7, the number of lymphoblasts also significantly surpassed the control values ($p<0.01$). The most marked changes were observed in stressed animals. The number of large lymphocytes tended to increase in animals resistant to ES and to decrease in rats predisposed to stress. In non-stressed ES-liable animals, the number of large lymphocytes did not significantly differ from the corresponding control value ($p>0.05$). In this case, the number of cells with mitotic figures was significantly decreased

compared to the corresponding control in all experimental groups ($p<0.01$).

These changes attest to stimulation of migration of T lymphocyte precursors from the bone marrow to the subcapsular zone of the thymus, activation of T lymphocytes differentiation in the subcapsular zone and cortex, enhanced migration of medullary thymocytes from the thymus. This is consistent with reported data on regulatory influence of the thymus on hemopoiesis during stress [1].

Thus, autoimmune and adaptation mechanisms, which are closely interrelated, occupy a special place in the pathogenesis of the experimental ICH. Against the background of involutive changes in the thymus, signs of its functional activation are clearly seen.

Wistar rats resistant and predisposed to ES react to experimental ICH with different severity of changes in the cellular composition in all layers of the thymus. Stress before surgerical modeling of ICH also affects the severity of changes in the thymus.

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