Parameters of Cell Immune Response in Wistar and OXYS Rats and Their Behavior in the Open Field Test

E. V. Markova, L. A. Obukhova*, and N. G. Kolosova**

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Comparative analysis of the state of the thymus, activity of cell component of the immune system, and open field behavior of Wistar and OXYS rats showed early involution of the thymus in OXYS rats. By morphological picture this involution corresponded to accidental involution and was paralleled by low level of delayed hypersensitivity reaction and by inhibition of motor and exploratory behavior.

Key Words: *thymus; delayed hypersensitivity; motor and exploratory behavior*

It is obvious that neurochemical setting of the brain determining individual behavior essentially modulates immune reactions in the body. Our previous studies on mice demonstrated a close relationship between the behavior and activity of cell-mediated immunity: modulation of cell-mediated immune reactions can modify exploratory activity of animals [4, 10]. In order to clear out whether this relationship is universal, we compared some parameters of cell immunity in Wistar and OXYS rats. The latter rat strain is characterized by changes in the cognitive and emotional spheres typical of aging animals and humans, including decreased level of exploratory behavior [3]. Now we compared intensity of delayed-type hypersensitivity (DTH) reaction and the state of the thymus as one of the central organs of the immune system responsible for cell-mediated immunity in these rat strains. The individual differences in the rat behavior were evaluated using the open field (OF) test.

MATERIALS AND METHODS

The study was carried out on 3-month-old male Wistar and OXYS rats (n=60) at the Laboratory of Experimental Animal Breeding, Institute of Cytology and Genetics, where the strain of early aging OXYS rats was bred [9]. The animals were kept on standard vivarium ration with free access to water. Experiments were carried out from10.00 to15.00. Individual differences in OF behavior were recorded. OF was a large (100×100 cm) cage divided into 100 squares with plastic 40-cm walls. OF was illuminated with a shadowless 100 W lamp positioned at a height of 1 m above the center of the field. The animal was put into the corner of the field and motor and exploratory behavior (number of crossed squares, rearing postures) was evaluated for 5 min; emotional strain was evaluated by the number of grooming reactions, fecal boluses, and the latency of the first entry into the center of the field [1].

In some animals the thymus was examined by light microscopy. The thymuses were weighed for estimating the weight index (1 mg thymus/100 g body weight), fixed in Telesnitskii fixative at 4°C, and embedded in histoplast. Serial sections were stained with hematoxylin and eosin, azur B, and eosin Y. Morphometry of structural components of the thymus was carried out by the point counting method and subsequent estimation of the absolute volumes [7].

In order to evaluate DTH reaction, the animals were immunized intraperitoneally with sheep erythrocytes (0.5%, 5 ml). The challenge dose of the antigen (50%, 0.5 ml) was injected under the hind paw aponeurosis after 96 h. The formation of DTH reaction was evaluated 24 h after challenge by the severity of

State Institute of Clinical Immunology, Siberian Division of Russian Academy of Medical Sciences; *State Institute of Clinical and Experimental Lymphology, Siberian Division of Russian Academy of Medical Sciences; **Institute of Cytology and Genetics, Siberian Division of Russian Academy of Sciences, Novosibirsk. ***Address for correspondence:*** evgeniya_markova@mail.ru. Markova E. V.

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TABLE 1. Parameters of Behavioral Activity of Wistar and OXYS Rats in Open Field Test (*M*±*m*, *n*=20)

Parameter	Wistar	OXYS	*p*
Latency of visiting the center	261.0±17.9	265.0±25.3	0.59
Number of crossed squares	106.9±15.8	42.6±7.4*	0.0007
Number of rearings	10.9±1.4	4.1±0.7*	0.0002
Grooming	3.4±0.6	1.0±0.2*	0.0007
Defecations	3.6±0.5	5.1±0.6	0.055

Note. * significant differences from Wistar rats.

edema (alteration of paw thickness in comparison with hind paw injected with RPMI 1640). The reaction index (RI) was estimated for each animal by the formula: $RI=(R_E-R_C)/R_C$, where R_E was reaction in experimental paw and R_C reaction in the control paw, and expressed in percent [11].

The results were statistically processed using ANOVA factorial analysis in the STATGRAPHICS software. The differences were significant at $p<0.05$.

RESULTS

The study confirmed previous data on appreciable inhibition of motor and exploratory behavior in OXYS rats (Table 1). This was seen from their 2.5 times lower horizontal and vertical activities (number of crossed squares and rearings) in comparison with Wistar rats. The number of defecations was 1.4 times higher and the number of grooming acts 3.4 times lower in OXYS rats than in Wistar rats, which indicated high anxiety of these animals. Latency of the first entry into OF center was virtually the same in rats of both strains, which, considering chaotic trajectory of movements in OXYS rats, could be explained by their disordered spatial orientation.

Progressive involution of the thymus, inhibition of its function, and gradual loss of autotolerance are typical manifestations of aging. Gravimetric and morphometric studies revealed significant differences in the state of the thymus between OXYS and Wistar rats. The weight index of the thymus in OXYS rats was 1.8 times lower, the total volume of the thymus 1.9 times, the volume of the cortical matter 2.1 times, that of medullary substance 1.9, and the volume of connective-tissue stroma 1.3 times lower (Table 2) than in Wistar rats. Microscopy of thymus sections from OXYS rats revealed decreased density of lymphoid cells in the cortical matter and medulla and decreased (in comparison with Wistar rats) number of blasts in the subcapsular zone of the cortical matter. The thymus cortex in OXYS rats more often contained cells with pyknotic nuclei, macrophages containing pigment inclusions and/or fragments of dead lymphocyte nuclei, plasma cells. The capsule and interlobular septae of the thymus in OXYS rats contained more degranulating mast cells than in Wistar rats. These results indicate earlier involution of the thymus in OXYS rats, which by the type of morphological changes corresponded to accidental involution of the thymus in humans. Since accidental involution usually develops as the result of stress exposure [5], it seems that in OXYS rats the factor triggering accidental involution of the thymus (in the absence of external stimuli) is high sensitivity to oxidative stress characteristic of this rat strain.

Decreased reactivity of cell immunity is a manifestation of changes in the thymus in OXYS rats. The development of DTH reaction to sheep erythrocytes in OXYS rats was 2.4 times less pronounced than in Wistar rats $F(1.30)=251.42$; $p<0.01$; Fig. 1).

Brain structures modulating the intensity of immune response are the posterior and anterior hypothalamic fields, hippocampus, reticular formation of the midbrain, raphe nucleus, and amygdala. Increased

TABLE 2. Morphometric Parameters of the Thymus in Wistar and OXYS Rats (*M*±*m*, *n*=5)

Parameter	Wistar	OXYS
Total volume of thymus, mm^3	99.54±4.84	51.59±9.86**
Volume of cortical matter, mm^3	71.85±4.69	34.58±6.81**
Volume of medulla, mm^3	20.05±1.32	10.56±2.25**
Volume of capsule and interlobular septae, mm^3	9.44±0.78	6.44±0.94**
Weight index, mg/100 g	110.03±6.91	62.11±5.95*

Note. *$p<0.001$, **$p<0.01$, ***$p<0.05$ compared to Wistar rats.

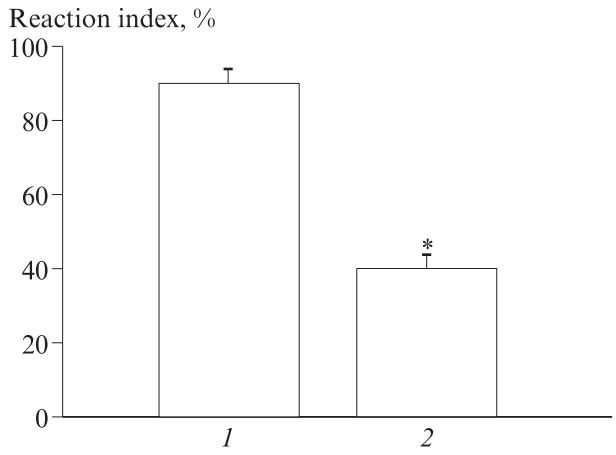

Fig. 1. Intensity of delayed hypersensitivity reaction in response to injection of sheep erythrocytes in Wistar (*1*) and OXYS (*2*) rats. **p*<0.01 compared to Wistar rats.

activity of the serotoninergic system of the midbrain raphe nuclei realized through the hypothalamic-pituitary-adrenal axis can lead to suppression of the immune system [8]. As was shown previously, OXYS rats are characterized by significant changes in activity of the serotoninergic system, including increased serotonin content in the midbrain of young animals [6] and appreciable decrease in activity of monoaminoxidase A in the brainstem [2]. We hypothesize that the detected behavioral characteristics in OXYS rats can be due to the above-mentioned changes in the neurochemical settings of the brain and to the specific features of the immune system functioning in these animals.

Hence, the relationship between activity of cell-mediated immunity and behavioral characteristics detected previously for mice is also characteristic of early aging OXYS rats. Apart from decreased exploratory activity these animals are characterized by early involution of the thymus paralleled by inhibition of cell immunity.

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