BIOGERONTOLOGY

Stimulation of Cell Component of the Immune Response Activates Exploratory Behavior in Senescence Accelerated OXYS Rats

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Suppression of the cell component of the immune system and open-field behavior developing in OXYS rats by the age of 3 months are regarded as manifestations of accelerated aging. Stimulation of cell-mediated immune response with BCG vaccine caused a dose-dependent increase of orientation and exploratory activity of OXYS rats in the open field test to virtually the same level as in Wistar rats.

Key Words: *orientation and exploratory behavior; immune response; delayed-type hypersensitivity; senescence accelerated OXYS rats*

Changes in cognitive and emotional spheres are typical of aging. Their prevention is an obligatory condition providing active longevity of elderly people. The identity of cognitive, neurobiological, and neurochemical changes in aging human and animal brain permits the use of biological models for developing new means correcting these changes. OXYS rats bred at Institute of Cytology and Genetics is an adequate model of accelerated aging and aging-related neurodegenerative processes [3,4]. Increased anxiety (compared to Wistar rats), disturbances in associative learning, decreased exploratory activity in the open field test develop in OXYS rats by the age of 3 months and are regarded as manifestations of accelerated aging [5,6]. It was previously shown that changes in exploratory activity in OXYS rats [8], similarly as in (CBA×C57Bl/6)F_1 mice [14], are significantly related to activity of cellular immune reactions. It was also shown that stimulation of cellular component of immune response activated parameters of exploratory behavior in passive (CBA×C57Bl/6)F_1 mice, but did not modify behavioral status of active animals [8].

The aim of our study was to clear out whether it was possible to modify the behavior of OXYS rats by stimulating cellular immune reactions.

MATERIALS AND METHODS

The study was carried out on 3-month-old male OXYS (n=40) and Wistar (n=10) rats from laboratory of experimental animals of Institute of Cytology and Genetics. The animals were placed into cages (5 per cage) at least 2 weeks before the experiment and kept at natural illumination on standard ration with free access to fodder and water. Experiments were carried out from 12.00 to 15.00. All animals were tested in an "open field" [2], after which some animals were injected with dry tuberculous BCG vaccine (Allergen Firm) for stimulating delayed-type hypersensitivity

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reaction. The vaccine was injected intraperitoneally in doses of 50 or 600 μg/kg in 0.5 ml RPMI-1640. Controls were injected with the same volume of the solvent (RPMI-1640) under similar conditions. Two weeks after injection exploratory activity in the open field was repeatedly evaluated.

Open field was a 100 cm^2 chamber with 40-cm plastic walls, illuminated with a 100 W shadowless lamp hanging above the center of the field (100 cm). The animal was placed into the corner of the chamber and its orientation and exploratory behavior was evaluated by counting crossed squares and rearings over 5 min. Emotional strain was evaluated by the number of fecal boluses, grooming reactions, and latency of exit into the center of the field.

The results were statistically processed using ANOVA analysis in the Statgraphics software.

RESULTS

During the first open-field testing horizontal ($F_{1.47}$= 12.2, p<0.001) and vertical ($F_{1.47}$=5.6, p<0.022) motor activities of OXYS rats were, as expected, markedly lower than of Wistar rats (Table 1). The number of defecation boluses and grooming reactions was virtually the same; the latency of the first exit into the center was also similar in animals of both strains. During repeated testing in the open field horizontal and vertical activities of control OXYS rats were lower than in Wistar rats (6.7 times ($F_{1.18}$=7.5, p<0.013) and 2.6 times ($F_{1.18}$=4.7, p<0.044), respectively). High anxiety characteristic of OXYS rats manifested in a 3-fold lower number of grooming reactions in comparison with repeatedly tested Wistar rats ($F_{1.18}$=7.2, p=0.015) and 5-fold greater number of boluses ($F_{1.18}$=18.2, p<0.0001).

Stimulation of the cellular component of the immune response by injection of BCG vaccine significantly modified only horizontal motor activity of animals ($F_{2.25}$=3.45, p<0.0473). The effect was dose-dependent and peaked after injection of 600 μg/kg vaccine: in this case horizontal motor activity increased 3-fold (p<0.033) and did not differ from that of Wistar rats. Changes in vertical motor activity and number of grooming reactions were insignificant; however, animals receiving the maximum dose of the vaccine did not differ from Wistar rats by these parameters. Immunization did not reduce anxiety of animals, which remained high: the number of defecation acts in all OXYS rats was higher.

Injection of BCG vaccine caused pronounced delayed-type hypersensitivity reaction, characterized by selective activation of macrophages and $CD4^+$ lymphocytes belonging mainly to type I T-helpers [1,13]. The key elements of BCG-induced immune response were cytokines produced by these cells (IFN-γ, TNF-α, and IL-12 [11,12]. It seems that these cytokines are mainly responsible for the detected changes in the orientation and exploratory behavior of animals. The fact that after BCG vaccination IFN-γ inhibits the development of humoral immune response [10] con-

TABLE 1. Effect of BCG Vaccine on the Orientation and Exploratory Activity of OXYS Rats in the Open Field Test (*M*±*m*)

Test, drug		n	Latency of exit into the center	Horizontal motor activity (crossed squares)	Rearings	Grooming	Defecations
Wistar	test 1	10	212±33	119.0±19.6	15.2±3.0	4.20±0.82	2.10±0.73
	test 2	10	253±31	57.9±17.0	3.60±0.94	2.90±0.65	0.60±0.22
OXYS	test 1	30	251±33	60.6±6.9* $F_{1,47}$=12.2 p<0.001	9.50±0.96* $F_{1,47}$=5.6 p<0.022	4.90±0.52	2.62±0.35
	test 2 control	10	300±0	8.7±1.4* $F_{1,18}$=7.5 p<0.013	1.40±0.37* $F_{1,18}$=4.7 p<0.044	0.90±0.34* $F_{1,18}$=7.2 p=0.015	3.00±0.51* $F_{1,18}$=18.2 p<0.0001
BCG, 50 μg/kg		10	300±0.0	16.6±5.7	1.10±0.35	1.00±0.23* $F_{1,18}$=6.8 p<0.019	3.89±0.86* $F_{1,18}$=15 p<0.001
BCG, 600 μg/kg		10	298.0±2.5	28.8±9.1$^+$ $F_{1,18}$=4.7 p<0.043	2.6±1.1	1.9±0.7	2.80±0.81* $F_{1,18}$=6.8 p<0.018

Note. *n*: number of animals. Significant differences compared to: *Wistar rats, $^+$controls and BCG-immunized OXYS rats.

firms our previous conclusion on close relationship between activity of the cellular component of the immune response and exploratory behavior in experimental animals [7,14].

Parallel age-specific changes in functional activity of the immune system and orientation and exploratory behavior were demonstrated in a number of papers [10,15]. However, even the presence of correlations cannot be regarded as a proof of the direct effect of changes in the immune system on the manifestations of neuronal aging. The possibility of correction of behavioral changes associated with early aging of OXYS rats by stimulation of cellular immune reactions is a direct proof of the involvement of neuroimmune relationships in the process of aging. As was previously shown, stimulation of the cellular immune response activates exploratory activity in mice with passive behavioral type [7].

Hence, the results indicate a universal stimulatory effect of the cellular component of immune response on parameters of orientation and exploratory behavior of animals.

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