Immune Response in Wistar Rats with High and Low Level of Situational Anxiety

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A large sample of Wistar rats was divided into 2 groups of high-anxiety and low-anxiety animals by the time spent in the open arms of the elevated plus-maze. This selection was based on the criterion of time (low-anxiety animals, not less than 10 sec; high-anxiety animals, not more than 2 sec). Immunization with T-dependent antigen was performed on the day of behavioral testing. The number of rosette-forming cells in high-anxiety rats significantly decreased on day 5 after immunization. A genetically determined relationship probably exists between low activity of the immune response and high level of reactive anxiety.

Key Words: *neuroimmunomodulation; anxiety; rosette-forming cells; rats*

A large body of evidence demonstrates the dependence of the immune status on psychoemotional state of humans and animals [3,7,14]. Depressive and panic disorders and stress exposure are accompanied by immune dysfunction, hypersensitivity to infectious agents, and high risk of tumor development [7,10,11]. Anxiety serves as a symptom of other diseases and forms a class of mental disorders, which ranks second after depressions. The decrease in several immunological parameters is observed not only in patients with panic disorders, but also in practically healthy subjects with reactive anxiety [6,9,11].

Little is known about the interrelation between basic anxiety and immune response in animals. Our previous experiments revealed this interrelation under conditions of chronic social stress of subordinate behavior. C57Bl/6J and CBA mice differing in basic anxiety and development of a depression-like state [1] exhibited various immune reactions to the T-dependent antigen (sheep erythrocytes) [4]. As distinct from low-anxiety CBA mice, changes in the number of $CD8^+$ and $CD4^+$ T lymphocytes in submissive C57Bl/6J mice were observed after 10-day confrontation. The immune response was suppressed in the follow-up period (up to 20 days) [2,4,5]. Hence, the rate of transformation into a depression-like state increased with an increase in individual anxiety of animals.

Here we studied the immune response in Wistar rats differing in anxious behavior under conditions of the elevated plus-maze.

MATERIALS AND METHODS

Experiments were performed on 50 male Wistar rats aging 3.5-4.0 months and obtained from the Laboratory of Animal Breeding (Institute of Cytology and Genetics). The animals obtained from the vivarium were housed in plastic cages (2 rats per cage) under standard conditions and natural light/dark cycle and had free access to water and food. Our study was started after 1-week adaptation of animals to new laboratory conditions. The rats were divided into groups of low-anxiety (LA) and high-anxiety (HA) animals by their behavior in the elevated plus-maze test [12]. Behavioral parameters of anxiety were evaluated for 5 min. They included

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the time spent in the open arms, closed arms, and central area and the number of transitions between the open and closed arms of the maze. We recorded various ethological parameters, including the number of overhangs from the open arms and time of behavioral suppression (freezing). The time spent in the open arms of the maze served as a criterion to select the animals with extreme levels of anxiety. This parameter for LA animals was not less than 10 sec (complete entry). HA animals were characterized by the absence of complete entries or exhibited an incomplete entry for 2-3 sec (forelimbs in the 1st section of the arm, hindlimbs in the central area). Intermediate animals were excluded from a further study. On the day of behavioral testing, LA and HT animals and control rats (n=20) were intraperitoneally immunized with sheep erythrocytes (5×10^8 in 0.5 ml physiological saline). Rosette-forming cells (RFC) in the spleen were counted at the peak of the immune response (day 5 after antigen treatment) [4].

Intergroup differences were evaluated by analysis of variance (ANOVA).

The experiment was conducted in accordance with the principles of humanity stated in the Directives of the European Community (86/609/EC) and approved by the Committee of Biomedical Ethics (Institute of Physiology).

RESULTS

One-way ANOVA revealed significant inter-group differences in the percent of time spent in the open arms ($F_{1,28}$=54.04, p<0.001), number of transitions into the closed ($F_{1,28}$=90.01, p<0.001) and open arms ($F_{1,28}$=57.5, p<0.001), and additional ethological parameters for anxiety in the maze (Table 1). Exploratory activity decreased in HA rats (lower number of transitions between the arms, absence of overhangs from the open arms). Freezing behavior (complete immobility) was observed in 89% HA animals. The mean time of freezing behavior in these rats was 196.7 sec. This reaction was not revealed in LA animals. The immune response differed in rats with various levels of anxiety (Fig. 1). At the peak of the immune response (day 5 after immunization), the number of RFC in HA rats was much lower than in controls ($F_{1,37}$= 12.67, p<0.01) and LA animals ($F_{1,28}$=7.48, p<0.05). The immune response in LA rats did not differ from that in control animals. It should be emphasized that the number of RFC tended to increase in LA rats, which was probably related to emotional homogeneity of the sample. A strong correlation was found between the number of RFC and percent of time spent in the open (r=0.59, n=30, p<0.05) and closed arms (r=-0.60). Moreover, the count of RFC correlated with the number of transitions into the open (r=0.62) and closed arms (r=0.69).

These data and the correlations between behavioral parameters for anxiety and number of RFC illustrate the cause-effect relation between emotiogenic and immune systems (at least in the immune reaction to T-dependent antigens, including sheep erythrocytes). It remains unclear whether these features are typical of T-independent antigens. The T-dependent and T-independent immune responses may differ in sensitivity to similar psychoemotional factors [15].

It is important to evaluate the analogy between this model of anxiety and nosological classification. The plus-maze is used to study situational anxiety [8], which serves as an analogue of reactive (situational) anxiety in humans. It reflects the degree of emotional strain in case of typical events. HA rats

TABLE 1. Behavior of Rats in the Elevated Plus-Maze (M±m)

Parameter	Group	
	HA rats (n=19)	LA rats (n=11)
Number of entries into open arms	0.3±0.1	2.7±0.3*
Time spent in open arms, sec	0.9±0.5	23.5±3.9*
Percent of time spent in open arms	0.4±0.1	7.8±1.3*
Time spent in central area, sec	12.7±3.1	58.3±7.2*
Percent of time spent in central area	4.2±1.0	19.4±2.4*
Number of transitions into closed arms	1.05±0.05	4.3±0.5*
Time spent in closed arms, sec	286.7±3.1	200.3±21.3*
Percent of time spent in closed arms	95.5±1.0	73.2±2.6*
Number of overhangs	0.0	6.2±0.9*

Note. *p<0.01: differences between the groups.

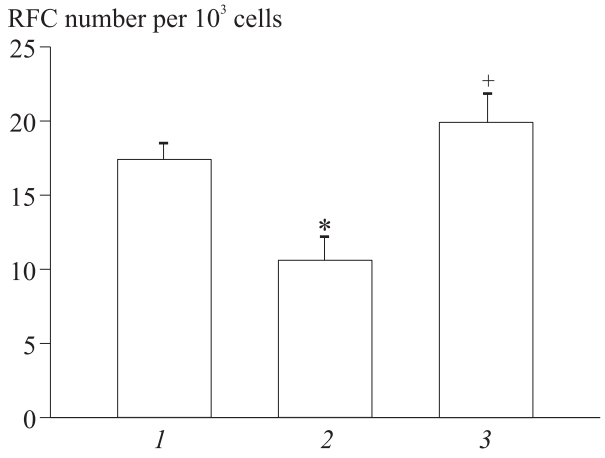

Fig. 1. Immune response in Wistar rats differing in anxiety in elevated plus-maze. Intact control (*1*), HA rats (*2*), and LA rats (*3*). **p*<0.01 compared to the control; +*p*<0.01 compared to HA rats.

avoid open arms of the maze, which suggests hyperreactivity of animals to new situations of this type. It is unlikely that severe suppression of the immune response results from short-term testing in the maze. For example, the number of leukocytes and other parameters of the immune response in healthy students remain unchanged during severe acute stress before examination [13].

Clinical and experimental trials showed that acute stress is often followed by activation of the immune response [3]. Probably, acute stress does not cause suppression of the immune system in emotionally resistant subjects. We also revealed no changes in the immune response in LA rats. The decrease in the number of RFC in HA rats probably results from psychoneuroimmunomodulation in these animals. Our results are consistent with published data on suppression of the immune response in healthy volunteers with high level of reactive anxiety (according to the Spielberger—Khanin questionnaire) [6]. It remains unknown which of these two systems plays a primary role.

Another situation is observed during chronic exposure (isolation or confrontation) accompanied by social stress and changes in the immune response. Negative influences under these conditions are long-lasting and contribute to the development of depressive anxiety, which always results in dysfunction of the immune system [2,4,5,10]. Different states of depressive and reactive anxiety are provided by various anatomical and neurotransmitter systems.

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