EXPERIMENTAL BIOLOGY

Diurnal Variations in Lymphocyte Subpopulations in Lymphoid Organs of Rats with Genetic Catalepsy and Wistar Rats

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Parameters of the immune status of the thymus and spleen in rats with genetic catalepsy were lower compared to those in Wistar rats. Diurnal variations in cell subpopulations of lymphoid organs were different in animals of these strains. Behavioral characteristics and neuroendocrine state in rats with genetic catalepsy were associated with specific changes in the immune system and neuroimmune interactions.

Key Words: *catalepsy; lymphocyte subpopulations; lymphoid organs*

Mental disorders in humans and animals are accompanied by changes in the immune system, which decreases general resistance, causes a variety of infections, increases the risk of tumor growth, and results in preterm aging [9]. Moreover, cytokines and neuromodulators produced by immunocompetent cells modulate functions of the brain [2]. Abnormal production of neurotropic bioactive substances during psychoneuroimmune disorders closes the vicious cycle, which plays a role in the development and progression of mental disorders. Study of psychoneuroimmune interactions in animals with mental disorders is an urgent scientific problem.

The rats genetically predisposed to catalepsy (GC) were bred from Wistar rats at the Institute of Cytology and Genetics (V. G. Kolpakov). These animals are used to study functional psychoses. Previous experiments showed that GC rats are characterized by behavioral disorders (nervosity), high excitable aggressiveness, low locomotor, orientation, and volitional activity, disturbances in interhemispheric asymmetry, delta-sleep deficiency, and reduced content of neurotransmitters and protein fractions in brain structures [4,8].

A correlation was found between activities of the monoaminergic and pituitary-adrenal systems and immune state [2]. Functional activity of the serotoninergic [3,4], catecholaminergic [4], gonadal, and adrenal systems is impaired in GC rats [7]. Here we compared immune indexes in GC rats and Wistar rats with normal behavioral and neuroendocrine activity.

MATERIALS AND METHODS

Experiments were performed on adult male Wistar and GC rats weighing 200 g. The animals were decapitated at 9.00-10.00 and 18.00-19.00. Cell suspensions were obtained from the thymus and spleen. Smears were prepared on slides by the dried-drop method and fixed

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with acetone. Subpopulations of $CD4^+$, $CD8^+$, and activated lymphocytes were studied immunocytochemically using monoclonal antibodies (Sigma) [6]. Peroxidase-labeled ExtrAvidin-biotin kit (Sigma) served as the second layer. Cell nuclei were stained with 1% methyl green.

Preparations were studied under a light microscope (oil immersion, ×1000). The percentage of peroxidase-positive cells was estimated.

The results were analyzed by Mann--Whitney test.

RESULTS

GC rats differed from the parent strain of Wistar rats in the ratio of lymphocyte subpopulations in the thymus and spleen (Table 1).

No diurnal differences were found in the ratio of $CD4^+$ lymphocytes in the thymus of GC and Wistar rats. In the evening the percentage of $CD4^+$ lymphocytes in GC rats was lower than in Wistar rats (Table 1). In the morning the percentage of $CD8^+$ lymphocytes in GC rats was much lower than in the evening. This index underwent no diurnal variations in Wistar rats. In the morning the percentage of $CD8^+$ lymphocytes in GC rats was lower than in Wistar rats. We revealed no diurnal and interstrain differences in the ratio of activated lymphocytes.

In contrast to Wistar rats, the content of $CD4^+$ lymphocytes in the spleen of GC rats in the morning was higher than in the evening. Significant interstrain differences were found in the evening. The percentage of $CD4^+$ lymphocytes in GC rats was lower than in Wistar rats. No diurnal and interstrain differences were revealed in the ratio of $CD8^+$ splenocytes. In the morning the percentage of activated lymphocytes in the spleen of GC rats was higher than in the evening. We revealed significant interstrain differences in this index. In GC rats the percentage of activated lymphocytes in the morning and evening was lower than in Wistar rats.

The study of T lymphocyte subpopulations has high diagnostic and prognostic significance during congenital and acquired immunodeficiencies, autoimmune disorders, and allergic, infectious, and malignant diseases. Clinical trials showed that T cell immunity is impaired in patients with various mental disorders, including schizophrenia, depression, and alcohol dependence [2,8,10]. This is accompanied by a decrease in interleukin-2 production and inactivation of T suppressors. Changes in the quantitative ratio between T helpers and T suppressors are followed by stimulation of B cell immunity and accumulation of autoantibodies (including brain antibodies). Similar changes in T cell immunity were revealed in GC rats: decrease in the content of $CD8^+$ lymphocytes in the thymus and number of $CD4^+$ and activated lymphocytes in the spleen. These data suggest that GC rats are characterized by disturbances in central differentiation of T lymphocytes and decrease in functional activity of T cells in secondary lymphoid organs (compared to the parent strain of Wistar rats). Published data show that serotoninergic function increases in GC rats. Treatment with serotonin synthesis blocker *p*-chlorophenylalanine increases activity of striatal tryptophan hydroxylase and shortens the time of catalepsy in these animals [3]. It could be hypothesized that immune deficiency in GC rats is related to high activity of the serotoninergic system. Since these relationships are mediated by the adrenal glands, our assumption disagrees with low corticosterone level in GC rats (compared to Wistar rats). Immune deficiency in GC rats is more likely associated with dopamine deficiency [8]. Dopamine directly stimulates immune function via the thymus without affecting the adrenal glands. A relationship was found between the number of dopamine D2 receptors and immune reactivity. Moreover, the dopaminergic system is involved in the redistribution of T cells [2]. These data suggest that neurotransmitter imbalance affects the immune system by modulating activity of the neuroendocrine axis (serotonin—hypothalamus—pituitary—adrenal glands).

TABLE 1. Diurnal Variations in Lymphocyte Subpopulations in Lymphoid Organs of Wistar and GC Rats (%, *M*±*m*)

Organ, rat strain		Sampling, h	$CD4^+$	$CD8^+$	Activated lymphocytes
Spleen	GC	10.00	44.75±2.60	60.4±8.2	40.0±3.2$^+$
		18.00	29.0±3.7*	64.3±2.9	12.33±0.90*$^+$
	Wistar	10.00	55.66±7.60	69.6±5.3	69.66±6.80
		18.00	60.8±6.3	52.56±6.00	63.2±0.4
Thymus	GC	10.00	50.0±7.8	36.75±3.60	41.25±8.40
		18.00	33±2$^+$	60.0±5.3*	50.33±1.20
	Wistar	10.00	66.8±9.7	67.4±8.1	37.67±1.10
		18.00	61.8±9.7	68.4±7.3	57.4±9.4

Note. $p<0.05$: *compared to 10.00; $^+$compared to Wistar rats.

Our results indicate that behavioral characteristics and neuroendocrine state in rats genetically predisposed to catalepsy are associated with specific changes in the immune system and neuroimmune interactions.

Parameters of the immune status of the thymus and spleen in GC rats are lower than in Wistar rats. Therefore, the effector function and central differentiation of T cells in GC rats are reduced compared to Wistar rats.

GC and Wistar rats are characterized by different diurnal variations in the ratio of cell subpopulations in lymphoid organs. It amplifies published data on different diurnal changes in blood lymphocyte enzymes and cell count in lymphoid organs [5]. Monoamine oxidase activity in the brain of GC rats undergoes diurnal variations [11]. Therefore, temporal organization of the neuroimmune interactions differs in the parent strain of Wistar rats and GC rats genetically predisposed to catalepsy.

Interstrain differences in various indexes were observed in the morning or evening, which necessitates the use of chronobiological approach in detailed study of the immune system.

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