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Level of Cytokines and C3 Complement in the Blood of Rats under Conditions of Chronic Unpredictable Stress of Different Durations

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The parameters of the cytokine profile and functional activity of the complement system in the blood of rats were studied during different time periods of chronic unpredictable mild stress using a model of sequentially alternating low-intensity stress effects for 1, 2, 3, and 4 weeks. In the dynamics of observation, a general tendency towards multidirectional fluctuations in the concentration of cytokines was revealed: an increase in IL-10, but a decrease in IL-4 in comparison with the control. Statistically significant changes in the level of IL-10 were noted after 2, 3, and 4 weeks, IL-4 — after 2 and 4 weeks of stress loads. The percentage of lysis of the C3 component in rats gradually increased by the 2nd week of chronic stress, but then decreased and practically did not differ from the control values (intact animals) by the end of the study. These results illustrate the specificity of changes in the indicators of the C component of the complement system and the cytokine profile of the blood reflecting activity of the cellular and humoral components of the immune response in rats exposed to repeated stress factors of different origins and duration.

Key Words: *rats; chronic unpredictable mild stress; blood cytokines; C3 component of complement system*

According to Selye's definition (Selye H., 1956), stress is a nonspecific response of the body characterized by a number of successively changing stages — anxiety, resistance, and exhaustion. Numerous studies in the field of stressology have obtained data that complement classical ideas about the nature of the influence of negative emotiogenic factors on the physiological systems of the body, as well as the dependence of the observed consequences on the type, duration, and strength of the stress load.

It is known that psychoemotional stress affects the functional activity of the immune system in mammals, being one of the leading factors in the pathogenesis of pathological processes, such as inflammation and immunodeficiency states of various origins [1]. In particular, changes in the cytokine profile of the blood were discovered under the influence of various stress factors [2,3]. Cytokines are polypeptide mediators of intercellular interactions. These bioactive substances are involved in the regulation of many vital processes, for example, the development of the immune response, which ensures the formation of a specific reaction of the body depending on the type of pathological effect.

The complement system in mammals is one of the most important humoral links of innate immunity. The central component of the complement system, C3, is present in large quantities in the blood and is involved in the implementation of the classical, alternative, and lectin pathways of complement activation [4]. Thus, the C3 component is the most informative factor re-

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flecting the functional activity of this system; however, the specifics of its changes under stress conditions remain poorly studied.

Mammals are constantly exposed to a variety of stressors. To simulate similar living conditions that exclude the development of adaptation and/or resistance to stress factors, appropriate experimental models have been developed that involve the sequential use of different stressors. One of these models is chronic unpredictable mild stress (CUMS) proposed by P. Willner, *et al.* [5]. It is important that studying the nature of changes in the systemic organization of physiological functions under repeated stress loads of various types allows us to most correctly extrapolate the results of studies on animals in the field of stress to humans.

The purpose of this work was to study the indicators of the blood cytokine profile and the functional activity of the C3 component of the complement system in the blood of rats during different time periods of long-term mild unpredictable stress load.

MATERIALS AND METHODS

Experiments were carried out on male Wistar rats (n=42) weighing 180-200 g, obtained from the Stolbovaya nursery. For 5 days before the start of the study, the animals were kept in a vivarium in cages (450×360×160 mm; 4 rats each per cage) with free access to water and food, under artificial lighting conditions (12/12 h day/night) at air temperature 20-22°C. All rats underwent the handling procedure. The experiment was performed in accordance with the Rules for Carrying out Work Using Experimental Animals, approved at a meeting of the Ethical Commission of the P. K. Anokhin Research Institute of Normal Physiology (Protocol No. 1 of September 3, 2005), the requirements of the World Society for the Protection of Animals (WSPA) and the European Convention for the Protection of Experimental Animals.

The rats were divided into 5 groups: group 1 consisted of intact controls (n=10) that were decapitated after a period of adaptation to the conditions of the vivarium and the blood was sampled; experimental groups 2-5 (n=8 in each) included rats exposed to stress factors for 1, 2, 3, and 4 weeks, respectively.

Rats in the experimental groups were subjected to 7-day cycles of sequentially alternating low-intensity stressors using a modified CUMS model [5,6]. The stressors used included cage tilt (30°) for 7 h, daylight (17 h); soiled cage (12 h); water deprivation (12 h); drinking bowl without water (12 h); food deprivation (12 h); small cage (300×150×150 mm, 4 rats) (12 h); the absence of bedding (12 h). At the end of the experiment, the animals were decapitated under light ether anesthesia, followed by blood collection.

Serum was isolated from the blood collected after decapitation and stored at -70°C. The concentrations of cytokines IL-4 and IL-10 were measured by ELISA using mono- and polyclonal antibodies. The optical density of the solution was measured using an HTI ImmunoChem-2100 microplate reader. The functional activity of the C3 component of the complement system in rat blood serum was determined using an appropriate screening test in the complement-dependent lysis reaction of human erythrocytes [7] on a Multiskan MCC ELISA microplate reader (LabSystem).

Statistical and analytical processing of the results was carried out using the Statistica 12.0 (StatSoft, Inc.) and Microsoft Excel 2021 software. Since the distribution of the obtained values differed from normal (according to the Shapiro–Wilk test), the differences between the variables were analyzed using the nonparametric Mann–Whitney U test. The data are presented as median (Me) and quartiles (Q1; Q3). The differences were considered statistically significant at $p<0.05$.

RESULTS

CUMS in rats was accompanied by a pronounced increase in the concentration of IL-10 in the peripheral blood (Table 1). An increase in the level of IL-10 was detected after 1 week (by 95.6%) and after 2 weeks (by 159.2%, $p<0.005$). After 3 and 4 weeks of repeated stress loads, the IL-10 content decreased slightly in comparison with the previous period, but exceeded that in intact animals by 142 and 124%, respectively ($p<0.005$).

Chronic stress led to a decrease in the concentration of IL-4 in the peripheral blood in comparison with the control: by 45.5% after 1 week, by 47% after 2 weeks ($p<0.05$), by 73.8% after 3 weeks, and by 72.4% after 4 weeks ($p<0.05$).

The percentage of lysis of the C3 component in rats after 1 week of CUMS increased slightly in comparison with that in intact animals (by 16.6%; Table 1). By the end of the 2nd week of repeated stress exposure, it exceeded the control values by 17.3% ($p<0.05$). After 3 and 4 weeks of repeated stress loads, this indicator gradually decreased, approaching control values. By the end of the 4th week, the percentage of lysis of the C3 component in animals was significantly lower than after 2 weeks (by 14.3%, $p<0.05$).

The Figure 1 shows a generalized diagram illustrating the direction of changes in the studied cytokines and the C3 component of the complement system in the peripheral blood of rats in the dynamics of CUMS of different durations.

Discussing the data obtained, it should be noted that IL-4 and IL-10 are anti-inflammatory cytokines

TABLE 1. Concentration of Cytokines (pg /ml) and Percentage of Lysis of the C3 Component of the Complement System in the Blood Serum of Rats of the Study Groups (Me (Q1; Q3))

Group		Cytokines		C3 component of complement
		IL-4	IL-10	
Intact		2.18 (2.18; 2.38)	4.03 (2.90; 7.26)	75.0 (56.0; 78.0)
Stress	1 week	1.19 (0.73; 2.18)	7.89 (5.53; 11.58)	87.5 (75.0; 92.0)
	2 weeks	1.16 (0.66; 1.65)*	10.45 (7.99; 10.55)**	88.0 (86.0; 92.0)*
	3 weeks	0.57 (0.24; 0.84)	9.76 (9.35; 11.05)**	85.0 (71.5; 92.0)
	4 weeks	0.60 (0.36; 1.14)*	9.03 (8.39; 10.89)**	77.0 (69.0; 81.5)$^{+}$

Note. *p<0.05, **p<0.005 in comparison with the intact group; ^{+}p<0.05 in comparison with 2 weeks of stress.

that have a regulatory effect on the functional activity of both the immune system and CNS.

The production of IL-10 by macrophages is enhanced under the influence of catecholamines, which are actively released under stress [8]. Apparently, this process contributes to the increase in IL-10 concentration that we detected starting from the 1st week of chronic stress loads. Our findings are consistent with previously described changes in IL-10 in the blood of rats during daily 4-h immobilization for 8 days [2].

One of the main functions of IL-10 is regulation of cytokinogenesis. It has been shown that IL-10 has a modulating effect on the threshold of T-cell activation by suppressing cytokine production (including IL-4) by these cells, reducing eosinophil survival rates and IL-4-induced IgE synthesis. Thus, IL-10 inhibits cytokines associated with cellular immunity and allergic inflammation, but stimulates the humoral response [9]. It can be assumed that the observed decrease in the concentration of IL-4 in the blood of rats under conditions of chronic stress is partly due to the above-mentioned effects of IL-10.

IL-4 participates in the regulation of immunoglobulin synthesis and is also actively involved in the processes of inflammation and fibrosis, allergic reactions, and antitumor activity [10]. In addition to immunomodulatory properties, this cytokine has a number of specific functions. It was revealed that IL-4 suppresses functional activity of the serotonin transporter, acting similarly to antidepressants. A decrease in IL-4 levels contributes to the development of depressive-like behavior [11]. Thus, the detected fluctuations in the concentration of IL-4 contribute to behavioral disorders often observed during stress exposure [12].

We have shown that the functional activity of the blood complement system in rats increases after 1 week and especially after 2 weeks of CUMS. It should be noted that the complement system provides a number of immune processes, such as cell lysis, attraction of leukocytes to the site of inflammation, facilitation of phagocytosis, stimulation of inflammation and hypersensitivity reactions [4]. It was previously established that the C3 component of complement responds to non-antigenic stressor stimuli [13]. The increase in the functional activity of the complement system in response to a nonspecific stimulation (alternating stress factors) revealed by us may be due to the functional readiness syndrome and mediates the preparation of the body for the subsequent implementation of a specific immune response in appropriate conditions.

In addition to the above, we note that the synthesis of the C3 component in various tissues in mammals is enhanced by the action of IL-1β [14]. In turn, IL-10 suppresses the processing of IL-1β [1,15]. These processes may underlie the revealed decrease in the functional activity of the C3 component in the blood of rats against the background of high levels of IL-10 after 3 and 4 weeks of repeated stress exposure.

The results of the study indirectly indicate that changes in the studied immune parameters in rats subjected to CUMS for 4 weeks have a different character during certain periods of observation. The fluctuations in the concentration of cytokines IL-4 and IL-10 that we identified in the early stages of repeated stress exposure, accompanied by an increase in the percentage of lysis of the C3 component, illustrate the formation of a stress response to the presentation

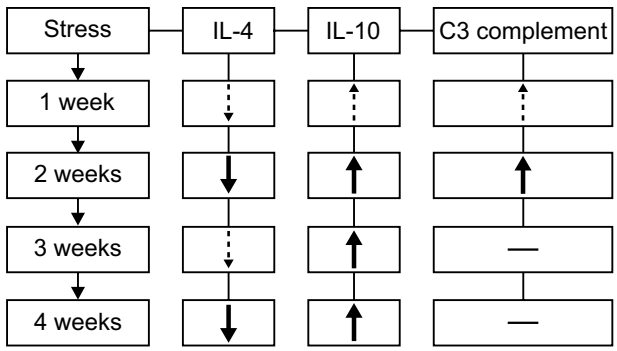

Fig. 1. The direction of changes in the concentration of the studied cytokines and the percentage of lysis of the C3 component of the complement system in the blood of rats in the dynamics of CUMS (relative to the control group). Heavy arrows indicate statistically significant changes (p<0.05). Dash: no difference from control.

of various stress factors. However, the gradual restoration of the functional activity of the complement system starting from the 3rd week, with subsequent return to normal values by the end of the experiment, probably reflects the development of the adaptive reactions at the later stages of chronic stress.

The data obtained expand the understanding of the features of physiological processes in the body of mammals that underlie the immune mechanisms of adaptation to changing environmental conditions, in particular those of a stressful nature, and also illustrate the specificity of changes in the indicators of the C-component of the complement system and the cytokine profile of the blood, reflecting the activity of cellular and humoral components of the immune response, under repeated stress loads of different origins and duration.

Conflict of interest. The authors have no conflicts of interest to declare.

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