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# Blood Cytokine Profile in Rats with Different Behavioral Characteristics after Metabolic Stress

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Changes in the blood cytokine profile in rats with various parameters of behavior were studied under conditions of 5-day starvation not followed by the recovery period or with subsequent normalization of food intake. Metabolic stress had no effect on the content of most cytokines. The concentration of pro- and anti-inflammatory cytokines in blood plasma of rats was shown to increase significantly by the end of a 5-day recovery period after food deprivation. These changes in behaviorally passive specimens were more pronounced than in active animals. Therefore, differences in the strain of immune reactions in mammals with different prognostic resistance to extreme factors are most pronounced during the post-stress period. These data illustrate the necessity of an individual approach to studying the systemic organization of physiological functions under normal and pathological conditions.

**Key Words:** *rats; behavioral activity; blood cytokines; metabolic stress*

Modern concepts of stress are based on studying the behavioral, hormonal, neurochemical, and immunological processes of vital activity, as well as on the evaluation of systemic interrelations [8]. A variety of conflict situations occurs in life, which is followed by the summation of emotional stress-related autonomic and neurological disorders. It should be emphasized that various specimens have different resistance to the development of pathological consequences of negative emotiogenic exposures [10].

The open-field test is extensively used in experimental researches to evaluate the relationship between behavioral activity of animals and with their resistance to stress [4]. Our studies revealed the existence of significant individual differences in the reaction of behaviorally passive and active rats on various models of acute and chronic emotional stress. For example, specimens with various parameters of behavior are characterized by the specific changes in circadian rhythms of activity [5], state of stress-marker organs [6], and oxidative status of brain tissues during stress loads [7]. Special studies were devoted to the evaluation of some proteomic features of brain structures and identification of protein markers for the resistance to stress [12].

Much attention is paid to studying the involvement of immune molecules in systemic organization of physiological functions in mammals [9]. The pathogenesis of stress-related diseases is associated with immune disturbances (*e.g.*, change in the cytokine profile of biological fluids) [15]. Some others believe that these processes are mainly related to stressogenic changes in the ratio of Th1 and Th2 cells, which produce immunomodulatory cytokines [13]. Therefore, cytokine indices serve as one of the markers for oxidative stress, as a component of the systemic reaction under adverse conditions.

Changes in immune parameters of the human body often result from insufficient intake of nutrient substances and energetic compounds. Exogenous alimentary deficiency is followed by the development of

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alimentary dystrophy. This state is characterized by severe body weight loss, reduction of subcutaneous fat, decrease in protein content in the blood and tissues, progressives change in all types of metabolic processes, and atrophy of skeletal muscles and internal organs [11]. It should be noted that even fasting therapy can produce the negative effects. The majority of them are related to an inadequate course of the post-starvation period. Physiological dysfunction under these conditions illustrates the development of metabolic stress.

Starvation and related pathological states are the urgent problem. However, many problems in this field remain to be solved. Little is known about variations in the cytokine profile of biological tissues in mammals during metabolic stress.

This work was designed to study a change in the content of pro- and anti-inflammatory cytokines in the blood of specimens with various parameters of behavior during starvation and recovery period.

## MATERIALS AND METHODS

Experiments were performed on 48 male Wistar rats weighing 210.2±36.7 g. The experiment was conducted in accordance with the Rules of Studies on Experimental Animals (approved by the Ethics Committee of the P. K. Anokhin Research Institute of Normal Physiology; protocol No. 1, September 3, 2005), requirements of the World Society for the Protection of Animals (WSPA), and European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes.

The animals were housed in cages (4 specimens per cage) at 20-22°C under the artificial light/dark cycle (09.00-21.00, lightness; 21.00-09.00, darkness) and had free access to water and food. After the delivery from a nursery, the rats were adapted to laboratory conditions for 5 days. They were daily subjected to repeated handling over 15 min to prevent the stress reaction to a researcher.

The initial behavioral characteristics of rats were estimated in the open-field test for 3 min [2]. To calculate the index of activity, the sum of crossed peripheral and central sectors, peripheral and central rearing postures, and explored objects was divided by the sum of the latencies of the first movement and entry into the center of the open field. Depending on the initial parameters of behavior, the rats were divided into passive ($n$=24) and active specimens ($n$=24), which differed in the average index of activity (0.46±0.02 and 4.50±0.50, respectively). The study was conducted with 6 groups of 8 animals each (Table 1).

Metabolic stress was induced by 5-day starvation (water *ad libitum*) not followed by the recovery period (groups 3 and 4) or with subsequent normalization of food intake for 5 days (groups 5 and 6). Control animals (groups 1 and 2) had free access to water and food over the period of study.

The rats were decapitated by the end of observations. The blood was collected, placed into serum separating tubes (Bio-Plex serum diluent kit), maintained for 30 min, and centrifuged on an ELMI-Multi Centrifuge-CM 6M at 4°C and 3000 rpm for 10 min. Blood serum samples were put into Eppendorf tubes and stored in a low-temperature chamber at -70°C. The samples were then defrosted. The cytokine profile of peripheral blood serum was studied on a Bio-Plex device (Bio-Rad Laboratories) with rat cytokine assay kits (Bio-Plex Pro Rat Cytokine Th1/Th2 Assay). We measured the concentration of the following cytokines: IL-1α, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12p70, IL-13, IFNγ, and granulocyte-macrophage CSF (GM-CSF).

The results were analyzed by the corresponding statistical and analytical methods (Statistica 8.0, Microsoft Office Excel 2007) and Bio-Plex Manager software (version 4.1). The statistical significance of between-group differences in the concentration of rat blood cytokines was evaluated by nonparametric Wilcoxon's $t$ test and Mann—Whitney $U$ test. The significance level was 5%. The data are presented as the $M$±$SD$.

## RESULTS

Under basal conditions (control), the concentration of most cytokines in the peripheral blood of behaviorally passive rats was lower than in active specimens. However, these differences were statistically insignificant (Table 2).

The level of cytokines in animals with various behavioral parameters in the open-field test tended to decrease after experimental metabolic stress. Under these conditions, statistically significant changes were observed only for IL-5. The concentration of this cytokine in blood serum of passive specimens decreased by 11.5 times compared to the control ($p$<0.05).

**TABLE 1.** Characteristics of Experimental Groups

| Group | Type of behavioral activity in the open field | Experimental conditions |
|---|---|---|
| 1 | Active | Intact specimens (control) |
| 2 | Passive | |
| 3 | Active | Starvation |
| 4 | Passive | |
| 5 | Active | Starvation→recovery |
| 6 | Passive | |

**TABLE 2.** Cytokine Profile of the Peripheral Blood in Behaviorally Active and Passive Rats of Various Groups (*M*±*SD*)

| Cytokines | Control | | Starvation | | Starvation→recovery | |
|---|---|---|---|---|---|---|
| | active | passive | active | passive | active | passive |
| IL-1α | 0.205±0.169 | 0.163±0.057 | 0.145±0.069 | 0.125±0.035 | 0.183±0.037 | 0.203±0.052 |
| IL-1β | 0.104±0.167 | 0.340±0.633 | 0.056±0.018 | 0.078±0.203 | 0.086±0.029 | 0.077±0.032 |
| IL-2 | 0.245±0.318 | 0.177±0.139 | 0.229±0.234 | 0.165±0.112 | 0.309±0.115* | 0.316±0.092* |
| IL-4 | 0.035±0.050 | 0.018±0.014 | 0.021±0.015 | 0.014±0.011 | 0.061±0.017** | 0.069±0.023** |
| IL-5 | 0.252±0.128 | 0.204±0.061 | 0.135±0.120 | 0.018±0.015* | 0.340±0.027** | 0.315±0.041** |
| IL-6 | 0.227±0.352 | 0.121±0.093 | 0.159±0.056 | 0.076±0.077 | 0.544±0.166** | 0.572±0.278** |
| IL-10 | 0.094±0.149 | 0.046±0.034 | 0.071±0.048 | 0.032±0.043 | 0.167±0.052** | 0.140±0.049** |
| IL-12p70 | 0.050±0.094 | 0.016±0.012 | 0.028±0.017 | 0.012±0.010 | 0.072±0.014** | 0.065±0.035** |
| IL-13 | 0.049±0.066 | 0.080±0,144 | 0.033±0.010 | 0.043±0.033 | 0.106±0.019** | 0.093±0,032* |
| IFNγ | 0.093±0.144 | 0.055±0.035 | 0.061±0.048 | 0.037±0.033 | 0.217±0.076** | 0.220±0.126** |
| GM-CSF | 0.016±0.027 | 0.006±0.006 | 0.009±0.007 | 0.002±0.011 | 0.049±0.015** | 0.047±0.025** |

**Note.** *$p<0.05$ and **$p<0.01$ in comparison with the control.

In the recovery period after starvation, behaviorally active and passive animals were characterized by a significant increase in blood concentration of the following proinflammatory cytokines synthesized by Th1 cells (Table 2): IL-2 (by 26.1 and 78.5%, respectively; $p<0.05$), IL-12p70 (by 44.0 and 305.3%, respectively; $p<0.01$), and IFNγ (by 133.3 and 300.0%, respectively; $p<0.01$). The rats of these experimental groups demonstrated an increase in the level of typical anti-inflammatory cytokines from Th2 cells: IL-4 (by 74.3 и 283.3%, respectively; $p<0.01$), IL-10 (by 77.7 и 204.4%, respectively; $p<0.01$), and IL-13 (by 116.3% [$p<0.01$] and 16.3% [$p<0.05$], respectively). During the recovery of food intake after metabolic stress, active and passive specimens also exhibited an increase in blood levels of IL-5 (by 34.9 and 54.4%, respectively; $p<0.01$), IL-6 (by 139.7 and 372.7%, respectively; $p<0.01$), and GM-CSF (by 206.3 and 6833%, respectively; $p<0.01$).

Therefore, metabolic stress due to 5-day starvation has little effect on the blood cytokine profile in animals with various parameters of behavior in the open-field test. It can be suggested that variations in the intensity of metabolism under these conditions are not accompanied by profound changes in immune functions, which results from adaptive processes in mammals.

The concentration of pro- and anti-inflammatory cytokines in peripheral blood serum from animals significantly increases by the end of a 5-day recovery period after food deprivation. It should be emphasized that these changes were particularly significant in stress-predisposed passive rats. Hence, differences in the strain of immune reactions in mammals with different prognostic resistance to extreme factors are most pronounced during the post-stress period. Reparative processes under these conditions are accompanied by the reactions, which resemble the cellular immune response. This feature is manifested in the increased production of cytokines by various subpopulations of cells, which are involved in the Th1- and Th2-mediated response. The observed changes serve as a compensatory reaction to stress factors.

These data complement the results of our previous experiments. Specific changes in the blood leukocyte profile of rats with various types of behavior were revealed on the model of acute emotional stress [3]. Active animals were characterized by an increase in the count of neutrophils, while passive specimens demonstrated a decrease in the content of eosinophils during the post-stress period. These changes were accompanied by specific variations in cytokine concentration in the peripheral blood [1]. Acute stress was followed by a decrease in the level of study cytokines in behaviorally active rats. As distinct from active animals, passive specimens exhibited the accumulation of a proinflammatory cytokine IL-1β and anti-inflammatory cytokine IL-4 in blood serum. It should be emphasized that administration of exogenous interleukins (*e.g.*, IL-1β) can produce a modulatory effect on the cellular composition [3] and blood cytokine profile in mammals [14].

These data suggest that the state of stress is not a nonspecific reaction of living organisms to extreme exogenous or endogenous factors. The nature of stress-induced changes depends on the type of stress and peculiarities of the post-stress period. It is important that mammals with various parameters of behavior are characterized by significant differences in the systemic organization of physiological functions under normal and pathological conditions.

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