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Blood Cytokine Profile in Rats with Various Behavioral Characteristics after a Single Exposure to Long-Term Stress

S. S. Pertsov[1,2], A. Yu. Abramova[1,2], V. V. Chekhlov[2], and E. V. Nikenina[1]

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Changes in the blood cytokine profile of rats with different behavioral activity were evaluated in various periods after stress exposure on the model of 24-h immobilization. Behaviorally active animals exhibited only a tendency to a change in the concentration of study cytokines in the dynamics after experimental stress. Stress exposure in passive specimens was accompanied by a decrease in the content of pro- and anti-inflammatory cytokines. These changes were most pronounced at the early stages of the post-stress period and persisted until the end of observations. After a single exposure to long-term immobilization, cytokine level in the peripheral blood of behaviorally passive animals was much lower than in active rats. Variations in immune indexes of mammals depend on the initial parameters of their behavior and duration of the post-stress period. Differences in the blood cytokine profile during negative emotiogenic exposures in passive and active rats are probably related to the specifics of immune reactivity in specimens with various sensitivities to stress.

Key Words: *blood cytokines; rats; behavioral parameters; long-term stress; post-stress period*

The basic works of H. Selye formulate the notions of stress as a general adaptation syndrome of the body [15]. Stress was believed to be the nonspecific response of the body, which is characterized by successive stages of alarm, resistance, and exhaustion.

A paradoxical situation exists in numerous studies of emotional stress and associated psychosomatic disease [5]. In the majority of researches, a study of physiological, biochemical, genetic, and other indexes of stress is based on the analysis of averaged data. However, the real nature of various manifestations of emotional stress, as well as the systemic mechanisms of the body's stress response cannot be understood only from the mean values.

A large body of evidence indicates that the severity of physiological dysfunction during negative emotiogenic exposures differs significantly in various individuals [5,10]. In experimental researches, studying the open-field behavior of rats is used to predict their sensitivity to stress factors. For example, the survival rate of behaviorally active specimens after experimental stress is higher than that of passive animals [3]. These data suggest that stress is not the nonspecific reaction of living organisms to extreme exposures. Under similar stress conditions, these reactions differ in stress-resistant and stress-predisposed specimens.

In modern medicine, there is a tendency toward the individual evaluation of similar nosological forms of diseases (*e.g.*, stress-induced disorders) and corresponding approaches to the pharmacological treatment [6]. Previous studies showed that psychoemotional stress can be accompanied by a change in the immune status [8,14]. Special attention in this regard is paid to cytokines. These polypeptide transmitters of the cell-to-cell interaction have high immunomodulatory activity. Previous studies revealed that stress exposures of various types are followed by a change in the ratio of pro- and anti-inflammatory cytokines in mammalian tissues [9,12].

[1]P. K. Anokhin Research Institute of Normal Physiology; [2]A. I. Evdokimov Moscow State Medical and Dental University, Ministry of Health of the Russian Federation, Moscow, Russia. ***Address for correspondence:*** s.pertsov@mail.ru. S. S. Pertsov

Many adverse consequences of stress are formed after the termination of extreme loads. Therefore, studying the processes at various stages after negative emotiogenic exposures is important to develop new approaches to the prevention of post-stress dysfunction. It is necessary to evaluate the specifics of stress-induced disturbances in specimens with various parameters of behavior, which are characterized by differences in the systemic organization of functions under normal and pathological conditions.

Previous studies showed that immune compounds are involved in the regulation of physiological functions in mammals. However, there are contradictory data on the specifics of cytokine production and secretion at various stages of the post-stress period. Differences in the cytokine profile of biological tissues in specimens with various prognostic resistances to similar stress factors at the early and late stages after a single exposure to long-term emotiogenic loads remain unknown.

This work was designed to study a change in the concentration of pro- and anti-inflammatory cytokines in the blood of rats with different behavioral activity in various periods after a single exposure to long-term stress.

MATERIALS AND METHODS

Experiments were performed on 68 male Wistar rats weighing 255.6±2.8 g. The animals were housed in a vivarium under the artificial light/dark cycle (9.00-21.00, lightness; 21.00-9.00, darkness) and fed a standard diet. 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 Experimental Animals.

Behavioral characteristics of animals were evaluated in the open-field test for 3 min [3]. To calculate the index of activity, the sum of crossed peripheral and central squares, peripheral and central rearing postures, and explored objects was divided by the sum of the latency 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=36) and active specimens (n=32), which differed in the average index of activity (0.39±0.03 and 3.00±0.36, respectively).

In the follow-up period, they were randomized into 4 groups of passive animals and 4 groups of active animals. Each of these groups consisted of 9 and 8 rats, respectively. Behaviorally passive and active specimens of groups 1 and 2 served as the control. These animals were handled and maintained in home cages for 24 h before decapitation. Other rats were subjected to long-term stress on the model of immobilization in individual plastic tubes for 24 h. The animals were decapitated 1 day (groups 3 and 4), 3 days (groups 5 and 6), or 8 days (groups 7 and 8) after restraint stress. The model of stress was selected since this exposure is accompanied by the appearance of some signs for cell atrophy and necrobiosis, as well as by the depletion of lipids in all zones of the adrenal glands [1]. Moreover, 24-h immobilization is followed by general manifestations of the body's stress response (involution of the thymus and spleen; and pronounced increase in plasma cortisol level) [13].

The blood was collected after decapitation of rats. Blood plasma was stored at -70°C. The concentration of proinflammatory (IL-1α, IL-1β, IL-2, IL-5, IL-6, and IFNγ) and anti-inflammatory cytokines (IL-4 and IL-10) in rat blood plasma was measured on a Bio-Plex device (Bio-Rad Laboratories) with cytokine assay kits (Bio-Plex Pro Rat Cytokine Th1/Th2 Assay).

The results were analyzed by the corresponding statistical and analytical methods (Statistica 8.0, Microsoft Excel 2007) and Bio-Plex Manager software (version 4.1). The statistical significance of 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 numerical data are shown as M±SEM.

RESULTS

Under basal conditions, the concentration of pro- and anti-inflammatory cytokines in blood plasma of behaviorally active rats was slightly higher than in passive specimens (Tables 1, 2). However, these between-group differences were not statistically significant.

In stage I, variations in the level of blood plasma cytokines in behaviorally active specimens were analyzed at various periods after a single exposure to 24-h restraint stress (Table 2). It should be emphasized that active animals exhibited only a tendency to a change in cytokine concentration under these conditions (not statistically significant).

Proinflammatory cytokines of the blood in active rats. The content of IL-1β and IL-5 in behaviorally active rats remained unchanged on days 1, 3, and 8 after long-term acute stress. The concentrations of IL-2 and IL-6 in the peripheral blood of these specimens increased 1 day after restrain stress (by 31.2 and 68.2%, respectively, in comparison with the control). The level of these cytokines returned to the baseline on day 3 after immobilization and remained unchanged to the end of observations (day 8). The content of IL-1α

TABLE 1. Cytokine Concentration in Blood Plasma of Behaviorally Passive Rats at Various Periods after Long-Term Acute Stress (pg/ml, *M*±*SEM*)

Cytokines	Control (intact)	Time after 24-h restraint stress		
		day 1	day 3	day 8
IL-1α	343.32±64.37	191.19±43.94*°	252.12±84.38	181.56±57.35
IL-1β	2434.43±66.37	2350.09±47.72	2339.59±94.40	2304.69±54.27
IL-2	684.08±170.12	515.91±99.49°	586.13±174.15	433.42±131.98
IL-5	1676.02±137.18	1385.54±115.40*	1404.87±145.47	1378.76±143.30
IL-6	181.86±47.42	101.67±26.22*°	162.25±75.08	80.91±22.10°
IFNγ	344.56±95.49	146.41±43.86*°°	343.28±113.11	159.89±40.04°
IL-4	736.96±270.01	302.88±45.05*°	181.78±45.08*°	337.58±138.55°
IL-10	805.45±207.21	503.99±110.76*°	415.16±72.47°	430.64±118.49

Note. *$p<0.05$ in comparison with the control; °$p<0.05$ and °°$p<0.01$ in comparison with active rats.

TABLE 2. Cytokine Concentration in Blood Plasma of Behaviorally Active Rats at Various Periods after Long-Term Acute Stress (pg/ml, *M*±*SEM*)

Cytokines	Control (intact)	Time after 24-h restraint stress		
		day 1	day 3	day 8
IL-1α	381.82±120.43	472.25±152.25	337.51±112.96	413.62±146.09
IL-1β	2510.16±120.34	2581.47±119.89	2435.51±90.86	2502.37±132.60
IL-2	862.66±298.53	1131.97±276.34	746.60±251.05	760.25±203.68
IL-5	1834.06±271.62	1850.52±243.85	1582.70±234.59	1559.93±226.63
IL-6	223.42±102.00	375.78±151.36	237.68±94.23	247.96±89.72
IFNγ	359.80±148.59	839.42±295.28	239.74±101.69+	622.94±209.31x
IL-4	993.63±459.19	1267.51±529.63	785.26±408.16	1025.42±460.88
IL-10	1270.19±465.21	1226.86±392.55	743.26±232.85	826.23±338.59

Note. $p<0.05$ in comparison with +day 1 after stress, xday 3 after stress.

in blood samples from active animals was slightly elevated 1 day after stress (by 23.7%), decreased below the baseline on day 3, but then increased and did not differ from the control on day 8. IFNγ concentration in blood plasma of behaviorally active specimens was characterized by the following waveform changes after 24-h immobilization: day 1, more than a 2-fold increase in comparison with the control; day 3, 71.4% decrease in comparison with that in the previous stage ($p<0.05$); and day 8, repeated rise above the baseline (by 73.1%).

Anti-inflammatory cytokines of the blood in active rats. Immobilization of behaviorally active rats for 24 h induced the following waveform changes of IL-4 concentration in the peripheral blood: day 1, rise by 27.6%; day 3, decrease below the baseline; and day 8, increase to the control level. IL-10 content in the blood of these animals did not change on day 1 after stress exposure, but decreased by day 3 (by 41.5% in comparison with the control) and remained low until the end of observations (day 8).

Then we studied a change in blood cytokine concentration in behaviorally passive rats at various periods after long-term acute stress (Table 1).

Proinflammatory cytokines of the blood in passive rats. The content of IL-1β and IL-2 in behaviorally passive rats remained unchanged in the dynamics after 24-h restrain stress. Stress exposure was accompanied by similar variations in the level of other proinflammatory cytokines in animals of this group. The concentrations of IL-1α, IL-5, IL-6, and IFNγ in blood plasma from passive specimens decreased by day 1 after experimental stress (by 44.3, 17.3, 44.1, and 57.5%, respectively; $p<0.05$ in comparison with the control).

The content of study cytokines slightly increased by day 3, but then decreased (day 8) and practically did not differ from that on day 1 (47.1, 17.7, 55.5, and 53.6% below the baseline, respectively).

At all stages of the post-stress period, the level of proinflammatory cytokines in blood plasma of behaviorally passive rats was lower than in active specimens (Tables 1 and 2). Statistically significant between-group differences were found for the concentrations of the following cytokines: IL-1α, IL-2, IL-6, and IFNγ on day 1 after stress (by 2.5, 2.2, 3.7, and 5.7 times, respectively, $p<0.05$-0.1); and IL-6 and IFNγ on day 8 (by 3.1 and 3.9 times, respectively, $p<0.05$).

Anti-inflammatory cytokines of the blood in passive rats. Plasma IL-4 concentration in passive rats progressively decreased on days 1 and 3 after restraint stress (by 58.9 and 75.3%, respectively; $p<0.05$ in comparison with the control). The content of this cytokine increased by the 8th day, but remained 54.2% below the baseline. Long-term acute stress was accompanied by a decrease in IL-10 concentration in the blood of behaviorally passive specimens on day 1 of the study (by 37.4%, $p<0.05$ in comparison with the control). In the follow-up period, the level of this cytokine did not change and remained below the baseline.

On days 1, 3, and 8 after the exposure to stress, the concentration of anti-inflammatory cytokines in the peripheral blood of passive rats was much lower than in active animals (Tables 1 and 2): IL-4, by 4.2, 4.3, and 3.0 times, respectively ($p<0.05$); and IL-10, by 2.4, 1.8, and 1.9 times, respectively ($p<0.05$).

Table 3 shows the general scheme, which illustrates the direction of changes in the level of proinflammatory and anti-inflammatory cytokines in the peripheral blood of behaviorally passive and active rats at various periods after a single exposure to long-term stress.

We conclude that stress exposure on the model of 24-h immobilization has a specific effect on the blood cytokine profile in rats with various behavioral parameters in the open-field test. Behaviorally active animals exhibit only a tendency to a change in the concentration of study cytokines in the dynamics after experimental stress. However, passive specimens are characterized by severe post-stress disturbances in the blood cytokine profile. Stress exposure in these rats is accompanied by a decrease in the content of proinflammatory and anti-inflammatory cytokines. These changes are most pronounced at the early stages of the post-stress period (day 1) and persist until the end of observations (day 8). After a single exposure to long-term immobilization, cytokine level in the peripheral blood of behaviorally passive animals was much lower than in active specimens.

Our results complement the data on immune dysfunction in mammals under negative emotiogenic conditions. The exposure to various stress factors can be followed not only by a significant decrease in functional activity of the immune system, but also by activation of its components. The direction of changes in immune indexes strongly depends on the intensity and duration of stress exposure. Generally, weak and short exposure to stressors has an immunostimulatory effect. By contrast, strong and long-term stress exposures produce an inhibitory effect, which is manifested in a decrease in immune activity [7,11]. We found that immunosuppression (decrease in blood cytokine level

TABLE 3. Direction of Changes in the Concentration of Proinflammatory and Anti-Inflammatory Cytokines in the Blood of Behaviorally Passive and Active Rats at Various Periods after a Single Exposure to 24-h Restraint Stress (Relative to the Baseline)

Cytokines	Proinflammatory						Anti-inflammatory	
	IL-1α	IL-1β	IL-2	IL-5	IL-6	IFNγ	IL-4	IL-10
Passive rats								
Day 1 after stress	↓↓	↓↓	—	↓↓	—	↓↓	↓↓	↓↓
Day 3 after stress	↓	↓	—	↓	—	↓	↓↓	↓
Day 8 after stress	↓	↓	—	↓	–	↓	↓	↓
Active rats								
Day 1 after stress	↑	↑	↑	—	—	↑	↑	—
Day 3 after stress	↓	↓	—	—	—	—	↓	—
Day 8 after stress	—	↑	—	—	—	—	—	↓

Note. — , no differences from the control; single arrows, tendency to a change compared to the control ($p>0.05$); double arrows, statistically significant changes compared to the control ($p<0.05$).

after 24-h immobilization) occurs only in behaviorally passive specimens, which are prognostically predisposed to adverse consequences of stress.

This study continues our previous researches on the type of changes in the cytokine profile of biological tissues in mammals under extreme conditions. For example, we showed that cytokine level in the plasma of behaviorally active rats decreases immediately after 1-h immobilization with a simultaneous delivery of subthreshold electrocutaneous stimulation. Under these conditions, passive specimens were characterized by the accumulation of a proinflammatory cytokine IL-1β and anti-inflammatory cytokine IL-4 in the peripheral blood [2]. Experiments on another model of acute stress (12-h immobilization in the nighttime period) revealed that a decrease in the concentration of most pro- and anti-inflammatory cytokines in passive animals is most pronounced immediately and particularly 3 days after the exposure [4]. Changes in the blood cytokine profile at various periods after experimental stress were less significant in active specimens. They were characterized by a decrease in the concentration of a proinflammatory cytokine IL-1α and anti-inflammatory cytokines IL-4 and IL-13 (compared to the control). As differentiated from passive rats, these changes in active animals were observed 1 day after negative emotiogenic exposure.

Our results indicate that variations in immune indexes of mammals depend on the type of stress and duration of the post-stress period. Differences in the blood cytokine profile during negative emotiogenic exposures in behaviorally passive and active rats are probably related to the specifics of immune reactivity in specimens with various sensitivities to stress.

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