Blood Cytokines in Rats with Various Behavioral Characteristics during Emotional Stress and Treatment with Interleukin-1β

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We studied the effect of acute emotional stress and exogenous IL-1β (5 μg/kg intraperitoneally) on the cytokine profile of blood serum in Wistar rats with various behavioral characteristics in the open-field test. Blood level of proinflammatory cytokine IL-1β decreased in behaviorally passive rats, but increased in active animals after simultaneous immobilization and electrocutaneous stimulation. These changes reflect the opposite immune responses to a similar stress exposure in rats with different emotional reactivity. Poststress variations in the concentration of circulating IL-1β differed in rats receiving exogenous IL-1β. Blood cytokine concentration decreased in behaviorally active rats, but remained unchanged in passive animals that were exposed to immobilization and electrocutaneous stimulation after pretreatment with IL-1β. Emotional stress and injection of IL-1β had no effect on blood level of an anti-inflammatory cytokine IL-4 in rats. Our results indicate that rats with various behavioral parameters are characterized by significant differences in the cytokine profile of blood serum under conditions of emotional stress and treatment with IL-1β. These data illustrate the specific functional features of immune mechanisms, which provide an individual resistance of rats to the same stress exposure.

Key Words: *blood cytokines; interleukin-1β; emotional stress; rats; individual resistance to stress*

A large body of evidence indicates that psychoemotional stress is accompanied by impairment of immune functions in mammals [9]. Immune dysfunction contributes to individual predisposition to stress factors.

The pathogenesis of immune dysfunction during emotional stress involves changes in the cytokine profile. Cytokines are polypeptide transmitters of cell-cell interaction playing a regulatory role in normal physiological functions and progression of the defense response to foreign factors and impairment of tissue integrity [1]. Much attention is paid to the superfamily of IL-1. IL-1β plays a trigger role and induces the cascade of cytokine secretion in the organism. IL-1β is one of the transmitters of the acute phase of the stress response. This cytokine modulates functional activity of the hypothalamic—pituitary—adrenal axis [8]. Our previous studies demonstrated an antistress effect of IL-1β on rats. Intracerebroventricular injection of IL-1β to animals prevents gastric ulceration [3] and involution of the spleen [5] and modulates the oxidative status of the hypothalamus and sensorimotor cortex of the brain under conditions of water-immersion stress [4].

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The state of immune functions in mammals depends on the relationship between proinflammatory (IL-1β, IL-2, IL-6, IFN-γ, TNF-α, *etc*.) and anti-inflammatory cytokines (IL-4, IL-10, IL-13, and transforming growth factor-β).

Significant differences were found in individual resistance of subjects to negative consequences of emotional stress [6,7]. Previous studies showed that behavioral activity of rats in the open-field test serves as a reliable prognostic criterion for their resistance to stress [2]. Behaviorally active animals are prognostically more resistant to stress exposure than passive specimens.

Considerable evidence exists that immune compounds are involved in the regulation of physiological functions in mammals. However, there are contradictory data on the production and secretion of cytokines during emotional stress. We found no data on possible variations in the amount of proinflammatory and anti-inflammatory cytokines in specimens with different prognostic resistance to the same stress exposure. Little is known about the effect of exogenous IL-1β on changes in the cytokine profile of the organism during psychoemotional stress.

Here we compared the concentrations of proinflammatory cytokine IL-1β and anti-inflammatory cytokine IL-4 in the blood of rats with various behavioral characteristics during the same stress exposure and administration of IL-1β.

MATERIALS AND METHODS

Experiments were performed on 40 male Wistar rats weighing 346.7±5.2 g. The experiment was conducted in accordance with the "Rules of Studies with Experimental Animals" (approved by the Ethics Committee of the P. K. Anokhin Institute of Normal Physiology; protocol No. 1, 3.09.2005), requirements of the World Society for the Protection of Animals (WSPA), and European Convention for the Protection of Experimental Animals. The animals were housed in cages (5 rats per cage) at 20-22°C and artificial light/dark cycle (8.00-20.00, lightness; 20.00-8.00, darkness). They had free access to water and food. The rats were adapted to laboratory conditions for 5 days after delivery to the laboratory.

Individual and typological characteristics of rats were evaluated in the open field for 3 min [2]. 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.

The open-filed test allowed us to reveal 20 active rats (prognostically resistant to stress) and 20 passive rats (prognostically predisposed to stress). Passive and active animals differed by the index of activity (0.51±0.04 and 4.21±0.59, respectively). Behaviorally active and passive rats were divided into 8 groups of 5 specimens each (Table 1). IL-1β in a dose of 5 μg/kg (activity 10^8 U/mg) was dissolved in 1 ml physiological saline. Physiological saline (1 ml) or IL-1β was injected intraperitoneally to animals 1 h before stress exposure. Control (unstressed) rats received injection of physiological saline or IL-1β 2 h before decapitation. Human recombinant IL-1β was obtained from the State Research Institute of Highly Pure Biopreparations (Federal Medical and Biological Agency of Russia).

Simultaneous immobilization and electrocutaneous stimulation of rats served as a model of acute emotional stress. The animals were placed in individual plastic cages (length 16.5 cm, diameter 5.5 cm). Metal needle electrodes were inserted into the skin of the back to deliver stochastic electrocutaneous stimulation with alternating current (1 msec duration, 4-6 V voltage, 50 Hz frequency). The strength of stimulation was selected individually by the vocalization threshold during electrostimulation. The duration of one session of electrostimulation was 30 or 60 sec. The animals were subjected to 12 sessions of 30-sec stimulation and 5 sessions of 60-sec stimulation over 1 h of stress exposure. The time between repeated sessions of electrical stimulation was 90-180 sec. Control (unstressed) rats were maintained in home cages during this period.

Stressed rats and control animals were decapitated immediately after the experiment. The blood was collected, maintained in tubes for 45-60 min, and centrifuged to obtain the serum. Serum samples were frozen at -20°C. The concentrations of IL-1β and IL-4 in rat blood serum were measured by enzyme immunoassay with ELISA kits for rat cytokines (IL-1-beta, BMS630; and IL-4, BMS628, Sigma).

Experimental data were analyzed by statistical and analytical methods. The significance of intergroup differences was evaluated by the nonparametric Mann—Whitney test. The data are presented as means and standard errors.

RESULTS

In the initial state, behaviorally passive and active rats did not differ by the concentrations of IL-1β and IL-4 in blood serum (Table 2).

Intraperitoneal injection of IL-1β was accompanied by an increase in serum IL-1β concentration in active rats (by 1.85 times, $p<0.05$ compared to the initial level; Table 2).

Stress of simultaneous immobilization and electrocutaneous stimulation was followed by various

changes in the concentration of proinflammatory cytokine IL-1β in blood serum from behaviorally passive and active rats (Table 2). After acute stress the concentration of IL-1β in blood serum decreased in passive animals (by 3.35 times), but increased in active specimens (by 2.54 times, $p<0.05$ compared to unstressed rats). These changes contribute to the appearance of significant differences in IL-1β concentration in the blood of rats. After immobilization and electrocutaneous stimulation, serum IL-1β concentration in active rats was 8.28-fold higher than in passive animals ($p<0.01$).

Exogenous IL-1β had a modulatory effect on stress-induced changes in the cytokine profile of rat blood (Table 2). The concentration of this cytokine in the blood decreased by 6.25 times in behaviorally active animals that were exposed to immobilization and electrocutaneous stimulation after pretreatment with IL-1β ($p<0.01$ compared to unstressed specimens). Serum IL-1β level in passive rats remained unchanged under these conditions.

Stress exposure (simultaneous immobilization and electrocutaneous stimulation) and administration of IL-1β had little effect on the concentration of an anti-inflammatory cytokine IL-4 in blood serum of behaviorally passive and active rats (Table 2).

Much attention is paid to studying the immune processes in mammals. However, there is no general agreement about a change in the cytokine profile under conditions of emotional stress. Previous experiments revealed that *in vitro* production of IL-2 and IFN-γ by rat lymphocytes significantly decreases after stress of intermittent electrocutaneous stimulation [15]. Other authors reported that stress exposure does not necessarily inhibit the immune system. For example, plasma IL-6 concentration in rats increases over 30 min after the open-filed test (model of psychological stress) [10]. Besides this, the production of IL-1β and TNF-α by isolated alveolar macrophages of rats increases after stress [14].

Stress exposure and physical load are followed by an increase in cytokine production in CNS [11]. Clinical observations of depressive patients revealed a relationship between psychological stress and elevated content of proinflammatory cytokines [12]. A stress-induced increase in the concentration of proinflammatory cytokines is accompanied by the resistance of neuroendocrine and immune tissues to glucocorticoids, which serves as one of the mechanisms for adaptation to stress factors [13].

Our experiments showed that simultaneous immobilization and electrocutaneous stimulation do not affect the content of IL-4, but modulate the concentration of IL-1β in rat blood serum. Therefore, the formation of a negative emotional state in rats on this model of acute stress is mainly accompanied by variations of proinflammatory IL-1β, but not of anti-inflammatory IL-4 in the blood.

We found that simultaneous immobilization and electrocutaneous stimulation are followed by various changes in blood IL-1β concentration in rats with different emotional reactivity. The concentration of this cytokine decreases in behaviorally passive animals, but increases in active specimens. These changes reflect the opposite immune responses to stress in rats with various behavioral characteristics. This model of stress is probably accompanied by inhibition of the immune function in passive animals (prognostically predisposed to stress). By contrast, active specimens (prognostically resistant to stress) exhibit activation of immune processes under conditions of acute stress. It is manifested in increased production of IL-1β by monocytes, macrophages, T and B lymphocytes, and other cells.

The decrease in serum IL-1β concentration in active rats after cytokine pretreatment and stress expo-

TABLE 1. Experimental Groups of Rats (n=5)

Group	Behavioral activity	Injected solutions	Experimental conditions
1	Active	PS	Control (unstressed)
2	Active	IL-1β	Control (unstressed)
3	Active	PS	Stress exposure
4	Active	IL-1β	Stress exposure
5	Passive	PS	Control (unstressed)
6	Passive	IL-1β	Control (unstressed)
7	Passive	PS	Stress exposure
8	Passive	IL-1β	Stress exposure

Note. Here and in Table 2: PS, physiological saline.

TABLE 2. Serum Cytokine Concentration in Rats of Various Experimental Groups (pg/ml, *M*±*m*)

Parameter, conditions		Active rats (*n*=20)		Passive rats (*n*=20)	
		PS	IL-1β	PS	IL-1β
IL-1β	control (unstressed)	188.68±53.67	349.63±61.21^{x+}	194.29±50.44	164.61±60.13
	stress exposure	479.63±135.78^{*++}	55.96±34.46^{**xx}	57.96±34.84*	143.47±68.27
IL-4	control (unstressed)	25.05±4.73	25.16±3.56	25.94±3.12	26.27±1.67
	stress exposure	21.05±4.48	31.87±5.80	23.95±2.55	23.40±2.35

Note. $^{*}p<0.05$ and $^{**}p<0.01$ compared to unstressed rats; $^{+}p<0.05$ and $^{++}p<0.01$ compared to passive rats; $^{x}p<0.05$ and $^{xx}p<0.01$ compared to PS.

sure can be related to several reasons. The production and secretion of IL-1β by immune cells are probably mediated by a negative feedback mechanism. We showed that injection of IL-1β is followed by an increase in blood IL-1β concentration in unstressed active rats. These changes can be followed by inhibition of endogenous IL-1β production.

Our results suggest that the exogenous cytokine is "consumed" in rats after pretreatment with IL-1β and exposure to acute stress. Under these conditions, the concentration of circulating cytokine is not recovered due to inhibition of endogenous IL-1β production via a negative feedback mechanism. Hence, the concentration of IL-1β in blood serum decreases in active rats.

These changes were not observed in behaviorally passive animals. Administration of IL-1β before stress exposure prevented the decrease in the concentration of IL-1β in blood serum from rats of this group. Pretreatment of stress-predisposed passive animals with IL-1β probably prevents inhibition of immune function under conditions of emotional stress.

We conclude that rats with different behavioral parameters are characterized by significant intergroup differences in the cytokine profile of blood serum during the same stress exposure and administration of IL-1β. Acute emotional stress and treatment with exogenous IL-1β are mainly accompanied by changes in proinflammatory IL-1β, but not in anti-inflammatory IL-4 in the blood of animals. These data illustrate the specific functional features of immune mechanisms, which provide individual resistance of rats to the same stress exposure.

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REFERENCES

1. S. A. Ketlinskii and A. S. Simbirtsev, *Cytokines* [in Russian], St. Petersburg (2008).
2. E. V. Koplik, *Vestn. Nov. Med. Tekhnol*., **9**, No. 1, 16-18 (2002).
3. S. S. Pertsov, V. M. Abramov, A. S. Sosnovskii, *et al*., *Byull. Eksp. Biol. Med*., **117**, No. 3, 238-239 (1994).
4. S. S. Pertsov, T. S. Balashova, A. A. Kubatiev, *et al*., *Ibid*., **120**, No. 9, 244-247 (1995).
5. S. S. Pertsov, A. S. Sosnovskii, and G. V. Pirogova, *Ibid*., **124**, No. 7, 32-35 (1997).
6. K. V. Sudakov, A. V. Kotov, and S. S. Pertsov, *Vestn. Ural'sk. Med. Akademich. Nauki*, No. 1, 51-57 (2004).
7. S. K. Sudakov, *Byull. Eksp. Biol. Med*., **89**, No. 6, 662-664 (1980).
8. A. Gadek-Michalska, A. J. Bugajski, and J. Bugajski, *J. Physiol. Pharmacol*., **59**, No. 3, 563-575 (2008).
9. J. P. Godbout and R. Glaser, *J. Neuroimmune Pharmacol*., **1**, No, 4, 421-427 (2006).
10. L. B. LeMay, A. J. Vander, and M. J. Kluger, *Physiol. Behav*., **47**, 957-961 (1990).
11. H. A. Marques, G. Cizza, and E. Sternberg, *Rev. Bras. Psiquiatr*., **29**, Suppl. 1, S27-S32 (2007).
12. T. W. Pace, T. C. Mletzko, O. Alagbe, *et al*., *Am. J. Psychiatry*, **163**, No 9, 1630-1633 (2006).
13. W. W. Pace, F. Hu, and A. H. Miller, *Brain Behav. Immun*., **21**, No. 1, 9-19 (2007).
14. J. H. Persoons, K. Schornagel, J. Breve, *et al*., *Am. J. Respir. Crit. Care Med*., **152**, 619-624 (1995).
15. J. M. Weiss, S. K. Sundar, K. J. Becker, *et al*., *J. Clin. Psychiatry*., **50**, 43-53 (1989).