Cytokine Profile of Peripheral Blood in Rats with Various Behavioral Characteristics during Acute Emotional Stress

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The cytokine profile of peripheral blood plasma was studied in rats with various behavioral characteristics after acute emotional stress (1-hour immobilization with simultaneous electrocutaneous stimulation). Under basal conditions the concentrations of pro-inflammatory (IL-1α, IL-1β, IL-2, IFN-γ, and granulocyte-monocyte CSF) and anti-inflammatory cytokines (IL-4 and IL-10) in the blood of active animals were higher than in passive specimens. Acute stress was accompanied by a decrease in the level of plasma cytokines in behaviorally active rats. Stressed passive specimens were characterized by the accumulation of a pro-inflammatory cytokine IL-1β and anti-inflammatory cytokine IL-4 in the peripheral blood. The observed differences in the cytokine profile of the blood in behaviorally passive and active rats under basal conditions and after a negative emotiogenic exposure can be related to the specifics of immune reactions and metabolic processes in animals with different prognostic resistance to similar stress factors.

Key Words: *emotional stress; pro- and anti-inflammatory cytokines; blood; rats with various behavioral characteristics*

Psychoemotional stress in mammals is accompanied by immune dysfunction, which includes a change in the cellular composition of the blood and functional activity of immunocompetent cells, decrease in the immune resistance of the body, development of immunodeficiency, and progression of pathological and infectious processes [2,4,7]. The pathogenesis of stress disorders is associated with a change in the cytokine profile of biological fluids in the body [2,8].

Cytokines are polypeptides mediators of cell–cell interaction that regulate physiological processes under normal and pathological conditions. Immune functions of the body are primarily determined by the ratio between pro-inflammatory and anti-inflammatory cytokines [2,9]. Previous studies showed that stress in mammals is accompanied by variations in the content of immunomodulatory cytokines and affinity of receptors in biological fluids and brain tissues [5,9,10]. However, individual features of blood cytokine profile in animals with various behavioral characteristics that exhibit different prognostic resistance to stress exposures are poorly understood.

Here we studied the effect of acute emotional stress on the ratio between pro-inflammatory and anti-inflammatory cytokines in the peripheral blood from rats with various behavioral characteristics.

MATERIALS AND METHODS

Experiments were performed on 35 male Wistar rats weighing 241.0±2.7 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, 3.09.2005), requirements of the World Society for the Protection of Animals (WSPA), and European Convention for the Protection of Experimental Animals.

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The animals were housed in cages (5-10 specimens per cage) at 20-22°C and artificial light/dark cycle (8.00-20.00, lightness; 20.00-8.00, darkness) and had free access to water and food. After delivery to the laboratory, the rats were adapted to laboratory conditions for 5 days.

Individual and typological characteristics of animals were evaluated in the open-field test for 3 min. Our previous studies showed that various specimens have different resistance to the development of negative consequences of emotional stress [6]. Behavioral activity of rats in the open-field test serves as a reliable prognostic criterion for their resistance to stress exposure. Behaviorally active animals are more resistant to stress than passive specimens [3].

To calculate the index of activity, the sum of crossed squares, 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 [3]. Depending on the index of activity, the rats were divided into active (n=15) and passive (n=20) specimens. These animals differed in the average index of activity (passive rats, 0.45±0.02; active rats, 5.94±0.85). In the follow-up period, active and passive rats were divided into 2 groups (control and stress) of 5-10 animals each.

Immobilization (1 h) of rats in individual plastic cages with simultaneous delivery of subthreshold stochastic electrocutaneous stimulation served as a model of acute emotional stress. This standard method of stress exposure was described previously [1,5]. Control (non-stressed) animals were maintained in home cages during this period.

Stressed and non-stressed rats were decapitated immediately after the experiment. The blood was collected during decapitation. Blood plasma was stored at -20°C. The concentration of pro-inflammatory (IL-1β, IL-1α, IL-2, IFN-γ, and granulocyte-monocyte CSF [GM-CSF]) 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 significance of between-group differences was evaluated by nonparametric Mann–Whitney test. The data are presented as the means and standard errors of the means.

RESULTS

In the initial state, the concentrations of pro-inflammatory and anti-inflammatory cytokines in the peripheral blood of active rats were higher than in passive specimens (Table 1). Statistically significant differences were found for the level of IL-1α, IL-1β, and GM-CSF (by 2.00 [p<0.01], 3.71 [p<0.05], and 1.50 times [p<0.05], respectively).

Acute emotional stress was accompanied by a decrease in the content of pro-inflammatory cytokines in blood plasma from active rats. The concentrations of IL-1α, IFN-γ, IL-1β, IL-2, and GM-CSF in these animals were reduced by 4.71 (p<0.05), 9.35 (p<0.01), 1.18, 2.42, and 1.42 times, respectively (as compared to non-stressed specimens).

Similar changes in the level of pro-inflammatory cytokines in the peripheral blood were revealed in behaviorally passive rats after stress exposure. Under these conditions, we observed a decrease in the concentrations of IL-1α (by 1.80 times, p<0.01), IL-2 (by 1.75 times), IFN-γ (by 1.65 times), and GM-CSF

TABLE 1. Cytokine Concentration in Peripheral Blood Plasma of Non-stressed and Stressed Rats with Various Behavioral Characteristics (pg/ml, $M±m$)

Cytokine	Active rats		Passive rats	
	control	stress	control	stress
Pro-inflammatory cytokines				
IL-1α	61.50±7.34^{++}	13.07±1.65*	30.80±6.64	17.07±4.20**
IL-1β	85.34±17.09^{+}	72.54±13.31	23.03±6.40	55.63±11.28**
IL-2	75.49±50.60	31.25±10.95	40.77±26.68	23.36±12.08
IFN-γ	573.49±182.26	61.33±7.14^{+**}	355.82±107.28	215.18±67.77
GM-CSF	2.05±0.08^{+}	1.44±0.19	1.37±0.19	1.13±0.30
Anti-inflammatory cytokines				
IL-4	5.03±1.50	3.08±0.65	2.83±0.68	4.53±2.30
IL-10	362.29±76.83	249.27±29.87	272.24±31.60^{++}	151.82±34.49

Note. *p<0.05 and **p<0.01 in comparison with control rats; ^{+}p<0.05 and ^{++}p<0.01 in comparison with passive rats.

(by 1.21 times). It should be emphasized that stressed passive animals were characterized by a 2.42-fold increase in the content of IL-1β in blood plasma ($p<0.01$; Table 1).

IL-1β is one of the major pro-inflammatory cytokine. This cytokine not only triggers the cascade of secretion of other cytokines in the body, but also plays a key role in the development of emotional stress. IL-1β contributes to the induction of the stress response in mammals and increases functional activity of the hypothalamic-pituitary-adrenal complex [2,4,9]. Some authors reported that the stress reaction in animals is accompanied by an increase in the content of IL-1β in biological tissues [2,8-10]. It should be emphasized that these experiments were performed on the total population of animals without taking into account the individual resistance or predisposition to stress exposures. We revealed that a post-stress accumulation of IL-1β in peripheral blood plasma is typical only for behaviorally passive rats (prognostically predisposed to the development of negative consequences of emotional stress). It can be suggested that active animals are characterized by rapid adaptation to emotional stress. Activation of adaptive and compensatory processes in these specimens contributes to the absence of significant variations in plasma IL-1β concentration or normalization of cytokine content by the 1st hour of stress exposure. It cannot be excluded that stress in behaviorally active animals is accompanied not only by a change in the concentration of IL-1β in biological fluids, but also by an increase in the synthesis of specific trap receptors for this cytokine [10]. These structures bind IL-1β, which abolishes the biological effects of study cytokine.

We found that immobilization with simultaneous electrocutaneous stimulation was accompanied by a slight decrease in the concentration of anti-inflammatory cytokines IL-4 and IL-10 in peripheral blood plasma of behaviorally active rats (by 1.63 and 1.45 times, respectively, compared to non-stressed specimens). Stressed passive animals were characterized by opposite changes in the content of anti-inflammatory cytokines in the blood. After stress exposure of these rats the concentration of IL-4 increased by 1.60 times, while the level of IL-10 decreased by 1.79 times.

It should be emphasized that variations in the concentration of nearly all cytokines in the peripheral blood after acute stress were more pronounced in behaviorally active rats than in passive specimens. Differences in the cytokine profile of the blood in intact animals with various behavioral characteristics became less significant after stress exposure.

There are two major pathways for the realization of the stress response. The first pathway is associated with an increase in the secretion of corticotropin-releasing hormone in the hypothalamus, stimulation of ACTH production in the pituitary gland, and elevation of glucocorticoid release from the adrenal glands. These changes are accompanied by activation of type II T helper cells and increase in the secretion of IL-4, IL-5, IL-10, and IL-13. The second pathway is related to a change in neurotransmitter metabolism (*e.g.*, norepinephrine synthesis) in brain structures with the corresponding activation of the sympathetic nervous system. These processes lead to a decrease in functional activity of type II T helper cells and reduction of the secretion of IL-2, IL-12, IFN-γ, and TNF-α. Therefore, glucocorticoids and catecholamines whose production increases significantly under stress conditions contribute to a shift in the ratio of type I and II T helper cells toward the last-named cells and stimulation of cytokine synthesis by type II T helper cells [7-9].

Our experiments revealed changes in the cytokine profile of the peripheral blood after emotional stress. We observed a decrease in the level of IL-2 and IFN-γ (produced by type I T helper cells) in behaviorally active rats, but increase in the concentration of IL-4 (synthesized by type II T helper cells) in passive animals. These data are consistent with modern notions of stress-induced changes in the concentration of cytokines in biological tissues.

We conclude that the cytokine profile of the peripheral blood differs in non-stressed rats with various individual and typological characteristics. Acute emotional stress on the model of immobilization with simultaneous electrocutaneous stimulation was accompanied by a decrease in the level of pro-inflammatory and anti-inflammatory cytokines in blood plasma of behaviorally active animals. Similar changes in cytokine content in the blood were found in passive specimens after stress exposure. As differentiated from behaviorally active rats, stressed passive animals were characterized by the accumulation of a pro-inflammatory cytokine IL-1β and anti-inflammatory cytokine IL-4 in the peripheral blood. The observed differences in the cytokine profile of blood plasma in behaviorally passive and active rats under basal conditions and after a negative emotiogenic exposure can be related to the specifics of immune reactions and metabolic processes in animals with different prognostic resistance to similar stress factors.

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