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Effect of Interleukin-4 on Peripheral Blood Leukocytes in Rats with Various Behavioral Characteristics during Acute Stress

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We studied the effect of anti-inflammatory cytokine IL-4 (5 μg/kg intraperitoneally) on peripheral blood leukocytes in Wistar rats with various behavioral characteristics during acute emotional stress (1-h immobilization with simultaneous subthreshold electrocutaneous stimulation). IL-4 reduced the differences in blood leukocyte count in rats with various behavioral characteristics, which was related to a significant decrease in this parameter in active animals. IL-4 injection to active animals was accompanied by changes in the leukogram (development of neutrophilia, monocytopenia, and lymphopenia) and had a modulatory effect on leukocyte indexes of cell reactivity. Blood leukocyte count in cytokine-treated animals did not change after stress exposure. IL-4 prevented shifts in leukocyte indexes of cell reactivity, which was found after acute stress exposure. Our results expand current notions on the specific involvement of endogenous immunomodulatory compounds in the realization of adaptive and compensatory processes in mammals during negative emotiogenic exposures.

Key Words: *emotional stress; interleukin-4; peripheral blood leukocytes; behaviorally passive and active rats*

Stress in mammals is accompanied by complex neurohumoral reactions that produce generalized effect on peripheral organs and tissues. Among a wide range of physiological dysfunctions after negative emotiogenic exposures, much attention is paid to cardiovascular disorders and associated pathological changes in the blood [14,15].

Previous studies revealed the existence of individual differences in the sensitivity of animals to negative consequences of stress [7]. Behavioral activity of rats in the open-field test serves as a prognostic criterion of their resistance to stress. Active animals have greater prognostic resistance to stress factors than passive ones [4].

The pathogenesis of stress-induced dysfunction in mammals is strongly related to variations in the immune status, including changes in the cytokine profile [13]. Our previous experiments showed that stress is followed by changes in the concentration and ratio of pro- and anti-inflammatory cytokines in the peripheral blood [1]. We found that behaviorally passive and active animals with different sensitivity to stress are characterized by specific features of the blood cytokine profile. Hence, endogenous immunoactive compounds hold much promise for the correction of poststress disorders in mammals. Anti-inflammatory cytokine IL-4 is one of these compounds. Our previous studies have demonstrated that intraperitoneal injection of IL-4 to rats was followed by a decrease in serum corticosterone level (one of the major hormones in the stress response) [3]. Moreover, IL-4 had a modulatory effect on antioxidant defense enzymes in the brain during stress conditions [2].

Little is known about the effect of IL-4 on white blood cells in specimens with different prognostic resistance to stress exposure.

Here we studied the influence of IL-4 on blood leukocyte indexes in rats with various behavioral characteristics during acute emotional stress.

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MATERIALS AND METHODS

Experiments were performed on 28 male Wistar rats weighing 251.4±6.7 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 Experimental Animals.

The rats were housed in cages (7 specimens per cage) under standard vivarium conditions. Individual and typological characteristics of the animals were evaluated in 3-min open-field test [4]. To calculate 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 latencies of the first movements and entries into the center of the open field. Depending on the initial open-field behavior, the animals were divided into passive (n=14; activity index 0.49±0.05) and active specimens (n=14; activity index 3.57±0.44). In the follow-up period, active and passive rats were divided into 8 groups of 7 specimens each.

IL-4 (5 μg/kg, Sigma) was dissolved in 1 ml sterile physiological saline. IL-4 or physiological saline (1 ml) was injected intraperitoneally 1 h before stress exposure. Control (non-stressed) rats received these injections 2 h before decapitation.

Immobilization of rats in individual plastic cages with simultaneous delivery of subthreshold stochastic electrocutaneous stimulation (1 h) served as the model of acute stress [10]. Control animals were handled and placed in home cages for 1 h.

The blood of rats was obtained during decapitation. Heparin (10 U/ml) served as an anticoagulant. The number and ratio of various types of leukocytes were estimated in the whole blood [6]. Differential counting of leukocytes was performed in blood smears after Romanovsky–Giemsa staining. Leukocyte indexes were calculated by evaluating the ratio of white blood cells in leukogram.

The Kalf-Kalif leukocyte index of intoxication (LII) reflects the intensity of intoxication processes in the body, particularly during chronic stress. This index was calculated as follows:

$$LII=(2B+S)/(M+L)\times(E+1),$$

where B and S are band and segmented neutrophils, respectively, L, M, and E are lymphocytes, monocytes, and eosinophils, respectively [11].

The Shaganin lymphocyte index (LI) was calculated as follows:

$$LI=L/N,$$

where L are lymphocytes and N are segmented and band neutrophils.

Granular and nongranular leukocytes of the blood replace each other and play an important role in the stress response. The blood leukocyte shift index (BLSI) is calculated for a rapid analysis of the body's response to irritant or inflammatory agents [13]. The Yabluchanskii BLSI was calculated as follows:

$$BLSI=(E+Bp+N)/(M+L),$$

where E are eosinophils, Bp are basophils, N are segmented and band neutrophils, M are monocytes, and L are lymphocytes [12].

The results of our previous experiments are shown in tables and figures for a comparative analysis. These data illustrate a change in the number of leukocytes, leukogram, and leukocyte indexes of the blood in control and stressed rats after injection of physiological saline [5].

The significance of between-group differences was evaluated by nonparametric Mann–Whitney test.

RESULTS

Our previous studies showed that behaviorally passive rats under basal conditions are characterized by lower of leukocyte count per blood volume unit than active specimens (Table 1). Active animals had a greater percentage of lymphocytes, while passive specimens demonstrated a higher number of band and segmented

TABLE 1. Peripheral Blood Leukocyte Count in Rats (thousands/ml, $M\pm m$; n=28)

Administered solution	Active rats		Passive rats	
	control	stress	control	stress
Physiological saline	14.83±2.91	5.14±0.40**	7.68±1.62^{xx}	5.79±0.72
IL-4	7.28±1.06^{++}	5.25±0.16	7.10±0.60	8.88±2.21^{x}

Note. Here and in Table 2: *p<0.05 and **p<0.01 in comparison with non-stressed rats; ^{+}p<0.05 and ^{++}p<0.01 in comparison with rats receiving physiological saline; ^{x}p<0.05 and ^{xx}p<0.01 in comparison with active rats.

neutrophils (Table 2). These peculiarities contributed to significant between-group differences in LI (higher index in active rats than in passive specimens; Fig. 1, *a*). Statistically significant differences in LII (Fig. 2, *a*) and BLSI (Fig. 3, *a*) were not observed in non-stressed rats with various behavioral characteristics.

Intraperitoneal injection of IL-4 to active rats was followed by a significant decrease in the number of blood leukocytes (by 2 times, $p<0.01$ compared to animals receiving physiological saline; Table 1). This index in passive specimens remained practically unchanged under similar conditions (Table 2).

Studying the leukogram of rats (Table 2) showed that under basal conditions an intraperitoneal injection of IL-4 to behaviorally active specimens is followed by a significant increase in the content of segmented neutrophils (by 1.7 times, $p<0.05$), but decrease in the percentage of blood lymphocytes (by 1.5 times, $p<0.05$ compared to animals receiving physiological saline). The number of band neutrophils in passive rats was reduced by 5.1 times ($p<0.01$) under these conditions. As differentiated from control specimens, blood monocytes were not found in passive and active rats receiving IL-4 ($p<0.01$).

Intraperitoneal injection of IL-4 to non-stressed active rats was followed by a significant increase in BLSI (by 2.4 times, $p<0.01$; Fig. 3, *b*), but decrease in LI (by 2.5 times, $p<0.01$ compared to specimens receiving physiological saline; Fig. 1, *b*). No significant changes in LII were observed in animals of this group (Fig. 2, *b*). As distinct from active rats, behaviorally passive specimens did not demonstrate statistically significant changes in these indexes after IL-4 administration.

Then we studied the effect of IL-4 on leukocyte indexes of the peripheral blood in rats during acute stress on the model of immobilization with simultaneous electrocutaneous stimulation. Our previous experiments showed that stress is accompanied by a decrease in the total number of peripheral blood leukocytes in animals with various behavioral characteristics in the open field (Table 1) [5]. Active specimens were char-

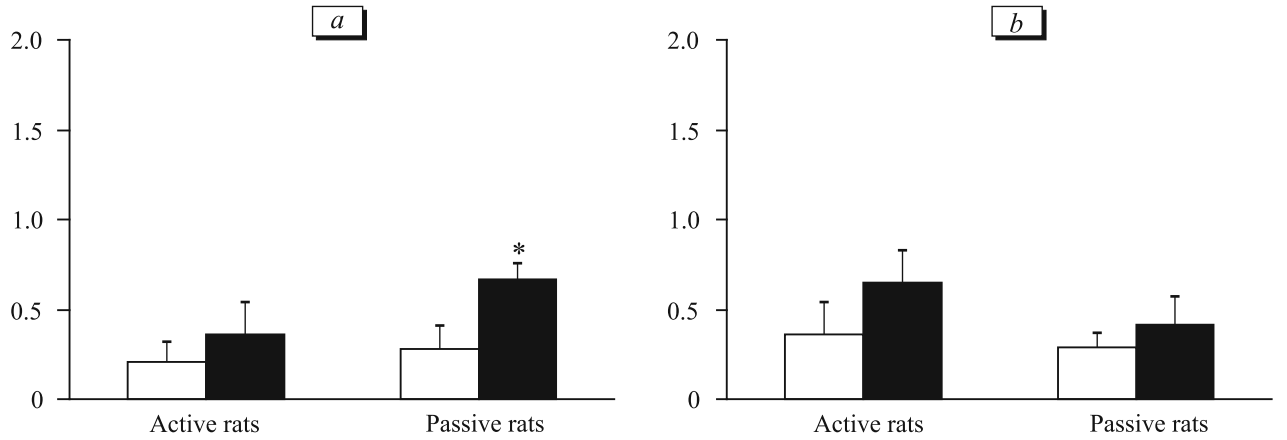

Fig. 1. LI in rats with various behavioral characteristics under control conditions (light bars) and acute stress (dark bars) after administration of physiological saline (*a*) or IL-4 (*b*). Here and in Figs. 2 and 3: $^{*}p<0.01$ compared to the control; $^{+}p<0.05$ and $^{++}p<0.01$ in comparison with rats receiving physiological saline; $^{x}p<0.01$ in comparison with active rats.

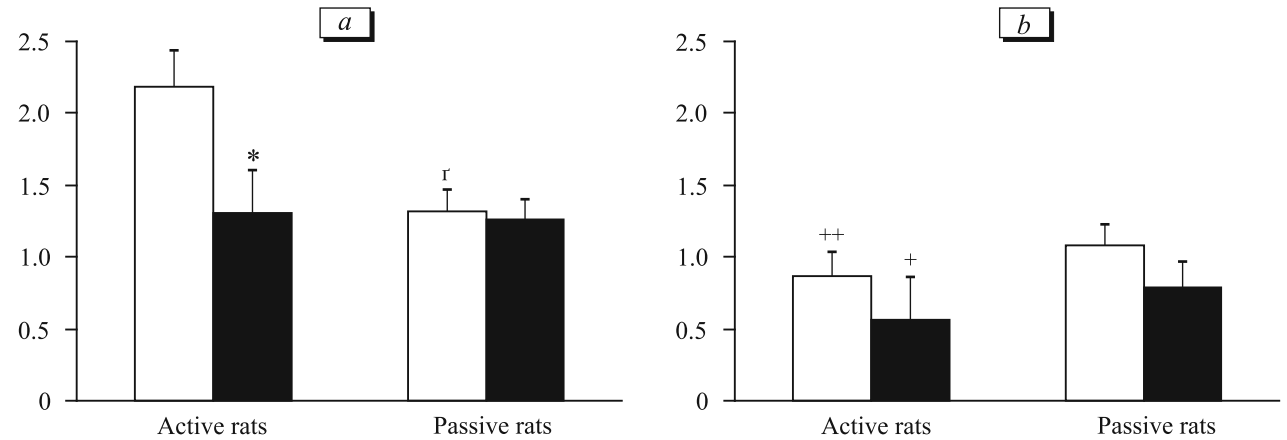

Fig. 2. LII in rats with various behavioral characteristics under control conditions (light bars) and acute stress (dark bars) after administration of physiological saline (*a*) or IL-4 (*b*).

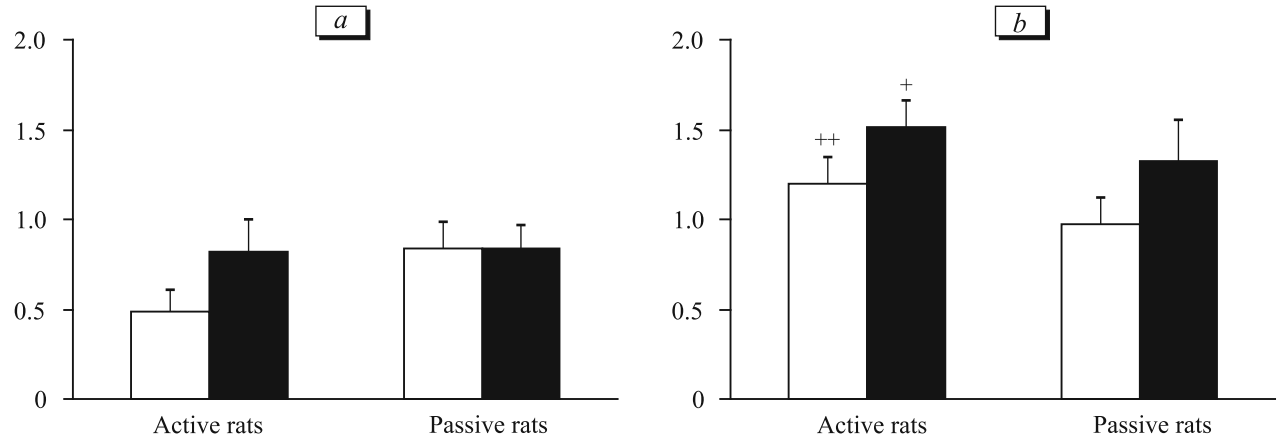

Fig. 3. BLSI in rats with various behavioral characteristics under control conditions (light bars) and acute stress (dark bars) after administration of physiological saline (*a*) or IL-4 (*b*).

acterized by the increase in neutrophil count during stress. The number of eosinophils in passive animals was shown to decrease under these conditions (Table 2). Specific features of the leukogram in stressed rats were manifested in variations of leukocyte indexes for cell reactivity. Emotional stress was followed by a decrease in LI of active rats (Fig. 1, *a*), but increase in LII of passive specimens (Fig. 2, *a*).

Acute stress after intraperitoneal injection of IL-4 had little effect on the total number of peripheral blood leukocytes in rats (Table 1). Behaviorally active and passive specimens were characterized by an increase in the content of band neutrophils under these conditions (by 3.3 and 2.7 times, respectively; $p<0.05$ compared to non-stressed animals; Table 2). Statistically significant changes in other parameters of the leukogram and leukocyte indexes of cell reactivity in rats with various behavioral characteristics in the open field were not found after IL-4 injection and subsequent exposure to stress (Figs. 1-3, *b*).

These data indicate that systemic administration of IL-4 reduces the differences in blood leukocyte count in rats with various behavioral characteristics, which is related to a significant decrease in this parameter in active animals. It should be emphasized that IL-4 injection to active specimens produces leukogram changes: neutrophilia, monocytopenia, and lymphopenia and leftward shift in the leukogram. As differentiated from behaviorally passive rats, IL-4 had a modulatory effect on leukocyte indexes of cell reactivity in active animals (increase in LII, but decrease in LI). Similar changes in leukocyte indexes of the peripheral blood were revealed previously under the influence of proinflammatory cytokine IL-1β [5]. Our results are

TABLE 2. Leukogram of Rats (%, *M*±*m*; *n*=14)

Group	Band neutrophils	Segmented neutrophils	Eosinophils	Monocytes	Lymphocytes
Active rats, physiological saline					
Control	0.71±0.47	30.57±2.80	1.29±0.99	0.57±0.37	66.57±3.31
Stress	2.14±0.55*	41.86±3.80*	1.29±0.42	0.29±0.20	55.00±3.40
Active rats, IL-4					
Control	1.00±0.41	51.25±7.23^{+}	2.25±1.03	0.00^{++}	45.50±7.60^{+}
Stress	3.33±1.20*	60.00±3.86^{+}	1.33±0.67	0.00^{+}	35.67±3.59^{+}
Passive rats, physiological saline					
Control	2.57±1.21	40.43±3.00^{x}	2.00±0.62	0.43±0.30	53.41±3.80^{x}
Stress	2.14±0.46	43.14±5.70	0.29±0.20*^{xx}	0.14±0.12	54.43±5.70
Passive rats, IL-4					
Control	0.50±0.29^{++}	46.50±3.66	2.25±1.31	0.00^{+}	50.75±4.48
Stress	1.33±0.33*^{x}	53.83±4.66	2.17±0.48^{++}	0.00	43.33±5.11

consistent with published data that IL-1β and IL-4 have similar effects on various physiological parameters in rats, including the functional state of stress-marker organs [6] and binding sites of blood albumin [9].

Stress-induced changes in leukocyte indexes of the peripheral blood from rats were shown to differ after intraperitoneal injection of IL-4. As distinct from poststress leukopenia, the number of blood leukocytes practically did not change after stress exposure in cytokine-treated animals. Moreover, pretreatment with IL-4 abolishes the changes in leukocyte indexes of cell reactivity after acute stress. These data indicate that adaptive processes in mammals after negative emotiogenic exposures are partially mediated by specific immunomodulatory compounds.

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