Effect of Neutrophilokine on the Blood System Reaction under Various Extremal Influences

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The effect of neutrophilokine, a secretory product of neutrophils, on the blood system response to 9-h immobilization and zymosan-induced peritonitis is examined. Pretreatment with neutrophilokine (7×10^{-7} mg per animal, 3 times) diminishes the stress-induced changes in the blood and has no effect on the blood reaction to zymosan-induced peritonitis.

Key Words: *neutrophilokine; immobilization stress; inflammation; blood system*

Stress induces specific changes in the blood system manifesting themselves as neutrophilia and lymphopenia [1]. Similar changes (neutrophilia) have been observed in inflammation. Recently, it has been found that latex-activated neutrophils produce biologically active substances (neutrophilokines, NK) which stimulate the immune response, activate phagocytizing cells, and inhibit growth of persistent tumors [3-5]. Previously, we showed that a single administration of the secretory products of neutrophils induces the stress syndrome [6]. The aim of the present study was to assess the effect of NK on immobilization stress and inflammation.

MATERIALS AND METHODS

Experiments were performed on outbred albino rats weighing 180-220 g (n=109). The animals were divided into 6 groups. Rats of group 1 (immobilization control), group 2 (9-h immobilization), group 4 (peritonitis control), and group 5 (zymosan-induced peritonitis) were injected 0.5 ml 0.9% NaCl (3 times, subcutaneously, at 24-h intervals). Rats of group 3 (NK+9-h immobilization) and group 6 (NK+ zymosan-induced peritonitis) were injected NK in a dose of 7×10^{-7} mg according to the same scheme. After the last injection, all the rats wered starved for 24 h with free access to water. Then group 2 and 3 rats were immobilized for 9 h on the back fastened by four limbs, group 5 and 6 rats were injected with zymosan intraperitoneally in a dose of 100 mg/kg, and group 4 rats received an equal volume of 0.9% NaCl [7].

At the end of immobilization or 6 h after zymosan injection, blood was collected from the retroorbital sinus under ether anesthesia, after which the rats were killed by cervical dislocation. The karyocyte spectrum and count in the blood, bone marrow (BM), and spleen were determined by conventional methods [1]. In some rats given NK or normal saline, serum contents of haptoglobin and α_2-macroglobulin were determined in a REVIEWPROTEIN-SYSTEM apparatus (Beckman).

Neutrophilokine was isolated from fraction 5 of the culture medium conditioned by latex-stimulated neutrophils as described previously [4]. The results were analyzed using serial, Student, Wilcoxon—Mann—Whitney, and other tests [2].

RESULTS

Hematological manifestations of the stress syndrome were observed in rats after a 9-h immobilization (Table 1). Neutrophilia and lymphopenia occurred in the blood, the BM count of poorly differentiated myelocytes increased, and that of neutrophils

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TABLE 1. Effect of NK on the Blood System after a 9-h Immobilization Stress

Parameter	Group 1 (immobilization control)	Group 2 (9-h immobilization)	Group 3 (NK+9-h immobilization)
Peripheral blood leukocytes, $\times 10^9$/liter	9.55±0.63 (25)	12.6±0.88 (23)**	13.16±0.90 (25)
Peripheral blood neutrophils, %	11.59±1.15 (27)	61.94±2.67 (23)*	37.14±4.28 (25)+
Peripheral blood lymphocytes, %	87.90±1.14 (27)	37.31±2.77 (23)*	62.02±4.43 (25)+
Lymphocytes/neutrophils	10.30±1.20 (27)	0.69±0.10 (23)*	4.65±1.63 (25)+
BM karyocytes, $\times 10^6$/femur	80.75±6.67 (30)	80.26±6.01 (26)	79.55±7.06 (28)
BM poorly differentiated myelocytes, %	45.89±1.62 (30)	56.05±1.68 (26)*	47.00±1.61 (28)+
BM neutrophils, %	18.56±1.14 (30)	6.85±0.72 (26)*	13.80±1.13 (28)+
Spleen karyocytes, $\times 10^6$	521.56±31.05 (30)	309.99±35.85 (26)**	332.14±33.39 (28)
Spleen neutrophils, %	1.23±0.13 (30)	6.66±0.87 (26)*	2.47±0.36 (28)+
Spleen lymphocytes, %	98.52±0.14 (30)	92.49±0.80 (26)*	97.29±0.37 (28)+

Note. *$p<0.001$, **$p<0.01$ compared with group 1, +$p<0.001$ compared with group 2. Here and in Table 2: the number of animals is given in parentheses.

TABLE 2. Effect of NK on the Blood System after Zymosan-Induced Peritonitis

Parameter	Group 4 (peritonitis control)	Group 5 (zymosan-induced peritonitis)	Group 6 (NK+zymosan-induced peritonitis)
Peripheral blood leukocytes, $\times 10^9$/liter	13.59±1.76 (6)	10.38±1.86 (9)	8.36±1.25 (8)
Peripheral blood neutrophils, %	11.77±3.77 (6)	21.53±3.13 (9)*	28.54±4.96 (8)
Peripheral blood lymphocytes, %	78.76±4.27 (6)	72.92±3.88 (9)	64.62±4.80 (8)
Lymphocytes/neutrophils	10.27±2.55 (6)	4.38±1.00 (9)*	2.92±0.59 (8)
BM karyocytes, $\times 10^6$/femur	116.25±22.24 (6)	104.38±12.83 (9)	93.28±11.46 (8)
BM poorly differentiated myelocytes, %	37.95±2.31 (6)	44.57±3.06 (9)	50.62±2.21 (8)
BM neutrophils, %	28.63±10.10 (6)	5.20±0.92 (9)*	5.61±1.27 (8)
Spleen karyocytes, $\times 10^6$	755.63±168.15 (6)	536.88±36.32 (9)	544.22±73.55 (8)
Spleen neutrophils, %	2.39±0.50 (6)	1.04±0.37 (9)*	0.55±0.15 (8)
Spleen lymphocytes, %	97.33±0.44 (6)	98.46±0.42 (9)	99.13±0.22 (8)

Note. *$p<0.05$ compared with groups 4 and 6.

decreased. In the spleen the neutrophil count increased, while the lymphocyte count decreased. Administration of NK markedly diminished the stress-induced changes in the blood system (Table 1). The neutrophil count dropped 40%, while the lymphocyte count increased 66.23% in comparison with group 2 rats. The count of poorly differentiated myelocytes in the BM decreased by 16.07%, while the neutrophil count increased by 101.46%. In the spleen the neutrophil count decreased by 62.91%, and the lymphocyte count increased by 5.19%. Thus, administration of NK induces the development of tolerance to the immobilization stress. Previously, we showed that NK lowers the sensitivity to stress hormones (glucocorticoids and epinephrine) and increases the sensitivity to insulin. This phenomenon may be associated with the "tolerogenic" effect of NK.

Six hours after administration of zymosan, blood neutrophil count increased by 82.43%, the lymphocyte/neutrophil ratio decreased by 57.35%, and the neutrophil content in the BM and spleen dropped by 81.84 and 56.49%, respectively, compared with the corresponding controls (Table 2). Administration of NK prior to zymosan induced no significant changes in the blood system compared with group 5 (Table 2). It should be noted that during inflammation the stimulated phagocytizing cells secrete a number of biologically active substances (interleukin-1, tumor necrosis factor, leukotriene B_4, etc.). These substances induce neurophilia and lymphopenia in the blood and decrease the neutrophil count in the BM [8,10, 11]. Consequently, NK does not alter the activation of phagocytes during inflammation.

However, NK by itself stimulates phagocytes, as evidenced by the increase in the serum content of haptoglobin to 20.18±4.85 mg/dl (n=5) *vs.* 11.84± 4.41 mg/dl in the control (n=6, $p<0.05$) and α_2-macroglobulin to 5.50±0.35 mg/dl (n=6) *vs.* 3.60±

0.29 mg/dl in the control (n=5, p<0.01). It is known that synthesis of the acute phase proteins in the liver is induced by the products of activated phagocytes [9].

These results suggest that the previously described NK-induced changes in the blood system are associated not only with the stress-realizing systems but also with activation of phagocytizing cells.

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Immunotherapy of Experimental Drug Addiction with Antibodies to Serotonin and Dopamine

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Systemic administration of antiserotonin and antidopamine antibodies to chronically morphinized animals reduces the major manifestations of the withdrawal syndrome induced by naloxone injection or discontinuation of morphine.

Key Words: *antibodies; neurotransmitters; immunization; withdrawal syndrome*

Immunization of chronically morphinized animals with protein conjugates of serotonin (5-HT) and dopamine (DA) suppresses to different extents the manifestations of the withdrawal syndrome [7]. Experimental data suggest that systemic administration of antibodies to the above-mentioned neurotransmitters is a physiologically adequate therapy for drug addiction. The detection of autoantibodies to neurotransmitters in drug addicts [1] and in chronically morphinized animals [2] supports this suggestion. In addition, it was demonstrated that antiserotonin antibodies have a pronounced protective effect in alcoholism, a pathology similar to drug addiction [6].

Our objective was to explore the possibility of suppressing the major manifestations of withdrawal syndrome in chronically morphinized animals by systemic administration of antibodies to 5-HT and DA.

MATERIALS AND METHODS

Experiments were performed on male C57Bl/6 mice weighing 20 g. Two models of withdrawal syndrome were used. In the first series, 40 mice were chronically morphinized by injecting rising doses of morphine (from 20 to 80 mg/kg body weight, subcuta-

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