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SUPPRESSION OF NORMAL KILLER ACTIVITY IN EMOTIONAL-PAINFUL STRESS AND ITS ABOLITION BY INTERFERON INDUCER

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Reactions to stress may have a suppressor effect on the immunogenesis system [6, 9] and, in particular, on antitumor immunity [7, 8]. At the same time it has been shown that activity of the normal killer cell (NK) system, as an essential factor of antitumor resistance, largely depends on synthesis of interferon and its level in the body [3].

The aim of the present investigation was accordingly to study the effect of emotional-painful stress (EPS) on activity of the NK system in animals and to study whether the stress-induced depression of activity of this system can be abolished by means of interferon inducer.

EXPERIMENTAL METHOD

Male August rats weighing 120-160 g were used. The model of EPS was produced in the form of an "anxiety neurosis" by the method in [2].

Activity of NK was tested immediately after exposure to stress for 1.5, 3, and 6 h, and also at various times from 1 to 9 days after the end of a 6-h period of stress.

Spleen cells, adjusted to a final concentration of 20×10^6/ml, were used as the source of NK. These effector cells were transferred to a culture medium prepared from Eagle's medium with 10% fetal calf serum, 2 mM glutamine, 1 mM HEPES buffer, and monomycin in a concentration of 100 U/ml. The targets for NK were human erythroid leukemia cells of line K 562, maintained by serial passages *in vitro*.

Activity of NK (i.e., their ability to cause lysis of target cells) was judged by the outflow of ^{51}Cr from destroyed target cells into the culture medium.

To label the targets, 100 μ Ci of ^{51}Cr (specific activity 350-600 mCi/mg Cr) was added to 5×10^6 K 562 cells in 0.9 ml of culture medium and the sample was incubated for 60 min at 37°C on a water bath. The labeled cells were rinsed vigorously three times to remove free chromium and made up to a concentration of 2×10^5 cells/ml. To 100 μl of the effectors in wells in roundbottomed plates 2×10^4 target cells in 100 μl of medium were added. All experiments were carried out with three different ratios of effectors to targets (100:1, 50:1, and 25:1). The cells were incubated for 14 h at 37°C in a CO_2 incubator, sedimented at 200g for 3 min, after which 100 μl of supernatant was withdrawn from each well. The samples were counted on a gamma-counter. To assess spontaneous liberation of ^{51}Cr, instead of effectors,

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TABLE 1. Effect of EPS on NK Activity in Rat Spleen

Experimental conditions	Mean values of normal killer activity, %		Mean values of NK activity, lytic units/ spleen	
	control	stress	control	stress
Exposure to stress, h				
1½	16,7±1,8	34,7±3,2*	392	1142
3	16,7±1,8	29,0±2,7*	392	815
6	19,5±1,7	13,6±1,2*	523	217
After exposure to stress, days				
1	16,5±1,8	10,2±1,5*	413	172
2	19,5±1,7	14,6±1,5*	523	363
3	19,5±1,7	18,4±1,6	523	556
5	15,5±1,6	39,5±3,2*	413	1502
9	19,5±1,7	21,9±1,9	523	680

Legend. Here and in Table 2, asterisk denotes parameters differing significantly from control. Number of animals at each point not less than six.

TABLE 2. Abolition of Depression of NK Activity Due to Stress by Interferon Inducer Tilorone

Ratio of effectors to target	Control	Tilorone	Stress	Stress + tilorone
100:1	20,7±2,0	25,1±2,2	12,2±1,8*	18,0±1,9
50:1	6,1±0,8	10,6±1,7	3,0±0,2*	8,2±1,0
25:1	3,0±0,4	7,3±1,3	2,5±0,3	2,7±0,2

100 µl of medium was added to the targets, and when determining maximal release of ^{51}Cr, 100 µl of 4% Triton X-100 solution was added. The percentage of lysis was determined by the equation:

$$CTI = \frac{\text{Experimental } ^{51}Cr \text{ release} - \text{spontaneous } ^{51}Cr \text{ release}}{\text{Maximal } ^{51}Cr \text{ release} - \text{spontaneous } ^{51}Cr \text{ release}} \times 100.$$

Spontaneous ^{51}Cr release usually did not exceed 10-15%. Besides the cytotoxicity index (CTI) of the natural killer cells, to express NK activity lytic units also were used. One lytic unit was taken to be the number of effector cells capable of producing lysis of 10% of the target cells. NK activity was calculated in lytic units, both per 10^6 spleen cells and per total spleen cell population. By means of this quantitative parameter it was possible to characterize the total NK activity of the spleen.

Tilorone, an inducer of endogenous interferon, was injected intraperitoneally immediately after exposure to stress for 6 h in a dose of 50 mg per rat, and NK activity was then tested 24 h later.

EXPERIMENTAL RESULTS

It follows from Table 1 that in animals exposed to stress for 1.5 h there was a twofold increase in NK activity. If stress lasted 3 h, CTI rose by only 70%. Meanwhile prolonged exposure (6 h) to stress, which gave rise to ulcers of the gastric mucosa in the animals, accompanied by disturbance of cardiac function, and a decrease in the number of thymus and spleen cells [1], gave the opposite result. In the rats of this group a regular decrease in NK activity by 1.5-2 times was observed.

The dynamics of natural killer activity at different times after prlonged (for 6 h) EPS is given in Table 1. NK activity 24 h after exposure to severe stress remained depressed by

about one-third. This tendency was still present by the end of the 2nd day, and was followed by restoration of activity of the test system to the control level by the end of the 3rd day.

An essential increase in NK activity, to 2.6 times the control values, was observed 5 days after stress. Later (the 9th day after stress) natural killer activity was almost back to the control level.

A short exposure to stress thus activates the NK system, whereas prolonged exposure to the same stress situation has the opposite effect. This fact agrees with the general rule that an increase in the duration of exposure to stress is a factor which frequently plays a definite role in conversion of the stress reaction from an element of adaptation into an element of the pathogenesis of bodily injury [1].

The considerable depression of natural killer activity during prolonged exposure to stress, which the writers have discovered for the first time, agrees with recently published observations to the effect that NK activity is depressed by the action of adrenocortical hormones [5]. It can thus be tentatively suggested that the action of an excess of glucocorticoids is one mechanism by which stress depresses NK activity.

Another possible factor involved in the depression of natural killer activity during stress may be a decrease in the content of interferon, an endogenous NK activator [3]. This hypothesis is all the more realistic because previously, during exposure to stress, a considerable decrease in the serum interferon concentration was demonstrated under experimental conditions [4].

Data on the effect of tilorone, a synthetic interferon inducer, on poststress depression of NK activity are given in Table 2. Injection of tilorone immediately after exposure to stress for 6 h almost completely abolished the poststress depression of NK activity by 24 h after the reaction to stress.

It can be concluded that a rather long exposure to EPS causes a marked decrease in activity of one component of the antitumor system of the body, namely natural killer cells, whereas administration of interferon inducer abolishes this effect.

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