LECTIN-INDUCED CELLULAR CYTOTOXICITY AND PRODUCTION OF CYTOTOXIC FACTOR OF NORMAL KILLER CELLS IN MICE EXPOSED TO IMMOBILIZATION STRESS

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The system of natural (or normal) killer (NK) cells is the first line of defense against tumor growth [9]. The writers previously demonstrated a decrease in NK cell activity after stress [2, 4] and analyzed the causes of this phenomenon. It was found that production of interleukin 2 [3] and of the system of interferons [5], participating in maintenance of the activity of this class of cytotoxic cells [8], is inhibited during stress, and interferon and its inducers, if injected into mice after exposure to stress, help to restore the cytotoxic function of the NK cells [2]. One of the mechanisms of action of interferon on NK cells is through an increase in synthesis and activation and release of the cytotoxic factor of NK cells (CFNK) by effectors [14].

On the basis of these data, the present investigation was carried out with the aim of determining CFNK in animals exposed to immobilization stress. The effect of stress on lectin-induced cellular cytotoxicity (LICT), mediated by NK cells, also was investigated.

EXPERIMENTAL METHOD

Experiments were carried out on male CBA mice aged 12 weeks. The mice were exposed to immobilization stress for 6 h by fixation by the limbs in the supine position. Activity of NK cells and of effectors of LICT was determined 24 h after the end of exposure to stress. Ability of the mouse splenocytes to produce CFNK also was investigated at the same time. To obtain CFNK, spleen cells of control mice and of animals exposed to stress were incubated, in the number of $5 \cdot 10^6$ in 1 ml, with YAC-1 stimulator cells (10^5 in 1 ml) in medium RPMI 1640 with 1% L-glutamine, 1% sodium pyruvate, 1% of a mixture of essential amino acids, and 50 μg/ml of gentamicin in a CO_2 incubator at 37°C. After incubation (from 24 to 72 h) the supernatants were collected after centrifugation at 1500 g for 30 min and frozen in 1-ml ampuls at −20°C [6].

CFNK activity was determined in a 16-h test of release of radioactive Cr from YAC-1 or K 562 target cells [13]. To each well 100 μl of supernatant, containing CFNK and $2 \cdot 10^4$ YAC-1 or K 562 cells, also in a volume of 100 μl, was introduced. To reduce spontaneous outflow of ^{51}Cr from YAC-1 target cells the plates were kept for 14 h at 4°C, then incubated for a further 2 h in a CO_2 incubator at 37°C. CFNK activity was judged by the radioactivity of the supernatant, collected in a volume of 100 μl from each well after culture of the CFNK for 16 h with target cells.

To assess the effect of corticosteroids on CFNK production splenocytes (10^7 cells in 1 ml) were incubated with $5 \cdot 10^{-7}$ M dexamethasone (from Sigma, USA) for 4 h at 37°C. Before addition of the YAC-1 targets the cells were washed three times, otherwise the method was the same as that described above. NK cell activity was determined in the cytotoxic test with release of ^{51}Cr from YAC-1 target cells [1].

The increase in activity of the mouse NK, induced by lectin, was determined against human erythromyeloleukosis K 562 cells in the presence of agglutinin from the mollusk *Helix pomatia*

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TABLE 1. Stress-Induced Changes in LICT and NK Cell Activity in Mouse Spleen 24 h after Immobilization Stress (M ± m)

Parameter	Target cells	Ratio of effectors to targets	Control	Stress
NK cell activity, %	YAC-1	100 : 1	34,6±2,9	18,9±1,6
		50 : 1	33,0±3,0	15,0±1,2
		25:1	20,3±1,8	11,2±0,9
LICT	K 562	100:1	54,0±4,1	20,0±2,1
		50:1	37,5±3,8	17,0±1,4
		25:1	15,0±1,6	9,0±0,8

Legend. No fewer than nine animals used for each determination.

[6], which was added directly to the system for the whole period of incubation. The final concentration of lectin in the wells of the culture panels was 125 μg/ml.

EXPERIMENTAL RESULTS

Table 1 gives data on the character of the change in activity of NK cells and LICT effectors in the population of spleen cells from the control and stressed mice 24 h after exposure to immobilization stress. The results showed that exposure to stress causes not only marked depression of activity of the classical NK cells relative to sensitive YAC-1 target cells, but also, by a no lesser degree, a fall in activity of LICT effectors against K 562 targets. Considering that the increase in activity of the LICT effectors, unlike NK cell activity, is independent of interferon, but nevertheless was reduced in mice in the poststress period, a more complex mechanism for stress-induced depression of the natural cytotoxicity system can be postulated, but evidently not limited to interferon deficiency in animals exposed to stress. The stress-induced fall of LICT is perhaps connected with disturbance of recognition of the tumor target cells by the effectors or with weakening of the triggering action of lectin on the lytic apparatus of the effector cells themselves.

When the probable causes of stress-induced depression of NK cell activity are analyzed, the possible role of disturbance of the synthesis and secretion of CFNK in the pathogenesis of depression of the cytolytic function of cells of natural resistance in animals exposed to extremal conditions must first be determined. A study of the kinetics of CFNK production showed that this reaches a maximum in the splenocyte population of intact mice after 48 h relative to YAC-1 targets.

K 562 cells exhibited low sensitivity to the action of CFNK. A similar picture was found when the kinetics of CFNK was determined in mice after stress. The level of production of the factor was the same in animals of these two groups. Dexomethasone, in a concentration of $5 \cdot 10^{-7}$ M, significantly inhibited CFNK secretion in a culture of spleen cells in vitro (Fig. 1). Thus correlation was not found between CFNK production by splenocytes of mice after stress and of animals whose spleen cells were incubated with dexamethasone. The absence of such correlation could indicate that the poststress changes in the NK cell population are more complex than those taking place after treatment with corticosteroids. The identical ability of splenocyte populations of intact animals and those exposed to stress to produce CFNK, despite differences in NK cell activity, may be indirect evidence of a disturbance of another postulated mechanism in stress, namely recycling, i.e., the abiliity of NK cells to produce lysis of several target cells in succession. It will be noted that glucocorticoids do not impair the ability of NK cells to recognize and bind target cells, but they prevent CFNK production [6].

Meanwhile, the data showing a considerable decrease in activity of NK cells and inhibition of LICT after exposure to stress, obtained in these experiments, in the absence of any difference in secretion of the cytotoxic factor by NK cells in the control and experimental mice may be evidence of profound disturbances of the regulatory and effector cell components associated with production and secretion of some important soluble cell products. This is confirmed by recent findings showing a decrease in secretion of colony-stimulating factor [11], of interleukin 1 [10], and also in the production of lymphotoxin by T cells [12], and in the release of histamine from mast cells [7] under the influence of glucocorticoids. It

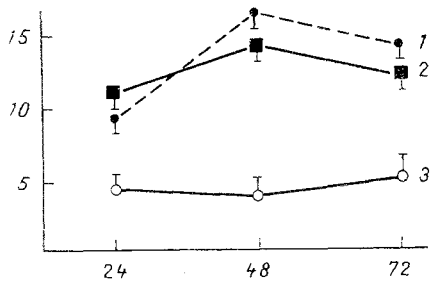

Fig. 1. Kinetics of CFNK production in culture of splenocytes of CBA mice under normal conditions, in immobilization stress, and after treatment with dexamethasone. Abscissa, time of culture of splenocytes with YAC-1 (in h); ordinate, lysis of YAC-1 target cells (in %). 1) Splenocytes of control mice; 2) splenocytes of mice exposed to stress; 3) splenocytes of intact mice treated for 4 h with dexamethasone ($5 \cdot 10^{-7}$ M).

is evident that during exposure to stress and in the subsequent stress reaction we are justified in expecting larger scale involvement and more profound disturbances both of the regulatory immunocompetent cells and of monokines and lymphokines, and also, probably, of lymphotoxins and other soluble factors, participating directly or indirectly in the mechanism of lysis of tumor target cells than after administration of glucocorticoid hormones.

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