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RELATIONS BETWEEN NATURAL KILLER CYTOTOXICITY AND INTERFERON SYSTEMS IN IMMOBILIZATION STRESS

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Normal killer (NK) cells constitute a lymphoid cell population with cytotoxic activity against various tumor cells and also cells infected with viruses or microbial agents [6]. Consequently, NK cells may play an important role in resistance to many diseases. Since these cells do not require preliminary antigenic sensitization for their function and, consequently, do not require time to develop their specific immune response, NK cells are rightly considered to be the "first line of defense" against malignant or virus-infected cells [7].

The writers showed previously that emotional-painful and immobilization stress regularly induce phasic changes in NK cell activity [2]. The first phase of deep depression of NK cell activity, corresponding to the stage of inhibition of nucleic acid and protein synthesis in stress, is followed by a second phase of increased NK cell activity, corresponding to the anabolic stage, i.e., to poststressor activation of nucleic acid and protein synthesis [1]. Since interferons and their inducers play a decisive role in the regulation of NK cell activity [5, 10] and since the serum interferon level falls in the initial phase of the stress response [9], it has been suggested that changes in interferon formation in the body may participate to some degree in the mechanism of the change in NK cell activity observed in stress. One way of testing this hypothesis is evidently to study the dynamics of the serum level of interferon and its formation by lymphocytes during development of the stress response.

The aim of this investigation was accordingly to study the dynamics of the serum interferon level and of interferon production by splenocytes and to compare them with depression of NK cell activity in immobilization stress.

EXPERIMENTAL METHOD

Experiments were carried out on inbred male CBA mice weighing 16-18 g. Immobilization stress was produced in animals in the supine position with their limbs fixed for 6 h. NK cell activity was determined 1, 3, 5, 7, 14, and 21 days after exposure to stress. The mice were

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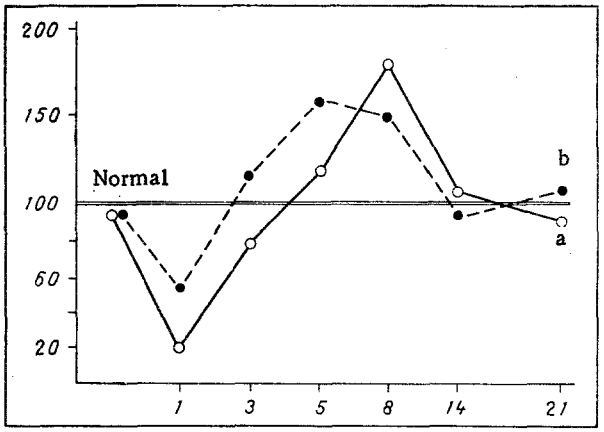

Fig. 1. Relationship between NK cell activity (a) and serum interferon level (b) at different times after immobilization stress. Abscissa, time after stress (in days); ordinate, changes in NK cell activity and serum interferon titer relative to normal (in %).

TABLE 1. Interferon Response of CBA Mouse Splenocytes at Different Times after Immobilization Stress (at least 10 animals used at each point)

Time after stress, days	α- and β-interferons	γ-Interferon		
		PHA	Con A	SEA
Control	80	16	64	32
1	20	8	32	10
3	80	16	64	32
5	80	32	64	64
8	160	64	64	64
14	160	64	64	32
21	80	16—32	64	32

decapitated, and a suspension of splenocytes, obtained by the method described previously [4], was used as the source of NK cells. NK cell activity was determined in the test based on liberation of ^{51}Cr from labeled YAC-1 target cells (mouse T-cell lymphoma maintained by subculture *in vitro*). The method of determination of NK cell activity was described in detail previously [3, 4].

The serum interferon level was determined at the same times after stress as NK cell activity. Interferon was titrated by a micromethod on a culture of mouse fibroblasts based on determination of the protective action against 100 CPD_{50} of mouse encephalomyocarditis (EMC) virus. The unit of interferon activity was the reciprocal of the final dilution of interferon at which 50% of cells were protected against 100 CPD_{50} of the test virus. To study the interferon response of the splenocytes, spleen cells obtained from stressed mice were placed in incubation medium (medium RPMI-1640 + 10% fetal calf serum + 1 mM HEPES-buffer + 2 mM glutamine + 100 U/ml of penicillin), adjusted to a final concentration of $(1\text{-}3)\cdot10^6$ cells/ml, and then incubated in a 24-well plate at 37°C in a CO_2 incubator for 24 h, when Newcastle disease virus (NDV) was used, and 72 h for work with mitogens. NDV was used as interferon inducer, and phytohemagglutinin (PHA), concanavalin A (Con A), and staphylococcal enterotoxin A (SEA) were used to obtain γ-interferon [8]. The level of the interferon response of the splenocytes to the inducers titrated in the same way as for serum interferon.

The results were subjected to statistical analysis by Student's t test.

EXPERIMENTAL RESULTS

The dynamics of NK cell activity of the mouse splenocytes and the serum interferon concentration at different times of the poststress period are shown in Fig. 1. These data in-

dicate that the greatest decrease in NK cell activity on the first day after stress corresponded to the greatest fall in the serum interferon level (by half). On the 2nd day the interferon titer returned to control values, and by the 3rd day there was no sign of depression of NK cell activity. On the 5th-8th day of the poststress period an increase in NK cell activity developed, and at the same time, the serum interferon titer in the mice exposed to stress increased compared with its initial value.

Ability of the splenocytes to produce interferon followed approximately the same time course after immobilization stress (Table 1). A fourfold decrease in production of NDV-induced α- and β-interferons was observed in the first 24 h after stress, after 2 days these parameters returned to normal, and on the 8th-14th days they showed a twofold increase. After 3 weeks the concentrations of α- and β-interferons had returned again to the control level.

Production of γ-interferon by splenocytes of animals exposed to immobilization stress, in the case of induction by SEA and mitogens (PHA and Con A) was the same in principle as that of α- and β-interferons. The most significant fall in the γ-interferon concentration also was observed during the first 24 h after the end of stress. By 72 h it reached control values, by the 5th-8th day it was higher than the level of the interferon response in intact animals, but by the end of the second week after stress it had returned to its initial levels.

The dynamics of stressor depression and subsequent poststressor activation of the NK cell system is thus sufficiently closely linked with the corresponding dynamics of the changes in serum interferon level and the interferon response of the splenocytes of these animals to mitogens. This is evidence of the leading role of the level of endogenous interferon or its endogenous inducers in the mechanism of disturbance of NK cell activity during stress. This conclusion indicates that exogenous interferons or their inducers may be used to prevent or abolish depression of activity of the natural killer cell cytotoxicity system during exposure to stress.

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