STRESS-INDUCED INHIBITION OF MOUSE LEUKEMIA IMMUNOCYTOTHERAPY AND ITS POSSIBLE ABOLITION

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UDC 616.155.392-085.373-035.2-02:613.863]-092.9

KEY WORDS: stress; immunocytotherapy of leukemia.

It is now generally accepted that the system of natural cell-mediated resistance, involving normal killer (NK) cells, plays an important role in defense of the organism against tumors [9]. Numerous investigations have demonstrated the extremely important role of the interferon system in the regulation of NK activity [10]. The writer previously showed that marked depression of NK cell activity occurs in the spleen of rats and mice subjected to emotional-painful and immobilization types of stress [5, 6], demonstrated a poststress decline in the serum interferon level and the interferon-forming response of splenocytes to mitogens [7], and accordingly postulated an essential role of depression of activity of the interferon system in the pathogenesis of stress-induced inhibition of NK cell function. This hypothesis is confirmed by experiments which showed that NK cell activity can recover rapidly in the poststress period after injection of leukocytic interferon or its inducers [3].

Adequate biological models suitable for analysis both of the influence of effector cell systems themselves and of various pathophysiological factors on the antitumor resistance of the organism have recently been described. They include a promising model of immunocytotherapy, using cyclophosphamide and adoptive transfer of syngeneic splenocytes into tumor-bearing mice [8].

The aim of the present investigation was to study the effect of immunobilization stress on the ability of splenocytes to exert their antitumor action, on a model of adoptive transfer and to study the possibility of poststress correction of the immunotherapeutic activity of splenocytes.

EXPERIMENTAL METHOD

Inbred male C57BL/6 ($H\text{-}2^b$) mice weighing 18-20 g, obtained from the "Stolbovaya" Nursery, Academy of Medical Sciences of the USSR, were used. Leukemic strain EL-4 ($H\text{-}2^b$) was maintained by subculture through syngeneic C57BL/6 mice. Mice were subjected to immobilization stress by keeping them in the supine position with their limbs fixed for 6 h.

Mice were inoculated intraperitoneally with EL-4 leukemic cells in a dose of 2×10^4 per mouse. After 5 days the animals were given an intraperitoneal injection of cyclophosphamide in a dose of 180 mg/kg, followed 5 h later by an intraperitoneal injection of 2×10^7 splenocytes from syngeneic mice. The antitumor resistance of the animals and functional efficiency of adoptively transferred immunocompetent cells were judged by the mean survival time and the survival rate of tumor-bearing mice.

The animals as a whole were divided into eight groups: 1) control; 2) animals receiving cyclophosphamide on the 5th day; 3) mice receiving splenocytes of intact animals; 4) recipient mice into which spenocytes were transferred from donors exposed to stress; 5) tumor-bearing mice exposed to stress, inoculated 24 h later with tumor cells, given cyclophosphamide 5 days later, then protected with splenocytes of intact animals; 6) both recipients and donors of splenocytes, subjected to immobilization stress; 7) splenocyte donor mice receiving interferon after exposure to stress; 8) donor mice exposed to stress and then treated with polyI·polyC in liposomes. Leukocytic interferon, generously provided by V. V. Malnov-

Institute of Human Morphology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR A. P. Avtsyn.) Translated from Byulleten' Eksperimental'noi Biologii i Medisiny, Vol. 98, No. 12, pp. 694-696, December, 1984. Original article submitted February 29, 1984.

 0007-4888/84/9812-1676$08.50

TABLE 1. Influence of Immobilization Stress on Effect of Splenocytes Used in a System of Adoptive Immunocytotherapy of EL-4 Leukemia

Group of animals	Conditions to which mice receiving splenocytes were exposed	EL-4 leukemia cells	Cyclophosphamide (180 mg/kg)	Conditions to which mice donating splenocytes were exposed	Mean survival period, days (M±m)	No. of surviving mice/number of mice used in expt.
1	Control (normal)	+	—	—	16±2	0/30
2	Normal	+	+	—	25±3	0/30
3	»	+	+	$2 \cdot 10^7$ (Normal)	60±7	9/30
4	»	+	+	$2 \cdot 10^7$ (Stress)	35±5*	0/30
5	Stress	+	+	$2 \cdot 10^7$ (Normal)	40±6*	0/30
6	»	+	+	$2 \cdot 10^7$ (Stress)	30±4*	0/30
7	Normal	+	+	$2 \cdot 10^7$ (Stress + interferon)	60±5	11/30
8	»	+	+	$2 \cdot 10^7$ (Stress + polyI·polyC)	60±6	10/30

Legend. Asterisk indicates groups of animals in which results differed statistically significantly ($P < 0.05$) from group 3 (normal).

skaya (N. F. Gamaleya Research Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR), was injected intraperitoneally in a dose of 1200 U/mouse immediately after the end of stress. PolyI·PolyC, a synthetic interferon inducer, in liposomes (the technique of preparation and details of administration were described previously [1]), was obtained from A. M. Poverennyi (Research Institute of Medical Radiology, Academy of Medical Sciences of the USSR) and also injected intraperitoneally immediately after the end of stress in a dose of 50 µg per mouse. Splenocytes of these donors, 24 h after stress and injection of the abovementioned preparations, were transplanted into mice of group 7 (with interferon) and group 8 (with polyI·polyC).

EXPERIMENTAL RESULTS

The results are given in Table 1. They show that injection of 2×10^4 EL-4 leukemia cells into mice was followed by death of all the animals by the 16th day. Injection of cyclophosphamide increased the mean survival period of the mice: They all died by the 25th day after injection of the tumor cells. Meanwhile, adoptive transfer of intact syngeneic spleen cells into tumor-bearing mice prevented death of 30% of the animals (nine of 30 mice remained alive after more than 60 days). However, in group 4, in which tumor-bearing mice were protected by splenocytes from donors exposed to stress, immunocytotherapy had no protective effect and all the animals died by the 35th day after inoculation of the tumor. Animals of group 5, exposed to stress 24 h before inoculation of leukemia cells, but protected by syngeneic intact splenocytes, also died by the 35th-40th day of observation. Mice of group 6, subjected to immobilization stress before inoculation of the tumor and receiving splenocytes from stressed animals, also all died by the 30th day after injection of the tumor cells. Only 35-37% of the mice of groups 7 and 8 were free from tumors by the 60th day of observation.

It can be concluded from analysis of these results that stress, first, considerably disturbs the system of antitumor resistance of the tumor-bearing mice and, second, substantially reduces the antitumor activity of splenocytes of mice dontating syngeneic effector cells used to immunocytotherapy on an adoptive transfer model. Injection of interferon or its inducer, after exposure to stress, into mice donating splenocytes abolished the immunosuppressor effect of stress on adoptively transferred splenocytes.

When the possible mechanisms of the immunocytotherapeutic action of splenocytes are analyzed, a number of suggestions regarding the possible role of certain types of effector cells in the realization of the protective mechanism of antitumor resistance of animals during stress must be put forward. The fact that injection of interferon or of its inducer into splenocyte donor mice previously exposed to stress caused recovery of their immunotherapeutic action evidently indicates that this effect can be attributed to the eradication of stress-induced depression of activity of the cells of natural resistance and, in particular, of NK cells. Further evidence in support of the involvement of NK cells in the antitumor effect of adoptively transferred immunocompetent cells is given by data published by the writer previously on disturbance of NK cell activity during stress [2], and on the possibility of operative restoration of function of the system of natural cell-mediated resistance by the use of interferon or of inducers of its synthesis [2, 7].

The use of a model of adoptive immunocytotherapy thus demonstrated, on the one hand, the immunosuppressor effect of excessively strong stimuli on the state of systems responsible for protecting the animal against growth of tumors. On the other hand, the arguments presented confirm the important role of NK cells in tumor immunocytotherapy. However, the possible role of regulatory cells (amplifiers) in the sensitivity of splenocytes to stress-induced depression of their therapeutic activity and the correction of these disturbances must be investigated in the future. The writer showed recently [4] that ability of splenocytes to produce interleukin-2 is sharply depressed in stress. This lymphokine is directly concerned in interferon production and NK cell activation.

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EFFECT OF BONE MARROW MYELOPEPTIDE MEDIATORS ON THE THRESHOLD SUMMATION INDEX AND BEHAVIORAL RESPONSE IN RATS

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UDC 612.419.018:577.175.82]-08:612.884

KEY WORDS: myelopeptides; threshold summation index; behavioral responses; analgesia.

The bone marrow cells of man and animals produce a mediator which causes a two-three-fold increase in antibody production at the peak of the immune response [1, 8]. This mediator is a thermostable substance of peptide nature with molecular weight of about 2 kilodaltons [5, 6]. Studies of the biological and physicochemical properties of this substance demonstrated its structural and functional heterogeneity [6, 9]. Besides immunostimulating activity, the bone marrow mediator also possesses analgesic and endorphin-like properties. Like morphine and certain endogenous opiates, it interacts with μ- and β- opiate receptors of the brain [10] and selectively inhibits cortical responses to nociceptive stimulation [9]. By analogy with neuropeptides, produced by cells of nerve tissue, the bone marrow mediators have been called myelopeptides.

The aim of this investigation was to continue the study of the analgesic action of myelopeptides, as reflected in their effect on the threshold-summation index (TSI) and behavioral responses in rats.

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(Presented by Academician of the Academy of Medical Sciences of the USSR R. V. Petrov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 98, No. 12, pp. 696-698, December 1984. Original article submitted February 16, 1984.

0007-4888/84/9812-1678$08.50