IMMUNOMODULATING ACTION OF MYELOPEPTIDES IN SEVERE EXPERIMENTAL CLOSED CRANIOCEREBRAL TRAUMA IN RATS

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UDC 617.51+616.831]-001-036.17-092.9-092:[612.017.1.014.46:615.362.833:]547.95:547.943

KEY WORDS: craniocerebral trauma; myelopeptides; immunomodulation; lymphoid organs; rosette formation.

It is now generally accepted that carniocerebral trauma (CCT) causes a complex series of disturbances not only directly in the CNS, but also in other systems of the body and, in particular, in the immune system. In recent years ever-increasing attention has been paid to its changes, as a system which largely determines homeostasis of the body as a whole. Considerable disturbances of both the cellular and the humoral immune response have been found in victims of CCT [3, 4, 10, 11]. There would thus appear to be a promising case for the use of immunomodulators in CCT, more especially because in disturbances of the immune system of other etiology, attempts have already been made to use preparations based on hormones and mediators of the immune system such as T-activin, splenin, γ-interfron, etc. [5].

A preparation based on bone marrow peptides, or myelopeptides (MP), may be promising in this respect. Besides stimulating antibody production [8], MP also have a marked analgesic, endorphin-like action [9] and, consequently, they possess affinity not only for the functional cells of the immunity system, but also for the CNS.

The aim of this investigation was to study the cellular composition of subpopulations of the thymus, spleen, and bone marrow during the early postraumatic period after severe closed CCT in rats without immunocorrection and receiving MP.

EXPERIMENTAL METHOD

Noninbred male rats weighing 155-255 g were used in the experiments. CCT was inflicted by means of a spring-operated striker in the left parieto-temporal region. The severity of the trauma was selected in preliminary experiments, and it corresponded morphologically to mild or moderately severe concussion.

MP were isolated from a supernatant of hog bone marrow cell cultures by gel-chromatography on Sephadex G-25 [7]. The protein content of the preparation was determined by Lowry's method. The MP were injected intramuscularly in a dose of 500 μg/kg three times, on the 1st, 3rd, and 5th days of the experiment.

At intervals during the early posttraumatic period (on the 1st, 5th, 15th, and 30th days after CCT) the number of nucleated cells was counted in the thymus, spleen, and femoral bone marrow, and the number of cells in these organs forming rosettes with sheep red blood cells (RFC-SRBC) [13], forming autorosettes (AU-RFC) [15], and EA- and EAC-rosettes [12, 14] also was determined.

The effect of MP on the resistance of the animals to infection was studied in experiments in which the animals were infected intraperitoneally 1 h after trauma with LD_{50} of *Staphylococcus aureus* (reference strain 8325; $2 \cdot 10^{10}$ microbial cells in 1 ml of physiological saline). The 10-day survival rate of the rats after infection and persistence of *Staph. aureus* in the animals were analyzed. For seeding a homogenate of the animals' kidneys, 5% salt agar was used. Colonies in the agar were counted after incubation for 48 h at 37°C.

EXPERIMENTAL RESULTS

Data were obtained on the change in cell composition of the thymus, spleen, and bone marrow at intervals during the posttraumatic period of severe CCT. Throughout the period of

Research Institute of Neurosurgery, Kiev. Institute of Immunology, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR A. P. Romodanov). Translated from Byulleten' Eksperimental'noi Biologii i Meditsiny, Vol. 105, No. 3, pp. 310-311, March, 1988. Original article submitted December 1, 1986.

observation (30 days) a significant fall was found in the absolute number of thymus and bone marrow cells, accompanied by an increase in the number of splenic karyocytes on the 15th and 30th days of the experiment. No such changes were observed after exposure to stress of other etiology [1, 2]. Considering changes found in the cell subpopulations of these organs and changes in immunity after CCT described in the literature, it is possible that factors of CCT have a specific action on the immunity system and on the formation of a secondary immunodeficiency. This largely explains the high frequency of infectious complications after CCT (from 18 to 80%) and the ineffectiveness of antibiotic treatment of these complications.

The investigations showed that injection of MP has the most marked effect during the first day after severe CCT, when it can prevent the cellular depopulation of the thymus and bone marrow. On the 15th and 30th days of the experiment, the normal number of nucleated spleen cells also was restored under the influence of MP.

In the early posttraumatic period of CCT, MP had a marked corrective action on individual cell populations of these organs. For instance, MP prevented the fall in the number of AU-RFC, which are postthymic precursor T cells [6] in the thymus and bone marrow on the 1st and 5th days of experiment. Meanwhile their number in the spleen remained low.

MP also had different effects on the population of EA-RFC. In the thymus their number was virtually unchanged, whereas in the bone marrow they accumulated during the first 5 days of the experiment, their number decreased on the 15th day, after which it was restored by the 30th day of observation. This parameter in the control animals was significantly depressed at all times of observation after CCT.

Injection of MP had no significant effect on the level of antigen-reacting cells (RFC-SRBC) in the thymus. Meanwhile changes in the same direction were discovered in the spleen and bone marrow, with a decrease in the number of RFC-SRBC on the 1st day, an increase in their number on the 5th day, a second decrease on the 15th day, and virtual restoration of the normal level on the 30th day of observation.

Significant differences were observed between the experimental and control groups of animals with respect to the number of EAC-RFC also in the thymus, spleen, and bone marrow. For instance, in response to injection of MP the EAC-RFC level in the thymus was significantly lowered only by the 30th day of the experiment, whereas in the spleen, a decrease in the number of these cells was observed during the 15 days after infliction of CCT. A corrective action of MP was observed in the bone marrow only on the 5th day, when the number of EAC-RFC was restored up to the control level.

The study of the immunocorrective action of MP in the early posttraumatic period after severe closed CCT in rats thus showed that by means of this preparation the cellular depopulation of the thymus and bone marrow and also the hyperplasia of the spleen can be largely prevented. MP have various effects on the functional and migrational properties both of the cell populations of the lymphoid organs as a whole, and of their individual subpopulations.

A corrective action of MP also was discovered in experiments to assess the resistance of animals after CCT to infection with *Staphylococcus aureus*. It will be clear from Fig. 2 that MP completely prevented death of the animals and reduced by more than two-thirds the level of persistence of *Staph. aureus* in rats. The use of splenin, a preparation of pharmacopieal status (injection into rats in doses of 0.1 ml on the 1st, 3rd, and 5th days after infection) also reduced the mortality among the animals, but in this case persistence of the microorganisms remained at the same level as in untreated animals.

Consequently, MP exert a marked antistressor action and prevent cellular depopulation of the central organs of immunity and posttraumatic redistribution of lymphoid cells, thereby ultimately increasing the resistance of the experimental animals to infection.

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EFFECT OF INTERLEUKIN 1 ON ADRENAL FUNCTION IN MICE

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UDC 612.45.018-06:[612.112.94.015.2:612.6

KEY WORDS: macrophages; corticosterone; interleukin 1; prostaglandin E_2.

The functions of the immune and endocrine systems are known to be closely interconnected. There is abundant experimental evidence of the influence of the endocrine glands on lymphoid cells and macrophages [1, 3, 4, 6]. Meanwhile, investigations have shown that the plasma glucocorticoid level rises in animals of various species during the development of an immune response [3, 4, 6]. However, the nature of the signal from the activated immune system to the adrenals is not yet known. It has been found [10] that macrophages, which perform an important function in the formation and regulation of the immune response, produce a spectrum of biologically active substances, including interleukin 1 (IL-1) and prostaglandin E_2 (PGE_2), which play an essential role in the regulation of immune reactions [13]. There is also evidence that IL-1 acts on the hypothalamus [7] and that this effect is mediated by PGE_2.

The aim of this investigation was to study the effect of factors of macrophagal nature, secreted during antigen processing, on adrenal function.

EXPERIMENTAL METHOD

Male (CBA × C57Bl)F_1 mice aged 2-3 months, obtained from the Stolbovaya Nursery, Academy of Medical Sciences of the USSR, were used in all the experiments. The animals were given intravenous injections of purified supernatants of macrophages, containing IL-1, in a volume of 0.2 ml. Mice of the control group received injections of the same volume of physiological saline. Adrenal function was judged from the corticosterone level in plasma from peripheral blood. The corticosterone concentration was determined by competitive protein binding with modifications [2]. IL-1 was isolated from supernatant of peritoneal macrophages, activated by lipopolysaccharide (LPS, 25 μg/ml) in vitro in (CBA × C57Bl)F_1 mice. The collected supernatant was dialyzed, concentrated by means of an Amicon membrane (RM-10; from LKB, Sweden), and applied to a "Toyoperl 50F" column. Fractions containing material with mol. wt. of 10-20, 20-30, 30-40, and 40-60 kilodaltons were dialyzed against medium 199, filtered through a filter with pore diameter of 0.2 μ (Millipore), frozen at −20°C, and kept until use. IL-1 was tested on a mouse thymocyte culture by the method in [11].

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 0007-4888/88/0003-0366$12.50