IMMUNOMODULATING ACTION OF MYELOPEPTIDES IN ANIMALS WITH HYPOXIC HYPOXIA

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When the body is exposed to the action of unfavorable factors and, in particular, acute hypoxic hypoxia, which is a nonspecific stress factor, considerable changes take place in lymphoid tissue function [3]. Hematopoietic stem cells are redistributed and migration of T and B lymphocytes and their precursors from the central organs to the periphery is observed. These cellular responses are evidently aimed at maintaining immune homeostasis during hypoxia. However, in the general mechanism of immunogenesis, under the influence of the hypoxic factor, they do not ensure a full and complete immune response to different antigens [4, 6].

By inhibiting the immune response, hypoxia increases the vulnerability of the body to certain diseases, especially to those whose onset is closely linked with a disturbance of immunologic mechanisms of protection, namely infections, malignant tumors, and autoimmune diseases.

In clinical practice attempts have been made to use preparations based on hormones and mediators of the immune system as immunotherapeutic agents. Thymic factors, used for disturbances of the T system of immunity are well known [1].

Research is currently in progress to create a preparation based on immunoregulatory peptides produced by intact bone marrow cells — myelopeptides (MP). These substances can stimulate antibody production in various forms of immunodeficiency [2, 5]. The results of previous investigations [2] suggest that MP are involved in the development of stress-induced immunodeficiency states.

The aim of this investigation was to study the effect of hypoxic stress on natural production of MP and to assess their immunocorrective effect in this type of stress.

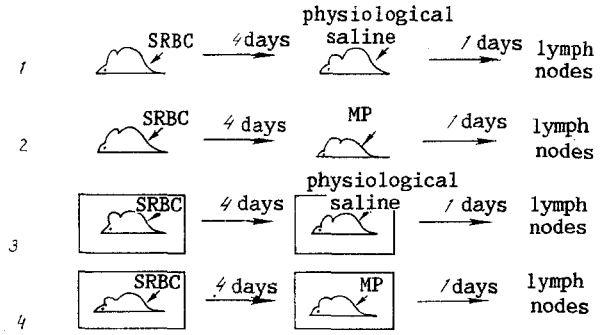

Fig. 1. Scheme of experiment. On left — No. of group of animals: 1) control mice immunized twice with SRBC; 2) animals receiving MP intravenously in a dose of 200 μg per mouse on 4th day of secondary immune response; 3) mice subjected to hypoxic stress by daily lifting for 22 h in a pressure chamber to an "altitude" of 7000 m for 3 days; 4) the same + intravenous injection of MP in a dose of 200 μg per mouse on 4th day of development of secondary immune response.

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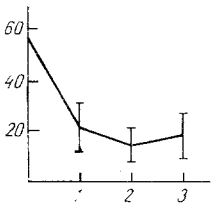

Fig. 2. Effect of hypoxic stress on level of MP production in bone marrow of animals exposed to hypoxia for 1, 2, and 3 days. Abscissa, duration of stay in pressure chamber (in days); ordinate, protein content (in mg).

EXPERIMENTAL METHOD

(CBA × C57BL)F_1 hybrid mice, males and females weighing 18-22 g, were used. The mice were immunized with a 5% suspension of sheep's red blood cells (SRBC) subcutaneously, in a dose of 0.1 ml into each of the four footpads. Reimmunization with the same antigen and by the same method was carried out 2 weeks later. Hypoxic stress was created by lifting the animals daily to an "altitude" of 7000 m in a pressure chamber for 22 h for 3 days. The experimental scheme is shown in Fig. 1.

To study the effect of hypoxic stress on the level of MP production, bone marrow cells were obtained from unimmunized mice which had been exposed to hypoxia for 3 days. The cells (10^7) were cultured at 37°C for 20 h in medium 199 with the addition of glutamine (200 mM), HEPES (1 M), and penicillin (100 U/ml). After culture for 20 h the supernatant was separated by centrifugation (20 min, 8000 rpm) and lyophilized. Fractions with stimulating activity were separated by gel-chromatography on Sephadex G-25. Distilled water, adjusted to pH 7.2 with 1N NaOH, was used for elution.

The level of MP production in the bone marrow was estimated as the protein concentration, determined by Lowry's method.

The second fraction of myelopeptides, with mol. wt. of about 2 kilodaltons, obtained by gel-chromatography of cultures of hog bone marrow cells on Sephadex G-25, was used as the immunomodulating agent. MP were injected intravenously into the animals in a dose of 200 μg per mouse on the 4th day of the secondary immune response to SRBC.

The effect of stimulation of antibody formation in the lymph node cells was assessed on the 5th day of the secondary immune response as the number of antibody-forming cells (AFC), determined by Jerne's method in the modification for IgG-forming cells [7]. The coefficient of stimulation of antibody formation was calculated as the ratio of the number of AFC in the experiment and control. The results were subjected to statistical analysis by Student's test.

EXPERIMENTAL RESULTS

The level of MP production by bone marrow cells was estimated after the animals had been kept in the pressure chamber for 24, 48, and 72 h. The results are given in Fig. 2.

It will be clear that after a stay of 24 h in the pressure chamber the experimental animals produced 3 times less MP. The deficiency of MP production continued on the 2nd and 3rd days of exposure to hypoxic stress.

The number of AFC in the lymph nodes of the immune animals was not significantly altered after a stay of 1 or 2 days in the pressure chamber. On the 3rd day of exposure to hypoxic stress a significant decrease in the secondary immune response was observed. The development of this immunodeficiency state could be effectively prevented by injection of MP.

The results of comparison of stimulation of antibody formation under the influence of MP in mice kept under normoxic and hypoxic conditions are given in Table 1.

Keeping the animals for 3 days in the pressure chamber reduced the immune response on average by 47%. The immunostimulating action of MP was manifested under both normoxic and hypoxic conditions. The coefficient of stimulation was 1.7 in the first case and 3.4 in the second. Thus intravenous injection of MP completely restores the immune response to SRBC, when depressed by hypoxic stress.

TABLE 1. Effect of MP on Immune Response in Mice Kept under Normoxic Conditions and Exposed to Hypoxic Stress

Group of animals	Number of animals	Number of AFC per 10^6 cells (M ± m)	K	p
1	66	177±15		
2	46	293±28,8	1,7	<0,05
3	61	96±10		
4	48	326±39,7	3,4	<0,05

Legend. K) Coefficient of stimulation of antibody formation, calculated as ratio between number of AFC per 10^6 nucleated cells in animals of experimental and control groups. Details of groups indicated in caption to Fig. 1.

The results of these investigations confirm the hypothesis that MP have a protective function against stress. It can be postulated that one cause of depression of the immune response in hypoxic hypoxia is inhibition of natural MP production. Artificial restoration of their body level by exogenous administration prevents the development of an immunodeficiency state caused by hypoxia.

Hypoxia plays an important role in the pathogenesis of most stress-induced and pathological states. Experimental proof of the immunocorrective action of MP in hypoxic stress therefore offers good prospects for their extensive therapeutic and prophylactic use in various clinical forms of secondary immunodeficiencies.

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