EFFECT OF BONE MARROW OPIOID PEPTIDES ON ANTIBODY FORMATION IN THE PRODUCTIVE PHASE OF THE IMMUNE RESPONSE

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Bone marrow cells are known to produce a group of biologically active peptides, known as myelopeptides (MP), which are functionally heterogeneous [3, 5, 8]. MP, with a molecular weight of about 2 kD, stimulate antibody formation at the peak of the immune response [3, 8]. MP not only have an immunostimulating action, but they also give rise to analgesic and endorphin-like effects, which are abolished by the opiate antagonist naloxone [1, 6]. The presence of endorphin-like substances in the composition of MP has been confirmed by experiments with displacement of radioactively labeled opiates from their specific binding sites [7]. Substances closely related or identical to α-, β-, and γ-endorphins have been discovered among MP by radioimmunoassay [2].

The aim of this investigation was to study the effect of bone marrow opioid peptides on antibody formation in the productive phase of the immune respone. Experiments were carried out to compare the antibody-stimulating action of opioid peptides and MP, and the effect of naloxone on this action.

EXPERIMENTAL METHOD

MP were isolated from the supernatant of hog bone marrow cell cultures by gel-chromatography on Sephadex G-25 [4]. The dose of the substances which are components of the bone marrow mediators was estimated as the protein content, which was determined by Lowry's method. MP were used in the experiments in a dose of 60 μg/ml of solution.

According to the results of the radioimmunologic investigation 1 mg of MP contains 71 ± 4 fmoles of α-lipotrophin, 124 ± 46 fmoles of α-endorphin, 39 ± 16 fmoles of β-endorphin, and 33 ± 10 fmoles of γ-endorphin [2]. We used a mixture of synthetic opioid peptides (Serva, West Germany): β-lipotrophin:α-endorphin:β-endorphin:γ-endorphin in the ratio of 71:124:39:33 in the experiments. The working dose of MP, which was 60 μg protein, corresponds to 16 fmoles of the mixture of opioid peptides.

Naloxone (Endo Laboratories, USA) was used as the opiate antagonist.

Activity of MP and endogenous opioid substances was assessed in culture in vitro by the method described previously [5]. The number of antibody-forming cells (AFC) in the culture was determined by Jerne's method in the modification for IgG AFC [9].

The coefficient of stimulation of antibody formation was calculated as the ratio of the number of AFC in the experiment to the number of AFC in the control.

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

EXPERIMENTAL RESULTS

In the experiments of series I the effect of naloxone on the antibody-stimulating activity of MP was studied. As a preliminary step in this model the dose in which naloxone itself did not affect antibody formation was determined. Naloxone was used within a dose range of 0.4 to 30 μg/ml per 10^6 nucleated cells. On the addition of naloxone to the immune lymph nodes (LN) in doses of 0.4-10 μg/ml the number of AFC in the culture was unchanged.

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TABLE 1. Effect of Naloxone on Immunostimulating Activity of MP

Components of cultures	Number of experiments	Number of AFC per 10^6 nucleated cells	Coef. of stimulation	p
Immune LN	13	183±5	—	—
LN + MP	13	366±17	2,0	<0,001
LN + naloxone	13	200±9	1,1	>0,05
LN + MP + naloxone	13	206±6	1,1	>0,05

TABLE 2. Effect of Naloxone on Antibody-Stimulating Activity of a Mixture of Opioid Peptides

Components of cultures	Number of experiments	Number of AFC per 10^6 nucleated cells	Coef. of stimulation	p
LN	4	189±5	—	—
LN + mix. of opiates	4	350±24	1,9	<0,001
LN + naloxone	4	198±5	1,0	>0,05
LN + mix. of opiates + naloxone	4	222±18	1,2	>0,05

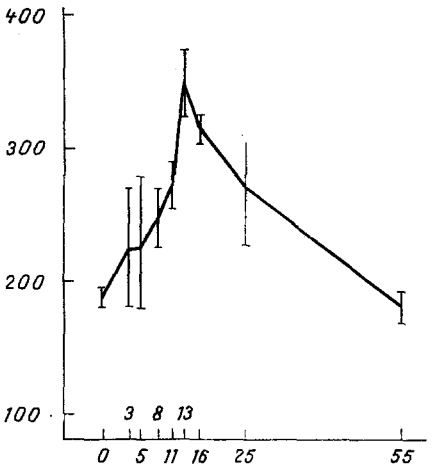

Fig. 1. Dependence of effect of stimulation of antibody formation on dose of opioid peptides. Abscissa, dose of opioid peptides (in fmoles); ordinate, number of AFC per 10^6 nucleated cells.

Doses of over 10 μg/ml per 10^6 nucleated cells depressed antibody formation. On the basis of these results, a dose of 0.4 μg/ml was used in all the subsequent experiments.

The results of investigations of combined culture of immune LN with MP and/or naloxone are given in Table 1.

The results show that MP doubled the intensity of antibody formation. Naloxone, added together with MP to the culture, abolished their stimulating action.

In the experiments of series II the effect of a mixture of synthetic opioid peptides (α-, β-, and γ-endorphins) on antibody formation was studied in the production phase of the immune response. The mixture of opiates was used in concentrations of 3 to 55 fmoles/ml (Fig. 1).

It will be clear from Fig. 1 that the mixture of opioid peptides in concentrations of 11 to 16 fmoles stimulated antibody formation. The greatest effect was observed when a concentration of 13 to 16 fmoles/ml was used (coefficient of stimulation 1.7-1.9), i.e., amounts corresponding to the content of these substances in MP. The remaining doses of opiates used had no such effect.

The effect of naloxone on the antibody-stimulating activity of the mixture of opioid peptides was next studied. Peptides were used in a dose of 13 fmoles. The results of these experiments are given in Table 2.

It will be clear from Table 2 that naloxone abolished the antibody-stimulating effect of the mixture of opiates.

In the experiments of series III one of the components of the mixture was used, namely synthetic β-endorphin. β-Endorphin was added in doses of between $2.4 \cdot 10^{-6}$ and $2.4 \cdot 10^{2}$

TABLE 3. Effect of Naloxone on Antibody-Stimulating Activity of β-Endorphin

Components of cultures	Number of experiments	Number of AFC per 10^6 nucleated cells	Coef. of stimulation	p
LN	5	171±15	—	—
LN + β-endorphin	5	368±22	2,2	<0,001
LN + naloxone	5	209±19	1,2	>0,05
LN + β-endorphin + naloxone	5	196±18	1,2	>0,05

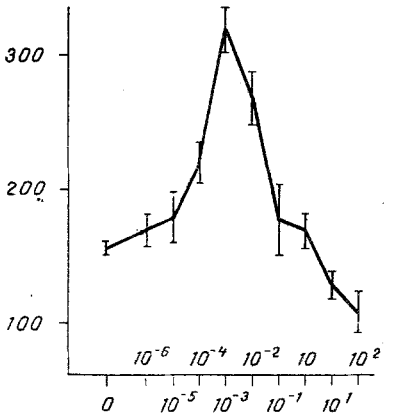

Fig. 2. Dose-dependent effect of β-endorphin on antibody production. Abscissa, dose of β-endorphin (in fmoles); ordinate, number of AFC per 10^6 nucleated cells.

fmoles/ml to LN cells obtained from mice on the 4th day of the secondary immune response to sheep's red blood cells (Fig. 2).

It will be clear from Fig. 2 that β-endorphin, in doses of $2.4 \cdot 10^{-4}$ to $2.4 \cdot 10^{-2}$ fmoles/ml increased antibody production at the peak of the immune response. The maximal effect was observed with β-endorphin in a concentration of $2.4 \cdot 10^{-3}$ fmoles/ml (coefficient of stimulation 2.1), i.e., in a dose 1000 times less than its content in MP. Doses of $2.4 \cdot 10^{-6}$ to $2.4 \cdot 10^{-5}$ and $2.4 \cdot 10^{-1}$ fmoles/ml did not affect antibody production (coefficient of stimulation 1.0-1.1). With an increase in the doses of β-endorphin to 24-240 fmoles/ml, inhibition of antibody formation was observed (coefficient of stimulation 0.7-0.8). The antibody-stimulating effect of β-endorphin was abolished by naloxone (Table 3). The effect of Met- and Leu-enkephalins on antibody production also was investigated. Neither Met- nor Leu-enkephalin, used in doses of $6 \cdot 10^{-3}$ to $6 \cdot 10^{5}$ fmoles/ml, had any effect on antibody production (coefficient of stimulation 0.8-1.2).

The mixture of opioid peptides in quantities corresponding to their content in MP, and also β-endorphin thus evoke an antibody-stimulating effect similar to that of MP. The opiate antagonist naloxone blocks antibody-stimulating activity not only of added opiates, but also of MP.

The results indicate close connection between the antibody-stimulating and analgesic and endorphin-like activities of MP. However, the question of which molecules are responsible for these effects can be solved only after elucidation of the structure of individual MP. The possibility cannot be ruled out that opioid peptides exert their influence on the humoral immune response in a complex manner. By binding with opiate receptors of immunocompetent cells they activate a chain of signals essential for its normal development [10].

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EFFECT OF HUMAN REGULATORY MYELOPEPTIDES ON PRODUCTION OF LEUKOCYTE MIGRATION INHIBITION FACTOR

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One of the key factors in the development of the normal immune respone is lymphocyte–macrophage interaction, which is mediated by a group of humoral factors, one of which is that known as leukocyte migration inhibition factor (LMIF). In several pathological states LMIF synthesis is disturbed. This may arise for various reasons: disturbance of functions of effector cells, a change in the mechanisms regulating its synthesis at cellular, genetic, and molecular levels [1, 4].

In recent years the role of cells as regulators of the thymic and medullary circulation in the development of various immunologic processes, including LMIF synthesis, has been actively studied [6-8, 10, 11]. The study of the mechanisms of this regulation would shed light on the possible stages of development of a number of immunopathological states.

For the reasons given above it was decided to study the regulatory action of human medullary myelopeptides (MP), namely B-activin and suppressor factor, under normal conditions and in patients with agammaglobulinemia (AGG) and multiple myeloma, in the LMIF production system.

EXPERIMENTAL METHOD

Bone marrow cells (BMC) were obtained during diagnostic trephine biopsy. BMC were cultured in complete RPM 1640 medium with the addition of 0.1 ml glutamine, 0.1 ml HEPES buffer, and 5% fetal serum to 10 ml of complete medium. After culture for 48 h the supernatants, containing a factor with suppressor activity, was obtained. B-Activin was obtained in R. N. Stepanenko's laboratory [9].

Soluble factors were tested as follows. The target cells in all cases were healthy human lymphocytes. The lymphocytes were treated with B-activin or suppressor factors for 1 h, after which the cells were washed twice by centrifugation. To determine the action of the factors on spontaneous LMIF production by lymphocytes, the latter were cultured in RPMI medium with additives for 18-24 h.

To determine the effect on induced LMIF production, lymphocytes were incubated for 3 h with the mitogen phytohemagglutinin (PHA, Difco, USA) in a dose of 10 μg/ml. The cells

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