EFFECT OF MYELOPEPTIDES ON DNA AND PROTEIN SYNTHESIS AND ON ANTIBODY SECRETION BY MOUSE B-CELL HYBRIDOMAS

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It was shown previously that bone marrow cells from animals of various species and from man produce regulatory peptides with mol. wt. of ~2.0 kilodaltons (kD), to which the name of myelopeptides (MP) has been given [1, 3, 7]. MP possess immunoregulatory and opiate-like activity [1, 2, 7, 8]. One of the best studied functions of MP is their ability to stimulate antibody production at the peak of the immune response. Stimulation of antibody production at the peak of the immune response under the influence of MP takes place through an increase in the number of antibody-producers without any change in the level of antibody secretion by each single cell. It has been suggested that, under the influence of MP, cells known as "silent" antibody-forming cells are switched to antibody production [5]. It has been shown that the switching in the work of "silent" cells is the result of the modulating effect of MP on helper-suppressor interactions of T lymphocytes [4]. It has been shown that MP have no appreciable effect on the level of DNA and total protein synthesis in cell populations of the thymus, immune lymph nodes, and spleen [6].

The aim of this investigation was to study the effect of MP on DNA and protein synthesis and on secretion of antibodies by B-cell mouse hybridoma clones, constituting homogeneous cell populations.

EXPERIMENTAL METHOD

Two clones of hybridoma cells obtained after fusion of AgX63.P653 myeloma cells with spleen cells of BALB/c mice, immunized twice with capsular F1-antigen of Yersinia pestis, were used. Clone 2G6B3 secreted antibodies of the IgM class and clone 3C7C7 – of the IgG2b class.

Hybridoma cells in a concentration of 10^5/ml were incubated in a volume of 0.2 ml for 24 and 48 h at 37°C, in the presence of 7% CO_2 in medium RPMI-1640 with the addition of 15% fetal calf serum, 50 μg/ml gentamycin, and 2 mM L-glutamine. At the beginning of incubation different doses of MP, obtained by the method described previously [1], were added to the cells. ^{3}H-thymidine (1 μCi/0.2 ml) or ^{14}C-glycine (1 μCi/0.2 ml) was added to the wells 4 h before the end of incubation. Incorporation of the label was determined by the traditional method on a "Mark III" scintillation counter.

The quantity of antibodies secreted in supernatants of hybridoma cell cultures was determined after 48 h by enzyme immunoassay (EIA). For this purpose, F1-antigen was introduced in a concentration of 10 μg/ml in a volume of 0.06 ml into each well of 96-well micropanels. The micropanels were incubated with the agent for 1 h at 37°C, then carefully washed with distilled water containing Tween (0.02%). Test supernatants were applied to the panels with antigen after frequent dilution with phosphate buffer, with the addition of 0.1% bovine serum albumin and 0.02% Tween. After incubation for 1 h the micropanels were washed 3 times with distilled water containing Tween. Into each well 50 μl of a conjugate of rabbit antibodies to mouse immunoglobulins with horseradish peroxidase was added in a dilution of 1:4000. After incubation for 1 h at 37°C the micropanels were thoroughly washed with distilled water and 100 μl of orthophenylenediamine

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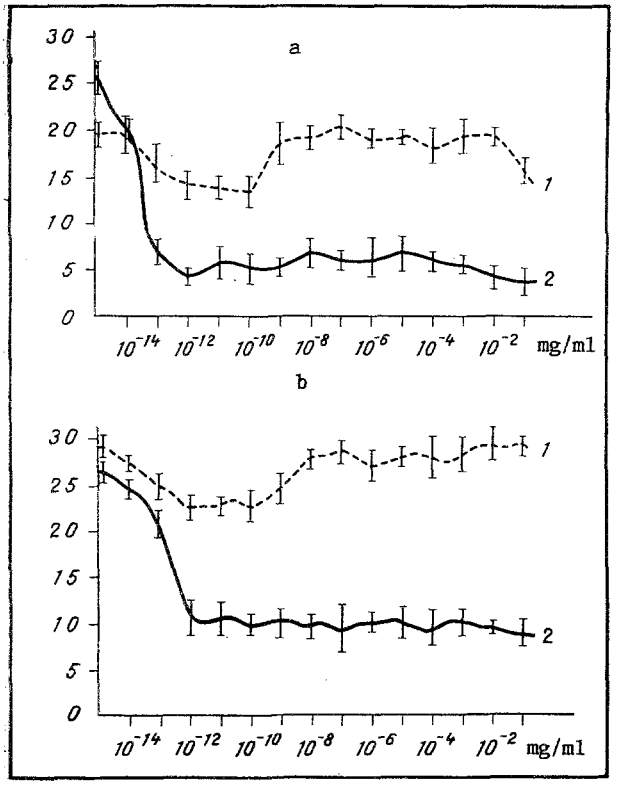

Fig. 1. Effect of MP on DNA synthesis by hybridoma clone 2G6B3 (a) and by clone 3C7C7 (b). Abscissa, concentration of MP (in mg/ml); ordinate, number of counts per minutes ($\cdot 10^{-3}$). 1) After culture for 24 h, 2) for 48 h.

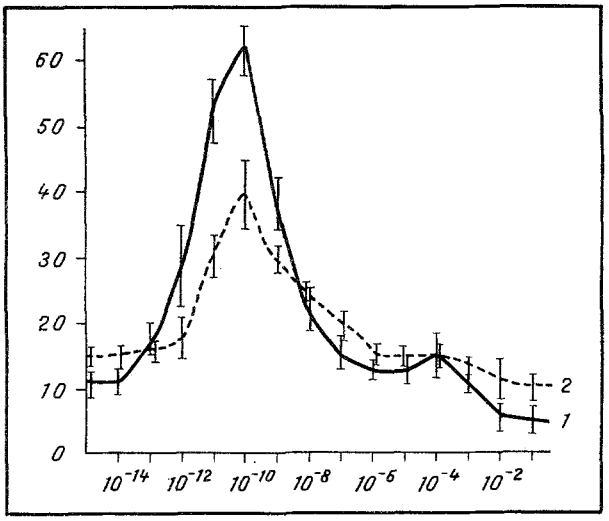

Fig. 2. Effect of MP on protein synthesis by B-cell hybridoma clones. Abscissa, concentration of myelopeptides (in mg/ml); ordinate, content of ^{14}C-label (in cpm$\cdot 10^{-3}$). 1) Clone 2G6B3; 2) clone 3C7C7.

(at the rate of 20 mg orthophenylenediamine to 50 ml of buffer solution consisting of citric acid and phosphate buffer) and 10 µl of 30% H_2O_2 were added into each well. After incubation for 30 min at 37°C the reaction was stopped by the addition of 100 µl 10% H_2SO_4. The intensity of the reaction was assessed by means of the reader of the "Titertek Multiscan MCC/340" at a wavelength of 492 nm. Serum of mice immunized with Fl-antigen was used as the positive control and serum of intact mice or supernatants of cultures of the parental strain AgX63.P653 as the negative control.

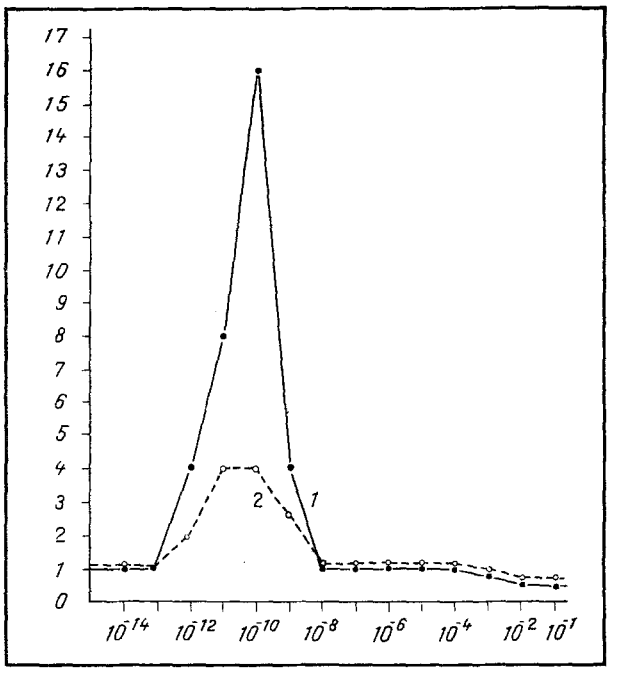

Fig. 3. Effect of MP on antibody secretion by B-cell hybridoma clones. Abscissa, concentration of myelopeptides (in mg/ml); ordinate, index of stimulation (experiment/control). 1) Clone 2G6B3; 2) clone 3C7C7.

EXPERIMENTAL RESULTS

The result of the action of MP on incorporation of ^{3}H-thymidine into cells of two B-cell hybridoma clones, producing antibodies of the IgM- and IgG2b-classes, is shown in Fig. 1. It is clear that MP, over a wide dose range from 10^{-13} to 10^{-1} mg/kg, inhibited by 2-5 times the proliferation of the two hybridoma clones when cultured for 48 h. However, the effect of MP on proliferation of the 2G6B3-clone of the hybrid B cells producing antibodies of the IgM-isotype was stronger than their effect of proliferation of the 3C7C7 clones, producing antibodies of the IgG2b isotype. In the course of culture of hybridoma cells for 24 h in the presence of MP, proliferation was depressed by not more than 25-30%. No significant difference was observed in the number of cells in the control and experimental wells. This was evidently due to the fact that maximal inhibition of ^{3}H-thymidine incorporation into the cells was observed at the end of 48 h of culture.

Parallel assessment of ^{14}C-glycine incorporation showed that inhibition of proliferation of the hybridoma B cells was accompanied by increased protein synthesis (Fig. 2). In that case the 2G6B3 clone also responded more strongly to the action of MP than the 3C7C7 clone. Under the influence of MP incorporation of ^{14}C-glycine into the hybridoma cells was increased by 6-12 times. As a result of treatment of the hybridoma B cells with MP the ratio of DNA synthesis to protein synthesis changed by more than an order of magnitude. A change in this parameter also was observed on treatment of intact bone marrow cells with MP: in this case by not more than 25% [6].

However, the titer of specific monoclonal antibodies to the F1 capsular antigen of Y. pestis in cultural supernatants of hybrid cells showed that MP in a concentration of 10^{-12}-10^{-9} mg/ml stimulated antibody production by 4-16 times.

In high concentrations (10^{-1}-10^{-2} mg/ml) MP were evidently toxic for the hybrid B cells, because simultaneous inhibition of proliferation, protein synthesis, and secretion of specific monoclonal antibodies was observed. Within the dose range from 10^{-8} to 10^{-3} mg/ml inhibition of proliferation of hybridoma B cells under the influence of MP was accompanied by a very small increase in protein synthesis without any significant change in antibody secretion. In a concentration of 10^{-12}-10^{-9} mg/ml inhibition of proliferation of hybridoma B cells was accompanied by marked stimulation of protein synthesis, which correlated with an increase in secretion of specific antibodies.

Whereas in a culture of mouse lymph node cells isolated at the peak of the secondary immune response MP give a stimulating effect through modulation of helper–suppressor interactions of T lymphocytes [4], in the present case a direct influence of MP in cells secreting antibodies is observed; moreover, the increase in the intensity of antibody secretion under the influence of MP was accompanied by inhibition of cell proliferation. Thus, under the influence of MP the hybridoma cells switched from the proliferative into the secretory cycle. On the basis of the data given above, it can be postulated that different mechanisms controlling antibody secretion exist in the case of a pure homogeneous population of hybridoma B cells and a heterogeneous population of immune lymph node cells, for in the latter case MP probably have no direct action on B lymphocytes [4]. At the present time clone 2G6B3 of hybridoma B cells is used to evaluate the functional activity of MP and of the immunoregulatory preparation myelopide that has been created on their basis.

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PREPARATION OF SPECIFIC ANTISERA TO BRADYKININ AND THEIR INVESTIGATION BY ELISA

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Bradykinin (BK) is involved in many physiological processes and also in the pathogenesis of acute and chronic diseases [1]. Specific antibodies to BK and its analogs are known to be antagonists of BK, blocking some of its biological functions [5, 6]. For that reason, immunoneutralization of kinins by active or passive immunization can be regarded as an important technical approach to the correction of BK-dependent pathological states. For this purpose, it is essential to have available a set of specific antisera and a reliable method of their identification.

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