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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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TABLE 1. Effect of Suppressor Factor and B-Activin on LMIF Production by Peripheral Blood Lymphocytes (M ± m)

Group of factors tested	MII, %	
	spontaneous production	induced production
Medium (control)	1,3±0,9	54,4±1,3
Supressor factor	—4,0±1,0	—6,0±4,1
B-Activin, μg/ml		
5	50,0±2,3	24,0±7,3
25	—20,0±4,7	—52,0±2,1
50	—12,0±1,4	10,4±3,8

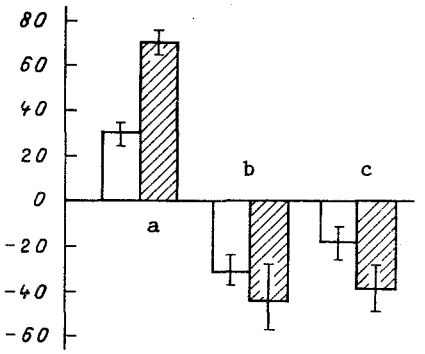

Fig. 1. Effect of MP of patients with AGG on LMIF production. Ordinate, value of MII (in %). a) Control, b) B-activin, c) suppressor factor. Unshaded columns) spontaneous; shaded) induced production.

were then washed off and cultured with the test factors for 1 h. After washing to remove the factors the cells were cultured in medium for 18-24 h. LMIF activity was determined in the resulting cell-free supernatants in the migration inhibition capillary test. As the quantitative parameter a migration inhibition index (MII) was used: this was calculated by the equation

$$MII = 100 - \frac{\text{area of migration zone in experiment}}{\text{area of migration zone in control}} \times 100.$$

EXPERIMENTAL RESULTS

It was shown previously that human BMC, on direct contact with peripheral blood lymphocytes, modify LMIF production, and completely abolish it [2]. However, the possibility cannot be ruled out that cytotoxic effects and production of various lymphokines may be observed in this system of combined culture of allogeneic BMC and lymphocytes. Accordingly, in this investigation we used cell-free supernatant of 48-h BMC cultures, for experiments have shown that this supernatant has a suppressor action [7].

Data on the effect of B-activin and the suppressor factor from healty human blood on LMIF production by peripheral blood lymphocytes are given in Table 1.

Human lymphocytes, both intact and stimulated by PHA, are known to produce LMIF. LMIF production by intact lymphocytes is called spontaneous, and determined quantitatively it has an MII value of (10-15) ± 0.4%. Lymphokine production under the influence of PHA is called induced, and its MII is (50-60) ± 0.6%. Under the influence of suppressor factor on peripheral lymphocytes, complete abolition of LMIF production is observed. The action of B-activin was shown to be dose-dependent. For instance, in a dose of 5 μg/ml B-activin had different actions on spontaneous and induced LMIF production: it suppressed induced but stimulated spontaneous production. Higher doses abolished both spontaneous and induced LMIF production.

It can be postulated on the basis of these results that B-activin may have a dose-dependent immunoregulatory action. It is also known that spontaneous LMIF production is effected by B-lymphocytes [12]. The action of B-activin can accordingly be linked not only

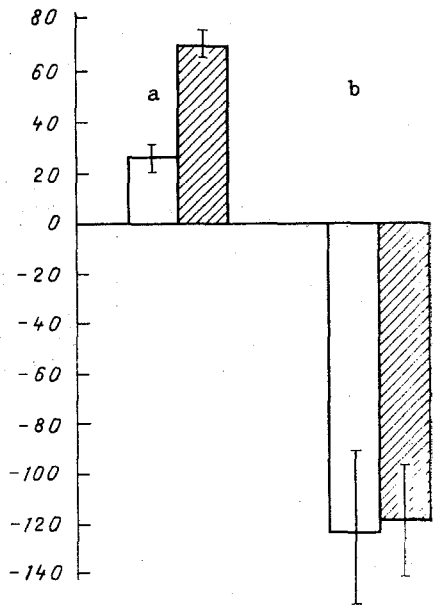

Fig. 2. Effect of suppressor factor from patients with multiple myeloma on LMIF production. a) Control, b) suppressor factor. Remainder of legend as to Fig. 1.

with stimulation of a silent AFC population to synthesize antibodies, but also with activation of a lymphocyte function, namely lymphokine production. It was impossible to draw a more precise line between the action of B-activin and suppressor factor on LMIF production. It can be tentatively suggested that the action of these factors on the cellular component of immunity is similar in type, and a difference is exhibited only in their action on the humoral component. Moreover, the action of low doses of B-activin on spontaneous production also confirms that B-lymphocytes are involved in LMIF production.

It can be postulated on the basis of these results that B-activin and suppressor factor, which are polypeptides in nature, are in fact the same substance. Depending on certain as yet unidentified conditions and influences, certain changes may take place as a result of which one of the effects (either suppressor or stimulating) will predominate. This relationship may perhaps be changed in the healthy organism under certain physiological conditions, and also in pathological states, leading to the development of a definite clinical picture.

Accordingly, it was decided next to investigate the action of MP of patients with AGG and multiple myeloma. These diseases were chosen because of their different levels of detectable B-activin activity. The peripheral blood of patients with AGG was characterized by complete absence of immunoglobulins of the different classes [3].

The patients' BMC were cultured in vitro and two soluble factors were obtained: B-activin [9] and the supernatant after incubation for 48 h (suppressor factor). Testing was carried out by the standard scheme. The action of B-activin from patients and healthy blood donors was tested in parallel in an antibody forming system. In the course of the investigation no B-activin activity could be found in the system of secondary immunity [9].

The two soluble factors had similar actions in the LMIF production system: they abruptly suppressed both spontaneous and induced LMIF production (Fig. 1).

In this disease, B-activin production is thus disturbed and suppressor activity predominates among MP. This raises the question of the possible clinical use of B-activin as an immunocorrective preparation in the treatment of patients with immunodeficiency diseases.

In the case of multiple myeloma, the cell-free supernatant of BMC cultures had a marked action on lymphocyte function (Fig. 2). Treatment of lymphocytes with bone marrow factor from patients with multiple myeloma led to the production of a lymphokine with stimulating action. In the presence of this lymphokine the target cells migrated twice as fast as in its absence. Several investigators have demonstrated the possible presence of a factor with stimulating activity [13].

It can thus be tentatively suggested on the basis of these different activities of MP in health and disease that a definite and adequate relationship is found in healthy individuals between the stimulating and suppressor activity of MP, but in disease one of the effects may become predominant. In AGG, for example, the suppressor effect predominates, whereas in multiple myeloma, besides an increase in B-activin activity, increased mobility of the target cells also is observed under the influence of the patients' BMC.

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MONOCLONAL ANTIBODIES AGAINST THE α-CHAIN OF HUMAN LYMPHOCYTE FUNCTION-ASSOCIATED ANTIGEN (LFA-1)

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Human lymphocytic functional-associative antigen (LFA-1) is a noncovalently bound heterodimer membrane glycoprotein consisting of α- and β-chains with molecular weights of 175-180 and 93-95 kD respectively, which is a structural analog of the mouse LFA-1 antigen described previously [4, 5, 8].

A study of the function of mouse LFA-1 antigen revealed its important role in the initial recognition-adhesive phase of the reaction of cytotoxic T lymphocytes (CTL) [6]. The α-chain is unique for LFA-1 whereas the β chain is also a component of the CR3 antigen, which is a receptor for the inactivated C3b component of complement (C3bi) and of antigen

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