NATURE OF IMMUNE LYMPH NODE FACTORS STIMULATING HUMORAL AND CELLULAR IMMUNE RESPONSES

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It was shown previously that peripheral lymph node cells, after short-term contact with an antigen in vivo, secrete an antigen-specific factor [2-4]. Other workers found antigen-specific complexes in the serum of animals 3-6 h after immunization, which contained determinants of the antigen, of histocompatibility class II molecules, and of immunoglobulin [7, 8]. Injection of immune lymph node factors (ILNF), discovered by the present writers, into intact animals led to changes similar to those following injection of the antigens themselves. Like the antigen, ILNF caused a decrease in antigen-specific stimulating activity and enhancement of antigen-nonspecific suppressor activity of bone marrow cells [3, 4]. Simultaneous injection of ILNF and antigen led to a 3- to 6-fold increase in the intensity of humoral and cellular reactions [2].

The aim of this investigation was to study the nature of ILNF and possible ways of their purification.

EXPERIMENTAL METHOD

(CBA × C57BL/6)F_1 hybrid mice aged 2-3 months and DBA/2 mice, obtained from the "Stolbovaya" nursery, Academy of Medical Sciences of the USSR, were used in the experiments.

ILNF were obtained by the method described previously [3, 4]. A suspension of mastocytoma P815 cells in a concentration of $20 \cdot 10^6$/ml, transplanted in DBA/2 mice, was injected in a volume of 0.1 ml into the footpads of F_1 mice. Cells from the axillary, inguinal, and popliteal lymph nodes were isolated 6 h after the injection and cultured in serum-free medium in a concentration of $5 \cdot 10^6$/ml for 18-20 h. The supernatants thus obtained were concentrated by lyophilization and fractionated on a column with Sephadex G-200. The molecular weight of the $ILNF_{P815}$ (obtained after immunization with P815 tumor cells) was determined on a column measuring 3 × 73 cm, equilibrated with 0.2 M phosphate buffer, pH 7.2, with elution at the rate of 2 ml/h. The column was calibrated before hand with γ-globulin and with the albumin fraction of mouse serum [1]. The supernatant containing $ILNF_{P815}$ was adsorbed by the panning method [9] on Petri dishes, treated beforehand with anti-I-A^k-antibodies or with rabbit antibodies to mouse IgG. Anti-I-A^k-antibodies were obtained from supernatants of hybridoma cells of clone 10.2.16, generously provided by staff of the N. V. Medunitsin Laboratory, Institute of Immunology, Ministry of Health of the USSR, and were applied to Petri dishes 40 mm in diameter, in 5 ml with a concentration of 5-20 μg/ml. Rabbit anti-mouse-IgG antibodies, obtained from the hybridoma laboratory, Institute of Immunology, Ministry of Health of the USSR, by I. A. Nikolaeva, were applied in a concentration of 50 μg/ml in a volume of 5 ml. As the control, buffer solution or culture medium was applied to the dishes, depending on the solvent used to dilute the antisera. The dishes were incubated for 16-18 h at 4°C. After incubation the dishes were washed 4 times with phosphate buffer and supernatants of lymph node cells (containing higher $ILNF_{P815}$) were applied to them in a volume of 5 ml. After remaining for 2 h at 37°C the supernatants were poured off and their biological activity tested. Supernatants of intact lymph node cells served as the control.

Biological activity of fractions not fractionated on Sephadex G-200 and of those obtained after gel-filtration, and also of supernatants after adsorption on antisera, was

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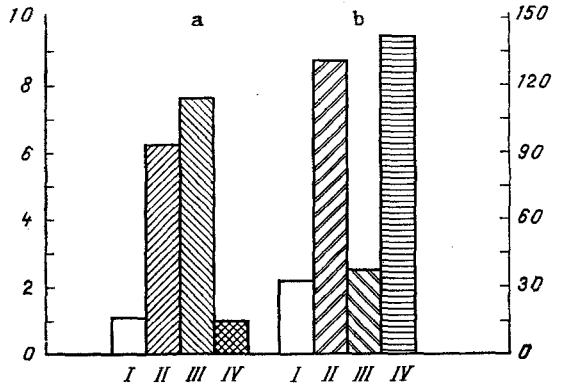

Fig. 1. Effect of fractionation of supernatant of immune lymph nodes proliferative activity of popliteal lymph node cells (a) and on killer activity of peritoneal lymphocytes (b). a) Ordinate (here and in Fig. 2): coefficient of stimulation of proliferation – incorporation of ^3H in experimental lymph node/incorporation of ^3H in control lymph node (in relative units); abscissa: I) control (medium for supernatant of intact lymph node cells); II) unfractionated supernatant, III) fraction with mol. wt. of 110-120 kD; IV) fraction with mol. wt. of 1-2 kD. b) Ordinate (here and in Fig. 2): LU_{30} (lytic units of killer activity); abscissa: I) control, II) unfractionated supernatant, III) fraction 2, IV) fraction 1 (mol. wt. 110-120 kD).

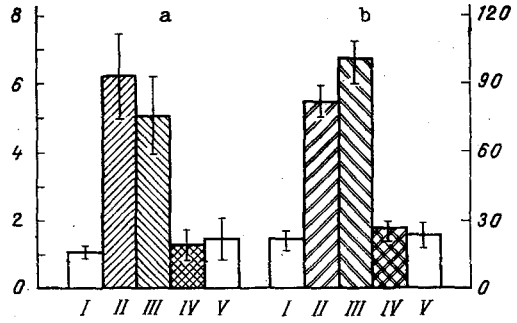

Fig. 2. Effect of antisera on activity of immune lymph node factor. Abscissa: I) control (medium for supernatant of intact lymph node cells), II) $ILNF_{P815}$, III) $ILNF_{P815}$, adsorbed on control medium or buffer, IV) $ILNF_{P815}$, adsorbed on antiimmunoglobulin antibodies, V) $ILNF_{P815}$, adsorbed on antibodies to $I\text{-}A^k$.

determined by testing their ability to enhance the proliferative activity of popliteal lymph node cells and to enhance the killer activity of peritoneal lymphocytes [2, 6]. The results were subjected to statistical analysis by Student's t test.

EXPERIMENTAL RESULTS

In the experiments of series I the molecular weight of $ILNF_{P815}$ was determined. After gel-filtration on Sephadex G-200 two peaks eluted in the region of mol. wt. 110-120 kD and 1-2 kD (fractions 1 and 2) were obtained. The results of testing the biological activity of fractions 1 and 2 on a model of stimulation of proliferation of popliteal lymph node cells (determined at the peak of activity on the 6th day) and the increase in killer activity of peritoneal lymphocytes are given in Fig. 1. An increase in the proliferative activity of popliteal lymph node cells and the killer activity of peritoneal lymphocytes was observed under the influence of fraction 1 compared with the unfractionated supernatant. As will be clear from Fig. 1, fraction 2 had no stimulating effect on the parameters tested. Consequently, the material with activity of $ILNF_{P815}$ has a molecular weight of 110-120 kD.

In the experiments of series II supernatants containing $ILNF_{P815}$ were adsorbed on Petri dishes covered with antibodies to mouse immunoglobulins and on dishes covered with antibodies to the I-A^k-determinants of the major histocompatibility complex. As Fig. 2 shows, the active factor of $ILNF_{P815}$ was effectively adsorbed on dishes covered both with antibodies to immunoglobulins and with antibodies to I-A-^{k} determinants. The proliferative activity of the popliteal lymph node cells after injection of supernatant adsorbed on these antibodies into mice was abolished virtually completely (Fig. 2). Similar results were obtained on testing the adsorbed supernatants for killer activity of the peritoneal lymphocytes (Fig. 2). In the control, i.e., after adsorption of the supernatants on dishes treated with culture medium or with buffer solution, the $ILNF_{P815}$ activity was virtually unchanged. Thus it can be concluded from the previously established antigen-specific nature of the immune lymph node factors and the results of the present investigation that ILNF include at least three components: determinants of class II of the major histocompatibility complex, determinants of the antigen, and determinants of immunoglobulin; in other words, the factor we are describing is identical with the complex discovered in mouse serum 6 h after immunization by other workers in [7, 8]. However, it is worth noting that experiments in culture in vitro have shown that macrophages and T-lymphocytes are essential for the formation of this complex. It can be postulated that lymph nodes which receive up to 99% of the antigen [5], are the same organ whose cells are the first to interact with the antigen and the first to secrete the complexes discovered. The detection of these molecules in the serum is evidently the result of their arrival from lymph flowing from the lymph nodes and entering the blood stream. The study of the mechanisms of formation of ILNF and their migration within the body and the investigation of the functions of these mediators will help to broaden our ideas on the mechanisms of development of immune reactions.

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