EFFECT OF IMMUNE LYMPH NODE FACTORS ON IMMUNOLOGIC MEMORY FORMATION

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It was shown previously that peripheral lymph node cells secrete an antigen-specific factor after short-term contact with antigens in vivo [2, 3]. Similar mediators were found in the serum of animals 3-6 h after immunization or in a cell culture in vitro by other workers [7, 8]. Injection of immune lymph node factors (ILNF) into intact animals led to changes similar to those found after injection of the antigens themselves. Just like antigen, ILNF induced a decrease in antigen-specific stimulating activity and an increase in antigen-nonspecific suppressor activity in bone marrow cells [2, 3]. Meanwhile injection of ILNF and antigens (sheep's red blood cells or P-815 mastocytoma cells) led to a three to sixfold increase in the intensity of humoral and cellular reactions [1].

The aim of this investigation was the preliminary biochemical purification of ILNF and determination of its effect on the formation of immunologic memory cells during humoral immune reactions.

EXPERIMENTAL METHOD

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

The ILNF was obtained by the method described previously [2, 3]. An injection of 0.1 ml of a 5% suspension of sheep's red blood cells (SRBC) or of 0.1 ml of a suspension of 2×10^6 P-815 mastocytoma cells was given into the footpads of all four limbs of the mice. Cells of the axillary, inguinal, and popliteal lymph nodes were isolated after 6 h and cultured in a concentration of 5×10^6 cells/ml in serum-free medium for 18-20 h. Supernatants of these cultures obtained by centrifugation and filtration through membrane filters with a paw diameter of 0.45 μ were concentrated by lyophilization and fractionated on Sephadex G-200 [4]. The molecular weight of the ILNF was determined on a column measuring 3 × 73 cm in 0.2 M phosphate buffer, pH 7.2. The column was calibrated with the γ-globulin and albumin fractions of mouse serum. The rate of elution from the column was 2 ml/h. The biological activity of the unfractionated supernatants and of the fractions obtained after gel filtration was determined by their ability to stimulate the proliferative activity of cells of the popliteal lymph nodes and to enhance the killer activity of peritoneal lymphocytes [1].

TABLE 1. Effect of ILNF on Induction of Primary Immune Response to SRBC in Culture in Vitro

Conditions of culture	Number of AFC per culture	Incorporation of ^{3}H-thymidine, cpm
Spleen cells	26±3	26 684±1 260
Spleen cells + SRBC	190±15	40 795±3 634
Spleen cells + SRBC + 2% ILNF	712±32	14 899±5 684
Spleen cells + SRBC + 5% ILNF	704±26	30 747±6 204
Spleen cells + SRBC + 10% ILNF	544±38	31 248±5 207

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TABLE 2. Effect of ILNF on Induction of Primary Immune Response to SRBC in a System in Vivo

Number of cells in spleen 10^{-6}		Number of AFC in spleen		Number of AFC per 10^6 spleen cells	
control	experiment	control	experiment	control	experiment
117±10	150±13,6	10,500±730	18 250±530	82,6±13,6	150±9,5

TABLE 3. Effect of ILNF on Formation of Immunologic Memory Cells during Humoral Immune Response to SRBC

Group of animals	Number of cells in spleen $\times 10^{-6}$	Number of AFC per 10^6 nucleated spleen cells	Number of AFC in spleen	Incorporation of ^{3}H-thymidine, cpm
Control	125,5±9,25	33±4	5583±893	13650±1240
Priming with control ILNF*	123,5±10,6	48±17	8608±2152	19500±3650
Priming with SRBC and ILNF (110-120 kD)	195,0±11,67	66±1,5	28065±3752	39500±2700

Legend. Asterisk indicates that control ILNF consisted of supernatant of intact lymph node cells.

The effect of ILNF on the production of antibody-forming cells (AFC) was assessed as follows. The development of a primary immune response in vitro was induced by means of SRBC in a culture of mouse spleen cells. For this purpose, 5×10^6 SRBC were added to a suspension of 15×10^6 spleen cells in 1.5 ml. The cells were incubated for 4 days in complete RPMI-1640 medium with the addition of 20% fetal calf serum, glutamine, 2-mercaptoethanol, and antibiotics in 24-well panels at 37°C in an atmosphere containing 7% CO_2. At the end of incubation the number of AFC to SRBC was estimated [6] and the proliferative activity of the cells determined by measuring incorporation of ^{3}H-thymidine into DNA. Preparations containing ILNF were added to the cell cultures simultaneously with antigen up to a final concentration of 2, 5, and 10%. Parallel determinations were made of the effect of ILNF on induction of the immune response to SRBC in a system in vivo. Mice of two groups were immunized by intraperitoneal injection of 0.2 ml of 2% SRBC. Simultaneously with the antigen, animals of one group received an intraperitoneal injection of 0.2 ml of ILNF, whereas those of the other group received the same volume of culture medium or supernatant of intact lymph node cells by way of a control. The number of AFC to SRBC in the spleens of the mice of both groups was determined separately on the 5th day.

To assess the effect of ILNF on the formation of immunologic memory cells, animals were immunized intraperitoneally with 0.2 ml of SRBC with or without addition of 0.2 ml of ILNF, or with a fraction possessing ILNF activity. Immunization was repeated 4 weeks later with 0.3 ml of a 5% suspension of SRBC. On the 4th day after the 2nd injection of antigen the number of IgG-secreting AFC was determined in the spleens of the mice by a modified Jerne's method [5]. The proliferative activity of the cells was estimated from incorporation of ^{3}H-thymidine. For this purpose, 1 uCi of ^{3}H-thymidine was introduced into the wells of the panels containing 5×10^6 cells in 1 ml, for 4 h at 37°C, after which the contents of the wells were transferred to glass fiber filters by means of a cell harvester. The amount of label incorporated was estimated in a liquid scintillation counter. The number of cells in the organs and cultures and their viability were determined with the aid of 0.1% trypan blue solution. The results were subjected to statistical analysis by Student's t test.

EXPERIMENTAL RESULTS

In this series of experiments preliminary biochemical purification of the ILNF contained in supernatants of immune lymph node cells was carried out. After gel-filtration on Sephadex G-200 two peaks eluting in the region of molecular weights of 110-120 and 1-2 kD (fractions 1 and 2) were obtained. The biological activity of the isolated fractions was estimated on models of stimulation of proliferation of popliteal lymph node cells and from enhancement of the killer activity of peritoneal lymphocytes [1]. Material possessing ILNF activity was found in fraction 1, eluting in the region of mol. wt. of 110-120 kD. The addition of ILNF to a culture of spleen cells simultaneously with the antigen in vitro, and also simultaneous injection of them with the antigen during induction of the immune response to SRBC in vivo led to marked strengthening of the humoral immune reactions. The results are given in Tables 1 and 2. It will be clear from Table 1 that the addition of 2-5% ILNF to the culture medium simultaneously

with SRBC led to a more than threefold strengthening of the immune response. However, the proliferative activity of the cells in cultures with 2% and 5% ILNF was lower than without addition of the mediator. Similar results were obtained in the system in vivo.

It follows from the data given in Table 2 that ILNF not only stimulate AFC formation to the antigen injected with them, but they also significantly increase the absolute number of cells in the spleen. Such a marked enhancement of the primary immune response under the influence of ILNF is evidently reflected in the formation of immunologic memory cells. To test this hypothesis, primary immunization of animals was carried out with a suboptimal dose of antigen, with simultaneous injection of ILNF. After 1 month the animals were reimmunized with an optimal dose of antigen. As Table 3 shows, the use of ILNF with antigen for priming the animals leads to a more than fivefold increase in intensity of the secondary immune response to the same dose of antigen as in mice immunized twice with antigen alone. The results are evidence that ILNF potentiate the primary humoral immune response and thereby facilitate the formation of a larger number of immunologic memory cells, which take part in antibody production in response to repeated contact with the antigen. This adjuvant action of ILNF may be of great importance for the practical use of this mediator.

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