NATURE OF A HUMORAL FACTOR OF BONE MARROW STIMULATING ANTIBODY PRODUCTION

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A fraction doubling the quantity of antibodies synthesized in a culture of cells from immune lymph nodes was isolated by gel chromatography from the supernatant obtained after culture of bone marrow cells from intact donors. The stimulator of antibody producers (SAP) was eluted in the region of emergence of cytochrome C with a molecular weight of 13,000 daltons and it was thermostable. Synthesis of RNA and protein was essential for its production, but DNA synthesis was not necessary. Incorporation of radioactive label showed that the SAP contained ribonucleotides and amino acids. It is suggested that this factor plays an important role in the regulation of antibody synthesis.
KEY WORDS: *productive phase of the immune response; stimulator of antibody producers; inhibitor analysis.*

The writers showed previously that bone marrow cells produce a humoral factor which increases by two or three times the production of antibodies in a population of lymph node cells obtained at the peak of the secondary immune response [1, 2, 8]. This factor is secreted by living, actively metabolizing cells in the absence of antigenic or mitogenic stimulation and it leads to the appearance of additional numbers of antibody producers in the immune population [5, 7].

The object of this investigation was to study the nature of the humoral factor of bone marrow which has been called stimulator of antibody production (SAP).

EXPERIMENTAL METHOD

(CBA × C57BL)F_1 mice were used in the experiments. Lymph node cells were obtained from immune mice on the fourth day after the second injection of antigen (horse γ globulin) and the bone marrow cells were flushed out of the long bones of nonimmune donors [3, 10]. The cells were cultured in RPMI-1640 medium or minimal Eagle's medium with 10% fetal calf serum, 2-mercaptoethanol (5×10^{-5} M), glutamine (200 mM), and Hepes-buffer (1 M). The concentration of cells was 25×10^6 cells/ml. In some experiments radioactive label was added to the medium: glycine-^{14}C, uridine-^{14}C, or thymidine-^{3}H, in a concentration of 1 μCi/ml.

The supernatants obtained after culture of bone marrow or immune lymph node cells were fractionated on a column with Sephadex G-200 or G-100, equilibrated with 0.031% bicarbonate buffer. The column was first calibrated with markers of known molecular weight.

In the experiments using inhibitor analysis bone marrow cells were cultured for 3 h at 37°C with different doses of cyclohexamide, actinomycin D, or mitomycin C. The effectiveness of action of the inhibitors was assessed from the change in incorporation of glycine-^{14}C into protein, uridine-^{14}C into RNA, and thymidine-^{3}H into DNA. Bone marrow cells washed to remove the inhibitors were added to lymph node cells from immune donors and cultured together for 18-20 h. The intensity of antibody synthesis in the mono- and mixed cultures was determined from the incorporation of glycine-^{14}C, after removal of the antibodies from the medium with the aid of immunosorbents [2, 3, 10]. The coefficient of stimulation on antibody formation and the ratio between the intensities of antibody synthesis in the mixed cultures and in monoculture were calculated.

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TABLE 1. Effect of Stimulation of Antibody Formation in Mixed Culture of Cells from Immune Lymph Nodes and Intact Bone Marrow on Treatment of Bone Marrow Cells with Inhibitors of Protein, RNA, and DNA Synthesis.

Inhibitor	Number of experiments	Concentration, μg/ml	Percentage inhibition of synthesis of			Coefficient of stimulation
			protein	RNA	DNA	
Cycloheximide	3	—	—	—	—	2,52±0,16
	3	10	75,5±2,3	—	—	2,74±0,41
	3	25	82,4±2,0	—	—	1,62±0,11
	3	500	97,2±0,8	—	—	1,22±0,02
	3	—	—	—	—	2,53±0,30
Actinomycin	3	1	18,0±1,5	23,9±2,8	—	1,13±0,09
	3	4	41,0±3,2	60,0±1,2	—	0,93±0,13
Mitomycin	3	—	—	—	—	2,52±0,16
	3	33	—	—	91±1,8	2,51±0,21

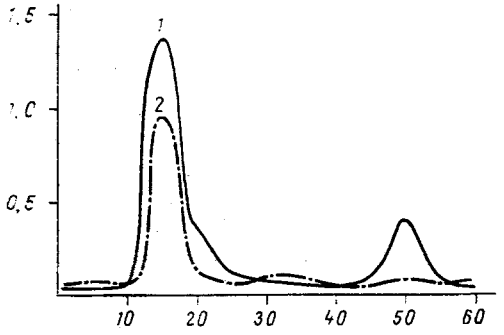

Fig. 1

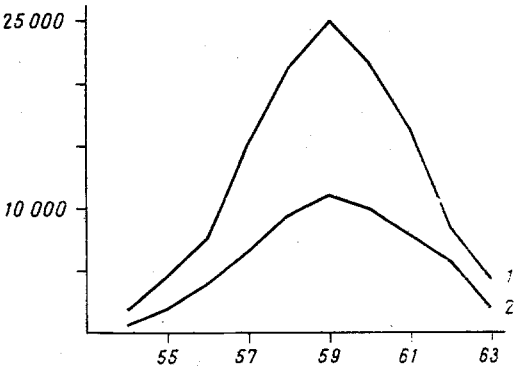

Fig. 2

Fig. 1. Gel chromatography on Sephadex G-200 of supernatants obtained from cultures of bone marrow cells (1) or lymph node cells (2) from nonimmune donors. Abscissa, No. of fractions; ordinate, optical density at 280 nm.

Fig. 2. Incorporation of urdine-^{14}C (1) and glycine-^{14}C (2) into fraction isolated from supernatant of cultures of bone marrow cells during fractionation on Sephadex G-100. Abscissa, No. of fractions; ordinate, radioactivity (in cpm).

EXPERIMENTAL RESULTS

In the experiments of series I the need for synthesis of protein, RNA, or DNA for SAP production was estimated. For this purpose the effect of stimulation of antibody formation in a mixed culture of cells of immune lymph nodes and intact bone marrow was studied after treatment of the bone marrow cells with various inhibitors.

The results of these experiments are given in Table 1. They show that a reduction of protein synthesis by less than 80% (dose of cycloheximide 10 μg/ml) does not affect the stimulation of antibody formation. A reduction of the coefficient of stimulation was observed after inhibition of protein synthesis by 83% (dose of cycloheximide 25 μg/ml). Complete suppression of protein synthesis in the bone marrow cells (dose of cycloheximide 500 μg/ml) abolished the stimulation effect.

Inhibition of RNA synthesis by actinomycin D by 20-40% (doses of actinomycin D 1 and 4 μg/ml) abolished the stimulation of antibody formation. Protein synthesis under these circumstances was depressed by not more than 20%, and this did not change the stimulating effect of the bone marrow cells.

By contrast with the inhibitors of protein and RNA synthesis, the inhibitor of DNA synthesis (mitomycin C) did not change the stimulating action of the bone marrow cells. The stimulating effect on antibody formation in mixed culture was the same regardless of whether the bone marrow cells were treated with this inhibitor or not.

TABLE 2. Stimulation of Antibody Synthesis in Culture of Immune Lymph Node Cells on Addition of Heated Supernatant Obtained from Cultures of Bone Marrow Cells to It

Temperature of heating, °C	Number of experiments	Coefficient of stimulation
36	4	1,74±0,11
56	4	1,57±0,09
80	4	1,41±0,06

The results are evidence that RNA and protein synthesis, but not DNA synthesis, is necessary for SAP production.

In the experiments of series II an attempt was made to isolate a biologically active fraction from the supernatant of cultures of bone marrow cells by gel filtration on Sephadex G-200. In parallel experiments supernatant obtained from cultures of cells of intact lymph nodes, which had no stimulating activity [3, 8], was fractionated. A fraction not present in the supernatant of cultures of lymph node cells was discovered in the supernatant of cultures of bone marrow cells, and it was eluted in the region of emergence of cytochrome C with a molecular weight of 13,000 daltons (Fig. 1). This fraction doubled antibody production when added to a culture of lymph node cells from immune donors.

During gel chromatography on Sephadex G-100 of the supernatant of bone marrow cells cultured in the presence of glycine-^{14}C or uridine-^{14}C, incorporation of the radioactive label into the active fraction was observed (Fig. 2).

It can be postulated on the basis of these results that the SAP molecule includes ribonucleotides and amino acids. Other evidence in support of the ribonucleotide nature of SAP is given by its thermostability. Heating the supernatant to 56°C for 30 min reduced its stimulating activity by 15%, whereas heating to 80°C for 30 min reduced its activity by 30% (Table 2).

The partial reduction in the activity of the supernatant on heating was evidently connected with destruction of certain components of the nutrient medium and not to inactivation of the stimulating substance.

The experimental data on the soluble factors of cellular and humoral immunity which have now been accumulated are considerable [4, 6, 9]. Most of these factors are produced by lymphocytes under the influence of antigenic or mitogenic stimulators. The factor now discovered is secreted by bone marrow cells without any special antigenic or mitogenic stimulation rather like thymosine, which is produced by the epithelial cells of the thymus in the course of their normal metabolic activity, and which promotes the development of the T-cell population [4]. The writers suggest that SAP is a mediator of cellular interaction at the level of mature antibody producers, and that it participates in the mechanism of the regulatory function of the bone marrow cells in the productive phase of the immune response.

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