STIMULATION OF IMMUNOGLOBULIN SYNTHESIS IN MIXED CULTURES OF LYMPH GLAND AND BONE MARROW CELLS

TIMES OF STIMULATION AND ROLE OF CELL INTEGRITY

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The effect of stimulation of immunoglobulin synthesis in mixed cultures of lymph gland cells from immune mice and intact bone marrow cells was studied by a radioisotope method using immunosorbents. The stimulation effect was observed during the first few hours of combined incubation and it continued for 21 h. The increase in synthesis in mixed cultures was shown to take place uniformly through the same classes of immunoglobulins as are synthesized in monoculture. The stimulation effect takes place in the presence of intact, metabolizing cells. On the addition of supernatant from cultures of intact bone marrow cells to the cells of immune lymph glands a slight increase in antibody synthesis was observed.

Previous investigations showed that antibody synthesis is increased by about 3 times and the synthesis of nonspecific immunoglobulins is increased by 1.5 times compared with the levels observed in monocultures in mixed cultures of lymph gland cells obtained from immune animals at the peak of the productive phase of antibody formation with bone marrow or spleen cells from intact donors [2, 3]. This effect of cellular interaction is a new phenomenon in immunology, for it operates not at the level of the precursor cells initiating immunogenesis, but at the level of mature antibody-producers [4]. The mechanisms of this effect have so far received very little study.

This paper describes experiments to study some aspects of interaction between immune lymphocytes and intact bone marrow cells in mixed culture. The times of appearance of the effect, the possibility of its production by humoral factors, and the relationship between the various classes of immunoglobulins synthesized after stimulation were investigated.

EXPERIMENTAL METHOD

Inbred CBA, A, and C57BL male and female mice weighing 18-22 g were used. The animals were immunized by subcutaneous injection of horse γ-globulin in a dose of 5 mg per mouse in Freund's complete adjuvant and they were reimmunized by intravenous injection of a dose of 3 mg per mouse 1-1.5 months later. To prepare lymph gland cell suspensions the mice were decapitated on the fourth day after reimmunization and the axillary, inguinal, and mesenteric lymph glands were removed with sterile precautions. The tissue was minced with scissors and forced through needles with successively decreasing diameters. The bone marrow cells were washed out with cold Eagle's medium by means of a syringe from the long bones of the hind limbs of intact animals. The resulting cell suspensions were passed through a double layer of sterile gauze and washed twice with cold Eagle's medium. The washed cells were suspended in Eagle's medium in a proportion of $3 \cdot 10^7$–$4 \cdot 10^7$ cells to 1 ml medium, to which 20% bovine serum, glycine-C^{14} (1 μCi/ml), and penicillin (10 units/ml) with streptomycin (50 mg/ml) had first been added. The resulting suspension was poured in volumes of 3 ml into flasks for incubation. In the case of mixed cultures, 1.5 ml of a suspension of each of the two cell suspensions for testing was poured into the flasks. The cells were incubated for 3-22 h at 37°C. After the end of incubation the cells were disintegrated with

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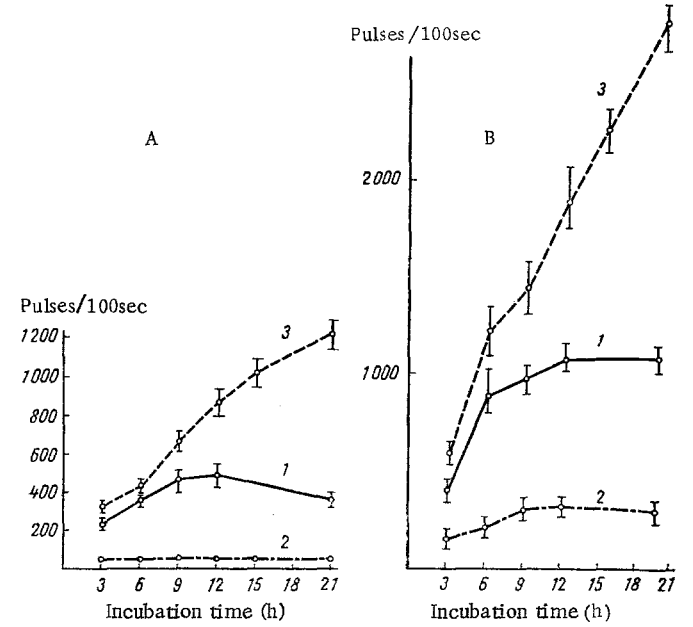

Fig. 1. Synthesis of antibodies (A) and nonspecific γ-globulins (B) in cultures of immune lymph gland cells and in mixed cultures of cells from immune lymph glands and intact bone marrow at different times of incubation: 1) synthesis of immunoglobulins by immune lymph gland cells; 2) by intact bone marrow cells; 3) in mixed cultures of cells from immune lymph glands and intact bone marrow.

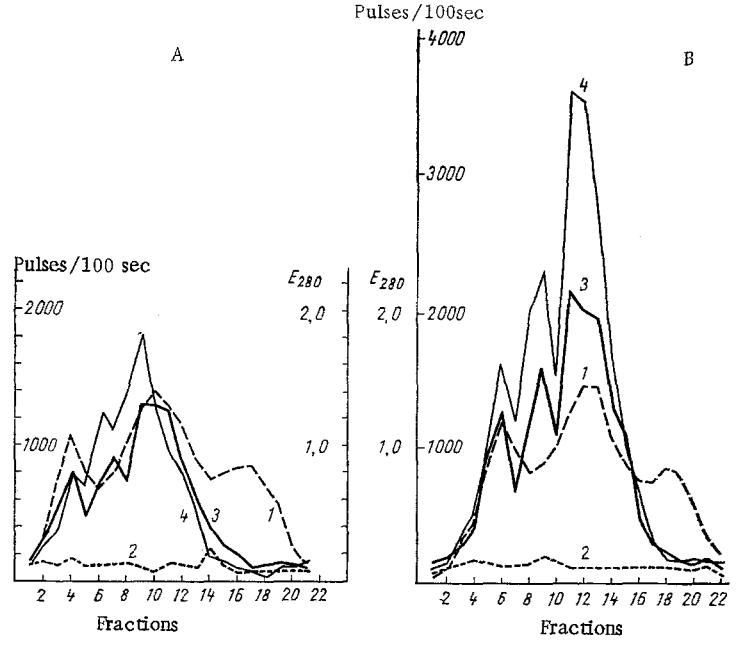

Fig. 2. Gel filtration of immunoglobulins synthesized by lymphocytes in monocultures (A) and in mixed cultures (B) through column with Sephadex G-200: 1) absorption at 280 nm; 2) nonspecific sorption of radioactivity; 3) intensity of antibody synthesis; 4) intensity of synthesis of nonspecific γ-globulins.

TABLE 1. Synthesis of Antibodies and Nonspecific γ-Globulins in Mixed Culture of Immune Lymph Gland and Intact Bone Marrow Cells with Disintegrated Cells of One Component of the Mixture (M ± m)

Mixture of cells investigated	No. of expts.	Coefficient of stimulation	
		antibodies	nonspecific γ-globulins
Cells of immune lymph glands and cells of intact bone marrow	3	3,46±0,47	2,98±0,13
Cells of immune lymph glands and disintegrated cells of intact bone marrow	3	0,74±0,16	0,48±0,096
Cells of intact bone marrow and disintegrated cells of immune lymph glands	3	No synthesis	1,05±0,13

Note. Here and in Table 2: Coefficient of stimulation implies ratio between synthesis of antibodies or nonspecific γ-globulins in mixed culture and synthesis of these proteins in corresponding monocultures.

TABLE 2. Synthesis of Antibodies and Nonspecific γ-Globulins in Mixed Cultures of Immune Lymph Gland and Intact Bone Marrow Cells with Incubation Fluid Obtained from These Same Cells

Mixture of cells investigated	No. of expts.	Coefficient of stimulation (M ± m; P)			
		antibodies	P_1	nonspecific γ-globulins	P_2
Cells of immune lymph glands and cells of intact bone marrow	8	2,23±0,28	<0,01	1,55±0,16	<0,001
Cells of immune lymph glands and supernatant from intact bone marrow cells	9	1,20±0,16	>0,05	0,69±0,008	<0,001
Cells of intact bone marrow and supernatant from immune lymph gland cells	7	0,88±0,06	<0,05	0,92±0,01	<0,001
Cells of immune lymph glands and supernatant from a mixture of immune lymph gland and intact bone marrow cells	7	0,65±0,008	<0,01	0,65±0,1	<0,05
Cells of intact bone marrow and supernatant from mixture of immune lymph gland and intact bone marrow cells	5	0,78±0,1	<0,05	0,78±0,1	<0,05

Note: P_1 and P_2) significance of stimulation of synthesis of antibodies and nonspecific γ-globulins respectively, determined from the Fisher–Student table.

0.5% Triton X-100 solution or by freezing and thawing three times in the incubation medium, and the cell membranes were then sedimented by centrifugation (10,000 g, 20 min).

The synthesis of antibodies and of nonspecific immunoglobulins in the mixed cultures was compared with the theoretically expected value calculated from the corresponding unmixed controls. The level of immunoglobulin synthesis was determined from the incorporation of radioactive label into these proteins extracted specifically by means of immunosorbents [1].

The ratio between the synthesis of immunoglobulins IgM, IgG, and IgA was studied by gel filtration on a column (2 × 60 cm) with Sephadex G-200, equilibrated with 0.039 M Tris-phosphate buffer, pH 7.5, with 0.1 M NaCl solution.

EXPERIMENTAL RESULTS

The dynamics of synthesis of antibodies and nonspecific immunoglobulins in cultures of lymph gland cells from immune donors grown separately or in combined culture with bone marrow cells taken from intact mice of the same line was studied in the experiments of series I (Fig. 1).

It is clear from Fig. 1 that in monocultures of lymph gland cells from immune donors the titers of synthesized antibodies and nonspecific γ-globulins rose gradually during the first 9 h of incubation, after which the production of these proteins ceased. In mixed cultures, on the other hand, the production of both specific and nonspecific immunoglobulins continued throughout the period of incubation and reached a maximum after 21 h.

To discover which class of immunoglobulins was responsible for the increase in protein synthesis in the mixed culture, the incubation medium either from cultures of immune lymph gland cells or from a mixture of immune lymph gland cells and intact bone marrow cells was passed through a column with Sephadex G-200. This showed that the same classes of immunoglobulins are synthesized in the mixed culture as in the monoculture of immune lymph glands, and that the increase in γ-globulin synthesis took place uniformly on account of all classes of immunoglobulins: IgM, IgA, and IgG (Fig. 2).

In the next series (II) of experiments the problem of whether immunoglobulin synthesis is increased in mixed cultures of immune lymph gland and intact bone marrow cells following destruction of the cells of one component of the mixture was studied. The results showed that intact cell suspensions must be cultivated in order to obtain intensification of globulin synthesis (Table 1).

The increase in immunoglobulin synthesis in the mixed cultures evidently takes place either through direct contact between the interacting cells or on account of a humoral factor secreted by one of the mixed populations into the incubation fluid during metabolism of the cultured cells.

To examine the role of a humoral factor in the stimulation of immunoglobulin synthesis in the mixed culture experiments were carried out in which supernatant from intact bone marrow cells grown for 7-10 h was added to lymph gland cells of immune donors or, on the contrary, supernatant from cultures of immune lymph gland cells was added to intact bone marrow cells. In addition, supernatant from mixed cultures giving the stimulation effect, i.e., from a mixture of immune lymph gland and intact bone marrow cells, was added to these same cells (Table 2).

It will be clear from Table 2 that on mixing the supernatant from a culture of immune lymph gland cells and also from mixed cultures with the bone marrow or immune lymph gland cells no stimulation effect was obtained, and indeed, immunoglobulin synthesis was inhibited. This phenomenon could be due to the presence of certain inhibitors secreted into the culture fluid by the cells in the course of their metabolism. Only in one case, in which supernatant from intact bone marrow cells was mixed with cells from immune lymph glands, was a small increase in synthesis observed. It must be emphasized that, although this stimulation effect was not statistically significant, the increase in immunoglobulin synthesis in that case was significant compared with the lowered level of production of these proteins in cultures to which supernatant from immune lymph glands and from the mixture of cells was added.

The possibility thus cannot be ruled out that, parallel with direct contact between cells of immune lymph glands and intact bone marrow, in order to obtain the stimulation effect certain humoral factors must be present. These factors are evidently secreted by intact bone marrow cells and they exert their effect on mature antibody-producing cells which are responsible for the increase in antibody synthesis.

LITERATURE CITED

1. A. E. Gurvich, G. I. Drizlikh, and E. V. Sidorova, in: Immunochemical Analysis [in Russian], Moscow (1968), p. 234.
2. A. A. Mikhailova, R. V. Petrov, and L. A. Zakharova, Dokl. Akad. Nauk SSSR, 197, No. 4, 209 (1971).
3. A. A. Mikhailova, R. V. Petrov, and L. A. Zakharova, Dokl. Akad. Nauk SSSR, 203, No. 4, 948 (1972).
4. R. V. Petrov and A. A. Mikhailova (Mihhajlova), Cell. Immunol., 5, 3 (1972).