INTERACTION IN VITRO BETWEEN CELLS OF IMMUNE LYMPH GLANDS AND INTACT BONE MARROW SEPARATED BY A MILLIPORE MEMBRANE

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The effect of stimulation of immunoglobulin synthesis by combined cultivation of lymph gland cells of immune mice and intact bone marrow cells, separated by membrane filters, was studied with the use of radioisotopes and immunosorbents. An increase in immunoglobulin synthesis was observed in the case of separation of the cell populations by Millipore membranes with a pore size of 25 nm and of 1.0-0.3 μ. No stimulation effect was observed if the cells were separated by cellophane membranes. Intensification of immunoglobulin synthesis was evidently the result of a humoral factor secreted by the cells of one of the adjacent populations.

KEY WORDS: immune response; intercellular interaction; bone marrow; lymph glands.

Previous experiments showed that if lymph gland cells of hyperimmune animals are mixed with bone marrow cells the synthesis of immunoglobulins is increased by twice to three times compared with that observed if the cells are grown separately [2, 3, 7]. This intensification of synthesis of antibodies and not specific immunoglobulins in mixed cultures was observed throughout the period of incubation (21 h) and on account of the same classes of immunoglobulins as in monocultures. Special experiments showed that neither the disintegrated cell populations nor the incubation medium in which the cells were grown gave an increase in immunoglobulin synthesis on addition to immune and intact cells.

In this investigation an attempt was made to discover whether direct contact between interacting cells of immune lymph glands and intact bone marrow is necessary for the effect of stimulation of immunoglobulin synthesis to be manifested. For this purpose the cell populations were separated from each other by membrane filters with different pore sizes.

EXPERIMENTAL METHOD

Inbred C57BL/6 male mice weighing 18-22 g were used. The animals were immunized subcutaneously with horse γ-globulin in a dose of 5 mg per mouse in Freund's complete adjuvant and they were reimmunized intravenously (2 mg protein per mouse) after 1-1.5 months. For preparation of the cell suspension from the lymph glands and bone marrow, mice were killed by decapitation on the 4th day after reimmunization and the axillary, inguinal, and mesenteric lymph glands were removed with sterile precautions. The tissue was minced with scissors and expelled through needles of successively diminishing diameter. The bone marrow cells were washed out with cold Eagle's medium with a syringe from the long bones of the 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 placed in medium at the rate of 3.5×10^7 cells per ml medium. Minimal Eagle's medium (MEM) (British Drug Houses) was used for the cultures, with the addition of essential and nonessential amino acids (JBL), pyruvate, glutamine, antibiotics, and 10% calf embryonic serum [4]. The cultures were grown in Petri dishes 5 cm in diameter and

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TABLE 1. Increase in Immunoglobulin Synthesis during Combined Cultivation of Cells of Immune Lymph Glands and Intact Bone Marrow, Separated by Membrane Filters

Membrane filters used	Number of experiments	Conditions of culture					
		Upper chamber – lymph gland cells; lower chamber – bone marrow cells		Lower chamber – lymph gland cells; upper chamber – bone marrow cells		mixed cultures of cells without membrane	
		antibodies	nonspecific γ-globulins	antibodies	nonspecific γ-globulins	antibodies	nonspecific γ-globulins
Cellophane membranes	3	0,98 ± 0,02	0,8 ± 0,07	0,99 ± 0,04	0,87 ± 0,07	1,72 ± 0,36	1,27 ± 0,12
Millipore membranes with a pore size of 25 nm	2	3,4 ± 1,9	1,35 ± 0,25	1,79 ± 0,76	1,08 ± 0,05	1,38 ± 0,06	1,02 ± 0,01
Synpore membranes with pore size of 0.1-0.3μ	5	2,01 ± 0,16	1,88 ± 0,25	1,87 ± 0,28	1,42 ± 0,32	1,77 ± 0,12	1,05 ± 0,01

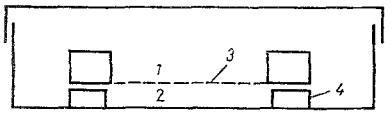

Fig. 1. Diagram showing system for culturing cells separated by membrane filters: 1) upper chamber; 2) lower chamber; 3) Millipore filter; 4) plastic ring.

1 cm deep (Fig. 1). Two curved platforms made of transparent plastic, 1 mm high and 1.5 cm long, were placed on the bottom of each dish. Plastic rings 4 cm in diameter and 0.7 cm high, to which the membrane filters with different pore size were fixed with acetone, were placed on the platform.

The Petri dish thus formed the lower chamber, whereas the ring with the attached membrane served as the upper chamber. Millipore membranes with a pore size of 25 nm (Millipore, France) and also Synpore membranes with a pore size of 0.1-0.3 μ (Chemapol, Prague, Czechoslovakia) were used. In some experiments this type of membrane was replaced by cellophane. Before use the membrane filters were boiled in distilled water three times for 15 min each time. The cells of the immune lymph glands and intact bone marrow, each in a volume of 1.5 ml, were placed separately in the lower and upper chambers. In the control experiments the corresponding cells were placed in the upper chamber and incubation medium without cells was poured into the lower chamber. A mixture of cells of immune lymph glands and intact bone marrow in Petri dishes without separation of the cells by a membrane was incubated in parallel experiments.

Incubation was carried out for 21 h in an exsiccator with an atmosphere containing 3% CO_2. After the end of incubation the cells were sedimented by spinning at 10,000 g for 20 min. The level of synthesis of antibodies and nonspecific immunoglobulins was determined from the incorporation of radioactive label into these proteins and extracting them specifically by means of immunosorbents [1]. The degree of stimulation of immunoglobulin synthesis was estimated by means of a coefficient consisting of the ratio between the intensity of synthesis of antibodies and nonspecific immunoglobulins in the mixed culture and the intensity of synthesis of these proteins in corresponding individual cultures.

EXPERIMENTAL RESULTS

In the experiments of series I, cells of immune lymph glands and intact bone marrow were separated by a cellophane membrane. In this case no increase in the synthesis of antibodies and nonspecific immunoglobulins was observed by comparison with the synthesis of these proteins in monocultures (Table 1).

It was concluded from this series of experiments that for the effect of stimulation of immunoglobulin synthesis to be manifested in a mixed culture either direct contact between the interacting cells was necessary or the humoral factor responsible for the intensity of immunoglobulin synthesis must have a molecular size larger than the pores in the cellophane membrane.

In the next series of experiments the interacting cell populations were separated by membrane filters with different pore sizes. In this case an increase in the synthesis of immunoglobulins of the same order as in mixed cultures incubated without the membranes was obtained (Table 1).

These experiments thus showed that an undialyzable humoral factor participates in the stimulation of immunoglobulin synthesis in a mixed cell culture from immune lymph glands and intact bone marrow. The data do not show which of the mixed populations secretes the factor. Some workers [5, 6, 8] have

shown an increase in the immune response in vitro on account of a factor secreted by thymus-dependent lymphocytes. However, the factor having a stimulant action in the present system evidently differs from the factors described by these other workers. In earlier experiments we obtained some increase in antibody synthesis by the addition of incubation fluid from intact bone marrow cells, virtually free from thymus-dependent lymphocytes, to cells of immune lymph glands. Incubation fluid from immune lymph gland cells had no such action. The increase in antibody synthesis in experiments with the addition of incubation fluid from bone marrow cells to immune lymph gland cells was not always observed. This factor is perhaps quickly destroyed.

In the system used in the present investigation, the stimulating factor was constantly secreted by the incubated cells and it thus gave rise to significant intensification of immunoglobulin synthesis.

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