ANTIBODY FORMATION IN ORGAN CULTURES OF LYMPH GLANDS AFTER PRIMARY IMMUNIZATION

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The appearance of antibody-forming cells is described in organ cultures of lymph glands of intact rabbits after addition of complete antigen from Salmonella enteritidis to the culture. The number of antibody-forming cells reached a maximum when the culture was immunized on the 20th day after its explantation from the donor.

The problem of the formation of specific antibodies in tissue cultures has interested immunologists for a long time. As a result of many investigations of antibody production in tissue culture, it has been shown that this method can be used to detect antibodies in cultures of fragments of lymph glands and spleen of immunized animals.

In the last decade, in connection with the study of mechanisms of antibody formation, interest in reproduction of the primary immune response in vitro has increased [1-4, 5-8, 10-13].

In this investigation antibody synthesis was studied in organ cultures of lymphoid tissue after primary immunization with complete antigen from Salmonella enteritidis.

EXPERIMENTAL METHOD

Experiments were carried out on adult rabbits weighing 2.5-3 kg, serologically negative to S. enteritidis antigen (by the indirect hemagglutination test). As nonspecific stimulator, the rabbits received an injection of a mixture of 2% peptone solution with 0.2% gelatin solution, in a volume of 1 ml, subcutaneously or intramuscularly in the lower third of the hind limb.

Complete antigen was prepared from S. enteritidis cells by Boivin's method. The antigen was sterilized by brief treatment with alcohol and ether.

The popliteal lymph glands were excised under aseptic conditions, freed from fat, washed three times with Hanks's solution, cut up with pointed scissors to a size of 2-3 mm, and transferred to No. 6 filters of Soviet manufacture. Before the experiment the filters were cut into pieces of the required size and sterilized by boiling three times in distilled water. Cultivation was carried out in transparent plastic chambers contained in sterile Petri dishes. The chambers contained medium No. 199 with 20% inactivated homologous serum, 4 mg/ml glucose, and 100 units each of penicillin and streptomycin.

The dishes with moistened, sterile filter paper were placed in an exsiccator into which a gas mixture (5% CO_2 + 95% O_2) was introduced every day, and incubated at 37°. The medium was changed every 3-4 days. Antigen was added in vitro at different times after explantation of the pieces (at the time of starting cultivation and 7 and 20 days thereafter) in a dose of 0.01 μg/ml. After 24 h the pieces were washed twice with medium and cultivation was continued in fresh medium without antibiotics for 10 days. Cultures to which no antigen was added acted as the control.

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TABLE 1. Dynamics of Increase in Number of Antibody-Forming Cells in Relation to Times of Addition of Antigen (number of plaques per 10^6 cells)

Time of addition of antigen	Time after addition of antigen									
	1st day		2nd day		4th day		7th day		10th day	
	control	experiment	control	experiment	control	experiment	control	experiment	control	experiment
Simultaneously with explantation of tissue (series I)	2	0.03	8	9	10	23	8	14	0	40
On 7th day of organ culture (series II)	2	18	1.7	27	1	38	0.8	49	0	10
On 20th day of organ culture (series III)					3.6	125	0.5	189	0	26

Antibody production was determined by the method of local indirect hemolysis in agar [9] with sheep's erythrocytes sensitized with hapten from complete S. enteritidis antigen [2].

For performance of Jerne's test, pieces were taken from the filters, cut up with scissors, and treated with 0.1% trypsin solution in a water bath at 37°, and then centrifuged for 3 min at 750 rpm. The residues were diluted with 0.2 ml Hanks's solution with 10% bovine serum. The number of cells was counted in a Goryaev's chamber. Next, 0.1 ml of each cell suspension was added to 1.5 ml melted 0.7% agar (48°), containing 0.1 ml 10% suspension of sensitized sheep's erythrocytes. After gentle mixing, the warm agar was layered above in a thin layer of 1.4% agar in the bottom of a Petri dish. The dishes were incubated for 2 h at 37°, after which complement was added in the ratio of 1:10 for 1 h.

EXPERIMENTAL RESULTS

Three series of experiments were carried out to investigate the effect of the time of addition of antigen to the culture on antibody production.

In the experiments of series I, antigen was added to the culture at the same time as the fragments were placed on the filters.

The results given in Table 1 show that the number of antibody-synthesizing cells on the 4th day was double that in the control, and on the 9th day after addition of antigen to the tissue there were 40 plaques per million cells, while the control was negative.

In the experiments of series II, in which antigen was added to the organ culture on the 7th day, an increase in the number of hemolytic plaques compared with the control (not stimulated) tissue was observed from the 2nd to the 10th day, the increase being greatest (by 38-49 times) on the 4th-7th day after addition of antigen.

A steep rise in the number of antibody-forming cells was observed when the tissue cultures were immunized on the 20th day after explantation (experiments of series III). As Table 1 shows, in response to addition of antigen in vitro, the largest number of hemolytic plaques was found on the 7th day after antigenic stimulation. By the 10th day the number of antibody-producing cells had fallen sharply.

Titration of the culture fluids in all three series of experiments for the presence of antibodies by means of the passive hemagglutination reaction as a rule failed to reveal any positive immunologic response.

Comparison of the results of the three series of experiments shows that the degree of increase in the number of antibody-producing cells varied very considerably, depending on the time when the antibody was added.

It can thus be concluded from these experiments that after primary immunization with complete antigen from cells of S. enteritidis, organ cultures of lymphoid organs can carry out primary antibody synthesis.

The number of plaques and the dynamics of their appearance varied with the time of addition of the antigen to the organ culture. The results given in Table 1 show that the largest number of plaques was obtained when the tissue was immunized on the 20th day after explantation. The greatest accumulation of antibody-forming cells in this case (mean 189 plaques) was observed on the 7th day after antigenic stimulation. The lowest values were obtained in the experiments of series I, in which antigen was added simultaneously with explantation.

It can be assumed for the analysis of these results that the low level of antibody synthesis by the immunized cultures, especially in the experiments of series I, was due to the state of the organ cultures of lymph glands in the early periods of cultivation. If lymph glands are grown for a long period in organ cultures [3], changes take place in their morphological picture in a series of stages. During the first week degenerative and necrobiotic changes take place, with necrosis of the medullary zone and preservation of viable lymphocytes at the periphery. Later, starting from the 7th and continuing to the 11th day, complete regeneration of the lymphoid tissue in the cortex takes place, with the formation of structures resembling secondary lymphoid follicles. This tissue remains viable for 30-45 days. These results were confirmed in experiments carried out jointly by L. D. Safronova and the writer, in which organ cultures were studied histologically. During regeneration of lymphoid tissue in 20-day cultures, this tissue is thus capable of giving an immunologic response to primary addition of antigen in vitro.

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