INDUCTION OF ANTIBODY SYNTHESIS IN ORGAN CULTURES OF LYMPH GLANDS

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Antibody production may be observed in tissue cultures in vitro if the lymphoid tissue synthesizing antibodies are taken from a preliminarily immunized animal [5, 6]. Until recently, however, attempts to induce the synthesis of antibodies in cultures by primary exposure to the antigen in vitro had proved unsuccessful [7, 8].

In ordinary cultures of lymphoid tissue in vitro the tissue structures participating in the immunologic response are lost. It can be assumed that this is the reason for the failure to induce antibodies in experiments with ordinary tissue cultures.

In the present investigation an attempt was made to induce antibody synthesis in organ cultures of lymphoid tissue in which its structure was preserved.

EXPERIMENTAL METHOD

Whole inguinal, axillary, and popliteal lymph glands of adult rabbits and guinea pigs were used for cultivation. The lymph glands were cultivated by the method of multiple organ cultures advocated by the authors [3].

The lymph glands were placed on HA (pore size 0.45 μ) or Rufs (pore size 1.2 μ) millipore filters which were placed at the boundary between two phases: the liquid phase of the nutrient medium and the gaseous phase, consisting of 5% CO_2 in oxygen. The nutrient mediem was made up from 80-85% medium No. 199, 10-15% of heated homologous serum, and in some cases, 5% homologous embryonic extract. To 1 ml of medium, 4 mg glucose and 100 units each of penicillin and streptomycin were added. The medium was changed every 48-72 h.

Immunization of the lymph glands was carried out by adding the antigen, consisting of horse γ-globulin (HGG), to the medium in doses of 0.1 and 0.5 mg/ml medium. The cultures were incubated with the antigen for several hours or for 2-3 days. In most experiments the cultures were then washed in two portions of medium to remove the antigen. In two cases (experiments Nos. 4 and 5) washing to remove antigen was not carried out and the cultures were transferred to a medium not containing the antigen.

Between 24 and 48 h before the end of the experiment C^{14}-glycine dissolved in physiological saline or in Ringer's solution was added to the medium. The final concentration of radioactive glycine in the culture medium was 0.4-4.0 μCi/ml. After the end of incubation with label, in most experiments the lymph glands were ground in a mortar, mixed with incubation medium, and centrifuged for 20 min at 9000 g. Antigens in the supernatant were determined by means of cellulose-protein copolymers by the method of Gurvich and Sidorova [1]. Two copolymers of cellulose with egg albumin (e-cellulose), and with horse γ-globulin (HGG-cellulose) were used. The supernatant was first treated with an immunosorbent containing nonspecific protein (e-cellulose), mixed, and the sorbent was removed by centrifugation. The supernatant was transferred to another tube, a sorbent containing the protein with which the cultures were immunized (HGG-cellulose) was added, the contents of the tube were mixed, and the sorbent was separated by centrifugation. The two sorbents were transferred to separate disks of filter paper. The disks were washed with 1% glycine in physiological saline, pH 7.0, to remove nonspecifically bound label. The radioactivity of the washed and dried targets was then determined with a T-25-BFL end-type counter. The target with

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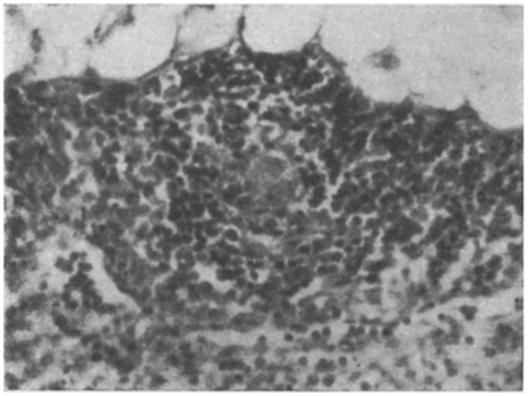

Fig. 1. Culture of a guinea pig's lymph gland. Secondary lymphoid follicle. Time 8 days. Section. Objective 60×.

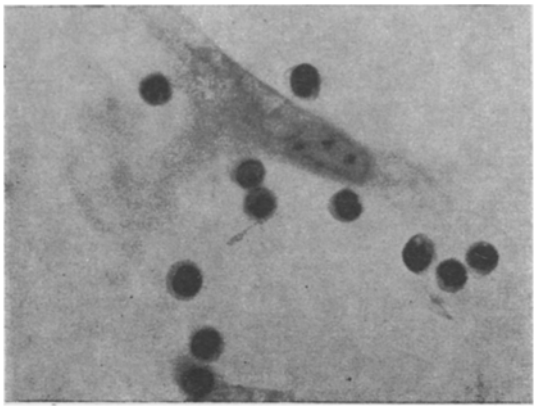

Fig. 2. Culture of a guinea pig's lymph gland. Reticular "fodder cell" surrounded by lymphocytes. Time 2 days. Total preparation. Objective 10×.

an immunosorbent containing specific antigen (HGG-cellulose) was used to determine the amount of antibody synthesis taking place from the increase in radioactivity on the sorbent.

The target with the immunosorbent containing nonspecific protein (e-cellulose) was used to determine the nonspecific increase in radioactivity.

EXPERIMENTAL RESULTS

Six experiments were carried out, two of them on lymph glands of the rabbit and four on lymph glands of the guinea pig. The morphology of the cultures of the lymph glands from these animals was similar. Just as was described previously [2, 3], during the first 4 days in vitro, the lymphocytes in the central part of the glands underwent degeneration, and only at the periphery were isolated groups and entire zones of lymphoid cells still present under the capsule. On the 5th-7th day intensive regeneration of the cortical layer took place and the medulla was freed from cell debris. By the 8th-12th day circular lymphoid structures of the secondary follicle type were restored in the cortical layer (Fig. 1). They were composed of large, medium sized, and small lymphocytes. Between the follicles a very few plasma cells were observed. The medulla did not regenerate and remained filled with reticular tissue with a few lymphocytes. The same morphological pattern persisted in the 2- or 3-week old cultures. Migration of lymphoid and reticular cells on to the surface of the filter took place. They formed elementary structures consisting of lymphocytes, which surrounded the individual reticular cells (Fig. 2).

After 10 days, no lymphocytes could be found on the filter, and a zone of growth consisting of reticular cells remained around the piece of tissue. When the lymph nodes were cultivated on the Rufs filters the degree of preservation of the lymphoid tissue in the later periods of cultivation was better than when HA filters were used.

In most experiments the addition of antigen and C^{14}-glycine to the medium caused no visible morphological changes. However, in experiment No. 4, in which C^{14}-glycine was added to the medium on the 10th day at the rate of 2 μCi/ml, pycnosis of the nuclei of the lymphocytes was observed. In this experiment no antibody synthesis could be demonstrated. In 3 of the other 5 experiments moderate antibody synthesis was observed. In all 3 experiments, the increase of radioactivity on the immunosorbent containing the antigen with which the cultures were immunized was rather greater than the increase on the immunisorbent containing the other antigen (see Table 1).

The greatest increase of radioactivity was observed in the case of immunization of the lymph glands of the rabbit with HGG. The antigen was added on the fourth day of explantation in vitro in a dose of 0.5 mg/ml/h. The cultures were then washed in two changes of nutrient medium and placed in culture medium not containing antigen. Four days later radioactive C^{14}-glycine was added to the medium in a dose of 2 μCi/ml, and after incubation for 24 h the medium was treated with the immunosorbent. The increase in

TABLE 1. Increase in Radioactivity on Immunosorbent

Expt. No.	Conditions of cultivation	Day of addition of antigen	Conditions of immunization	Day of addition of C^{14}-glycine (from beginning of cultivation)	Incubation time with C^{14}-glycine (in hours)	C^{14}-activity type of sorbent	
						e-cellulose	HGG-cellulose
1	Rabbit's lymph glands in medium with 10% homologous serum and 5% homologous embryonic extract	4th	Incubation for 1 h with HGG in a dose of 0.5 mg/ml	8th	24	9	94
3	Guinea pig's lymph glands in medium with 15% homologous serum	7th	Incubation for 1.5 h with HGG in a dose of 0.5 mg/ml	17th	48	0	17
5	Guinea pig's lymph glands in medium with 10% homologous serum	Immunized with HGG in a dose of 0.5 mg/ml 3 times: antigen was added to the medium for 1 day on transplantation, 6 days later for 2 days, and 24 days later for 3 days		28th	48	11	39

Note: Experiments in which positive results were obtained are included in the table.

radioactivity was 85 pulses/min. In the experiment in which triple immunization of the lymph glands of guinea pigs was carried out without subsequent washing to remove antigen, the radioactivity was lower, namely 28 pulses/min.

These results show that in certain conditions when an antigen acts on an organ culture of lymphoid tissue in vitro antibody synthesis may be induced. Incorporation of the label, although observed in these experiments, was slight; however, it evidently afforded reliable evidence of synthesis of the corresponding antibodies. The low level of antibody synthesis in the cultures in these experiments correlated with the very few plasma cells that were formed, whether without antigen or with its addition. This may be associated with the poorly developed degeneration of the medullary layer, in which most of the plasma cells are located in the normal lymph gland. Meanwhile, the cortical layer of the lymph glands in organ cultures regenerated well and contained many living, proliferating lymphocytes. In contrast to the ordinary tissue cultures, the lymph gland in organ cultures is an organ in which several of the tissue structures are preserved. This is true primarily of the secondary lymphoid follicles. The presence of these structures is evidently responsible for the fact that induction of antibody synthesis can occur in such cultures.

The possibility of using organ cultures for the induction of antibodies was demonstrated by Globerson and Auerbach, who cultivated fragments of mouse spleen in organ cultures after injection of phytochemagglutinin into donors [4]. In these experiments low antibody titers were obtained in the hemagglutination reaction. In the experiments undertaken by the present authors true synthesis of antibody protein was proved by the incorporation of labeled amino acids in the lymph gland cultures without preliminary treatment with phytochemagglutinin.

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