IMMUNE RESPONSE OF A SPLEEN CELL CULTURE TO ANTIGENIC STIMULATION IN VITRO

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Introduction of sheep's erythrocytes into a culture of rabbit spleen cells leads to the appearance of cells in the culture synthesizing specific antibodies detectable by the reaction of local hemolysis in gel. The number of antibody-forming cells reaches a maximum 48 h after introduction of erythrocytes into a culture of spleen cells from intact rabbits and 24 h after their introduction into a culture of spleen cells from rabbits preliminarily sensitized with sheep's erythrocytes.

Until recently it was considered impossible to obtain a primary response by immunizing a tissue culture obtained from the spleen of intact animals, despite individual investigations [1, 3, 4, 6] in which primary immunization was reproduced in vitro.

Successful induction of antibody formation in vitro is possible if a highly sensitive model in used and cells are obtained from lymphoid organs by a gentle procedure. The quality of the nutrient medium and sensitivity of the method used to detect antibodies in the cells are also of considerable importance.

In the present investigation we studied the possibility of obtaining an immune response in a culture of rabbit spleen cells after treatment with antigen in vitro.

EXPERIMENTAL METHOD

To reproduce primary antigenic stimulation in vitro, cultures of cells were obtained from the spleens of intact rabbits or of rabbits receiving 50-75 ml 2% peptone with 0.2% gelation by intraperitoneal injection 72 h before sacrifice. In the experiments with secondary stimulation in vitro the spleen was taken from rabbits 72 h after intravenous immunization with sheep's erythrocytes (0.5 ml of a 20% suspension).

Pieces of spleen, chopped up with pointed scissors, were washed with medium No. 199 and treated with 0.25% trypsin solution for 1 min. The cell suspension obtained by washing and trypsinization of the fragments was discarded. Next 4-5 ml of medium No. 199 was added to the fragments which were sucked up and down with a pipet in the medium; after standing for a short time the cells contained in the supernatant were removed by centrifugation and resuspended in medium No. 199. The pieces of spleen were again trypsinized and washed. Next, cells were carefully expressed from the remaining fragments in an unground homogenizer, filtered, centrifuged (2 min at 700 rpm), and resuspended. All the portions of cells were

TABLE 1. Number of Plaques (per 10^6 cells) after Primary Antigenic Stimulation in Vitro

Rabbit no.	Number of plaques				Rabbit no.	Number of plaques			
	Original	After incubation				Original	After incubation		
		24 h	48 h	72 h			24 h	48 h	72 h
1	—	—	3 141	—	9	14	4	313	286
2	—	—	3 000	—	10	19,5	7	115	—
3	—	—	1 048	275	11	20	—	15	—
4	5	444	725	35	12	—	15	8	0
5	—	—	620	385	13	22	—	7,6	—
6	1	507	611	83	14	6,5	—	0	—
7	—	—	465	—	15	10,0	0	0	0
8	3,3	—	398	261					

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TABLE 2. Number of Plaques (per 10^6 cells) during Cultivation of Spleen Cells on Films of Macrophages and without Them.

Culture	Experiment No.					
	1	2	3	4	5	6
Spleen cells + sheep's erythrocytes	7	15	115	313	398	620
Spleen cells + macrophages + sheep's erythrocytes	88	33	367	1 453	2 918	758

TABLE 3. Number of Plaques (per 10^6 Cells) 24 and 48 h after Antigenic Stimulation In Vitro of Spleen Cells from an Immune Rabbit

Rabbit No.	Number of plaques				
	Original	after 24 h		after 48 h	
		expt.	control	expt.	control
1	465	1 737	600	670	—
2	158	16 743	662	3 870	60
3	38	1 888	101	777	10

cooled, counted in a Goryaev counting chamber, and poured into test tubes at the rate of 3 million cells in 1.5 ml medium. The medium in the tubes was changed after incubation for 24 h.

In some parallel experiments spleen cells were added to test tubes onto layers of macrophages (10,000 cells per test tube), preliminarily allowed to spread for 30 min, to which sheep's erythrocytes were added. Medium No. 199 containing 10% inactivated rabbit serum, but without antibiotics, was used for cultivation.

Washed sheep's or rat's erythrocytes were added to the cell culture at the rate of 1 erythrocyte per 50 spleen cells immediately after production of the culture.

Antibody-synthesizing cells were detected by Jerne's method 24, 48, and 72 h after addition of antigen. The cells, which had spread out over the glass walls, were removed mechanically and investigated along the cells freely floating in the culture fluid. In contrast to Jerne's orginal method, to detect the plaques more clearly, cells with sheep's erythrocytes in 0.7% agar were placed on a thin agar base layer (2.5-3 mm agar poured into a dish with radius 4.5 cm). By this modification of the method it was possible to detect very small plaques, which would be impossible with the usual agar plates.

EXPERIMENTAL RESULTS

In a culture of spleen cells obtained from intact rabbits, hemolytic plaques detected by Jerne's method were observed 24, 48, and 72 h after addition of sheep's erythrocytes to the cell culture (Table 1). The number of plaques reached a maximum 48 h after primary stimulation of the cells (Table 1).

In 10 of the 15 experiments (each experiment consisting of investigation of spleen cells from one rabbit) numerous plaques were observed (in 3 cases more than 1000 and in 7 between 100 and 1000), in 3 experiments there were fewer than 50 plaques, and in 2 experiments cells synthesizing antibodies were completely absent. The number of cells synthesizing antibodies in vitro evidently depends on the immunologic reactivity of the rabbit and the functional state of the cells.

In the control experiments, when spleen cells were cultivated without the addition of sheep's erythrocytes or when other antigens were added to the culture (rat's erythrocytes or LPA), only a few cells (not more than 20 per 10^6 cells) giving plaques in the agar were found.

Hemolytic plaques found after primary antigenic stimulation were very small and were not always circular.

In 9 experiments spleen cells were added to macrophages spread over the tube walls. In 6 of these experiments there was a considerable (2-7 times) increase in the number of cells producing antibodies compared with the same spleen culture incubated with sheep's erythrocytes but without addition of macrophages (Table 2).

After secondary antigenic stimulation of the cell culture of spleen obtained from an immunized rabbit, a considerable increase (3-30 times) was observed in the number of cells producing antibodies compared with the same unstimulated culture (control).

In contrast to primary antigenic stimulation, the largest number of hemolytic plaques in the case of secondary antigenic stimulation of the cells was observed after 24 h (Table 3).

The results described show that if spleen cell suspensions are obtained by a gentle method the cells retain high functional activity in vitro, as shown by the possibility of reproduction of antibody synthesis by isolated spleen cells during both primary and secondary antigenic stimulation.

In their cell composition the cell cultures used contained cells of lymphoid type, macrophages, and reticulum cells. Primary and secondary antigenic stimulation in vitro show a number of special features: very slight variation in the number of cells in experimental and control samples together with a sharp increase in the number of plaques in the experimental samples, absence of mitoses in the first 48 h, and the early (24 h) appearance of antibody-producing cells. These facts suggest that antibody synthesis in vitro is independent of cell proliferation, in agreement with views expressed by other investigators [2, 5].

Considerable stimulation of synthesis in some experiments on the addition of peritoneal macrophages may perhaps be attributable to the more rapid absorption of antigen by cells of the inflammatory exudate (not traumatized as during fragmentation of the spleen) and to active resorption of cell debris which is inevitably present in the cultures.

LITERATURE CITED

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