COMPARISON OF THE ACTION OF AN ANTIGEN IN VIVO AND IN VITRO AT DIFFERENT STAGES OF THE IMMUNE PROCESS

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Curves showing the change in the number of IgM-antibody-forming (plaque-forming) cells (PFCs) against sheep's red cells in C57BL/6 mice in vivo and in a suspension of spleen cells obtained at different stages of the immune process and cultured for 4 days in vitro were compared. After 4 days the number of PFCs obtained by culture in vitro could be increased by many more times than in vivo. In response to repeated injection of antigen into mice the response obtained was always less intensive than in vitro.

KEY WORDS: antibody formation in vivo and in vitro; regulation of the immune response.

In most cases the intensity of antibody biosynthesis changes rapidly after immunization: initially it rises exponentially, then falls sharply [1, 4, 5, 8]. The reasons for this fall have received little study. Recently, however, it has been shown not to take place in cell suspensions or in fragments of lymphoid organs cultured in vitro [3, 9].

To discover whether this difference between the course of antibody formation in vivo and in vitro is connected with the rapid elimination of the antigen from the body, the action of an antigen was compared in different stages of the immune process in vivo and during culture of immunocompetent cells.

EXPERIMENTAL METHOD

Experiments were carried out on C57BL/6 mice. Sterile sheep's red cells (SRC) in 7B preservative were used as the antigen.

The animals were immunized by intravenous injection of 500×10^6 or 50×10^6 SRC in a volume of 0.2 ml.

The action of the antigen in vitro was studied by the method of Mishell and Dutton [9], modified by Click et al. [6] and also slightly by ourselves. This last modification was to incubate the cells in silicone-treated flat-bottomed glass flasks (35 × 45 × 30 mm) or in penicillin flasks 22-24 mm in diameter. For the silicone treatment the flasks were rinsed with a 3% solution of "Antifoam Silane" (Riga Pharmaceutical Chemical Factory) in ether, heated to 120°C for 1 h, then washed 3 times with hot distilled water and sterilized by dry heat at 120°C for 2 h. The spleens of the unimmunized mice and of mice immunized 1-7 days before the experiment were removed with sterile precautions. The cells were shelled out of the spleens, suspended from a pipet in double Eagle's medium, and washed twice in the cold with the same medium in the centrifuge at 700 g. The washed cells were suspended in the medium described by Click et al. [6], to which insulin (0.24 unit/ml) and glucose (1 mg/ml) were added. The suspension of cells (10 million) was poured in volumes of 2 ml into the large flasks and 0.5 ml into the penicillin flasks. The flasks were filled

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TABLE 1. Comparison of Action of Antigen in Vivo in Situ and in a Culture of Spleen Cells in Vitro

In situ				Cell culture in vitro	
primary immunization		reimmunization		beginning and end of incubation (days after primary immunization in situ)	number of PFCs per 10^6 nucleated cells after incubation for 4 days in vitro
day after immunization	number of PFCs per 10^6 nucleated spleen cells	number of PFCS per 10^6 nucleated spleen cells on the 4th day after reimmunization of mice			
		50×10^6 SRC	500×10^6 SRC		
Unimmunized	1,2 (0,8–1,5) [4]	—	—	(0–4)	558 (286–1 086) [44]
1	1,2 (0,13–10,9) [3]	—	914 (802–1042) [5]	1–5	524 (375–731) [12]
2	6,8 (4,0–11,7) [11]	267 (144–496) [6]	586 (414–830) [14]	2–6	1 426 (1 208–1 683) [37]
3	86 (63–119) [8]	527 (384–723) [5]	498 (256–966) [4]	3–7	2 009 (1 119–3 606) [20]
4	273 (224–333) [12]	500 (219–1140) [5]	452 (206–993) [5]	4–8	6 353 (3 793–10 640) [6]
5	467 (306–711) [5]	—	—	—	—
6	306 (180–520) [6]	—	—	—	—
7	60 (41–89) [15]	98 (50–192) [7]	564 (455–698) [7]	7–11	1 560 (1 102–2 208) [33]
8	49 (21–112) [5]	—	—	—	—
11	16,7 (6,2–45,2) [7]	—	—	—	—

Legend. Mean value and confidence limits ($P < 0.05$) given in parentheses; number of observations between square brackets.

with an atmosphere consisting 0f 5% CO_2, 10% O_2, and 85% N_2, closed with rubber stoppers, and incubated for 4 days at 37°C.

The number of IgM-antibody-forming (plaque-forming) cells (PFCs) was determined by Jerne's method [8] and the number of living cells by staining with eosin and trypan blue [2].

EXPERIMENTAL RESULTS

Under the experimental conditions used a well-marked primary response of the spleen cells of the unimmunized mice and a sharp increase in the number of PFCs in the cell suspension from the immunized animals could be obtained. This increase took place only in the presence of 2-mercaptoethanol and only in the silicone-treated flasks.

In the principal series of experiments the ability of spleen cells taken at different stages of the immune process to respond to the antigen in vitro was compared. The spleen was removed with sterile precautions from the mice 1, 2, 3, 4, 6, and 7 days after immunization with 500×10^6 SRC and cell suspensions were prepared as described above. The original number of PFCs in each suspension was determined. As the data given in Table 1 show, injection of SRC into the mice induced the usual immune response which reached a maximum on the 5th day and then declined rapidly. The greater part of each of the suspensions was poured into flasks and incubated at 37°C for 4 days in the presence of antigen (1×10^6 SRC to 4×10^6 spleen cells). During the period of incubation the number of PFCs rose very sharply (by 23-440×) compared with initially. This increase was observed during incubation of spleen cells removed not only at the beginning of development of the immune process, but also at its climax and during its decline.

The increase in the number of PFCs in the suspensions of spleen cells of the immunized animals was much greater than in the cell suspension from the unimmunized animals. It reached a maximum on the 4th day after immunization, in good agreement with results obtained by Dutton and Mishell [7].

It follows from Table 1 that during culture of the cells in vitro the number of PFCs increased much faster than during the same period in vivo. For example, the spleen of an animal 8 days after immunization contained 130 times fewer PFCs than a cell suspension cultured from the 4th to the 8th day in vitro.

Even between the 7th and 11th days after immunization, when a sharp decline in antibody synthesis was observed in vivo, the number of PFCs in a suspension of cells cultured in vitro rose sharply and reached a level 93 times higher than in vivo.

The difference between the response in vivo and in vitro could be due to the action of antigen added again during culture of the cells. To study this problem a special series of experiments was carried out in which all the animals were immunized primarily with 500×10^6 SRC, and reimmunized after various intervals of time with 50×10^6 or 500×10^6 SRC. The smaller of these doses corresponded approximately (calculated per gram weight) to the concentration of antigen added in vitro. After a time interval equal to the period of incubation of the cultures (4 days) the animals were killed and the number of PFCs in their spleens determined.

It will be clear from Table 1 that the second injection of antigen in the early stages of development of the immune process caused an increase in the number of PFCs, but in the late stages it prevented the decrease in the number of PFCs. The action of the larger of the doses used was particularly effective. However, the increase in the number of PFCs during the action of the antigen in vivo was much less (5-16 times) than the increase in their number during the action of antigen on the cells cultured in vitro (Table 1).

It can be concluded from these results that the formation of many more PFCs in vitro than in vivo is evidently attributable to disturbance of intercellular interaction during preparation of the suspension or to removal of inhibitory factors acting in vivo.

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