SENSITIVITY OF LYMPHOID CELLS TO THE ACTION OF ANTILYMPHOCYTIC SERUM AT VARIOUS STAGES OF IMMUNOGENESIS

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Experiments on mice immunized with sheep's erythrocytes showed that the antibody-forming cells of the spleen in the early periods after immunization are more sensitive to the action of antilymphocytic serum than the cells in the later stages of immunogenesis. The total number of viable spleen cells fell in the early periods after injection of the antilymphocytic serum, and later recovered.

Antilymphocytic sera (ALS) have a depressant action on the total population of lymphoid cells in the body and also on cells participating in the immune response [4, 7, 8]. Cells responsible for immunogenesis are most sensitive to antiserum in its early stages, associated with reception of the antigenic stimulus [1-3, 6].

Sensitivity of lymphoid cells in general and of immunocompetent cells in particular to ALS in various stages of immunogenesis were studied in the investigation described below.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred and inbred (CC57BR) mice weighing 18-20 and 12 g. The animals were immunized by a single intravenous injection of sheep's erythrocytes ($5 \cdot 10^8$). Each experiment was repeated 3 times, and material from 3 animals was used in each test. The results were subjected to statistical analysis, and the arithmetic mean and its mean error were determined. Antibodies in the sera were detected by the hemolysis and agglutination tests. Antibody-producing cells were detected by the local hemolysis in gel test [5]. The total number of cells was counted in a Goryaev's chamber, and nonviable cells were detected by staining with 0.5% trypan blue solution. ALS were prepared by injecting rabbits intravenously 10 times with lymphocytes from mouse lymph glands. The sera were exhausted with mouse erythrocytes. Their titers in the complement fixation test were 1 : 640-1 : 1280, and in the leuko-agglutination test 1 : 512-1 : 1024.

EXPERIMENTAL RESULTS

The action of ALS in vitro was studied as follows. The ALS were added in various concentrations to tubes containing about $30 \cdot 10^6$ nucleated spleen cells from mice immunized 4 days before sacrifice. The mixture was incubated at 37° in the presence of 10% guinea pig complement, after which the total number of lymphoid cells, the number of viable lymphoid cells, and the number of antibody-forming cells (AFC) were determined.

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TABLE 1. Characteristics of Splenic Lymphoid Population of Normal and Immune Mice after Injection of ALS

Animals	Time after ALS inject. (in h)	Nucleated cells in spleen (in millions)		Number of living cells (in millions)		AFC (absolute number)	
		NS*	ALS	NS*	ALS	NS*	ALS
Immunized	0	543±64		471±61		7 258±1 000	
	3—5	591±160	451±81	505±137	275±54	14 413±2 037	11 683±1 700
	24	439±115	533±166	396±83	424±127	7 216±2 281	21 033±5 000
Unimmunized	0	17,5±3,0		14,0±3,3		62±17	
	3—5	75±10,8	52±6,8	49,5±7,0	21,6±4,1	77±22	95±12,8
	24	55±10	50±5,5	39,8±7,2	39,4±10,0	339±26	448±50

*Normal serum.

TABLE 2. Characteristics of Splenic Lymphoid Population of Immunized Mice 4 Days after Immunization with Sheep's Erythrocytes

Time of injection of ALS relative to immunization	Total No. of cells (in millions)	No. of living cells (in millions)	No. of AFC (per 10^6 living nucleated spleen cells)
3 (4) days before	425±110	409±65	12,9±4,4
1 day before	737±108	429±68	0,83±0,16
At the same time	500±105	396±84	17,4±5,9
2 days after	486±84	366±74	22,9±6,8
Control	612±80	508±85	16,9±3,7
Normal serum 1 day before	595±140	595±130	21,6±4,7

The results of the tests with 4 series of ALS showed that after incubation of the spleen cells for 1 h with normal rabbit serum or with ALS in concentrations of 1.25, 2.5, or 10%, the number of viable nucleated cells was 29.8±4.0, 30.7±4.5, 21.6±2.5, 19.2±2.4, and 17.8±0.42 respectively. Under the same conditions the number of cells capable of forming antibodies was 2660±450, 621±172, 196±42, 40±10, and 8.3±1.0 respectively. The number of AFC after incubation with 1.25% ALS was thus 23% of its initial value, and after incubation with 10% ALS it was 0.3%. The total number of viable lymphoid cells was 60% of the control level even after incubation with 10% ALS. This indicated a much higher sensitivity of the AFC in vitro than of the splenic lymphoid cells as a whole.

Experiments in vivo showed that at the same time after immunization (4 days), ALS when injected intravenously into the animal in a dose of 0.15 ml gave a different effect (Table 1). The total number of cells in the spleen was substantially unchanged 3-5 and 24 h after the injection of ALS. However, 3-5 h after injection the number of viable cells had fallen from 471±61 million to 275±54 million ($P<0.05$). The number of nonviable spleen cells at this time was 37% compared with 16% in animals receiving normal serum. The number of AFC was not reduced at this time but, on the contrary, it rose until 24 h after injection of the serum. This could be due to stimulation by the protein of the injected serum, for an increase in the number of AFC also occurred in groups of animals receiving normal serum. However, a specific stimulant action of the ALS cannot be ruled out.

The ALS had a similar action on spleen cells of normal mice. For a brief period after its injection the ALS had a cytotoxic action on the total population of lymphoid cells (Table 1). This was reflected in the fact that the increase in number of lymphoid cells, which is observed constantly after injection of any serum, was not so marked, while the number of nonviable spleen cells after 3 h was 56% compared with 34% in the control. The number of AFC did not fall but, on the contrary, it rose until 24 h after injection of the serum by the same degree as after injection of normal serum.

These results were obtained on young, unimmunized mice weighing about 12 g. In the groups of older animals, in which the initial level of nucleated cells in the spleen was higher, the same general pattern was observed. However, the increase in the total number of nucleated cells 3-24 h after injection of ALS was slight.

In the groups of animals tested on the 4th day after immunization, the initial level of nucleated cells in the spleen was high, and did not increase substantially after injection of the sera. Meanwhile, in the nonimmunized mice the initial number of spleen cells was many times lower, and injection of the sera caused it to increase considerably.

The resistance of the AFC obtained at the height of the immune response to the action of ALS contrasted with the high sensitivity of cells participating in reception of the antigenic stimulus. After injection

of ALS 24 h before immunization, a definite depression of immunogenesis was observed (Table 2). On the 4th day after injection of sheep's erythrocytes, the number of AFC remained low (just as in the subsequent periods of observation). Injection of the ALS 3-4 days before immunization had a less marked action, while injections simultaneously with immunization or 48 h later were ineffective. The total number of cells in the spleen, like the number of viable cells, determined on the 4th day after immunization was constant, regardless of the times of injection of the ALS.

These results corresponded to two aspects of the action of ALS: their effect on viability of the lymphoid cells and on their immunologic function. Sensitivity of the total population of lymphoid cells in the organism to ALS at various stages of immunogenesis and outside the period of immunogenesis was shown to obey the same rule: soon after injection of the serum a transient cytotoxic effect is observed. The action of ALS on immunocompetent cells is different. Injection of the serum 24 h before antigenic stimulation sharply depresses the function of the immunocompetent cells. This indicates high sensitivity of lymphoid cells participating in the initial stages of immunogenesis to ALS. Cells in the stage of antibody production after antigenic stimulation, or producing "normal" antibodies, are more resistant to the action of ALS than the general mass of lymphoid cells. Moreover, after injection of ALS a definite increase is observed in the number of antibody-producing cells in the body. This indicates the stimulant action of ALS on the population of antibody-forming cells. This action of ALS in vivo differs from its effect in vitro. In the latter case, the sensitivity of the AFC was higher than the sensitivity of the general mass of lymphoid cells determined by the cytotoxic test. Differences between the action of ALS in vivo and in vitro may be due to the fact that in vivo the antiserum concentration obtained is not so high, or the toxic action of the serum is quickly compensated.

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