ACTION OF HETEROLOGOUS ANTILYMPHOCYTE SERUM ON IMMUNOLOGIC REACTIVITY OF MICE

R. K. Andreson

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Experiments on mice have shown that antilymphocyte serum considerably retards the development of transplantation immunity and also inhibits the formation of antibody-producing cells in response to primary injection of sheep's erythrocytes.

Recent investigations have shown that heterologous antilymphocyte serum (ALS) influences several immunologic phenomena in animals of different species and has a definite immunodepressive action. The survival period of a skin homograft in rats [13, 14] and mice [4, 8-10] was considerably prolonged by means of ALS. In conjunction with other immunodepressors, ALS has been used successfully in clinical trials during kidney homotransplantation operations [11, 12]. ALS also suppresses the formation of humoral antibodies against certain antigens in various animals, notably against sheep's erythrocytes in rats and mice [1, 4, 10]. The results of these experimental and clinical studies suggest that ALS is one of the most promising immunodepressants.

The object of this investigation was to examine the immunodepressive action of rabbit antiserum against mouse lymphocytes on transplantation immunity and on the primary response to injection of sheep's erythrocytes into adult mice.

EXPERIMENTAL METHOD

The experiments were carried out on adult male mice of lines A and CBA, differing genetically with respect to the powerful H-2 tissue incompatability locus.

Rabbit ALS was prepared by the method of Levey and Medawar [8] with slight modifications. Suspensions of thymus cells and lymph gland cells of adult line A mice, washed 3 times by centrifugation, and made up in Hanks' solution, were injected intravenously into rabbits in two doses at intervals of 14 days ($1 \cdot 10^9$ cells per course). Seven days after the second injection, blood was taken. The serum obtained from it was heated to 56° for 30 min and absorbed with thrice washed mouse erythrocytes in the proportion of 3:1. The titer of the ALS thus obtained was 1:256 in the leukoagglutination test.

In experiments to study the effect of ALS on transplantation immunity, the ALS was injected subcutaneously into mice in four doses, each of 0.25 ml, at intervals of 3 days, starting on the second day after skin grafting. The control mice received a similar course of injections of normal rabbit serum. The recipients of the skin were line A mice and the donors line CBA mice. Skin was grafted by the method of Billingham and Medawar [2].

In the experiments to study the effect of ALS on the formation of humoral antibodies against sheep's erythrocytes, ALS was injected into line A/He mice intraperitoneally in two doses, each of 0.25 ml, at an interval of two days, at different times before the test injection of sheep's erythrocytes (on days -24, -22,

Laboratory of Immunological Tolerance, Department of General and Radiation Immunology, N. F. Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR G. V. Vygodchikov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 69, No. 3, pp. 84-87, March, 1970. Original article submitted June 20, 1969.

TABLE 1. Effect of ALS on Production of Antibody-Forming Cells in Mice during Immunization with Sheep's Erythrocytes

Time of injection of ALS (days)*	Number of mice	Number of nucl. cells in spleen (millions)	Number of antibody-forming cells and confidence limits	Titer of antibodies†	
				homolysine	hemagglutinins
−24, −22	6	302±40	5 781 (2 301–14 520)	2,4±0,7	7,8±0,4
−16, −14	14	283±46	4 498 (3 076–6 577)	2,2±0,4	7,7±0,2
−2, −0	14	270±40	3 707 (2 576–5 333)	1,3±0,3	4,5±0,2
0, +2	8	229±55	19 320 (10 640–55 590)	6,5±0,3	5,2±0,7
Control	8	325±67	88 720 (57 280–137 400)	6,7±0,3	8,0±0

*The sign - denotes serum injected at the corresponding time before injection of antigen, the sign + denotes injection of serum after antigen.
†In logarithms to base 2. Initial dilution of serum (1:10) taken as 1.

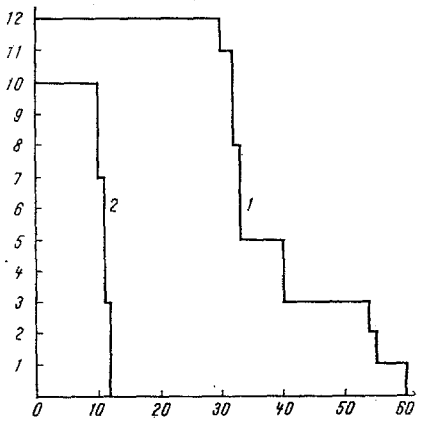

Fig. 1. Effect of ALS on rejection of allogenic skin graft in mice. Abscissa, days after transplantation; ordinate, number of mice retaining graft. 1) Animals receiving ALS; 2) control mice.

-16, -14, -2, and 0) or after the test injection (days 0 and + 2).* The control animals received 0.25 ml of normal rabbit serum on days -2 and 0. On day 0 all groups received the test injection of sheep's erythrocytes ($5 \cdot 10^8$ cells intravenously). On the 4th day after immunization the spleen was taken from the mice and the local hemolysis in gel test carried out by the method of Jerne and Nordin [6]. The titers of hemolysins and hemagglutinins in the blood sera were determined by parallel tests in the usual manner. The results of these experiments were subjected to statistical analysis.

EXPERIMENTAL RESULTS

The results are shown in Fig. 1 and Table 1. In the experiments to study the effect of ALS on transplantation immunity (Fig. 1), the life of the skin homograft was increased by a statistically significant amount in the mice treated with ALS compared with the control animals ($P < 0.001$). In most experimental animals the grafted skin was rejected between the 37th and 43rd days after transplantation. In some animals rejection of the skin was observed between the 50th and 60th days. In the control animals the mean time of rejection of the skin was on the 11th day after grafting.

In the experiments to study the effect of ALS on antibody formation against sheep's erythrocytes (Table 1), a significant decrease in the number of antibody-forming cells was obtained in the spleens of animals receiving ALS. Maximal suppresion was observed in animals receiving ALS on days -2 and 0 relative to the test injection of sheep's erythrocytes (mean 3707 antibody-forming cells per spleen; control 88,720). The titers of serum hemolysins and hemagglutinins were correspondingly reduced in the mice receiving ALS (Table 1).

It can be concluded from these results that ALS retards the development of transplantation immunity and also reduces the number of antibody-forming cells during immunization with a corpuscular antigen. It will be noted that the ALS which was used was given in a comparatively small dose (in the experiments of series I, 1 ml per course, and in those of series II, 0.5 ml per course).

It can be postulated on the basis of these results that ALS is most effective when given to animals shortly before the antigenic stimulus (on days -2 and 0 relative to injection of sheep's erythrocytes) or on the days immediately after antigenic stimulation (starting from the 2nd day in experiments to study transplantation immunity), in agreement with results obtained by other workers [8, 9].

*The signs - and + denote that the corresponding days are before or after immunization; 0 represents the day of immunization.

Several theories have been put forward to account for the mechanism of action of ALS, the most popular being that the immunodepressive properties of ALS are due to its direct cytotoxic action on lymphocytes (the cytotoxic theory) [5, 10].

According to the "blinding" theory, antilymphocyte antibodies cover the reactive zones of the lymphocytes and thus prevent identification of the antigen by the lymphocytes [8, 9]. This theory is closely related to the theory of the competing antigen, based on the rapid and selective uptake of antilymphocyte antibodies by the lymphoid tissue of the body. Since these antibodies are themselves antigens, the lymphoid tissue cells ingesting them are no longer able to respond to the action of other antigens with a less selective action [3, 7].

The total number of nucleated cells in the spleens of the experimental animals was only a little smaller than in the control (Table 1), and this index showed no correlation whatever with changes in immunologic reactivity in the various groups of experimental animals. This evidently indicates that the immunodepressive effect of the doses of ALS used is unconnected with the cytotoxic action of ALS on the spleen cells. The mechanism of action of ALS on the immunologic reactivity of the animals in this case can be considered to be based on temporary afferent blockade of reactive zones of the lymphoid tissue cells by antilymphocyte antibodies and with the inability of these "blocked" lymphocytes to receive the antigenic stimulus and to give the corresponding immune response. This hypothesis would explain the fact that injection of ALS before injection of antigen is more effective. However, effective suppression of transplantation immunity in cases when ALS was injected after skin grafting evidently requires further assumptions.

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