

# ACTION OF ANTILYMPHOCYTIC SERA ON THE PRIMARY AND SECONDARY IMMUNE RESPONSE IN EXPERIMENTS ON ANIMALS

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The action of rabbit antilymphocytic serum (ALS) against mouse lymphocytes on the development of the primary and secondary response was investigated in CBA mice. Injection of ALS was found to inhibit the immune response of the animals for a long period if given not only immediately before immunization but also 2-3 weeks after (sheep's red cells or egg albumin in Freund's adjuvant was used as the antigen). Injection of ALS before primary immunization reduces the ability of the animals to give a secondary response to injection of red cells at long periods after primary immunization. It is postulated that ALS injures cells which are capable of receiving antigenic stimulation for a period of at least three weeks, and also inhibits the formation of the immunological memory.

Antilymphocytic serum (ALS) is a powerful immunosuppressive agent which acts on the early stages of the immunological process [6, 7]. ALS is considered to be active for only a relatively short time after injection. For instance, its inhibitory action on antibody production has been detected if injected between 5 and 1 days before application of the antigen. Injection of the serum at various times after injection of the antigen does not significantly reduce the effect of immunization [2-5] or of revaccination with the same antigen [1].

This paper describes the results of a study of the action of ALS on primary and secondary immunity in mice immunized with various antigens.

## EXPERIMENTAL METHOD

Experiments were carried out on CBA mice weighing 16-18 g. The animals of this line proved to be more sensitive to ALS in preliminary experiments than mice of line CC57BR. Sheep's red cells and twice recrystallized egg albumin, injected in Freund's complete adjuvant containing 3 mg dried, killed Mycobacterium tuberculosis cells, were used as the antigens. Antibodies against sheep's red cells were detected by the agglutination test, and antibodies against egg albumin by the passive hemagglutination test.

The ALS was prepared by intravenous injection of lymphocytes from the lymph glands of mice or of thymocytes into rabbits in doses of 300 million cells three times a week for three weeks. The antisera were exhausted with mouse red cells and serum, after which their titers in the lymphocyte agglutination test were 1:1000-1:2000. The immunosuppressive activity of the sera was assayed from the inhibition of antibody production against sheep's red cells in mice receiving subcutaneous injections of the serum three days before immunization. The sera used in the experiments lowered the antibody titers compared with the control by at least 10 times in response to a single dose of 0.4-0.6 ml ALS.

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TABLE 1. Manifestation of Immunosuppressive Action of ALS Depending on Time of Its Administration before Injection of Antigen

Time of injection of serum before immunization (in days)	Antigen used for immunization	Reciprocals of blood antibody titers in mice receiving	
		ALS	normal serum
27 17 7	Sheep's red cells	320 40 0	1280 1280 1280
40,38 25,23 20 18,15 9,7	Egg albumin in Freund's adjuvant	320 20 13 0 0	160 160 320 480 160

Note: Titers of antibodies in a mixture of sera obtained from not less than 4 animals for each test are given in this table. Antibodies against sheep's red cells were detected on the 11th day, and against egg albumin on the 27th day after immunization.

TABLE 2. Antibody Level in Mice treated with ALS before Primary Immunization in Response to Injection of the Same Dose of Sheep's Red Cells

Dose of antigen	Serum	Mean antibody titers 5 days after	
		primary immunization	reimmunization
10 <sup>6</sup>	ALS	0	187±71,8
	Normal	0	1920±369,5
10 <sup>9</sup>	ALS	0	200±66
	Normal	320±0	8530±1735

TABLE 3. Revaccination Response to a Subimmunizing Dose of Sheep's Red Cells in Mice Receiving ALS 3 Days before Primary Immunization

Time of immunization (in days)	Titers of agglutinins in blood of mice receiving	
	ALS	normal serum
10	10±5,6	70±14
35—40	228±42,4	1330±320
60	80±0	440±120
90	85±75,7	480±154,3

## EXPERIMENTAL RESULTS

To determine the duration of the immunosuppressive effect of ALS it was injected once or twice on alternate days into mice at different times before immunization with a corpuscular antigen (sheep's red cells in a dose of  $1 \times 10^9$ ) or a soluble protein antigen (egg albumin in a dose of 2 mg) in 0.1 ml of Freund's adjuvant. As Table 1 shows, a single injection of ALS at various times within one month before immunization with sheep's red cells reduced to some extent the level of the immune response. ALS had a similar action on antibody production induced by a single immunization with egg albumin in Freund's adjuvant. Similar experiments with bovine serum albumin also showed that injection of the serum between 5-2 and 23-21 days before immunization inhibited the animals' immune response.

The duration of suppression of the immune response induced by injection of the serum before immunization is noteworthy. The results showed that the antibody level remained constantly reduced for more than 60 days. The antibody level in the experimental animals remained consistently low compared with the corresponding controls regardless of the time before immunization that the serum with the immunosuppressive action was injected.

To determine how the suppression of the primary response by ALS is reflected in the ability of the animal to respond to a second antigenic stimulation, the animals were prepared by two different doses of antigen and they were given the same dose of antigen at revaccination. For instance, one group of mice received  $1 \times 10^6$  sheep's red cells, i.e., a dose which, on primary injection, induced weak antibody production (not more than 1 : 20), while the other group of mice received  $1 \times 10^9$  red cells which induced a marked immunological response. In these experiments half the mice of each group received an injection of ALS

three days before the injection of the antigen. Revaccination was carried out six weeks later. It will be clear from Table 2 that in every case the increase in antibody titer was much weaker after the second injection of the antigen in the mice prepared with ALS before the first immunization than in the control animals.

Suppression of the ability to give a secondary response also was well-marked at different times after primary immunization (Table 3). This indicated that the effect was due, not to the direct action of the serum injected before the first immunization, but to a disturbance of the formation of the immunological memory.

The results described show that injection of ALS had a prolonged inhibitory action on immunologically competent cells which lasts about 3 weeks. The fact will be noted that the reactivity of cells not stimulated by antigen was suppressed. The suppressive action of ALS is also reflected on the formation of the immunological memory, as the result of which the effect of revaccination with different doses of antigen, injected 40 or even 90 days after the primary immunization, was sharply reduced.

This is the first time that such a prolonged effect of a single or double injection of ALS has been described in the accessible literature. This fact, in the writer's opinion, is of great interest both for the evaluation of the action of ALS and for the study of the properties of the immunocompetent cells participating in the immunological response of the organism.

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