PRODUCTION OF IMMUNOLOGICAL TOLERANCE IN ADULT MICE TO PROTEIN ANTIGEN WITH THE AID OF ANTILYMPHOCYTIC SERUM

O. A. Karasik and B. N. Sofronov

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Tolerance to egg albumin was reproduced in adult CBA mice after a single injection of antilymphocytic serum and a large dose (1-25 mg) of antigen. The tolerant animals were incapable of an immunological response for four weeks after preparation, and no antibodies appeared in them for 1.5-2 months after immunization with Freund's adjuvant. The resulting tolerance was specific, its duration was proportional to the dose of inducing antigen, and the reactivity of the lymphoid cells of the tolerant animal was suppressed if the material transferred was syngeneic.

The possibility of producing and analyzing tolerance to protein antigens of various types is of great interest to the discovery of the mechanisms of immunological tolerance. Only a few experiments to induce tolerance with the aid of antilymphocytic serum (ALS) to bovine serum albumin in mice [4] and to flagellin in rats [5] are known to have been successful.

The possibility of reproducing tolerance to egg albumin in CBA mice was studied. The production of tolerance in adult animals to this antigen is difficult because egg albumin is a highly immunogeneic and phylogenetically remote antigen for mice.

EXPERIMENTAL METHOD

Experiments were carried out on female CBA mice weighing 16 g. The ALS was prepared by intensive immunization of rabbits [1]. Tolerance was induced by subcutaneous injection of ALS a few days before the injection of antigen. To detect tolerance, 2 mg egg albumin in Freund's adjuvant was injected subcutaneously into the experimental and corresponding control mice at various times (14-35 days) after treatment. The absence of antibodies in the experimental animals when subsequently tested at various times, while they were present in the control mice, was used as the indicator of tolerance. Antibodies were determined in the blood sera by the passive hemagglutination method [2] using formalinized red cells and double dilutions of serum, starting with 1 : 40.

EXPERIMENTAL RESULTS

As Table 1 shows, immunological tolerance developed in mice receiving large doses of antigen (25, 10, or 1 mg) intraperitoneally after a single subcutaneous injection of ALS in a dose of 0.5-0.6 ml 2-3 days before the injection of antigen (the optimal time for obtaining an immunosuppressive effect). The duration of the ensuing tolerance was seen to increase with the size of the inducing dose of antigen and was maximal with a dose of 25 mg. In every case areactivity was superseded by the appearance of antibodies in late stages after the testing injection. However, their level was considerably lower than in the corresponding control animals. A further injection of antigen (10 mg) into mice after the loss of their tolerance led to an

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TABLE 1. Dependence of Development of Tolerance and Its Duration on Dose of Inducing Antigen (M±m)

Group of mice	Dose of antigen (in mg)	Reciprocals of antibody titers at various times (in days) after test injection of antigen			
		32	41	53	74
Experimental (ALS)	25	0	0	0	100±60
	10	0	0	220±50	370±143
	1,	0	960±323	3 200±2 000	640
	0,1	160	1040±500		
	0,01	100±60	960±323		
	—	130±34	1730±955	560±265	
Control (normal serum)	25	960±323	1920±646	10 240±0	20 000±12 000
	10	760±190	370±190	8 500±1 700	20 000±12 000
	1	640±0	1920±646	25 000±15 000	20 000
	0,1	480±161	4200±2250		
	0,01	400±242	1920±646		
	—	480±73	1600±750	3 840±750	3 500±1 700

TABLE 2. Result of Repeated Injection of Antigen into Animals That Have Lost Their Tolerance (M±m)

Preparation of animals		Blood antibody level (after creation of tolerance)		
Serum	Dose of antigen (in mg)	70th day	70th day	90th day
ALS	25	0	Repeated injection of egg albumin	60±20
	10	200±121		480±161
	—	960±323		3 840±1 285
Normal	25	10 000 ±0	(10 mg)	20 000±12 000
	10	10 000 ±0		20 000±12 000
	—	5 000 ±0		9 000±6 850

increase in their blood level of antibodies, but in this case also their titers were significantly lower than in the corresponding control animals (Table 2).

An important factor for the induction of tolerance using ALS was the interval between injection of the serum and antigen. Injection of ALS 2-7 days before injection of the antigen was found to be effective; injection of the serum 12 or more days before the antigen, on the other hand, at best only lowered the titer of antibodies in the experimental animals by comparison with the corresponding control.

To determine the duration of tolerance experiments were carried out in which individual groups of experimental mice were tested for tolerance 14, 21, 28, and 35 days after the inducing injection of antigen. In the first three cases all the animals were found to be tolerant; i.e., they did not respond to immunization.

The ability of the mice in the various series of experiments to produce antibodies against sheep's red cells in the same titers as the control animals is evidence of the specificity of tolerance to egg albumin.

An experiment was carried out in which lymphoid cells were transplanted from immune, normal, and tolerant animals into syngeneic recipients treated with a large dose of cyclophosphamide (2.5 mg/10 g body weight) to suppress their own immunological activity. The results showed that injection of $4.5 \cdot 10^7$ lymphoid cells from the lymph glands and spleen, mixed with 10 mg egg albumin, into the recipient led to a marked immunological response only if immune animals were used as donors. In the analogous experiment with cells from tolerant mice antibodies appeared in the recipients' blood, but their titer was 16 times lower than in recipients receiving the cells from an immune animal (1 : 80 and 1 : 1280, respectively). In the mice of the three control groups, which received cells from immune donors without the addition of antigen, antigen only, or cells of normal donors with antigen, no antibody production was observed.

These experiments showed that injection of ALS before antigen enabled tolerance of the animals to injection of antigen in Freund's adjuvant to be produced. The character and duration of the tolerance corresponded to indices of tolerance obtained to serum protein antigens in adult mice [3]. Tolerance occurred only if large doses of antigen were injected, so that it can be classed as "high-dose" tolerance. The possibility of creating tolerance with low doses of egg albumin is a problem requiring further study. The results so far obtained confirm the view that immunological tolerance can be obtained by means of ALS to any (including a powerful) phylogenetically remote antigen.

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