IMMUNOLOGIC TOLERANCE IN ADULT ANIMALS TO PROTEIN ANTIGENS TREATED BY BIOLOGICAL FILTRATION

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The blood serum of mice receiving bovine serum albumin by intravenous injection, when injected into fresh mice, led to the development of specific immunologic tolerance to this antigen in the recipients. The optimum dose of albumin injected into donor was 0.125-5 mg. The most marked effect was given by serum taken from the donors 1.5-3 h after injection of the protein. Tolerance in mice receiving the serum persisted up to 50 days. After this time antibody production was observed without additional injection of antigen.

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When studying the role of macrophages in the induction of the immunologic response, Frei and co-workers [3] showed in 1965 that bovine serum albumin (BSA), present in the blood serum of rabbits 48 h after its injection, can induce tolerance in normal animals. This confirmed the view that the immunologic response is induced by the phagocytosed antigen fraction. The phenomenon described was reproduced by Sofronov [1], who also showed that the period of circulation of the antigen in the organism necessary for the tolerance-inducing effect can be reduced to a few hours. The phenomenon was also produced in mice [4].

The object of the present investigation was to study the conditions for production of tolerance in adult mice, to determine the optimum period of circulation of the antigen in the donor and the optimum dose of antigen, and also to study the developing immunologic tolerance in greater detail.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred albino mice weighing 16-18 g. Groups of donor mice consisted of 8-12 animals. The antigen consisted of BSA (Koch-Light Laboratories, England). An active immunologic response was produced in the mice by subcutaneous injection of 5 mg albumin in Freund's adjuvant. Each experimental group consisted of 4 or 5 mice. The sera obtained from them were pooled.

To detect antibodies in the recipients' sera, Boyden's passive hemagglutination reaction [2] was carried out with formalinized sheep's erythrocytes [5]. Antigen in the donors' sera was determined by the inhibition reaction.

EXPERIMENTAL RESULTS

The experiments began with induction of tolerance by administration of antigen treated by biological filtration. The four groups of mice each received 5 mg BSA intravenously. The animals were exsanguinated 1.5, 3, 6, and 24 h later and their sera injected intravenously into experimental recipient mice. Control animals were injected with albumin mixed with normal mouse serum. Concurrently the donors' sera were tested for their content of BSA capable of reacting in vitro with the corresponding antiserum. It was found that the sera obtained 1.5, 3, and 6 h after injection of antigen into the donors contained the same concentration of antigen (2 mg/ml), rising after 24 h to 0.5 mg/ml. Consequently, the dose injected into the recipients in a volume of 0.5 ml serum was 1.0 and 0.25 mg,respectively (Table 1). On the 7th day after injection of sera containing the antigen, all the animals and also the group of mice not receiving preliminary injection of serum, were immunized with BSA in adjuvant. Blood samples thereafter were taken repeatedly

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TABLE 1. Antibody Titer in Mice Treated with BSA Present in Sera Obtained at Various Times after Injection of Antigen into Donors

Duration	Dose of BSA injected into recipient (in mg)	Time of investigation (in days)					
		21	33	39	45–50	70	78
1½	1	0	0	0	2,9	4,4	5,8
3	1	0	0	2,3	2,9	5,0	6,4
6	1		2,9	4,4	5,0	5,8	6,4
24	0,25	2,3	4,4	5,0	5,8	5,8	6,4
—	—	5,0	6,4		5,8		

Note. Here and in Tables 2 and 3, antibody titers in Boyden's reaction are given in $\log_2$.

TABLE 2. Antibody Titer in Sera of Mice Receiving Different Doses of "Filtered" and Native BSA

Dose of BSA injected into donor (in mg)	Dose of BSA injected into recipient (in mg)	Time of investigation (in days)					
		21	36	40	45–50	53	68–73
25	8	4,4	4,4	4,4	5,8		
5	0,8	0	0	0	2,9	3,7	4,4
1	0,1	0	0	0	0	3,7	4,4
0,125	0,02	0	0	0	2,3		5,0
—	Normal mouse serum	4,4	5,0	5,8	7,1	6,4	7,1
—							
—	25	5,8	6,4	6,4	5,8	6,4	
—	1	0	3,7	5,0	6,4	5,0	6,4
—	0,1	0	2,3	3,7	4,4	2,9	2,9
—	—	0	4,4	6,4	7,1	6,4	7,1

from the mice and antibodies determined. Antibodies appeared 21 days after immunization of the control mice and also of the animals receiving "24-h" serum. The time of appearance of antibodies was delayed up to 33 days in the case of mice receiving the "6-h" filtrate. The use of antigens exposed to a shorter "filtration" (3 and 1.5 h) led to absence of an immunologic response (tolerance) for 45-50 days. After this period, antibodies could be found in all the animals without additional immunization.

In the next series of experiments the effect of the dose of antigen injected into the donors on development of tolerance in recipients was studied. Donor mice were injected with different quantities of BSA – from 25 to 0.125 mg. The BSA level in the serum after 3 h was from 16 to 0.04 mg/ml. Recipients were injected with 0.5 ml of donors' sera, i.e., with 8.0, 0.8, 0.1, or 0.02 mg antigen (Table 2). Control animals received various doses of native BSA mixed with 0.5 ml normal mouse serum. Injection of "filtered" antigen into the recipients in a dose of 0.8-0.02 mg led to a state of tolerance for up to 45-50 days. In animals injected with donor's serum containing 8 mg BSA antibodies were formed on the 21st day after immunization. In the control animals they appeared after 21-29 days.

The results of experiments carried out to demonstrate the specificity of the developing tolerance are given in Table 3. They show that in mice receiving preliminary injections of "filtered" BSA no antibodies were produced against this antigen, but large quantities of antibodies were formed against egg albumin and the antigens of bovine serum except the albumin to which the animals were tolerant.

Antigen present in the blood sera of adult mice 1.5-3 h after injection thus differs essentially from the original antigen in its ability to induce an immunologic response. Injection of donors' serum containing such antigen produces a state of tolerance lasting 45-50 days, after which, without additional antigenic stimulation, it comes to an end and antibody production begins. This indicates that the organism in a state of tolerance possesses an adequate quantity of immunogenic substance, but the mechanisms of the immunologic response are temporarily suppressed.

Dose of antigen is the essential factor for production of tolerance. Injection of 0.125-5 mg BSA into a donor endows the donor's serum with the property of producing tolerance. The use of a large dose (25 mg) does not have the desired effect, because this dose of antigen is probably too much for the organism to absorb and to process.

TABLE 3. Specificity of Immunologic Tolerance Obtained by Biological Filtration of BSA

Antigen injected preliminarily into recipients	Antigen injected in adjuvant after 7 days (5 mg)		Time of investigation (in days)				
			18	28	33	43	48
"Filtered" BSA (0.1 mg per mouse)	BSA	BSA	0	0	0	0	0
	Bovine serum	BSA	0	0	0	0	0
		Bovine serum	4.4	5.8	7.1	6.4	5.0
	Egg albumin	BSA	0	0	0	0	0
		Egg albumin	5.0	5.8	6.4	5.8	5.8
Native BSA (0.1 mg per mouse)	BSA	BSA	0	2.3	5.0	6.0	
	Bovine serum	Bovine serum	5.8	5.8	7.1	6.4	
	Egg albumin	Egg albumin	4.4	5.8	5.8	5.0	

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