SHORT COMMUNICATIONS

ACQUIRED IMMUNOLOGICAL UNRESPONSIVENESS TO BOVINE PLASMA ALBUMIN IN MICE*

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It has been suggested by Cinader (1961) and Dresser (1961) that the failure to demonstrate specific unresponsiveness, or the production of partial unresponsiveness, by the injection of the antigen prior to or soon after birth could be due to the presence of impurities in the antigens used. An animal injected neonatally with sufficient principal antigen to induce unresponsiveness would retain reactive competence to the impurities. Subsequent challenge would elicit no antibodies to the principal antigen but only to the impurities. In order to test this simple hypothesis the following studies, using bovine plasma albumin (B.P.A.) (Armour Fraction V) as an antigen, were undertaken with two-dimensional gel diffusion analysis of the specific antigen/antibody reactions of the albumin and its impurities. The methods developed by Sobey and Magrath (unpublished data) were used to measure antibody response to the principal antigen (B.P.A.–albumin) and the impurities (B.P.A.–impurities). All titres are given in a $\log_2$ scale.

In attempting to induce unresponsiveness, mice were injected with antigen within 24 hr of birth, one-third of each dose being given in incomplete Freund's adjuvant and two-thirds in normal saline. All injections were subcutaneous. A group of control mice was injected with normal saline and with normal saline in incomplete Freund's adjuvant.

Two experiments, essentially similar in their neonatal treatment but differing in the method of sensitization, were run (Tables 1 and 2). In the first experiment animals at 15 weeks of age were sensitized with two injections of 5 mg B.P.A. in Freund's adjuvant spaced by 14 days, followed 3 weeks later by an intraperitoneal injection of 8 mg alum-precipitated B.P.A.; 7 days later they were bled. The same animals were re-injected intraperitoneally when 28 weeks old with 8 mg alum-precipitated B.P.A. and bled 1 week later. Animals in the second experiment were sensitized with two intraperitoneal injections of 8 mg alum-precipitated B.P.A. spaced by 14 days, and bled 7 days after the last injection. All sera were examined for the presence of antibodies to B.P.A.–albumin, B.P.A.–impurities, and for circulating antigen.

All the sera in the first experiment showed the presence of antibodies to B.P.A.–impurities. Only two of the sera failed to show the presence of antibodies against B.P.A.–albumin, and only one of these (the one from group 3) showed circulating B.P.A.–albumin. It is clear that the degree of unresponsiveness increases with increasing neonatal dose of B.P.A. up to 8 mg. The slightly lower mean titre in

* Manuscript received May 21, 1965.

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TABLE 1

TITRES OF SERA FROM MICE NEONATALLY INJECTED WITH B.P.A. AND SENSITIZED WITH B.P.A. IN FREUND'S ADJUVANT AND ALUM-PRECIPITATED B.P.A

Group No.	Neonatal Dose of B.P.A. (mg)	Age when Sensitized (weeks)	Log_2 Serum Titres against B.P.A.–Albumin																Final Mean Titre
			Female Mice							Mean	Male Mice								Mean	
1	0·4	15	7·0	7·0	4·0	4·0	7·0	5·5	4·0	5·5	3·0	6·0	3·0	7·0	4·5	5·0	5·0	6·0	4·9	5·2
		28	6·0	6·5	4·0	3·0	6·0	5·0	4·0	4·9	2·0	6·0	2·0	6·0	4·0	5·0	6·0	5·5	4·5	4·7
2	4·0	15	1·0	5·5	6·0	7·0	6·0	5·5		5·1	5·0	3·0	3·0	4·0	1·5	6·0			3·8	4·5
		28	1·0	3·5	4·0	7·0	5·0	5·0		4·3	4·0	3·0	3·0	3·0	1·0	4·0			3·0	3·6
3	8·0	15	3·5							3·5	4·5	2·0	3·0	2·0		0†			2·3	2·5
		28	4·0							4·0	5·0	2·5	2·0	1·0		0†			2·1	2·4
4	20·0	15	5·5	4·0	5·5	0				3·7	4·0	1·0	3·0	1·0					2·2	3·0
		28	5·5	5·0	4·0	*				3·6	4·0	1·0	2·0	1·0					2·0	3·1
5	None (saline)	15	5·0	7·0	5·0	6·0	6·0	6·0		5·9	6·0	6·0	6·0	6·0					6·0	5·9
		28	4·0	7·0	5·5	6·0	5·0	6·0		5·6	4·0	6·0	6·0	5·0					5·2	5·5

* This animal was not tested a second time since it had been mated to the "negative" male in group 3.

† This was the only animal to show circulating B.P.A.–albumin.

group 3 as compared to group 4 is probably due to the deficiency of females, it being generally found (Sobey and Adams 1955) that female mice have a better capacity than males for producing antibodies. The variation in antibody response of the neonatally treated mice is much greater than that in the controls (a variance ratio, V_t/V_c, for 35 treated (t) and 9 control (c) mice $= 8{\cdot}5$, $P<0{\cdot}01$).

In the second experiment (Table 2) the mice in the two groups neonatally injected with 20 and 4 mg respectively behaved similarly over all ages of sensitization, and showed about 45% (9 out of 20, and 8 out of 18, respectively) complete unresponsiveness. Those sensitized at 12 and 16 weeks of age and which produced antibodies to B.P.A.–albumin had a reduced titre as compared to controls. The titres to B.P.A.–albumin in the group neonatally injected with 13·5 mg of alum-precipitated B.P.A. are reduced with respect to the controls. Although the numbers in this group are limited, none became fully unresponsive; in contrast to the 20-mg and 4-mg groups and similar to the controls they produced a strong reaction to B.P.A.–impurities. While the 20-mg and 4-mg groups showed a generally weaker reaction to B.P.A.–impurities than the other two groups, it is nevertheless clear that some animals completely unresponsive to B.P.A.–albumin did produce antibodies to B.P.A.–impurities, giving as strong a reaction as controls in those sensitized at 16 weeks. The animals that died on the second sensitizing injection did so of anaphylactic shock, thus it can be assumed they were producing antibodies. While it is probable that the antibodies causing anaphylaxis were to B.P.A.–albumin, those to the impurities could also be implicated (see Sobey, Reisner, and Adams 1962).

Neonatally injected mice sensitized with B.P.A. in Freund's adjuvant and alum-precipitated B.P.A. at 15 weeks of age resulted in only two out of 41 mice becoming fully unresponsive to B.P.A.–albumin. The mean level of antibody response in all groups of neonatally injected mice was lower than that of controls, and inversely related to the size of the neonatal dose. All of the animals in this experiment produced antibodies to the impurities in B.P.A. A much higher proportion (17 out of 44) of the neonatally injected animals became completely unresponsive to B.P.A.–albumin when the sensitizing injection was alum-precipitated B.P.A.; and only 18 of the animals had impurity antibodies. The level of antibody response among those of the neonatally injected animals which did respond was markedly lower than controls. The differences in the number of mice rendered unresponsive, and the number producing impurity antibodies in these experiments, probably reflects the relative efficiencies of the two adjuvants in stimulating antibodies: this poses the question of what sensitizing regime may be considered adequate to reflect a true picture of the degree of unresponsiveness?

The mice used in the present work were randomly bred, and a variable antibody response would be expected (Sang and Sobey 1954; Sobey and Adams 1961). However, the control mice (Table 1) which did not receive neonatal antigen were much less variable than any of the groups that did. This suggests that animals were differentially affected by a given neonatal dose of antigen, i.e. that some animals are more likely to become unresponsive than others because of differences in sensitivity which could be genetic. A selection line was started with the "negative" mice from the first experiment (Table 1). Selection, which was based on adult animals which

TABLE 2

TITRES OF SERA FROM MICE NEONATALLY INJECTED WITH B.P.A. AND SENSITIZED WITH ALUM-PRECIPITATED B.P.A.

+, strong band formation; ±, weak band formation; −, negative; D, died following second sensitizing injection

Age at Sensitization (weeks)	Sera Tested for Antibodies to	Neonatal Treatment			
		Saline Controls	20 mg B.P.A. in Incomplete Freund's Adjuvant and Saline	4 mg B.P.A. in Incomplete Freund's Adjuvant and Saline	13·5 mg B.P.A. (alum-precipitated)
8	B.P.A.–albumin	1 D D D D	0 0 0 1 1 1	0 0 1 0 1 1	1 1·5
	B.P.A.–impurities	+	± − − ± − ±	− − − − + −	+ +
12	B.P.A.–albumin	3 4 4 4 4 D D	0 1 1 1 2 0 D	1 3 0 0 1 0	1·5 1
	B.P.A.–impurities	+ + + + +	− − − − + −	− + ± − + −	+ +
16	B.P.A.–albumin	3 3 5 1 D D D	0 0 2·5 0 0 D D	1·5 0 0 2 2 D	1 2
	B.P.A.–impurities	+ + + +	+ − + + −	+ + + + +	+ +

were *not* subjected to neonatal injection and which failed to produce antibodies to a sensitizing regime similar to that in the first experiment, has shown an increasing number of "genetic negatives".

Whatever the underlying mechanism of unresponsiveness may be, it is clear that the neonatal injection of relatively large amounts of B.P.A. (0·4–20 mg in this case) has a prolonged inhibitory effect (up to 16 weeks in this work) on the ability of animals to produce antibodies specific to B.P.A.–albumin. The degree of inhibition is dependent on the amount of B.P.A. neonatally injected, the type of sensitizing injection used (i.e. the type of adjuvant), and probably on the genetic constitution of the animal. Antibody response to the impurities in B.P.A. does not appear to be affected by the above neonatal treatment, and animals rendered completely unresponsive to B.P.A.–albumin in most cases remained immunologically competent to B.P.A.–impurities.

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