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BRIEF COMMUNICATION

Genetically Controlled Specific Immunological Unresponsiveness

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Summary. Evidence is presented that in rabbits and mice a relatively simple genetic mechanism exists which controls the animals' ability to produce antibody against bovine serum albumin.

Sang and Sobey (1954) reported the case of a rabbit failing to produce antibodies to bovine serum albumin Armour Fraction V (BSA) after two 20-mg intravenous injections. In addition they demonstrated the presence of circulating BSA 29 days after the last injection. A similar rabbit was found by Sobey and Reisner (unpublished data) during the routine production of BSA-antiserum in fifty rabbits. The rabbits were sensitized with BSA in Freund's adjuvant (25 mg in five sites) boosted after 3 weeks with 40 mg BSA given intravenously and bled after a further week. The 'negative' buck was mated to two does which produced eight offspring, all of which produced antibodies to BSA. Inter-crosses were made between these animals, and the does were later back-crossed to the 'negative' buck. The results of these matings are given in Table 1. These results suggest the 'negative' phenomenon to be genetically controlled. However, they do not fit a single gene model and it may be surmised that two or three genes are involved. Attempts to select for a 'negative' line of animals were abandoned when none of the 'negative' does succeeded in producing live offspring. The cause of the infertility was not determined. The 'negative' rabbits behaved normally in their response to stimulation with egg albumin, bovine γ-globulin and to the impurities in the BSA fraction.

TABLE 1

THE RESULTS OF INTER-CROSS AND BACK-CROSS MATINGS BETWEEN A 'NEGATIVE' RABBIT BUCK AND HIS OFFSPRING FROM TWO NORMAL DOES

Type of mating	No. of 'negative' (circulating BSA)	No. of 'positive' (no circulating BSA)	Total
Inter-cross	6 (4 males, 2 females)	50	56
Back-cross	10 (4 males, 6 females)	55	65

During experiments on immunological unresponsiveness to BSA in mice (Sobey and Magrath, 1965a) a mouse suspected of the above 'negative' phenomenon was found, and selection for a 'negative' line of mice was started. The mouse concerned, a buck, was one of fifty which had been injected with BSA, as previously described (Sobey and Magrath,

1965a). He was the only mouse to show circulating BSA, and one of two which failed to produce measurable antibodies to BSA. Testing for the presence of antibodies to BSA, to the impurities in BSA and for circulating BSA was carried out as described by Sobey and Magrath (1965b). The criterion for selection was the absence of circulating antibodies to BSA using the above injection regime of Sobey and Magrath (1965a) in mice 12–16 weeks old which had had no previous contact with the antigen. The presence of circulating BSA was noted but not used as a criterion for selection. The selection line was started from the progeny of five out-cross and four back-cross matings, of the original buck, shown in Table 2. These data do not fit a single gene model and suggest the involvement of two or three genes. In the original randomly bred strain of mice, no 'negative' mice were found in 200 tested.

TABLE 2

THE RESULTS FROM MATING BETWEEN A 'NEGATIVE' MALE MOUSE TO INBRED LINES, A RANDOM LINE FROM WHICH HE CAME, AND HIS F_1 OFFSPRING

Type of mating	No. of 'negatives' (no antibody to BSA)	No. of 'positives' (antibody to BSA)	Total	No. of matings
Out-cross to inbred lines Swiss and CBA	0	13	13	2
Out-cross within random line	1	19	20	3
Back-cross to original negative male	4	28	32	4

All subsequent matings were made between 'negative' animals.

The progress achieved by selection is illustrated in Table 3. All animals, including the 'negatives', produced antibodies to the impurities in BSA. Efforts were made to keep inbreeding to a minimum. This sometimes necessitated inter-generation matings resulting in half-generation matings resulting in half-generation scores. Advance to selection was rapid, as would be expected if a few genes were responsible. It may be seen from Table 3 that during the course of selection there were no significant changes in the sex-ratio of the offspring. There is evidence for some decrease in litter size, probably due to inbreeding. However, there was no indication of sterility, i.e. all matings produced offspring. It was felt that the plateau of about 90 per cent 'negatives' reached at the fifth generation of selection might be due to the method of selection. The absence of an antibody response is a subjective measurement since it is technically difficult to distinguish between a 'negative' and a very weak responder. To examine this point fourteen 'negatives' at the fifth generation of selection which had been boosted 14 days after sensitisation were re-boosted 44 days

TABLE 3

CHANGES IN THE NUMBER OF 'NEGATIVES' AND THE NUMBER WITH CIRCULATING BSA AS A RESULT OF SELECTION

Generation of selection	$\bar{x}$ litter size at weaning	Females	Males	No. tested	No. 'negative'	Percentage 'negative'	No. with circulating BSA	Percentage with circulating BSA
1·0	7·0	15	20	32	2	6·3	0	0·0
2·0	6·8	15	19	33	4	12·1	0	0·0
3·0	6·6	33	33	58	14	24·1	2	3·5
3·5	6·2	11	20	22	10	45·5	1	4·5
4·0	6·3	20	18	30	19	63·3	5	16·7
4·5	4·4	11	11	19	16	84·2	4	21·0
5·0	6·2	17	14	30	28	93·3	10	33·3
5·5	5·0	10	15	21	19	90·5	1	5·3
6·0	5·3	31	33	60	53	88·3	17	28·3

after sensitization. The results are shown in Table 4. Four of the 'negatives' became positive suggesting they had been very weak responders. The accumulation of weak responders with selection could reduce the selection differential for true 'negatives' to a point where no further selection progress could be achieved. Only one of the seven mice showing circulating BSA produced antibodies on re-injection. As might be expected, it would seem that circulating BSA is a better criterion for 'negativeness' in selected animals, than the absence of detectable antibodies. With the exception of generation 5·5, there is a correlated rise in the number of mice showing circulating BSA with the number showing no antibody response. Further advance to selection might be expected if circulating BSA were used as the criterion for selection.

TABLE 4

THE PROPORTION OF 'NEGATIVES' AND THOSE SHOWING CIRCULATING BSA WHEN BOOSTED 14 DAYS AND AGAIN 44 DAYS AFTER SENSITIZATION WITH BSA IN FREUND'S ADJUVANT

Time boosted (days)	No. 'negative'	No. with circulating BSA	No. 'positive'	Total
14	6	7	1	14
44	3	6	5	14

Genetic control of the failure to produce antibodies to synthetic antigens has been demonstrated in guinea-pigs by Benacerraf, Ojeda and Maurer (1963) and in mice by Pinchuck and Maurer (1965). They suggest that the failure to produce antibodies could be due to a failure in the breakdown of the antigen, or in the postulated recognition system at a level prior to the synthesis of immunoglobulin, i.e. the antigen is not getting to the antibody forming mechanism. Clearly the BSA 'negatives' could be failing to produce antibodies for similar reasons, particularly those showing circulating BSA. There are of course other possible explanations for 'negative' responders. Cinader (1960) suggested that animals are tolerant to their own circulating antigens and hence to a large number of antigenic determinants. Sufficient overlap of determinants between the host and the antigen could render the host partially or completely tolerant to the antigen. Further, it has been suggested by Sobey and Magrath (1965a) that sensitivity to tolerance induction is variable and may be genetically controlled. If at the extreme of the range of tolerance sensitivity, animals regardless of their developmental stage can be rendered tolerant by small injections of the antigen, it would be possible to select a line of animals unable to produce specific antibodies due entirely to their tolerance sensitivity to the specific antigen in question.

Whatever the underlying mechanism may be, it is clear that a specific genetically controlled block in the antibody forming mechanism to BSA exists in mice and probably in rabbits.

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