Clin. exp. Immunol. (1972) **12,** 231–241.

THE HETEROGENEITY OF ANTIBODY AFFINITY IN INBRED MICE AND ITS POSSIBLE IMMUNOPATHOLOGIC SIGNIFICANCE

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(*Received* 13 *April* 1972)

SUMMARY

The range of relative affinity of antibody to HSA in mice from ten strains is presented. Previously reported high and low affinity strains are not exceptional.

Age, sex, dose and time after immunization have little effect on relative affinity of antibody to HSA and HST.

Antibodies, raised by injecting DNP-HSA or DNP-RSA, and tested by ^{3}H-εDNP-L-lysine, show similar interstrain differences of affinity. This excludes recognition of only a limited proportion of the diverse antigenic sites on complex protein antigens as the sole explanation of this phenomenon.

INTRODUCTION

We have previously described interstrain differences in relative affinity of antibody in inbred mice (Soothill & Steward, 1971). The four strains studied were selected because of their reported susceptibility to soluble complex disease following chronic LCM virus infection (Oldstone & Dixon, 1969) and we suggested that this susceptibility might be due to inability to produce high affinity antibody.

The data presented in this paper extend our initial observations to include measurements of relative affinity of antibody to HSA and total antibody in ten strains of mice and include studies of affinity of antibody elicited by immunization with DNP-HSA and DNP-rabbit serum albumin conjugates. Antibody affinity is shown to be independent of age and sex of mouse, antigen dose and immunoglobulin class, and to be related to strain of mouse rather than to type of antigen.

MATERIALS AND METHODS

Mice

Eight inbred strains of mice (SWR/J, B10D2 new, C_3H, $C_{57}B1$, Simpson, AJAX, C/A and CBA) and two random bred strains, A_2G and TO_3 were studied. AJAX mice were

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supplied by the London Hospital Medical College and TO_3 mice were purchased from Animal Suppliers Ltd, London. The rest were maintained at the Animal House of the Institute of Child Health.

Antigens

Human serum albumin (HSA) was supplied by the Blood Products Laboratory of the Lister Institute of Preventive Medicine and was further purified by filtration through Sephadex G-200. Human serum transferrin (HST) was obtained from Sigma Chemical Co. Ltd. DNP-RSA and DNP-HSA were prepared from 2,4 dinitrobenzene sulphonic acid and RSA or HSA by the method of Eisen (1967). The degree of substitution was determined spectrophotometrically.

Immunization

Mice were immunized by intraperitoneal injection of the antigen in 0·1-ml sterile saline once a week for 4 weeks. Two weeks after the fourth injection, serum was obtained. For the determination of the effect of time on antibody affinity, mice were bled at intervals of from 2 to 20 weeks after the last of four weekly injections.

Affinity measurement

Measurement of relative affinity, K_R and of total antibody Ab_t was carried out by the technique previously described using ammonium sulphate precipitation to separate bound and free antigen (Steward & Petty, 1972a, b). HSA and HST were radioiodinated by the iodine monochloride method of McFarlane (1958). For measurement of anti-DNP antibody, radiolabelled ε-DNP lysine was used. This was prepared from ^{3}H-dinitrofluorobenzene (Radiochemical Centre, Amersham) and *N-t*-butyl oxycarbonyl-L-lysine (Sigma Chemical Co. Ltd) by the method of Eisen, Simms & Potter (1968).

Gel filtration

Pools of serum were obtained from groups of six SWR/J, B10D2 new AJAX, C_3H and Simpson mice which had been immunized with HSA. 250 μl of each pool was filtered through a column (57 cm × 1·2 cm) of Sephadex G-200. The column was equilibrated at room temperature in 0·1 M potassium phosphate buffer, pH 6·8 and eluted at 5 ml/hr. First peak (19S) and second peak (7S) fractions were pooled and concentrated by ultrafiltration. The presence of anti-HSA antibody was demonstrated by the ability of the concentrated fractions to bind iodinated HSA in the ammonium sulphate globulin precipitation test.

RESULTS

Affinity determinations in ten strains of mice

The results of affinity determinations of eight inbred and two random bred strains of mice are shown in Fig. 1. The range of values of K_R for antibody to HSA in individual animals was $1{\cdot}0 \times 10^5$ to $9{\cdot}0 \times 10^6$ L/mole. Within each strain, the values were restricted to a range of less than ten-fold. $C_{57}B1$, A_2G, CBA and the two strains known to be LCM nephritis-prone, B10D2 new and SWR/J, have antibody of low affinity; that is the mean value for K_R for each of these strains was less than $1{\cdot}0 \times 10^6$ L/mole. In contrast, C/A, TO_3, Simpson and the two strains known to be nephritis-resistant, AJAX and C_3H, have anti-

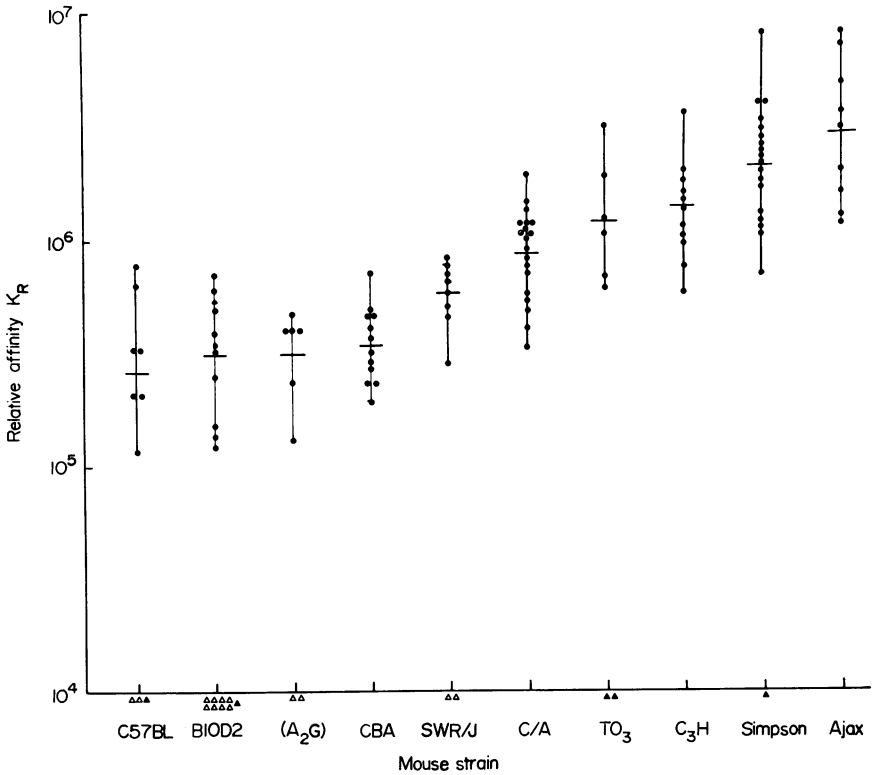

FIG. 1. Relative affinity values of antibody to HSA in ten mouse strains.
▲, Animals producing antibody but K_R incalculable;
△, animals not producing detectable antibody.

TABLE 1. *P* values in Student's *t*-test for the interstrain differences in K_R of anti-HSA antibody

Low affinity strains	High affinity strains				
	AJAX	Simpson	C_3H	TO_3	C/A
$C_{57}B1$	<0·001	<0·001	<0·001	<0·001	<0·001
B10D2 new	<0·001	<0·001	<0·001	<0·001	0·001
A_2G	<0·001	<0·001	<0·001	<0·001	0·001
CBA	<0·001	<0·001	<0·001	0·001	0·001–0·005
SWR/J	<0·001	<0·001	<0·001	0·01–0·02	0·30

HSA antibody with affinity above $1{\cdot}0 \times 10^6$ L/mole. The significance of the differences between affinity of antibody in different strains is tabulated in Table 1. Although the relative affinity values obtained previously were at either end of the range shown here, they were not exceptional. There was no evidence of greater scatter in the random bred strains A_2G and TO_3 than in the rest.

The variation in affinity of Anti-HSA antibodies with time

Antibody affinity has been shown to increase with time after immunization with a single dose of antigen in Freund's complete adjuvant (Eisen & Siskind, 1964). We have used a

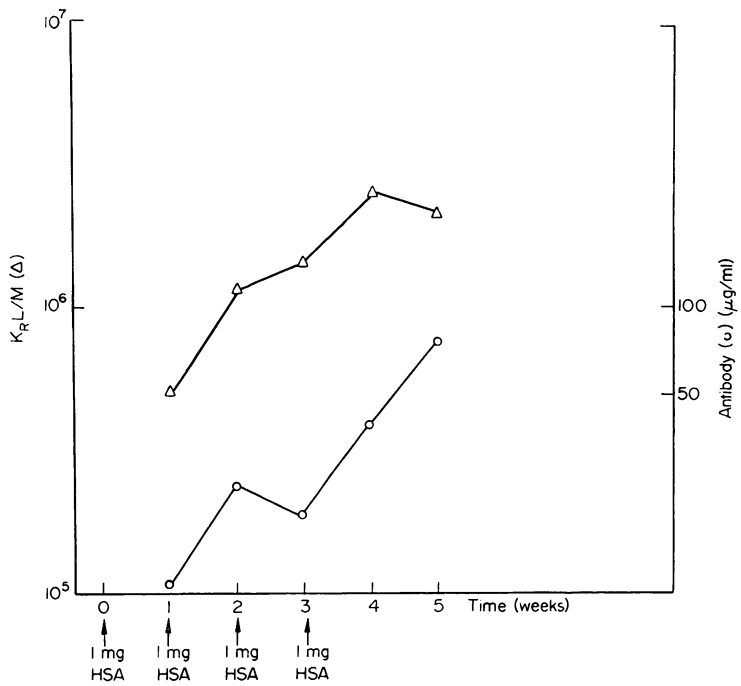

FIG. 2. The change in amount and relative affinity K_R of antibody produced to HSA in AJAX mice during immunization. Each point represents mean values from groups of three mice.

prolonged immunization course injecting antigen in saline at weekly intervals in order to attain a plateau at maximal response. Fig. 2 represents the mean K_R values in AJAX mice bled at intervals during the immunization procedure and up to 2 weeks after the last injection. As expected, both the affinity and amount of antibody rose during the course of this experiment.

The stability of affinity after this time was studied in separate experiments on Simpson and $C_{57}B1$ mice (Fig. 3). The differences between the high K_R (Simpson) and low K_R ($C_{57}B1$) antibody-producing mice persist after 4 weeks in both strains, but both the K_R and the Ab_t fell with time. The number of animals having no detectable antibody increased. Antibody was still detectable in C/A mice 19 weeks after the last of four injections, and the K_R fell

slowly. It appears, therefore, that the rise and fall of affinity with time after immunization is parallel in high and low affinity strains, that the difference in these strains is not due to differences in the rate of production of high K_R antibody, and that low affinity antibody-producing strains do not, ultimately, produce high K_R antibody with this type of immunization.

Effect of antigen dose on affinity

K_R was determined in four inbred strains of mice immunized with widely differing doses of two antigens, HSA and HST (Tables 2 and 3). Within the range used, there was no detect-

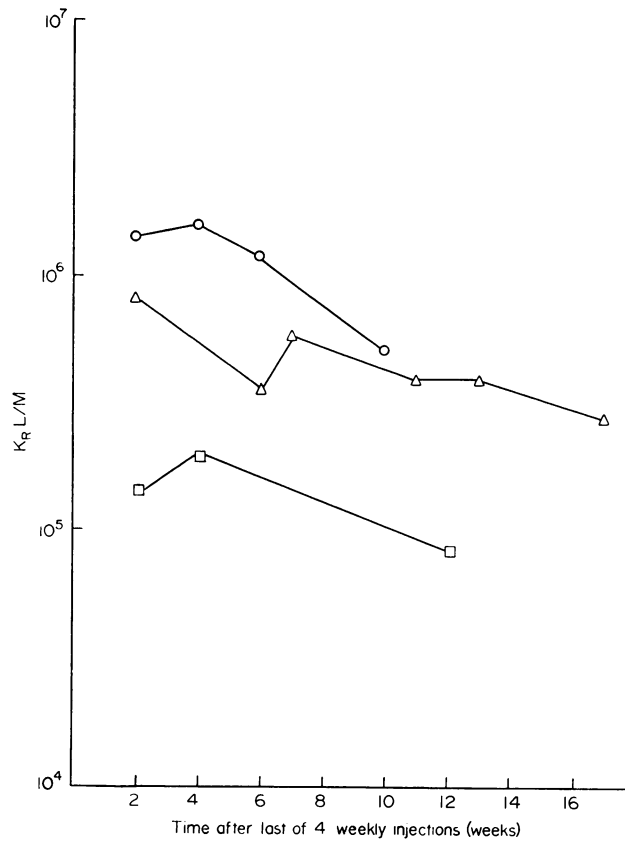

FIG. 3. Change in relative affinity with time after immunization with antigen in saline. (○) Simpson mice; (△) C/A mice; (□) C_{57}Bl mice. Each point represents the mean values from groups of three mice.

able effect of dose of antigen on the affinity of antibody produced. At low antigen doses, no antibody was detected. The proportion of such non-responding animals diminished as higher immunizing doses were used and the highest proportion of responders was produced by the highest antigen dose used (2·0 mg weekly for 4 weeks).

Effect of sex of mouse on affinity of antibody

The data presented in Fig. 1 include values of K_R for both male and female mice. Comparison of K_R and total antibody in each sex of three strains of mice is summarized in Table 4. The K_R of antibody produced by female Simpson mice is slightly higher than that produced by males but that of female B10D2 new and C/A mice is somewhat lower than

TABLE 2. Effect of dose of HSA on antibody level and relative affinity in four strains of mice

Strain	Dose (mg)	Number of mice	Number of responders	Log mean Ab (μg/ml)	Log mean K_R (L/M)
SWR/J	0·100	3	2(+1)	25	$6·0 \times 10^5$
	0·500	6	5	42	$3·5 \times 10^5$
	1·000	10	8	91	$5·7 \times 10^5$
B10D2 new	0·005	3	0	—	—
	0·020	3	0	—	—
	0·050	3	0	—	—
	0·100	4	0	—	—
	0·500	6	3(+2)	15	$5·6 \times 10^5$
	1·000	20	11(+1)	20	$3·1 \times 10^5$
	2·000	6	6	29	$5·5 \times 10^5$
AJAX	0·100	6	6	57	$2·3 \times 10^6$
	0·500	6	6	195	$3·7 \times 10^6$
	1·000	9	9	36	$2·9 \times 10^6$
	2·000	7	5(+1)	44	$1·9 \times 10^6$
C_3H	0·005	3	0	—	—
	0·020	3	1	400	$1·37 \times 10^6$
	0·050	3	0	—	—
	0·100	9	0	—	—
	0·500	9	0	—	—
	1·000	6	6	25	$1·2 \times 10^6$
	2·000	4	3(+1)	75	$0·8 \times 10^6$

Numbers in parentheses refer to animals producing antibody but the K_R values were not calculable.

that produced by males. It is apparent that the differences in K_R between the sexes are insufficient to account for the interstrain differences shown in Fig. 1, the data for which was obtained with mixed groups of both male and female mice.

Effect of age of mouse on affinity of antibody

Mice were immunized with HSA in saline at ages 2, 4, 6, 9 and 12 months in order to determine the effect of age at immunization on relative affinity of antibody produced. The interstrain differences in K_R persist throughout adult life and, with the exception of C_3H mice,

TABLE 3. Effect of dose of HST on antibody level and relative affinity in four strains of mice

Strain	Dose (mg)	Number of mice	Number of responders	Log mean Ab (μg/ml)	Log mean K_R (L/M)
SWR/J	0·100	6	1(+1)	40	$3·1 \times 10^5$
	0·500	4	0	—	—
	1·000	10	8(+2)	68	$9·5 \times 10^5$
B10D2	0·0001	4	0	—	—
	0·0010	4	0	—	—
	0·0050	4	0	—	—
	0·0020	4	0	—	—
	0·020	4	0	—	—
	0·050	4	0	—	—
	0·100	10	0	—	—
	0·500	9	4(+2)	20	$2·7 \times 10^5$
	1·000	9	2(+1)	26	$1·9 \times 10^5$
	2·000	12	5(+2)	14	$3·2 \times 10^5$
AJAX	0·0001	3	0	—	—
	0·0010	3	0	—	—
	0·0050	3	0	—	—
	0·020	3	0	—	—
	0·050	4	3(+1)	40	$4·9 \times 10^6$
	0·100	7	7	89	$6·5 \times 10^6$
	0·500	5	5	47	$5·8 \times 10^6$
	1·000	5	4(+1)	77	$5·8 \times 10^6$
	2·000	6	6	35	$2·5 \times 10^6$
C_3H	0·100	6	6	68	$1·2 \times 10^6$
	0·500	6	3(+3)	50	$2·0 \times 10^6$
	1·000	6	6	123	$3·7 \times 10^6$
	2·000	11	10(+1)	14	$1·9 \times 10^6$

Numbers in parentheses refer to animals producing antibody but the K_R values were not calculable.

TABLE 4. Variation in relative affinity (K_R) with sex in three strains of mice

		Strain		
		B10D2 new	C/A	Simpson
Male	Number	6	16	9
	Mean K_R	$4·3 \times 10^5$	$1·1 \times 10^6$	$1·6 \times 10^6$
Female	Number	6	16	10
	Mean K_R	$2·15 \times 10^5$	$5·4 \times 10^5$	$2·7 \times 10^6$
P (t-test)		$<0·05$	$<0·01$	$<0·05$

no change was noted in K_R of antibody to HSA or in the proportion of mice responding in the age range tested (Table 5). K_R of C_3H mice at 2 months of age was significantly higher than at 9 months ($P = 0{\cdot}01$, Student's *t*-test).

Immunoglobulin class of antibody studied

Anti-HSA antibody activity was detected in the 7S region on Sephadex G 200 gel filtration in pooled sera from SWR/J, B10D2 new, CBA, AJAX, C_3H and Simpson mice. Only one strain (AJAX) had demonstrable anti-HSA activity in the 19S region. The interstrain differences in K_R are therefore probably not due to differences in immunoglobulin class of antibody.

TABLE 5. Effect of age at time of immunization on antibody levels and relative affinity of antibody produced in four strains of mice

Strain	Age (months)	Number of mice	Number of responders	Antibody (μg/ml)	K_R (L/M)
SWR/J	2	10	8	91	$5{\cdot}7\times10^5$
	12	6	6	56	$3{\cdot}5\times10^5$
B10D2	2	5	3	12	$4{\cdot}8\times10^5$
	4	8	2	11	$4{\cdot}9\times10^5$
	9	4	4	26	$8{\cdot}0\times10^5$
	12	8	6	40	$2{\cdot}4\times10^5$
Simpson	2	5	5	327	$1{\cdot}6\times10^6$
	4	9	9	407	$4{\cdot}3\times10^6$
	6	5	5	331	$5{\cdot}2\times10^6$
	9	4	4	183	$3{\cdot}8\times10^6$
C_3H	2	5	5	11	$1{\cdot}6\times10^6$
	4	6	4	44	$4{\cdot}8\times10^5$
	6	5	4	16	$6{\cdot}1\times10^5$
	9	7	6	20	$4{\cdot}8\times10^5$

Affinity of antibody to DNP

SWR/J, B10D2 new, C_3H and AJAX strains were immunized with DNP_8HSA, $DNP_{15}HSA$ or $DNP_{15}RSA$, and antibody was determined by reaction with ^3H-ε-DNP lysine. No difference in affinity or amount of antibody was noted between the animals immunized with DNP_8HSA or $DNP_{15}HSA$, so these data are considered together DNP-HSA) (Table 6).

In a high proportion of mice, no antibody was detected. However, in those animals which responded, higher levels of anti-DNP antibody were produced when the immunogen was DNP-RSA rather than DNP-HSA, though there was no consistent difference in affinity. Mean affinity values of anti-DNP antibody were consistently higher in AJAX and C_3H mice than in B10D2 new and SWR/J mice but the differences were not as great as those seen with antibodies to protein antigens. A ten-fold difference in immunizing dose had no

TABLE 6. Amount and affinity of anti-DNP antibody in four strains of mice immunized with DNP-HSA and DNP-RSA

Strain	Dose (mg)	DNP-HSA*				$DNP_{15}RSA$			
		Number of mice	Number of responders	Antibody (μg/ml)	K (L/M)	Number of mice	Number of responders	Antibody (μg/ml)	K (L/M)
SWR/J	0·1	6	1	64	$1{\cdot}5\times10^5$	6	2	87	$1{\cdot}0\times10^5$
	1·0	14(10)†	2	38	$2{\cdot}1\times10^5$	ND	—	—	—
B10D2	0·1	4	1	29	$1{\cdot}0\times10^5$	7	4	318	$1{\cdot}3\times10^5$
	1·0	13(2)	7	30	$1{\cdot}9\times10^5$	4	2	138	$3{\cdot}4\times10^4$
C_3H	0·1	6(6)	0	—	—	6	3	214	$4{\cdot}0\times10^5$
	1·0	6	6	12	$1{\cdot}5\times10^6$	4	0	—	—
AJAX	0·1	6	6	91	$4{\cdot}3\times10^5$	3	3	58	$6{\cdot}3\times10^5$
	1·0	ND	—	—	—	3	3	208	$5{\cdot}2\times10^5$

* Animals were immunized with either DNP_8 or DNP_{15}-HSA (see text).

† Numbers in parentheses refer to number of animals which died during immunization procedure. ND = not done.

consistent effect on the affinity of antibody produced but considerable differences in antibody levels were noticed in some strains. There was no obvious relationship between affinity and amount of anti-DNP antibody.

DISCUSSION

Measurements of relative affinity of mouse anti-protein antibody previously reported (Soothill & Steward, 1971) have been confirmed and extended to include six other strains of mice. The four strains originally studied were selected because of their known susceptibility or resistance to chronic soluble complex disease induced by LCM virus infection (Oldstone & Dixon, 1969). The range of values obtained in this larger series of strains indicates that these four strains are not exceptional. It is surprising that the scatter for the two random bred strains was not greater than that for the inbred strains. Perhaps this is because their breeding has been very restricted over many generations. The failure of other workers to demonstrate interstrain differences in antibody affinity (Paul, Yoshida & Benacerraf, 1970) may be due to the use of adjuvants in their immunization procedure. If such differences in relative affinity are characteristic of the host rather than of the antigen, and if affinity of antibody has immunopathologic significance then inability of the host to make antibody of high affinity may represent a common immunodeficiency which contributes to disease susceptibility.

Published studies (Dixon, Feldman & Vazquez, 1961; Pincus, Haberkern & Christian, 1968; Christian, 1970) have described the induction of chronic soluble complex disease in a small proportion of animals immunized daily with protein antigens. Wilson & Dixon (1971) have shown that such disease may be induced in all animals making a sustained precipitating antibody response provided sufficient antigen is given, presumably to maintain the antibody in a state of antigen excess. Taking all these data into consideration it appears that

there is a 400-fold range in the dose of antigen required to produce this disease in different rabbits. We suggest that those animals in which a low daily dose of antigen is sufficient to cause chronic soluble complex disease are those which make low affinity antibody and which therefore fail to eliminate the antigen. We have shown that failure of some strains of mice to eliminate antigen is related to the affinity of antibody (Alpers, Steward & Soothill, 1972).

The apparent discrepancy between our observation that affinity of antibody decreased slightly with time after immunization, and the findings of Eisen & Siskind (1964) that it increased, may be an effect of their use of adjuvant. Urbain *et al.* (1972) have also reported a decrease in affinity of anti-BSA antibodies with time in rabbits immunized with antigen in saline.

A wide range of doses of two different protein antigens radically influenced the number of animals responding—none responded to less than 0·02 mg, but there was no fall in the number of responders at the highest dose used, 2 mg. There was no evidence that, when response occurred, either the K_R or the Ab_t was related to dose. The small sex differences noted were not consistent from strain to strain; with the exception of a fall in K_R with age in C_3H mice, affinity was not related to age in the range studied (2–12 months).

One possible explanation of this phenomenon is that the different strains respond to these antigens in the production of antibody of different classes or subclasses, and that these different immunoglobulins have different affinities. Our prolonged immunization course was planned to achieve a secondary response, and the demonstration that antibody was detected in the gel filtration fractions containing IgG, in all strains, and in only one strain was it detectable in the fractions containing IgM, suggests that the interstrain differences are not due to immunoglobulin class differences of response. They may be due to IgG subclass differences.

The protein antigens used in our previous studies were complex, and measurements of antigen binding by antibody raised to them represent the combined effect of reaction of antibodies with several antigenic determinants. The assay for K_R was done in gross antigen excess to achieve conditions in which one antigen molecule is bound to only one antibody-binding site. The use of hapten-protein antigens, while still eliciting a heterogeneous antibody response, permits the use of a single haptenic determinant in the assay of affinity. Thus the demonstration of interstrain differences in affinity of anti-hapten antibody, similar to though smaller than those observed with anti-protein antibody, indicates that these differences are not the result of a complete failure to react to some of the antigen sites on a complex antigen molecule.

Antigen processing may be important in determining the affinity of antibody. We have demonstrated that antigen presented in Freund's complete adjuvant elicits high affinity antibody in strains of mice which produce low affinity antibody when immunized with antigen in saline (Soothill & Steward, 1971; Steward & Petty, 1972c, in preparation; Petty & Steward, 1972, in preparation).

Protein carriers might influence the affinity of anti-hapten antibody. However, in our limited studies, we detected no carrier effect on affinity of anti-DNP antibody in a system which shows an effect on the amount of antibody produced.

ACKNOWLEDGMENTS

The authors thank Mr N. J. West for skilled technical assistance, Mr A. A. Weir, Chief

Animal Technician, Mr D. Cooper and Mr B. Gregory of the Institute of Child Health Animal House for invaluable assistance with the mice. R.E.P. is a recipient of a Canadian Arthritis and Rheumatism Society Research Fellowship.

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